

Adaptor proteins involved in polarized sorting

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Polarized cells such as epithelial cells and neurons exhibit different plasma membrane domains with distinct protein compositions. Recent studies have shown that sorting of transmembrane proteins to the basolateral domain of epithelial cells and the somatodendritic domain of neurons is mediated by recognition of signals in the cytosolic domains of the proteins by adaptors. These adaptors are components of protein coats associated with the trans-Golgi network and/or recycling endosomes. The clathrin-associated adaptor protein 1 (AP-1) complex plays a preeminent role in this process, although other adaptors and coat proteins, such as AP-4, ARH, Numb, exomer, and retromer, have also been implicated.

Cell polarity

Virtually all cells exhibit an asymmetric arrangement of cytoplasmic organelles and the cytoskeleton that is referred to as “cell polarity”. This asymmetry extends to the plasma membrane, which is differentiated into two or more domains with distinct protein and lipid compositions. Cell polarity is particularly critical for multicellular organisms, in which each cell must be perfectly integrated into a complex body plan. Polarity is manifested in many forms, depending on the cell type. For example, the plasma membrane of epithelial cells is differentiated into an apical domain that faces the exterior or lumen of organs, a lateral domain that contacts neighboring cells, and a basal domain that sits on a basement membrane (Fig. 1 A; Bryant and Mostov, 2008; Mellman and Nelson, 2008; Apodaca et al., 2012). The lateral and basal domains share many properties, for which they are often referred to as the “basolateral” domain. Neurons are another prominent type of polarized cell, in which the cytoplasm is organized into dendrites, soma, and axon (Fig. 1 B; Arimura and Kaibuchi, 2007; Lasiecka et al., 2009). Neuronal asymmetry can be extreme, as best exemplified by the axons of spinal motor neurons, which in humans can reach distances of up to one meter from the soma. In some respects, the dendrites and soma are also considered parts of

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Abbreviations used in this paper: AP, adaptor protein; KO, knock-out; LDLR, low density lipoprotein receptor; RE, recycling endosomes; SOP, sensory organ precursor; TfR, transferrin receptor.

a common “somatodendritic” domain. The establishment and maintenance of polarity in various cell types depend on many factors, including extracellular diffusible molecules and their surface receptors, interactions with the extracellular matrix and with other cells, intracellular landmark complexes, signal-transduction pathways, cytoskeletal structures, and protein traffic. These different aspects of cell polarity have been extensively reviewed in the literature (Arimura and Kaibuchi, 2007; Bryant and Mostov, 2008; Mellman and Nelson, 2008; Lasiecka et al., 2009; Apodaca et al., 2012). In this article I will focus on a specific question: how are transmembrane proteins sorted to different plasma membrane domains in various polarized cell types? The discussion will particularly center on the role of adaptor proteins (APs) in cargo recognition for polarized sorting.

Signals for basolateral sorting in epithelial cells

The mechanisms of polarized sorting have been studied in greater detail in epithelial cells (Fig. 1 A). Sorting of transmembrane proteins to the apical and basolateral surfaces is thought to occur by segregation into two types of vesicular transport carriers emanating from the trans-Golgi network (TGN) and/or recycling endosomes (RE; Fig. 1 A). While the TGN participates in the sorting of newly synthesized proteins, RE are involved in returning internalized proteins to the cell surface. Biosynthetic transport from the TGN to the cell surface via RE has also been demonstrated (Ang et al., 2004). Polarized sorting depends on recognition of specific determinants on the cargo proteins. Apical sorting determinants are highly diverse and vaguely defined (Weisz and Rodriguez-Boulan, 2009; Cao et al., 2012). For transmembrane proteins, they have been variously mapped to the extracellular, transmembrane, and cytosolic domains, but their exact nature and mode of action remain poorly understood. A leading theory is that apical determinants promote direct or indirect partitioning into glycosphingolipid- and cholesterol-rich membrane microdomains (i.e., lipid rafts) at the TGN and/or RE, from where apically bound carriers arise (Fig. 1 A). On the other hand, basolateral sorting determinants are often discrete and more precisely defined, earning them the more stringent designation of “signals”. Basolateral signals are generally located in the cytosolic domains of the proteins and in

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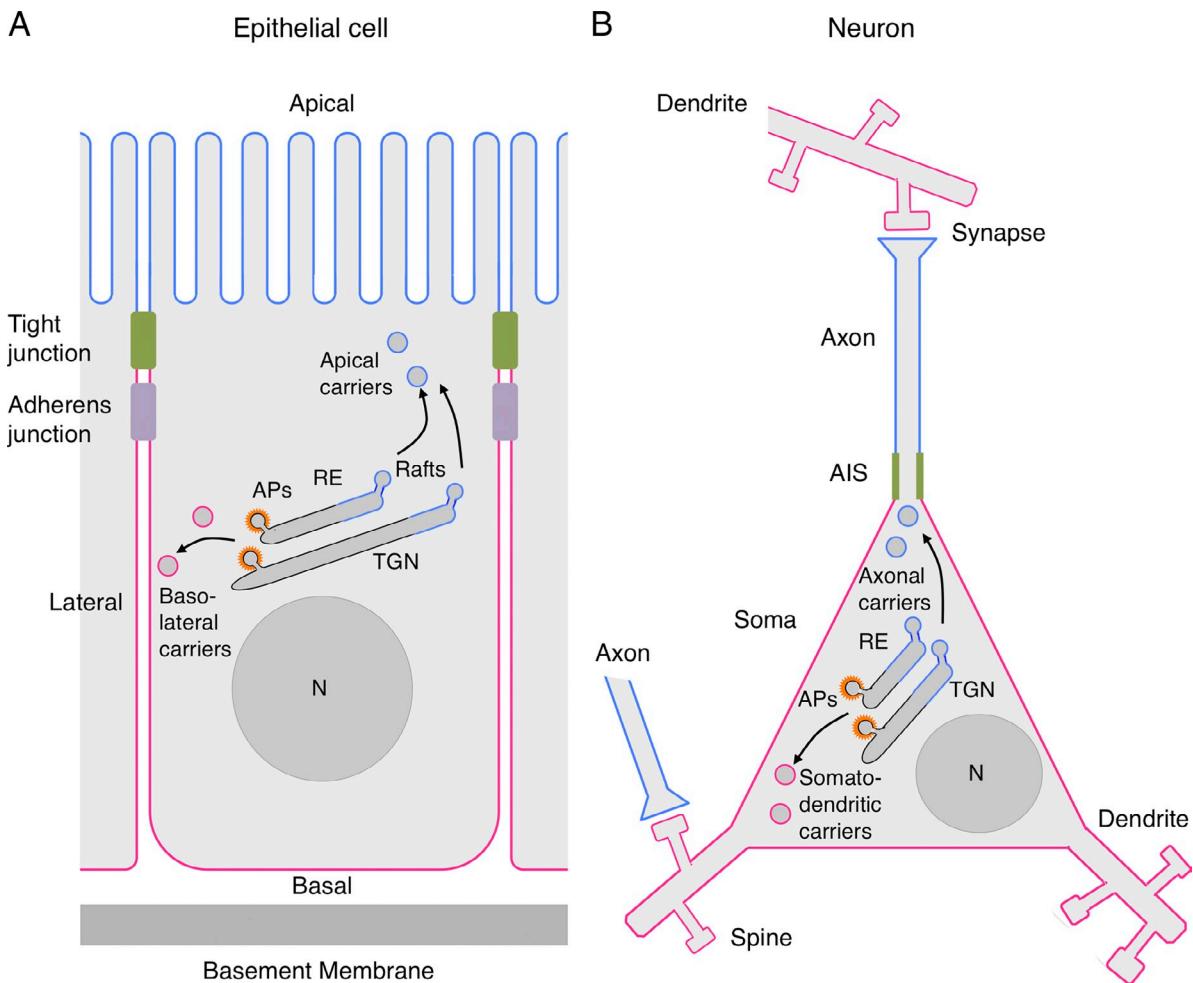


Figure 1. Schematic representation of a polarized epithelial cell and a neuron. (A) The scheme depicts a polarized epithelial cell flanked by two neighboring cells. Tight junctions block the passage of substances through the intercellular space and also serve as a boundary between the apical (blue) and basolateral (red) domains of the plasma membrane. Adherens junctions hold cells together and regulate their proliferation. Vesicular carriers that transport proteins to the apical and basolateral plasma membrane domains are shown to emanate from both the trans-Golgi network (TGN) and recycling endosomes (RE), reflecting polarized sorting in both the biosynthetic and recycling pathways, respectively. Apical carriers are shown to arise from cholesterol- and glycosphingolipid-enriched “raft” domains, whereas basolateral carriers arise from adaptor protein (AP)-enriched “coat” domains. (B) Depiction of a neuron making synapses with two other neurons. Synapses are shown as contacts between an axon terminal and a dendritic spine, but post-synaptic sites also occur on the dendritic shaft or soma. The boundary between the axonal (blue) and somatodendritic (red) domains of the plasma membrane is at the axon initial segment (AIS, green). Axonal and somatodendritic carriers are shown emanating from the TGN and RE, as in epithelial cells. Although both schemes depict polarized sorting occurring by segregation into two types of transport carriers at the TGN/RE, selective retention at or removal from a particular domain of the plasma membrane, as well as transcytosis, also determine polarized distribution of some proteins in both epithelial cells and neurons (Garrido et al., 2001; Gan et al., 2002; Sampo et al., 2003; Anderson et al., 2005; Yap et al., 2008). A trans-endosomal route involving transport from the TGN to RE in the biosynthetic pathway has also been demonstrated (Ang et al., 2004). N, nucleus.

some cases consist of amino acid arrays that fit canonical motifs such as YXXØ and [DE]XXXL[LI] (amino acids denoted in single letter code; X is any amino acid and Ø is a bulky hydrophobic amino acid; Fig. 2 A; Duffield et al., 2008; Gonzalez and Rodriguez-Boulan, 2009). Notably, sequences conforming to these motifs also mediate rapid endocytosis from the cell surface as well as sorting to lysosomes and lysosome-related organelles (Traub and Bonifacino, 2013). Some basolateral signals, however, do not fit any known motif and are functionally distinct from endocytic/lysosomal-sorting signals. For example, the basolateral signal of the transferrin receptor (Tfr) is not the endocytic signal YTRF (a YXXØ motif) but the noncanonical sequence GDNS (Fig. 2 A; Odorizzi and Trowbridge, 1997). Likewise, the basolateral sorting signal of the low density

lipoprotein receptor (LDLR) is not the endocytic signal FDNVPY (a [YF]XNPX[YF] motif) but a bipartite signal comprising a proximal determinant and a distal determinant, both having critical tyrosine and acidic amino acid residues (Fig. 2 A; Matter et al., 1992). Finally, the polymeric immunoglobulin receptor (pIgR) has a noncanonical basolateral signal consisting of a 17-amino acid sequence with a critical HRRNV core that is not involved in endocytosis (Fig. 2 A; Casanova et al., 1991; Aroeti et al., 1993). Mutations in these signals abrogate basolateral sorting, resulting in nonpolarized or apical distribution of the corresponding proteins. An example that is relevant to human disease is the mutation of glycine-823 to aspartic acid in the distal determinant of the LDLR in patients with familial hypercholesterolemia–Turku variant, which causes missorting of the

A

Coxsackie virus and adenovirus receptor (CAR) (YXXØ)
 TM-43-SMSPSNMEGYSKTQ_YNQV_ØPSEDFERTPQSPTLPPA-27

MHC-I-associated invariant chain p33 ([DE]XXXL[LI])
 MDDQRDLISNN_EQLPMLGRGRGAPESKCSR-TM

Transferrin receptor (TfR) (YXXØ and non-canonical)
 15-EPLSYTRF_SLARQVD_GDNSHVEMKLA_VDEEENADN-18-TM

Low density lipoprotein receptor (LDLR) (non-canonical)
 TM-12-FDNPV_YQKTT_EDEVHICHNQD_GYSYPSRQMVSLEDDVA

Polymeric immunoglobulin receptor (plgR) (non-canonical)
 TM-RARHRRNV_DRVSIGSYRTDISMSDLENSREFGAIDNPS-65

B

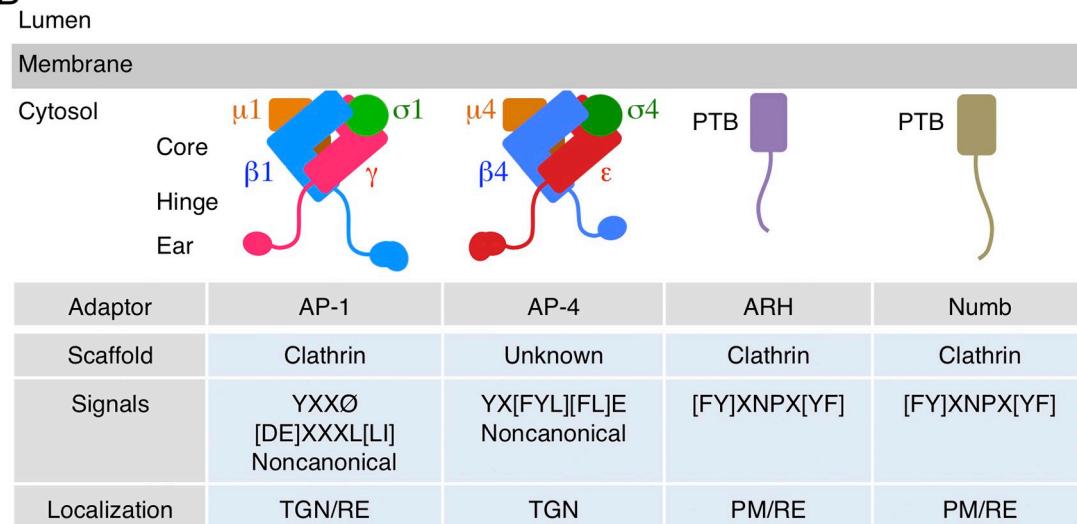


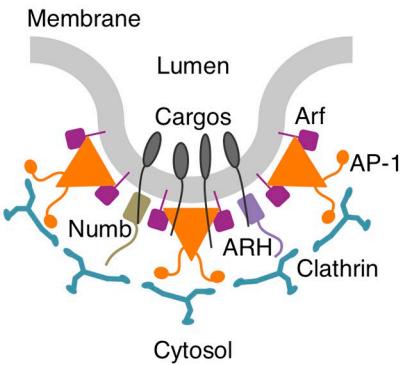
Figure 2. Signals and adaptor proteins involved in polarized sorting. (A) Sequences of the cytosolic tails of the indicated proteins, with critical residues of polarized sorting signals highlighted in red. Signals are categorized as YXXØ, [DE]XXXL[LI], or noncanonical. The transmembrane domain (TM) and the number of additional residues in each tail are indicated. For the TfR, the basolateral sorting signal is GDNS, but the somatodendritic sorting signal is YTRF. The LDLR has a proximal signal with one tyrosine residue and an acidic cluster (EDE) and a distal signal with two tyrosine residues and another acidic cluster (EED). (B) Adaptor proteins that have been implicated in polarized sorting. AP-1 and AP-4 are composed of four homologous subunits, as reflected by the color scheme. The core, hinge, and ear domains of the AP complexes and the PTB domain of ARH and Numb are indicated. Folded regions are represented by geometric shapes and disordered regions by wavy lines. The AP-2, AP-3, AP-5, and COPI-F complexes are homologous to AP-1 and AP-4, but to date they have not been directly implicated in polarized sorting. Three of the AP-1 subunits occur as isoforms encoded by different genes: $\gamma 1$ and $\gamma 2$; $\mu 1A$ and $\mu 1B$; and $\sigma 1A$, $\sigma 1B$, and $\sigma 1C$. PM, plasma membrane. (C) Schematic representation of a clathrin-coated bud on the TGN/RE containing AP-1, Numb, and ARH as adaptor proteins. Arf proteins recruit AP-1 to membranes and promote its conformational activation. Clathrin triskelia polymerize onto the adaptor proteins to form a polyhedral coat. Cargos are gathered into the coated buds by interaction of sorting signals with the adaptor proteins.

LDLR to the apical surface of epithelial cells and the bile canalicular surface of hepatocytes (Koivisto et al., 2001). Decreased basolateral endocytosis of LDL leads to reduced cholesterol clearance from the bloodstream and ensuing hypercholesterolemia.

Role of AP-1 in basolateral sorting

What is the nature of the sorting devices that decode basolateral signals? Before the identification of the basolateral-sorting adaptors, it was known that endocytic/lysosomal-sorting signals fitting the YXXØ and [DE]XXXL[LI] motifs were recognized by some, although not all, members of the family of heterotetrameric AP complexes, which includes AP-1, AP-2, AP-3, AP-4, AP-5, and COPI-F (Fig. 2 B; Robinson, 2004; Hirst et al., 2013). AP family complexes are components of protein coats that mediate cargo selection and coated vesicle formation at different stages of the endomembrane system (Fig. 2, B and C). It was then logical

C



to assume that basolateral sorting would be mediated by one or more of these complexes—but which one(s)? The first hint to the identity of a basolateral-sorting adaptor was provided by the identification of $\mu 1B$, an isoform of the $\mu 1$ subunit of AP-1 (Fig. 2 B) that is specifically expressed in a subset of polarized epithelial cells in mammals (Ohno et al., 1999). This was in contrast to the $\mu 1A$ isoform and the other subunits of AP-1 (γ , $\beta 1$, and $\sigma 1$, and their various isoforms; Fig. 2 B), which were expressed in all cell types. Initial analysis of the role of $\mu 1B$ in basolateral sorting was enabled by the use of the $\mu 1B$ -positive MDCK and the $\mu 1B$ -negative LLC-PK1 epithelial cell lines, derived from kidney distal and proximal tubule cells, respectively. Both cell lines can be cultured as monolayers of polarized columnar cells, but they sort some cargos differently (Roush et al., 1998; Fölsch et al., 1999). For instance, the TfR and LDLR are basolateral in MDCK cells but apical in LLC-PK1 cells (Fölsch et al., 1999). Strikingly,

expression of μ 1B in LLC-PK1 redirected the TfR and LDLR to the basolateral surface, thus demonstrating the critical role of this μ 1 isoform in basolateral sorting (Fölsch et al., 1999). Later studies showed that μ 1B knockdown also impaired basolateral sorting of several cargos, including the TfR and LDLR, in MDCK cells, and that this defect was exacerbated by simultaneous μ 1A knockdown (Gravotta et al., 2012; Carvajal-Gonzalez et al., 2012). These findings thus demonstrated that μ 1A and μ 1B play partly complementary roles in basolateral sorting as alternative subunits of two AP-1 complex variants named AP-1A and AP-1B, respectively. Consistent with the role of AP-1 as a clathrin adaptor (Fig. 2 C), the scaffolding protein clathrin was also found to be required for basolateral sorting (Deborde et al., 2008).

The μ 1B isoform is expressed in some mammalian epithelial cells (e.g., kidney distal and intestinal epithelial cells) but not in other epithelial cells (e.g., kidney proximal and retinal pigmented epithelial cells) or other polarized cell types (e.g., neurons, hepatocytes). This expression pattern is different from that of μ 1A, which is expressed in all cells (Ohno et al., 1999; Diaz et al., 2009; Schreiner et al., 2010). Why did some epithelial cells evolve to express μ 1B? Several studies showed predominant localization of μ 1A and μ 1B to the TGN and RE, respectively (Fölsch et al., 2001, 2003; Gravotta et al., 2012), leading to the notion that AP-1A may be mainly involved in biosynthetic sorting at the TGN and AP-1B in recycling to the basolateral surface from RE. Additionally, AP-1B was shown to be specifically regulated by the small GTP-binding protein Arf6 (Shteyn et al., 2011) and the phosphoinositide phosphatidylinositol 3,4,5-trisphosphate (PIP3; Fields et al., 2010), and to recognize the proximal basolateral sorting signal of the LDLR indirectly through interaction with ARH (Fig. 2 B; Kang and Fölsch, 2011). However, AP-1B was also shown to promote export of the LDLR from the TGN in the absence of AP-1A (Gravotta et al., 2012), and AP-1A was found to participate in endosomal sorting events in other cell types (Eskelin et al., 2002; Delevoye et al., 2009; Hirst et al., 2012). Consistent with these findings, a recent study using high-resolution microscopic and biochemical methods presented an alternative explanation to the μ 1A– μ 1B conundrum: both AP-1A and AP-1B localize indistinctly to the TGN and RE and exhibit similar regulation by Arf family members, but display preferential interactions with some cargos (X. Guo et al., 2013). In particular, AP-1B directly and preferentially binds to basolateral signals from cargos that are sorted in a μ 1B-dependent manner in some cell types. For example, AP-1B binds the noncanonical signals from the LDLR approximately fivefold more strongly than AP-1A (X. Guo et al., 2013). According to these findings, then, expression of μ 1B enables recognition of cargos that are not efficiently recognized by μ 1A. Differential expression of μ 1B may allow alternative sorting of some receptors in different epithelial cell types, as is the case for the LDLR, which is nonpolarized (i.e., both basolateral and apical) in μ 1B-negative proximal kidney tubule cells but exclusively basolateral in μ 1B-positive intestinal epithelial cells (Pathak et al., 1990; Hase et al., 2013). Because cargo binding stabilizes the conformationally active, membrane-associated form of AP-1 (Lee et al., 2008; Ren et al., 2013), AP-1A and AP-1B could function

at either the TGN or RE, depending on where they meet their preferred cargos.

Altered epithelial cell polarity in AP-1 mutant animals

The involvement of AP-1 in polarized sorting in epithelial cells has received strong confirmation from recent studies on whole animals. Knock-out (KO) of the genes encoding the γ 1 (AP1G1) or μ 1A (AP1M1) subunits of AP-1 in mouse caused early embryonic lethality (Zizioli et al., 1999; Meyer et al., 2000). In contrast, μ 1B (AP1M2) KO mice were born at term, although half died before eight weeks of age and the surviving half showed stunted growth (Hase et al., 2013). This reduced viability and growth retardation were due to severe defects in the intestinal epithelial cell layer, including hyperplasia and morphological abnormalities with ensuing nutrient malabsorption. In line with the cell culture studies discussed above, basolateral sorting of some cargos such as the LDLR, the ephrin receptor Eph2B, the adhesion protein E-cadherin, and the interleukin 6 signal transducer IL-6st was impaired in intestinal epithelial cells from the μ 1B KO mice (Takahashi et al., 2011; Hase et al., 2013). Misorting of E-cadherin disrupted its complex with β -catenin at adherens junctions, causing nuclear translocation of β -catenin and increased transcription of β -catenin-regulated genes (Hase et al., 2013). Activation of this signaling pathway is likely responsible for the hyperproliferative phenotype of the intestinal epithelial cells in the μ 1B KO mice. The presence of duodenal polyps in μ 1B KO mice and the reduced expression of μ 1B in some human colorectal tumors suggest that hyperproliferation caused by loss of μ 1B may contribute to the pathogenesis of intestinal cancer (Mimura et al., 2012; Hase et al., 2013). The μ 1B KO mice exhibited an additional pathology: chronic colitis due to misorting of cytokine receptors such as IL-6st, reduced expression of antimicrobial peptides, and impaired secretion of immunoglobulin A (IgA) in the intestine (Takahashi et al., 2011). These perturbations allowed increased penetration of commensal bacteria through the intestinal mucosa, triggering a strong inflammatory response (Takahashi et al., 2011). The inflammatory colitis in μ 1B KO mice is reminiscent of that in humans with Crohn's disease. Indeed, analysis of colonic mucosa from Crohn's patients showed reduced levels of μ 1B mRNA, possibly implicating changes in μ 1B expression in this pathology (Takahashi et al., 2011).

Studies using *Caenorhabditis elegans* (Shafaq-Zadah et al., 2012; Zhang et al., 2012) and zebrafish (Clemens Grisham et al., 2013) have provided additional support for the role of AP-1 and clathrin in the regulation of epithelial cell polarity. RNAi-mediated depletion of *C. elegans* AP-1 subunits, including the APM-1 μ 1 isoform, decreased the polarization of several basolateral transmembrane proteins (Shafaq-Zadah et al., 2012; Zhang et al., 2012). Mutations in the gene encoding the β 1 subunit of AP-1 in zebrafish also mislocalized the basolateral Na^+/K^+ ATPase to the apical surface in auditory and lateral-line hair cells, which are specialized epithelial cells (Clemens Grisham et al., 2013). Mislocalization of the Na^+/K^+ ATPase and other basolateral proteins likely underlie other phenotypes such as abnormal ion homeostasis and mechanotransduction as

well as degeneration of the mutant hair cells (Clemens Grisham et al., 2013).

Unexpectedly, $\mu 1B$ KO mice (Hase et al., 2013) and AP-1-deficient *C. elegans* (Shafaq-Zadah et al., 2012; Zhang et al., 2012) also displayed missorting of apical proteins to the basolateral surface or to intracellular vesicles in intestinal epithelial cells. These included transmembrane proteins (e.g., sucrase, NHX-2, PEPT-1, AQP-4) as well as cytoskeletal proteins (e.g., villin, actin) and the polarity-determinant protein PAR-6. Moreover, loss of AP-1 subunits in both organisms resulted in the appearance of ectopic apical-like membranes along the lateral surface or as invaginations from the lateral membrane (Shafaq-Zadah et al., 2012; Zhang et al., 2012; Hase et al., 2013). Similar phenotypes were observed upon clathrin depletion in *C. elegans* (Zhang et al., 2012). These apical abnormalities resembled those resulting from interference with glycosphingolipid biosynthesis, which affects the lipid raft mechanism of apical sorting (Cao et al., 2012; Zhang et al., 2012). Thus, AP-1 is required for the sorting of both basolateral and apical proteins in intestinal epithelial cells of whole animals. The role of AP-1 in basolateral sorting is most likely direct because of its ability to interact with basolateral sorting signals. How AP-1 mediates apical sorting is less clear. Direct recognition of apical sorting determinants seems unlikely because these determinants are most often found in the luminal or transmembrane domains. Instead, AP-1-dependent sorting of one or more key proteins to the basolateral membrane might be required for exclusion of apical proteins from this membrane domain. Alternatively, AP-1 defects could alter the global properties of TGN or RE membranes such that transport to the basolateral and apical membranes becomes randomized.

Role of AP-1 in cell fate specification during asymmetric cell division

The ability of AP-1 to regulate polarized sorting to different plasma membrane domains has been recently linked to Notch-dependent cell fate specification after asymmetric division of *Drosophila* sensory organ precursor (SOP) cells (Fig. 3 A; Benhra et al., 2011; Cotton et al., 2013). SOP cells arise in the dorsal neuroepithelium (i.e., notum) of *Drosophila* pupae and undergo a series of asymmetric divisions to generate the four specialized cells that make up the mature sensory organ. The first division generates two daughter cells named pIIa (posterior) and pIIb (anterior). Notch, a transmembrane signaling receptor for Delta family ligands, is activated in pIIa cells and inhibited in pIIb cells, leading each cell along a different specification pathway. Activation of Notch requires another transmembrane protein named Sanpodo. How is signaling by Notch/Sanpodo differentially regulated in pIIa and pIIb cells? During asymmetric division of the SOP cell, the monomeric PTB-containing adaptor Numb (Fig. 2 B) is unequally partitioned into the pIIb cell, where it prevents Notch and Sanpodo localization to the cell surface, thus suppressing Notch signaling (Fig. 3 A). Notch/Sanpodo are internalized in an AP-2-dependent manner, but expression of Numb has no effect on the rate of endocytosis (Berdnik et al., 2002; Cotton et al., 2013; Couturier et al., 2013). Instead, Numb regulates Notch/Sanpodo traffic through effects

on AP-1 (Benhra et al., 2011; Cotton et al., 2013). In the SOP and pIIb cells Sanpodo is mainly localized to endosomes, whereas in the pIIa cell it localizes to the basolateral plasma membrane. Silencing or mutation of AP-1 subunit genes causes Sanpodo redistribution to the apical surface of all of these cells, indicating that AP-1 mediates basolateral sorting as in other polarized epithelia (Benhra et al., 2011). In the pIIb cell, Numb inhibits the ability of AP-1 to return Sanpodo to the basolateral surface, shifting its steady-state localization to endosomes. This function is dependent on the YTNP sequence (fitting a [FY]XNPX[FY] motif) in the Sanpodo N-terminal tail, which binds to the Numb PTB domain (Tong et al., 2010; Benhra et al., 2011). Redistribution of Sanpodo to the plasma membrane by mutation of genes encoding Numb or AP-1 subunits causes Notch activation, resulting in pIIb-to-pIIa cell fate transformation (Benhra et al., 2011; Cotton et al., 2013). Regulation of Notch activation by this process starts during cytokinesis of the SOP cell (Couturier et al., 2013), suggesting that AP-1 exerts its role even before separation of the daughter cells. Thus, Numb functions in endosomes to prevent AP-1-mediated recycling of Notch/Sanpodo to the pIIb cell surface, in line with previous studies implicating Numb in the regulation of endosomal traffic in mammals (McGill et al., 2009) and *C. elegans* (Nilsson et al., 2008). These findings demonstrate that some adaptors (e.g., Numb) can counteract the ability of other adaptors (e.g., AP-1) to promote transport. The molecular mechanisms involved in such inhibitory effects, however, remain to be elucidated.

Function of AP-1 in sorting to the neuronal somatodendritic domain

The mechanisms of protein sorting to the axonal and somatodendritic domains of neurons are thought to be similar, although not identical, to those of sorting to the apical and basolateral domains of epithelial cells, respectively (Fig. 1 B; Dotti and Simons, 1990; Jareb and Bunker, 1998). The similarities are particularly apparent for somatodendritic sorting signals, which also map to the cytosolic domains of the proteins and in some cases are identical to basolateral sorting signals (Jareb and Bunker, 1998). For example, the sequence YNQV (a YXXØ motif) in the cytosolic tail of the Coxsackie and adenovirus receptor (CAR; Fig. 2 A) mediates both basolateral (Cohen et al., 2001; Carvajal-Gonzalez et al., 2012) and somatodendritic sorting (Farías et al., 2012). In other cases, somatodendritic and basolateral sorting signals differ. For instance, the somatodendritic sorting signal of the TfR is the sequence YTRF (a YXXØ motif) and not the basolateral signal GDNS (Fig. 2 A; Farías et al., 2012). Both the CAR and TfR somatodendritic signals are directly recognized by $\mu 1A$ (Carvajal-Gonzalez et al., 2012; Farías et al., 2012), the only $\mu 1$ subunit isoform expressed in the brain (Ohno et al., 1999). Expression of a $\gamma 1$ subunit of AP-1 abrogates the somatodendritic polarity of TfR and CAR, as well as that of metabotropic and NMDA-type glutamate receptor proteins, causing them to become evenly distributed between the somatodendritic and axonal domains (Farías et al., 2012). Analysis of TfR traffic showed that this loss of polarity upon AP-1 interference is due to misincorporation of

A

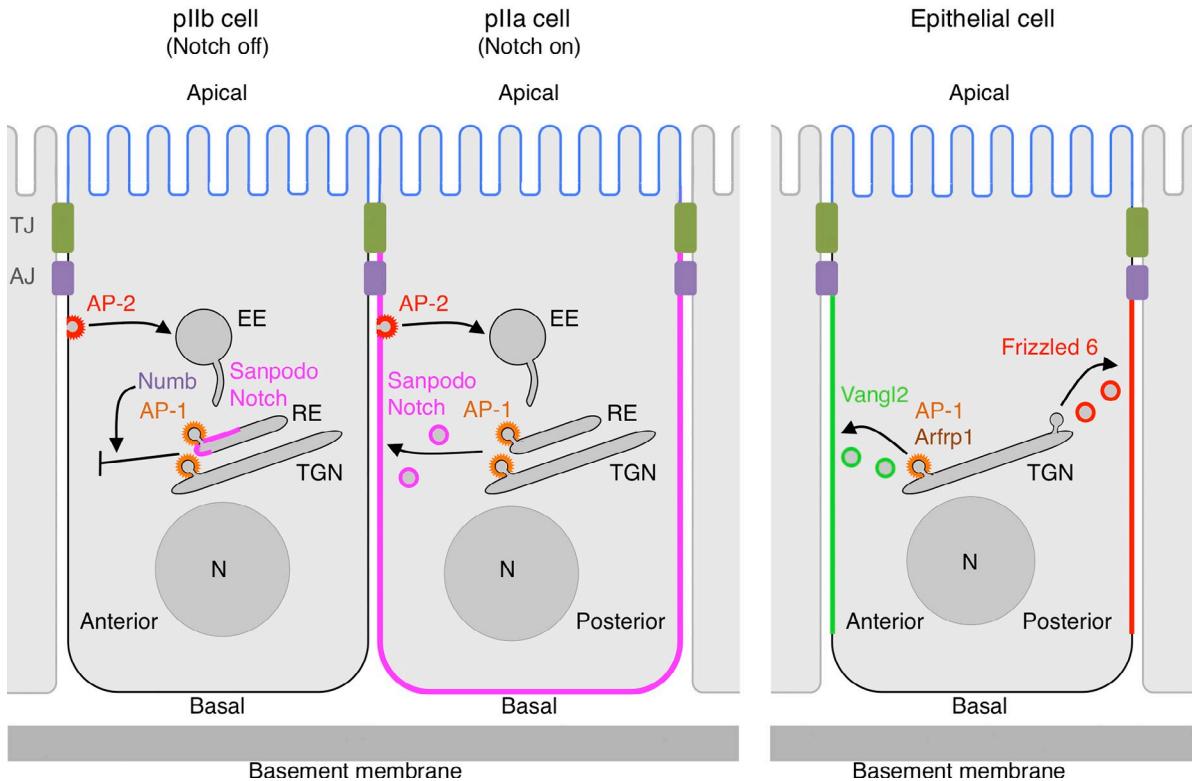


Figure 3. Role of AP-1 in cell fate specification and planar cell polarity. (A) Asymmetric inheritance of Numb in *Drosophila* sensory organ precursor (SOP) daughter cells determines AP-1-dependent inhibition of Sanpodo and Notch recycling in the pllb cell (Numb positive) and allows AP-1-dependent recycling of Sanpodo and Notch in the plla cell (Numb negative; Benhra et al., 2011; Cotton et al., 2013). Notch signaling is thus inhibited in the pllb cell and activated in the plla cell. The related AP-2 complex mediates Notch and Sanpodo internalization, but Numb does not appear to regulate this process (Cotton et al., 2013; Couturier et al., 2013). The anterior or posterior orientation of the SOP daughter cells in the plane of the notum is indicated. EE, early endosome. (B) AP-1 in association with the Arf-like protein Arfrp1 mediates export of the planar cell polarity (PCP) regulator Vangl2 from the TGN to the anterior (or proximal) surface of the lateral membrane domain in epithelial cells [Y. Guo et al., 2013]. Another PCP regulator, Frizzled 6, is targeted to the posterior (or distal) surface by an unknown mechanism. AJ, adherens junction; EE, early endosome; N, nucleus; TJ, tight junction.

TfR into axonal transport carriers, from which the receptor is normally excluded. Similar effects were elicited by expression of a clathrin dominant-negative mutant, indicating that, as in basolateral sorting, AP-1 and clathrin function together in signal-mediated cargo sorting to the somatodendritic domain (Farías et al., 2012).

AP-1 and clathrin have also been implicated in sorting of various receptors and transporters to dendrites in *C. elegans* neurons (Dwyer et al., 2001; Bae et al., 2006; Margeta et al., 2009). The $\mu 1$ isoform involved in dendritic sorting is UNC-101 and not the basolateral-sorting APM-1 (Shafiq-Zadah et al., 2012; Zhang et al., 2012), providing another example of the tissue-specific roles of different $\mu 1$ isoforms. Also in this organism, dendritic sorting is mediated by the cytosolic tails of proteins. Mutation of either UNC-101 or the cytosolic tails of the proteins resulted in even distribution between dendrites and axons. In some neurons (e.g., AWA) UNC-101 mutation blocked formation of dendritic carriers (Dwyer et al., 2001), whereas in others (e.g., RIA) it prevented retrieval of the proteins from axonal presynaptic sites (Margeta et al., 2009), suggesting that the mechanisms of AP-1 action may vary in different types of neurons. Another monomeric clathrin adaptor, UNC-11 (homologous to mammalian AP180), was implicated in axonal sorting of the synaptic vesicle protein synaptobrevin

(Dwyer et al., 2001) but not dendritic sorting of several receptors (Dwyer et al., 2001; Margeta et al., 2009) in *C. elegans*.

Defects in somatodendritic sorting may underlie two human neurodevelopmental disorders caused by mutations in isoforms of the $\sigma 1$ subunit of AP-1 (Fig. 2 B): the MEDNIK syndrome ($\sigma 1A$ mutation; Montpetit et al., 2008) and Fried-type X-linked mental retardation ($\sigma 1B$ mutation; Tarpey et al., 2006). Both diseases present with intellectual disability and other neurological abnormalities. In addition, MEDNIK patients exhibit enteropathy and skin conditions. The organs affected (i.e., brain, spinal cord, intestines, skin) are all consistent with the role of AP-1 in polarized sorting in neurons and epithelial cells. Because isoforms of $\sigma 1$ exhibit distinct preferences for subsets of [DE]XXXL[LI] signals (Mattera et al., 2011), it is likely that the pathogenesis of these diseases involves defective polarized sorting of select transmembrane cargos bearing [DE]XXXL[LI] or related signals.

Models for AP-1 function in epithelial and neuronal polarized sorting

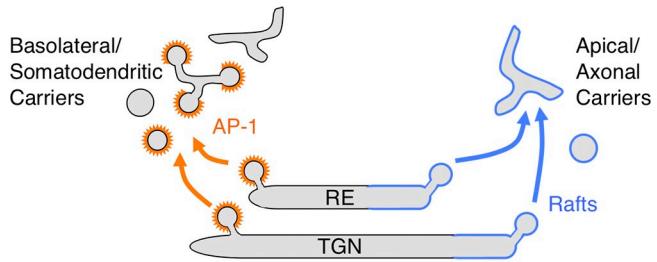
AP-1 has been localized to both the TGN and RE (Klumperman et al., 1993; Futter et al., 1998), where it likely functions to select basolateral or somatodendritic cargos for packaging into

a population of clathrin-coated vesicles (CCVs) or pleiomorphic transport carriers having clathrin-coated buds (Fig. 2 C and Fig. 4 A; Polishchuk et al., 2006). These intermediates could themselves deliver cargos to the corresponding plasma membrane domain. Alternatively, they could uncoat soon after budding and undergo homotypic or heterotypic fusion to generate the actual transport carriers. Importantly, in many cases AP-1 and clathrin are not strictly required for cargo transport to the basolateral or somatodendritic domain; rather, they are required for exclusion of these cargos from the apical and axonal domains (Deborde et al., 2008; Carvajal-Gonzalez et al., 2012; Farías et al., 2012; Gravotta et al., 2012). Therefore, the main role of AP-1 might be to capture cargos into CCVs so that they are excluded from apical/axonal carriers. AP-1 could also function to retrieve basolateral/somatodendritic cargos from apical/axonal carriers budding from the TGN/RE (Fig. 4 B) in a manner analogous to the retrieval of mannose 6-phosphate receptors from immature secretory vesicles (Klumperman et al., 1998). Finally, AP-1 could function not just to form CCVs but to differentiate a domain of the TGN/RE into which basolateral/somatodendritic cargos are segregated away from sites of apical/axonal-carrier formation (Fig. 4 C). In this latter model, interference with AP-1 or clathrin could disrupt not only the sorting of basolateral/somatodendritic cargos but also the membrane domain organization of the TGN/RE, leading to concomitant missorting of apical/axonal cargos, as observed in AP-1-deficient mice (Hase et al., 2013) and *C. elegans* (Shafaq-Zadah et al., 2012; Zhang et al., 2012).

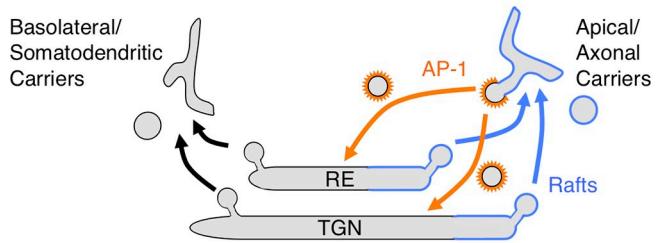
Role of AP-4 in polarized sorting

AP-4 is another member of the heterotetrameric AP family that is associated with the TGN (Fig. 2 B; Dell'Angelica et al., 1999; Hirst et al., 1999). Unlike AP-1, however, AP-4 does not associate with clathrin and might therefore be part of a nonclathrin coat. The $\mu 4$ subunit of AP-4 was shown to interact with the cytosolic tails of several basolateral cargos, including the LDR, and antisense-RNA-mediated knockdown of $\mu 4$ caused mislocalization of those cargos to the apical surface (Simmen et al., 2002). However, ablation of the gene encoding the $\beta 4$ subunit of AP-4 in mouse did not cause any obvious defects in epithelia (Matsuda et al., 2008; Hase et al., 2013). In fact, these mice were perfectly viable and only exhibited mild motor impairment (Matsuda et al., 2008). Histological analysis showed mislocalization of the normally dendritic AMPA-type glutamate receptors and their regulatory proteins, TARPs, to axonal autophagosomes in cerebellar Purkinje neurons (Matsuda et al., 2008). The dendritic Delta2 glutamate receptor and LDR were also mislocalized to axons, whereas metabotropic and NMDA-type glutamate receptor proteins were not affected (Matsuda and Yuzaki, 2008). Mutations in each of the four subunits of AP-4 have recently been identified as causes of a neurodevelopmental disorder with characteristics of hereditary spastic paraparesis in humans (Verkerk et al., 2009; Abou Jamra et al., 2011; Moreno-De-Luca et al., 2011). In contrast to the mild neurological impairment of $\beta 4$ KO mice, the symptoms of this disease in humans are very severe, including microcephaly, cerebral palsy, and profound intellectual disability. Post-mortem immunohistochemical analysis also showed increased accumulation of

A Formation of basolateral/somatodendritic transport carriers



B Retrieval from apical/axonal carriers



C Segregation to coated domains of the TGN/RE

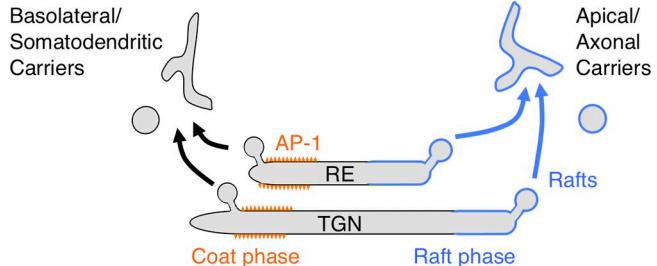


Figure 4. Models of AP-1 function in polarized sorting at the TGN and RE. The schemes depict three models for the function of AP-1 in sorting to the basolateral domain of epithelial cells and the somatodendritic domain of neurons. In model A, AP-1 sorts cargos into clathrin-coated vesicles or pleiomorphic transport carriers at the TGN/RE. These intermediates lose their coats and eventually deliver their cargos to the basolateral or somatodendritic plasma membrane domains. In model B, AP-1 removes basolateral or somatodendritic cargos from apical or axonal transport carriers to return them to the TGN/RE. In model C, AP-1 segregates basolateral or somatodendritic cargos into a "coat phase" of the TGN/RE, away from the "raft phase" that gives rise to apical or axonal carriers.

Delta2 glutamate receptors in the soma of Purkinje neurons from an affected patient (Verkerk et al., 2009), consistent with decreased transport to the dendrites. The $\mu 4$ subunit interacts with the cytosolic tails of the TARPs and the Delta2 glutamate receptor via noncanonical sequences containing phenylalanine and tyrosine residues (Yap et al., 2003; Matsuda et al., 2008), indicating that AP-4 may function in a manner similar to AP-1 to sort a subset of neuronal cargos into dendritic transport carriers (Figs. 1 A and 4).

Other sorting events, other coats

AP-1 has been implicated in many other sorting processes, some related, some unrelated, to cell polarity. In the related category is the requirement of AP-1 and clathrin for secretion of the signaling protein Wnt3a from the basolateral surface in epithelial cells, probably through a role of AP-1 and clathrin in

basolateral sorting of the Wntless receptor (Yamamoto et al., 2013). AP-1 is also required for lysosome exocytosis from the basolateral surface, likely due to its role in basolateral sorting of the target-SNARE syntaxin 4 (Reales et al., 2011; Xu et al., 2012). In addition, AP-1 promotes delivery of another syntaxin, KNOLLE, to the cell plate in dividing plant cells (Teh et al., 2013). Moreover, AP-1 may be involved in some forms of planar cell polarity (PCP; Y. Guo et al., 2013) in which cellular structures are asymmetrically distributed within the plane of the epithelium (Y. Guo et al., 2013). PCP involves further differentiation of the lateral membrane into anterior/proximal and posterior/distal domains (Fig. 3 B). In this regard, AP-1, in conjunction with the Arf-related protein Arfrp1/Arl3, has recently been shown to promote export of the anterior/proximal signaling protein Vangl2 from the TGN to the plasma membrane through recognition of a YYXXF motif (Y. Guo et al., 2013). Another TGN-associated coat complex named “exomer” mediates export of the chitin synthase Chs3p to the bud neck and the cell fusion protein Fus1p to mating projections in yeast (Wang et al., 2006; Barfield et al., 2009). In the absence of exomer, these cargos are retained in the TGN or RE, but mutation of AP-1 subunit genes restores their transport to the plasma membrane (Valdivia et al., 2002). Thus, under these conditions AP-1 mediates intracellular retention of Chs3p, through recognition of a [DE]XXXX[L/I] signal in the Chs3p tail (Starr et al., 2012). Taken together, these findings indicate that AP-1 can exert both positive and negative effects on cargo transport from the TGN/RE to different cell surface domains. The list of functions ascribed to AP-1 is much longer and also includes bidirectional transport between the TGN and endosomes (Hirst et al., 2012), sorting to maturing melanosomes (Delevoye et al., 2009), biogenesis of Weibel-Palade bodies (Lui-Roberts et al., 2005), and many others. How could AP-1 mediate so many different processes? A likely explanation is that the activity of AP-1 is modified by interactions with other adaptors or accessory proteins, as exemplified by ARH (Kang and Fölsch, 2011), Numb (Cotton et al., 2013), and Arfrp1 (Y. Guo et al., 2013) in the cases discussed in the previous sections. The outcome of a particular AP-1 signal-recognition event would thus depend on what other interacting proteins are involved. Another possibility is that AP-1 functions more generally as a domain organizer at the TGN/RE, enabling sorting to occur by additional mechanisms.

This discussion has focused mainly on AP-1 and related proteins because their roles in polarized sorting are the best understood. However, other coats are also likely to be involved. One of these is the “retromer”, a structurally distinct protein complex that primarily mediates retrograde transport from endosomes to the TGN (Seaman et al., 1998). Analyses of polarized sorting have shown that retromer is required for maintenance of the TGF- β receptor II at the basolateral surface (Yin et al., 2013), basolateral-to-apical transcytosis of the polymeric immunoglobulin receptor in polarized epithelial cells (Vergés et al., 2004), and trafficking of the polarity determinants Crumbs, Par6, and aPKC to the apical surface of epithelial cells (Pocha et al., 2011). Finally, the sorting nexin SNX17, which is structurally related to two of the retromer subunits, directs recycling of the LDLR-related protein LRP1 from endosomes to

the basolateral surface through interaction of an IXNPXY motif (a variant of the [FY]XNPX[YF] motif) in the LRP1 tail with the FERM domain of SNX17 (Donoso et al., 2009; Farfán et al., 2013).

Concluding remarks

The evidence reviewed here makes it abundantly clear that sorting of transmembrane proteins to the basolateral surface of epithelial cells, the somatodendritic surface of neurons, and various specialized plasma membrane domains in other cells is dependent on specific interactions of the transmembrane protein tails with adaptors or other coat proteins associated with the cytosolic aspect of the TGN and RE. The molecular mechanisms involved in this process are thus similar to those responsible for clathrin-mediated endocytosis from the cell surface (Traub and Bonifacino, 2013). The AP-1 clathrin adaptor appears to play a central role in polarized sorting in the same manner that AP-2 functions as a core component of the endocytic machinery. However, many outstanding issues remain to be investigated, including the exact compartment where polarized sorting takes place, the relationship of polarized sorting to other sorting events mediated by AP-1, how AP-1 could mediate both export from and retention at the TGN/RE, the mechanisms by which AP-4 and retromer participate in polarized sorting, and the potential involvement of other coat proteins.

Future work should also address whether adaptor and coat proteins influence events that follow the sorting of cargo into transport carriers, such as the translocation of the carriers through the cytoplasm and their fusion with the corresponding target organelles. In this regard, AP-1 has been shown to interact directly or indirectly with components of the microtubule motors kinesin-1 (Schmidt et al., 2009) and kinesin-3 (Nakagawa et al., 2000), although the role of these interactions in polarized sorting remains to be determined. Adaptors or other coat proteins are also likely to select the SNARE proteins that mediate fusion of transport carriers with their target organelles. For example, syntaxin 4 is sorted to the basolateral surface of epithelial cells by virtue of interaction with AP-1 (Xu et al., 2012). Assembly of the vesicle-SNARE cellubrevin/VAMP3 with syntaxin 4 and other cognate SNAREs then allows for the delivery of AP-1B cargos to the basolateral surface (Fields et al., 2007). The exocyst complex, which promotes tethering and fusion of transport carriers with the basolateral plasma membrane, is also recruited to recycling endosomes in an AP-1B-dependent manner (Fölsch et al., 2003). The molecular details of these interactions and their exact roles in polarized sorting are, however, poorly understood. The elucidation of these mechanisms should help explain how failure of polarized sorting underlies various genetic and acquired diseases. With powerful molecular, structural, genetic, and imaging tools now at hand, we can expect many exciting developments in this area of research in the coming years.

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