Protein Sorting at the *trans*-Golgi Network

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Abstract

The *trans*-Golgi network (TGN) is an important cargo sorting station within the cell where newly synthesized proteins are packaged into distinct transport carriers that are targeted to various destinations. To maintain the fidelity of protein transport, elaborate protein sorting machinery is employed to mediate sorting of specific cargo proteins into distinct transport carriers. Protein sorting requires assembly of the cytosolic sorting machinery onto the TGN membrane and capture of cargo proteins. We review the cytosolic and transmembrane sorting machinery that function at the TGN and describe molecular interactions and regulatory mechanisms that enable accurate protein sorting. In addition, we highlight the importance of TGN sorting in physiology and disease.

Contents

OVERVIEW

Approximately one-third of newly synthesized proteins pass though the Golgi apparatus en route to various cellular destinations. The Golgi complex in higher eukaryotes is composed of flattened and fenestrated membrane disks or cisternae (Ladinsky et al. 1999). Several cisternae are aligned in parallel to form a stack. The Golgi stack is compartmentalized into *cis*, *medial*, and *trans* compartments that are enriched with specific Golgi enzymes. The *trans*-most cisterna of the Golgi is continuous with a tubular, branching, and reticulating compartment termed the *trans*-Golgi network (TGN) (**Figure 1**) (Klumperman 2011). Newly synthesized secretory proteins enter the

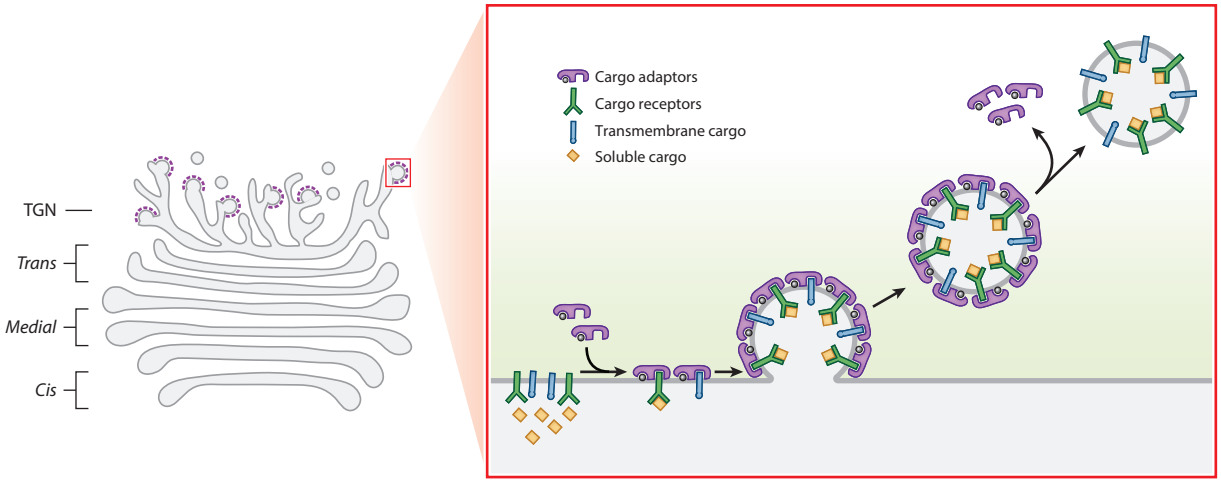


Figure 1

Schematic view of protein sorting at the *trans*-Golgi network (TGN). Diagram of the Golgi apparatus and TGN. (*inset*) The TGN protein sorting process mediated by cargo adaptors and cargo receptors.

Golgi complex at the *cis* face and exit at the TGN. TGN size and structure undergo dynamic changes depending on the level of incoming and outgoing cargo molecules (De Matteis & Luini 2008). Clathrin-coated buds and tubules are detected exclusively at the *trans*-most cisterna/TGN, whereas buds identified in other Golgi cisternae are not clathrin coated (**Figure 1**) (Ladinsky et al. 2002, Mogelsvang et al. 2004). COPI-coated buds are also identified in the TGN but with much lower frequency (Klumperman 2011).

The TGN has conventionally been viewed as the main cargo sorting station where proteins and lipids are sorted into distinct transport carriers that are targeted to various downstream destinations. These destinations include apical and basolateral plasma membrane, endosomal compartments, and additional compartments in specialized cells, such as secretory granules (SGs) (De Matteis & Luini 2008). To achieve high fidelity of protein transport in the complicated TGN exit routes, cells employ elaborate cargo sorting machineries to accurately package the right cargo molecules into the right transport carriers. Sorting is a process in which specific cargo molecules are concentrated in specific membrane microdomains from which other proteins, such as the resident proteins, may be excluded (**Figure 1**, inset). The key players in the sorting process include various cytosolic cargo adaptors that are recruited to the TGN membrane to directly or indirectly bind cargo molecules. Some proteins, in particular luminal proteins, are associated with cargo adaptors indirectly through transmembrane cargo receptors. Many of the cargo adaptors recruit clathrin, and polymerization of clathrin along with the associated cargo adaptors forms characteristic electron-dense membrane coat structures. Assembly of vesicle coat structures concentrates the associated cargo molecules into coated membrane patches and, with the help of other cellular factors, causes membrane deformation, leading to vesicle budding (**Figure 1**, inset). Once vesicles are released from the membranes, vesicle coat proteins disassociate to perform additional rounds of protein sorting. In this review, we describe the functional roles of various cargo adaptors and cargo receptors at the TGN, as well as molecular mechanisms that regulate the protein sorting process and roles of protein sorting at the TGN in various physiological processes.

CARGO ADAPTORS RECOGNIZING TRANSMEMBRANE PROTEINS AT THE TGN

Cytosolic cargo adaptors play a fundamental role in protein sorting at the TGN. Adaptors are recruited to the membrane to recognize specific sorting signals located within the cytosolic domains of transmembrane cargo molecules, thereby marking them for enrichment into nascent vesicles. In this section, we summarize examples of cargo adaptors, focusing on their physiological roles, mechanisms of binding to sorting signals, and functional relationships.

Adaptor Complexes

The heterotetrameric adaptor protein complexes (APs) are the most well-characterized cargo adaptors at the TGN. APs are composed of two large subunits (~100–160 kD), one medium subunit (~50 kD), and one small subunit (~20 kD) (**Figure 2*ai***). The N-terminal regions of the two large subunits encompass the small and medium subunits, forming the trunk or core domain, which binds Arf proteins, cargo molecules, and phospholipids (Owen et al. 2004). The C termini of the large subunits form appendage domains that bind several accessory proteins, such as epsins and AP180 (Owen et al. 2004). The trunk and appendage domains are separated by a flexible linker region.

Five APs have been identified in higher eukaryotes, and three of them (AP-1, AP-3, and AP-4) are involved in protein sorting at the TGN (**Figure 2*ai***). The flexible linker regions of AP-1 contain clathrin box motifs that recruit clathrin. AP-3 also contains a clathrin box motif in the appendage domain of its β subunit, but whether AP-3 recruits clathrin physiologically remains controversial (Newell-Litwa et al. 2007). AP-4 is not associated with clathrin. The medium (μ) subunit of AP-1 has two isoforms, μ1A and μ1B, which are incorporated into complexes termed AP-1A and AP-1B, respectively (**Figure 2*ai***). AP-1A is expressed ubiquitously, whereas AP-1B is expressed only in epithelial cells; both have important roles in regulating basolateral polarity, described below. Similarly, the β and μ subunits of AP-3 have two isoforms that are incorporated into complexes termed AP-3A and AP-3B (**Figure 2*ai***). AP-3A is expressed ubiquitously, and AP-3B is expressed only in neurons and neuroendocrine tissues (Newell-Litwa et al. 2007).

Mutagenesis and gene-knockout studies in model organisms and natural mutations in human patients demonstrate that the adaptor complexes play important roles in various physiological processes (summarized in **Table 1**). APs recognize cargo molecules bearing tyrosine-based sorting motifs (YXXΦ), where Φ is an amino acid containing a bulky hydrophobic side chain, and dileucine sorting motifs ([DE]XXXL[LI]). In the biosynthetic pathway, the tyrosine sorting motif is important for sorting to lysosomes, the basolateral surface of polarized epithelial cells, and the somatodendritic domain of neural cells (Farias et al. 2012, Owen et al. 2004). This motif is recognized by the C-terminal domain of the μ subunit of APs, which contains 16 or 17 β strands forming two β-sandwich subdomains (Mardones et al. 2013, Owen & Evans 1998). The peptides bearing the YXXΦ motif adopt an extended conformation with the Y and Φ residues recognized by the hydrophobic binding pocket of strand β1 and β16 within the μ subunit (**Figure 2*aii***) (Mardones et al. 2013, Owen & Evans 1998). The interaction is further stabilized by additional hydrogen bonds between the backbone of the peptide and the β sheet from the μ subunit (Mardones et al. 2013, Owen & Evans 1998).

Although the key residues on the μ subunit that bind the canonical YXXΦ are highly conserved in different APs (Carvajal-Gonzalez et al. 2012, Heldwein et al. 2004), different adaptor μ subunits have different affinities for particular YXXΦ motifs. This interaction is regulated by the identity of the X and Φ residues, the residues flanking the YXXΦ motif, and the position of the motif on

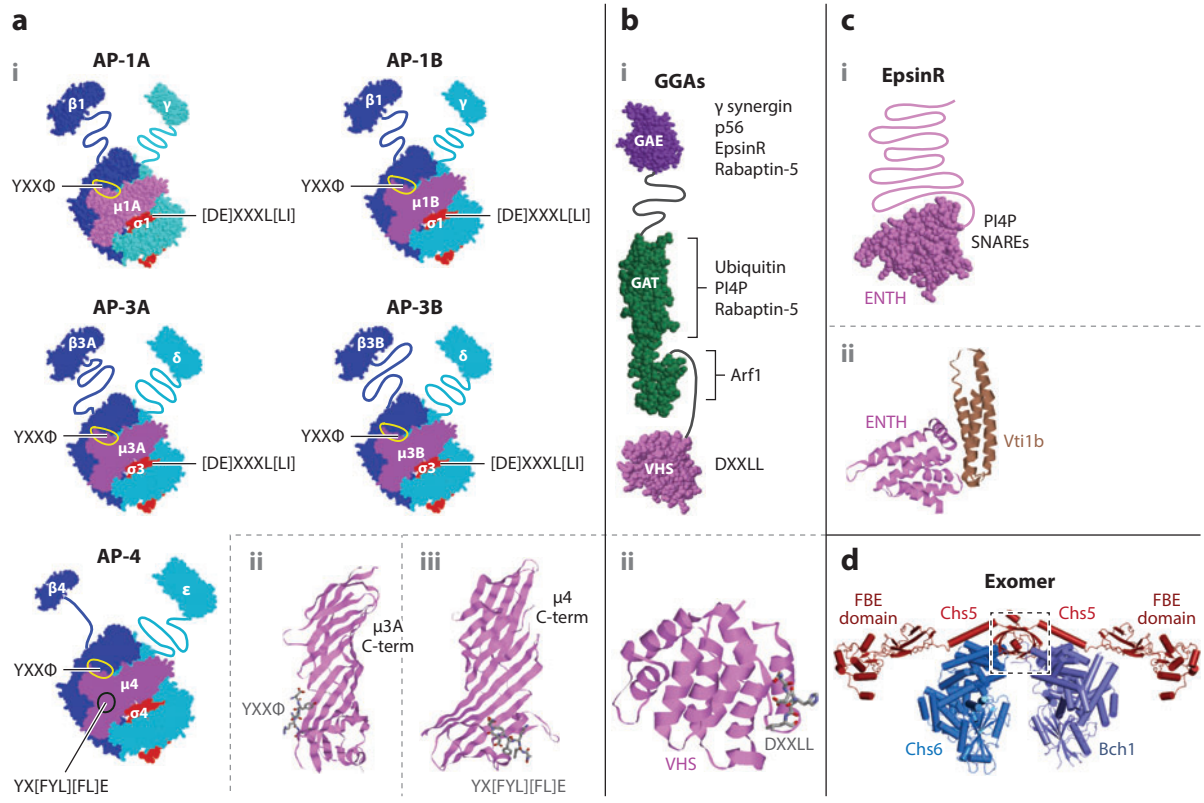


Figure 2

Cargo adaptors. (*a*) (*i*) Diagrams of adaptor protein complexes (APs) with the sorting-motif-binding sites highlighted. APs are composed of four subunits forming a central trunk domain, flexible linker regions, and two appendage domains. The diagram of the trunk domain of AP-1 is drawn based on the crystal structure of the rodent AP-1 trunk domain (PDB ID: 1W63) (Heldwein et al. 2004), the diagram of the appendage domain of γ-adaptin is drawn based on the crystal structures of the appendage domain of mouse γ1-adaptin (PDB ID: 1GYU) (Kent et al. 2002), and the diagram of the appendage domain of β1-adaptin originated from a computational model generated using ModBase based on sequence homology to the appendage domain of the α subunit of AP-2 (Brett et al. 2002, Pieper et al. 2011). (*ii*) Crystal structure of the rat μ3A C-terminal domain in complex with the TGN38 peptide SDYQRL (PDB ID: 4IKN) (Mardones et al. 2013). (*iii*) Crystal structure of the human μ4 C-terminal domain in complex with the APP peptide ENPTYKFFEQ (PDB ID: 3L81) (Burgos et al. 2010). (*b*) (*i*) Diagram of Golgi-localized, γ-ear-containing, Arf-binding proteins (GGAs). Domains of GGAs are taken from the crystal structures of the VHS domain of human GGA3 (PDB ID: 1JUQ), the GAT domain of human GGA1 (PDB ID: 1OXZ), and the GAE domain of human GGA3 (PDB ID: 1P4U) (Miller et al. 2003, Misra et al. 2002, Zhu et al. 2003). (*ii*) Crystal structure of the VHS domain of human GGA3 in complex with the sorting signal derived from the C-terminal region of CI-MPR (FHDDSDEDLLHI) (PDB ID: 1JPL) (Misra et al. 2002). (*c*) (*i*) Diagram of epsinR. The ENTH domain is drawn based on the crystal structure derived from the human protein (PDB ID: 2QY7) (Miller et al. 2007). (*ii*) Crystal structure of the Habc domain of Vti1b in complex with the ENTH domain of epsinR (PDB ID: 2V8S) (Miller et al. 2007). (*d*) Diagram of exomer contributed by Jon Paczkowski and Christopher Fromme (Cornell). The diagram derives from the crystal structure of the Chs5-Chs6 exomer complex (PDB ID: 4GNS) (Paczkowski et al. 2012) and the crystal structure of the Chs5-Bch1 exomer complex (PDB ID: 4IN3) (Richardson & Fromme 2013). The dashed box highlights the Chs5-dimerization domain.

the cytosolic domain (Ohno et al. 1996, 1998). For example, the μ subunit of AP-3 preferentially binds YXXΦ motifs with a glycine preceding the tyrosine residue and an acidic residue following the tyrosine residue, which is characteristic of the YXXΦ signal found in many lysosome-targeted proteins (Ohno et al. 1998, Rous et al. 2002), suggesting that AP-3 may be important for sorting these proteins.

An unconventional tyrosine sorting motif, YX[FYL][FL]E, in the cytosolic domain of the Alzheimer's disease–associated amyloid precursor protein (APP) interacts with the μ subunit of AP-4 (μ4), and this interaction regulates its transport from the TGN to endosomes (Burgos et al. 2010). Structural analysis indicates that this tyrosine sorting motif on APP binds in an extended conformation to a novel site on the surface of μ4 that is opposite to the conventional

Table 1 Genetic studies of physiological functions of adaptor protein complexes

Mutagenesis and knockout studies in mice		
Complex	**Phenotype**	**Reference**
AP-1		
γ	Embryonic lethal	Ohno 2006, Zizioli et al. 1999
μ1A	Embryonic lethal	Meyer et al. 2000, Ohno 2006
μ1B	Epithelial immune dysfunctions in colon, intestinal epithelial cell hyperplasia, missorting of basolateral proteins in colonic and intestinal epithelial cells	Hase et al. 2013, Takahashi et al. 2011
σ1B	Defects in synaptic vesicle biogenesis, deficits in motor control and spatial memory	Glyvuk et al. 2010
AP-3		
δ	Lysosomal abnormalities, inner ear degeneration, neurological deficits, prolonged bleeding times, pigmentation defects	Kantheti et al. 1998
β3A	Prolonged bleeding times, pigmentation defects, mistargeting of lysosomal proteins	Feng et al. 1999, Yang et al. 2000
β3B	Seizures, compromised synaptic zinc stores, and compromised synaptic vesicle targeting of membrane proteins involved in zinc and chloride transport	Seong et al. 2005
μ3B	Seizures, impairment of γ-aminobutyric acid (GABA) release, and reduction of vesicular GABA transporter	Nakatsu et al. 2004
AP-4		
β4	Mistargeting of normally somatodendritically localized receptors to axons of hippocampal neurons	Matsuda et al. 2008
Natural mutations in human patients		
Complex	**Phenotype**	**Reference**
AP-1		
σ1A	MEDNIK syndrome characterized by mental retardation, enteropathy, deafness, peripheral neuropathy, ichthyosis, and keratoderma	Montpetit et al. 2008
σ1B	Psychomotor delay, hypotonia, and X-linked Dandy-Walker malformation with intellectual disability, basal ganglia disease, and seizures	Saillour et al. 2007, Tarpey et al. 2006
AP-3		
β3A	Hermansky-Pudlak syndrome-2 characterized by prolonged bleeding times and pigmentation defects, missorting of the lysosomal membrane proteins	Dell'Angelica et al. 1999
AP-4		
ε, β4, μ4, σ4	Spastic paraplegia, severe intellectual disability and progressive spasticity	Hirst et al. 2013

(*Continued*)

Table 1 (*Continued*)

Mutagenesis studies in zebrafish (*Danio rerio*)		
Complex	**Phenotype**	**Reference**
AP-1		
β1	Missorting of the basolateral localized NA^+/K^+-ATPase pump in sensory hair cells of the inner ear	Clemens Grisham et al. 2013
μ1A, μ1C	Defects in central nervous system	Gariano et al. 2014
μ1B	Defects in kidney, gut, and liver formation	Gariano et al. 2014, Zizioli et al. 2010
σ1A	Perturbation in development of skin and spinal cord, reduced pigmentation	Montpetit et al. 2008
Mutagenesis studies in *Caenorhabditis elegans*		
Complex	**Phenotype**	**Reference**
AP-1		
μ1 (*apm-1*)	Larval lethality, synthetic embryonic lethal with UNC101	Shim et al. 2000
μ1 (*unc-101*)	~50% larval lethality, uncoordinated movement, mistargeting of dendritic- or cilia-localized transmembrane proteins, defects in neuronal dye uptake, male spicule, cilium formation, and defecation	Bonifacino 2014, Kaplan et al. 2010, Lee et al. 1994
σ1, γ, β1	Embryonic lethal, defects in apical-basolateral polarity	Bonifacino 2014, Shim et al. 2000
AP-3		
β3, μ3	Defects in gut granule biogenesis (mutagenesis)	Hermann et al. 2005
δ, β3, μ3, σ3	Embryonic lethal and larval lethal (RNAi)	Shim & Lee 2005
Mutagenesis studies in *Saccharomyces cerevisiae*		
Complex	**Phenotype**	**Reference**
AP-1		
γ, β1, μ1, σ1	Defects in retention of a subset of late Golgi membrane proteins	Valvidia et al. 2002
γ, β1	Defects in sorting ubiquitinated cargo to the vacuole lumen	Phelan et al. 2006
AP-3		
δ, β3, μ3, σ3	Missorting of alkaline phosphatase and the vacuolar t-SNARE, Vam3p	Cowles et al. 1997, Stepp et al. 1997
Mutagenesis studies in *Drosophila melanogaster*		
Complex	**Phenotype**	**Reference**
AP-1		
γ, μ1, σ1	Lethal; tissue-specific deletion causes defects in secretory granule biogenesis and intracellular trafficking of Notch and the Notch activator Sanpodo during development	Benhra et al. 2011, Burgess et al. 2011, Kametaka et al. 2012
AP-3		
δ, β3, μ3, σ3	Defects in biogenesis of eye pigment granules	Dell'Angelica 2009

tyrosine-sorting-motif-binding site, suggesting that multiple cargo-binding sites exist on μ4 (**Figure 2*aiii***) (Burgos et al. 2010). This novel binding site also contains hydrophobic pockets to accommodate the tyrosine and FL residues (Burgos et al. 2010). Key residues involved in the interaction are conserved in the μ subunit of other APs, indicating that other APs may use this binding domain to recognize a specific subset of cargo molecules bearing similar motifs (Burgos et al. 2010).

The dileucine sorting motif, [DE]XXXL[LI], is present in the cytosolic domain of many transmembrane proteins that are targeted to late endosomes, lysosomes, specialized endo-lysosomal

compartments, and the basolateral domain of polarized epithelial cells (Bonifacino & Traub 2003). This motif binds one of the hemicomplexes of the APs: γ-σ1 in AP-1, α-σ2 in AP-2, and δ-σ3 in AP-3 (Chaudhuri et al. 2007, Doray et al. 2007, Janvier et al. 2003). There is no evidence showing that AP-4 binds dileucine motifs. Both of the leucine/isoleucine residues are crucial for binding the hemicomplexes (Doray et al. 2007, Janvier et al. 2003). The two leucine residues in the so-called Q-peptide from the T cell surface antigen protein CD4 [RM(phosphoS)QIKRLLSE] bind two adjacent hydrophobic pockets on the σ2 subunit of the AP-2 α-σ2 hemicomplex, and the hydrophilic residue at the L-4 position binds the hydrophilic patch at the boundary of σ2 and α (Kelly et al. 2008). Although the Q-peptide does not fit the canonical [DE]XXXL[LI] motif, mutagenesis and protein-binding assays indicate that canonical dileucine motifs bind the same site on AP-2 and homologous sites on AP-1 and AP-3 (Mattera et al. 2011).

Different hemicomplexes show preference for particular dileucine motifs (Doray et al. 2007, Mattera et al. 2011). Moreover, APs assembled using different isoforms of the subunits are not functionally equivalent to recognize particular dileucine motifs (Mattera et al. 2011), which potentially increases the specificity of the cargo sorting process.

In addition to short linear motifs, APs can also recognize motifs embedded within the tertiary structure of cargo molecules. TGN export of the potassium channel Kir2.1 depends on conserved basic residues in the N-terminal cytosolic domain and a patch of residues in the C-terminal cytosolic domain (Ma et al. 2011, Stockklausner & Klocker 2003). In the tertiary structure of Kir2.1, these key residues on separate domains juxtapose each other, forming a signal patch for interacting with AP-1 during TGN export (Ma et al. 2011). Recognizing trafficking motifs within the tertiary structure of cargo molecules enables cargo adaptors to package cargo molecules that are correctly folded into budding vesicles.

Golgi-Localized, γ-Ear-Containing, Arf-Binding Proteins

Golgi-localized, γ-ear-containing, Arf-binding proteins (GGAs) form another class of clathrin-associated cargo adaptors that regulate sorting of proteins from the TGN to endosomes. GGAs are monomeric and ubiquitously expressed. Mammalian cells have three GGAs (GGA1, GGA2, GGA3), yeast has two (Gga1 and Gga2), and *Drosophila* has one GGA gene. Single knockout of GGA1 or GGA3 is tolerated in mice, whereas knockout of GGA2 causes embryonic or neonatal lethality depending on the genetic background (Govero et al. 2012). In yeast, deletion of either Gga1 or Gga2 causes no obvious changes in trafficking, whereas combined deletion of Gga1 and Gga2 causes defects in trafficking from TGN to vacuole (Bonifacino 2004). Knocking down the expression of GGA by >95% using transgenic RNA interference (RNAi) in *Drosophila* does not result in a discernible phenotype (Hirst & Carmichael 2011). However, another study showed that potent knockdown of *Drosophila* GGA causes lethality during early pupation, whereas weaker knockdown causes lysosomal dysfunction and retinal defects (Eissenberg et al. 2011).

All of the GGAs are composed of an N-terminal VHS (Vps27, Hrs, STAM) domain, a GAT (GGA and TOM) domain, and a C-terminal GAE (γ-adaptin ear) domain (**Figure 2*bi***). The GAT domain is connected to the VHS domain through a short proline-rich linker and to the GAE domain through a long flexible linker. The VHS domain binds acidic-cluster dileucine motifs (DXXLL) in the cytosolic tail of cargo molecules that traffic between the TGN and the endosomes (Owen et al. 2004). The VHS domain is composed of eight α-helices forming a right-handed super helix (**Figure 2*bii***), similar to the VHS domains of TOM1, which function in endosomal sorting, and Hrs, which mediate sorting of ubiquitinated proteins to MVBs (Misra et al. 2002, Shiba et al. 2002, Zhu et al. 2003). The adjacent sixth and eighth α-helices and the

loop following α6 within the VHS domain form a positively charged pocket and two hydrophobic pockets that accommodate the D and LL residues, respectively (**Figure 2*bii***) (Misra et al. 2002, Shiba et al. 2002). Several polar side chains adjoining a hydrophobic pocket near the C termini of the sixth and eighth α-helices can form electrostatic interactions with the C-terminal carboxyl group following the DXXLL motif (Misra et al. 2002). Moreover, residues in the cleft of α6 and α8 can form additional electrostatic interactions with a phosphoserine residue upstream of the DXXLL motif, strengthening the interaction (Misra et al. 2002). The structural analysis is consistent with the observation that the position of the DXXLL motif is normally one or two residues upstream of the C terminus (Bonifacino & Traub 2003), although internal DXXLL motifs can also bind GGAs (Doray et al. 2012). In addition, the presence of a phosphorylated serine residue upstream of the DXXLL motif facilitates the recognition of cargo molecules by GGAs (Kato et al. 2002).

In mammals, the key residues involved in cargo binding are conserved in GGAs but not in non-GGA VHS domain–containing proteins (Misra et al. 2002, Shiba et al. 2002). In yeast, these residues are only partially conserved, suggesting that yeast Ggas may not bind the canonical DXXLL motif (Misra et al. 2002). Indeed, Gga2p directly binds the cytosolic domain of Kex2p, which does not contain the canonical DXXLL motif, to mediate sorting of Kex2p from the TGN to the prevacuole compartment (De et al. 2013).

The GAT domain is composed of four α-helices that are folded to form two subdomains: the N-terminal helix-loop-helix region, which binds Arf1, and the C-terminal triple-helix bundle, which interacts with ubiquitin, phosphatidylinositol 4-phosphate (PI4P), and a Rab4/5 effector, rabaptin5 (Bonifacino 2004, Wang et al. 2007). Binding of GGAs to ubiquitin is required for the sorting of some ubiquitinated cargo molecules at the TGN (Puertollano & Bonifacino 2004, Scott et al. 2004, Shiba et al. 2004). The GAE domain, which is homologous to the appendage domain of the AP-1 γ subunit, binds many accessory molecules, including γ-synergin, p56, epsinR (Ent3 and Ent5 in yeast), and rabaptin5, and the long flexible linker contains a clathrin-binding box motif variant that recruits clathrin (Bonifacino 2004).

Both GGAs and AP-1 bind PI4P and Arf1 for their membrane recruitment, and both regulate trafficking of specific cargo proteins, such as the mannose-6-phosphate receptor (MPR), between the TGN and endosomes. What is the functional relationship between the two adaptors? Evidence suggests that GGAs and AP-1 cooperate in the packaging of cargo molecules into AP-1-containing clathrin-coated vesicles (CCVs). The long flexible linker region in GGA1 and GGA3 contains an internal DXXLL motif that binds the VHS domain, forming an autoinhibited state that prevents MPR binding (Doray et al. 2002a). The linker region also binds the appendage domain of the γ subunit of AP-1. AP-1-associated vesicles contain casein kinase-2 that phosphorylates Ser355, promoting GGAs to form an autoinhibited conformation that releases its associated MPR proteins, which may induce transfer of cargo from GGAs to AP-1 (Doray et al. 2002b). However, another report suggests that GGA1 binding to cargo proteins is unaffected by phosphorylation of Ser355, thus challenging the GGA autoinhibition model (Cramer et al. 2010). In yeast, two sequential waves of clathrin coat assembly marked by distinct clathrin adaptors (Gga2p and AP-1) are detected at the TGN (Daboussi et al. 2012). Gga2p is assembled in the first wave, which recruits Pik1p to synthesize PI4P, which subsequently promotes AP-1 assembly on the TGN membrane (Daboussi et al. 2012). The majority of the Gga2p structures are adjacent to, but distinct from, the AP-1 structures (Daboussi et al. 2012). Assembly of Gga2 and AP-1 is thus spatially and temporally separated, which ensures that clathrin-coated vesicles budded from the TGN are enriched with either Gga2- or AP-1-associated cargo molecules that are targeted to distinct destinations.

Contrary to the models suggesting cooperative roles in sorting at the TGN, other evidence indicates that AP-1 and GGAs function at least somewhat independently, in that both need to be depleted for a robust inhibition of sorting of lysosomal hydrolases to CCVs (Hirst et al. 2009). Acute inactivation of AP-1 in mammalian cells depletes GGA2 from CCVs but not vice versa (Hirst et al. 2012). Acute inactivation of AP-1 also depletes various luminal, peripheral, and transmembrane proteins, including SNAREs, lysosomal hydrolases, and their receptors from CCVs, whereas acute inactivation of GGA2 affects only lysosomal hydrolases and their receptors, suggesting that AP-1 plays a broader role in cargo sorting than GGA2 does (Hirst et al. 2012).

Epsin-Related Proteins

The epsin-related proteins constitute another class of monomeric clathrin adaptors at the TGN that recruits clathrin and regulates traffic between the TGN and endosomes. Yeast has two epsin-related proteins (Ent3p and Ent5p), and mammals have one functioning at the TGN, epsinR. The *Drosophila* homolog of epsinR is encoded by the liquid facets-Related (LqfR) gene. LqfR is essential for survival and is required for cell proliferation, insulin-independent cell growth, cell patterning, and egg chamber morphogenesis during oogenesis, suggesting its involvement in various signaling pathways (Lee et al. 2009, Leventis et al. 2011). Recently, however, it has been shown that exon 6 of LqfRa encodes the *Drosophila* Tel2 homolog, and the majority of the morphological defects of LqfR mutants can be rescued by exon 6 of LqfRa, suggesting that Tel2 is the essential protein regulating these signaling processes (Lee & Fischer 2012). In yeast, cells depleted of both Ent3p and Ent5p are viable but show defects in trafficking between TGN and endosomes and sorting of ubiquitinated cargo into MVBs (Duncan et al. 2003, Friant et al. 2003). In mammalian cells, epsinR is required for retrograde transport of shiga toxin, TGN46, and MPR from early endosomes to the TGN (Saint-Pol et al. 2004).

Epsins are composed of an ENTH domain at the N terminus followed by a long, unfolded domain (**Figure 2*ci***) (Owen et al. 2004). The ENTH domains of epsinR and Ent3p directly bind the N-terminal three-helix bundle (Habc domain) of various SNAREs that regulate trafficking between the TGN, endosomes, and lysosomes (Chidambaram et al. 2004, 2008; Hirst et al. 2004). Structural analysis indicates that the ENTH domain of epsinR binds surface patches of the folded domains of Vti1b (**Figure 2*cii***) (Miller et al. 2007). In yeast, the ENTH domain of Ent3p binds Vti1p on the surface of the Habc domain that is opposite to that of the mammalian homolog, suggesting a diversity of SNARE-adaptor recognition modes (Wang et al. 2011). Cargo adaptors functioning in other trafficking steps also recognize sorting signals embedded within the folded domain of SNAREs (Mancias & Goldberg 2007, Pryor et al. 2008). Such recognition is thought to allow cargo adaptors to specifically package correctly folded and assembled SNAREs into vesicles. As an example, binding of a SNARE protein, Sec22, to the COPII subunit Sec24 ensures that only the unassembled SNAREs are incorporated (Mancias & Goldberg 2007). In addition to binding cargo, the ENTH domain of epsinR also binds PI4P at the TGN, and this interaction may induce the unstructured N-terminal residues to form amphipathic helices that are inserted into the lipid bilayer, causing membrane deformation (Ford et al. 2002).

The long unfolded domain of epsinR contains binding sites for the appendage domain of AP-1 and GGAs (Owen et al. 2004), suggesting that these cargo adaptors may be functionally related. AP-1 and epsinR may depend on each other for optimal incorporation into clathrin coats, as knockdown of either causes reduction of the other in CCVs (Hirst et al. 2004). In yeast, the Ggas, Ent3p and Ent5p cooperate in regulating trafficking between the TGN and endosomes. Ent3p functions primarily in GGA-dependent transport, whereas Ent5p functions

with both AP-1 and GGAs (Costaguta et al. 2006). Ent3p and Ent5p redundantly act in parallel with AP-1 to mediate intracellular retention of Chs3p (Copic et al. 2007). In addition, Ent3p but not Ent5p facilitates binding of Gga2p to the endosomal syntaxin Pep12p and to clathrin (Copic et al. 2007). The clathrin-binding box region in Gga2p binds its appendage domain, forming an autoinhibited conformation that inhibits Ent5p binding (Hung et al. 2012). Mutations that release this autoinhibition enhance interaction of Gga2p with both clathrin and Ent5p, suggesting that binding of Gga2 and Ent5p is regulated during the coat assembly process, and such regulation may prevent premature recruitment of Ent5 to Gga2-containing structures (Hung et al. 2012).

Exomer

Exomer is a cargo adaptor protein complex identified in yeast that traffics proteins directly from the TGN to the plasma membrane. It is composed of five subunits: Chs5p and the ChAPs (Chs5-Arf1-binding proteins) family of proteins (Chs6, Bud7p, Bch1p, and Bch2p). Chs5p binds to the small GTPase Arf1, whereas the ChAPs are responsible for cargo binding and sorting (Barfield et al. 2009, Paczkowski et al. 2012, Starr et al. 2012). The ChAPs appear to bind specific sorting motifs in the cytosolic tails of cargo proteins (Barfield et al. 2009, Sanchatjate & Schekman 2006). Exomer is recruited to membranes by Arf1-GTP and forms a coated surface on synthetic liposomes (Wang et al. 2006), but exomer alone cannot deform lipid membranes, suggesting that additional factors are required for vesicle budding. Currently, no known homologs of exomer are found in metazoans.

Exomer regulates trafficking of chitin synthase III (Chs3p) and a fusion protein, Fus1p, from the TGN to the cell surface (Sanchatjate & Schekman 2006, Santos & Snyder 2003, Trautwein et al. 2006, Wang et al. 2006). Exomer binds directly to a novel sorting signal (IXTPK) on the cytosolic domain of Fus1p, whose trafficking is dependent upon the ChAPs Bud7 and Bch1 (Barfield et al. 2009). Conversely, exomer binds Chs3p through a DXE motif on the cytosolic domain of Chs3p (Starr et al. 2012). Transport of Chs3p to the bud neck is cell-cycle regulated (Chuang & Schekman 1996), whereas transport of Fus1p to the plasma membrane is not (Barfield et al. 2009, Zanolari et al. 2011).

Exomer and AP-1 are functionally correlated. Chs3p and Fus1p accumulate in the TGN/endosomes in chs6Δ and bch1Δbud7Δ mutants, respectively, whereas deletion of clathrin or AP-1 restores membrane localization of Chs3p and Fus1p, suggesting that AP-1 functions in retention of exomer-dependent cargo at the TGN, possibly by regulating the retrieval of these cargo molecules from endosomes to the TGN (Barfield et al. 2009, Valdivia et al. 2002). The DEESLL motif in the cytosolic domain of Chs3p mediates binding to AP-1 and is required for TGN retention (Starr et al. 2012).

In solution, exomer is a heterotetramer consisting of two copies of Chs5p and two copies of the ChAPs (**Figure 2*d***). Structural analysis of the complex composed of Chs5p and one ChAP family protein, Bch1, indicates that the heterotetramer is formed by dimerization of a Chs5p/Bch1 heterodimer through the Chs5p dimerization domain (dashed box in **Figure 2*d***) (Richardson & Fromme 2013). The Chs5p dimerization domain is a flexible molecular hinge that enables exomer to maintain interactions with cargo and membranes despite dynamic changes in membrane curvature (Richardson & Fromme 2013). The region distal to the N-terminal dimerization motif of Chs5p contains fibronectin type III and BRCT domains, altogether termed the FBE domain (Martín-García et al. 2011). Structural analysis indicates that the FBE domain of Chs5p extends from the exomer core complex and has an overall structure similar to the appendage domain of clathrin adaptors (Paczkowski et al. 2012). The FBE domain of Chs5p

binds directly to Arf1, whereas Chs6p can bind membranes independent of Arf1 (Paczkowski et al. 2012). The combination of these two low-affinity interactions, in addition to cargo binding, may facilitate the recruitment of exomer to the TGN.

CARGO RECEPTORS RECOGNIZING MEMBRANE AND LUMINAL PROTEINS AT THE TGN

Some transmembrane proteins can function as receptors at the TGN to recognize cargo molecules through their luminal domains. In addition, the cytosolic domains of these cargo receptors are often associated with the cargo adaptors described above. In this way, luminal cargo molecules are linked with the cargo sorting machineries on the cytoplasmic side of the membrane. Below, we describe various cargo receptors that regulate sorting of proteins into budding vesicles.

Mannose-6-Phosphate Receptor

Many soluble lysosomal enzymes become modified within the Golgi complex by the addition of mannose-6-phosphate (M6P) on their N-linked oligosaccharides. The M6P residues on these lysosomal enzymes are recognized by a type I integral membrane protein, the MPR, which transports these enzymes from the TGN to endosomes. The acidic pH of endosomes induces release of lysosomal enzymes from MPR, and subsequently, MPR is recycled back from endosomes to the TGN, a process mediated by retromer, Rab9, and its effector TIP47 (Braulke & Bonifacino 2009).

There are two MPRs: the cation-independent MPR (CI-MPR, or MPR300) and the cation-dependent MPR (CD-MPR, or MPR46). CI-MPR is 300 kD, and its luminal domain contains 15 homologous repeat domains, with each domain containing ~150 amino acids (**Figure 3*a***). Two M6P-binding sites are located in domains 3 and 9. M6P modification requires removal of GlcNAc by the uncovering enzyme (UCE), and lysosomal enzymes in mice deficient in UCE retain Man-P-GlcNAc phosphodiester-modified N-glycans (Boonen et al. 2009). CI-MPR but not CD-MPR recognizes the phosphodiester-containing lysosomal enzymes through domain 5 of its luminal domain (Bohnsack et al. 2009, Boonen et al. 2009, Chavez et al. 2007, Olson et al. 2010). Residues adjacent to domains 3 and 5 facilitate their binding to phosphomannosyl residues (Bohnsack et al. 2009, Hancock et al. 2002). Region 11 contains a binding site for insulin-like growth factor II (IGF II) and functions in clearance of IGF II by delivering it to lysosomes for degradation (Braulke & Bonifacino 2009). The CD-MPR exists in membranes primarily as a dimer, consisting of 46-kD subunits and one M6P-binding site (Braulke & Bonifacino 2009). The luminal domain of CD-MPR is homologous to each of the repeat regions within CI-MPR (**Figure 3*a***) (Braulke & Bonifacino 2009).

MPR dysfunction causes secretion of lysosomal enzymes. Serum from mice deficient in either form of MPR shows elevated levels of M6P-tagged glycoproteins (Koster et al. 1993, Ludwig et al. 1993, Qian et al. 2008, Sohar et al. 1998). Primary embryonic fibroblasts lacking either CI- or CD-MPR secrete largely nonoverlapping sets of phosphorylated lysosomal enzymes, and the presence of one MPR does not compensate for loss of the other, suggesting that the two MPRs have complementary functions (Ludwig et al. 1994, Munier-Lehmann et al. 1996).

The cytosolic tail of MPRs contains an acidic cluster dileucine motif (DXXLL) that is recognized by the GGA proteins. In addition, the cytosolic tail also contains motifs that are recognized by AP-1. Both AP-1 and GGAs have been demonstrated to influence trafficking of MPRs from the TGN to endosomes. In particular, GGAs have been suggested to deliver MPR to AP-1-containing CCVs (Doray et al. 2002b). However, some evidence suggests that GGAs and AP-1 function independent of each other to regulate the trafficking of MPR (Hirst et al.

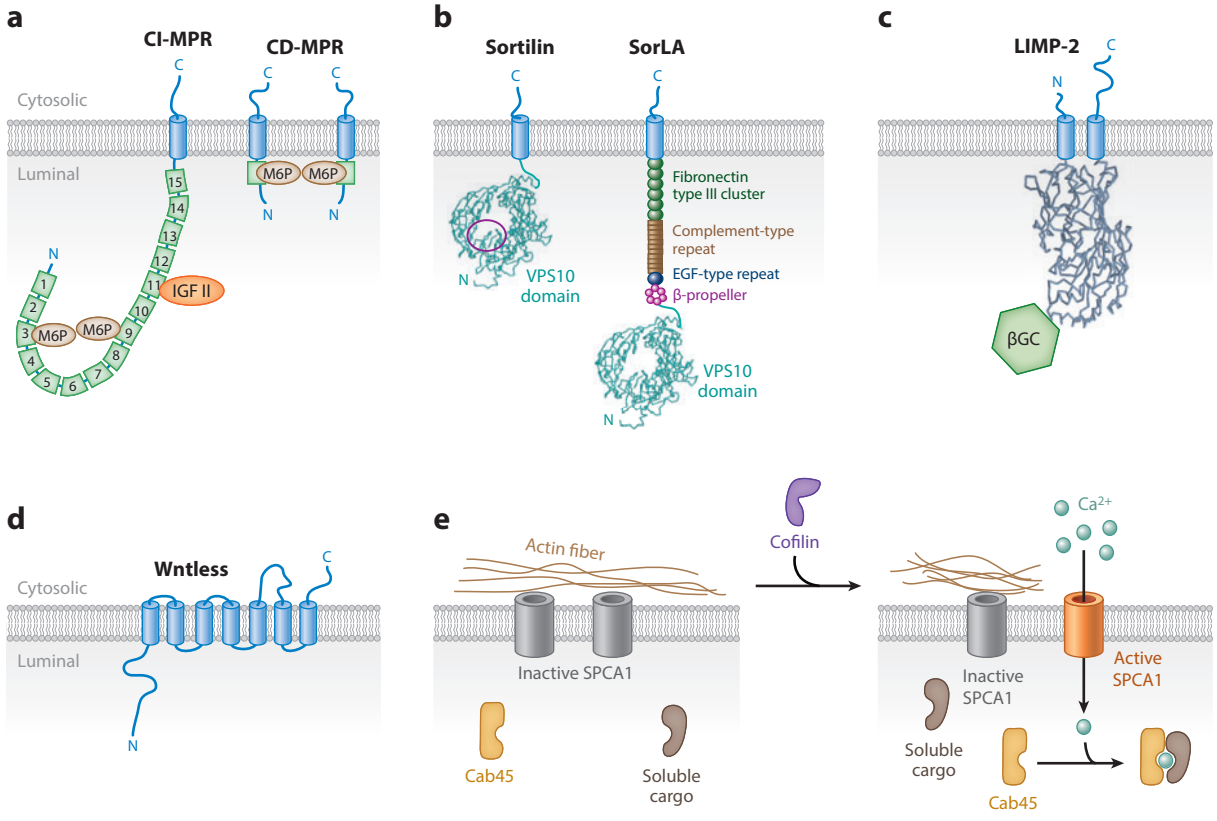


Figure 3

Cargo receptors. (*a–d*) Diagrams of cargo receptors. The VPS10 domain of sortilin and SorLA is drawn based on the crystal structure of human sortilin (PDB ID: 3F6K) (Quistgaard et al. 2009). The cargo-binding site on the VPS10 domain of sortilin is highlighted (*purple circle*). The luminal domain of LIMP-2 is drawn based on the crystal structure derived from the human protein (PDB ID: 4F7B) (Neculai et al. 2013). (*e*) Sorting of soluble proteins at the *trans*-Golgi network mediated by Ca^{2+}, actin, cofilin, SPCA1, and Cab45 (Curwin et al. 2012; von Blume et al. 2009, 2011). Abbreviations: CD-MPR, cation-dependent mannose-6-phosphate receptor; CI-MPR, cation-independent mannose-6-phosphate receptor.

2009). The functional relationship between GGAs and AP-1 in regulating the sorting of MPRs thus needs to be further investigated.

Sortilin

Sortilin is a member of the VPS10 family of proteins, which are type I integral membrane proteins with a luminal portion containing a VPS10 domain (**Figure 3*b***). Vps10p is a sorting receptor in yeast that binds lysosomal hydrolases and regulates their trafficking from the TGN to the vacuole (Cooper & Stevens 1996, Marcusson et al. 1994). Sortilin regulates sorting of lysosomal enzymes, including sphingolipid activator proteins, acid sphingomyelinase, and cathepsins D and H, whose lysosomal targeting is independent of M6P (Braulke & Bonifacino 2009, Canuel et al. 2008). Sortilin is predominantly localized to the TGN. Similar to that in MPRs, the cytosolic domain of sortilin contains motifs that enable binding to GGAs and APs. The luminal VPS10 domain

binds ligand and contains two structural features that are important for its function: an N-terminal propeptide and a C-terminal segment containing 10 conserved cysteines (Westergaard et al. 2004). The propeptide, which is cleaved in the TGN, prevents ligand binding to the immature form of sortilin and moreover facilitates transport of sortilin to the TGN in the biosynthetic pathway (Petersen et al. 1999, Westergaard et al. 2004). The N-terminal portion of the VPS10 domain following the propeptide is made up of a 10-bladed β-propeller fold, forming a wide, slightly conical tunnel (**Figure 3*b***) (Quistgaard et al. 2009). The binding sites for different ligands are clustered on a confined surface inside the tunnel, which ensures sortilin accommodates only one cargo molecule at a time (purple circle in **Figure 3*b***) (Quistgaard et al. 2009).

Sortilin also mediates trafficking of nonlysosomal cargoes functioning in a variety of biological processes. For example, overexpressing sortilin decreases intracellular and secreted levels of α-1 antitrypsin in a rat hepatoma cell line (Gelling et al. 2012). In neural cells, sortilin regulates anterograde transport of the tropomyosin-related kinase family of proteins, which are neurotrophin receptors, and also regulates sorting of brain-derived neurotrophic factor to the regulated secretory pathway (RSP) (Chen et al. 2005, Vaegter et al. 2011). Sortilin interacts with apoB100 and regulates hepatic export of apoB100-containing lipoprotein, thereby regulating plasma cholesterol levels (Kjolby et al. 2010, Strong et al. 2012). Sortilin is also associated with the precursors of TGF-β family proteins and transports them to lysosomes for degradation, thereby downregulating TGF-β signaling (Kwon & Christian 2011). Sortilin may function as a sorting receptor for proprotein convertase subtilisin/kexin type 9 (PCSK9), a protein that mediates degradation of LDLR, and appears to regulate the secretion of PCSK9 (Gustafsen et al. 2014). In *Tetrahymena*, sortilin homologs have recently been shown to be important for the biogenesis of SGs by mediating the sorting of a subset of nonaggregated cargo molecules (Briguglio et al. 2013).

Sortilin-Related Receptor with A-Type Repeats

Sortilin-related receptor with A-type repeats (SorLA) is another member of the VPS10-domain receptors (**Figure 3*b***) and is expressed mainly in the nervous system, including neuronal populations in the cortex, hippocampus, and cerebellum (Motoi et al. 1999). SorLA is genetically associated with Alzheimer's disease and is a central regulator of trafficking and processing of APP (Rogaeva et al. 2007, Willnow & Andersen 2013). Mice lacking SorLA show increased levels of endogenous amyloid-β peptide (Aβ), the major component of senile plaques found in the brain of Alzheimer's disease patients (Andersen et al. 2005). SorLA predominantly localizes to the TGN and early endosomes (Jacobsen et al. 2001, Offe et al. 2006). Overexpressing SorLA in neurons has been suggested to cause accumulation of APP within the Golgi, leading to a reduction in Aβ production (Andersen et al. 2005). However, several reports have developed evidence that the TGN is a major site of Aβ production (Burgos et al. 2010, Choy et al. 2012, Siman & Velji 2003, Xu et al. 1997). Because processing of APP to Aβ relies on an endocytic pool of APP that recycles from early endosomes to the TGN (Choy et al. 2012), overexpression of SorLA may impair the biosynthetic delivery of APP from the TGN to the cell surface, thus reducing the endocytic pool of APP and inhibiting Aβ production.

The cytosolic domain of SorLA contains motifs for binding various cargo adaptors, including APs, PACS1, retromer, and GGAs, and these interactions regulate shuttling of SorLA between the Golgi and endosomes (Willnow & Andersen 2013). The VPS10 domain in the luminal portion of SorLA binds neuropeptides and the receptor-associated protein (Jacobsen et al. 2001). Similar to sortilin, a propeptide at the N terminus of the VPS10 domain autoinhibits ligand binding to the VPS10 domain, and this propeptide is cleaved by furin in the late Golgi compartments (Jacobsen et al. 2001).

In addition to the VPS10 domain, the luminal portion of SorLA also contains a β-propeller domain, complement-type repeats, and a cluster of fibronectin type III repeats (**Figure 3*b***). The cluster of complement-type repeats in the SorLA luminal domain binds the carbohydrate-linked domain of the APP extracellular domain (Andersen et al. 2006) and also interacts with lipoprotein lipase (LPL), receptor-associated protein, and apolipoprotein E (Jacobsen et al. 2001). SorLA is proposed to transport LPL from the TGN to endosomes, from which LPL is further transported to lysosomes for degradation, causing a reduction of LPL activity (Klinger et al. 2011). A pool of SorLA also localizes to the cell surface, where it mediates the endocytosis of several proteins, including β-VLDL (Jacobsen et al. 2001, Taira et al. 2001).

Lysosomal Integral Membrane Protein Type 2

Lysosomal integral membrane protein type 2 (LIMP-2) acts as a cargo receptor to mediate the transport of β-glucocerebrosidase (βGC), an enzyme deficient in Gaucher disease, to the lysosomal compartment in an MPR-independent pathway (Gonzalez et al. 2013, Reczek et al. 2007). Mutations in the gene encoding LIMP-2 (SCARB2) may contribute to the phenotypic heterogeneity of Gaucher disease patients with the same genotype of the βGC gene (GBA1) (Velayati et al. 2011). Moreover, mutations in SCARB2 are the major cause of action myoclonus renal failure syndrome (Berkovic et al. 2008). Deficiency of LIMP-2 in mice causes ureteropelvic junction obstruction, deafness, peripheral neuropathy, secretion of βGC, and impaired apically localized potassium channel KCNQ1/KCNE1 (Gamp et al. 2003, Knipper et al. 2006, Reczek et al. 2007). LIMP-2 is also a receptor for several enteroviruses and supports virus propagation (Yamayoshi et al. 2009, 2012). These viruses include enterovirus 71 and coxsackieviruses A16, A14, and A7, which are associated with hand, foot, and mouth disease and neurological diseases (Yamayoshi et al. 2009, 2012). The molecular mechanisms underlying the roles of LIMP-2 in virus propagation remain unclear.

LIMP-2 is a type III glycoprotein with its N and C termini facing the cytoplasm, two transmembrane domains, and a large, highly glycosylated luminal domain (**Figure 3*c***). The luminal domain of LIMP-2 contains a coiled-coil motif that is important for binding to βGC at neutral pH but that dissociates from cargo at acidic pH in the late endosomal and lysosomal compartments (Reczek et al. 2007). pH-dependent binding is mediated by a histidine residue that is in close proximity to the coiled-coil motif. Protonation of this residue is proposed to promote dissociation of βGC from LIMP-2 (Zachos et al. 2012). This coiled-coil motif is part of a helical bundle on the head of the LIMP-2 luminal domain that binds βGC (**Figure 3*c***) (Neculai et al. 2013). The binding site of enterovirus 71 on LIMP-2 maps to the head of the luminal domain and overlaps the binding site of βGC (Yamayoshi & Koike 2011). Moreover, LIMP-2 contains an interconnected hydrophobic cavity that forms a tunnel through the luminal domain, but the function of this tunnel structure remains unclear (Neculai et al. 2013). The hydrophobic tunnel is also predicted to be present in a scavenger receptor, SR-BI, where it may mediate transfer of cholesterol from lipoproteins to the outer leaflet of the plasma membrane (Neculai et al. 2013). The C-terminal cytosolic domain of LIMP-2 contains a dileucine motif that interacts with AP-3 and is critical for sorting of LIMP-2 to lysosomes (Honing et al. 1998, Ogata & Fukuda 1994). The C-terminal region of LIMP-2 may also bind AP-1 and serve in the sorting of LIMP-2 (Fujita et al. 1999).

Wntless

Wntless, a highly conserved seven-pass transmembrane protein (**Figure 3*d***), is a putative cargo receptor that regulates the secretion of Wnt proteins from the Golgi to the cell surface (Banziger

et al. 2006, Bartscherer et al. 2006, Goodman et al. 2006), but it is unclear how Wntless itself is exported from the TGN. Wnt binding to Wntless depends on Porcupine-mediated lipidation of the serine 239–equivalent residue in Wnt (Herr & Basler 2012). At the plasma membrane, Wntless displays a YXXΦ motif in a cytoplasmic loop that is important for internalization and recycling back to the TGN, and this motif is possibly recognized by AP-2 and clathrin (Gasnereau et al. 2011). Retrieval of Wntless from the endosomal compartments back to the TGN depends on retromer (Harterink & Korswagen 2012).

Cab45

Von Blume et al. (2009, 2011) demonstrated that sorting of a subset of soluble cargo depends on a pathway regulated by Ca^{2+}, the actin-severing proteins ADF/cofilin, a soluble luminal Ca^{2+}-binding Golgi-resident protein (Cab45), and the Ca^{2+} ATPase (SPCA1) (**Figure 3*e***). The evidence suggests a model in which TGN-localized cofilin severs actin filaments, thus exposing and activating SPCA1, which imports Ca^{2+} into the TGN. This enables Cab45 to bind soluble cargo molecules in a Ca^{2+}-dependent manner and mediate the sorting of cargo out of the TGN (Curwin et al. 2012; von Blume et al. 2009, 2011). Because Cab45 is itself a soluble protein, it is currently unclear how it links cargo to the sorting machinery on the cytoplasmic side of the membrane.

ROLES OF SMALL GTPASES OF THE ADP-RIBOSYLATION FACTOR FAMILY AND PHOSPHOLIPIDS IN REGULATING PROTEIN SORTING AT THE TGN

Protein sorting at the TGN requires recruitment of cytosolic cargo adaptors and binding of cargo adaptors to cargo molecules. These processes are regulated by multivalent low-affinity interactions of cargo adaptors with multiple cellular components, including small GTPases of the ADP-ribosylation factor (Arf) family and phospholipids. Moreover, the activities of these components are regulated by a variety of other cellular factors. Such elaborate molecular machineries precisely regulate protein sorting at the TGN in a temporally and spatially dependent manner.

Small GTPases of the Arf Family

Small GTPases of the Arf family initiate membrane recruitment of a wide variety of cytosolic cargo adaptors. The activity of Arf proteins depends on the status of their bound nucleotide. In the GDP-bound state, Arf proteins are cytosolic. Switching to the GTP-bound state, a process catalyzed by Arf guanine nucleotide exchange factors (Arf GEFs), causes an N-terminal amphipathic helix, which is normally myristoylated or acetylated, to be exposed and inserted into the lipid bilayer (Donaldson & Jackson 2011, Gillingham & Munro 2007). The amphipathic helix induces membrane deformation, thereby facilitating the formation of nascent vesicles (Donaldson & Jackson 2011, Gillingham & Munro 2007). GTP binding also causes conformational changes in the switch regions of Arf proteins to recruit various cytosolic effectors, including cargo adaptors and lipid-modification enzymes. The amphipathic motif and switch regions reside close to each other, such that their effectors are recruited in very close proximity to the membrane (Gillingham & Munro 2007). Arfs have intrinsic GTP hydrolysis activity, which switches Arf proteins back to the GDP-bound, inactive state, and this process is stimulated by Arf GTPase-activating proteins (ArfGAPs).

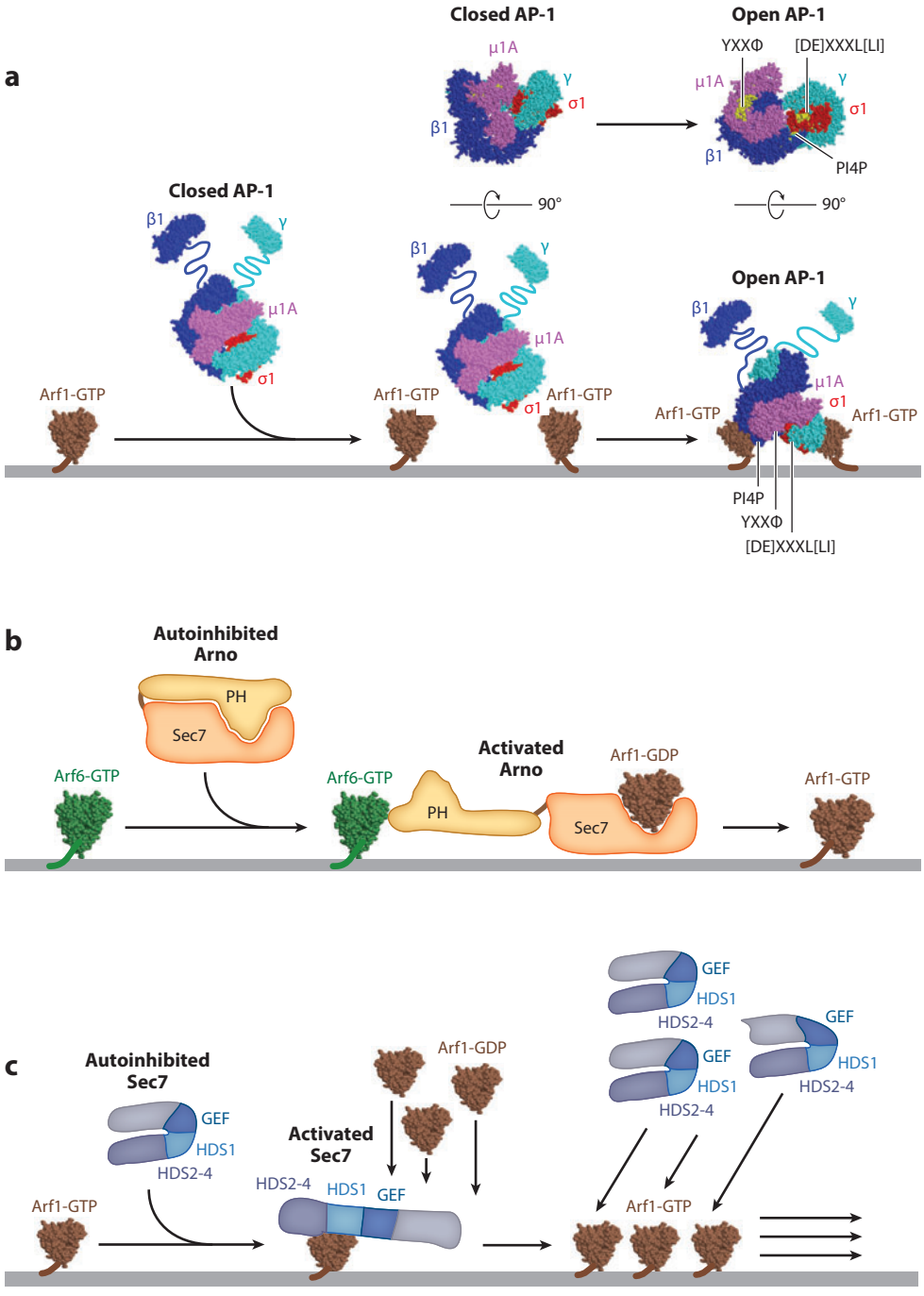
There are five Arf proteins and more than 20 Arf-like proteins in human. Several Arf proteins are localized to the TGN. siRNA knockdown analysis of some Arf proteins (Arf1, -3, -4, and -5)

suggests they are functionally redundant in some respects (Volpicelli-Daley et al. 2005). Arf1 is the most well-studied Arf, and its effectors include GGAs, AP-1, AP-3, AP-4, and exomer (Donaldson & Jackson 2011, Gillingham & Munro 2007). Arf3 shares 97% sequence identity with Arf1 and localizes specifically to the TGN (Manolea et al. 2010). Arf4 binds directly to the VXPX sorting motif in rhodopsin and regulates sorting of rhodopsin at the TGN (Mazelova et al. 2009). Arf-related protein 1 (Arfrp1) is essential for survival and mediates trafficking of VSVG, E-cadherin, Vangl2, and the glucose transporters GLUT4 and GLUT2 and also regulates lipid droplet growth (Guo et al. 2013b; Hesse et al. 2010, 2012; Hommel et al. 2010; Nishimoto-Morita et al. 2009; Shin et al. 2005; Zahn et al. 2008). Similar to Arf1, Arfrp1 also directly interacts with AP-1 and mediates membrane recruitment of AP-1 in a GTP-dependent manner (Guo et al. 2013b).

Arf-dependent membrane recruitment of cargo adaptors is stimulated by interaction with cargo molecules. Peptides containing dileucine or tyrosine motifs simulate AP-1 binding to Arf1 on synthetic liposomes (Crottet et al. 2002, Guo et al. 2013b, Lee et al. 2008a, Ren et al. 2013). Furthermore, cargo sorting signals induce the oligomerization of AP-1, which contributes to vesicle coat assembly and cargo sorting (Lee et al. 2008b, Meyer et al. 2005). In addition, overexpression of particular cargo molecules in mammalian cells enhances the membrane association of specific cargo adaptors in an Arf-dependent manner (Caster et al. 2013). Such stimulation of adaptor recruitment is thought to facilitate vesicle coat assembly. Similar stimulatory effects of cargo molecules have also been observed in the assembly of COPII coats during endoplasmic reticulum exit and assembly of clathrin coats during endocytosis (Aridor et al. 1999, Puthenveedu & von Zastrow 2006). Cargo sorting is thus linked to vesicle coat assembly, which ensures that the vesicle budding process is productive.

In addition to mediating the membrane recruitment of cargo adaptors, Arf proteins also induce conformational changes in the adaptors that enable their binding to cargo molecules. Structural analysis indicates that the cytosolic form of AP-1 exists in a closed conformation in which the tyrosine-motif-binding site on the μ subunit and the dileucine-motif-binding site on the γ-σ1 hemicomplex are blocked (Heldwein et al. 2004). Once recruited to the membranes, AP-1 undergoes a large-scale conformational change such that the cargo-binding sites are exposed, enabling recognition of cargo molecules (Lee et al. 2008a, Ren et al. 2013). Similar conformational changes have been detected in AP-2 (Jackson et al. 2010). Binding of Arf1 promotes an open conformational change in AP-1 (**Figure 4*a***) (Ren et al. 2013). Once in the open conformation, binding sites on AP-1 and AP-2 become coplanar to allow simultaneous interactions with cargo and phospholipids, which is thought to further stabilize membrane association (Jackson et al. 2010, Ren et al. 2013).

Arf1 is proposed as an allosteric activator of AP-1. Three Arf1-binding sites in AP-1 are involved in its membrane recruitment and cargo activation. Two are identified on the N terminus of each large subunit of the trunk domain, which bind the switch region of Arf1 and are important for the membrane association of AP-1. Compared with the much lower affinity in γ-adaptin, the site on β1 adaptin is the primary Arf1-binding site and is also important for the Arf1-mediated conformational change (Ren et al. 2013). Such bivalent binding to the switch region of Arf1 is also detected in the COPI coat, where each of the large subunits, β and γ, contains an Arf1-binding site (Lee & Goldberg 2010). The Arf1-binding sites on AP-1 are spatially correlated to the Arf1-binding sites on COPI and the PIP2-binding site on AP-2. During cargo activation, the third site in the central part of the γ trunk is proposed to make a novel contact with the back side of Arf1, which is distal to the switch regions. The back side of Arf1 is essential for AP-1/Arf1 2:2 dimer assembly, and full activation of AP-1 such that it can recognize the cargos (Ren et al. 2013). The synergistic effects of Arf1, cargo molecules, and phospholipids on the membrane association of AP-1 enable assembly of clathrin/AP-1 coats at the right place and with the right cargo.


a
Closed AP-1
Open AP-1
μ1A
γ
σ1
β1
YXXΦ
[DE]XXXL[LI]
PI4P
90°
Arf1-GTP
b
Autoinhibited
Arno
PH
Sec7
Arf6-GTP
Activated
Arno
Arf1-GDP
c
Autoinhibited
Sec7
GEF
HDS1
HDS2-4
Activated
Sec7

Another Arf family protein, Arfrp1, also mediates membrane recruitment of AP-1 in a GTP-dependent manner. This process is stimulated by the presence of the Vangl2 C-terminal cytosolic domain bearing a tyrosine sorting motif (YYXXF) that directly binds the μ subunit of AP-1 (Guo et al. 2013b). In contrast, Arf1-mediated AP-1 membrane recruitment cannot be stimulated by the Vangl2 C-terminal cytosolic domain (Guo et al. 2013b). This evidence suggests that the YYXXF sorting motif may bind noncanonical sites on the μ subunit, and AP-1 may have at least two active conformations, one activated by Arf1, which exposes the canonical cargo-binding site, and the other activated by Arfrp1, which exposes the noncanonical binding site to enable recognition of the tyrosine motif on Vangl2. The presence of multiple cargo-binding sites on μ1 has been suggested with the demonstration that μ1 binds the tyrosine sorting motif on transferrin receptor through a noncanonical site (Farias et al. 2012). In addition, the key residues on μ4 that are involved in binding the noncanonical tyrosine motif on APP are conserved in μ1 (Burgos et al. 2010). By influencing the conformational state of cargo adaptors, the Arf proteins thus not only promote cargo adaptors to bind cargo molecules but also mediate the specificity of cargo recognition.

The membrane association and activity of Arfs are dependent on Arf GEF proteins. Interestingly, some Arf family members mediate the membrane association of specific Arf GEFs, which subsequently recruit and activate other Arf isoforms. In mammalian cells, GTP-bound Arl1 mediates the membrane recruitment of BIG1 and BIG2, the TGN-localized Arf GEFs (Christis & Munro 2012). The Arf nucleotide-binding site opener (Arno), a GEF for Arf1 and Arf6, is recruited by GTP-bound Arf6 to the plasma membrane, where Arno promotes recruitment of Arf1 to dynamic plasma membrane ruffles (**Figure 4*b***) (Cohen et al. 2007). Similarly, Arno is also an effector of Arl4 (Hofmann et al. 2007). Arl3p, the yeast homolog of Arfrp1, is required for membrane recruitment of Arl1p, possibly by recruiting the GEF for Arl1p, thereby regulating the targeting of the Arl1p effector, Imh1p, to the TGN (Panic et al. 2003). Sequential activation of Arfs thus allows cells to spatially and temporally regulate vesicle coat assembly and other trafficking events.

The nucleotide exchange activity of Arf GEFs can be activated by their products, GTP-bound Arfs, forming positive feedback loops. The crystal structure of the Sec7 domain in tandem with the PH domain of a mammalian Arf GEF, Grp1, indicates that the nucleotide exchange activity of the Sec7 domain is potently autoinhibited by conserved elements proximal to the PH domain (DiNitto et al. 2007). Binding of the PH domain of Grp1 with its product, the GTP-bound Arf6, relieves this autoinhibition. Similarly, the GTP-bound Arf1 activates the GEF activity of Arno (Stalder et al. 2011). The HDS2-4 domains of Sec7, a TGN-localized Arf GEF in yeast, have an autoinhibitory function (**Figure 4*c***). The HDS1 domain of Sec7 binds GTP-bound Arf1, and this interaction stimulates GEF activity of Sec7 and mediates recruitment of Sec7 to the TGN (**Figure 4*c***) (Richardson et al. 2012). Arf GEFs are thus regulated by both autoinhibition and

Figure 4

Roles of Arf proteins in cargo sorting processes. (*a*) GTP-bound Arf1 has dual roles in recruiting AP-1 to the *trans*-Golgi network (TGN) membrane and in inducing a large-scale conformational change in the adaptor to enable AP-1 to directly recognize sorting motifs on cargo molecules (Ren et al. 2013). The 90° rotated view shows the membrane contact region of AP-1 with the cargo- and PI4P-binding sites highlighted in yellow. (*b*) Sequential activation of Arf proteins. GTP-bound Arf6 binds an Arf GEF, Arno, and this binding activates the GEF activity of Arno by releasing its autoinhibition, which subsequently activates Arf1 (Cohen et al. 2007). (*c*) Positive feedback loop between Arf and Arf GEF. In yeast, GTP-bound Arf1 binds an Arf GEF, Sec7p, to recruit Sec7p to the TGN membranes, which releases autoinhibition and thus activates the GEF activity of Sec7p, thereby generating more GTP-bound Arf1 (Richardson et al. 2012).

positive feedback. The positive feedback between Arf and Arf GEF is thought to accelerate vesicle coat assembly on membranes.

Phospholipids

Various cargo adaptors bind directly to specific phospholipids that are enriched in distinct organelles, and this binding is important for their membrane association. Two phospholipids play specific roles in the membrane recruitment of cargo adaptors at the TGN.

Phosphatidylinositol 4-phosphate. PI4P is enriched predominantly in the TGN and mediates membrane recruitment of a number of cargo adaptors, including AP-1, GGAs, and epsinR (Mills et al. 2003; Wang et al. 2003, 2007). The γ subunit of AP-1 interacts directly with PI4P. Mutations in the PI4P-binding sites inhibit the TGN membrane association of AP-1 (Heldwein et al. 2004). GGAs interact directly with PI4P through basic residues on the C-terminal triple-helix bundle of the GAT domain (Wang et al. 2007). A PI4P-binding site is also detected within the N-terminal VHS domain of Gga2p in yeast (Demmel et al. 2008). In addition to regulating the membrane association of GGAs, PI4P also promotes the binding of GGAs to ubiquitin sorting signals (Wang et al. 2007). The PI4P-binding site on epsinR is localized in its ENTH domain (Mills et al. 2003). PI4P also binds and mediates membrane recruitment of PH-domain-containing proteins, such as oxysterol-binding protein, ceramide transfer protein, four-phosphate adaptor protein 1 and 2 (FAPP1 and FAPP2), and BAR-domain-containing proteins arfaptin1 and arfaptin2 (Cruz-Garcia et al. 2013, De Matteis et al. 2005). These proteins are important for membrane deformation and lipid transport. Arfaptin1 binds Arf1, Arf3, and Arl1 in a GTP-dependent manner (Kanoh et al. 1997, Man et al. 2011) and regulates glucose-stimulated insulin secretion and secretion of chromogranin in the RSP (Cruz-Garcia et al. 2013, Gehart et al. 2012). Phosphorylation of arfaptin1 by protein kinase D (PKD) inhibits binding of arfaptin1 to PI4P-containing membranes (Cruz-Garcia et al. 2013).

The distribution and abundance of PI4P are regulated by PI4 kinases (PI4Ks) and phosphatases. In mammals, there are four PI4Ks: PI4KIIα, PI4KIIβ, PI4KIIIα, and PI4KIIIβ. Among them, PI4KIIα and PI4KIIIβ are predominantly TGN localized and are the major regulators of the level of PI4P at the Golgi. TGN localization of PI4KIIα is dependent on palmitoylation of a cysteine-rich motif located within its catalytic domain (Barylko et al. 2009). Knockdown of PI4KIIα by RNAi depletes PI4P at the TGN and causes dispersal of AP-1 and GGAs (Wang et al. 2003, 2007). PI4KIIIβ is required for the integrity of the Golgi (Godi et al. 1999). The activity of PI4KIIIβ is stimulated by Arf1 and an Arf1-binding partner, neuronal calcium sensor-1 (NCS-1) (Godi et al. 1999, Haynes et al. 2005). Frq1, the yeast ortholog of NCS-1, stimulates the activity of Pik1, the yeast ortholog of PI4KIIIβ (Hendricks et al. 1999). PI4KIIIβ is also activated by phosphorylation by PKD, and 14-3-3 protein protects PI4KIIIβ from dephosphorylation (Hausser et al. 2005, 2006).

The lipid phosphatase Sac1 is the only known phosphatase that regulates the PI4P levels. Sac1 is transported from the endoplasmic reticulum to the Golgi upon starvation (Faulhammer et al. 2005). In yeast, starvation-induced transport of Sac1p to the Golgi specifically eliminates a pool of PI4P generated by Pik1p (Faulhammer et al. 2007), suggesting that Sac1p may inhibit PI4P-dependent membrane recruitment of cargo adaptors at the TGN and thus arrest protein traffic in starved cells. Recruitment of Sac1 phosphatase to the Golgi in a rapamycin-inducible system causes acute loss of PI4P at the Golgi, induces release of clathrin and GGAs 1 and 2 from the Golgi, and impairs TGN export of cargo molecules destined for the plasma membrane and late endosomes (Szentpetery et al. 2010).

Phosphatidylserine. Phosphatidylserine (PS) is another phospholipid implicated in the regulation of vesicle budding at the TGN. PS stimulates Arf1-dependent recruitment of AP-1 to synthetic liposomes and regulates ferrichrome-induced sorting of Arn1 to the plasma membrane in yeast (Guo et al. 2010, Zhu et al. 1999). PS is localized to the luminal leaflet of the ER and Golgi and becomes exposed cytosolically at the TGN (Fairn et al. 2011). In yeast, flipping of PS from the luminal leaflet to the cytosolic leaflet at the TGN is mediated by the activity of the aminophospholipid translocase Drs2p, a type IV P-type ATPase (Sebastian et al. 2012). Drs2p has been demonstrated to enable AP-1/clathrin-coated vesicle budding from the TGN (Sebastian et al. 2012). The flippase activity of Drs2p is stimulated by PI4P and an Arf GEF, Gea2, suggesting that the phospholipid translocation and vesicle budding machinery are functionally coupled (Natarajan et al. 2009). Enriching PS at the cytosolic leaflet will induce curvature of the membranes, which is thought to facilitate the vesicle budding process. Because many cargo adaptors contain basic residues on their membrane-proximal surfaces, enrichment of negatively charged PS in the cytosolic leaflet will enhance membrane association of these adaptors via electrostatic interactions.

ROLES OF PROTEIN SORTING AT THE TGN IN PHYSIOLOGICAL PROCESSES

Proteins must be targeted to the correct location for them to perform their physiological functions. Defects in protein sorting at the TGN will inevitably lead to physiological defects. Here we describe protein sorting at the TGN in some physiological processes, including the maintenance of cell polarity, immunity, and regulated secretion.

Protein Sorting at the TGN Regulates Cell Polarity

Epithelial cells are polarized into apical and basolateral domains, and each domain contains distinct proteins that perform specific functions. In addition, many epithelial cells are also polarized along the plane of the epithelium, a process termed planar cell polarity (PCP). PCP is controlled by a group of conserved signaling receptors that are asymmetrically localized on opposing cellular boundaries. Evidence suggests that protein sorting at the TGN contributes to the polarized distributions of apical and basolateral proteins as well as the asymmetrically localized PCP signaling receptors.

Apical-Basolateral Polarity

The polarized distribution of apically or basolaterally targeted proteins depends on trafficking in the biosynthetic and endocytic recycling pathway (Rodriguez-Boulan et al. 2005). Live imaging analysis indicates that apical and basolateral proteins exit the TGN in separate transport carriers, suggesting that the TGN is an important sorting station for apical- and basolateral-targeted proteins (Keller et al. 2001).

Sorting of Basolateral-Targeted Proteins

Sorting of many basolateral proteins depends on sorting signals present in their cytosolic domains. These signals include tyrosine-based motifs (YXXΦ) and dileucine motifs ([DE]XXXL[LI]) that directly interact with clathrin adaptor complexes (Rodriguez-Boulan et al. 2005). Some basolateral cargo molecules contain noncanonical sorting motifs. These motifs include a single leucine residue

in CD147, the GDNS motif in the transferrin receptor, two tyrosine-based signals in the cytosolic domain of the LDL receptor, a tyrosine residue in F protein and dityrosine residues in G protein of Nipah virus, and several key residues (His656, Arg657, and Val660) in the juxtamembrane cytosolic region of the polymeric immunoglobulin receptor (Bonifacino 2014, Cao et al. 2012).

Clathrin and APs play important roles in sorting basolateral-targeted cargo molecules at the TGN, both in cultured cells and in whole organisms (Deborde et al. 2008, Gravotta et al. 2012, Guo et al. 2013a, Rodriguez-Boulan et al. 2005, Shafaq-Zadah et al. 2012). Roles of APs in basolateral sorting events have been reviewed recently (Bonifacino 2014). The μ subunit of AP-1 has two isoforms (μ1A and μ1B). μ1A is ubiquitously expressed, and μ1B is expressed specifically in epithelial cells. Although previous work indicates that μ1A and μ1B preferentially localize to the TGN and recycling endosomes, respectively, recent analysis with improved analytical tools suggests that they are largely colocalized with one another at the TGN and recycling endosomes (Guo et al. 2013a). Moreover, μ1A and μ1B show a similar pattern of regulation by Arf proteins (Guo et al. 2013a). μ1B regulates basolateral sorting of various transmembrane proteins at recycling endosomes (Rodriguez-Boulan et al. 2005) but also promotes TGN export of LDLR in cells depleted of μ1A (Gravotta et al. 2012). μ1A is proposed to mediate protein sorting at the TGN but is also demonstrated to mediate sorting at endosomes (Bonifacino 2014). Double knockdown of μ1A and μ1B causes more severe defects in the basolateral targeting of transferrin receptor, LDLR, and the coxsackie and adenovirus receptor than single knockdown, suggesting that μ1A and μ1B play compensatory roles in the sorting of some polarized cargo (Carvajal-Gonzalez et al. 2012, Gravotta et al. 2012). The cytosolic tail of LDLR, the interleukin-6 receptor β chain, and the poliovirus receptor are efficiently recognized by μ1B but not μ1A, and basolateral sorting of these cargo molecules is dependent on μ1B (Guo et al. 2013a). This evidence suggests that both μ1A and μ1B are important mediators for basolateral targeting and that μ1B extends the spectrum of sorting signal recognition (Gravotta et al. 2012, Guo et al. 2013a).

AP-4 has also been demonstrated to mediate basolateral polarity (Simmen et al. 2002). AP-4 binds to several basolaterally targeted proteins, including furin, LDLR, and CD-MPR, and knockdown of μ4 in MDCK cells causes mistargeting of these proteins to the apical surface (Simmen et al. 2002). However, mice lacking the gene encoding the β subunit of AP-4 do not show any detectable defects in epithelia (Bonifacino 2014). Instead, β4 knockout mice show axonal mislocalization of several neuronal membrane proteins that are normally somatodendritically localized, suggesting that AP-4 is crucial for somatodendritic polarity (Matsuda & Yuzaki 2008, Matsuda et al. 2008).

Sorting of Apical-Targeted Proteins

The sorting signals, cargo adaptors, and receptors that regulate the sorting of apical-targeted proteins are less well defined compared with those mediating basolateral sorting. Here, we summarize the apical sorting signals and the proposed sorting mechanisms.

Glycosylphosphatidylinositol

The sorting signals on apically targeted proteins have been shown to be localized in luminal, transmembrane, and cytosolic domains (Weisz & Rodriguez-Boulan 2009). One of the well-defined apical sorting signals is a glycosylphosphatidylinositol (GPI) anchor. At the TGN, GPI-anchored proteins (GPI-APs) are thought to be associated with lipid microdomains termed lipid rafts (or detergent-resistant membranes), which are enriched in cholesterol and glycosphingolipids. The evidence suggesting the presence of lipid rafts at the TGN in living cells remains controversial.

In yeast, ergosterol and sphingolipid species are specifically enriched in immunoisolated TGN-derived secretory vesicles containing FusMidGFP, a lipid raft–associated protein, suggesting that the sorting of lipid rafts occurs at the TGN (Klemm et al. 2009). Lipid rafts have lipid-ordered phase properties and have the tendency to self-segregate within the membrane, and this process can concentrate associated GPI-APs in membrane patches. Association of GPI-APs with lipid rafts has been shown to be important for apical targeting. However, MDCK cells deficient in the GPI lipid remodeling process produce an intermediate precursor, lysoGPI-APs, that is not incorporated into lipid rafts, and lysoGPI-APs are still delivered apically, suggesting that other pathways may redundantly regulate apical targeting (Castillon et al. 2013).

In addition to lipid rafts, oligomerization of GPI-APs is also important for apical sorting. Apically targeted GPI-APs form high–molecular weight complexes, and impairment of GPI-AP oligomerization causes protein missorting to the basolateral domain. Some GPI-APs are still localized basolaterally (Weisz & Rodriguez-Boulan 2009), and those proteins do not form high–molecular weight complexes (Paladino et al. 2004). Oligomerization of GPI-APs is influenced by the cellular cholesterol content, the GPI signal, and N-glycosylation (Imjeti et al. 2011, Lebreton et al. 2008, Paladino et al. 2008). Oligomerization may increase the affinity of GPI-APs for lipid rafts. Moreover, oligomerization of GPI-APs may drive clustering of small lipid rafts to form large lipid-ordered domains. This would in turn cause increased line tension at the phase boundary, which may induce budding of domains enriched in lipid rafts and their associated GPI-APs.

Sorting of some GPI-APs as well as other apical proteins is regulated by myelin and lymphocyte protein (MAL; VIP17) as well as annexin2 and annexin13b. MAL and the two annexins are associated with lipid rafts and have intrinsic lipid raft clustering properties (Magal et al. 2009, Schuck & Simons 2004). The Golgi-associated FAPP2 is also important for the sorting of some apical proteins. In particular, the PH domain of FAPP2 binds PI4P and Arf1 and induces tubule formation from membrane sheets, suggesting that FAPP2 mediates membrane deformation to facilitate the formation of apical transport carriers (Cao et al. 2009).

N- and O-Linked Glycans

N- and O-linked glycans are important for apical targeting of a variety of cargo molecules. The carbohydrate-binding proteins, galectins, are important players that regulate apical sorting of cargo molecules bearing N- or O-glycans. Most galectins form dimers or higher-order oligomers that possess multivalent carbohydrate-binding sites (Brewer et al. 2002). The multivalent galectin-carbohydrate interactions have the potential to induce clustering of glycoproteins (Brewer et al. 2002), and their associated lipid rafts may contribute to the sorting process. Some galectins may mediate apical sorting independent of lipid rafts. For example, galectin-3 interacts with various apically targeted glycoproteins that are not associated with rafts (Delacour et al. 2006, 2007). Galectin-4, however, associates with sulfatides, which are highly enriched in lipid rafts (Delacour et al. 2005). Moreover, galectin-4 directly interacts with lipid raft–associated glycoproteins and is required for association of apical glycoproteins, including glycoprotein dipeptidylpeptidase-IV and the GPI-anchored complement regulatory protein (CD59), with lipid rafts (Stechly et al. 2009). Galectin-9 has been shown to regulate the apical targeting of endolyn, and this process depends on sialylation of N-glycans on endolyn (Mo et al. 2012).

Other Apical Sorting Signals

The transmembrane domains of influenza virus hemagglutinin (HA) and neuraminidase (NA) are important for apical targeting and mediate the association of HA and NA with lipid rafts (Kundu

et al. 1996, Scheiffele et al. 1997), suggesting that apical sorting signals can be localized within the transmembrane domain. Moreover, many cargo molecules depend on sorting motifs in their cytosolic domain for proper apical targeting (Cao et al. 2012). For example, apical targeting of rhodopsin in polarized MDCK cells depends on its cytosolic domain (Chuang & Sung 1998). In retinal photoreceptors, retinal rhodopsin is transported into the rod outer segment, a specialized cilium, and this process depends on the VXPX sorting motif with its cytosolic domain (Deretic et al. 1998). The VXPX motif directly interacts with Arf4 and regulates the recruitment of Arf4 to the TGN. This process initiates the assembly of the cilia targeting complex composed of Arf4 and Rab11, the Rab11/Arf effector FIP3, and the ArfGAP ASAP1 (Deretic et al. 2005, Wang et al. 2012). The cilia targeting complex has been demonstrated to mediate the packaging of retinal rhodopsin into transport carriers at the TGN (Wang et al. 2012).

Planar Cell Polarity

The establishment of PCP is regulated by an evolutionarily conserved set of signaling receptors, including Vangl2 and Frizzled6 (Fzd6), which adopt characteristic asymmetric localizations on opposing cellular boundaries (**Figure 5*a***) (Klein & Mlodzik 2005). How the PCP asymmetry is established is unclear. GFP-tagged Frizzled is preferentially targeted to the distal site of cell boundaries in the *Drosophila* wing, indicating that polarized intracellular trafficking may regulate the PCP asymmetry (Shimada et al. 2006). Sorting of Vangl2 at the TGN is mediated by an Arf protein, Arfrp1, and its effector, AP-1, which directly interacts with the tyrosine sorting motif within the Vangl2 cytosolic domain (Guo et al. 2013b). In contrast, TGN export of Fzd6 is independent of Arfrp1 and AP-1, suggesting that Vangl2 and Fzd6 are sorted by different cargo

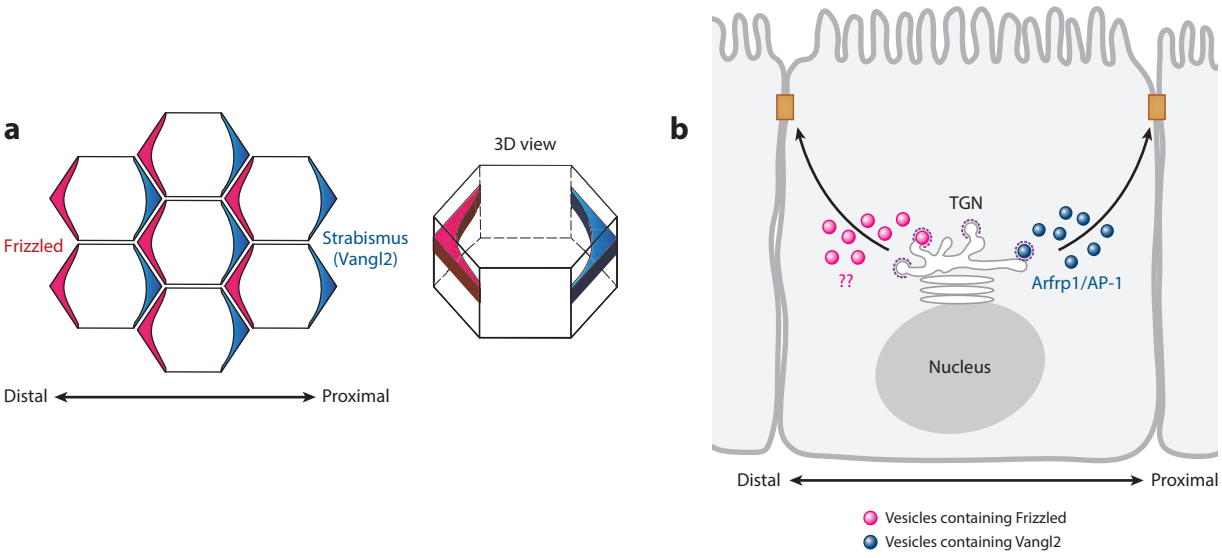


Figure 5

Sorting of planar cell polarity signaling receptors at the *trans*-Golgi network (TGN). (*a*) Diagrams of epithelial cells of *Drosophila* pupal wing revised from Shimada et al. (2006). Strabismus (the *Drosophila* homolog of Vangl2) and Frizzled, two PCP signaling receptors, are localized on opposing cellular boundaries: Strabismus is localized proximal to the *Drosophila* body, whereas Frizzled is localized distal to the body. (*b*) Vangl2 and Frizzled6 are sorted by distinct sorting machineries, which enables their packaging into distinct vesicles. Polarized trafficking of Vangl2- and Frizzled6-enriched vesicles may contribute to the asymmetric localizations of Vangl2 and Frizzled6 (Guo et al. 2013b).

sorting machineries at the TGN. Differential sorting may cause packaging of Vangl2 and Fzd6 into distinct vesicles at the TGN, and polarized transport of these vesicles to specific cell boundaries may contribute to the asymmetric localizations of Vangl2 and Fzd6 (**Figure 5*b***).

Protein Sorting at the TGN Regulates Immunological Processes

The human immunodeficiency virus type 1 (HIV-1) protein Nef disrupts the trafficking of MHC-I by recruiting AP-1 to the cytosolic domain of MHC-I at the TGN, thus diverting it to the lysosome for degradation (Le Gall et al. 1998, Roeth et al. 2004). Through this process, Nef decreases the surface expression of MHC-I to protect HIV-infected cells from killing by cytotoxic T lymphocytes (Roeth et al. 2004). A crystal structure of the ternary complex consisting of the Nef N terminus, the cytosolic domain of MHC-I, and the C-terminal domain of AP-1 μ1 established that a tyrosine residue on the MHC-I cytosolic domain binds the hydrophobic binding pocket within the conventional tyrosine-motif-binding site on μ1 (Jia et al. 2012). The MHC-I cytosolic domain lacks a hydrophobic residue at the Y + 3 position, suggesting that MHC-I is normally unable to bind AP-1. The proline-rich strand (PXXP repeats) of Nef, which runs along the MHC-I cytosolic domain, forms a side wall of the binding groove and promotes interaction between the MHC-I cytosolic domain and μ1 (Jia et al. 2012). In addition, Nef promotes a structural change in μ1 to complete the MHC-I binding groove (Jia et al. 2012). Thus, Nef compensates for the incomplete trafficking motif in MHC-I, enabling an interaction with AP-1.

BST2/tetherin inhibits the release of enveloped virus from the cell surface, and this antiviral activity is antagonized by another HIV-1 protein, the viral protein u (Vpu). One part of the Vpu-induced antagonistic mechanism appears to involve downregulating the delivery of BST2 from the TGN to the cell surface in the biosynthetic trafficking pathway (Dube et al. 2010, Hauser et al. 2010, Schmidt et al. 2011). BST2 binds to AP-1 through an unusual YXY motif on its cytosolic domain (Jia et al. 2014). Vpu simultaneously interacts with AP-1 through its canonical dileucine-sorting motif and with BST2 on the membrane (Jia et al. 2014). These interactions enhance the interaction between BST2 and AP-1, which presumably causes retention of BST2 at the TGN and eventually leads to lysosomal degradation, thereby antagonizing the antiviral activity of BST2 (Jia et al. 2014). A crystal structure of the ternary complex consisting of the AP-1 core, the cytosolic domain of Vpu, and BST2 demonstrates that BST2 and Vpu binds the canonical tyrosine-binding and dileucine-binding sites on AP-1, respectively (Jia et al. 2014). Moreover, AP-1 in the ternary complex adopts a conformation that is much more open than the Arf1-bound AP-1, suggesting that APs may adopt multiple active conformations to recognize distinct cargo molecules (Jia et al. 2014).

Protein Sorting at the TGN Is Important for the Regulated Secretory Pathway

Protein sorting at the TGN is particularly important for professional secretory cells, such as pancreatic β and adrenal chromaffin cells. In contrast to most eukaryotic cells that secrete proteins constitutively via TGN-derived vesicles, cells containing a RSP store physiologically important proteins, such as peptide hormones and neuropeptides, in SGs that undergo exocytosis in a stimulus-dependent manner. Unlike constitutive secretory vesicles, SGs contain an electron-dense core of cargo proteins that undergo aggregation within the lumen of the TGN prior to vesicle formation. Nascent SGs observed by thin-section electron microscopy do not contain an obvious coat structure, and SG formation has thus been proposed to be driven primarily by cargo aggregation at the TGN. However, cells that contain a RSP also possess a constitutive secretory pathway that operates in parallel, and it remains unclear if aggregation alone can account

for the highly specific TGN sorting that occurs in these cells. Indeed, careful immuno-electron microscopy observations have demonstrated the segregation of regulated and constitutive cargo at the level of the TGN (Orci et al. 1987), suggesting an active sorting mechanism.

Several transmembrane proteins contain cytosolic sorting signals that are crucial for their localization to SGs, suggesting the existence of cytosolic proteins involved in directing these membrane proteins into the RSP. For example, the vesicular monoamine transporter, which fills SGs with monoamine transmitters, contains a conserved dileucine-like motif with upstream acidic residues required for efficient sorting to the RSP (Li et al. 2005). In addition, the insulin granule-localized membrane protein phogrin (IA-2β) contains a closely related sorting signal required for its localization (Torii et al. 2005).

An RNAi screen for sorting to the RSP recently led to the identification of the adaptor protein AP-3 as an important sorting determinant (Asensio et al. 2010). In particular, AP-3 knockdown dysregulates secretion in neuroendocrine PC12 cells (Asensio et al. 2010), and complete loss of AP-3 in mouse adrenal chromaffin and pancreatic islet cells produces similar defects (Sirkis et al. 2013). Precisely how AP-3 promotes sorting to the RSP is unclear, but depletion of this adaptor by RNAi perturbs cargo sorting at the level of the TGN (Asensio et al. 2010). Importantly, mutant mice lacking AP-3 show defects in peptide hormone secretion and reduced storage of soluble SG cargo proteins (Sirkis et al. 2013). In addition, the AP-3-interacting protein VPS41 has recently been shown to play a critical role in sorting to the RSP, and loss of this protein produces a phenotype nearly identical to loss of AP-3 (Asensio et al. 2013). Because VPS41 contains a clathrin heavy-chain repeat domain, is capable of self-assembling in vitro, interacts with AP-3, and localizes in part to the TGN, it has been suggested that VPS41 may act as a coat protein involved in sorting to the RSP (Asensio et al. 2013). In addition, several BAR domain–containing proteins, including arfaptin, PICK1, and ICA69, have recently been suggested to promote the formation of SGs at the level of the TGN (Cao et al. 2013, Cruz-Garcia et al. 2013, Gehart et al. 2012, Holst et al. 2013), and mice lacking PICK1 and ICA69 indeed appear to have moderate defects in regulated secretion.

CONCLUSION AND PERSPECTIVE

Over the past several decades, a large number of cargo adaptors and receptors have been identified that specifically recognize a wide variety of cargo molecules at the TGN. Structural analysis has provided a clear view of the interactions between specific cargo adaptors/receptors and their associated cargo molecules. Moreover, past findings have provided mechanistic insights into the key steps of the sorting process, including membrane association of cargo adaptors, cargo recognition, and spatiotemporal regulation.

Despite significant advances in the understanding of protein sorting at the TGN, several important aspects of the TGN sorting process remain open for investigation. Are there novel cargo adaptors and receptors at the TGN? Do Arf proteins promote the binding of novel cargo adaptors to cargo molecules? Do Arf proteins other than Arfrp1 regulate the specificity of cargo recognition? TGN-derived vesicles should contain not only the correct cargo molecules but also the correct SNARE proteins that will ensure targeting to the correct destination. How the cargo sorting machinery is coupled to the vesicle targeting machinery is still unclear. Moreover, little attention has been given to the TGN sorting of proteins that are involved in cancer, metastasis, and other diseases.

To achieve a comprehensive view of protein sorting at the TGN, it is now important to develop in vitro assays that faithfully reconstitute the vesicular release of a variety of proteins from the TGN. Moreover, a better understanding of the protein composition of purified TGN-derived

vesicles containing specific cargo proteins will provide novel information about the sorting process. In addition, high-resolution fluorescence imaging and correlative light-electron microscopy studies will be essential for a further understanding of the protein sorting process at the TGN in vivo.

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LITERATURE CITED

Andersen OM, Reiche J, Schmidt V, Gotthardt M, Spoelgen R, et al. 2005. Neuronal sorting protein-related receptor sorLA/LR11 regulates processing of the amyloid precursor protein. *Proc. Natl. Acad. Sci. USA* 102:13461–66

Andersen OM, Schmidt V, Spoelgen R, Gliemann J, Behlke J, et al. 2006. Molecular dissection of the interaction between amyloid precursor protein and its neuronal trafficking receptor SorLA/LR11. *Biochemistry* 45:2618–28

Aridor M, Bannykh SI, Rowe T, Balch WE. 1999. Cargo can modulate COPII vesicle formation from the endoplasmic reticulum. *J. Biol. Chem.* 274:4389–99

Asensio CS, Sirkis DW, Edwards RH. 2010. RNAi screen identifies a role for adaptor protein AP-3 in sorting to the regulated secretory pathway. *J. Cell Biol.* 191:1173–87

Asensio CS, Sirkis DW, Maas JW Jr, Egami K, To TL, et al. 2013. Self-assembly of VPS41 promotes sorting required for biogenesis of the regulated secretory pathway. *Dev. Cell* 27:425–37

Banziger C, Soldini D, Schutt C, Zipperlen P, Hausmann G, Basler K. 2006. Wntless, a conserved membrane protein dedicated to the secretion of Wnt proteins from signaling cells. *Cell* 125:509–22

Barfield RM, Fromme JC, Schekman R. 2009. The exomer coat complex transports Fus1p to the plasma membrane via a novel plasma membrane sorting signal in yeast. *Mol. Biol. Cell* 20:4985–96

Bartscherer K, Pelte N, Ingelfinger D, Boutros M. 2006. Secretion of Wnt ligands requires Evi, a conserved transmembrane protein. *Cell* 125:523–33

Barylko B, Mao YS, Wlodarski P, Jung G, Binns DD, et al. 2009. Palmitoylation controls the catalytic activity and subcellular distribution of phosphatidylinositol 4-kinase IIα. *J. Biol. Chem.* 284:9994–10003

Benhra N, Lallet S, Cotton M, Le Bras S, Dussert A, Le Borgne R. 2011. AP-1 controls the trafficking of Notch and Sanpodo toward E-cadherin junctions in sensory organ precursors. *Curr. Biol.* 21:87–95

Berkovic SF, Dibbens LM, Oshlack A, Silver JD, Katerelos M, et al. 2008. Array-based gene discovery with three unrelated subjects shows SCARB2/LIMP-2 deficiency causes myoclonus epilepsy and glomerulosclerosis. *Am. J. Hum. Genet.* 82:673–84

Bohnsack RN, Song X, Olson LJ, Kudo M, Gotschall RR, et al. 2009. Cation-independent mannose 6-phosphate receptor: a composite of distinct phosphomannosyl binding sites. *J. Biol. Chem.* 284:35215–26

Bonifacino JS. 2004. The GGA proteins: adaptors on the move. *Nat. Rev. Mol. Cell Biol.* 5:23–32

Bonifacino JS. 2014. Adaptor proteins involved in polarized sorting. *J. Cell Biol.* 204:7–17

Bonifacino JS, Traub LM. 2003. Signals for sorting of transmembrane proteins to endosomes and lysosomes. *Annu. Rev. Biochem.* 72:395–447

Boonen M, Vogel P, Platt KA, Dahms N, Kornfeld S. 2009. Mice lacking mannose 6-phosphate uncovering enzyme activity have a milder phenotype than mice deficient for *N*-acetylglucosamine-1-phosphotransferase activity. *Mol. Biol. Cell* 20:4381–89

Braulke T, Bonifacino JS. 2009. Sorting of lysosomal proteins. *Biochim. Biophys. Acta* 1793:605–14

Brett TJ, Traub LM, Fremont DH. 2002. Accessory protein recruitment motifs in clathrin-mediated endocytosis. *Structure* 10:797–809

Brewer CF, Miceli MC, Baum LG. 2002. Clusters, bundles, arrays and lattices: novel mechanisms for lectin-saccharide-mediated cellular interactions. *Curr. Opin. Struct. Biol.* 12:616–23

Briguglio JS, Kumar S, Turkewitz AP. 2013. Lysosomal sorting receptors are essential for secretory granule biogenesis in *Tetrahymena*. *J. Cell Biol.* 203:537–50

Burgess J, Jauregui M, Tan J, Rollins J, Lallet S, et al. 2011. AP-1 and clathrin are essential for secretory granule biogenesis in *Drosophila*. *Mol. Biol. Cell* 22:2094–105

Burgos PV, Mardones GA, Rojas AL, daSilva LLP, Prabhu Y, et al. 2010. Sorting of the Alzheimer's disease amyloid precursor protein mediated by the AP-4 complex. *Dev. Cell* 18:425–36

Canuel M, Korkidakis A, Konnyu K, Morales CR. 2008. Sortilin mediates the lysosomal targeting of cathepsins D and H. *Biochem. Biophys. Res. Commun.* 373:292–97

Cao M, Mao Z, Kam C, Xiao N, Cao X, et al. 2013. PICK1 and ICA69 control insulin granule trafficking and their deficiencies lead to impaired glucose tolerance. *PLOS Biol.* 11:e1001541

Cao X, Coskun U, Rossle M, Buschhorn SB, Grzybek M, et al. 2009. Golgi protein FAPP2 tubulates membranes. *Proc. Natl. Acad. Sci. USA* 106:21121–25

Cao X, Surma MA, Simons K. 2012. Polarized sorting and trafficking in epithelial cells. *Cell Res.* 22:793–805

Carvajal-Gonzalez JM, Gravotta D, Mattera R, Diaz F, Perez Bay A, et al. 2012. Basolateral sorting of the coxsackie and adenovirus receptor through interaction of a canonical YXXΦ motif with the clathrin adaptors AP-1A and AP-1B. *Proc. Natl. Acad. Sci. USA* 109:3820–25

Caster AH, Sztul E, Kahn RA. 2013. A role for cargo in Arf-dependent adaptor recruitment. *J. Biol. Chem.* 288:14788–804

Castillon GA, Michon L, Watanabe R. 2013. Apical sorting of lysoGPI-anchored proteins occurs independent of association with detergent-resistant membranes but dependent on their N-glycosylation. *Mol. Biol. Cell* 24:2021–33

Chaudhuri R, Lindwasser OW, Smith WJ, Hurley JH, Bonifacino JS. 2007. Downregulation of CD4 by human immunodeficiency virus type 1 Nef is dependent on clathrin and involves direct interaction of Nef with the AP2 clathrin adaptor. *J. Virol.* 81:3877–90

Chavez CA, Bohnsack RN, Kudo M, Gotschall RR, Canfield WM, Dahms NM. 2007. Domain 5 of the cation-independent mannose 6-phosphate receptor preferentially binds phosphodiesters (mannose 6-phosphate *N*-acetylglucosamine ester). *Biochemistry* 46:12604–17

Chen ZY, Ieraci A, Teng H, Dall H, Meng CX, et al. 2005. Sortilin controls intracellular sorting of brain-derived neurotrophic factor to the regulated secretory pathway. *J. Neurosci.* 25:6156–66

Chidambaram S, Müllers N, Wiederhold K, Haucke V, von Mollard GF. 2004. Specific interaction between SNAREs and epsin N-terminal homology (ENTH) domains of epsin-related proteins in *trans*-Golgi network to endosome transport. *J. Biol. Chem.* 279:4175–79

Chidambaram S, Zimmermann J, von Mollard GF. 2008. ENTH domain proteins are cargo adaptors for multiple SNARE proteins at the TGN endosome. *J. Cell Sci.* 121:329–38

Choy RW, Cheng Z, Schekman R. 2012. Amyloid precursor protein (APP) traffics from the cell surface via endosomes for amyloid β (Aβ) production in the *trans*-Golgi network. *Proc. Natl. Acad. Sci. USA* 109:E2077–82

Christis C, Munro S. 2012. The small G protein Arl1 directs the *trans*-Golgi-specific targeting of the Arf1 exchange factors BIG1 and BIG2. *J. Cell Biol.* 196:327–35

Chuang JS, Schekman RW. 1996. Differential trafficking and timed localization of two chitin synthase proteins, Chs2p and Chs3p. *J. Cell Biol.* 135:597–610

Chuang JZ, Sung CH. 1998. The cytoplasmic tail of rhodopsin acts as a novel apical sorting signal in polarized MDCK cells. *J. Cell Biol.* 142:1245–56

Clemens Grisham R, Kindt K, Finger-Baier K, Schmid B, Nicolson T. 2013. Mutations in *ap1b1* cause mistargeting of the Na^+/K^+-ATPase pump in sensory hair cells. *PLOS ONE* 8:e60866

Cohen LA, Honda A, Varnai P, Brown FD, Balla T, Donaldson JG. 2007. Active Arf6 recruits ARNO/cytohesin GEFs to the PM by binding their PH domains. *Mol. Biol. Cell* 18:2244–53

Cooper AA, Stevens TH. 1996. Vps10p cycles between the late-Golgi and prevacuolar compartments in its function as the sorting receptor for multiple yeast vacuolar hydrolases. *J. Cell Biol.* 133:529–41

Copic A, Starr TL, Schekman R. 2007. Ent3p and Ent5p exhibit cargo-specific functions in trafficking proteins between the *trans*-Golgi network and the endosomes in yeast. *Mol. Biol. Cell* 18:1803–15

Costaguta G, Duncan MC, Fernández GE, Huang GH, Payne GS. 2006. Distinct roles for TGN/endosome epsin-like adaptors Ent3p and Ent5p. *Mol. Biol. Cell* 17:3907–20

Cowles CR, Odorizzi G, Payne GS, Emr SD. 1997. The AP-3 adaptor complex is essential for cargo-selective transport to the yeast vacuole. *Cell* 91:109–18

Cramer JF, Gustafsen C, Behrens MA, Oliveira CL, Pedersen JS, et al. 2010. GGA autoinhibition revisited. *Traffic* 11:259–73

Crottet P, Meyer DM, Rohrer J, Spiess M. 2002. ARF1·GTP, tyrosine-based signals, and phosphatidylinositol 4,5-bisphosphate constitute a minimal machinery to recruit the AP-1 clathrin adaptor to membranes. *Mol. Biol. Cell* 13:3672–82

Cruz-Garcia D, Ortega-Bellido M, Scarpa M, Villeneuve J, Jovic M, et al. 2013. Recruitment of arfaptins to the *trans*-Golgi network by PI(4)P and their involvement in cargo export. *EMBO J.* 32:1717–29

Curwin AJ, von Blume J, Malhotra V. 2012. Cofilin-mediated sorting and export of specific cargo from the Golgi apparatus in yeast. *Mol. Biol. Cell* 23:2327–38

Daboussi L, Costaguta G, Payne GS. 2012. Phosphoinositide-mediated clathrin adaptor progression at the *trans*-Golgi network. *Nat. Cell Biol.* 14:239–48

De M, Abazeed ME, Fuller RS. 2013. Direct binding of the Kex2p cytosolic tail to the VHS domain of yeast Gga2p facilitates TGN to prevacuolar compartment transport and is regulated by phosphorylation. *Mol. Biol. Cell* 24:495–509

De Matteis MA, Di Campli A, Godi A. 2005. The role of the phosphoinositides at the Golgi complex. *Biochim. Biophys. Acta* 1744:396–405

De Matteis MA, Luini A. 2008. Exiting the Golgi complex. *Nat. Rev. Mol. Cell Biol.* 9:273–84

Deborde S, Perret E, Gravotta D, Deora A, Salvarezza S, et al. 2008. Clathrin is a key regulator of basolateral polarity. *Nature* 452:719–23

Delacour D, Cramm-Behrens CI, Drobecq H, Le Bivic A, Naim HY, Jacob R. 2006. Requirement for galectin-3 in apical protein sorting. *Curr. Biol.* 16:408–14

Delacour D, Gouyer V, Zanetta JP, Drobecq H, Leteurtre E, et al. 2005. Galectin-4 and sulfatides in apical membrane trafficking in enterocyte-like cells. *J. Cell Biol.* 169:491–501

Delacour D, Greb C, Koch A, Salomonsson E, Leffler H, et al. 2007. Apical sorting by galectin-3-dependent glycoprotein clustering. *Traffic* 8:379–88

Dell'Angelica EC. 2009. AP-3-dependent trafficking and disease: the first decade. *Curr. Opin. Cell Biol.* 21:552–59

Dell'Angelica EC, Shotelersuk V, Aguilar RC, Gahl WA, Bonifacino JS. 1999. Altered trafficking of lysosomal proteins in Hermansky-Pudlak syndrome due to mutations in the β3A subunit of the AP-3 adaptor. *Mol. Cell* 3:11–21

Demmel L, Gravert M, Ercan E, Habermann B, Müller-Reichert T, et al. 2008. The clathrin adaptor Gga2p is a phosphatidylinositol 4-phosphate effector at the Golgi exit. *Mol. Biol. Cell* 19:1991–2002

Deretic D, Schmerl S, Hargrave PA, Arendt A, McDowell JH. 1998. Regulation of sorting and post-Golgi trafficking of rhodopsin by its C-terminal sequence QVS(A)PA. *Proc. Natl. Acad. Sci. USA* 95:10620–25

Deretic D, Williams AH, Ransom N, Morel V, Hargrave PA, Arendt A. 2005. Rhodopsin C terminus, the site of mutations causing retinal disease, regulates trafficking by binding to ADP-ribosylation factor 4 (ARF4). *Proc. Natl. Acad. Sci. USA* 102:3301–6

DiNitto JP, Delprato A, Gabe Lee MT, Cronin TC, Huang S, et al. 2007. Structural basis and mechanism of autoregulation in 3-phosphoinositide-dependent Grp1 family Arf GTPase exchange factors. *Mol. Cell* 28:569–83

Donaldson JG, Jackson CL. 2011. ARF family G proteins and their regulators: roles in membrane transport, development and disease. *Nat. Rev. Mol. Cell Biol.* 12:362–75

Doray B, Bruns K, Ghosh P, Kornfeld SA. 2002a. Autoinhibition of the ligand-binding site of GGA1/3 VHS domains by an internal acidic cluster-dileucine motif. *Proc. Natl. Acad. Sci. USA* 99:8072–77

Doray B, Ghosh P, Griffith J, Geuze HJ, Kornfeld S. 2002b. Cooperation of GGAs and AP-1 in packaging MPRs at the *trans*-Golgi network. *Science* 297:1700–3

Doray B, Lee I, Knisely J, Bu G, Kornfeld S. 2007. The γ/σ1 and α/σ2 hemicomplexes of clathrin adaptors AP-1 and AP-2 harbor the dileucine recognition site. *Mol. Biol. Cell* 18:1887–96

Doray B, Misra S, Qian Y, Brett TJ, Kornfeld S. 2012. Do GGA adaptors bind internal DXXLL motifs? *Traffic* 13:1315–25

Dube M, Roy BB, Guiot-Guillain P, Binette J, Mercier J, et al. 2010. Antagonism of tetherin restriction of HIV-1 release by Vpu involves binding and sequestration of the restriction factor in a perinuclear compartment. *PLOS Pathog.* 6:e1000856

Duncan MC, Costaguta G, Payne GS. 2003. Yeast epsin-related proteins required for Golgi-endosome traffic define a γ-adaptin ear-binding motif. *Nat. Cell Biol.* 5:77–81

Eissenberg JC, Ilvarsonn AM, Sly WS, Waheed A, Krzyzanek V, et al. 2011. *Drosophila* GGA model: an ultimate gateway to GGA analysis. *Traffic* 12:1821–38

Fairn GD, Schieber NL, Ariotti N, Murphy S, Kuerschner L, et al. 2011. High-resolution mapping reveals topologically distinct cellular pools of phosphatidylserine. *J. Cell Biol.* 194:257–75

Farias GG, Cuitino L, Guo X, Ren X, Jarnik M, et al. 2012. Signal-mediated, AP-1/clathrin-dependent sorting of transmembrane receptors to the somatodendritic domain of hippocampal neurons. *Neuron* 75:810–23

Faulhammer F, Kanjilal-Kolar S, Knödler A, Lo J, Lee Y, et al. 2007. Growth control of Golgi phosphoinositides by reciprocal localization of Sac1 lipid phosphatase and Pik1 4-kinase. *Traffic* 8:1554–67

Faulhammer F, Konrad G, Brankatschk B, Tahirovic S, Knödler A, Mayinger P. 2005. Cell growth-dependent coordination of lipid signaling and glycosylation is mediated by interactions between Sac1p and Dpm1p. *J. Cell Biol.* 168:185–91

Feng L, Seymour AB, Jiang S, To A, Peden AA, et al. 1999. The β3A subunit gene (*Ap3B1*) of the AP-3 adaptor complex is altered in the mouse hypopigmentation mutant pearl, a model for Hermansky-Pudlak syndrome and night blindness. *Hum. Mol. Genet.* 8:323–30

Ford MG, Mills IG, Peter BJ, Vallis Y, Praefcke GJ, et al. 2002. Curvature of clathrin-coated pits driven by epsin. *Nature* 419:361–66

Friant S, Pécheur EI, Eugster A, Michel F, Lefkir Y, et al. 2003. Ent3p is a PtdIns(3,5)P_2 effector required for protein sorting to the multivesicular body. *Dev. Cell* 5:499–511

Fujita H, Saeki M, Yasunaga K, Ueda T, Imoto T, Himeno M. 1999. *In vitro* binding study of adaptor protein complex (AP-1) to lysosomal targeting motif (LI-motif). *Biochem. Biophys. Res. Commun.* 255:54–58

Gamp AC, Tanaka Y, Lüllmann-Rauch R, Wittke D, D'Hooge R, et al. 2003. LIMP-2/LGP85 deficiency causes ureteric pelvic junction obstruction, deafness and peripheral neuropathy in mice. *Hum. Mol. Genet.* 12:631–46

Gariano G, Guarienti M, Bresciani R, Borsani G, Carola G, et al. 2014. Analysis of three μ1-AP1 subunits during zebrafish development. *Dev. Dyn.* 243:299–314

Gasnereau I, Herr P, Chia PZ, Basler K, Gleeson PA. 2011. Identification of an endocytosis motif in an intracellular loop of Wntless protein, essential for its recycling and the control of Wnt protein signaling. *J. Biol. Chem.* 286:43324–33

Gehart H, Goginashvili A, Beck R, Morvan J, Erbs E, et al. 2012. The BAR domain protein Arfaptin-1 controls secretory granule biogenesis at the *trans*-Golgi network. *Dev. Cell* 23:756–68

Gelling CL, Dawes IW, Perlmutter DH, Fisher EA, Brodsky JL. 2012. The endosomal protein-sorting receptor sortilin has a role in trafficking α-1 antitrypsin. *Genetics* 192:889–903

Gillingham AK, Munro S. 2007. The small G proteins of the Arf family and their regulators. *Annu. Rev. Cell Dev. Biol.* 23:579–611

Glyvuk N, Tsytsyura Y, Geumann C, D'Hooge R, Huve J, et al. 2010. AP-1/σ1B-adaptin mediates endosomal synaptic vesicle recycling, learning and memory. *EMBO J.* 29:1318–30

Godi A, Pertile P, Meyers R, Marra P, Di Tullio G, et al. 1999. ARF mediates recruitment of PtdIns-4-OH kinase-β and stimulates synthesis of PtdIns(4,5)P_2 on the Golgi complex. *Nat. Cell Biol.* 1:280–87

Gonzalez A, Valeiras M, Sidransky E, Tayebi N. 2013. Lysosomal integral membrane protein-2: a new player in lysosome-related pathology. *Mol. Genet. Metab.* 111:84–91

Goodman RM, Thombre S, Firtina Z, Gray D, Betts D, et al. 2006. Sprinter: a novel transmembrane protein required for Wg secretion and signaling. *Development* 133:4901–11
Govero J, Doray B, Bai H, Kornfeld S. 2012. Analysis of *Gga* null mice demonstrates a non-redundant role for mammalian GGA2 during development. *PLOS ONE* 7:e30184
Gravotta D, Carvajal-Gonzalez JM, Mattera R, Deborde S, Banfelder JR, et al. 2012. The clathrin adaptor AP-1A mediates basolateral polarity. *Dev. Cell* 22:811–23
Guo X, Mattera R, Ren X, Chen Y, Retamal C, et al. 2013a. The adaptor protein-1 μ1B subunit expands the repertoire of basolateral sorting signal recognition in epithelial cells. *Dev. Cell* 27:353–66
Guo Y, Au WC, Shakoury-Elizeh M, Protchenko O, Basrai M, et al. 2010. Phosphatidylserine is involved in the ferrichrome-induced plasma membrane trafficking of Arn1 in *Saccharomyces cerevisiae*. *J. Biol. Chem.* 285:39564–73
Guo Y, Zanetti G, Schekman R. 2013b. A novel GTP-binding protein-adaptor protein complex responsible for export of Vangl2 from the *trans* Golgi network. *eLife* 2:e00160
Gustafsen C, Kjolby M, Nyegaard M, Mattheisen M, Lundhede J, et al. 2014. The hypercholesterolemia-risk gene *SORT1* facilitates PCSK9 secretion. *Cell Metab.* 19:310–18
Hancock MK, Yammani RD, Dahms NM. 2002. Localization of the carbohydrate recognition sites of the insulin-like growth factor II/mannose 6-phosphate receptor to domains 3 and 9 of the extracytoplasmic region. *J. Biol. Chem.* 277:47205–12
Harterink M, Korswagen HC. 2012. Dissecting the Wnt secretion pathway: key questions on the modification and intracellular trafficking of Wnt proteins. *Acta Physiol.* 204:8–16
Hase K, Nakatsu F, Ohmae M, Sugihara K, Shioda N, et al. 2013. AP-1B-mediated protein sorting regulates polarity and proliferation of intestinal epithelial cells in mice. *Gastroenterology* 145:625–35
Hausser A, Link G, Hoene M, Russo C, Selchow O, Pfizenmaier K. 2006. Phospho-specific binding of 14–3–3 proteins to phosphatidylinositol 4-kinase III β protects from dephosphorylation and stabilizes lipid kinase activity. *J. Cell Sci.* 119:3613–21
Hauser H, Lopez LA, Yang SJ, Oldenburg JE, Exline CM, et al. 2010. HIV-1 Vpu and HIV-2 Env counteract BST-2/tetherin by sequestration in a perinuclear compartment. *Retrovirology* 7:51
Hausser A, Storz P, Märtens S, Link G, Toker A, Pfizenmaier K. 2005. Protein kinase D regulates vesicular transport by phosphorylating and activating phosphatidylinositol-4 kinase IIIβ at the Golgi complex. *Nat. Cell Biol.* 7:880–86
Haynes LP, Thomas GMH, Burgoyne RD. 2005. Interaction of neuronal calcium sensor-1 and ADP-ribosylation factor 1 allows bidirectional control of phosphatidylinositol 4-kinase β and *trans*-Golgi network-plasma membrane traffic. *J. Biol. Chem.* 280:6047–54
Heldwein EE, Macia E, Wang J, Yin HL, Kirchhausen T, Harrison SC. 2004. Crystal structure of the clathrin adaptor protein 1 core. *Proc. Natl. Acad. Sci. USA* 101:14108–13
Hendricks KB, Wang BQ, Schnieders EA, Thorner J. 1999. Yeast homologue of neuronal frequenin is a regulator of phosphatidylinositol-4-OH kinase. *Nat. Cell Biol.* 1:234–41
Hermann GJ, Schroeder LK, Hieb CA, Kershner AM, Rabbitts BM, et al. 2005. Genetic analysis of lysosomal trafficking in *Caenorhabditis elegans*. *Mol. Biol. Cell* 16:3273–88
Herr P, Basler K. 2012. Porcupine-mediated lipidation is required for Wnt recognition by Wls. *Dev. Biol.* 361:392–402
Hesse D, Hommel A, Jaschke A, Moser M, Bernhardt U, et al. 2010. Altered GLUT4 trafficking in adipocytes in the absence of the GTPase *Arfrp1*. *Biochem. Biophys. Res. Commun.* 394:896–903
Hesse D, Jaschke A, Kanzleiter T, Witte N, Augustin R, et al. 2012. GTPase ARFRP1 is essential for normal hepatic glycogen storage and insulin-like growth factor 1 secretion. *Mol. Cell. Biol.* 32:4363–74
Hirst J, Borner GH, Antrobus R, Peden AA, Hodson NA, et al. 2012. Distinct and overlapping roles for AP-1 and GGAs revealed by the "knocksideways" system. *Curr. Biol.* 22:1711–16
Hirst J, Carmichael J. 2011. A potential role for the clathrin adaptor GGA in *Drosophila* spermatogenesis. *BMC Cell Biol.* 12:22
Hirst J, Irving C, Borner GH. 2013. Adaptor protein complexes AP-4 and AP-5: new players in endosomal trafficking and progressive spastic paraplegia. *Traffic* 14:153–64
Hirst J, Miller SE, Taylor MJ, von Mollard GF, Robinson MS. 2004. EpsinR is an adaptor for the SNARE protein Vti1b. *Mol. Biol. Cell* 15:5593–602

Hirst J, Sahlender DA, Choma M, Sinka R, Harbour ME, et al. 2009. Spatial and functional relationship of GGAs and AP-1 in *Drosophila* and HeLa cells. *Traffic* 10:1696–710

Hofmann I, Thompson A, Sanderson CM, Munro S. 2007. The Arl4 family of small G proteins can recruit the cytohesin Arf6 exchange factors to the plasma membrane. *Curr. Biol.* 17:711–16

Holst B, Madsen KL, Jansen AM, Jin C, Rickhag M, et al. 2013. PICK1 deficiency impairs secretory vesicle biogenesis and leads to growth retardation and decreased glucose tolerance. *PLOS Biol.* 11:e1001542

Hommel A, Hesse D, Völker W, Jaschke A, Moser M, et al. 2010. The ARF-like GTPase ARFRP1 is essential for lipid droplet growth and is involved in the regulation of lipolysis. *Mol. Cell. Biol.* 30:1231–42

Honing S, Sandoval IV, von Figura K. 1998. A di-leucine-based motif in the cytoplasmic tail of LIMP-II and tyrosinase mediates selective binding of AP-3. *EMBO J.* 17:1304–14

Hung CW, Aoh QL, Joglekar AP, Payne GS, Duncan MC. 2012. Adaptor autoregulation promotes coordinated binding within clathrin coats. *J. Biol. Chem.* 287:17398–407

Imjeti NS, Lebreton S, Paladino S, de la Fuente E, Gonzalez A, Zurzolo C. 2011. N-glycosylation instead of cholesterol mediates oligomerization and apical sorting of GPI-APs in FRT cells. *Mol. Biol. Cell* 22:4621–34

Jackson LP, Kelly BT, McCoy AJ, Gaffry T, James LC, et al. 2010. A large-scale conformational change couples membrane recruitment to cargo binding in the AP2 clathrin adaptor complex. *Cell* 141:1220–29

Jacobsen L, Madsen P, Jacobsen C, Nielsen MS, Gliemann J, Petersen CM. 2001. Activation and functional characterization of the mosaic receptor SorLA/LR11. *J. Biol. Chem.* 276:22788–96

Janvier K, Kato Y, Boehm M, Rose JR, Martina JA, et al. 2003. Recognition of dileucine-based sorting signals from HIV-1 Nef and LIMP-II by the AP-1 γ-σ1 and AP-3 δ-σ3 hemicomplexes. *J. Cell Biol.* 163:1281–90

Jia X, Singh R, Homann S, Yang H, Guatelli J, Xiong Y. 2012. Structural basis of evasion of cellular adaptive immunity by HIV-1 Nef. *Nat. Struct. Mol. Biol.* 19:701–6

Jia X, Weber E, Tokarev A, Lewinski M, Rizk M, et al. 2014. Structural basis of HIV-1 Vpu-mediated BST2 antagonism via hijacking of the clathrin adaptor protein complex 1. *eLife* 3:e02362

Kametaka S, Kametaka A, Yonekura S, Haruta M, Takenoshita S, et al. 2012. AP-1 clathrin adaptor and CG8538/Aftiphilin are involved in Notch signaling during eye development in *Drosophila melanogaster*. *J. Cell Sci.* 125:634–48

Kanoh H, Williger BT, Exton JH. 1997. Arfaptin 1, a putative cytosolic target protein of ADP-ribosylation factor, is recruited to Golgi membranes. *J. Biol. Chem.* 272:5421–29

Kantheti P, Qiao X, Diaz ME, Peden AA, Meyer GE, et al. 1998. Mutation in AP-3 δ in the *mocha* mouse links endosomal transport to storage deficiency in platelets, melanosomes, and synaptic vesicles. *Neuron* 21:111–22

Kaplan OI, Molla-Herman A, Cevik S, Ghossoub R, Kida K, et al. 2010. The AP-1 clathrin adaptor facilitates cilium formation and functions with RAB-8 in *C. elegans* ciliary membrane transport. *J. Cell Sci.* 123:3966–77

Kato Y, Misra S, Puertollano R, Hurley JH, Bonifacino JS. 2002. Phosphoregulation of sorting signal-VHS domain interactions by a direct electrostatic mechanism. *Nat. Struct. Biol.* 9:532–36

Keller P, Toomre D, Diaz E, White J, Simons K. 2001. Multicolour imaging of post-Golgi sorting and trafficking in live cells. *Nat. Cell Biol.* 3:140–49

Kelly BT, McCoy AJ, Spate K, Miller SE, Evans PR, et al. 2008. A structural explanation for the binding of endocytic dileucine motifs by the AP2 complex. *Nature* 456:976–79

Kent HM, McMahon HT, Evans PR, Benmerah A, Owen DJ. 2002. γ-Adaptin appendage domain: structure and binding site for Eps15 and γ-synergin. *Structure* 10:1139–48

Kjolby M, Andersen OM, Breiderhoff T, Fjorback AW, Pedersen KM, et al. 2010. Sort1, encoded by the cardiovascular risk locus 1p13.3, is a regulator of hepatic lipoprotein export. *Cell Metab.* 12:213–23

Klein TJ, Mlodzik M. 2005. Planar cell polarization: An emerging model points in the right direction. *Annu. Rev. Cell Dev. Biol.* 21:155–76

Klemm RW, Ejsing CS, Surma MA, Kaiser HJ, Gerl MJ, et al. 2009. Segregation of sphingolipids and sterols during formation of secretory vesicles at the *trans*-Golgi network. *J. Cell Biol.* 185:601–12

Klinger SC, Glerup S, Raarup MK, Mari MC, Nyegaard M, et al. 2011. SorLA regulates the activity of lipoprotein lipase by intracellular trafficking. *J. Cell Sci.* 124:1095–105

Klumperman J. 2011. Architecture of the mammalian Golgi. *Cold Spring Harb. Perspect. Biol.* 3:a005181
Knipper M, Claussen C, Rüttiger L, Zimmermann U, Lüllmann-Rauch R, et al. 2006. Deafness in LIMP2-deficient mice due to early loss of the potassium channel KCNQ1/KCNE1 in marginal cells of the stria vascularis. *J. Physiol.* 576:73–86
Koster A, Saftig P, Matzner U, von Figura K, Peters C, Pohlmann R. 1993. Targeted disruption of the M(r) 46,000 mannose 6-phosphate receptor gene in mice results in misrouting of lysosomal proteins. *EMBO J.* 12:5219–23
Kundu A, Avalos RT, Sanderson CM, Nayak DP. 1996. Transmembrane domain of influenza virus neuraminidase, a type II protein, possesses an apical sorting signal in polarized MDCK cells. *J. Virol.* 70:6508–15
Kwon S, Christian JL. 2011. Sortilin associates with transforming growth factor-β family proteins to enhance lysosome-mediated degradation. *J. Biol. Chem.* 286:21876–85
Ladinsky MS, Mastronarde DN, McIntosh JR, Howell KE, Staehelin LA. 1999. Golgi structure in three dimensions: functional insights from the normal rat kidney cell. *J. Cell Biol.* 144:1135–49
Ladinsky MS, Wu CC, McIntosh S, McIntosh JR, Howell KE. 2002. Structure of the Golgi and distribution of reporter molecules at 20 degrees C reveals the complexity of the exit compartments. *Mol. Biol. Cell* 13:2810–25
Le Gall S, Erdtmann L, Benichou S, Berlioz-Torrent C, Liu L, et al. 1998. Nef interacts with the μ subunit of clathrin adaptor complexes and reveals a cryptic sorting signal in MHC I molecules. *Immunity* 8:483–95
Lebreton S, Paladino S, Zurzolo C. 2008. Selective roles for cholesterol and actin in compartmentalization of different proteins in the Golgi and plasma membrane of polarized cells. *J. Biol. Chem.* 283:29545–53
Lee J, Jongeward GD, Sternberg PW. 1994. *unc-101*, a gene required for many aspects of *Caenorhabditis elegans* development and behavior, encodes a clathrin-associated protein. *Genes Dev.* 8:60–73
Lee C, Goldberg J. 2010. Structure of coatomer cage proteins and the relationship among COPI, COPII, and clathrin vesicle coats. *Cell* 142:123–32
Lee I, Doray B, Govero J, Kornfeld S. 2008a. Binding of cargo sorting signals to AP-1 enhances its association with ADP ribosylation factor 1-GTP. *J. Cell Biol.* 180:467–72
Lee I, Drake MT, Traub LM, Kornfeld S. 2008b. Cargo-sorting signals promote polymerization of adaptor protein-1 in an Arf-1·GTP-independent manner. *Arch. Biochem. Biophys.* 479:63–68
Lee JH, Fischer JA. 2012. *Drosophila* Tel2 is expressed as a translational fusion with EpsinR and is a regulator of wingless signaling. *PLOS ONE* 7:e46357
Lee JH, Overstreet E, Fitch E, Fleenor S, Fischer JA. 2009. *Drosophila liquid facets-Related* encodes Golgi epsin and is an essential gene required for cell proliferation, growth, and patterning. *Dev. Biol.* 331:1–13
Leventis PA, Da Sylva TR, Rajwans N, Wasiak S, McPherson PS, Boulianne GL. 2011. Liquid facets-related (lqfR) is required for egg chamber morphogenesis during *Drosophila* oogenesis. *PLOS ONE* 6:e25466
Li H, Waites CL, Staal RG, Dobryy Y, Park J, et al. 2005. Sorting of vesicular monoamine transporter 2 to the regulated secretory pathway confers the somatodendritic exocytosis of monoamines. *Neuron* 48:619–33
Ludwig T, Munier-Lehmann H, Bauer U, Hollinshead M, Ovitt C, et al. 1994. Differential sorting of lysosomal enzymes in mannose 6-phosphate receptor-deficient fibroblasts. *EMBO J.* 13:3430–37
Ludwig T, Ovitt CE, Bauer U, Hollinshead M, Remmler J, et al. 1993. Targeted disruption of the mouse cation-dependent mannose 6-phosphate receptor results in partial missorting of multiple lysosomal enzymes. *EMBO J.* 12:5225–35
Ma D, Taneja TK, Hagen BM, Kim BY, Ortega B, et al. 2011. Golgi export of the Kir2.1 channel is driven by a trafficking signal located within its tertiary structure. *Cell* 145:1102–15
Magal LG, Yaffe Y, Shepshelovich J, Aranda JF, del Carmen de Marco M, et al. 2009. Clustering and lateral concentration of raft lipids by the MAL protein. *Mol. Biol. Cell* 20:3751–62
Man Z, Kondo Y, Koga H, Umino H, Nakayama K, Shin HW. 2011. Arfaptins are localized to the *trans*-Golgi by interaction with Arl1, but not Arfs. *J. Biol. Chem.* 286:11569–78
Mancias JD, Goldberg J. 2007. The transport signal on Sec22 for packaging into COPII-coated vesicles is a conformational epitope. *Mol. Cell* 26:403–14
Manolea F, Chun J, Chen DW, Clarke I, Summerfeldt N, et al. 2010. Arf3 is activated uniquely at the *trans*-Golgi network by brefeldin A-inhibited guanine nucleotide exchange factors. *Mol. Biol. Cell* 21:1836–49

Marcusson EG, Horazdovsky BF, Cereghino JL, Gharakhanian E, Emr SD. 1994. The sorting receptor for yeast vacuolar carboxypeptidase Y is encoded by the *VPS10* gene. *Cell* 77:579–86

Mardones GA, Burgos PV, Lin Y, Kloer DP, Magadan JG, et al. 2013. Structural basis for the recognition of tyrosine-based sorting signals by the mu3A subunit of the AP-3 adaptor complex. *J. Biol. Chem.* 288:9563–71

Martín-García R, de León N, Sharifmoghadam MR, Curto MA, Hoya M, et al. 2011. The FN3 and BRCT motifs in the exomer component Chs5p define a conserved module that is necessary and sufficient for its function. *Cell. Mol. Life Sci.* 68:2907–17

Matsuda S, Miura E, Matsuda K, Kakegawa W, Kohda K, et al. 2008. Accumulation of AMPA receptors in autophagosomes in neuronal axons lacking adaptor protein AP-4. *Neuron* 57:730–45

Matsuda S, Yuzaki M. 2008. AP-4: autophagy-four mislocalized proteins in axons. *Autophagy* 4:815–16

Mattera R, Boehm M, Chaudhuri R, Prabhu Y, Bonifacino JS. 2011. Conservation and diversification of dileucine signal recognition by adaptor protein (AP) complex variants. *J. Biol. Chem.* 286:2022–30

Mazelova J, Astuto-Gribble L, Inoue H, Tam BM, Schonteich E, et al. 2009. Ciliary targeting motif VxPx directs assembly of a trafficking module through Arf4. *EMBO J.* 28:183–92

Meyer DM, Crottet P, Maco B, Degtyar E, Cassel D, Spiess M. 2005. Oligomerization and dissociation of AP-1 adaptors are regulated by cargo signals and by ArfGAP1-induced GTP hydrolysis. *Mol. Biol. Cell* 16:4745–54

Meyer C, Zizioli D, Lausmann S, Eskelinen EL, Hamann J, et al. 2000. μ1A-adaptin-deficient mice: lethality, loss of AP-1 binding and rerouting of mannose 6-phosphate receptors. *EMBO J.* 19:2193–203

Miller GJ, Mattera R, Bonifacino JS, Hurley JH. 2003. Recognition of accessory protein motifs by the γ-adaptin ear domain of GGA3. *Nat. Struct. Biol.* 10:599–606

Miller SE, Collins BM, McCoy AJ, Robinson MS, Owen DJ. 2007. A SNARE-adaptor interaction is a new mode of cargo recognition in clathrin-coated vesicles. *Nature* 450:570–74

Mills IG, Praefcke GJ, Vallis Y, Peter BJ, Olesen LE, et al. 2003. EpsinR: an AP1/clathrin interacting protein involved in vesicle trafficking. *J. Cell Biol.* 160:213–22

Misra S, Puertollano R, Kato Y, Bonifacino JS, Hurley JH. 2002. Structural basis for acidic-cluster-dileucine sorting-signal recognition by VHS domains. *Nature* 415:933–37

Mo D, Costa SA, Ihrke G, Youker RT, Pastor-Soler N, et al. 2012. Sialylation of N-linked glycans mediates apical delivery of endolyn in MDCK cells via a galectin-9-dependent mechanism. *Mol. Biol. Cell* 23:3636–46

Mogelsvang S, Marsh BJ, Ladinsky MS, Howell KE. 2004. Predicting function from structure: 3D structure studies of the mammalian Golgi complex. *Traffic* 5:338–45

Montpetit A, Cote S, Brustein E, Drouin CA, Lapointe L, et al. 2008. Disruption of AP1S1, causing a novel neurocutaneous syndrome, perturbs development of the skin and spinal cord. *PLOS Genet.* 4:e1000296

Motoi Y, Aizawa T, Haga S, Nakamura S, Namba Y, Ikeda K. 1999. Neuronal localization of a novel mosaic apolipoprotein E receptor, LR11, in rat and human brain. *Brain Res.* 833:209–15

Munier-Lehmann H, Mauxion F, Bauer U, Lobel P, Hoflack B. 1996. Re-expression of the mannose 6-phosphate receptors in receptor-deficient fibroblasts. Complementary function of the two mannose 6-phosphate receptors in lysosomal enzyme targeting. *J. Biol. Chem.* 271:15166–74

Nakatsu F, Okada M, Mori F, Kumazawa N, Iwasa H, et al. 2004. Defective function of GABA-containing synaptic vesicles in mice lacking the AP-3B clathrin adaptor. *J. Cell Biol.* 167:293–302

Natarajan P, Liu K, Patil DV, Sciorra VA, Jackson CL, Graham TR. 2009. Regulation of a Golgi flippase by phosphoinositides and an ArfGEF. *Nat. Cell Biol.* 11:1421–26

Neculai D, Schwake M, Ravichandran M, Zunke F, Collins RF, et al. 2013. Structure of LIMP-2 provides functional insights with implications for SR-BI and CD36. *Nature* 504:172–76

Newell-Litwa K, Seong E, Burmeister M, Faundez V. 2007. Neuronal and non-neuronal functions of the AP-3 sorting machinery. *J. Cell Sci.* 120:531–41

Nishimoto-Morita K, Shin HW, Mitsuhashi H, Kitamura M, Zhang Q, et al. 2009. Differential effects of depletion of ARL1 and ARFRP1 on membrane trafficking between the *trans*-Golgi network and endosomes. *J. Biol. Chem.* 284:10583–92

Offe K, Dodson SE, Shoemaker JT, Fritz JJ, Gearing M, et al. 2006. The lipoprotein receptor LR11 regulates amyloid β production and amyloid precursor protein traffic in endosomal compartments. *J. Neurosci.* 26:1596–603

Ogata S, Fukuda M. 1994. Lysosomal targeting of Limp II membrane glycoprotein requires a novel Leu-Ile motif at a particular position in its cytoplasmic tail. *J. Biol. Chem.* 269:5210–17

Ohno H. 2006. Physiological roles of clathrin adaptor AP complexes: lessons from mutant animals. *J. Biochem.* 139:943–48

Ohno H, Aguilar RC, Yeh D, Taura D, Saito T, Bonifacino JS. 1998. The medium subunits of adaptor complexes recognize distinct but overlapping sets of tyrosine-based sorting signals. *J. Biol. Chem.* 273:25915–21

Ohno H, Fournier MC, Poy G, Bonifacino JS. 1996. Structural determinants of interaction of tyrosine-based sorting signals with the adaptor medium chains. *J. Biol. Chem.* 271:29009–15

Olson LJ, Peterson FC, Castonguay A, Bohnsack RN, Kudo M, et al. 2010. Structural basis for recognition of phosphodiester-containing lysosomal enzymes by the cation-independent mannose 6-phosphate receptor. *Proc. Natl. Acad. Sci. USA* 107:12493–98

Orci L, Ravazzola M, Amherdt M, Perrelet A, Powell SK, et al. 1987. The *trans*-most cisternae of the Golgi complex: a compartment for sorting of secretory and plasma membrane proteins. *Cell* 51:1039–51

Owen DJ, Collins BM, Evans PR. 2004. Adaptors for clathrin coats: structure and function. *Annu. Rev. Cell Dev. Biol.* 20:153–91

Owen DJ, Evans PR. 1998. A structural explanation for the recognition of tyrosine-based endocytotic signals. *Science* 282:1327–32

Paczkowski JE, Richardson BC, Strassner AM, Fromme JC. 2012. The exomer cargo adaptor structure reveals a novel GTPase-binding domain. *EMBO J.* 31:4191–203

Paladino S, Lebreton S, Tivodar S, Campana V, Tempre R, Zurzolo C. 2008. Different GPI-attachment signals affect the oligomerisation of GPI-anchored proteins and their apical sorting. *J. Cell Sci.* 121:4001–7

Paladino S, Sarnataro D, Pillich R, Tivodar S, Nitsch L, Zurzolo C. 2004. Protein oligomerization modulates raft partitioning and apical sorting of GPI-anchored proteins. *J. Cell Biol.* 167:699–709

Panic B, Whyte JR, Munro S. 2003. The ARF-like GTPases Arl1p and Arl3p act in a pathway that interacts with vesicle-tethering factors at the Golgi apparatus. *Curr. Biol.* 13:405–10

Petersen CM, Nielsen MS, Jacobsen C, Tauris J, Jacobsen L, et al. 1999. Propeptide cleavage conditions sortilin/neurotensin receptor-3 for ligand binding. *EMBO J.* 18:595–604

Phelan JP, Millson SH, Parker PJ, Piper PW, Cooke FT. 2006 . Fab1p and AP-1 are required for trafficking of endogenously ubiquitylated cargoes to the vacuole lumen in *S. cerevisiae*. *J. Cell Sci.* 119:4225–34

Pieper U, Webb BM, Barkan DT, Schneidman-Duhovny D, Schlessinger A, et al. 2011. ModBase, a database of annotated comparative protein structure models, and associated resources. *Nucleic Acids Res.* 39:D465–74

Pryor PR, Jackson L, Gray SR, Edeling MA, Thompson A, et al. 2008. Molecular basis for the sorting of the SNARE VAMP7 into endocytic clathrin-coated vesicles by the ArfGAP Hrb. *Cell* 134:817–27

Puertollano R, Bonifacino JS. 2004. Interactions of GGA3 with the ubiquitin sorting machinery. *Nat. Cell Biol.* 6:244–51

Puthenveedu MA, von Zastrow M. 2006. Cargo regulates clathrin-coated pit dynamics. *Cell* 127:113–24

Qian M, Sleat DE, Zheng H, Moore D, Lobel P. 2008. Proteomics analysis of serum from mutant mice reveals lysosomal proteins selectively transported by each of the two mannose 6-phosphate receptors. *Mol. Cell. Proteomics* 7:58–70

Quistgaard EM, Madsen P, Grøftehauge MK, Nissen P, Petersen CM, Thirup SS. 2009. Ligands bind to Sortilin in the tunnel of a ten-bladed β-propeller domain. *Nat. Struct. Mol. Biol.* 16:96–98

Reczek D, Schwake M, Schröder J, Hughes H, Blanz J, et al. 2007. LIMP-2 is a receptor for lysosomal mannose-6-phosphate-independent targeting of β-glucocerebrosidase. *Cell* 131:770–83

Ren X, Farias GG, Canagarajah BJ, Bonifacino JS, Hurley JH. 2013. Structural basis for recruitment and activation of the AP-1 clathrin adaptor complex by Arf1. *Cell* 152:755–67

Richardson BC, Fromme JC. 2013. The exomer cargo adaptor features a flexible hinge domain. *Structure* 21:486–92

Richardson BC, McDonold CM, Fromme JC. 2012. The Sec7 Arf-GEF is recruited to the *trans*-Golgi network by positive feedback. *Dev. Cell* 22:799–810

Rodriguez-Boulan E, Kreitzer G, Müsch A. 2005. Organization of vesicular trafficking in epithelia. *Nat. Rev. Mol. Cell Biol.* 6:233–47

Roeth JF, Williams M, Kasper MR, Filzen TM, Collins KL. 2004. HIV-1 Nef disrupts MHC-I trafficking by recruiting AP-1 to the MHC-I cytoplasmic tail. *J. Cell Biol.* 167:903–13

Rogaeva E, Meng Y, Lee JH, Gu Y, Kawarai T, et al. 2007. The neuronal sortilin-related receptor SORL1 is genetically associated with Alzheimer disease. *Nat. Genet.* 39:168–77

Rous BA, Reaves BJ, Ihrke G, Briggs JA, Gray SR, et al. 2002. Role of adaptor complex AP-3 in targeting wild-type and mutated CD63 to lysosomes. *Mol. Biol. Cell* 13:1071–82

Saillour Y, Zanni G, Des Portes V, Heron D, Guibaud L, et al. 2007. Mutations in the *AP1S2* gene encoding the sigma 2 subunit of the adaptor protein 1 complex are associated with syndromic X-linked mental retardation with hydrocephalus and calcifications in basal ganglia. *J. Med. Genet.* 44:739–44

Saint-Pol A, Yelamos B, Amessou M, Mills IG, Dugast M, et al. 2004. Clathrin adaptor epsinR is required for retrograde sorting on early endosomal membranes. *Dev. Cell* 6:525–38

Sanchatjate S, Schekman R. 2006. Chs5/6 complex: a multiprotein complex that interacts with and conveys chitin synthase III from the *trans*-Golgi network to the cell surface. *Mol. Biol. Cell* 17:4157–66

Santos B, Snyder M. 2003. Specific protein targeting during cell differentiation: polarized localization of Fus1p during mating depends on Chs5p in *Saccharomyces cerevisiae*. *Eukaryot. Cell* 2:821–25

Scheiffele P, Roth MG, Simons K. 1997. Interaction of influenza virus haemagglutinin with sphingolipid-cholesterol membrane domains via its transmembrane domain. *EMBO J.* 16:5501–8

Schmidt S, Fritz JV, Bitzegeio J, Fackler OT, Keppler OT. 2011. HIV-1 Vpu blocks recycling and biosynthetic transport of the intrinsic immunity factor CD317/tetherin to overcome the virion release restriction. *mBio* 2:e00036-11

Schuck S, Simons K. 2004. Polarized sorting in epithelial cells: raft clustering and the biogenesis of the apical membrane. *J. Cell Sci.* 117:5955–64

Scott PM, Bilodeau PS, Zhdankina O, Winistorfer SC, Hauglund MJ, et al. 2004. GGA proteins bind ubiquitin to facilitate sorting at the *trans*-Golgi network. *Nat. Cell Biol.* 6:252–59

Sebastian TT, Baldridge RD, Xu P, Graham TR. 2012. Phospholipid flippases: building asymmetric membranes and transport vesicles. *Biochim. Biophys. Acta* 1821:1068–77

Seong E, Wainer BH, Hughes ED, Saunders TL, Burmeister M, Faundez V. 2005. Genetic analysis of the neuronal and ubiquitous AP-3 adaptor complexes reveals divergent functions in brain. *Mol. Biol. Cell* 16:128–40

Shafaq-Zadah M, Brocard L, Solari F, Michaux G. 2012. AP-1 is required for the maintenance of apico-basal polarity in the *C. elegans* intestine. *Development* 139:2061–70

Shiba T, Takatsu H, Nogi T, Matsugaki N, Kawasaki M, et al. 2002. Structural basis for recognition of acidic-cluster dileucine sequence by GGA1. *Nature* 415:937–41

Shiba Y, Katoh Y, Shiba T, Yoshino K, Takatsu H, et al. 2004. GAT (GGA and Tom1) domain responsible for ubiquitin binding and ubiquitination. *J. Biol. Chem.* 279:7105–11

Shim J, Lee J. 2005. The AP-3 clathrin-associated complex is essential for embryonic and larval development in *Caenorhabditis elegans*. *Mol. Cells* 19:452–57

Shim J, Sternberg PW, Lee J. 2000. Distinct and redundant functions of μ1 medium chains of the AP-1 clathrin-associated protein complex in the nematode *Caenorhabditis elegans*. *Mol. Biol. Cell* 11:2743–56

Shimada Y, Yonemura S, Ohkura H, Strutt D, Uemura T. 2006. Polarized transport of Frizzled along the planar microtubule arrays in *Drosophila* wing epithelium. *Dev. Cell* 10:209–22

Shin HW, Kobayashi H, Kitamura M, Waguri S, Suganuma T, et al. 2005. Roles of ARFRP1 (ADP-ribosylation factor-related protein 1) in post-Golgi membrane trafficking. *J. Cell Sci.* 118:4039–48

Siman R, Velji J. 2003. Localization of presenilin-nicastrin complexes and γ-secretase activity to the *trans*-Golgi network. *J. Neurochem.* 84:1143–53

Simmen T, Honing S, Icking A, Tikkanen R, Hunziker W. 2002. AP-4 binds basolateral signals and participates in basolateral sorting in epithelial MDCK cells. *Nat. Cell Biol.* 4:154–59

Sirkis DW, Edwards RH, Asensio CS. 2013. Widespread dysregulation of peptide hormone release in mice lacking adaptor protein AP-3. *PLOS Genet.* 9:e1003812

Sohar I, Sleat D, Gong Liu C, Ludwig T, Lobel P. 1998. Mouse mutants lacking the cation-independent mannose 6-phosphate/insulin-like growth factor II receptor are impaired in lysosomal enzyme transport: comparison of cation-independent and cation-dependent mannose 6-phosphate receptor-deficient mice. *Biochem. J.* 330(Pt. 2):903–8

Stalder D, Barelli H, Gautier R, Macia E, Jackson CL, Antonny B. 2011. Kinetic studies of the Arf activator Arno on model membranes in the presence of Arf effectors suggest control by a positive feedback loop. *J. Biol. Chem.* 286:3873–83

Starr TL, Pagant S, Wang CW, Schekman R. 2012. Sorting signals that mediate traffic of chitin synthase III between the TGN/endosomes and to the plasma membrane in yeast. *PLOS ONE* 7:e46386

Stechly L, Morelle W, Dessein AF, André S, Grard G, et al. 2009. Galectin-4-regulated delivery of glycoproteins to the brush border membrane of enterocyte-like cells. *Traffic* 10:438–50

Stepp JD, Huang K, Lemmon SK. 1997. The yeast adaptor protein complex, AP-3, is essential for the efficient delivery of alkaline phosphatase by the alternate pathway to the vacuole. *J. Cell Biol.* 139:1761–74

Stockklausner C, Klocker N. 2003. Surface expression of inward rectifier potassium channels is controlled by selective Golgi export. *J. Biol. Chem.* 278:17000–5

Strong A, Ding Q, Edmondson AC, Millar JS, Sachs KV, et al. 2012. Hepatic sortilin regulates both apolipoprotein B secretion and LDL catabolism. *J. Clin. Investig.* 122:2807–16

Szentpetery Z, Varnai P, Balla T. 2010. Acute manipulation of Golgi phosphoinositides to assess their importance in cellular trafficking and signaling. *Proc. Natl. Acad. Sci. USA* 107:8225–30

Taira K, Bujo H, Hirayama S, Yamazaki H, Kanaki T, et al. 2001. LR11, a mosaic LDL receptor family member, mediates the uptake of ApoE-rich lipoproteins in vitro. *Arterioscler. Thromb. Vasc. Biol.* 21:1501–6

Takahashi D, Hase K, Kimura S, Nakatsu F, Ohmae M, et al. 2011. The epithelia-specific membrane trafficking factor AP-1B controls gut immune homeostasis in mice. *Gastroenterology* 141:621–32

Tarpey PS, Stevens C, Teague J, Edkins S, O'Meara S, et al. 2006. Mutations in the gene encoding the sigma 2 subunit of the adaptor protein 1 complex, *AP1S2*, cause X-linked mental retardation. *Am. J. Hum. Genet.* 79:1119–24

Torii S, Saito N, Kawano A, Zhao S, Izumi T, Takeuchi T. 2005. Cytoplasmic transport signal is involved in phogrin targeting and localization to secretory granules. *Traffic* 6:1213–24

Trautwein M, Schindler C, Gauss R, Dengjel J, Hartmann E, Spang A. 2006. Arf1p, Chs5p and the ChAPs are required for export of specialized cargo from the Golgi. *EMBO J.* 25:943–54

Vaegter CB, Jansen P, Fjorback AW, Glerup S, Skeldal S, et al. 2011. Sortilin associates with Trk receptors to enhance anterograde transport and neurotrophin signaling. *Nat. Neurosci.* 14:54–61

Valdivia RH, Baggott D, Chuang JS, Schekman RW. 2002. The yeast clathrin adaptor protein complex 1 is required for the efficient retention of a subset of late Golgi membrane proteins. *Dev. Cell* 2:283–94

Velayati A, DePaolo J, Gupta N, Choi JH, Moaven N, et al. 2011. A mutation in SCARB2 is a modifier in Gaucher disease. *Hum. Mutat.* 32:1232–38

Volpicelli-Daley LA, Li Y, Zhang CJ, Kahn RA. 2005. Isoform-selective effects of the depletion of ADP-ribosylation factors 1–5 on membrane traffic. *Mol. Biol. Cell* 16:4495–508

von Blume J, Alleaume AM, Cantero-Recasens G, Curwin A, Carreras-Sureda A, et al. 2011. ADF/cofilin regulates secretory cargo sorting at the TGN via the Ca^{2+} ATPase SPCA1. *Dev. Cell* 20:652–62

von Blume J, Duran JM, Forlanelli E, Alleaume AM, Egorov M, et al. 2009. Actin remodeling by ADF/cofilin is required for cargo sorting at the *trans*-Golgi network. *J. Cell Biol.* 187:1055–69

Wang CW, Hamamoto S, Orci L, Schekman R. 2006. Exomer: a coat complex for transport of select membrane proteins from the *trans*-Golgi network to the plasma membrane in yeast. *J. Cell Biol.* 174:973–83

Wang J, Gossing M, Fang P, Zimmermann J, Li X, et al. 2011. Epsin N-terminal homology domains bind on opposite sides of two SNAREs. *Proc. Natl. Acad. Sci. USA* 108:12277–82

Wang J, Morita Y, Mazelova J, Deretic D. 2012. The Arf GAP ASAP1 provides a platform to regulate Arf4- and Rab11-Rab8-mediated ciliary receptor targeting. *EMBO J.* 31:4057–71

Wang J, Sun HQ, Macia E, Kirchhausen T, Watson H, et al. 2007. PI4P promotes the recruitment of the GGA adaptor proteins to the *trans*-Golgi network and regulates their recognition of the ubiquitin sorting signal. *Mol. Biol. Cell* 18:2646–55

Wang YJ, Wang J, Sun HQ, Martinez M, Sun YX, et al. 2003. Phosphatidylinositol 4 phosphate regulates targeting of clathrin adaptor AP-1 complexes to the Golgi. *Cell* 114:299–310

Weisz OA, Rodriguez-Boulan E. 2009. Apical trafficking in epithelial cells: signals, clusters and motors. *J. Cell Sci.* 122:4253–66

Westergaard UB, Sorensen ES, Hermey G, Nielsen MS, Nykjaer A, et al. 2004. Functional organization of the sortilin Vps10p domain. *J. Biol. Chem.* 279:50221–29

Willnow TE, Andersen OM. 2013. Sorting receptor SORLA—a trafficking path to avoid Alzheimer disease. *J. Cell Sci.* 126:2751–60

Xu H, Sweeney D, Wang R, Thinakaran G, Lo AC, et al. 1997. Generation of Alzheimer β-amyloid protein in the *trans*-Golgi network in the apparent absence of vesicle formation. *Proc. Natl. Acad. Sci. USA* 94:3748–52

Yamayoshi S, Iizuka S, Yamashita T, Minagawa H, Mizuta K, et al. 2012. Human SCARB2-dependent infection by coxsackievirus A7, A14, and A16 and enterovirus 71. *J. Virol.* 86:5686–96

Yamayoshi S, Koike S. 2011. Identification of a human SCARB2 region that is important for enterovirus 71 binding and infection. *J. Virol.* 85:4937–46

Yamayoshi S, Yamashita Y, Li J, Hanagata N, Minowa T, et al. 2009. Scavenger receptor B2 is a cellular receptor for enterovirus 71. *Nat. Med.* 15:798–801

Yang W, Li C, Ward DM, Kaplan J, Mansour SL. 2000. Defective organellar membrane protein trafficking in Ap3b1-deficient cells. *J. Cell Sci.* 113(Pt. 22):4077–86

Zachos C, Blanz J, Saftig P, Schwake M. 2012. A critical histidine residue within LIMP-2 mediates pH sensitive binding to its ligand β-glucocerebrosidase. *Traffic* 13:1113–23

Zahn C, Jaschke A, Weiske J, Hommel A, Hesse D, et al. 2008. ADP-ribosylation factor-like GTPase ARFRP1 is required for *trans*-Golgi to plasma membrane trafficking of E-cadherin. *J. Biol. Chem.* 283:27179–88

Zanolari B, Rockenbauch U, Trautwein M, Clay L, Barral Y, Spang A. 2011. Transport to the plasma membrane is regulated differently early and late in the cell cycle in *Saccharomyces cerevisiae*. *J. Cell Sci.* 124:1055–66

Zhu G, Zhai P, He X, Terzyan S, Zhang R, et al. 2003. Crystal structure of the human GGA1 GAT domain. *Biochemistry* 42:6392–99

Zhu Y, Drake MT, Kornfeld S. 1999. ADP-ribosylation factor 1 dependent clathrin-coat assembly on synthetic liposomes. *Proc. Natl. Acad. Sci. USA* 96:5013–18

Zizioli D, Forlanelli E, Guarienti M, Nicoli S, Fanzani A, et al. 2010. Characterization of the AP-1 μ1A and μ1B adaptins in zebrafish (*Danio rerio*). *Dev. Dyn.* 239:2404–12

Zizioli D, Meyer C, Guhde G, Saftig P, von Figura K, Schu P. 1999. Early embryonic death of mice deficient in γ-adaptin. *J. Biol. Chem.* 274:5385–90