© 2016. Published by The Company of Biologists Ltd | Journal of Cell Science (2016) 129, 3971-3982 doi:10.1242/jcs.185702

COMMENTARY

Bidirectional traffic between the Golgi and the endosomes – machineries and regulation

Cinzia Progida* and Oddmund Bakke*

ABSTRACT

The bidirectional transport between the Golgi complex and the endocytic pathway has to be finely regulated in order to ensure the proper delivery of newly synthetized lysosomal enzymes and the return of sorting receptors from degradative compartments. The high complexity of these routes has led to experimental difficulties in properly dissecting and separating the different pathways. As a consequence, several models have been proposed during the past decades. However, recent advances in our understanding of endosomal dynamics have helped to unify these different views. We provide here an overview of the current insights into the transport routes between Golgi and endosomes in mammalian cells. The focus of the Commentary is on the key molecules involved in the trafficking pathways between these intracellular compartments, such as Rab proteins and sorting receptors, and their regulation. A proper understanding of the bidirectional traffic between the Golgi complex and the endolysosomal system is of uttermost importance, as several studies have demonstrated that mutations in the factors involved in these transport pathways result in various pathologies, in particular lysosome-associated diseases and diverse neurological disorders, such as Alzheimer's and Parkinson's disease.

KEY WORDS: Golgi, Endolysosomal system, Endocytic pathway, Rab protein, Trans-Golgi network, Sorting receptor

Introduction

The bidirectional transport between the trans-Golgi network (TGN) and endosomes is one of the key vesicular trafficking pathways in the cell. Several different molecules, including newly synthesized enzymes, and sorting receptors and lipids, as well as bacterial toxins, have been described to travel between these compartments by using distinct routes. Obtaining a proper distinction and clear separation between all these routes has been challenging for a long time and different models have been suggested. For example, two main retrograde pathways have been described, one originating from early endosomes and the other from late endosomes, but the elucidation of the transport routes in the opposite direction (that is from the Golgi to endosomes) has been even more arduous. However, recent technologies and advances in microscopy techniques have heavily contributed to the recent progress in further elucidating these pathways (Box 1).

The TGN acts as the hub of these pathways, representing both the starting point for the sorting of newly synthesized proteins and the arrival of proteins from the endosomal pathway. A plethora of proteins and lipids are delivered from the TGN to the endosomes. These include transmembrane sorting receptors, such as mannose 6-phosphate receptors (MPRs) and sortilins (Vps10p domain receptor family), which deliver newly synthesized enzymes to the endosomes, lysosomal membrane proteins, for example, lysosome-associated membrane proteins (LAMPs) and lysosomal integral membrane protein 2 (LIMP-2, also known as SCARB2), nutrients and ion transporters. Vice versa, the transport route from endosomes to the TGN is used by sorting receptors that are recycled back and by some toxins, including Shiga, cholera and pertussis toxins and ricin (Plaut and Carbonetti, 2008; Sandvig and van Deurs, 2005; Utskarpen et al., 2006; Wernick et al., 2010). All these molecules use a number of different routes to reach the TGN by interacting with different protein complexes, including those containing Rab proteins and their effectors, the retromer complex, clathrin and adaptor protein-1 (AP-1), (Matsudaira et al., 2015). Other proteins that are transported between endosomes and TGN include the endopeptidase furin, TGN46 (also known as TGOLN2, the human homolog of the rat TGN38) and certain SNAREs (Burd, 2011).

Previously, the routes between TGN and endosomes have been divided according to the type of endosomes involved into the early-endosome-to-TGN pathway with the retromer complex as the main regulator (Arighi et al., 2004; Mallard et al., 2002; Seaman, 2004), and the late-endosome-to-TGN pathway, which is mediated by Rab9 (the Rab9a isoform) and TIP47 (also known as PLIN3) (Carroll et al., 2001; Pfeffer, 2009). This division was mostly based on the notion of early and late endosomes as being static compartments that are connected by vesicular carriers. However, a more recent view of the endosomal pathway acknowledges endosomes as a continuum of maturing vesicular membranes, and provides new insights into the different routes that connect the endosomal system to the TGN (Hutagalung and Novick, 2011; Rink et al., 2005; Skjeldal et al., 2012). In this Commentary, we present the most recent findings on the factors and mechanisms that regulate the bidirectional transport between the continuum of maturing endosomes and TGN. We will in particular focus on Rab proteins, which are central regulators of intracellular transport (Fig. 1).

Sorting receptors and lysosomal membrane proteins

The best-characterized transport cargo proteins that have been used to study the pathways between endosomes and TGN are the sorting receptors, mannose-6-phosphate receptors (MPRs). They are transmembrane proteins mainly localized in the TGN where they bind to the newly synthesized lysosomal enzymes and mediate their transport to the endolysosomal pathway. In addition to MPRs, other sorting receptors or lysosomal membrane proteins mediate the transport of lysosomal enzymes to the endosomal pathway. Although much is known regarding their structures and the sorting motifs presents in their cytoplasmic domains, which binds to adaptor proteins (APs) for the targeting to specific membrane domains (i.e. Golgi, plasma membrane or endosomes; see Box 2), it is less clear how their transport dynamics are coordinated in space and time. Below, we will briefly summarize what is known about these transport proteins. For more details, many excellent reviews

Department of Biosciences, Centre for Immune Regulation, University of Oslo, Oslo, Norway.

*Authors for correspondence (c.a.m.progida@ibv.uio.no; oddmund.bakke@ibv.uio.no)

Journal of Cell Science (2016) 129, 3971-3982 doi:10.1242/jcs.185702

Box 1. Novel techniques for the study of transport pathways between endosomes and the TGN

The majority of the studies traditionally performed to dissect intracellular traffic routes have involved biochemical techniques, such as *in vitro* studies in cell-free systems or immunofluorescence analysis (Espinosa et al., 2009; Seaman, 2004). The recent advances in microscopy, which allow the detection and the quantitative analysis of fast dynamic live events at high resolution, have provided new tools for the study of complex intracellular pathways, such as those occurring between the endosomes and TGN. The simultaneous use of different fluorescent proteins to visualize both cargo and endosomal compartments in live cells now allows experimenters to precisely define the timing and sequence of trafficking events and to therefore solve issues that for many years have been the subject of debates and controversies (Chia and Gleeson, 2013; Kucera et al., 2016; van Weering et al., 2012). In addition, several high-throughput methods have been developed in the past few years. For example, pulse shape analysis (PulSA) is based on the measurement of a pulse width by flow cytometry and has been used to track the internalization and transport of fluorescent proteins that follow the retrograde pathway. Here, differences in pulse width measurements indicate a difference in fluorescence patterns and therefore in protein localization (Chia et al., 2013; Ramdzan et al., 2012). This approach has the advantage of being able to very rapidly analyze changes in the intracellular distribution of fluorescently labeled molecules in single cells; this allows experimenters to monitor thousands of cells, as well as to sort and recover cells for further analysis. In addition, high-throughput small interfering RNA (siRNA) screens have made it possible to identify the multiple kinases and phosphatases that regulate MPR trafficking, as well as new components of the endosome-to-Golgi retrieval pathway including the multipass membrane proteins SFT2D2, ZDHHC5 and GRINA (Anitei et al., 2014; Breusegem and Seaman, 2014). Another recent method is the so-called 'knock sideways' approach, which has been used to unravel the role of AP-1 in endosome-to-TGN trafficking (Hirst et al., 2012). This method is based on depletion of the protein of interest from its functional sites by mislocalizing it to mitochondria (Robinson and Hirst, 2013). Finally, correlative light and electron microscopy and super-resolution fluorescence imaging are essential to further understand the sorting processes that occur at the intersection between the endocytic and biosynthetic pathways. These methods are in constant development, and lattice light-sheet microscopy, in particular, bears great promise for live imaging of cells (Chen et al., 2014; Legant et al., 2016).

are available (see, for example, Braulke and Bonifacino, 2009; Coutinho et al., 2012; Hermey, 2009; Wang et al., 2016).

MPRs

Newly synthesized soluble lysosomal enzymes are modified in the Golgi with mannose 6-phosphate (M6P) residues. These tags are recognized and bound by MPRs and so are transported to the endosomal pathway. There are two different MPRs in humans, the cation-independent (CI)-MPR (~300 kDa, also known as IGF2R) and the cation-dependent (CD)-MPR (~46 kDa, also known as M6PR), which requires divalent cations to bind to M6P residues. The vesicles containing MPRs, which are formed at the TGN, are sorted by Golgi-localized, gamma-ear-containing, ADP-ribosylation-factor-binding proteins (GGAs) and AP-1 into clathrin-coated vesicles, which are directed towards the endosomes (Doray et al., 2002).

A small fraction of newly synthesized lysosomal enzymes escape this pathway and these are secreted after being included into vesicles that fuse with the plasma membrane. There, MPRs that are present in small amounts recapture and thus re-internalize the secreted enzymes into the endosomal compartments.

Once MPRs reach the endolysosomal pathway, the acidic pH of the endosomes promotes the dissociation of the ligand from the receptor. The ligand will then continue its journey, which ends in the lysosomes, where the acidic environment activates the degradative activity of the enzyme, whereas MPRs will be recycled to the TGN to begin a new transport cycle. Retrieval of

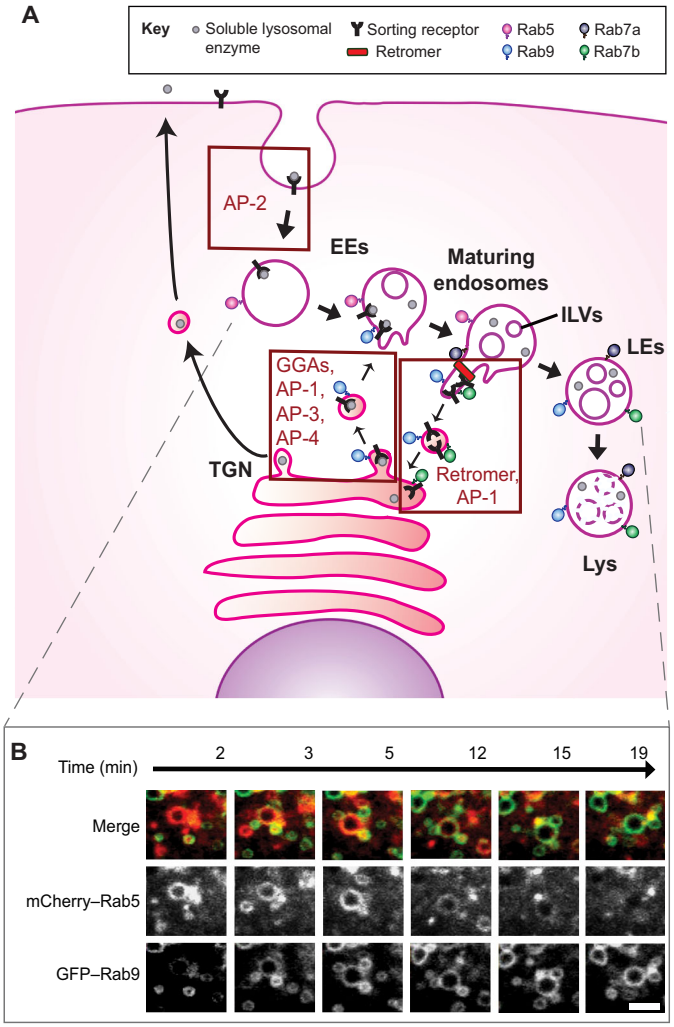

Fig. 1. Anterograde and retrograde pathways between maturing endosomes and the TGN. (A) Schematic overview of the vesicles and factors involved. Rab5 is present on early endosomes (EEs), and gradually lost during endosomal maturation. Rab7a is subsequently recruited at this transition stage, and endosomes become late endosomes (LEs). Maturation involves the formation of intraluminal vesicles (ILVs) and ends in lysosomes (Lys). At the TGN, newly synthesized lysosomal enzymes bind to sorting receptors, which are transported to the maturing endosomes. Rab9 and the retromer are responsible for the transport of sorting receptors and are recruited on endosomes at the transition between early Rab5-positive and late Rab7a-positive endosomes. When the endosomal pH becomes acidic, sorting receptors release the lysosomal enzymes in the endosomal lumen, and are recycled back to the TGN. Rab7b mediates the retrograde transport of sorting receptors. A fraction of newly synthesized lysosomal enzymes escapes the TGN and reaches the plasma membrane, where they can be captured by sorting receptors and internalized by the endocytic pathway. Adaptor proteins involved in the different transport pathways are indicated in the boxes. (B) Precise intracellular spatio-temporal dynamics of Rabs as determined by live imaging. mCherry–Rab5 (in red) present on early endosomes is released during endosome maturation. Simultaneously, GFP–Rab9 (in green) is acquired on the same endosome. By analyzing a large set of such movies with several fluorescently labeled proteins, the various intracellular vesicular trafficking pathways can be visualized. Scale bar: 2 µm. Image courtesy of Ana Kucera, Department of Biosciences, University of Oslo, Norway.

Journal of Cell Science

Journal of Cell Science (2016) 129, 3971-3982 doi:10.1242/jcs.185702

Box 2. Adaptor proteins

The cytosolic tail of transmembrane cargo proteins contains sorting signals which are responsible for the recruitment of specific adaptor protein complexes (APs), heterotetrameric coat protein complexes. At present, five different APs have been identified that regulate the sorting of cargos in a compartment-specific manner. AP-1 mediates the transport from the TGN to endosomes. In addition, AP-1 has also been shown to function on endosomes, where it is involved in retrograde transport to the TGN (Hirst et al., 2012; Matsudaira et al., 2015). AP-2 mediates clathrin-mediated endocytosis. Although both AP-1 and AP-2 can bind to clathrin, AP-1 can also interact with additional proteins, such as PACS-1 and EpsinR (also known as CLINT1). The precise functions of PACS-1 and EpsinR are not well established, even though PACS-1 has been shown to be involved in the transport of furin and CI-MPR, whereas EpsinR appears to function as a cargo adaptor for SNAREs (Chidambaram et al., 2008; Crump et al., 2001; Miller et al., 2007; Mills et al., 2003; Scott et al., 2006). The ability of AP-1 to interact with different proteins might explain its effect on multiple transport pathways. AP-3 mediates the transport towards lysosomes and lyososome-related organelles. It has been suggested that AP-3 not only works on endosomes but also at the TGN (Anitei et al., 2010; Gupta et al., 2006). AP-4 mediates TGN-to-endosome transport (Barois and Bakke, 2005; Burgos et al., 2010), whereas AP-5 is present on late endosomes where it has a role in endosomal and lysosomal homeostasis (Hirst et al., 2015). However, as AP-5 has only been identified recently, its exact function is still unclear. Another class of adaptors are the monomeric clathrin adaptors GGA1, GGA2 and GGA3. The Vps27p, Hrs and STAM (VHS) domain of GGAs binds to acidic-cluster-dileucine motifs present in the cytosolic tail of sorting receptors, thereby recruiting MPRs and other transmembrane proteins to newly formed carriers and thus mediating their transport between the TGN and endosomes (Braulke and Bonifacino, 2009). GGAs also contain a GAT domain that is responsible for their interaction with Arf, as well as a 'hinge and ear' domain that binds to clathrin (Puertollano et al., 2001). GGAs function together with AP-1 at the TGN for the sorting of MPRs and sortilin (Braulke and Bonifacino, 2009; Canuel et al., 2008; Doray et al., 2002).

MPRs from the endosomal pathway is mediated by the retromer complex (Arighi et al., 2004; Seaman, 2004).

It has been long debated where exactly MPRs enter the endocytic pathway, with some studies suggesting the early endosomes, whereas others point to late endosomes (Ganley et al., 2008; Mari et al., 2008; Medigeshi and Schu, 2003; Rohn et al., 2000; Varki and Kornfeld, 2009). Our recent findings now help to unify the discussion, as by using live imaging, we have been able to show that MPRs reach early endosomes just before their Rab5 (the Rab5a isoform) coat is lost and the Rab7a coat is acquired (Kucera et al., 2016). This is in line with earlier work that demonstrated that the recruitment of the retromer to endosomal membranes is regulated by the Rab5-to-Rab7a switch (Rojas et al., 2008).

However, it should be noted that MPR-independent pathways for TGN-to-endosome transport have also been described (Blanz et al., 2010; Coutinho et al., 2012). Indeed, studies on cells from patients with I-cell disease (a lysosomal storage disorder) have suggested the existence of an additional class of sorting receptors. This disease is characterized by mutations in the N-acetylglucosamine-1-phosphotransferase, the enzyme responsible for adding M6P residues to lysosomal hydrolases (see Table 1). As a consequence, enzymes lacking the M6P tag are secreted, instead of being transported to lysosomes. However, the lysosomal enzyme content is normal in some cell types from these patients, such as hepatocytes, Kupffer cells and lymphocytes, indicating the existence of MPR-independent pathways (Kollmann et al., 2010).

Vps10p-domain-containing receptors

A more recently identified family of sorting receptors is the Vps10p domain family, named after their homology to the luminal domain of the yeast sorting protein Vps10p (Marcusson et al., 1994). Vps10p proteins are conserved throughout evolution from yeast to man, and in mammals they consist of five members: sortilin (also known as SORT1), SorCS1, SorCS2, SorCS3 and SorLA (also known as SORL1) (Coutinho et al., 2012; Willnow et al., 2008).

The intracellular pathways by which sortilin and SorLA are trafficked between the Golgi and the endosomes are best characterized; they are synthesized as inactive precursors, which are unable to bind to their ligand until they are activated by furin-mediated propeptide cleavage in the TGN (Munck Petersen et al., 1999). In the TGN, both sortilin and SorLA are able to bind to ligands, which include neurotensin and lysosomal proteins, such as acid sphingomyelinase and sphingolipid activator proteins (SAPs), as well as cathepsin D and cathepsin H, and mediate their transport to endosomes after recruitment of GGAs and AP-1 through their cytoplasmic tails (Canuel et al., 2008; Coutinho et al., 2012; Lefrancois et al., 2003; Nielsen et al., 2001). The ability of sortilin and SorLA to bind such a variety of diverse ligands explains why defects in their associated pathways are linked to different disorders that range from neurodegenerative to cardiovascular diseases (see Table 1).

Sortilin and SorLA are transported to endosomes in the same transport vesicles that contain MPRs (Mari et al., 2008). The fact that sortilins and MPRs are localized in the same intracellular compartments and follow the same intracellular pathways is not surprising, considering that the sorting motifs in the cytoplasmic tail of sortilin and SorLA are closely related to those of CI-MPR (Mari et al., 2008; Nielsen et al., 2001). Sortilin and SorLA are also present at the plasma membrane, from where they can be internalized after binding to AP-2 and transported via the endosomal pathway to the TGN in a retromer-dependent way (Canuel et al., 2008; Nielsen et al., 2007; Willnow et al., 2008).

Lysosomal membrane proteins

Another group of proteins that are delivered to the endosomal pathway from the Golgi and TGN are LAMP-1, LAMP-2 and LAMP-3 (also known as CD63), and LIMP-2. These so-called lysosomal membrane proteins (LMPs) have multiple roles in lysosome biogenesis and maintenance, as well as in lysosomal transport (Schwake et al., 2013). LMPs are targeted to lysosomes by one of two alternative pathways: a direct one, from the TGN to lysosomes via endosomes, or an indirect one, in which they are first transported from the TGN to the plasma membrane and then internalized in the endosomal pathway (Braulke and Bonifacino, 2009).

In contrast to the sorting receptors, which avoid the lysosomes and are retrieved from endosomes to the TGN, LMPs do not recycle back to the TGN. Indeed, LMPs contain different sorting signals in their cytoplasmic tails, either a tyrosine or di-leucine motif, and the adaptor responsible for their sorting at the TGN is AP-3 (Anitei et al., 2010; Chapuy et al., 2008).

It is worth noting that the sorting motifs of LAMP-1 and LAMP-2 are also recognized by adaptor protein complexes AP-1 and AP-2 (Honing et al., 1996; Janvier and Bonifacino, 2005). However, the role of AP-1 in the direct transport of LAMPs from TGN to endosomes has been the subject of debate. Indeed, even though LAMP-1 has been found in TGN-derived vesicles positive for AP-1 and clathrin (Honing et al., 1996), LAMPs are also transported from the TGN to late endosomes in an AP-1-independent manner through carriers that are not coated with clathrin (Pols et al., 2013).

Journal of Cell Science (2016) 129, 3971-3982 doi:10.1242/jcs.185702

Table 1. Main disorders associated with alterations in molecular factors involved in the bidirectional endosome-Golgi transport

Pathway	Gene(s)	Associated diseases	References
MPRs	*GNPTA* (GlcNAc-phosphotransferase – enzyme responsible for adding M6P residues to enzymes directed to lysosomes)	I-cell disease (or mucolipidosis II) and mucolipidosis III	Kollmann et al., 2010; Paik et al., 2005; Tiede et al., 2005
Vps10p domain receptors	*SORL1* (SorLA)	Alzheimer's disease	Lee et al., 2008; Rogaeva et al., 2007; Willnow and Andersen, 2013
	SORT1 (sortilin)	Frontotemporal lobar degeneration (FTLD)	Hu et al., 2010
		Cardiovascular disease	Kjolby et al., 2015; Ogawa et al., 2016
		Essential tremor	Sanchez et al., 2015
LIMP-2	*SCARB2*	Action myoclonus – renal failure syndrome (AMRF)	Balreira et al., 2008; Berkovic et al., 2008
		Gaucher disease (GD)	Gonzalez et al., 2014; Velayati et al., 2011
Retromer	*VPS35*	Alzheimer's disease	Muhammad et al., 2008; Rovelet-Lecrux et al., 2015; Small et al., 2005
		Parkinson's disease	Follett et al., 2014; McGough et al., 2014; Vilarino-Guell et al., 2011; Zavodszky et al., 2014; Zimprich et al., 2011
AP-1	*AP1S2*	X-linked mental retardation	Borck et al., 2008; Tarpey et al., 2006
AP-4	*AP4B1*, *AP4E1*, *AP4S1*, *AP4M1*	Progressive spastic paraplegia	Bauer et al., 2012; Hirst et al., 2013; Moreno-De-Luca et al., 2011
GARP I	*VPS53*	Progressive cerebello-cerebral atrophy type 2 (PCCA2)	Feinstein et al., 2014; Frohlich et al., 2015

Commonly used protein names are given in the Genes column in parentheses where these are different.

LIMP-2 contains a di-leucine residue that is responsible for recruitment of AP-3 and targeting to lysosomes (Honing et al., 1998; Ogata and Fukuda, 1994). It has been shown that inserting the LIMP-2 residues that are responsible for AP-3 binding into proteins that are transported via the plasma membrane, for instance CD74, is sufficient to re-route them to direct endosomal sorting (Gupta et al., 2006).

LIMP-2 is involved in the transport of β-glucocerebrosidase (β-GC) from TGN to the late endosomes or lysosomes, where the enzyme is released due to the acidic pH (Zachos et al., 2012). β-GC is defective in patients with Gaucher disease (see Table 1), one of the most common lysosomal storage disorders (Gonzalez et al., 2014; Hruska et al., 2008). In addition, mutations in the human gene encoding LIMP-2 are responsible for action myoclonus – renal failure syndrome (AMRF), highlighting the importance of LMP-mediated transport (Balreira et al., 2008; Berkovic et al., 2008; Gonzalez et al., 2014).

Rab proteins

Sorting receptors and LMPs are transport cargos that traffic between the TGN and endosomes in tubular or vesicular transport carriers whose movement and specificity is ensured by a family of small GTPases, the Rab proteins. Rabs are master regulators of intracellular vesicular transport and more than 60 Rabs have been identified in humans, pointing to the complexity in the endomembrane system and trafficking pathways that has evolved in higher eukaryotes (Zhen and Stenmark, 2015).

Rab proteins regulate all steps involved in membrane transport, from cargo recruitment to coat assembly, vesicle budding, motor recruitment and vesicle motility along the cytoskeletal filaments, as well as vesicle tethering and fusion with target membranes. As with other GTPases, Rabs cycle between an inactive (cytosolic) GDP-bound state, to an active membrane-associated GTP-bound state. Several factors regulate the conversion into the GTP-bound or GDP-bound form, including guanine nucleotide exchange factors (GEFs) and GTPase-activating proteins (GAPs), respectively. In their active form, Rabs normally typically interact with effector molecules, such as tethering factors, cytoskeleton motors, kinases, phosphatases and sorting adaptors (Hutagalung and Novick, 2011; Wandinger-Ness and Zerial, 2014).

Rab GTPases are localized in different compartments, ensuring the functional identity of intracellular membrane compartments and regulating the specificity and directionality of the transport between different organelles. For examples, Rab5 regulates endocytosis and formation of early endosomes, whereas Rab7a enters the endocytic pathway at later time points to regulate the early-to-late endosomal transition, as well as lysosomal biogenesis and fusion (Bucci et al., 2000; Rink et al., 2005).

Some Rabs also regulate the bidirectional pathways between Golgi and endosomes as the cell requires a tight regulation of the enormous flux of cargos between these compartments; these are discussed in detail below.

Rab9

Rab9 was identified more than two decades ago and was one of the first Rab proteins to be characterized (Chavrier et al., 1990). Work from the Pfeffer group has suggested that the main role of Rab9 is to mediate the retrograde transport of MPRs from late endosomes to the Golgi, as Rab9 stimulates the transport between these two compartments in a cell-free system (Lombardi et al., 1993). They have also demonstrated that a dominant-negative mutant of Rab9 decreases the delivery of cathepsin D to lysosomes, and increases the secretion of newly synthesized lysosomal enzymes. This is in line with altered receptor-mediated delivery of newly synthesized lysosomal enzymes from the TGN to lysosomes, supporting a model in which Rab9 is required for efficient lysosomal enzyme targeting (Riederer et al., 1994). Indeed, Rab9 appears to be important for the proper morphology of late endosomes and lysosomes and their localization, as Rab9 silencing causes clustering of late endosomes and a reduction in their size (Ganley et al., 2004), again pointing to a defect in the membrane flux towards the endocytic pathway.

Several Rab9 effectors have been identified, including p40 (also known as RABEPK), a factor that stimulates the retrograde transport of MPR *in vitro* (Diaz et al., 1997). TIP47 was also suggested to be a

Rab9 effector that is involved in MPR recycling (Carroll et al., 2001). However, more recent data argue against the involvement of TIP47 in Rab9-mediated MPR transport, as an exclusive role of TIP47 in lipid droplet biogenesis has been demonstrated (Bulankina et al., 2009). The golgin GCC185 (also known as GCC2), a tethering protein of the trans-Golgi network, is another Rab9 effector that has been suggested to be involved in MPR transport (Reddy et al., 2006). Rab9, together with GCC185, has been shown to be required for an efficient late endosome-to-TGN retrieval of the endopeptidase furin (which cycles between the cell surface and the TGN through endosomes), but not of TGN38 (the rat homolog of human TGN46, also known as TGOLN2) (Chia et al., 2011). Rab9 also binds directly to RhoBTB3, an atypical member of the Rho GTPase family, and mediates the docking of transport vesicles at the Golgi complex (Espinosa et al., 2009).

RUTBC1 and RUTBC2 (also known as SGSM1) are other Rab9-binding proteins; however, the function of their interaction with Rab9 has not been fully characterized (Nottingham et al., 2011, 2012). Interestingly, a recent report has demonstrated that the Rab9–RUTBC1 complex is required for the trafficking of melanogenic enzymes in melanocytes (Marubashi et al., 2016). In line with this, Rab9 has been shown to be involved together with the biogenesis of lysosome-related organelles complex 3 (BLOC-3), another Rab9 effector, in syntaxin-13-mediated cargo transport from early endosomes to maturing melanosomes (Mahanty et al., 2016). Therefore, a new role of Rab9 as a mediator of the transport towards lysosomes or lysosome-related organelles, such as melanosomes, is now beginning to emerge. Indeed, our recent live-imaging studies have allowed us to detect Rab9 on maturing endosomes before they have lost Rab5 and gained a Rab7a coat, therefore suggesting an additional role for Rab9 in the transport from Golgi to maturing endosomes (Kucera et al., 2016). In agreement with this, it has been previously demonstrated that Rab9 binds to the Rab5 GAP SGSM3 (Gillingham et al., 2014). As GAPs regulate the lifetime of the activated state of the Rabs, the interaction of Rab9 with a Rab5 GAP also points to an involvement of Rab9 in the early-to-late endosomal transition, possibly by contributing to the promotion of the Rab5-to-Rab7a conversion.

Rab7b

Rab7b has only been identified more recently and was initially named after Rab7 (which later was renamed Rab7a) owing to its sequence similarity with this protein (Yang et al., 2004). However, we have since demonstrated that Rab7b is not an isoform of Rab7a and that it has a different cellular function in mediating endosome-to-Golgi transport. Indeed, Rab7b localizes to both late endosomes and the TGN and Golgi (Progida et al., 2010). Furthermore, silencing of Rab7b increases the levels of late endosomal markers, inhibits cathepsin D maturation, and delays the retrograde transport of sorting receptors (sortilin and CI-MPR) and of the cholera toxin B-subunit (Bucci et al., 2010; Progida et al., 2010, 2012). All these effects are in line with the role of Rab7b in the endosome-to-Golgi transport and support the notion that, in general, Rab7b and Rab9 work in pathways with an opposite directionality, rather than in the same retrograde pathway.

Indeed, constitutively active mutants of Rab7b (Rab7bQ67L) and Rab9 (Rab9Q66L) not only localize to different target compartments (Golgi and late endosomes, respectively), but also induce opposite effects on carrier formation from the TGN with Rab9Q66L increasing and Rab7bQ67L decreasing the number of TGN-derived vesicles (Kucera et al., 2016; Progida et al., 2010, 2012). However, Rab7b has been much less characterized compared to Rab9, and only a few effectors have been identified, including sortilin and myosin II (Borg et al., 2014; Progida et al., 2012). Therefore, further studies are required to fully elucidate the differences in the pathways regulated by these two Rabs.

Rab6

Rab6 is another Rab protein that functions in the bidirectional transport at the crossroad between the biosynthetic and the endocytic pathway. There are four Rab6 isoforms in mammalian cells: Rab6A, Rab6A′, Rab6B and Rab6C. Rab6A and Rab6A′ are produced by alternate splicing of the *Rab6a* gene and differ in only three amino acids. Rab6B, is preferentially expressed in the brain and encoded by a different gene, whereas Rab6C is a retrogene derived from the RAB6A′ transcript and is expressed in a limited number of human tissues where it is involved in the regulation of cell cycle progression (Liu and Storrie, 2012; Young et al., 2010).

Rab6A, Rab6A′ and Rab6B, are localized at the Golgi and TGN and, when active, recruit Rab6IP2 onto Golgi membranes. In cells that overexpress the Rab6-binding domain of Rab6IP2 (also known as ERC1) or a Rab6 dominant-negative mutant, or that are silenced for Rab6A′, the retrograde transport of the Shiga toxin B subunit (used as transport marker for the early endosome-to-Golgi pathway) is partly inhibited, suggesting that the Rab6–Rab6IP2 complex is involved in endosome-to-TGN transport (Del Nery et al., 2006; Mallard et al., 2002; Monier et al., 2002). Furthermore, the recycling of CD-MPR to the TGN is dependent on Rab6 (Medigeshi and Schu, 2003). Rab6A′ is involved in endosome-to-TGN transport, whereas Rab6A regulates the traffic between the Golgi and endoplasmic reticulum (ER) (Del Nery et al., 2006; Mallard et al., 2002). However, the transport of ricin from endosomes to the Golgi appears to be dependent on both Rab6A and Rab6A′, indicating that Rab6A can also regulate the retrograde transport to the Golgi (Utskarpen et al., 2006).

It has been suggested that, by being present on the TGN, Rab6 functions in regulating the targeting and docking of endosomes with the TGN. Indeed, Rab6 binds to dynactin and mediates its recruitment to Golgi membranes, thereby exerting a tethering function (Short et al., 2002). In addition, by binding to myosin II, Rab6 controls the fission of Rab6-positive vesicles from Golgi membranes; depletion of Rab6 or myosin II impairs both this fission process and the trafficking of anterograde and retrograde cargo from the Golgi (Miserey-Lenkei et al., 2010).

Rab6 is not only involved in endosome-to-Golgi transport, but also in other trafficking pathways that take place at the Golgi, such as retrograde transport from Golgi to ER, as well as intra-Golgi and Golgi-to-plasma-membrane transport (Del Nery et al., 2006; Grigoriev et al., 2011). The function of Rab6 in both tethering vesicles to acceptor membranes and in their fission from donor compartments explains the multiple membrane trafficking pathways that are regulated by Rab6 at the Golgi.

Other Rabs

In addition to Rab9, Rab7b and Rab6, other Rabs have also been shown to have effects on the bidirectional transport between TGN and the endosomal compartments. However, their role in the regulation of this pathway is less clear. An interesting question that still remains unanswered is why several Rabs are involved in the same trafficking pathways. Do these Rabs regulate the exact same transport route, or do they contribute to different steps in the pathway? As different Rabs are characterized by different protein–protein interaction networks, one could speculate that more than one Rab is needed to successfully complete a given transport step.

It is also possible that a number of Rabs that are localized both at endosomes and TGN are required to regulate the trafficking routes

Journal of Cell Science (2016) 129, 3971-3982 doi:10.1242/jcs.185702

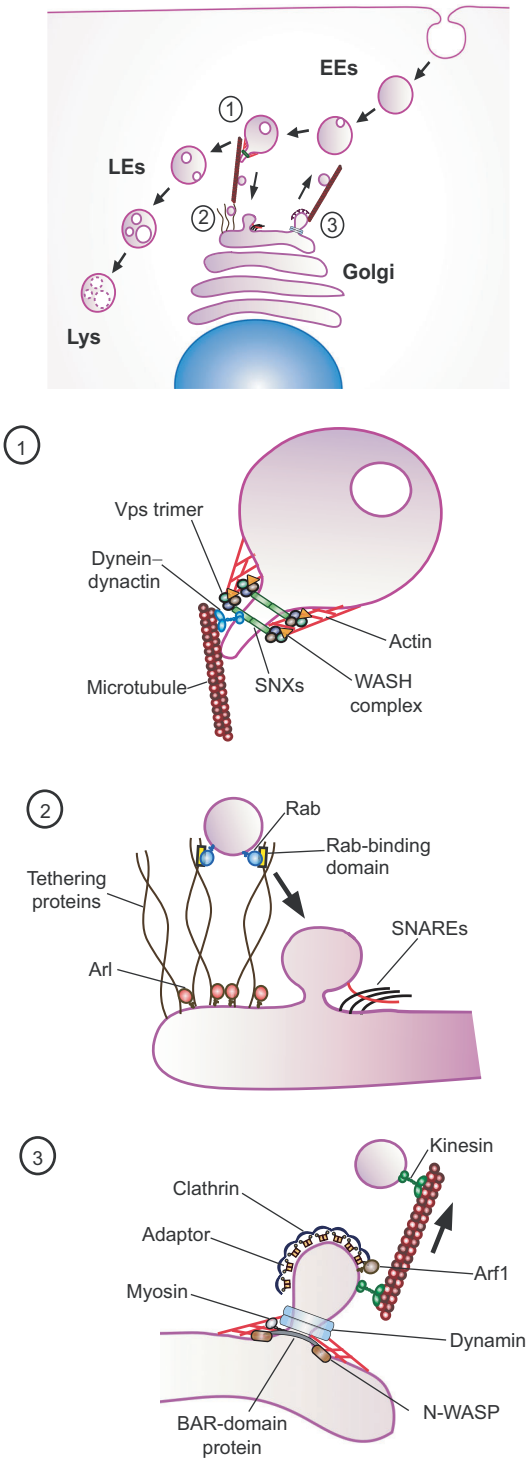

Fig. 2. Schematic model of the different transport steps in the bidirectional transport between maturing endosomes and the TGN. The bidirectional transport between the endosomal system and the Golgi comprises three main steps: (1) formation of transport carriers from the maturing endosomes towards the TGN; (2) tethering and fusion of these vesicles with the TGN membrane and (3) formation of transport carriers originating from the TGN and moving towards the maturing endosomes. (1) The retromer complex is present on the membranes of maturing endosomes. It consists of a Vps trimer responsible for the recruitment of the cargo to be delivered to the TGN and of a SNX dimer. SNXs sense and bind to endosomal tubular membranes. The retromer recruits the WASH complex, which mediates the formation of tubular carriers by promoting actin polymerization. Finally, SNXs bind to the dynein–dynactin motor complex that is directed towards the microtubule minus end. (2) Once an endosomal carrier reaches the proximity of the TGN, it is recognized and captured by tethering molecules (e.g. golgins), which extend from the TGN membrane. The Rabs present on the vesicle interact with tethering factors through specific Rab-binding sites in the tethers. Movement of the vesicle towards these Rab-binding sites will bring it into closer proximity with the TGN membrane for SNARE-mediated fusion. (3) At the TGN, carrier formation is mediated by the small GTPase Arf1, which recruits adaptor proteins, such as GGAs, AP-1 and clathrin, to TGN membranes, thereby inducing membrane curvature. Here, N-WASP promotes actin polymerization, which, together with myosin motors, provides the force necessary to initiate carrier formation. Actin, together with dynamin, then mediates the fission of carriers from the TGN. Nascent post-TGN carriers are transported by kinesins to the endosomal pathway along microtubule tracks.

between these compartments in different cell types. For example, Rab7b is highly expressed in immune cells such as dendritic cells and shows highly variable expression levels during differentiation (Berg-Larsen et al., 2013; Progida et al., 2010). Conversely, another Rab protein, Rab31 (also known as Rab22b) is highly expressed in brain tissue (Ng et al., 2009).

Live-imaging studies have demonstrated that Rab31 has a role in vesicle formation and in tubulo-vesicular transport from the TGN to endosomes (Ng et al., 2007; Rodriguez-Gabin et al., 2001, 2009). Rab31 interacts with Lowe oculocerebrorenal syndrome protein (OCRL) in oligodendrocytes (Rodriguez-Gabin et al., 2010). OCRL is a phosphatidylinositol 4,5-biphosphate [PtdIns(4,5)P_2] 5-phosphatase, which regulates the levels of PtdIns(4,5)P_2 and phosphatidylinositol 4-phosphate [PtdIns(4)P], both lipids involved in vesicular transport at the Golgi (Suchy et al., 1995). Rab31 and OCRL colocalize in the TGN and endosomes, as well as in carriers that bud from the TGN. Interestingly, post-Golgi carriers positive for Rab31 and OCRL contain MPRs, suggesting that Rab31 recruits OCRL-1 to the TGN to mediate the formation and sorting of MPRs carriers (Rodriguez-Gabin et al., 2009, 2010). However, a possible involvement of Rab31 in the transport from early to late endosomes has also been suggested (Chua and Tang, 2014; Ng et al., 2009; Rodriguez-Gabin et al., 2001). Therefore, further studies are necessary to clearly define the intracellular pathway(s) regulated by Rab31 and to understand how the different Rab31-dependent functions integrate with each another.

In addition, the more recently identified Rab29 (also referred to as Rab7L1) has also been reported to mediate endosome-to-Golgi transport of MPRs (Wang et al., 2014). Finally, another set of Rabs including Rab11, Rab13 and Rab14, are involved in transport between the TGN and recycling endosomes, a specific subset of endosomes (Jing et al., 2010; Junutula et al., 2004; Nokes et al., 2008; Ullrich et al., 1996; Wilcke et al., 2000). However, it is unclear which roles these Rabs have in the transport between endosomes and Golgi and whether they are actively involved in its regulation or whether the reported effects are indirect and caused by alterations in normal Rab functions. It should also be noted that most of the work on Rab proteins and other components of the bidirectional transport between endosomes and Golgi, such as that involving MPRs, has been conducted in cell lines, and this might not necessarily reflect the trafficking in more specialized cells *in vivo*.

Transport steps between endosomes and the TGN

The bidirectional trafficking between endosomes and the TGN comprises multiple transport steps, which involve formation of carriers and their transport from the endosomal pathway to the TGN, followed by tethering and fusion at the Golgi and TGN, as well as

Journal of Cell Science

carrier formation at the TGN and their transport to the endosomes (Fig. 2). These steps are not only mediated by Rab proteins, but also by specialized protein complexes, such as the retromer, tethering complexes and SNAREs, as discussed below.

Endosomal carrier formation

One of the main factors in the trafficking from endosomes to the TGN is the retromer complex (for recent reviews, see Burd and Cullen, 2014; Gallon and Cullen, 2015; Mukadam and Seaman, 2015; Seaman, 2012). The retromer is a multisubunit complex originally identified in yeast that is involved in the retrograde trafficking of sorting receptors (Seaman et al., 1998). Indeed, silencing of retromer subunits, such as Vps26 (which has two isoforms in mammals, Vps26a and Vps26b), Vps29 or sorting nexin 1 (SNX1), inhibits the retrieval of the CI-MPR from the endosomes for its reuse in the TGN, thereby targeting the receptor to lysosomes for degradation (Arighi et al., 2004; Carlton et al., 2004; Seaman, 2004).

The mammalian retromer complex consists of a dimer of sorting nexins (a combination of SNX1, SNX2, SNX5 and SNX6) and the Vps26–Vps29–Vps35 trimer. SNXs contain phox homology (PX) and BAR (Bin, amphiphysin, Rvs) domains, which are responsible for the association of the retromer with endosomal membranes. Indeed, the PX domain binds to phosphatidylinositol 3-phosphate [PtdIns(3)*P*], a phosphoinositide present in the membranes of endosomes, whereas the BAR domains bind to highly curved membranes, such as those in tubular endosomes. The Vps26–Vps29–Vps35 trimer is in turn responsible for cargo recruitment by binding the cytosolic tail of transmembrane receptors (Arighi et al., 2004; Mukadam and Seaman, 2015). Recently, it has been demonstrated that Vps35 recruits retromer to membrane by recognizing SNX3 and Rab7a (Harrison et al., 2014). The key role of Vps35 in retromer recruitment is further supported by the evidence that mutations in the *VPS35* gene are associated with neurodegenerative diseases, such as Alzheimer's or Parkinson's (see Table 1). However, whether SNX3 associates with the entire pentameric retromer complex or only with the Vps trimer as an alternative to the SNX dimer in order to mediate the transport of distinct carriers, as previously suggested (Cullen and Korswagen, 2012; Harterink et al., 2011), needs to be further investigated.

The next step following cargo recruitment is the formation of tubular carriers. To that end, the WASH complex is recruited by the retromer to the base of the endosomal tubules and, together with Arp2/3, promotes actin nucleation. It has been suggested that, by promoting actin polymerization, the WASH complex is responsible for generating the F-actin-driven forces that are necessary to catalyze endosomal membrane fission (Derivery et al., 2009; Gomez and Billadeau, 2009). Finally, the interaction of the SNX5–SNX6 dimer with the $p150^{glued}$ (also known as DCTN1) subunit of the dynein–dynactin motor complex promotes the minus-end-directed transport of vesicles along microtubules until they reach the TGN where the high concentration of PtdIns(4)*P* in the membrane promotes the dissociation of SNX6 from $p150^{glued}$ (Niu et al., 2013; Wassmer et al., 2009).

It is noteworthy that the retromer complex is present on maturing endosomes that correspond to intermediates in the early-to-late endosomal transition, and here Rab7a is responsible for the recruitment of the retromer trimer to the endosomal membrane (Bonifacino and Rojas, 2006; Rojas et al., 2008; Seaman, 2012). As a consequence, the retromer mediates the budding of tubular cargo from endosomal membranes as soon as Rab7a has been acquired. The direct link between retromer activity and Rab conversion during endosomal maturation is in line with its main function, which is to rescue cargos from lysosomal degradation by directing them towards the TGN (Burd and Cullen, 2014; van Weering et al., 2012).

Interestingly, different SNXs can associate with the retromer Vps trimer, and it has been suggested that these alternative retromer components mediate the recycling of cargos through different transport pathways (Cullen and Korswagen, 2012).

Tethering at the TGN

Once the endosome-derived carriers reach the proximity of the TGN, tethering factors, such as the Golgi-associated retrograde protein (GARP I) complex and golgins capture these incoming transport vesicles to promote their fusion to the TGN membrane. The final fusion step is then mediated by SNAREs (Bonifacino and Rojas, 2006; Gillingham and Munro, 2016).

Golgins form homodimers that are characterized by long coiled-coil regions that allow them to capture incoming vesicles owing to protruding extensively from the acceptor membranes (i.e. at the TGN). The capture of specific vesicles is ensured by the presence of multiple Rab-binding sites in the golgin coiled-coil regions (Sinka et al., 2008). Golgins include GCC88 (also known as GCC1), GCC185, golgin-245 (also known as GOLGA4) and golgin-97 (also known as GOLGA1), which contain a C-terminal golgin-97–RanBP2α–Imh1–p230 (GRIP) domain that is responsible for the recruitment of the Arl1 and Arl3 GTPases to the TGN. Mutations in Arl1 or Arl3, or depletion of GCC88, GCC185, golgin-245 or golgin-97 impair endosome-to-TGN transport (Bonifacino and Rojas, 2006; Lieu et al., 2007; Lu et al., 2004; Panic et al., 2003; Reddy et al., 2006; Yoshino et al., 2005). Golgins also contribute to the specificity of membrane recognition, as golgins with different localizations at the Golgi membrane have been shown to be able to capture vesicles of different origins (Wong and Munro, 2014).

The GARP I complex comprises four subunits (Vps51, Vps52, Vps53 and Vps54) and is responsible for the tethering of endosome-derived vesicles at the TGN by binding to the Arl5 GTPase (Rosa-Ferreira et al., 2015). Silencing of any of the GARP subunits inhibits the retrograde transport of MPRs, TGN46 and the B subunit of Shiga toxin (Perez-Victoria et al., 2008). More recent evidence also suggests the involvement of additional tethering complexes in the endosome-to-Golgi transport pathway, such as the COG and Dsl1 complexes (Arasaki et al., 2013; Hierro et al., 2015; Laufman et al., 2011).

SNAREs are membrane-bound proteins containing coiled-coil motifs (SNARE motifs). A total of 38 different SNAREs exist in humans, and these localize to different intracellular compartments where they regulate fusion events (for more information about SNAREs and their mechanisms of action, see the following reviews: Bombardier and Munson, 2015; Hong and Lev, 2014; Malsam and Sollner, 2011). They are classically divided into t-SNAREs, present on the target membrane, and v-SNAREs, which are localized on transport vesicles. The interaction of one SNARE motif in a v-SNARE with three t-SNAREs leads to the formation of a twisted parallel four-helix bundle that brings the two membranes in close proximity. The energy released during this assembly promotes membrane fusion (Li et al., 2007). After fusion, ATPase N-ethylmaleimide-sensitive fusion protein (NSF) and α-soluble NSF attachment protein (α-SNAP) catalyze the disassembly of the SNARE complex, releasing the SNAREs for new fusion events. A different classification has been introduced because some SNAREs are present on both vesicles and target membranes. This newer classification, which divides SNAREs into R- and Q-SNAREs, is based on their crystal structure (Fasshauer et al., 1998).

Several SNARE complexes and regulators are involved in the endosome-to-TGN transport, including syntaxin-6–syntaxin-16–Vti1a–Vamp4, syntaxin-6–syntaxin-16–Vti1a–Vamp3, syntaxin-10–syntaxin-16–Vti1a–Vamp3, and syntaxin-5–GS28–Ykt6–GS15 (GS28 and GS15 are also known as GOSR1 and BET1L, respectively) (Ganley et al., 2008; Mallard et al., 2002; Tai et al., 2004; Wang et al., 2005). In addition, tethering factors interact with SNAREs and promote their assembly. Indeed, it has been shown that the GARP I complex specifically interacts with the SNAREs syntaxin 6, syntaxin 16 and Vamp4 to promote endosome-to-TGN transport by mediating both vesicle tethering and assembly of SNARE complexes (Perez-Victoria and Bonifacino, 2009). In addition, the COG complex interacts with SNAREs, including the syntaxin-6–syntaxin-16–Vti1a–Vamp4 complex, thereby regulating SNARE complex assembly and the associated endosome-to-TGN transport steps (Laufman et al., 2011, 2013).

The interaction between tethering factors and SNAREs is also important for the efficiency, specificity and spatio-temporal coordination of membrane fusion. However, how these events are regulated is still unclear.

Cargo vesicle formation at the TGN

As discussed above, the retromer complex, together with accessory proteins, actin filaments and microtubules, is involved in the formation of cargo vesicles being transported from the endosome to the TGN. Similarly, the formation of carriers from the TGN that are directed towards the endosomal pathway is facilitated by coat and adaptor proteins, actin and the microtubule cytoskeleton.

Carrier formation at the TGN is initiated by the assembly of a coat that bends the membrane and generates a bud. Several proteins are involved in this process. Upon activation, the small GTPase Arf1 is recruited to Golgi membranes, where it recruits coat protein complexes (Puertollano et al., 2001; Ren et al., 2013). The cytosolic tail of the cargo contains sorting signals, which, together with Arf1, recruit AP-1, GGAs and clathrin (Baust et al., 2006). Clathrin and Arf1 induce membrane curvature, which is further regulated by the lipid composition of the surrounding membrane and the action of lipid-modifying enzymes (Krauss et al., 2008). Indeed, both GGAs and AP-1 bind to PtdIns(4)*P* and Arf1 (Ren et al., 2013; Wang et al., 2007, 2003). Furthermore, the activation of enzymes that synthesize the conical lipids phosphatidic acid and diacylglycerol promotes membrane budding (Asp et al., 2009; Schmidt and Brown, 2009). However, whether GGAs and AP-1 cooperate in the process of carrier formation at the TGN, or whether they function independently, is still debated (Daboussi et al., 2012; Doray et al., 2002; Hirst et al., 2012).

BAR-domain-containing proteins that sense membrane curvature are recruited to the budding sites by binding to phosphoinositides or proteins associated with TGN membranes (Itoh and De Camilli, 2006; Prouzet-Mauleon et al., 2008). BAR domain proteins are also able to bind to neural Wiskott–Aldrich syndrome protein (N-WASP, also known as WASL), which in turn promotes actin polymerization through Arp2/3. Actin polymerization, combined with the action of myosin motors, provides the force that is necessary to elongate membrane buds (Almeida et al., 2011; Anitei and Hoflack, 2012). The fission of carriers from the TGN occurs through the coordinate action of actin, myosin II, Rab6 and dynamin, which constricts the neck of the elongated carrier (Kessels et al., 2006; Miserey-Lenkei et al., 2010). Microtubules assist in carrier biogenesis and also constitute the tracks that are followed by the nascent carriers after they leave the TGN (Anitei et al., 2010; Miserey-Lenkei et al., 2010).

Taken together, although the key components of the carrier formation at the TGN have been identified, it nevertheless remains unclear how these factors are coordinated and what are their spatio-temporal dynamics.

Conclusions

In the past few years, the development of high-resolution live-cell imaging has made it possible to better define the endocytic pathway as a continuum of dynamically maturating endosomes rather than a set of static compartments with clearly defined boundaries. In light of this emerging notion, the bidirectional transport between the TGN and the endosomal system has to be reconsidered as a continuous exchange of material that occurs at different times during endosomal maturation rather than at specific distinct compartments. Such a model of exchange between maturing endosomal compartments is also able to consolidate the ongoing debates regarding the entry point of factors such as sorting receptors into the intersection of TGN and endosomes.

Although there has been a great progress in deciphering the main players involved in these transport pathways, several key questions remain, including how the different machineries are coordinated to regulate the same transport step, what is the precise spatio-temporal regulation of carrier formation at the TGN and how can the tethering factors coordinate the specific carrier recognition with membrane fusion at the TGN. Furthermore, an increasing number of diseases are associated with defects in factors or mechanisms that regulate the bidirectional transport between endosomes and Golgi. These include not only lysosomal storage disorders such as mucolipidosis or Gaucher disease, which are caused by defects in the transport of lysosomal enzymes, but also neurodegenerative diseases, such as Parkinson's or Alzheimer's disease (see Table 1). The study of genetic disorders affecting these pathways can provide important information regarding the specific function of the associated factors and clinical effects. Therefore, a full understanding of these processes is of utmost importance for the design of therapeutic strategies to combat these conditions.

Acknowledgements

We thank Bernard Hoflack for helpful comments on the manuscript and Ana Kucera in the Bakke laboratory for providing the live-imaging picture of the Rab5- and Rab9-positive endosomes.

Competing interests

The authors declare no competing or financial interests.

Funding

Work done in the author's laboratories is supported by the Kreftforeningen (Norwegian Cancer Society) [grant numbers 5760850 to C.P., 4604944 to O.B.], the Norges Forskningsråd (Research Council of Norway) [grant 239903 to C.P. and 230779 to O.B.] and through its Centers of Excellence funding scheme [project number 179573].

References

Almeida, C. G., Yamada, A., Tenza, D., Louvard, D., Raposo, G. and Coudrier, E. (2011). Myosin 1b promotes the formation of post-Golgi carriers by regulating actin assembly and membrane remodelling at the trans-Golgi network. *Nat. Cell Biol.* **13**, 779-789.

Anitei, M. and Hoflack, B. (2012). Bridging membrane and cytoskeleton dynamics in the secretory and endocytic pathways. *Nat. Cell Biol.* **14**, 11-19.

Anitei, M., Wassmer, T., Stange, C. and Hoflack, B. (2010). Bidirectional transport between the trans-Golgi network and the endosomal system. *Mol. Membr. Biol.* **27**, 443-456.

Anitei, M., Chenna, R., Czupalla, C., Esner, M., Christ, S., Lenhard, S., Korn, K., Meyenhofer, F., Bickle, M., Zerial, M. et al. (2014). A high-throughput siRNA screen identifies genes that regulate mannose 6-phosphate receptor trafficking. *J. Cell Sci.* **127**, 5079-5092.

Journal of Cell Science

Arasaki, K., Takagi, D., Furuno, A., Sohda, M., Misumi, Y., Wakana, Y., Inoue, H. and Tagaya, M. (2013). A new role for RINT-1 in SNARE complex assembly at the trans-Golgi network in coordination with the COG complex. *Mol. Biol. Cell* **24**, 2907-2917.

Arighi, C. N., Hartnell, L. M., Aguilar, R. C., Haft, C. R. and Bonifacino, J. S. (2004). Role of the mammalian retromer in sorting of the cation-independent mannose 6-phosphate receptor. *J. Cell Biol.* **165**, 123-133.

Asp, L., Kartberg, F., Fernandez-Rodriguez, J., Smedh, M., Elsner, M., Laporte, F., Barcena, M., Jansen, K. A., Valentijn, J. A., Koster, A. J. et al. (2009). Early stages of Golgi vesicle and tubule formation require diacylglycerol. *Mol. Biol. Cell* **20**, 780-790.

Balreira, A., Gaspar, P., Caiola, D., Chaves, J., Beirao, I., Lima, J. L., Azevedo, J. E. and Miranda, M. C. S. (2008). A nonsense mutation in the LIMP-2 gene associated with progressive myoclonic epilepsy and nephrotic syndrome. *Hum. Mol. Genet.* **17**, 2238-2243.

Barois, N. and Bakke, O. (2005). The adaptor protein AP-4 as a component of the clathrin coat machinery: a morphological study. *Biochem. J.* **385**, 503-510.

Bauer, P., Leshinsky-Silver, E., Blumkin, L., Schlipf, N., Schroder, C., Schicks, J., Lev, D., Riess, O., Lerman-Sagie, T. and Schols, L. (2012). Mutation in the AP4B1 gene cause hereditary spastic paraplegia type 47 (SPG47). *Neurogenetics* **13**, 73-76.

Baust, T., Czupalla, C., Krause, E., Bourel-Bonnet, L. and Hoflack, B. (2006). Proteomic analysis of adaptor protein 1A coats selectively assembled on liposomes. *Proc. Natl. Acad. Sci. USA* **103**, 3159-3164.

Berg-Larsen, A., Landsverk, O. J. B., Progida, C., Gregers, T. F. and Bakke, O. (2013). Differential regulation of Rab GTPase expression in monocyte-derived dendritic cells upon lipopolysaccharide activation: a correlation to maturation-dependent functional properties. *PLoS ONE* **8**, e73538.

Berkovic, S. F., Dibbens, L. M., Oshlack, A., Silver, J. D., Katerelos, M., Vears, D. F., Lullmann-Rauch, R., Blanz, J., Zhang, K. W., Stankovich, J. 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**, 673-684.

Blanz, J., Groth, J., Zachos, C., Wehling, C., Saftig, P. and Schwake, M. (2010). Disease-causing mutations within the lysosomal integral membrane protein type 2 (LIMP-2) reveal the nature of binding to its ligand beta-glucocerebrosidase. *Hum. Mol. Genet.* **19**, 563-572.

Bombardier, J. P. and Munson, M. (2015). Three steps forward, two steps back: mechanistic insights into the assembly and disassembly of the SNARE complex. *Curr. Opin. Chem. Biol.* **29**, 66-71.

Bonifacino, J. S. and Rojas, R. (2006). Retrograde transport from endosomes to the trans-Golgi network. *Nat. Rev. Mol. Cell Biol.* **7**, 568-579.

Borck, G., Molla-Herman, A., Boddaert, N., Encha-Razavi, F., Philippe, A., Robel, L., Desguerre, I., Brunelle, F., Benmerah, A., Munnich, A. et al. (2008). Clinical, cellular, and neuropathological consequences of AP1S2 mutations: further delineation of a recognizable X-linked mental retardation syndrome. *Hum. Mutat.* **29**, 966-974.

Borg, M., Bakke, O. and Progida, C. (2014). A novel interaction between Rab7b and actomyosin reveals a dual role in intracellular transport and cell migration. *J. Cell Sci.* **127**, 4927-4939.

Braulke, T. and Bonifacino, J. S. (2009). Sorting of lysosomal proteins. *Biochim. Biophys. Acta* **1793**, 605-614.

Breusegem, S. Y. and Seaman, M. N. J. (2014). Genome-wide RNAi screen reveals a role for multipass membrane proteins in endosome-to-golgi retrieval. *Cell Rep.* **9**, 1931-1945.

Bucci, C., Thomsen, P., Nicoziani, P., McCarthy, J. and van Deurs, B. (2000). Rab7: a key to lysosome biogenesis. *Mol. Biol. Cell* **11**, 467-480.

Bucci, C., Bakke, O. and Progida, C. (2010). Rab7b and receptors trafficking. *Commun. Integr. Biol.* **3**, 401-404.

Bulankina, A. V., Deggerich, A., Wenzel, D., Mutenda, K., Wittmann, J. G., Rudolph, M. G., Burger, K. N. J. and Honing, S. (2009). TIP47 functions in the biogenesis of lipid droplets. *J. Cell Biol.* **185**, 641-655.

Burd, C. G. (2011). Physiology and pathology of endosome-to-Golgi retrograde sorting. *Traffic* **12**, 948-955.

Burd, C. and Cullen, P. J. (2014). Retromer: a master conductor of endosome sorting. *Cold Spring Harb. Perspect. Biol.* **6**, pii: a016774.

Burgos, P. V., Mardones, G. A., Rojas, A. L., daSilva, L. L. P., Prabhu, Y., Hurley, J. H. and Bonifacino, J. S. (2010). Sorting of the Alzheimer's disease amyloid precursor protein mediated by the AP-4 complex. *Dev. Cell* **18**, 425-436.

Canuel, M., Lefrancois, S., Zeng, J. and Morales, C. R. (2008). AP-1 and retromer play opposite roles in the trafficking of sortilin between the Golgi apparatus and the lysosomes. *Biochem. Biophys. Res. Commun.* **366**, 724-730.

Carlton, J., Bujny, M., Peter, B. J., Oorschot, V. M. J., Rutherford, A., Mellor, H., Klumperman, J., McMahon, H. T. and Cullen, P. J. (2004). Sorting nexin-1 mediates tubular endosome-to-TGN transport through coincidence sensing of high- curvature membranes and 3-phosphoinositides. *Curr. Biol.* **14**, 1791-1800.

Carroll, K. S., Hanna, J., Simon, I., Krise, J., Barbero, P. and Pfeffer, S. R. (2001). Role of Rab9 GTPase in facilitating receptor recruitment by TIP47. *Science* **292**, 1373-1376.

Chapuy, B., Tikkanen, R., Mühlhausen, C., Wenzel, D., von Figura, K. and Höning, S. (2008). AP-1 and AP-3 mediate sorting of melanosomal and lysosomal membrane proteins into distinct post-Golgi trafficking pathways. *Traffic* **9**, 1157-1172.

Chavrier, P., Vingron, M., Sander, C., Simons, K. and Zerial, M. (1990). Molecular cloning of YPT1/SEC4-related cDNAs from an epithelial cell line. *Mol. Cell. Biol.* **10**, 6578-6585.

Chen, B.-C., Legant, W. R., Wang, K., Shao, L., Milkie, D. E., Davidson, M. W., Janetopoulos, C., Wu, X. S., Hammer, J. A., III, Liu, Z. et al. (2014). Lattice light-sheet microscopy: imaging molecules to embryos at high spatiotemporal resolution. *Science* **346**, 1257998.

Chia, P. Z. C. and Gleeson, P. A. (2013). Imaging and quantitation techniques for tracking cargo along endosome-to-Golgi transport pathways. *Cells* **2**, 105-123.

Chia, P. Z. C., Gasnereau, I., Lieu, Z. Z. and Gleeson, P. A. (2011). Rab9-dependent retrograde transport and endosomal sorting of the endopeptidase furin. *J. Cell Sci.* **124**, 2401-2413.

Chia, P. Z. C., Gunn, P. and Gleeson, P. A. (2013). Cargo trafficking between endosomes and the trans-Golgi network. *Histochem. Cell Biol.* **140**, 307-315.

Chidambaram, S., Zimmermann, J. and von Mollard, G. F. (2008). ENTH domain proteins are cargo adaptors for multiple SNARE proteins at the TGN endosome. *J. Cell Sci.* **121**, 329-338.

Chua, C. E. L. and Tang, B. L. (2014). Engagement of the small GTPase Rab31 protein and its effector, early endosome Antigen 1, is important for trafficking of the ligand-bound epidermal growth factor receptor from the early to the late endosome. *J. Biol. Chem.* **289**, 12375-12389.

Coutinho, M. F., Prata, M. J. and Alves, S. (2012). A shortcut to the lysosome: the mannose-6-phosphate-independent pathway. *Mol. Genet. Metab.* **107**, 257-266.

Crump, C. M., Xiang, Y., Thomas, L., Gu, F., Austin, C., Tooze, S. A. and Thomas, G. (2001). PACS-1 binding to adaptors is required for acidic cluster motif-mediated protein traffic. *EMBO J.* **20**, 2191-2201.

Cullen, P. J. and Korswagen, H. C. (2012). Sorting nexins provide diversity for retromer-dependent trafficking events. *Nat. Cell Biol.* **14**, 29-37.

Daboussi, L., Costaguta, G. and Payne, G. S. (2012). Phosphoinositide-mediated clathrin adaptor progression at the trans-Golgi network. *Nat. Cell Biol.* **14**, 239-248.

Del Nery, E., Miserey-Lenkei, S., Falguières, T., Nizak, C., Johannes, L., Perez, F. and Goud, B. (2006). Rab6A and Rab6A′ GTPases play non-overlapping roles in membrane trafficking. *Traffic* **7**, 394-407.

Derivery, E., Sousa, C., Gautier, J. J., Lombard, B., Loew, D. and Gautreau, A. (2009). The Arp2/3 activator WASH controls the fission of endosomes through a large multiprotein complex. *Dev. Cell* **17**, 712-723.

Diaz, E., Schimmoller, F. and Pfeffer, S. R. (1997). A novel Rab9 effector required for endosome-to-TGN transport. *J. Cell Biol.* **138**, 283-290.

Doray, B., Ghosh, P., Griffith, J., Geuze, H. J. and Kornfeld, S. (2002). Cooperation of GGAs and AP-1 in packaging MPRs at the trans-Golgi network. *Science* **297**, 1700-1703.

Espinosa, E. J., Calero, M., Sridevi, K. and Pfeffer, S. R. (2009). RhoBTB3: a Rho GTPase-family ATPase required for endosome to Golgi transport. *Cell* **137**, 938-948.

Fasshauer, D., Sutton, R. B., Brunger, A. T. and Jahn, R. (1998). Conserved structural features of the synaptic fusion complex: SNARE proteins reclassified as Q- and R-SNAREs. *Proc. Natl. Acad. Sci. USA* **95**, 15781-15786.

Feinstein, M., Flusser, H., Lerman-Sagie, T., Ben-Zeev, B., Lev, D., Agamy, O., Cohen, I., Kadir, R., Sivan, S., Leshinsky-Silver, E. et al. (2014). VPS53 mutations cause progressive cerebello-cerebral atrophy type 2 (PCCA2). *J. Med. Genet.* **51**, 303-308.

Follett, J., Norwood, S. J., Hamilton, N. A., Mohan, M., Kovtun, O., Tay, S., Zhe, Y., Wood, S. A., Mellick, G. D., Silburn, P. A. et al. (2014). The Vps35 D620N mutation linked to Parkinson's disease disrupts the cargo sorting function of retromer. *Traffic* **15**, 230-244.

Frohlich, F., Petit, C., Kory, N., Christiano, R., Hannibal-Bach, H.-K., Graham, M., Liu, X., Ejsing, C. S., Farese, R. V., Jr and Walther, T. C. (2015). The GARP complex is required for cellular sphingolipid homeostasis. *Elife* **4**, e08712.

Gallon, M. and Cullen, P. J. (2015). Retromer and sorting nexins in endosomal sorting. *Biochem. Soc. Trans.* **43**, 33-47.

Ganley, I. G., Carroll, K., Bittova, L. and Pfeffer, S. (2004). Rab9 GTPase regulates late endosome size and requires effector interaction for its stability. *Mol. Biol. Cell* **15**, 5420-5430.

Ganley, I. G., Espinosa, E. and Pfeffer, S. R. (2008). A syntaxin 10-SNARE complex distinguishes two distinct transport routes from endosomes to the trans-Golgi in human cells. *J. Cell Biol.* **180**, 159-172.

Gillingham, A. K. and Munro, S. (2016). Finding the Golgi: Golgin coiled-coil proteins show the way. *Trends Cell Biol.* **26**, 399-408.

Gillingham, A. K., Sinka, R., Torres, I. L., Lilley, K. S. and Munro, S. (2014). Toward a comprehensive map of the effectors of rab GTPases. *Dev. Cell* **31**, 358-373.

Gomez, T. S. and Billadeau, D. D. (2009). A FAM21-containing WASH complex regulates retromer-dependent sorting. *Dev. Cell* **17**, 699-711.

Gonzalez, A., Valeiras, M., Sidransky, E. and Tayebi, N. (2014). Lysosomal integral membrane protein-2: a new player in lysosome-related pathology. *Mol. Genet. Metab.* **111**, 84-91.
Grigoriev, I., Yu, K. L., Martinez-Sanchez, E., Serra-Marques, A., Smal, I., Meijering, E., Demmers, J., Peranen, J., Pasterkamp, R. J., van der Sluijs, P. et al. (2011). Rab6, Rab8, and MICAL3 cooperate in controlling docking and fusion of exocytotic carriers. *Curr. Biol.* **21**, 967-974.
Gupta, S. N., Kloster, M. M., Rodionov, D. G. and Bakke, O. (2006). Re-routing of the invariant chain to the direct sorting pathway by introduction of an AP3-binding motif from LIMP II. *Eur. J. Cell Biol.* **85**, 457-467.
Harrison, M. S., Hung, C.-S., Liu, T.-T., Christiano, R., Walther, T. C. and Burd, C. G. (2014). A mechanism for retromer endosomal coat complex assembly with cargo. *Proc. Natl. Acad. Sci. USA* **111**, 267-272.
Harterink, M., Port, F., Lorenowicz, M. J., McGough, I. J., Silhankova, M., Betist, M. C., van Weering, J. R. T., van Heesbeen, R. G. H. P., Middelkoop, T. C., Basler, K. et al. (2011). A SNX3-dependent retromer pathway mediates retrograde transport of the Wnt sorting receptor Wntless and is required for Wnt secretion. *Nat. Cell Biol.* **13**, 914-923.
Hermey, G. (2009). The Vps10p-domain receptor family. *Cell. Mol. Life Sci.* **66**, 2677-2689.
Hierro, A., Gershlick, D. C., Rojas, A. L. and Bonifacino, J. S. (2015). Formation of tubulovesicular carriers from endosomes and their fusion to the trans-Golgi network. *Int. Rev. Cell Mol. Biol.* **318**, 159-202.
Hirst, J., Borner, G. H. H., Antrobus, R., Peden, A. A., Hodson, N. A., Sahlender, D. A. and Robinson, M. S. (2012). Distinct and overlapping roles for AP-1 and GGAs revealed by the "knocksideways" system. *Curr. Biol.* **22**, 1711-1716.
Hirst, J., Irving, C. and Borner, G. H. H. (2013). Adaptor protein complexes AP-4 and AP-5: new players in endosomal trafficking and progressive spastic paraplegia. *Traffic* **14**, 153-164.
Hirst, J., Edgar, J. R., Esteves, T., Darios, F., Madeo, M., Chang, J., Roda, R. H., Durr, A., Anheim, M., Gellera, C. et al. (2015). Loss of AP-5 results in accumulation of aberrant endolysosomes: defining a new type of lysosomal storage disease. *Hum. Mol. Genet.* **24**, 4984-4996.
Hong, W. and Lev, S. (2014). Tethering the assembly of SNARE complexes. *Trends Cell Biol.* **24**, 35-43.
Honing, S., Griffith, J., Geuze, H. J. and Hunziker, W. (1996). The tyrosine-based lysosomal targeting signal in lamp-1 mediates sorting into Golgi-derived clathrin-coated vesicles. *EMBO J.* **15**, 5230-5239.
Honing, S., Sandoval, I. V. and 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**, 1304-1314.
Hruska, K. S., LaMarca, M. E., Scott, C. R. and Sidransky, E. (2008). Gaucher disease: mutation and polymorphism spectrum in the glucocerebrosidase gene (GBA). *Hum. Mutat.* **29**, 567-583.
Hu, F., Padukkavidana, T., Vaegter, C. B., Brady, O. A., Zheng, Y., Mackenzie, I. R., Feldman, H. H., Nykjaer, A. and Strittmatter, S. M. (2010). Sortilin-mediated endocytosis determines levels of the frontotemporal dementia protein, progranulin. *Neuron* **68**, 654-667.
Hutagalung, A. H. and Novick, P. J. (2011). Role of Rab GTPases in membrane traffic and cell physiology. *Physiol. Rev.* **91**, 119-149.
Itoh, T. and De Camilli, P. (2006). BAR, F-BAR (EFC) and ENTH/ANTH domains in the regulation of membrane-cytosol interfaces and membrane curvature. *Biochim. Biophys. Acta* **1761**, 897-912.
Janvier, K. and Bonifacino, J. S. (2005). Role of the endocytic machinery in the sorting of lysosome-associated membrane proteins. *Mol. Biol. Cell* **16**, 4231-4242.
Jing, J., Junutula, J. R., Wu, C., Burden, J., Matern, H., Peden, A. A. and Prekeris, R. (2010). FIP1/RCP binding to Golgin-97 regulates retrograde transport from recycling endosomes to the trans-Golgi network. *Mol. Biol. Cell* **21**, 3041-3053.
Junutula, J. R., De Maziére, A. M., Peden, A. A., Ervin, K. E., Advani, R. J., van Dijk, S. M., Klumperman, J. and Scheller, R. H. (2004). Rab14 is involved in membrane trafficking between the Golgi complex and endosomes. *Mol. Biol. Cell* **15**, 2218-2229.
Kessels, M. M., Dong, J., Leibig, W., Westermann, P. and Qualmann, B. (2006). Complexes of syndapin II with dynamin II promote vesicle formation at the trans-Golgi network. *J. Cell Sci.* **119**, 1504-1516.
Kjolby, M., Nielsen, M. S. and Petersen, C. M. (2015). Sortilin, encoded by the cardiovascular risk gene SORT1, and its suggested functions in cardiovascular disease. *Curr. Atheroscler. Rep.* **17**, 18.
Kollmann, K., Pohl, S., Marschner, K., Encarnacao, M., Sakwa, I., Tiede, S., Poorthuis, B. J., Lubke, T., Muller-Loennies, S., Storch, S. et al. (2010). Mannose phosphorylation in health and disease. *Eur. J. Cell Biol.* **89**, 117-123.
Krauss, M., Jia, J.-Y., Roux, A., Beck, R., Wieland, F. T., De Camilli, P. and Haucke, V. (2008). Arf1-GTP-induced tubule formation suggests a function of Arf family proteins in curvature acquisition at sites of vesicle budding. *J. Biol. Chem.* **283**, 27717-27723.
Kucera, A., Borg Distefano, M., Berg-Larsen, A., Skjeldal, F., Repnik, U., Bakke, O. and Progida, C. (2016). Spatiotemporal resolution of Rab9 and CI-MPR dynamics in the endocytic pathway. *Traffic* **17**, 211-229.
Laufman, O., Hong, W. and Lev, S. (2011). The COG complex interacts directly with Syntaxin 6 and positively regulates endosome-to-TGN retrograde transport. *J. Cell Biol.* **194**, 459-472.
Laufman, O., Hong, W. and Lev, S. (2013). The COG complex interacts with multiple Golgi SNAREs and enhances fusogenic assembly of SNARE complexes. *J. Cell Sci.* **126**, 1506-1516.
Lee, J. H., Barral, S. and Reitz, C. (2008). The neuronal sortilin-related receptor gene SORL1 and late-onset Alzheimer's disease. *Curr. Neurol Neurosci. Rep.* **8**, 384-391.
Lefrancois, S., Zeng, J., Hassan, A. J., Canuel, M. and Morales, C. R. (2003). The lysosomal trafficking of sphingolipid activator proteins (SAPs) is mediated by sortilin. *EMBO J.* **22**, 6430-6437.
Legant, W. R., Shao, L., Grimm, J. B., Brown, T. A., Milkie, D. E., Avants, B. B., Lavis, L. D. and Betzig, E. (2016). High-density three-dimensional localization microscopy across large volumes. *Nat. Methods* **13**, 359-365.
Li, F., Pincet, F., Perez, E., Eng, W. S., Melia, T. J., Rothman, J. E. and Tareste, D. (2007). Energetics and dynamics of SNAREpin folding across lipid bilayers. *Nat. Struct. Mol. Biol.* **14**, 890-896.
Lieu, Z. Z., Derby, M. C., Teasdale, R. D., Hart, C., Gunn, P. and Gleeson, P. A. (2007). The golgin GCC88 is required for efficient retrograde transport of cargo from the early endosomes to the trans-Golgi network. *Mol. Biol. Cell* **18**, 4979-4991.
Liu, S. and Storrie, B. (2012). Are Rab proteins the link between Golgi organization and membrane trafficking? *Cell. Mol. Life Sci.* **69**, 4093-4106.
Lombardi, D., Soldati, T., Riederer, M. A., Goda, Y., Zerial, M. and Pfeffer, S. R. (1993). Rab9 functions in transport between late endosomes and the trans Golgi network. *EMBO J.* **12**, 677-682.
Lu, L., Tai, G. and Hong, W. (2004). Autoantigen Golgin-97, an effector of Arl1 GTPase, participates in traffic from the endosome to the trans-golgi network. *Mol. Biol. Cell* **15**, 4426-4443.
Mahanty, S., Ravichandran, K., Chitirala, P., Prabha, J., Jani, R. A. and Setty, S. R. G. (2016). Rab9A is required for delivery of cargo from recycling endosomes to melanosomes. *Pigment Cell Melanoma Res.* **29**, 43-59.
Mallard, F., Tang, B. L., Galli, T., Tenza, D., Saint-Pol, A., Yue, X., Antony, C., Hong, W., Goud, B. and Johannes, L. (2002). Early/recycling endosomes-to-TGN transport involves two SNARE complexes and a Rab6 isoform. *J. Cell Biol.* **156**, 653-664.
Malsam, J. and Sollner, T. H. (2011). Organization of SNAREs within the Golgi stack. *Cold Spring Harb. Perspect. Biol.* **3**, a005249.
Marcusson, E. G., Horazdovsky, B. F., Cereghino, J. L., Gharakhanian, E. and Emr, S. D. (1994). The sorting receptor for yeast vacuolar carboxypeptidase Y is encoded by the VPS10 gene. *Cell* **77**, 579-586.
Mari, M., Bujny, M. V., Zeuschner, D., Geerts, W. J. C., Griffith, J., Petersen, C. M., Cullen, P. J., Klumperman, J. and Geuze, H. J. (2008). SNX1 defines an early endosomal recycling exit for sortilin and mannose 6-phosphate receptors. *Traffic* **9**, 380-393.
Marubashi, S., Shimada, H., Fukuda, M. and Ohbayashi, N. (2016). RUTBC1 functions as a GTPase-acitvating protein for Rab32/38 and regulates melanogenic enzyme trafficking in melanocytes. *J. Biol. Chem.* **291**, 1427-1440.
Matsudaira, T., Niki, T., Taguchi, T. and Arai, H. (2015). Transport of the cholera toxin B-subunit from recycling endosomes to the Golgi requires clathrin and AP-1. *J. Cell Sci.* **128**, 3131-3142.
McGough, I. J., Steinberg, F., Jia, D., Barbuti, P. A., McMillan, K. J., Heesom, K. J., Whone, A. L., Caldwell, M. A., Billadeau, D. D., Rosen, M. K. et al. (2014). Retromer binding to FAM21 and the WASH complex is perturbed by the Parkinson disease-linked VPS35(D620N) mutation. *Curr. Biol.* **24**, 1670-1676.
Medigeshi, G. R. and Schu, P. (2003). Characterization of the in vitro retrograde transport of MPR46. *Traffic* **4**, 802-811.
Miller, S. E., Collins, B. M., McCoy, A. J., Robinson, M. S. and Owen, D. J. (2007). A SNARE-adaptor interaction is a new mode of cargo recognition in clathrin-coated vesicles. *Nature* **450**, 570-574.
Mills, I. G., Praefcke, G. J. K., Vallis, Y., Peter, B. J., Olesen, L. E., Gallop, J. L., Butler, P. J. G., Evans, P. R. and McMahon, H. T. (2003). EpsinR: an AP1/clathrin interacting protein involved in vesicle trafficking. *J. Cell Biol.* **160**, 213-222.
Miserey-Lenkei, S., Chalancon, G., Bardin, S., Formstecher, E., Goud, B. and Echard, A. (2010). Rab and actomyosin-dependent fission of transport vesicles at the Golgi complex. *Nat. Cell Biol.* **12**, 645-654.
Monier, S., Jollivet, F., Janoueix-Lerosey, I., Johannes, L. and Goud, B. (2002). Characterization of novel Rab6-interacting proteins involved in endosome-to-TGN transport. *Traffic* **3**, 289-297.
Moreno-De-Luca, A., Helmers, S. L., Mao, H., Burns, T. G., Melton, A. M. A., Schmidt, K. R., Fernhoff, P. M., Ledbetter, D. H. and Martin, C. L. (2011). Adaptor protein complex-4 (AP-4) deficiency causes a novel autosomal recessive cerebral palsy syndrome with microcephaly and intellectual disability. *J. Med. Genet.* **48**, 141-144.
Muhammad, A., Flores, I., Zhang, H., Yu, R., Staniszewski, A., Planel, E., Herman, M., Ho, L., Kreber, R., Honig, L. S. et al. (2008). Retromer deficiency observed in Alzheimer's disease causes hippocampal dysfunction, neurodegeneration, and Abeta accumulation. *Proc. Natl. Acad. Sci. USA* **105**, 7327-7332.

Journal of Cell Science

Mukadam, A. S. and Seaman, M. N. J. (2015). Retromer-mediated endosomal protein sorting: the role of unstructured domains. *FEBS Lett.* **589**, 2620-2626.
Munck Petersen, C., Nielsen, M. S., Jacobsen, C., Tauris, J., Jacobsen, L., Gliemann, J., Moestrup, S. K. and Madsen, P. (1999). Propeptide cleavage conditions sortilin/neurotensin receptor-3 for ligand binding. *EMBO J.* **18**, 595-604.
Ng, E. L., Wang, Y. and Tang, B. L. (2007). Rab22B's role in trans-Golgi network membrane dynamics. *Biochem. Biophys. Res. Commun.* **361**, 751-757.
Ng, E. L., Ng, J. J., Liang, F. and Tang, B. L. (2009). Rab22B is expressed in the CNS astroglia lineage and plays a role in epidermal growth factor receptor trafficking in A431 cells. *J. Cell. Physiol.* **221**, 716-728.
Nielsen, M. S., Madsen, P., Christensen, E. I., Nykjaer, A., Gliemann, J., Kasper, D., Pohlmann, R. and Petersen, C. M. (2001). The sortilin cytoplasmic tail conveys Golgi-endosome transport and binds the VHS domain of the GGA2 sorting protein. *EMBO J.* **20**, 2180-2190.
Nielsen, M. S., Gustafsen, C., Madsen, P., Nyengaard, J. R., Hermey, G., Bakke, O., Mari, M., Schu, P., Pohlmann, R., Dennes, A. et al. (2007). Sorting by the cytoplasmic domain of the amyloid precursor protein binding receptor SorLA. *Mol. Cell. Biol.* **27**, 6842-6851.
Niu, Y., Zhang, C., Sun, Z., Hong, Z., Li, K., Sun, D., Yang, Y., Tian, C., Gong, W. and Liu, J.-J. (2013). PtdIns(4)P regulates retromer-motor interaction to facilitate dynein-cargo dissociation at the trans-Golgi network. *Nat. Cell Biol.* **15**, 417-429.
Nokes, R. L., Fields, I. C., Collins, R. N. and Fölsch, H. (2008). Rab13 regulates membrane trafficking between TGN and recycling endosomes in polarized epithelial cells. *J. Cell Biol.* **182**, 845-853.
Nottingham, R. M., Ganley, I. G., Barr, F. A., Lambright, D. G. and Pfeffer, S. R. (2011). RUTBC1 protein, a Rab9A effector that activates GTP hydrolysis by Rab32 and Rab33B proteins. *J. Biol. Chem.* **286**, 33213-33222.
Nottingham, R. M., Pusapati, G. V., Ganley, I. G., Barr, F. A., Lambright, D. G. and Pfeffer, S. R. (2012). RUTBC2 protein, a Rab9A effector and GTPase-activating protein for Rab36. *J. Biol. Chem.* **287**, 22740-22748.
Ogata, S. and 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**, 5210-5217.
Ogawa, K., Ueno, T., Iwasaki, T., Kujiraoka, T., Ishihara, M., Kunimoto, S., Takayama, T., Kanai, T., Hirayama, A. and Hattori, H. (2016). Soluble sortilin is released by activated platelets and its circulating levels are associated with cardiovascular risk factors. *Atherosclerosis* **249**, 110-115.
Paik, K. H., Song, S. M., Ki, C. S., Yu, H.-W., Kim, J. S., Min, K. H., Chang, S. H., Yoo, E. J., Lee, I. J., Kwan, E. K. et al. (2005). Identification of mutations in the GNPTA (MGC4170) gene coding for GlcNAc-phosphotransferase alpha/beta subunits in Korean patients with mucolipidosis type II or type IIIA. *Hum. Mutat.* **26**, 308-314.
Panic, B., Whyte, J. R. C. and 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**, 405-410.
Perez-Victoria, F. J. and Bonifacino, J. S. (2009). Dual roles of the mammalian GARP complex in tethering and SNARE complex assembly at the trans-golgi network. *Mol. Cell. Biol.* **29**, 5251-5263.
Perez-Victoria, F. J., Mardones, G. A. and Bonifacino, J. S. (2008). Requirement of the human GARP complex for mannose 6-phosphate-receptor-dependent sorting of cathepsin D to lysosomes. *Mol. Biol. Cell* **19**, 2350-2362.
Pfeffer, S. R. (2009). Multiple routes of protein transport from endosomes to the trans Golgi network. *FEBS Lett.* **583**, 3811-3816.
Plaut, R. D. and Carbonetti, N. H. (2008). Retrograde transport of pertussis toxin in the mammalian cell. *Cell. Microbiol.* **10**, 1130-1139.
Pols, M. S., van Meel, E., Oorschot, V., ten Brink, C., Fukuda, M., Swetha, M. G., Mayor, S. and Klumperman, J. (2013). hVps41 and VAMP7 function in direct TGN to late endosome transport of lysosomal membrane proteins. *Nat. Commun.* **4**, 1361.
Progida, C., Cogli, L., Piro, F., De Luca, A., Bakke, O. and Bucci, C. (2010). Rab7b controls trafficking from endosomes to the TGN. *J. Cell Sci.* **123**, 1480-1491.
Progida, C., Nielsen, M. S., Koster, G., Bucci, C. and Bakke, O. (2012). Dynamics of Rab7b-dependent transport of sorting receptors. *Traffic* **13**, 1273-1285.
Prouzet-Mauleon, V., Lefebvre, F., Thoraval, D., Crouzet, M. and Doignon, F. (2008). Phosphoinositides affect both the cellular distribution and activity of the F-BAR-containing RhoGAP Rgd1p in yeast. *J. Biol. Chem.* **283**, 33249-33257.
Puertollano, R., Randazzo, P. A., Presley, J. F., Hartnell, L. M. and Bonifacino, J. S. (2001). The GGAs promote ARF-dependent recruitment of clathrin to the TGN. *Cell* **105**, 93-102.
Ramdzan, Y. M., Polling, S., Chia, C. P. Z., Ng, I. H. W., Ormsby, A. R., Croft, N. P., Purcell, A. W., Bogoyevitch, M. A., Ng, D. C. H., Gleeson, P. A. et al. (2012). Tracking protein aggregation and mislocalization in cells with flow cytometry. *Nat. Methods* **9**, 467-470.
Reddy, J. V., Burguete, A. S., Sridevi, K., Ganley, I. G., Nottingham, R. M. and Pfeffer, S. R. (2006). A functional role for the GCC185 golgin in mannose 6-phosphate receptor recycling. *Mol. Biol. Cell* **17**, 4353-4363.
Ren, X., Farias, G. G., Canagarajah, B. J., Bonifacino, J. S. and Hurley, J. H. (2013). Structural basis for recruitment and activation of the AP-1 clathrin adaptor complex by Arf1. *Cell* **152**, 755-767.
Riederer, M. A., Soldati, T., Shapiro, A. D., Lin, J. and Pfeffer, S. R. (1994). Lysosome biogenesis requires Rab9 function and receptor recycling from endosomes to the trans-Golgi network. *J. Cell Biol.* **125**, 573-582.
Rink, J., Ghigo, E., Kalaidzidis, Y. and Zerial, M. (2005). Rab conversion as a mechanism of progression from early to late endosomes. *Cell* **122**, 735-749.
Robinson, M. S. and Hirst, J. (2013). Rapid inactivation of proteins by knocksideways. *Curr. Protoc. Cell Biol.* **61**, 15.20.1-7.
Rodriguez-Gabin, A. G., Cammer, M., Almazan, G., Charron, M. and Larocca, J. N. (2001). Role of rRAB22b, an oligodendrocyte protein, in regulation of transport of vesicles from trans Golgi to endocytic compartments. *J. Neurosci. Res.* **66**, 1149-1160.
Rodriguez-Gabin, A. G., Yin, X., Si, Q. and Larocca, J. N. (2009). Transport of mannose-6-phosphate receptors from the trans-Golgi network to endosomes requires Rab31. *Exp. Cell Res.* **315**, 2215-2230.
Rodriguez-Gabin, A. G., Ortiz, E., Demoliner, K., Si, Q., Almazan, G. and Larocca, J. N. (2010). Interaction of Rab31 and OCRL-1 in oligodendrocytes: its role in transport of mannose 6-phosphate receptors. *J. Neurosci. Res.* **88**, 589-604.
Rogaeva, E., Meng, Y., Lee, J. H., Gu, Y., Kawarai, T., Zou, F., Katayama, T., Baldwin, C. T., Cheng, R., Hasegawa, H. et al. (2007). The neuronal sortilin-related receptor SORL1 is genetically associated with Alzheimer disease. *Nat. Genet.* **39**, 168-177.
Rohn, W. M., Rouille, Y., Waguri, S. and Hoflack, B. (2000). Bi-directional trafficking between the trans-Golgi network and the endosomal/lysosomal system. *J. Cell Sci.* **113**, 2093-2101.
Rojas, R., van Vlijmen, T., Mardones, G. A., Prabhu, Y., Rojas, A. L., Mohammed, S., Heck, A. J. R., Raposo, G., van der Sluijs, P. and Bonifacino, J. S. (2008). Regulation of retromer recruitment to endosomes by sequential action of Rab5 and Rab7. *J. Cell Biol.* **183**, 513-526.
Rosa-Ferreira, C., Christis, C., Torres, I. L. and Munro, S. (2015). The small G protein Arl5 contributes to endosome-to-Golgi traffic by aiding the recruitment of the GARP complex to the Golgi. *Biol. Open* **4**, 474-481.
Rovelet-Lecrux, A., Charbonnier, C., Wallon, D., Nicolas, G., Seaman, M. N. J., Pottier, C., Breusegem, S. Y., Mathur, P. P., Jenardhanan, P., Le Guennec, K. et al. (2015). De novo deleterious genetic variations target a biological network centered on Abeta peptide in early-onset Alzheimer disease. *Mol. Psychiatry* **20**, 1046-1056.
Sanchez, E., Bergareche, A., Krebs, C. E., Gorostidi, A., Makarov, V., Ruiz-Martinez, J., Chorny, A., Lopez de Munain, A., Marti-Masso, J. F. and Paisan-Ruiz, C. (2015). SORT1 mutation resulting in sortilin deficiency and p75(NTR) upregulation in a family with essential tremor. *ASN Neuro.* **7**, pii: 1759091415598290.
Sandvig, K. and van Deurs, B. (2005). Delivery into cells: lessons learned from plant and bacterial toxins. *Gene Ther.* **12**, 865-872.
Schmidt, J. A. and Brown, W. J. (2009). Lysophosphatidic acid acyltransferase 3 regulates Golgi complex structure and function. *J. Cell Biol.* **186**, 211-218.
Schwake, M., Schroder, B. and Saftig, P. (2013). Lysosomal membrane proteins and their central role in physiology. *Traffic* **14**, 739-748.
Scott, G. K., Fei, H., Thomas, L., Medigeshi, G. R. and Thomas, G. (2006). A PACS-1, GGA3 and CK2 complex regulates CI-MPR trafficking. *EMBO J.* **25**, 4423-4435.
Seaman, M. N. J. (2004). Cargo-selective endosomal sorting for retrieval to the Golgi requires retromer. *J. Cell Biol.* **165**, 111-122.
Seaman, M. N. J. (2012). The retromer complex - endosomal protein recycling and beyond. *J. Cell Sci.* **125**, 4693-4702.
Seaman, M. N. J., McCaffery, J. M. and Emr, S. D. (1998). A membrane coat complex essential for endosome-to-Golgi retrograde transport in yeast. *J. Cell Biol.* **142**, 665-681.
Short, B., Preisinger, C., Schaletzky, J., Kopajtich, R. and Barr, F. A. (2002). The Rab6 GTPase regulates recruitment of the dynactin complex to Golgi membranes. *Curr. Biol.* **12**, 1792-1795.
Sinka, R., Gillingham, A. K., Kondylis, V. and Munro, S. (2008). Golgi coiled-coil proteins contain multiple binding sites for Rab family G proteins. *J. Cell Biol.* **183**, 607-615.
Skjeldal, F. M., Strunze, S., Bergeland, T., Walseng, E., Gregers, T. F. and Bakke, O. (2012). The fusion of early endosomes induces molecular-motor-driven tubule formation and fission. *J. Cell Sci.* **125**, 1910-1919.
Small, S. A., Kent, K., Pierce, A., Leung, C., Kang, M. S., Okada, H., Honig, L., Vonsattel, J.-P. and Kim, T.-W. (2005). Model-guided microarray implicates the retromer complex in Alzheimer's disease. *Ann. Neurol.* **58**, 909-919.
Suchy, S. F., Olivos-Glander, I. M. and Nussbaum, R. L. (1995). Lowe Syndrome, a deficiency of a phosphatidyl-inositol 4,5-bisphosphate 5-phosphatase in the Golgi apparatus. *Hum. Mol. Genet.* **4**, 2245-2250.
Tai, G., Lu, L., Wang, T. L., Tang, B. L., Goud, B., Johannes, L. and Hong, W. (2004). Participation of the syntaxin 5/Ykt6/GS28/GS15 SNARE complex in transport from the early/recycling endosome to the trans-Golgi network. *Mol. Biol. Cell* **15**, 4011-4022.

Tarpey, P. S., Stevens, C., Teague, J., Edkins, S., O'Meara, S., Avis, T., Barthorpe, S., Buck, G., Butler, A., Cole, J. 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**, 1119-1124.

Tiede, S., Storch, S., Lubke, T., Henrissat, B., Bargal, R., Raas-Rothschild, A. and Braulke, T. (2005). Mucolipidosis II is caused by mutations in GNPTA encoding the alpha/beta GlcNAc-1-phosphotransferase. *Nat. Med.* **11**, 1109-1112.

Ullrich, O., Reinsch, S., Urbe, S., Zerial, M. and Parton, R. G. (1996). Rab11 regulates recycling through the pericentriolar recycling endosome. *J. Cell Biol.* **135**, 913-924.

Utskarpen, A., Slagsvold, H. H., Iversen, T.-G., Walchli, S. and Sandvig, K. (2006). Transport of ricin from endosomes to the Golgi apparatus is regulated by Rab6A and Rab6A′. *Traffic* **7**, 663-672.

van Weering, J. R. T., Verkade, P. and Cullen, P. J. (2012). SNX-BAR-mediated endosome tubulation is co-ordinated with endosome maturation. *Traffic* **13**, 94-107.

Varki, A. and Kornfeld, S. (2009). P-type Lectins. In *Essentials of Glycobiology* (ed. A. Varki, R. D. Cummings, J. D. Esko, H. H. Freeze, P. Stanley, C. R. Bertozzi, G. W. Hart and M. E. Etzler), pp. 425-438. Cold Spring Harbor Press, NY.

Velayati, A., DePaolo, J., Gupta, N., Choi, J. H., Moaven, N., Westbroek, W., Goker-Alpan, O., Goldin, E., Stubblefield, B. K., Kolodny, E. et al. (2011). A mutation in SCARB2 is a modifier in Gaucher disease. *Hum. Mutat.* **32**, 1232-1238.

Vilarino-Guell, C., Wider, C., Ross, O. A., Dachsel, J. C., Kachergus, J. M., Lincoln, S. J., Soto-Ortolaza, A. I., Cobb, S. A., Wilhoite, G. J., Bacon, J. A. et al. (2011). VPS35 mutations in Parkinson disease. *Am. J. Hum. Genet.* **89**, 162-167.

Wandinger-Ness, A. and Zerial, M. (2014). Rab proteins and the compartmentalization of the endosomal system. *Cold Spring Harb. Perspect. Biol.* **6**, a022616.

Wang, Y. J., Wang, J., Sun, H. Q., Martinez, M., Sun, Y. X., Macia, E., Kirchhausen, T., Albanesi, J. P., Roth, M. G. and Yin, H. L. (2003). Phosphatidylinositol 4 phosphate regulates targeting of clathrin adaptor AP-1 complexes to the Golgi. *Cell* **114**, 299-310.

Wang, Y., Tai, G., Lu, L., Johannes, L., Hong, W. and Tang, B. L. (2005). Trans-Golgi network syntaxin 10 functions distinctly from syntaxins 6 and 16. *Mol. Membr. Biol.* **22**, 313-325.

Wang, J., Sun, H.-Q., Macia, E., Kirchhausen, T., Watson, H., Bonifacino, J. S. and Yin, H. L. (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**, 2646-2655.

Wang, S., Ma, Z., Xu, X., Wang, Z., Sun, L., Zhou, Y., Lin, X., Hong, W. and Wang, T. (2014). A role of Rab29 in the integrity of the trans-Golgi network and retrograde trafficking of mannose-6-phosphate receptor. *PLoS ONE* **9**, e96242.

Wang, Y., MacDonald, R. G., Thinakaran, G. and Kar, S. (2016). Insulin-like growth factor-II/cation-independent mannose 6-phosphate receptor in neurodegenerative diseases. *Mol. Neurobiol.* [Epub].

Wassmer, T., Attar, N., Harterink, M., van Weering, J. R. T., Traer, C. J., Oakley, J., Goud, B., Stephens, D. J., Verkade, P., Korswagen, H. C. et al. (2009). The retromer coat complex coordinates endosomal sorting and dynein-mediated transport, with carrier recognition by the trans-Golgi network. *Dev. Cell* **17**, 110-122.

Wernick, N. L. B., Chinnapen, D. J.-F., Cho, J. A. and Lencer, W. I. (2010). Cholera toxin: an intracellular journey into the cytosol by way of the endoplasmic reticulum. *Toxins* **2**, 310-325.

Wilcke, M., Johannes, L., Galli, T., Mayau, V., Goud, B. and Salamero, J. (2000). Rab11 regulates the compartmentalization of early endosomes required for efficient transport from early endosomes to the trans-golgi network. *J. Cell Biol.* **151**, 1207-1220.

Willnow, T. E. and Andersen, O. M. (2013). Sorting receptor SORLA–a trafficking path to avoid Alzheimer disease. *J. Cell Sci.* **126**, 2751-2760.

Willnow, T. E., Petersen, C. M. and Nykjaer, A. (2008). VPS10P-domain receptors - regulators of neuronal viability and function. *Nat. Rev. Neurosci.* **9**, 899-910.

Wong, M. and Munro, S. (2014). Membrane trafficking. The specificity of vesicle traffic to the Golgi is encoded in the golgin coiled-coil proteins. *Science* **346**, 1256898.

Yang, M., Chen, T., Han, C., Li, N., Wan, T. and Cao, X. (2004). Rab7b, a novel lysosome-associated small GTPase, is involved in monocytic differentiation of human acute promyelocytic leukemia cells. *Biochem. Biophys. Res. Commun.* **318**, 792-799.

Yoshino, A., Setty, S. R. G., Poynton, C., Whiteman, E. L., Saint-Pol, A., Burd, C. G., Johannes, L., Holzbaur, E. L., Koval, M., McCaffery, J. M. et al. (2005). tGolgin-1 (p230, golgin-245) modulates Shiga-toxin transport to the Golgi and Golgi motility towards the microtubule-organizing centre. *J. Cell Sci.* **118**, 2279-2293.

Young, J., Menetrey, J. and Goud, B. (2010). RAB6C is a retrogene that encodes a centrosomal protein involved in cell cycle progression. *J. Mol. Biol.* **397**, 69-88.

Zachos, C., Blanz, J., Saftig, P. and Schwake, M. (2012). A critical histidine residue within LIMP-2 mediates pH sensitive binding to its ligand beta-glucocerebrosidase. *Traffic* **13**, 1113-1123.

Zavodszky, E., Seaman, M. N. J., Moreau, K., Jimenez-Sanchez, M., Breusegem, S. Y., Harbour, M. E. and Rubinsztein, D. C. (2014). Mutation in VPS35 associated with Parkinson's disease impairs WASH complex association and inhibits autophagy. *Nat. Commun.* **5**, 3828.

Zhen, Y. and Stenmark, H. (2015). Cellular functions of Rab GTPases at a glance. *J. Cell Sci.* **128**, 3171-3176.

Zimprich, A., Benet-Pages, A., Struhal, W., Graf, E., Eck, S. H., Offman, M. N., Haubenberger, D., Spielberger, S., Schulte, E. C., Lichtner, P. et al. (2011). A mutation in VPS35, encoding a subunit of the retromer complex, causes late-onset Parkinson disease. *Am. J. Hum. Genet.* **89**, 168-175.