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COMMENTARY

Multiple activities of Arl1 GTPase in the trans-Golgi network

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ABSTRACT

ADP-ribosylation factors (Arfs) and ADP-ribosylation factor-like proteins (Arls) are highly conserved small GTPases that function as main regulators of vesicular trafficking and cytoskeletal reorganization. Arl1, the first identified member of the large Arl family, is an important regulator of Golgi complex structure and function in organisms ranging from yeast to mammals. Together with its effectors, Arl1 has been shown to be involved in several cellular processes, including endosomal trans-Golgi network and secretory trafficking, lipid droplet and salivary granule formation, innate immunity and neuronal development, stress tolerance, as well as the response of the unfolded protein. In this Commentary, we provide a comprehensive summary of the Arl1-dependent cellular functions and a detailed characterization of several Arl1 effectors. We propose that involvement of Arl1 in these diverse cellular functions reflects the fact that Arl1 is activated at several late-Golgi sites, corresponding to specific molecular complexes that respond to and integrate multiple signals. We also provide insight into how the GTP-GDP cycle of Arl1 is regulated, and highlight a newly discovered mechanism that controls the sophisticated regulation of Arl1 activity at the Golgi complex.

KEY WORDS: GTPase, Arf, Golgi complex, Golgin, Endosome, Membrane trafficking, GEF, GAP

Introduction

The ADP-ribosylation factors (Arfs) are members of a small GTPase family within the Ras superfamily that regulate membrane transport, organelle integrity, membrane lipid modifications, cytoskeletal dynamics and signal transduction (Boman and Kahn, 1995; Gillingham and Munro, 2007; Jackson and Bouvet, 2014). More than 20 Arf-like proteins (Arls), which share 40–60% sequence identity with Arfs (Stearns et al., 1990; Van Valkenburgh et al., 2001), have been identified in humans (Gillingham and Munro, 2007; Kahn et al., 2006). Similar to other Ras-related GTP-binding proteins, both Arf and Arl proteins are cycled between active GTP-bound and inactive GDP-bound conformations. The hydrolysis of bound GTP is mediated by GTPase-activating proteins (GAPs), whereas the exchange of GDP for GTP is mediated by guanine nucleotide-exchange factors (GEFs) (Casanova, 2007; East and Kahn, 2011). Arl1 was originally discovered in *Drosophila* in 1991 (Tamkun et al., 1991) and is ubiquitously expressed in most eukaryotic cells, including mammals, budding yeast, plants and protozoa (Lee et al., 1997, 2011; Lowe et al., 1996; Lu et al., 2001; Munro, 2005; Price et al., 2005; Setty et al., 2003). Arl1 has all the typical features of an Arf-family GTPase, including an amphipathic N-terminal helix and a consensus site for N-myristoylation (Lu et al., 2001; Price et al., 2005). In yeast, recruitment of Arl1 to the Golgi complex requires a second Arf-like GTPase, Arl3 (Behnia et al., 2004; Setty et al., 2003). Yeast Arl3 lacks a myristoylation site and is, instead, N-terminally acetylated; this modification is required for its recruitment to the Golgi complex by Sys1. In mammalian cells, ADP-ribosylation-factor-related protein 1 (Arfrp1), a mammalian ortholog of yeast Arl3, plays a pivotal role in the recruitment of Arl1 to the trans-Golgi network (TGN) (Behnia et al., 2004; Panic et al., 2003b; Setty et al., 2003; Zahn et al., 2006). GTP-bound Arl1 recruits several effectors, such as golgins, arfaptins and Arf-GEFs to the TGN, and modulates their functions at the Golgi complex (Burd et al., 2004; Christis and Munro, 2012; Derby et al., 2004; Huang et al., 2015; Lu and Hong, 2003; Man et al., 2011; Panic et al., 2003b; Torres et al., 2014; Wong et al., 2017; Wong and Munro, 2014). Arl1 is also involved in the intracellular transport of vesicular stomatitis virus G protein (VSVG) and bacterial toxin (Lu et al., 2001, 2004). However, knockdown of Arfrp1 but not Arl1 causes inhibition of VSVG exit from the TGN, suggesting that Arfrp1 and Arl1 have different roles in the regulation of anterograde transport (Nishimoto-Morita et al., 2009). Studies in yeast have also shown that Arl1-GTP is localized to the Golgi complex and facilitates the exit of vesicles from the TGN (Behnia et al., 2004; Bonangelino et al., 2002; Jochum et al., 2002; Liu et al., 2006; Munro, 2005; Panic et al., 2003b; Rosenwald et al., 2002; Setty et al., 2003). In this Commentary, we provide an overview of the multiple cellular and physiological functions of Arl1 at the TGN, focusing on how Arl1 participates in selective cargo transport, and works together with its effectors and associated regulators to modulate membrane remodeling and Arl1 activity at the TGN.

The physiological roles of Arl1

Cellular functions of Arl1 have been investigated in various cell types and organisms. Genetic alterations of Arl1 as well as its effectors were used to characterize developmental and physiological phenotypes (summarized in Fig. 1) (Chang et al., 2015; Cruz-Garcia et al., 2013; Eiseler et al., 2016; Gehart et al., 2012; Hesse et al., 2013; Hsu et al., 2016; Labbaoui et al., 2017; Lieu et al., 2008; Liu et al., 2006; Lock et al., 2005; Marešová and Sychrová, 2010; Marešová et al., 2012; Murray and Stow, 2014; Price et al., 2005; Torres et al., 2014; Yang and Rosenwald, 2016). In mammalian cells, these functions affect a wide range of fundamental cellular processes, including cell polarity (Lock et al., 2005), innate immunity (Bremond et al., 2009; Lieu et al., 2008; Murray and Stow, 2014; Stanley et al., 2012), lipid droplet and chylomicron formation (Hesse et al., 2013; Jaschke et al., 2012), as well as the secretion of insulin (Gehart et al., 2012), chromogranin A (Cruz-Garcia et al., 2013) and matrix metalloproteinases (MMPs) (Eiseler et al., 2016). In yeast, Arl1 and its effectors are also involved in numerous functions, such as maintenance of cell wall integrity (Liu et al., 2006), ion homeostasis (Marešová and Sychrová, 2010; Munson et al., 2004; Rosenwald et al., 2002), tolerance to stress

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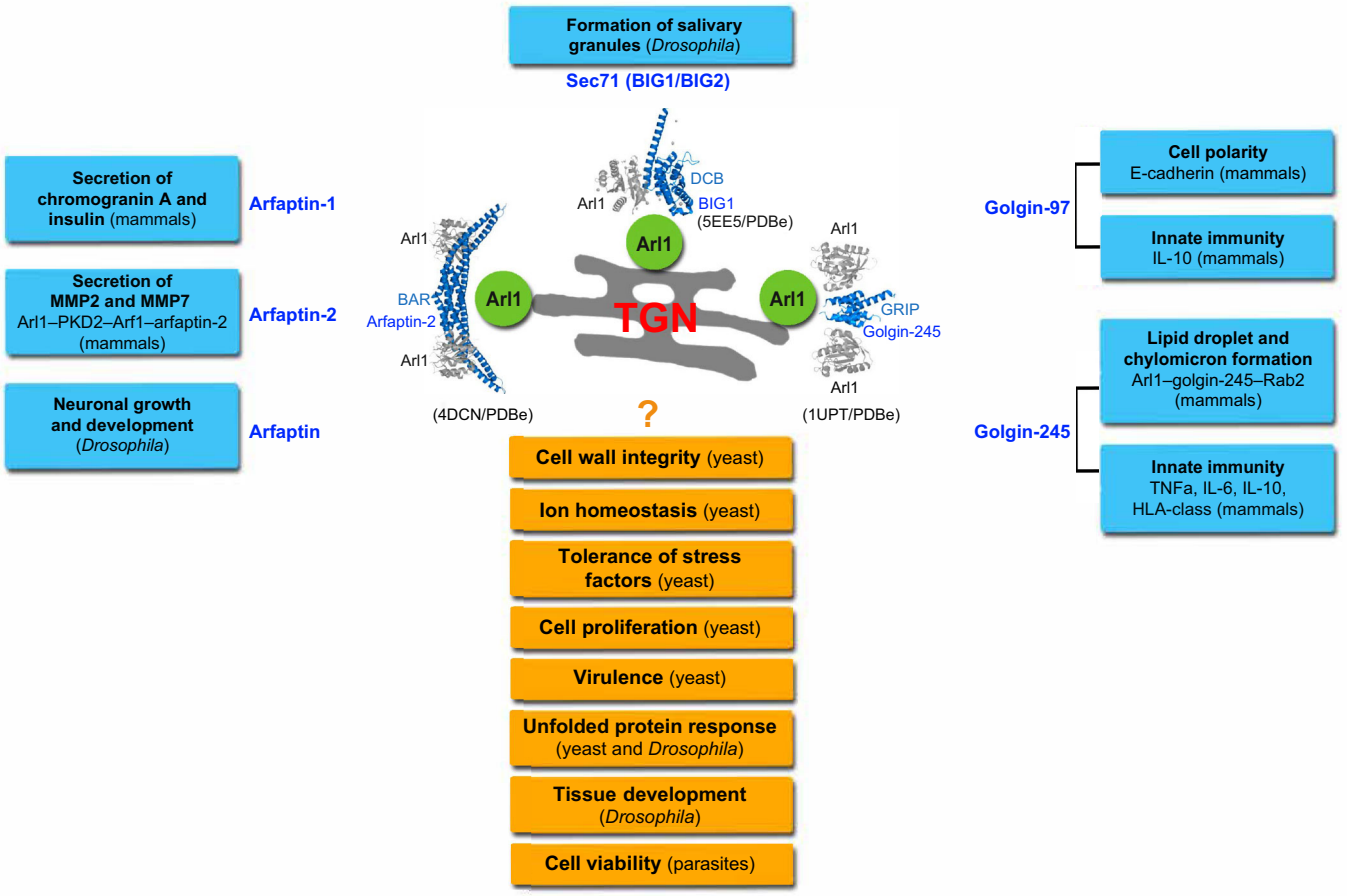

Fig. 1. Cellular functions of Arl1 at the TGN. Arl1 is involved in many cellular functions through specific or yet-unidentified effectors in specialized cell types and organisms. The physiological roles of Arl1 rely on the formation of functional complexes between Arl1 and its effectors, including GRIP-domain golgins, arfaptins, and Arf-GEFs, with the indicated functions in various cellular processes. Three domains, GRIP, BAR and DCB (structures of complexes shown here are from the Protein Data Bank Europe, PDBe), represent the Arl1-binding domains on its distinct effectors (shown here are golgin-245, arfaptin and BIG1), and the respective functional complexes are indicated. Arl1 also exerts other functions through, thus far, unidentified effectors (illustrated by the question mark) as indicated.

factors (Jaime et al., 2012; Marešova et al., 2012; Yang and Rosenwald, 2016), regulation of cell proliferation (Benjamin et al., 2011), the unfolded protein response (UPR) (Hsu et al., 2016) and also in the ability of yeast to cause disease (Labbaoui et al., 2017). In addition, these factors regulate the quality control of the endoplasmic reticulum (ER) (Lee et al., 2011), tissue development (Eisman et al., 2006; Torres et al., 2014) and neuronal growth in *Drosophila* (Chang et al., 2015), and control cell viability in parasites (Price et al., 2005). Altogether, the data from these functional studies indicate that Arl1 controls the trafficking of numerous cargos at the TGN in a variety of organisms.

Mammals

The role of mammalian Arl1 in vesicular trafficking at the TGN was revealed in several studies that investigated the interaction of Arl1 with GRIP domain proteins (after golgin-97, RanBP2α, Imh1, p230/golgin-245 – the four proteins in which it is found), which regulate the transport of several types of cargo, such as E-cadherin, tumor necrosis factor alpha (TNFα), interleukins 6 and 10 (IL-6 and IL-10), and human leukocyte antigen (HLA)-class I (Bremond et al., 2009; Lieu et al., 2008; Lock et al., 2005; Murray and Stow, 2014; Stanley et al., 2012). E-cadherin has an important role in cell adhesion and forms adherens junctions between cells that are crucial for tissue integrity (Gumbiner, 1996). Secretion of cytokines from innate immune cells and surface expression of HLA-class I are fundamental responses to injury and infection in the body (Arango Duque and Descoteaux, 2014; Moretta et al., 2005). Therefore, Arl1 – together with its GRIP-domain partner proteins – is able to modulate both cell polarity and innate immunity (see below for further details).

Arfrp1 regulates the activation of Arl1 in mammalian cells (Zahn et al., 2006), and its knockdown has revealed a potential role for Arl1 in the assembly and lipidation of chylomicrons (Hesse et al., 2013; Jaschke et al., 2012). For example, ApoA-I was found to accumulate at Golgi membranes of intestinal *Arfrp1*$^{vil-/-}$ cells. The authors of this work further showed that knockdown of Arl1, golgin-245 or Rab2 reduced triglyceride release from intestinal Caco-2 cells, suggesting that Arl1 and its downstream effector golgin-245 are required for the formation of lipid droplets and chylomicrons in intestinal epithelial cells (Jaschke et al., 2012).

Human Arl1 also has a role in protein secretion mediated by arfaptins, which were shown to play a role in maintaining insulin secretion from pancreatic β cells (Gehart et al., 2012) and are required for trafficking of the acidic secretory glycoprotein chromogranin A (Cruz-Garcia et al., 2013) from the TGN in neuroendocrine cells. Interestingly, a multi-protein complex comprising Arl1, PKD2, Arf1 and arfaptin-2 was found to regulate the constitutive secretion of endogenous MMP7 and MMP2 cargos in pancreatic cells (Eiseler et al., 2016). Thus, these findings reveal the physiological significance of Arl1 in the secretion of signaling proteins and enzymes.

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Yeast

Although deletion of Arl1 is not lethal in yeast (Lee et al., 1997), an Arl1-deletion mutant shows mild defects in mislocalizing carboxypeptidase Y to vacuoles and defects in K^+ uptake (Bonangelino et al., 2002; Marešová and Sychrová, 2010; Munson et al., 2004; Rosenwald et al., 2002). Furthermore, the Arl1-deletion mutant shows defects in cell wall integrity (Liu et al., 2006) and is more sensitive to salts (KCl, NaCl and LiCl), high temperatures, increased pH, hygromycin B and chitosan oligosaccharide than cells that possess the wild-type Arl1 (Jaime et al., 2012; Marešová and Sychrová, 2010; Marešová et al., 2012), supporting a role for Arl1 in tolerance to extracellular stress and/or stimulation. Arl1 also functions in cell proliferation, as demonstrated by the observation that dysregulation of active yeast Arl1 in response to a lack of its GAP Gcs1 prevents cell growth and impairs endosomal transport at low temperatures (Benjamin et al., 2011). Arl1 also shows synthetic lethality and physical interactions with Vps53, one of the subunits of the Golgi-associated retrograde protein (GARP) complex (Panic et al., 2003b; Tong et al., 2004), indicating that at least some functions of the GARP complex in endosomal TGN trafficking overlap with those of Arl1.

In addition to acting as a transport regulator between endosomes and the Golgi, yeast Arl1 – together with the Rab GTPase Ypt6 – is required for starvation-induced autophagy under high-temperature stress (Yang and Rosenwald, 2016). Interestingly, a novel role of Arl1 in regulation of fungal morphogenesis and virulence has recently been revealed in a *Candida-albicans*-infected oropharyngeal candidiasis mouse model (Labbaoui et al., 2017). These studies collectively suggest that yeast Arl1 has important physiological roles in the regulation of anterograde transport from the TGN to the cell surface, which in turn impacts upon the first line of cellular defense, and modulates endosomal trafficking required for cell growth and virulence.

Drosophila and parasites

As mentioned above, Arl1 also plays a role in *Drosophila* (Chang et al., 2015; Eisman et al., 2006; Lee et al., 2011; Torres et al., 2014) and in parasites (Price et al., 2005). In *Drosophila*, arf72A, the fly ortholog of mammalian Arl1, was shown to be required for *Drosophila* development and the centrosome cycle (Eisman et al., 2006). Furthermore, Lee et al. reported a role for Arl1 in the quality control machinery of the ER that distinguishes the cargos destined for secretion from those intended for degradation (Lee et al., 2011). In this context, loss of Arl1 activity changes the membrane characteristics of the ER, and shifts the membrane balance between ER and Golgi complex towards the Golgi complex, resulting in increased proliferation of Golgi complexes and accelerated protein secretion. In contrast to mammals, Arl1 is not required for E-cadherin trafficking or for cell polarity in *Drosophila* (Torres et al., 2014). Interestingly, loss of Arl1 induces cell death in imaginal discs and reduces the size of secretory granules in the larval salivary gland, supporting the notion that Arl1 is involved in normal wing development and salivary granule formation (Torres et al., 2014). Recently, Arl1, Arfaptin and the Arf-GEF Gartenzwerg (the *Drosophila* ortholog of the Arf-GEF GBF1) were found to colocalize at the Golgi in *Drosophila* motor neurons and to function in a newly described signaling pathway during synapse growth (Chang et al., 2015).

The role of Arl1 has also been investigated in some parasites. In the *Trypanosoma brucei* genome TbArl1 is present as a single-copy gene, and a single TbArl1 transcript has been detected in the mammalian bloodstream form but not in the insect procyclic form of the parasite (Price et al., 2005). TbArl1 is essential for the viability of the bloodstream form of *T. brucei*, as its depletion results in abnormal morphology, including disintegration of the Golgi structure and defects in exocytic flux (Price et al., 2005).

Taken together, there is clear evidence that Arl1 controls the trafficking of a number of different cargos in the TGN; however, how Arl1 regulates such diverse cellular functions is a key question for future studies.

Arl1 promotes selective vesicle trafficking at the TGN through distinct effectors

As discussed above, Arl1 regulates specific retrograde and anterograde transport routes through the recruitment of different effectors, which in turn regulate membrane transport, membrane dynamics and membrane architecture. Below, we summarize our current understanding of how Arl1 promotes selective vesicle trafficking.

The Arl1–GRIP-domain golgin complex

Golgins are a family of coiled-coil proteins associated with the Golgi complex; they act as tethering factors during membrane fusion and are required for maintaining the Golgi architecture (Barr and Short, 2003; Cheung and Pfeffer, 2016; Gillingham and Munro, 2016; Goud and Gleeson, 2010; Ramirez and Lowe, 2009; Witkos and Lowe, 2015). There are four GRIP-domain-containing golgins in mammalian cells, golgin-97 (also known as GOLGA1), golgin-245 (also known as GOLG4A), GCC185 (also known as GCC2), and GCC88 (also known as GCC1), whereas Imh1 is the only GRIP-domain golgin in yeast (Brown et al., 2001; Jackson, 2003; Munro and Nichols, 1999). The four mammalian GRIP-domain proteins differ in their membrane-binding properties and are recruited to distinct domains of the TGN (Derby et al., 2004). Structural studies of the Arl1-GTP complex together with the GRIP-domain of golgin-245 have revealed that the isolated GRIP-domain forms a homodimer that interacts with two Arl1-GTP molecules (Fig. 1) (Panic et al., 2003a; Wu et al., 2004). By contrast, GCC88 and GCC185 bind Arl1 poorly (Derby et al., 2004; Lu et al., 2004; Reddy et al., 2006; Torres et al., 2014), and the molecular basis of their membrane recruitment remains controversial (Burguete et al., 2008; Houghton et al., 2009; Torres et al., 2014). The Arl1-interacting TGN golgins have been proposed to act as scaffold molecules to establish a molecular platform for a number of distinct processes that might influence the specificity and efficiency of vesicle transport (Cheung and Pfeffer, 2016; Chia and Gleeson, 2011; Gillingham and Munro, 2016; Goud and Gleeson, 2010; Witkos and Lowe, 2015; Wong et al., 2017; Wong and Munro, 2014). For example, depletion of Arl1 or Golgin-97 has been reported to impair the transport of the Shiga toxin B-subunit from early endosomes to the TGN, and GCC185 has been shown to be involved in regulating the transport of mannose 6-phosphate receptors from recycling endosomes (Lu et al., 2004; Reddy et al., 2006). Furthermore, knockdown of golgin-97 blocked the exit of E-cadherin cargo from the TGN (Lock et al., 2005). Overexpression of the GRIP domain of golgin-245 induced the downregulation of HLA class I at the cell surface (Bremond et al., 2009). Further, golgin-245 but not golgin-97 has been identified as an essential regulator of TNFα and IL-6 trafficking from the TGN to the cell surface and of their secretion *in vitro* and *in vivo* (Lieu et al., 2008; Lock et al., 2005; Murray and Stow, 2014). A current area of investigation is the mechanisms by which golgins and factors regulate specific cargo selection and transport. According to the model proposed by Goud and Gleeson, the particular scaffold

involved (the coiled-coil regions and the set of interactive partners) defines the functions of different golgins (Goud and Gleeson, 2010). For example, GRIP-domain golgins contain multiple Rab-binding sites along their long N-terminal coiled-coil domain (Gillingham et al., 2014; Goud and Gleeson, 2010; Hayes et al., 2009; Sinka et al., 2008). Therefore, they might, together with Arl1, establish membrane 'hot spots' for the specific docking and fusion of Rab-decorated transport carriers, as well as being closely associated with the biogenesis of tubular transport carriers. This working hypothesis has been demonstrated by Wong and Munro who used a mitochondrial relocation assay to demonstrate that the specificity of vesicle traffic to the Golgi is encoded in the golgin coiled-coil proteins (Wong and Munro, 2014). In a follow-up study, the authors have recently shown that the capture of carriers is mediated by a short region consisting of 20−50 amino acid residues at the N-terminus of golgins, through which golgin-97 and golgin-245 capture a class of endosome-to-Golgi carriers that might be distinct from the carriers that are tethered by GCC88 (Wong et al., 2017).

In yeast, Arl1 is also involved in regulating vesicle transport at the Golgi complex, including recruitment of the GRIP-domain golgin Imh1 to the late Golgi (Behnia et al., 2004; Panic et al., 2003b; Pasqualato et al., 2002; Setty et al., 2003), transport of the GPI-anchored protein Gas1 to the plasma membrane (Liu et al., 2006), and regulation of the association between the clathrin adaptor protein Gga2 and the late Golgi (Singer-Kruger et al., 2008). At the molecular level, deletion of Arl1 or of its regulators Sys1 or Arl3 results in delocalization of the golgin Imh1 from the late Golgi (Behnia et al., 2004; Panic et al., 2003b; Setty et al., 2003). Accordingly, the Sys1-Arl3-Arl1-Imh1 signaling cascade participates in enhancing Ytp6-dependent vacuole fragmentation (Liu et al., 2006). Thus, Arl1 regulates selective retrograde and anterograde transport through the recruitment of golgins comprising different GRIP-domain.

The Arl1−arfaptin complex

Human Arl1 also interacts with arfaptin-1 and arfaptin-2 (Man et al., 2011), which are Bin−amphiphysin−Rvs (BAR)-domain-containing proteins associated with the sensing and/or induction of membrane curvature (Peter et al., 2004). Arfaptins bind to Arl1 through their BAR domains and are recruited to Golgi membranes where they induce membrane tubules (Man et al., 2011). Additional crystal structure analyses have shown that two molecules of Arl1 bind symmetrically to each side of the crescent-shaped homodimer of the BAR domain in arfaptin-2, leaving the concave face open for membrane association (Fig. 1) (Nakamura et al., 2012). Arfaptin-1b − the long isoform of arfaptin-1 − is phosphorylated by protein kinase D1 (PKD1); this results in the removal of arfaptin-1b from the Golgi, a pivotal step for maintaining insulin secretion from pancreatic β cells (Gehart et al., 2012). Non-phosphorylated arfaptin-1b inhibits the interaction between Arl1 and the Arf-GEF BIG2 (also known as ARFGEF2), thus blocking Arf1 activation. Glucose-stimulated insulin secretion might, thus, be regulated by modulating arfaptin-1b phosphorylation and by controlling Arf-mediated insulin granule fission at the Golgi (Gehart et al., 2012). In contrast, arfaptin-1a − the short isoform of arfaptin-1 − is phosphorylated by PKD2 and required for the regulatory secretion of chromogranin A in neuroendocrine cells, a process in which arfaptin-2 does not have a major role (Cruz-Garcia et al., 2013). Interestingly, PKD2, but not PKD1, assembles in a multi-protein complex consisting of Arl1, PKD2, Arf1 and arfaptin-2 at the TGN. Here, inactive PKD2 is required for the recruitment of an Arl1−arfaptin-2 complex to the TGN, and the establishment of Arf1-containing complexes that regulate the constitutive secretion of MMP7 and MMP2 in Panc-1 pancreatic cancer cells (Eiseler et al., 2016). Collectively, these studies suggest that Arl1 together with its specific effectors acts to modulate regulatory and constitutive protein secretion at the TGN.

Arl1−Arf-GEF complex

It has been reported that Arl1 facilitates the recruitment of the two Arf-GEFs BIG1 (also known as ARFGEF1) and BIG2 to the TGN in mammalian cells (Christis and Munro, 2012). Yeast Arl1 was also found to bind to Sec7 (the yeast ortholog of BIG1) and was proposed to regulate the Golgi recruitment and activation of Sec7 (McDonold and Fromme, 2014; Richardson et al., 2012). Arl1 also directly interacts with the Arf-GEF Sec71 (the *Drosophila* ortholog of BIG1 and BIG2) in *Drosophila* S2 cells (Christis and Munro, 2012). More recently, two groups characterized the structural basis of Arl1 binding to the dimerization and cyclophilin binding (DCB) domain located in the N-terminal region of BIG1 (Fig. 1), concluding that this interaction is required for targeting BIG1 to the Golgi (Galindo et al., 2016; Wang et al., 2016). Notably, the residues of Arl1 that bind to the DCB domain exhibit a conformation that is distinct from those present in the other known Arl1-effector complexes, suggesting that this plasticity enables Arl1 to interact with different effectors that have unrelated structures (Galindo et al., 2016).

In addition, Torres et al. demonstrated that both Arl1 and Sec71 are required for AP-1 recruitment and secretory granule maturation in larval salivary glands. Accordingly, a model was proposed, whereby the interaction between Arl1 and Sec71 enhances Arf1 activation on the TGN to promote the recruitment of AP-1, which is required for normal salivary granule formation (Torres et al., 2014).

These structural studies of the Arl1-GTP complex with its effectors in combination with functional studies of different types of signaling stimulation and/or regulation further support the notion that different Arl1-containing complexes form in distinct domains of the Golgi and are responsible for different functions.

Regulation of Arl1 activity at the TGN

Although the activation and Golgi localization of Arl1 are regulated by Arl3 in yeast and by Arfrp1 in mammalian cells (Behnia et al., 2004; Panic et al., 2003b; Zahn et al., 2006), how Arl1 is regulated by GEFs and GAPs remains unknown. To date, there have not been any GEFs or GAPs identified for human Arl1. In yeast, the Arf-GEF Syt1 and the Arf GAP Gcs1 are the best-characterized regulators of Arl1 activation in the TGN (Chen et al., 2010; Liu et al., 2005).

Arf-GEFs and their regulators

All Arf-GEFs contain a region of approximately 200 amino acids, known as the Sec7 domain, which is responsible for their GEF activity (Jackson and Casanova, 2000; Shin and Nakayama, 2004). Regions outside of the Sec7 domain that interact with partner proteins, also have important biological roles and are involved in membrane localization, substrate specificity and upstream regulation (Cox et al., 2004). In yeast, there are five Sec7-domain GEFs (Sec7, Gea1, Gea2, Syt1 and Yel1) and Mon2, a related GEF without a Sec7 domain, (Efe et al., 2005; Gillingham et al., 2006; Jochum et al., 2002). Sec7, Gea1 and Gea2 all have roles in secretion (Peyroche et al., 1996; Wolf et al., 1998). For instance, Sec7 localizes to the *trans*-Golgi complex and is involved in COPI-mediated intra-Golgi transport (Deitz et al., 2000). Almost every Arf has been assigned one or more GEFs based on either predictions

or experimental data from yeast and mammals (Cox et al., 2004). Importantly, we have shown that the Arf-GEF Syt1 acts as an Arl1-GEF in yeast where it regulates Golgi localization and activation of Arl1 (Chen et al., 2010) (Fig. 2). Syt1-dependent Arl1 activation results in the recruitment of Imh1 to the Golgi but does not affect other Arl1-mediated roles, such as cell wall integrity, vacuolar biogenesis or Gas1 transport. This suggests that Arl1 can be activated by more than one GEF to exert distinct roles in regulating the structure and function of the late Golgi. (Chen et al., 2010). Syt1 contains a Sec7 domain that is flanked by an N-terminal region and a PH domain. This study also showed that the PH domain of Syt1 is required for its Golgi localization and that the N-terminal region is necessary for Syt1 function (Chen et al., 2010). Previous studies have suggested that Mon2 is a GEF for yeast Arl1 based on its sequence homology to the Sec7 family of Arf-GEFs and on the genetic and protein interactions between Arl1 and Mon2 (Jochum et al., 2002). However, several lines of evidence have demonstrated that Mon2 is not a GEF for either yeast or human Arl1. First, multiple bioinformatics analyses revealed that Mon2 does not contain a Sec7 domain (Efe et al., 2005; Gillingham et al., 2006). Second, neither the Golgi localization of Arl1 nor the amount of active Arl1 changes significantly in Mon2-depleted yeast or human cells (Behnia et al., 2004; Gillingham et al., 2006; Mahajan et al., 2013). Third, mammalian Mon2 does not possess any GEF activity towards Arl1 *in vitro* (Mahajan et al., 2013). Last, yeast Mon2 was shown to be a negative regulator of the GTP-restricted allele of Arl1 (Manlandro et al., 2012). Thus, yeast Syt1 remains the only confirmed GEF to activate Arl1 at the Golgi complex.

Although Arl3 can regulate Syt1-dependent Arl1 activation, our previous work indicated that Arl3 does not interact directly with Syt1 or recruit Syt1 to the Golgi (Chen et al., 2010). Therefore, the regulation of the GEF activity of Syt1 remains unclear. Recently, we have shown that Ire1–Hac1 signaling regulates Arl1 activity by controlling Syt1 phosphorylation (Fig. 2) (Hsu et al., 2016). Ire1 is a kinase/endoribonuclease that localizes to the ER membrane and is activated by ER stress (Kimata and Kohno, 2011; Parmar and Schröder, 2012). The accumulation of unfolded proteins in the ER triggers the UPR, which selectively activates the expression of ER-resident chaperones (Kaufman et al., 2002). As shown in our recent study, ER stress induces the phosphorylation of Syt1 at S416 through the Ire1-Hac1 signaling pathway, which then promotes interaction of Syt1 with Arl1 in the late Golgi, resulting in Arl1 activation and Golgi targeting of Imh1. Importantly, both phosphorylation of Syt1 at S416 and Arl1 activity are increased upon treatment with the UPR inducer tunicamycin (Hsu et al., 2016). A better understanding of how the UPR influences the role of the Syt1–Arl1–Imh1 cascade in Golgi transport could also provide important mechanistic insights into how the Golgi contributes to mitigating proteotoxic stress from the ER.

Arf-GAPs and their regulators

Arf-GAPs regulate the GTPase cycle of Arfs by mediating their dissociation from membranes. In mammalian cells, Arf-GAPs have been shown to link cell signaling and morphogenesis to vesicular transport (Randazzo et al., 2000; Sabe et al., 2006). Thus, Arf-GAPs might facilitate the temporal and spatial coordination of the Arf GTPase cycle (Donaldson, 2000). ARFGAP1 localizes to the Golgi complex and contains a zinc-finger motif that is important for GAP activity (Cukierman et al., 1995). Mammalian ARFGAP1 is similar to the yeast zinc-finger proteins Gcs1, Glo3 and Age2 that can function as GAPs for yeast Arf1 *in vitro* (Poon et al., 1999, 2001, 1996). Gcs1 and Glo3 exhibit overlapping functions in ER-to-Golgi transport (Andreev et al., 1999; Dogic et al., 1999; Poon et al., 1999), and Gcs1 and Age2 overlap in functions in the transport from

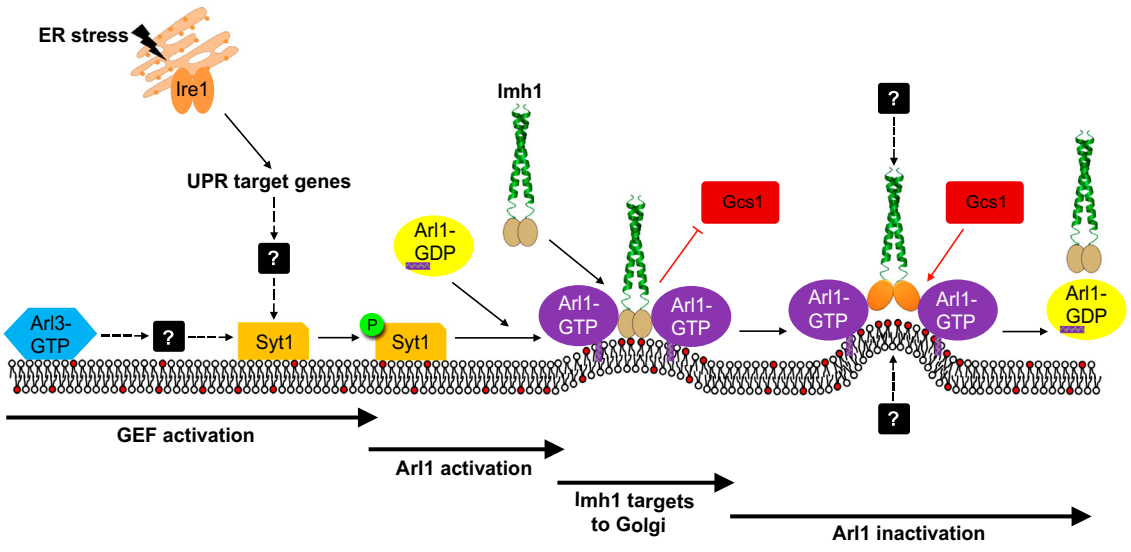

Fig. 2. Regulation of Arl1 activity at the TGN. Illustrated here are the conditional and sequential regulation of Arl1 activation and inactivation at the TGN by several factors, including the small GTPase Arl3, the Arf-GEF Syt1, the golgin Imh1and the GAP protein Gcs1. Arl3 and, possibly, other unknown factors activate Syt1 GEF by facilitating its phosphorylation that, in turn, promotes GDP-GTP exchange and Arl1 activation. Arl1 then associates with the TGN through its N-terminal myristoylation (illustrated by the small purple bar) and recruits Imh1 to the late Golgi complex. Unfolded-protein-response (UPR) signaling also induces Syt1 phosphorylation, which results in in activation of Arl1. Late Golgi-associated Imh1 inhibits access of the GAP Gcs1 to Arl1-GTP prior to any vesicle formation. Any changes in membrane curvature upon vesicle emergence or changes in the conformation of Imh1 might allow Gcs1 to access Arl1-GTP and promote hydrolysis of its bound GTP. Both Imh1 and Arl1-GDP are subsequently released from the Golgi complex to undergo another cycle. The red circles within the lipid membrane represent phosphatidylserine. The figure has been modified from Chen et al., 2010 and Chen et al., 2012 with permission.

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the yeast TGN (Poon et al., 2001). Gcs1 has also been implicated in the regulation of actin cytoskeletal organization (Blader et al., 1999) and mitochondrial morphology (Huang et al., 2002). Importantly, there is both *in vivo* and *in vitro* evidence that Gcs1 acts as an Arl1 GAP; it promotes Arl1 GTP hydrolysis and causes the dissociation of Arl1 from the late Golgi (Chen et al., 2010; Liu et al., 2005). In mutant cells lacking Gcs1, Arl1 is not re-distributed from late Golgi structures to the cytosol, indicating that failure of Arl1 to hydrolyze GTP in a Gcs1-deleted mutant might result in the accumulation of Arl1-GTP on late Golgi (Liu et al., 2006). The interaction of Gcs1 with Arl1 and Arf1 suggests that it acts at different intracellular membranes to regulate their membrane dynamics. Consistent with this notion, Glo3 has been identified as the main Arf1 GAP *in vivo* and the effectiveness of Golgi-to-ER retrieval of membrane proteins in a Glo3-deleted mutant is not limited by the amount of Gcs1 (Eugster et al., 2000). Therefore, Gcs1 might be the main GAP for Arl1 in *trans*-Golgi vesicular trafficking.

As noted above, the yeast golgin Imh1 is targeted to the TGN through the interaction between its GRIP-domain and GTP-bound Arl1 (Fig. 2). Recycling of Arl1 and Imh1 to the cytosol requires the hydrolysis of Arl1-bound GTP; however, the point at which GTP hydrolysis occurs remains unknown. We proposed a new, feedback-based mechanism whereby modulating the inactivation of Arl1 through its effector Imh1 determines the timing of Arl1 GTP hydrolysis by Gcs1 (Fig. 2) (Chen et al., 2012). We found that deletion of Imh1 reduces the amount of Golgi-localized GTP-bound Arl1 and that, at the molecular level, purified Imh1 competes with Gcs1 for binding to Arl1, thus interfering with the GAP activity of Gcs1 towards Arl1. Furthermore, homodimerization of Imh1 attenuates Gcs1-dependent GTP hydrolysis of Arl1. Although Imh1 has extensive coiled-coil stretches in its native state, the predicted coiled regions might unfold under physiological conditions through alterations in post-translational modifications, putative regulatory factors or the membrane environment. Therefore, we hypothesize that the regulation of Imh1 is crucial for modulating the Gcs1-dependent Arl1 inactivation at the TGN (Chen et al., 2012).

Taken together, these findings provide a link between Syt1-dependent activation and Imh1-Gcs1-dependent inactivation of Arl1. It would be of particular interest to investigate the mechanisms controlling GEF and GAP activity and recruitment, which enable the GEFs and GAPs to fine-tune Arl1 activity and functions at the TGN.

Mechanism underlying Arl1-dependent membrane remodeling at the TGN

Membrane asymmetry, curvature and dynamics all greatly affect cellular processes, including vesicle transport. Arfs and a lipid translocase (flippase) have been shown to be crucial for membrane reorganization during vesicle formation (Donaldson and Jackson, 2011; Graham, 2004). Arfs can recruit and activate enzymes, such as PLD, phosphatidylinositol 4-phosphate, 5-kinase (PIP5K) and phosphatidylinositol 4-kinase (PI4K), that alter the membrane lipid composition. Arl1 can also interact with and recruit arfaptins to induce membrane curvature and tubule formation (Man et al., 2011). However, direct evidence that Arfs and flippase work in concert in the modulation of membrane transformation and/or architecture is lacking. Phospholipid flippases are members of a family of P4-type ATPases that induce membrane bending by selectively flipping phospholipids from the luminal leaflet to the cytosolic leaflet of the membrane bilayer, thus, altering the phospholipid composition of each leaflet (Donaldson and

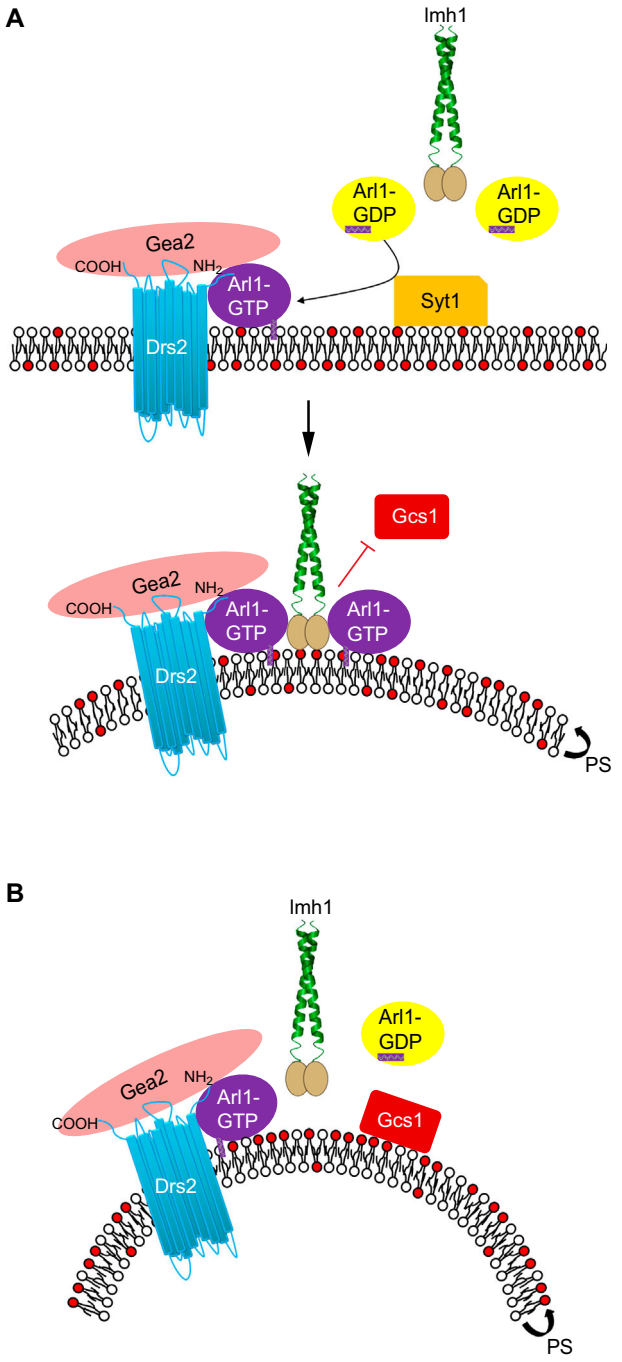

Fig. 3. Arl1 modulates membrane remodeling at the TGN. (A) Syt1-dependent Arl1 activation results in binding of Arl1-GTP to the Golgi (its N-terminal myristoylation, illustrated by the small purple bar, targets Arl1 to the membrane), where it recruits or activates effectors to promote vesicle formation. Gea2, Arl1 and Drs2 form a ternary complex that regulates the membrane dynamics and lipid asymmetry of the Golgi complex. Specifically, Arl1 directly interacts with Drs2 and activates its flippase activity through the formation of an Arl1–Gea2–Drs2 complex to generate an optimal membrane environment, thereby inducing translocation of phosphatidylserine (PS; phosphatidylserine, indicated by the red circles within the lipid membrane) for vesicle maturation and Imh1 targeting. Membrane-bound Imh1 interacts with active (GTP-bound) Arl1 and impedes GTP hydrolysis by sterically blocking access of Gcs1. (B) An increase in membrane curvature, such as upon vesicle maturation, allows for binding of Gcs1 that then exerts its GAP activity toward Arl1. Gcs1 might access Arl1 through curvature-induced Imh1 dissociation or a stochastic process, which induces Arl1 inactivation and its removal from the membrane. This figure has been modified from Hsu et al., 2014 with permission.

Jackson, 2011; Graham, 2004). Recently, we have shown that activated Arl1 interacts with the Arf-GEF Gea2 and the phosphatidylserine flippase Drs2 to form a ternary complex that is required for maintaining lipid asymmetry and Arl1 function at the Golgi (Tsai et al., 2013) (Fig. 3). Our study also implies a requirement for Arl1-GTP binding and the presence of phosphatidylserine in the cytosolic leaflet for membrane association of Imh1 at the late Golgi. This finding represents a previously missing piece of the puzzle in our current understanding of Arf-mediated membrane remodeling. Although we have shown that Imh1 prolongs Arl1 activity at the late Golgi, it remains unclear how and when Gcs1 accesses and stimulates Arl1-GTP hydrolysis. Hints on how to answer these questions came from a recent study demonstrating that Drs2 is required for Gcs1 localization to the TGN and early endosomes (Xu et al., 2013). Subsequently, we were able to show that, by altering the lipid environment, Drs2 facilitates the targeting of Gcs1 to the TGN and, hence, its interaction with Arl1 (Hsu et al., 2014). Together, these studies point to a sophisticated feedback regulation of Arl1 activity that is mediated by effector activity and binding, with Drs2 and Imh1 determining the GTP-GDP switch of Arl1 at the Golgi (Fig. 3). This type of feedback regulation is likely to also exists for other Arfs and small GTPases (Hsu et al., 2014).

Conclusions and future perspectives

Arl1 plays a fundamental and evolutionarily conserved role in the vesicle trafficking of diverse cargos from the TGN to the plasma membrane and from endosomes to the TGN. Research spanning more than two decades has shown that Arl1 exhibits multiple functions in many different organisms. However, how exactly Arl1 performs these multiple functions in different regions of the TGN remains unclear. The localization of GEFs and GAPs to distinct regions of the TGN might provide insight of specific domains for Arf1 activities coupled with different effectors. To date, no GEFs or GAPs have been identified for human Arl1, and the identity of these regulators must be determined in order to explore how Arl1 performs its multiple functions in different regions of the TGN within more-complex mammalian cells. Although studies in yeast suggest that Arl1 activation at several TGN sites can be carried out by Syt1 and probably other unidentified GEFs, it is important to identify these new GEFs as they might form specific molecular complexes that are important for Arl1 function. Until recently, Gcs1 appeared to be the only GAP for Arl1 in the TGN in yeast. However, there are indications of an additional Arf GAP that acts in the TGN. Furthermore, Gcs1 has many phosphorylation sites that might regulate the spatial and temporal activity of this GAP in the TGN, where Arl1 is known to exert numerous functions. In addition to its localization in the TGN, Arl1 is also present in the Rab4-subdomain of early endosomes, where it is required for the recruitment of BIG1 and BIG2 (D'Souza et al., 2014), suggesting that it acts downstream of Rab4 to promote the assembly of the early endosomal sorting machinery. The detailed mechanism responsible for Rab4-mediated Arl1 targeting to early endosomes, however, remains unclear.

Activated Arf can recruit numerous proteins to the membrane or activate lipid-modifying enzymes to facilitate aspects of vesicle formation, such as assembly of the vesicle coat, generation of membrane curvature, alteration of the lipid composition and final membrane fission (Donaldson and Jackson, 2011). Indeed, activated Arl1 interacts with the flippase Drs2 within the context of a ternary complex with Gea2, which is important for lipid asymmetry and Arl1 function at the Golgi (Tsai et al., 2013). This raises the question of why the activity of the Golgi phospholipid flippase needs to be regulated in a complex formed with proteins that have other functions (Graham, 2013). Another question is whether Gea2 is also capable of activating Arf as part of the Arl1-Gea2-Drs2 complex, or whether these two activities are mutually exclusive. It will be interesting to determine how closely intertwined the Arl, Arf-GEF and P4-type ATPase (flippase) family trees have been throughout eukaryotic evolution with regard to their role in membrane trafficking. Our appreciation of the important roles of the Arl GTPases in cell biology and biomedicine is poised to increase as we elucidate the regulatory mechanisms of these proteins and their regulators and effectors.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

C.-J.Y. and F.-J.S.L. drafted and edited the manuscript.

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