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# Intermediate Compartment: A Sorting Station between the Endoplasmic Reticulum and the Golgi Apparatus

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## Glossary

**Anterograde transport** Transport from endoplasmic reticulum (ER) toward the cell surface.
**ER exit sites** ER subdomains specialized in the export of newly synthesized molecules.
**Golgi stacks** Stacks of flat membrane-bound cisternae. The mammalian Golgi stacks contain 4–8 cisternae.
**Lumen** The space inside a membrane-bound structure.
**Microtubules** Cytoskeletal filaments that provide tracks for motor-dependent long-distance transport of organelles and transport carriers.
**Pleiomorphic** Variable in size and shape.
**Retrograde transport** Transport from post-ER compartments toward the ER.
**Saccule** Membrane-bound structure with a large lumen.
**Vesicle** Membrane-bound structure with a small lumen.

## Introduction

Newly synthesized proteins and lipids leave the endoplasmic reticulum (ER) at specialized transitional regions called ER exit sites (ERES) (Jamieson and Palade, 1967; Sesso *et al.*, 1994; Bannykh *et al.*, 1996; Hammond and Glick, 2000; Tang *et al.*, 2005) and enter the intermediate compartment (IC) that has been shown to operate as an obligatory a post-ER sorting station in the early biosynthetic-secretory trafficking of mammalian cells. From the IC they are typically transported to the cisternal stacks of the Golgi apparatus, prior to their delivery to the different organelles of the endomembrane system or secretion to the extracellular space. Bidirectional ER–Golgi trafficking involves the sequential operation of membrane-bound coat protein II (COPII) and COPI coats (Aridor and Balch, 1996; Scales *et al.*, 1997; Stephens *et al.*, 2000). ER-derived COPII vesicles mediate forward (anterograde) transport, while IC- and Golgi-derived COPI vesicles are thought to act in the opposite (retrograde) direction (Lee *et al.*, 2004; Rabouille and Klumperman, 2005). Retrograde transport also involves COPI-independent routes (Kano *et al.*, 2009).

Despite the conservation of the transport machineries (such as the COP coats), the organization of the ER–Golgi interface varies in different eukaryotic cells. For example, in plants, certain yeasts, and the fruit fly *Drosophila melanogaster*, the individual Golgi stacks lie next to the widespread ERES, establishing units for short range ER–Golgi communication. By contrast, in animal cells the Golgi stacks are linked together into a continuous ribbon around the microtubule-organizing center (MTOC)/centrosome, whereas the ER extends throughout the cytoplasm. Hence, a large proportion of the ERES reside at the cell periphery and ER–Golgi trafficking depends on the long distance movements of the IC elements along MT tracks (Saraste and Svensson, 1991; Presley *et al.*, 1997; Scales *et al.*, 1997; Brandizzi and Barlowe, 2013; Day *et al.*, 2013). Thus, it has been proposed that the IC represents a late evolutionary invention, which developed to meet the special sorting, transport and recycling requirements of the large-sized animal cells, but lacks lower eukaryotes. However, results showing that the IC constitutes an extensive membrane system that can be compared with the endosomal network in complexity, are questioning this view.

## Historical Perspective

### 15 °C Compartment

Electron microscopic (EM) studies using a temperature-sensitive mutant of Semliki Forest virus (SFV ts-1) to synchronize the transport of viral membrane glycoproteins from ER to the plasma membrane (PM) showed that when the cells are shifted from 39 °C to 15 °C the proteins exit the ER, but accumulate in vacuoles/saccules (up to 0.5 μm in diameter), tubules, and vesicles in the *cis*-Golgi region and more peripheral locations (Saraste and Kuismanen, 1984). By light microscopy (LM) the proteins were localized at 15 °C to scattered globular structures in the cytoplasm, a pattern distinct from that of ER or Golgi (Kuismanen and Saraste, 1989). The transport block was readily reversible, and the proteins entered the Golgi stacks and reached the PM following the transfer of cells from 15 °C to 28 °C, showing that these structures are normal transport intermediates.

Studies employing a similar mutant of vesicular stomatitis virus (VSV tsO45) showed that the '15 °C compartment' also acts as a way station during the transport of the VSV G glycoprotein (Balch *et al.*, 1986; Bonatti *et al.*, 1989; Schweizer *et al.*, 1990; Duden *et al.*, 1991; Lotti *et al.*, 1992). Like the SFV proteins, the G-protein was found to maintain its ER-type high-mannose glycans at 15 °C, indicating lack of processing by Golgi enzymes. Furthermore, cell fractionation experiments showed that newly synthesized secretory proteins are arrested in a post-ER location when pancreatic exocrine cells are kept at 16 °C (Saraste *et al.*, 1986). Live cell imaging of green fluorescent protein (GFP) equipped with an ER targeting signal (ssGFP) and EM studies of procollagen and growth hormone constructs verified that the transport of membrane and secretory proteins is similarly affected at 15–16 °C (Blum *et al.*, 2000; Volchuk *et al.*, 2000; Trucco *et al.*, 2004). In addition, the transport of virus glycoproteins and cholesterol appears to

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be blocked at the same site at this temperature (Urbani and Simoni, 1990; Heino *et al.* 2000).

### Coronavirus Budding Compartment

EM of mouse hepatitis virus (MHV)-infected cells showed that the budding of progeny virus initially takes place at tubulovesicular membranes located in the transitional region between the ER and the Golgi apparatus (Tooze *et al.*, 1984). The first step of *O*-glycosylation, the attachment of *N*-acetyl-galactosamine (GalNAc) to the viral membrane (M) glycoprotein, was suggested to occur in this compartment, causing its reactivity with the lectin Helix pomatia, which specifically binds GalNAc (Tooze *et al.*, 1988; Krijnse-Locker *et al.*, 1994). Subsequent studies showed that the intracellular maturation of various coronaviruses occurs at the same budding site, which corresponds to the IC (Klumperman *et al.*, 1994).

### Salvage Compartment

The discovery of the lys-asp-glu-leu tetra-peptide (KDEL)-motif in abundant, lumenal ER proteins lead to the proposal that the 15 °C/budding compartment is the post-ER site from which these proteins are retrieved to the ER (Munro and Pelham, 1987; Warren, 1987; Pelham, 1989). The first mammalian KDEL-receptor, a multispanning membrane protein, was identified and shown to predominantly localize to the IC and *cis*-Golgi (Lewis and Pelham, 1990; Tang *et al.*, 1993; Griffiths *et al.*, 1994; Orci *et al.*, 1997). Also, KDEL proteins, such as the immunoglobulin binding protein (BiP/GRP78), glucose-regulated protein of 94 kDa (GRP94), protein disulphide isomerase (PDI) and calreticulin (CR), are present in the IC (Griffiths *et al.*, 1994; Connolly *et al.*, 1994; Zuber *et al.*, 2001; Ying *et al.*, 2002; Breuza *et al.*, 2004). Following binding to their receptor, the proteins are retrieved to the ER in COPI vesicles (Orci *et al.*, 1997; Martínez-Menárguez *et al.*, 1999; Majoul *et al.*, 2001). Attachment of the KDEL-motif to lysosomal enzymes and the use of the 15 °C block suggested that the enzyme that initiates the formation of their lysosomal targeting signal (mannose-6-phosphate) resides in the IC (Pelham, 1988; Lazzarino and Gabel, 1988; Dittmer and von Figura, 1999). The cytoplasmic tails of certain type I integral membrane proteins were shown to contain dilysine (KKXX)-motifs, which by directly interacting with COPI coats result in their retrieval from the IC/*cis*-Golgi to the ER (Nilsson *et al.*, 1989; Jackson *et al.*, 1990, 1993; Cosson and Letourneur, 1997).

### p58/ERGIC-53/LMAN1

The first endogenous IC markers rat p58 and human ER–Golgi intermediate compartment (ERGIC)-53 (89% homology) were identified by the generation of antibodies against the 16 °C post-ER fraction isolated from pancreatic acinar cells (Saraste *et al.*, 1987) and a Golgi fraction derived from epithelial Caco-2 cells (Schweizer *et al.*, 1988), respectively. The cytoplasmic *C*-terminal tails of these non-glycosylated, hexameric, type-1 integral membrane proteins (Schindler *et al.*, 1993; Lahtinen *et al.*, 1992, 1996; Neve *et al.*, 2005) contain a KKFF-motif, which interacts with COPII and COPI coats and gives rise to their continuous cycling between ER, IC, and *cis*-Golgi (Kappeler *et al.*, 1997; Tisdale *et al.*, 1997). At 15 °C the recycling of p58/ERGIC-53 to the ER is inhibited and the proteins pile up in the same pre-Golgi structures that contain the SFV or VSV membrane proteins (Schweizer *et al.*, 1990; Saraste and Svensson, 1991; Plutner *et al.*, 1992), verifying that the p58/ERGIC-53 compartment (**Figures 1** and **4**) is equivalent to the site where cargo is arrested at low temperature.

ERGIC-53 and the related VIP36 were shown to share homology with leguminous plant lectins (Fiedler and Simons, 1994) and to be identical with a mannose-binding protein MR60 of human myelomonocytic (HL60) cells (Arar *et al.*, 1995). The *N*-terminal domain of p58/ERGIC-53 binds mannose in a calcium-dependent manner (Itin *et al.*, 1996; Velloso *et al.*, 2003; Zheng *et al.*, 2013); hence the name lectin mannose-binding protein 1 (LMAN1). It is the best characterized mammalian cargo receptor, facilitating the COPII vesicle-mediated export of a subset of soluble glycoproteins from the ER (Nichols *et al.*, 1998; Appenzeller *et al.*, 1999; Hauri *et al.*, 2000).

## Compositional Aspects

Most well-characterized IC proteins are components of the transport machinery. Many of them cycle at the ER–Golgi boundary and are also found in *cis*-Golgi, supporting the view of the IC as a transient structure (see below). In fact, EM studies showing the specific metal (osmium) staining of the IC and *cis*-Golgi provided the first indication of their compositional similarity (Friend and Murray, 1965; Rambourg *et al.*, 1974). However, the fungal compound brefeldin A (BFA) helps to discriminate between IC and Golgi components. It releases COPI coats, disassembles the Golgi stacks and redistributes Golgi components to the ER, whereas cycling proteins (such as p58/ERGIC-53/LMAN1 and the KDEL-receptor) are arrested in the drug-resistant IC elements (Lippincott-Schwartz *et al.*, 1990; Saraste and Svensson, 1991; Tang *et al.*, 1995b; Füllekrug *et al.*, 1997; Ward *et al.*, 2001; Marie *et al.*, 2009). The BFA resistance of the IC has been utilized for its proteomics analysis (Breuza *et al.*, 2004), and indicates its stability.

### The p24 Protein Family

Like p58/ERGIC-53, the p24 family proteins contain motifs for COPII and COPI binding, resulting in their cycling between the ER, IC, and *cis*-Golgi (Rojo *et al.*, 1997; Dominguez *et al.*, 1998; Blum *et al.*, 1999; Gommel *et al.*, 1999; Strating and Martens, 2009). As abundant type-1 transmembrane proteins they participate in the biogenesis of COPI vesicles (Stamnes *et al.*, 1995; Majoul *et al.*, 2001; Beck *et al.*, 2009), but have also been implicated in tubulation (Simpson *et al.*, 2006) and the formation of membrane domains (Lavoie *et al.*, 1999; Emery *et al.*, 2003). Studies in yeast first suggested their function as receptors for the exit of

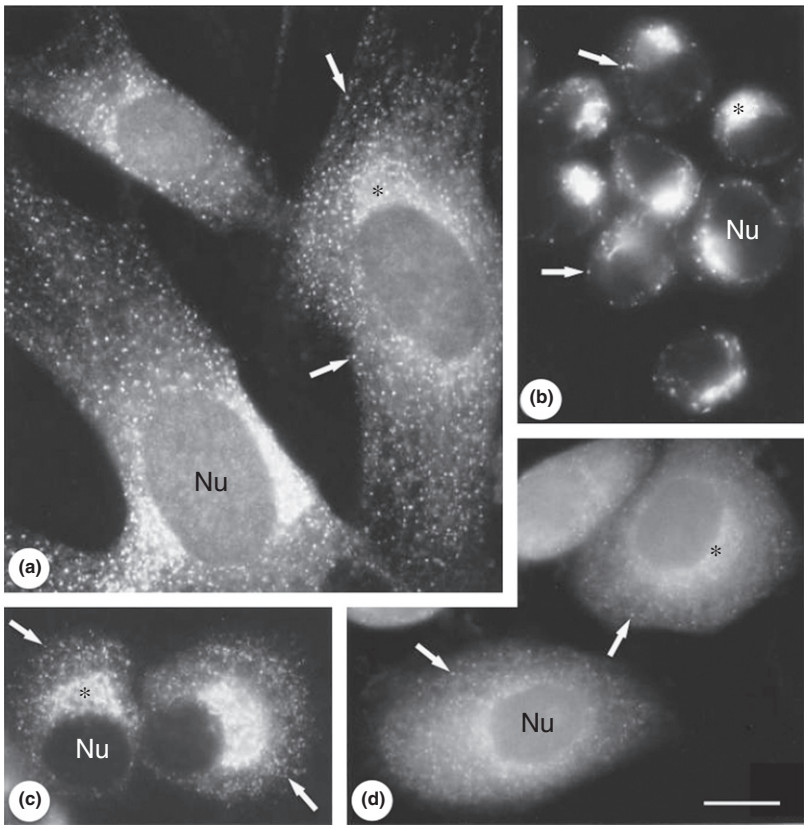


**Figure 1** (a) Immunofluorescence LM localization of p58/ERGIC-53 in baby hamster kidney, (b) mouse myeloma, (c) rat neuroendocrine PC12, and (d) human HeLa cells. The protein marks the IC elements accumulating in the perinuclear Golgi region (asterisks), scattered throughout the cytoplasm, and located close to the PM (arrows). The reticular staining indicates the ER pool of p58/ERGIC-53, which varies in the different cell types. Nu, nucleus. Bar: 10 μm.

glycosylphosphatidylinositol (GPI)-anchored proteins from the ER (Schimmöller *et al.*, 1995).

## COPI Coats

The COPI coats are mainly recruited to the cytoplasmic surface of IC and *cis*-Golgi membranes, but also associate with the lateral rims of the Golgi stacks (Duden *et al.*, 1991; Oprins *et al.*, 1993; Griffiths *et al.*, 1995; Orci *et al.*, 1997; Klumperman *et al.*, 1998). COPI vesicle budding depends on the activation of small GTPases of the ADP-ribosylation factor (Arf) family (Popoff *et al.*, 2011). The guanine nucleotide exchange factor (GEF) of these Arfs, GBF1, is the master regulator of COPI recruitment and the target of BFA (Niu *et al.*, 2005). It localizes to the IC and *cis*-Golgi and plays a key role in ER-to-Golgi trafficking (Kawamoto *et al.*, 2002; Garcia-Mata *et al.*, 2003; Zhao *et al.*, 2006; Szul *et al.*, 2007). GBF1 knock down or specific inhibition by Golgicide A releases COPI coats and arrests the VSV G-protein in the IC, indicating its involvement in anterograde IC-to-Golgi transport (Manolea *et al.*, 2008; Sáenz *et al.*, 2009). However, the prevailing view is that the main function of COPI vesicles is to recycle membrane and selected proteins to the ER. In pancreatic exocrine cells approximately 70% of the coats associate with the VTCs, suggesting that the IC represents the main point for recycling (Martínez-Menárguez *et al.*, 1999).

## Rab1 and Rab2

The roles of two GTPases of the large Rab family, Rab1 and Rab2, in ER–Golgi trafficking are relatively well understood (Plutner *et al.*, 1991; Tisdale *et al.*, 1992). Both interact with multiple effectors suggesting that they coordinate successive transport steps (Barnekow *et al.*, 2009). The association of Rab1 with the cytoplasmic surface of IC and *cis*-Golgi membranes, and its absence from the ER, has been demonstrated by EM (Griffiths *et al.*, 1994; Saraste *et al.*, 1995; Satoh *et al.*, 2003; Marie *et al.*, 2012; **Figure 2**). The two Rab1 isoforms, Rab1A and RAB1B (93% homology), are recruited to IC membranes at ERES, show similar localizations by LM (Mochizuki *et al.*, 2013; **Figure 3(a)**), but seem to play distinct roles in tubular and vesicular (long and short range) trafficking within the IC. Accordingly, live cell imaging and cell fractionation have shown that Rab1A mainly associates with IC tubules (Sannerud *et al.*, 2006; Marie *et al.*, 2009), while Rab1B interacts with GBF1 and evidently modulates COPI recruitment to globular IC domains (Alvarez *et al.*, 2003; Monetta *et al.*, 2007; see below). Also, Rab1A is specifically

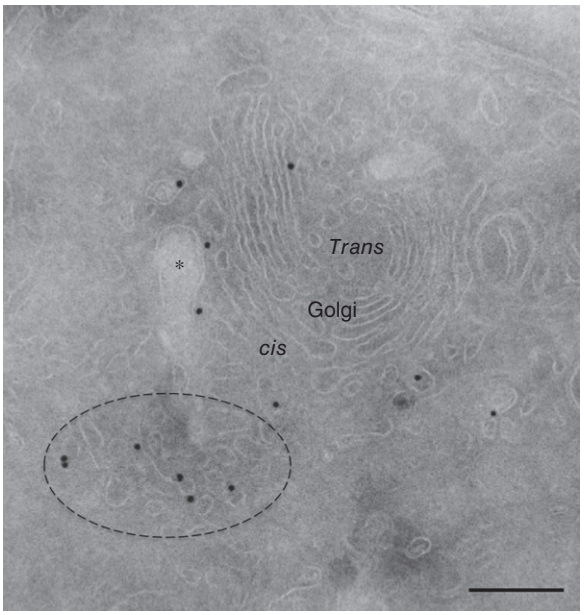


**Figure 2** Immunogold EM localization of Rab1 in the Golgi region of a normal rat kidney (NRK) cell. The 15 nm gold particles predominantly label pleiomorphic IC elements at the *cis*-face of the Golgi stacks. A saccule (asterisk) and a vesicular tubular cluster (VTC; dashed area) are indicated. The micrograph was kindly provided by Dr. Karin Pernet-Gallay (University Joseph Fourier, Grenoble, France). Bar: 3 μm.

phosphorylated at mitosis (Bailly *et al.*, 1991), correlating with the cessation of tubular IC dynamics, whereas COPI-mediated vesicular transport continues (Marie *et al.*, 2012). Rab1A can functionally replace Ypt1, which coordinates two-way ER–Golgi trafficking in the yeast *Saccharomyces cerevisiae* (Haubruck *et al.* 1989; Segev, 2001; Kamena *et al.*, 2008).

Rab2 has been localized to IC and *cis*-Golgi membranes (Chavrier *et al.*, 1990; Lotti *et al.*, 1992) and proposed to regulate the formation of retrograde COPI vesicles that contain p58/ERGIC-53 (Tisdale, 1999). It has also been implicated in the recruitment of the motor protein dynein to IC membranes and the association of IC elements with MTs (Tisdale *et al.*, 2009; see below).

### Tethers and Soluble NSF Attachment Protein Receptors

Most of the known Rab1 and Rab2 effectors are cytoplasmically oriented, long coiled-coil proteins, which function in the tethering of vesicle and organelle membranes preceding their soluble NSF attachment protein receptor (SNARE)-mediated fusion (Barnekow *et al.*, 2009; Sztul and Lupashin, 2009). Besides the IC/*cis*-Golgi tethers GM130 and GMAP-210 (Rios *et al.*, 2004), Rab2 has been shown to interact with *medial*- and *trans*-Golgi tethers, suggesting a more widespread function (Short *et al.*, 2001; Sinka *et al.*, 2008). The Rab1 effector p115 is functional already at the peripheral ERES (Alvarez *et al.*, 1999), while GM130 (bound to its membrane anchor GRASP65) cycles between central IC and *cis*-Golgi (Marra *et al.*, 2001) and regulates membrane tethering at a later transport step (Alvarez *et al.*, 2001; Marra *et al.*, 2007). Rab1 also interacts with the transmembrane tethers golgin-84 and giantin, which appear to act in COPI vesicle trafficking at the lateral rims of the Golgi stacks (Diao *et al.*, 2003; Malsam *et al.*, 2005; Barnekow *et al.*, 2009; Munro, 2011).

The membrane fusion machinery (SNARE proteins) operating in ER–Golgi trafficking in the yeast *Saccharomyces cerevisiae* has been well characterized (Cai *et al.*, 2007; Barlowe and Miller, 2013), whereas the fusion events that take place in mammalian cells remain enigmatic. The determination of the exact fusion steps is complicated by the continuous cycling of the SNAREs in COPI vesicles (Hay *et al.*, 1998; Martinez-Menárguez *et al.*, 2001). In analogy to yeast, Rab1 (Ypt1) has been suggested to recruit p115 (Uso1p) to COPII vesicles at ERES (Allan *et al.*, 2000), followed by the formation of a SNARE complex (Sec22B, membrin, Bet1 and syntaxin 5), which mediates either homotypic fusion of COPII vesicles or their heterotypic fusion with the stationary IC (Zhang *et al.*, 1997; Xu *et al.*, 2000; see below). Another SNARE complex (syntaxin 5, GS28, Bet1 and Ykt6) is involved in a later *cis*-Golgi transport step (Zhang and Hong, 2001).

## Structure, Distribution, and Dynamics

By EM the IC elements can be readily distinquished from the ER and Golgi, but share structural similarity with endosomes. They reside close to peripheral and central ERES as clusters of vesicles and tubules (VTCs; **Figure 4(f)**) that frequently contain COPI coats (Balch *et al.*, 1994; Bannykh *et al.*, 1996; Martínez-Menárguez *et al.*, 1999). However, they display considerable size heterogeneity (Ying *et al.*, 2000) and also include large saccules that are found within the membrane clusters, free in the cytoplasm, or at the *cis*-face of the Golgi stacks (Saraste and Svensson, 1991; Lahtinen *et al.*, 1992, Connolly *et al.*, 1994; Stinchcombe *et al.*, 1995; Ladinsky *et al.*, 1999; Fan *et al.*, 2003; **Figures 4(a)–4(e)**). These pleiomorphic saccules can accommodate large-sized cargo, such as procollagen or lumenal protein aggregates (Volchuk *et al.*, 2000; Trucco *et al.*, 2004; Zuber *et al.*, 2004), and based on correlative microscopy (LM/EM) correspond to many of the mobile carriers that are visible in living cells (Mironov *et al.*, 2003). Like endosomes, they extend narrow tubules and also bind COPI coats, indicating that they represent sites for vesicle budding (Saraste and Kuismanen, 1984; Volchuk *et al.*, 2000; Horstman *et al.*, 2002; **Figures 4(c)** and **4(d)**). The hypertrophy of the saccular domains most likely gives rise to the pre-Golgi vacuoles seen in cells kept at 15 °C (Saraste and Kuismanen, 1992; Trucco *et al.*, 2004).

LM shows the division of the IC into globular and tubular domains (Presley *et al.*, 1998). The former contain anterograde cargo, cargo receptors and COPI coats, most likely corresponding to individual VTCs or free saccules, while the latter are highlighted by Rab1A (Sannerud *et al.*, 2006). The tubules extend from the globular domains (**Figure 5**). Some of them contain recycling proteins, but lack anterograde cargo, indicating that they function in retrograde transport (Palokangas *et al.*, 1998; Simpson *et al.*, 2006). However, under synchronized conditions cargo is also detected in the tubular domain, due to overload or the existence of different types of

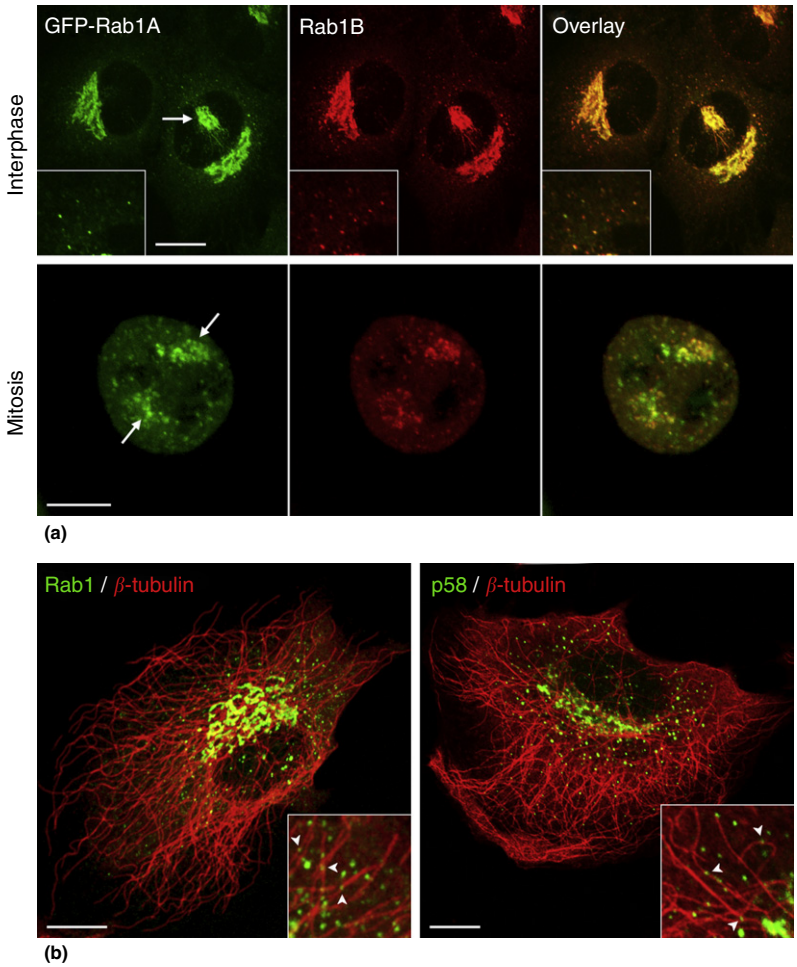


**Figure 3** (a) Immunofluorescence LM localization of the two Rab1 isoforms at different phases of the cell cycle. NRK cells stably expressing GFP-Rab1A were stained with monoclonal antibodies against Rab1B (kindly provided by Prof. Angelika Barnekow, University of Münster, Germany). At interphase the proteins colocalize in the Golgi ribbon, the separated pcIC (arrow) and peripheral IC elements (insets). During mitosis (at metaphase), following Golgi disassembly, the isoforms maintain their pericentrosomal co-localization at the spindle poles (arrows). (b) Association of IC elements with MTs. NRK cells were stained for the IC markers Rab1 or p58 and $\beta$-tubulin (to visualize MTs). Only the image overlays are shown. The insets show coalignment of IC elements with MTs (arrowheads). See also Marie *et al.*, 2012. Bars: 10 μm.

IC tubules (Figure 6). For example, incubation of cells at 15–16 °C inhibits the formation of IC tubules, but causes the expansion of the globular domain, while the shift of cells to 37 °C generates tubular networks containing both antero- and retrograde markers (Blum *et al.*, 2000; Ben-Takaya *et al.*, 2005; Simpson *et al.*, 2006). Proliferation of the tubules is induced when COPI function is compromised (Szul *et al.*, 2007; Marie *et al.*, 2009; Ben-Takaya *et al.*, 2010; Tomás *et al.*, 2010; Hamlin *et al.*, 2014), but also occurs under physiological conditions. In differentiating neuroendocrine PC12 cells the Rab1A-positive tubular IC domain expands and the tubules move from the cell body to the forming neurites accumulating in their growth cones (Sannerud *et al.*, 2006; Figure 5). An analogous pathway connects the IC with the leading edge of fibroblasts (Figure 6).

Live imaging of various fluorescent IC markers indicates that the tubules are highly dynamic, while the globular domains are typically more stationary. Due to their differential localization within these domains, the constructs highlight different aspects of IC dynamics (see below). Long distance ER-to-Golgi transport involves the movement of IC elements from the cell periphery to the central *cis*-Golgi region, resulting in the division of the IC into spatially distinct early (ERES-adjacent) and late (*cis*-Golgi-adjacent) domains (Saraste and Svensson, 1991; Presley *et al.*, 1997; Scales *et al.*, 1997; Marra *et al.*, 2001). Two types of anterogradely moving IC elements can be resolved by LM, narrow tubules and large pleiomorphic structures. Some of the latter appear to represent saccular elements that transform into elongated structures ('thick tubules') (Presley *et al.*, 2002; Marie *et al.*, 2009). In addition, narrow tubules establish dynamic connections between the globular IC domains (Ben-Tekaya *et al.*, 2005; Sannerud *et al.*, 2006).

## Association with the Cytoskeleton

The long distance movements of the IC elements depend on MTs (Murshid and Presley, 2004; Palmer *et al.*, 2005; **Figures 3**

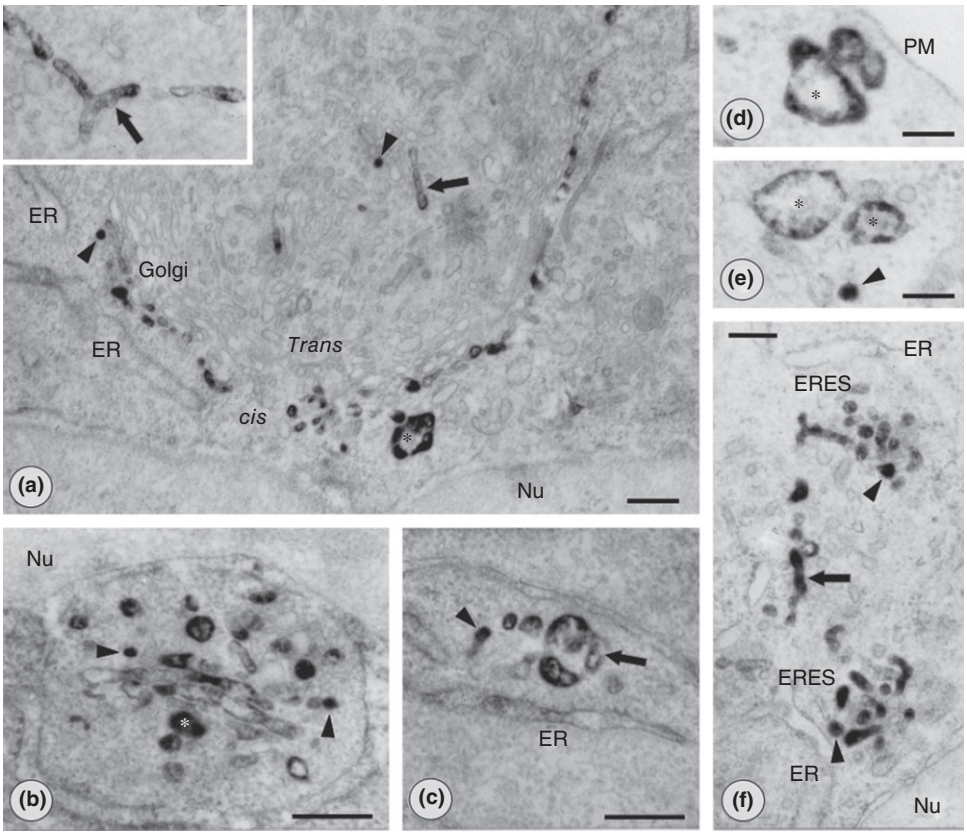


**Figure 4** Immunoperoxidase EM analysis of the ultrastructure of p58-containing IC elements in NRK (a; inset) and mouse myeloma (b–f) cells. The protein is present the *cis*-most Golgi cisterna (a) and in vesicular (arrowheads; ca. 80 nm in diameter), tubular (arrows) and saccular (asterisks; up to 0.5 μm in diameter) IC elements. Panels (a–c) adapted from Ying, M., Flatmark, T., Saraste, J., 2000. The p58-positive pre-Golgi intermediates consist of distinct subpopulations of particles that show differential binding of COPI and COPII coats and contain vacuolar $H^+$-ATPase. Journal of Cell Science 113, 3623–3638; (f) from Saraste, J., Palade, G.E., Farquhar, M.G., 1987. Antibodies to rat pancreas Golgi subfractions: Identification of a 58 kDa *cis*-Golgi protein. Journal of Cell Biology 105, 2021–2029. Nu; nucleus. Bars: 0.5 μm (a–c), 0.25 μm (d–f).

(b) and 6). The plus- and minus-end directed motor proteins kinesin and dynein associate with the IC elements (Lippincott-Schwartz *et al.*, 1995; Roghi and Allan, 1999; Stauber *et al.*, 2006), explaining their bidirectional movements even along the same MT tracks (Sannerud *et al.*, 2006). When the MTs in mammalian cells are depolymerized by nocodazole the IC elements become immobile and accumulate close to ERES (Saraste and Svensson, 1991; Hammond and Glick, 2000; Ben-Tekaya *et al.*, 2005; Sannerud *et al.*, 2006). Although the central Golgi ribbon breaks down, the formation of Golgi ministacks in the vicinity of ERES re-establishes ER–Golgi communication as a short range process (as in plants), explaining the ongoing Golgi modification and secretion of proteins (Saraste and Svensson, 1991; Cole *et al.*, 1996; Storrie *et al.*, 1998).

The Rho family GTPase cdc42, which regulates actin dynamics, interacts with COPI coats and affects dynein function, suggesting functional coupling between the actin filament system and MT-dependent motility of IC/*cis*-Golgi carriers (Luna *et al.*, 2002; Chen *et al.*, 2005). Similarly, WHAMM, which promotes actin nucleation and interacts with MTs, has been localized to the IC and implicated in the formation and transport of pre-Golgi tubules (Campellone *et al.*, 2008).

## Different IC Models

### Transport Complexes

Based on imaging of fluorescent cargo, such as the VSV G-protein and procollagen, in living cells (Presley *et al.*, 1997; Scales *et al.*, 1997; Stephens and Pepperkok, 2002), it has been proposed that the IC represents a collection of large, pleiomorphic transport complexes (TCs) (Bannykh and Balch, 1997; Stephens and Pepperkok, 2001), which form via homotypic fusion of COPII vesicles, or protrude directly from the ER (Mironov *et al.*, 2003; Xu and Hay, 2004; Yu *et al.*, 2006). Thereafter, they move in a MT- and dynein-dependent (Burkhardt *et al.*, 1997) 'stop-and-go' fashion to the Golgi region, where they either fuse with or transform into *cis*-Golgi cisternae (**Figure 7(a)**). In other words, the IC elements are transient structures which are first formed *de novo* at ERES and then consumed at *cis*-Golgi. These mobile structures (speed of

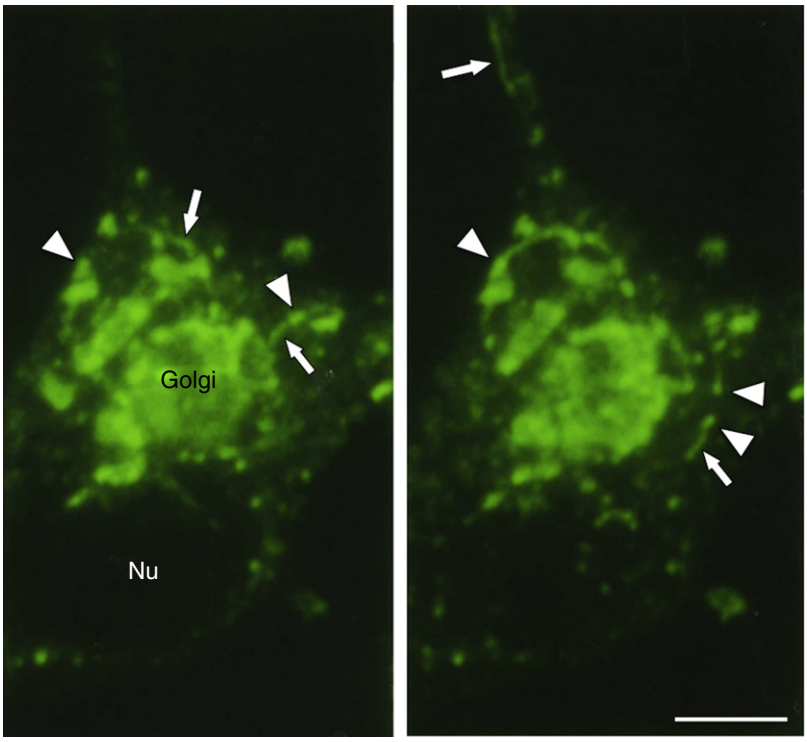


**Figure 5** Immunofluorescence LM localization of Rab1 in differentiating PC12 cells, illustrating the interconnected globular (arrowheads) and tubular (arrows) domains of the IC. Two confocal sections of the same cell are shown. The IC has expanded in response to a 24 h treatment with the nerve growth factor (NGF) and the tubules are found both in the cell body and the neurite-like extensions (right panel). Nu, nucleus; Golgi, perinuclear Golgi region. Bar: 5 μm.

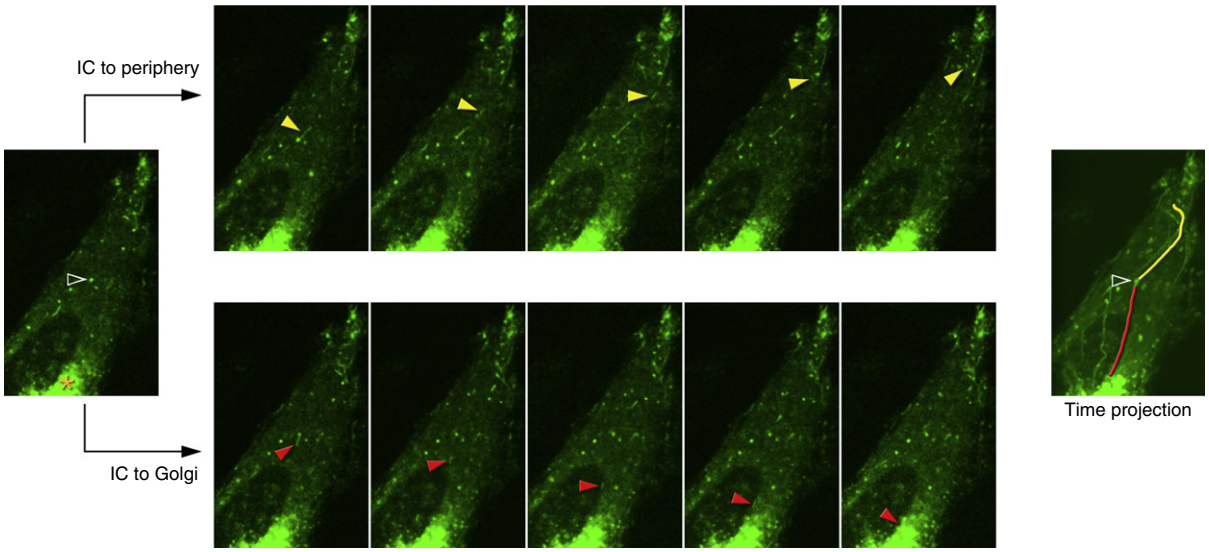


**Figure 6** Bidirectional movements of GFP-Rab1A-positive tubules in a living NRK cell. Selected confocal images from a movie obtained by time-lapse microscopy are shown. Tubules extending from the same relatively stationary globular IC element (black arrowhead) pinch off and move either in the direction of the cell's leading edge (upper panels; yellow arrowheads), or toward the Golgi indicated by an asterisk (lower panels; red arrowheads). The paths taken by the tubules are highlighted in the time projection on the right. Adapted from Sannerud, R., Marie, M., Nizak, C., *et al.*, 2006. Rab1 defines a novel pathway connecting the pre-Golgi intermediate compartment with the cell periphery. Molecular Biology of the Cell 17, 1514–1526.

about 1 μm/sec) corresponding to saccules or more complex, pleiomorphic elements (Mironov *et al.*, 2003) appear to consist of subdomains enriched in antero- or retrograde cargo, or COPI (Shima *et al.*, 1999; Stephens *et al.*, 2000). They have also been shown to contain other machinery proteins, such as p23/24, p58, VIP36, membrin and Rab1A (Blum *et al.*, 1999;

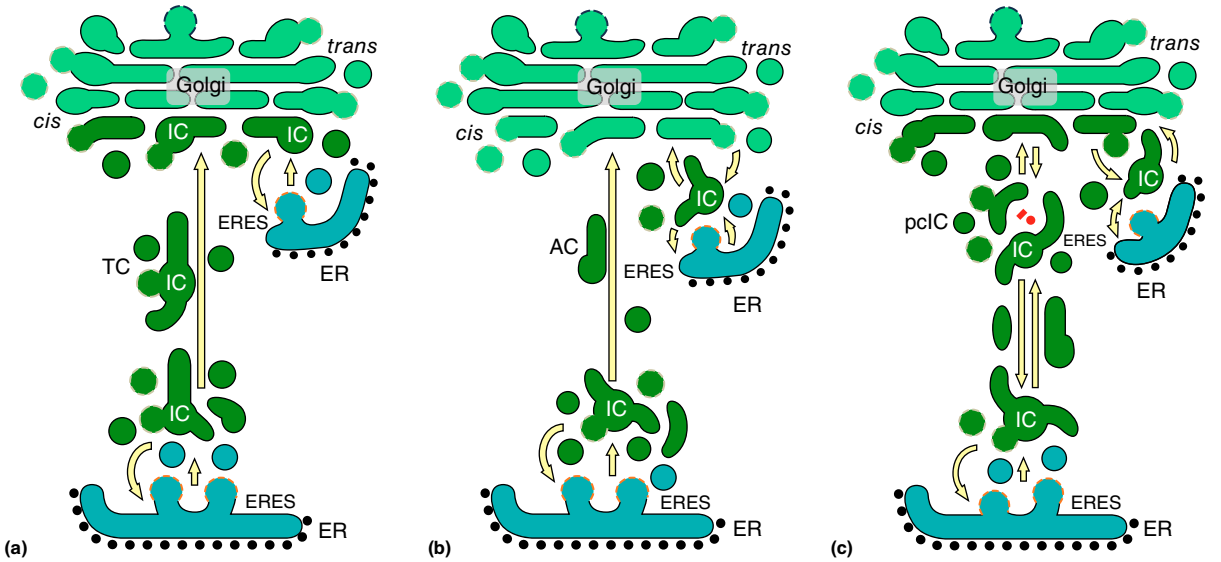


**Figure 7** Three views of the IC. All models take into account the existence of two types of ERES in mammalian cells, peripheral and Golgi-adjacent. For simplicity, lateral communication between ERES-adjacent IC elements is not illustrated. (a) The IC elements form *de novo* at ERES (via homotypic fusion of COPII vesicles), giving rise to pleiomorphic transport complexes (TC) that move along MTs to the *cis*-Golgi region where they either transform into *cis*-Golgi cisternae (as shown here) or fuse with the stationary Golgi stacks. (b) The IC consists of stationary membrane clusters located close to ERES. In this case ER-to-Golgi trafficking involves two distinct transport steps, since the IC recieves cargo from the ER via heterotypic fusion of COPII vesicles and forms special anterograde carriers (AC) that move along MTs to *cis*-Golgi. (c) The IC represents a stable interconnected network that is anchored both next to ERES and the centrosome. Bidirectional transport between these sites and between the central IC elements and the Golgi stacks involves vesicular, tubular or saccular carriers. ERES-IC communication via COPII vesicles occurs as in model b. COPI, COPII and clathrin coats are shown in gray, orange, and blue, respectively, and the centrosome in red.

Chao *et al.*, 1999; Dahm *et al.*, 2001; Sannerud *et al.*, 2006; Monetta *et al.*, 2007; Tomás *et al.*, 2010).

### Stationary Compartment

Another model of the IC (**Figure 7(b)**) is largely based on the imaging of GFP-ERGIC-53 dynamics in living cells (Ben-Tekaya *et al.*, 2005). It proposes that the IC consists of stationary, long-lived membrane clusters, located close to the ERES, which communicate with the ER and Golgi via distinct transport carriers (Appenzeller-Herzog and Hauri, 2006). Thus, in a two-step transport process cargo is first transfered from ER to the IC via heterotypic fusion of COPII vesicles. The IC then generates novel (possibly COPI-coated) anterograde carriers (ACs) that move along MTs to the *cis*-Golgi. The recycling of GFP-ERGIC-53 to the ER evidently occurs mainly from the ERES-associated IC, since it was not detected in the ACs containing soluble, Golgi-destined cargo. The identification of a motif in the neuronal GABA transporter, that seems to be required for its exit from the IC, supports the stable nature of the IC (Farhan *et al.*, 2008).

### Interconnected Membrane System

Visualization of IC dynamics using GFP-Rab1A showed that the pleiomorphic transport carriers arriving along MTs from the cell periphery do not move directly to *cis*-Golgi, but are targeted to the MTOC/centrosome that is normally positioned next to the Golgi ribbon. In cells displaying centrosome motility, for example, cells that are migrating or entering mitosis, the centrosome-targeted membranes can be resolved as a separate compartment, called the pericentrosomal IC (pcIC), which is distinct from the *cis*-Golgi-adjacent IC domain (Marie *et al.*, 2009, 2012; Mochizuki *et al.*, 2013; **Figure 3(a)**). Live imaging further showed that the separated pcIC and the Golgi communicate via tubular and globular carriers. The pcIC contains its own pool of COPI coats, and mediates the BFA-induced, COPI-independent backflow of Golgi enzymes to the ER, suggesting that forward pcIC-to-Golgi transport depends on COPI coats, and thus is blocked by BFA (Marie *et al.*, 2009).

Both the peripheral IC elements and the pcIC persist upon Golgi disassembly by BFA and maintain their communication with via dynamic tubules (Marie *et al.*, 2009). Accordingly, the IC has been proposed to constitute a dynamic, but stable membrane network due to its anchoring next to the ERES and the centrosome (Saraste *et al.*, 2009; **Figure 7(c)**). This model is supported by studies of mitotic cells, showing that the IC persists despite Golgi breakdown and the rearrangement of the MTs, and maintains its spatial organization due to its ongoing association with the spindle MTs and the centrosomes at the spindle poles (Marie *et al.*, 2012; **Figure 3(a)**).

## Functional Aspects

### Sorting and Transport

In the endocytic pathway, soluble proteins and particles bound to lysosomes accumulate in the lumen of vacuolar endosomes, while many membrane proteins are sorted into narrow tubules for recycling to the PM. The observed major

concentration of soluble secretory proteins within the IC (Oprins *et al.*, 2001) could be explained by similar geometry-based sorting, namely, their exclusion from vesicles and tubules, which recycle lipids and membrane-bound proteins back to the ER (Martínez-Menárguez *et al.*, 1999). Membrane recycling is a major function of both the IC and endosomes in mammalian cells. Due to the similarity of the tubular domain of the IC with the endocytic recycling compartment (ERC), its 'mirror compartment' next to the centrosome, it has been designated as the biosynthetic recycling compartment (BRC; Saraste and Goud, 2007; Marie *et al.*, 2009).

*Lumenal conditions*: Receptor-mediated retrieval of KDEL proteins from the IC requires that it is discontinuous with the ER and maintains special lumenal conditions (Pelham, 1989). The finding of low pH-dependent binding of KDEL-ligands to their receptor *in vitro* suggested the existence of a pH gradient between the ER and *cis*-Golgi (Wilson *et al.*, 1993). The pH-sensitive interactions of the low density lipoprotein (LDL)-receptor-related protein (LRP) and procollagen with the chaperones RAP and Hsp47, respectively (Bu *et al.*, 1995; Satoh *et al.*, 1996), and partial co-localization of DAMP (a marker for acidic compartments) and p58/ERGIC-53 in central IC elements (Palokangas *et al.*, 1998), are in accordance with this idea. The pH of the ER and *cis*-Golgi has been estimated to be about 7.2 and 6.5–6.7, respectively (Paroutis *et al.*, 2004; Vavassori *et al.*, 2013).

Although the effect pH on the binding of mannose-containing cargo to p58/ERGIC-53/LMAN1 remains unclear, it appears that cargo release is caused by a drop in free calcium concentration between the ER and IC lumen (Appenzeller-Herzog *et al.*, 2004; Bentley *et al.*, 2010; Zheng *et al.*, 2013). On the other hand, the depletion of lumenal calcium stores affects the morphology of the IC and the recycling of cargo receptors at the ER–Golgi boundary, and the IC has been reported to contain the calcium-ATPase SERCA, as well as the calcium-binding proteins BiP, GRP94, CR, and CALNUC (Ying *et al.*, 2002; Breuza *et al.*, 2004; Howe *et al.*, 2009; Bentley *et al.*, 2010), suggesting its role in intracellular calcium storage.

*Role of COPI coats*: There are at least three subtypes of COPI coats (Beck *et al.*, 2009), and four Arf GTPases that regulate their membrane binding (Popoff *et al.*, 2011). Three Arfs appear to act at the ER–Golgi boundary and two of these associate with membranes in a BFA-resistant manner (Volpicelli-Daley *et al.*, 2005; Chun *et al.*, 2008; Duijsings *et al.*, 2009; Ben-Tekaya *et al.*, 2010; Hamlin *et al.*, 2014), suggesting that different types of COPI vesicles mediate two-way trafficking at the level of the IC. The role of COPI in anterograde transport has been considered for some time (Hosobuchi *et al.*, 1992; Pepperkok *et al.*, 1993; Peter *et al.*, 1993; Orci *et al.*, 1997; Malsam *et al.*, 2005). In addition, although both p58/ERGIC-53 and KDEL-receptor employ COPI vesicles in their recycling, their transport itineraries differ (Tang *et al.*, 1995a; Marie *et al.*, 2009, 2012). In addition to acting in vesicle budding the COPI coats may form membrane domains (Presley *et al.*, 2002).

*Golgi bypass*: Besides mediating bidirectional ER–Golgi trafficking, the IC has been suggested to be involved in Golgi-independent pathway(s) (Marie *et al.*, 2008; Saraste *et al.*, 2009). The route between the IC and the leading edge or growth cone of motile fibroblasts or neurons, respectively (**Figures 5** and **6**), could participate in cholesterol and integrin trafficking (Urbani and Simoni, 1990; Sannerud *et al.*, 2006; Wang *et al.*, 2010) or correspond to the BFA-resistant ER-IC-PM route that supports phagocytosis (Becker *et al.*, 2005; Saraste and Goud, 2007; see below). Direct pericentrosomal communication between the IC and the endosomal system seems to be used by the cystic fibrosis transmembrane conductance regulator (CFTR) during its Golgi-independent transport to the cell surface (Yoo *et al.*, 2002; Marie *et al.*, 2009; Gee *et al.*, 2011). Further, the presence of the IC in neuronal dendrites may provide a Golgi-independent satellite pathway for local dendritic trafficking (Krijnse-Locker *et al.*, 1995; Sannerud *et al.*, 2006; Ehlers, 2013).

## Posttranslational Modification

Questioning the studies on coronavirus maturation, which suggested that *O*-glycosylation is initiated in the IC (see above), subsequent EM studies showed that the GalNAc-transferases are predominantly found in the Golgi. However, the activation of cells (by epidermal growth factor) causes their incorporation into COPI vesicles and relocation to the IC and ER, which consequently become positive for the lectin Helix pomatia (Gill *et al.*, 2010). The cycling of *cis*-Golgi proteins to the IC could be a constitutive event (Lin *et al.*, 1999; Marra *et al.*, 2001; Jarvela and Linstedt, 2012). Moreover, the construction of the sugar chains on proteoglycans has been suggested to begin in the IC (Jönsson *et al.*, 2003).

## Protein Maturation

The KDEL-containing molecular chaperones present in the IC (BiP, PDI, and GRP94; see above) could be cycling while still bound to their unfolded client proteins, opening for a post-ER level of protein folding and quality control. The presence of quality control machinery in the IC and the finding that the ER-associated degradation of certain proteins requires their ER export is in accordance with this idea (Zuber *et al.*, 2001; Anelli and Sitia, 2008). The PDI family member ERp44 and p58/ERGIC-53 cooperate in the IC/*cis*-Golgi in sequential assembly of IgM polymers (Anelli and Sitia, 2008). Unassembled IgM or T-cell antigen receptor subunits bound to ERp44 or BiP, respectively, can be retrieved to the ER by the KDEL-receptor in a pH-dependent manner (Yamamoto *et al.*, 2001; Vavassori *et al.*, 2013), similarly as the overexpressed, misfolded VSV G-protein (Hammond and Helenius, 1994) and mutant V2 vasopressin receptors (Hermosilla *et al.*, 2004). Additional proof for a post-ER checkpoint is provided by studies showing the accumulation of the deletion mutant ΔF508 of CFTR (Gilbert *et al.*, 1998), misfolded MHC class I proteins (Hsu *et al.*, 1991; Raposo *et al.*, 1995), and proinsulin (Zuber *et al.*, 2004) in expanded IC elements.

## Signaling

The level of p58/ERGIC-53 mRNA is up-regulated by the unfolded protein response (UPR), which also requires yeast Ypt1 function, supporting a link between this signaling pathway and ER–Golgi trafficking (Nyfeler *et al.*, 2003; Tsvetanova *et al.*, 2012). Protein kinases Src, aPKC, and Scyl1 have been

implicated in COPI function at the level of the IC (Tisdale and Artalejo, 2006; Hamlin *et al.*, 2014) and PKC and its downstream effectors appear to control IC morphology (Ben-Tekaya *et al.*, 2010; Sugawara *et al.*, 2012). The activation of neuronal Trk receptor tyrosine kinases in the IC initiates signaling via the MEK pathway leading to Golgi fragmentation (Schecterson *et al.*, 2010).

### Autophagy

Membranes enriched in IC markers (p58/ERGIC-53/LMAN1, KDEL-receptor, and Sec22B) play a role in the biogenesis of autophagosomes by representing the primary membrane source for the lipidation of LC3, which triggers this process (Ge *et al.*, 2013). Moreover, Ypt1/Rab1 is a key regulator of autophagy in yeast and mammals (Lynch-Day *et al.* 2010; Winslow *et al.*, 2010; Zoppino *et al.*, 2010; Huang *et al.*, 2011; Lipatova *et al.*, 2012). A phosphatidylinositol 3-phosphatase (MTMR6) acting in vesicle transport and autophagy is regulated by Rab1B and localizes predominantly to the pcIC (Mochizuki *et al.*, 2013), further supporting the role of the IC in autophagy.

### Phagocytosis and Antigen Presentation

Supporting the existence of an unconventional pathway that connects the ER with the PM-derived phagosome (Gagnon *et al.*, 2002), the IC-enriched SNARE Sec22B/ERS-24 (Zhang *et al.*, 1999) was shown to influence phagocytosis independently of its role in ER–Golgi trafficking (Becker *et al.*, 2005; Hatsuzawa *et al.*, 2009). In dendritic cells the delivery of certain proteins – such as the peptide transporter (TAP), CR and tapasin – from ER to the phagosome is required for antigen cross-presentation. This pathway depends on the interaction between Sec22B and the PM SNARE syntaxin 4 and involves efficient recruitment of the integral IC components sec22B and p58/ERGIC-53 to the phagosomes (Cebrian *et al.*, 2011), in accordance with the idea that the IC provides an important membrane source for their formation. The presence of CR, tapasin, and functional TAP in the IC/*cis*-Golgi support this possibility (Kleijmeer *et al.*, 1992; Howe *et al.*, 2009; Ghanem *et al.*, 2010).

## Links to Disease

The IC plays a role in the Golgi-independent trafficking of CFTR (see above). Mutations in p58/ERGIC-53/LMAN1, or its partner MCFD2 (multiple coagulation factor deficiency protein 2) result in an autosomal recessive bleeding disorder called combined deficiency of coagulation factors V and VIII. They disrupt the lectin mannose-binding protein 1 (LMAN1)-MCFD2 receptor complex, thereby inhibiting the secretion of these factors and reducing their serum levels (Nichols *et al.*, 1998; Zheng and Zhang, 2013). Parkinson's disease-related cytotoxic protein, $\alpha$-synuclein, has been suggested to interfere with ER–Golgi trafficking and to arrest autophagy by inhibiting Rab1 function (Cooper *et al.*, 2006; Winslow *et al.*, 2010). Many phagocytosed bacterial pathogens, such as *Legionella*, hijack the ER-to-IC-to-phagosome pathway during their intracellular replication (Isberg *et al.*, 2009; Arasaki *et al.*, 2012; see above). In addition to coronaviruses, the IC has been implicated in the replication of bunya-, entero-, flavi-, picorna, and vacciniaviruses (Jäntti *et al.*, 1997; Risco *et al.*, 2002; Miller and Krijnse-Locker, 2008; Hsu *et al.*, 2010). Surprisingly, p58/ERGIC-53 interacts in a lectin-independent manner with fusion proteins of a number of membrane viruses and participates in different stages of their life cycle (Klaus *et al.*, 2013).

*See also*: Interorganellar Communication: Interplay and Processes: Endoplasmic Reticulum Stress in Disease; ER–Golgi Transport; Regulation of the Secretory Pathway; Unconventional Protein Secretion: Fibroblast Growth Factor 2 and Interleukin-1$\beta$ as Examples. Intracellular Infectiology: Cell Processes: Phagocytosis. Organelles: Structure and Function: At the Center of Autophagy: Autophagosomes; Golgi and TGN; The Endoplasmic Reticulum

## References

Allan, B.B., Moyer, B.D., Balch, W.E., 2000. Rab1 recruitment of p115 into a *cis*-SNARE complex: Programming budding COPII vesicles for fusion. Science 289, 444–448.

Alvarez, C., Fujita, H., Hubbard, A., Sztul, E., 1999. ER to Golgi transport: Requirement for p115 at a pre-Golgi VTC stage. Journal of Cell Biology 147, 1205–1222.

Alvarez, C., Garcia-Mata, R., Brandon, E., Sztul, E., 2003. COPI recruitment is modulated by a Rab1b-dependent mechanism. Molecular Biology of the Cell 14, 2116–2127.

Alvarez, C., Garcia-Mata, R., Hauri, H.-P., Sztul, E., 2001. The p115-interactive proteins GM130 and giantin participate in endoplasmic reticulum-Golgi traffic. Journal of Biological Chemistry 276, 2693–2700.

Anelli, T., Sitia, R., 2008. Protein quality control in the early secretory pathway. EMBO Journal 27, 315–327.

Appenzeller, C., Andersson, H., Kappeler, F., Hauri, H.P., 1999. The lectin ERGIC-53 is a cargo transport receptor for glycoproteins. Nature Cell Biology 1, 330–334.

Appenzeller-Herzog, C., Hauri, H.P., 2006. The ER-Golgi intermediate compartment (ERGIC): In search of its identity and function. Journal of Cell Science 119, 2173–2183.

Appenzeller-Herzog, C., Roche, A.C., Nufer, O., Hauri, H.P., 2004. pH-induced conversion of the transport lectin ERGIC-53 triggers glycoprotein release. Journal of Biological Chemistry 279, 12943–12950.

Arar, C., Carpentier, V., Le Caer, J.P., *et al.*, 1995. ERGIC-53, a membrane protein of the endoplasmic reticulum-Golgi intermediate compartment, is identical to MR60, an intracellular mannose-specific lectin of myelomonocytic cells. Journal of Biological Chemistry 270, 3551–3553.

Arasaki, K., Toomre, D.K., Roy, C.R., 2012. The *Legionella pneumophila* effector DrrA is sufficient to stimulate SNARE-dependent membrane fusion. Cell Host & Microbe 11, 46–57.

Aridor, M., Balch, W.E., 1996. Principles of selective transport: Coat complexes hold the key. Trends in Cell Biology 6, 315–320.

Bailly, E., McCaffrey, M., Touchot., N., *et al.*, 1991. Phosphorylation of two small GTP-binding proteins of the Rab family by $p34^{cdc2}$. Nature 350, 715–718.

Balch, W.E., Elliott, M.M., Keller, D.D., 1986. ATP-coupled transport of vesicular stomatitis virus G protein between the endoplasmic reticulum and Golgi. Journal of Biological Chemistry 261, 14681–14689.

Balch, W.E., McCaffery, J.M., Plutner, H., Farquhar, M.G., 1994. Vesicular stomatitis virus glycoprotein is sorted and concentrated during export from the endoplasmic reticulum. Cell 76, 841–852.

Bannykh, S.I., Balch, W.E., 1997. Membrane dynamics at the endoplasmic reticulum-Golgi interface. Journal of Cell Biology 138, 1–4.

Bannykh, S.I., Rowe, T., Balch, W.E., 1996. The organization of endoplasmic reticulum export complexes. Journal of Cell Biology 135, 19–35.

Barlowe, C.K., Miller, E.A., 2013. Secretory protein biogenesis and traffic in the early secretory pathway. Genetics 193, 383–410.

Barnekow, A., Thyrock, A., Kessler, D., 2009. Rab proteins and their interaction partners. International Review of Cell and Molecular Biology 274, 235–274.

Beck, R., Rawet, M., Wieland, F.T., Cassel, D., 2009. The COPI system: Molecular mechanisms and function. FEBS Letters 583, 2701–2709.

Becker, T., Volchuk, A., Rothman, J.E., 2005. Differential use of endoplasmic reticulum membrane for phagocytosis in J774 macrophages. Proceedings of the National Academy of Sciences of the United States of America 102, 4022–4026.

Ben-Tekaya, H., Kahn, R.A., Hauri, H.P., 2010. ADP ribosylation factors 1 and 4 and group VIA phospholipase $A_2$ regulate morphology and intraorganellar traffic in the endoplasmic reticulum-Golgi intermediate compartment. Molecular Biology of the Cell 21, 4130–4140.

Ben-Tekaya, H., Miura, K., Pepperkok, R., Hauri, H.P., 2005. Live imaging of bidirectional traffic from the ERGIC. Journal of Cell Science 118, 357–367.

Bentley, M., Nycz, D.C., Joglekar, A., *et al.*, 2010. Vesicular calcium regulates coat retention, fusogenicity, and size of pre-Golgi intermediates. Molecular Biology of the Cell 21, 1033–1046.

Blum, R., Pfeiffer, F., Feick, P., *et al.*, 1999. Intracellular localization and *in vivo* trafficking of p24A and p23. Journal of Cell Science 112, 537–548.

Blum, R., Stephens, D.J., Schulz, I., 2000. Lumenal targeted GFP, used as a marker of soluble cargo, visualises rapid ERGIC to Golgi traffic by a tubulo-vesicular network. Journal of Cell Science 113, 3151–3159.

Bonatti, S., Migliaccio, G., Simons, K., 1989. Palmitylation of viral membrane glycoproteins takes place after exit from the endoplasmic reticulum. Journal of Biological Chemistry 264, 12590–12595.

Brandizzi, F., Barlowe, C., 2013. Organization of the ER-Golgi interface for membrane traffic control. Nature Reviews Molecular Cell Biology 14, 382–392.

Breuza, L., Halbeisen, R., Jenö, P., *et al.*, 2004. Proteomics of endoplasmic reticulum-Golgi intermediate compartment (ERGIC) membranes from brefeldin A-treated HepG2 cells identifies ERGIC-32, a new cycling protein that interacts with human Erv46. Journal of Biological Chemistry 279, 47242–47253.

Bu, G., Geuze, H.J., Strous, G.J., Schwartz, A.L., 1995. Binding and endocytosis of 39 kDa protein by MDBK cells. EMBO Journal 14, 2269–2280.

Burkhardt, J.K., Echeverri, C.J., Nilsson, T., Vallee, R.B., 1997. Overexpression of the dynamitin (p50) subunit of the dynactin complex disrupts dynein-dependent maintenance of membrane organelle distribution. Journal of Cell Biology 139, 469–484.

Cai, H., Reinisch, K., Ferro-Novick, S., 2007. Coats, tethers, Rabs, and SNAREs work together to mediate the intracellular destination of a transport vesicle. Developmental Cell 12, 671–682.

Campellone, K.G., Webb, N.J., Znameroski, E.A., Welch, M.D., 2008. WHAMM is an Arp2/3 complex activator that binds microtubules and functions in ER to Golgi transport. Cell 134, 148–161.

Cebrian, I., Visentin, G., Blanchard, N., *et al.*, 2011. Sec22b regulates phagosomal maturation and antigen crosspresentation by dendritic cells. Cell 147, 1355–1368.

Chao, D.S., Hay, J.C., Winnick, S., *et al.*, 1999. SNARE membrane trafficking dynamics *in vivo*. Journal of Cell Biology 144, 869–881.

Chavrier, P., Parton, R.G., Hauri, H.-P., Simons, K., Zerial, M., 1990. Localization of low molecular weight GTP binding proteins to exocytic and endocytic compartments. Cell 62, 317–329.

Chen, J.L., Fucini, R.V., Lacomis, L., *et al.*, 2005. Coatomer-bound Cdc42 regulates dynein recruitment to COPI vesicles. Journal of Cell Biology 169, 383–389.

Chun, J., Shapovalova, Z., Dejgaard, S.Y., Presley, J.F., Melançon, P., 2008. Characterization of class I and class II ADP-ribosylation factors in live cells: GDP-bound class II Arfs associate with the ER-Golgi intermediate compartment independently of GBF1. Molecular Biology of the Cell 19, 3488–3500.

Cole, N.B., Sciaky, N., Marotta, A., Song, J., Lippincott-Schwartz, J., 1996. Golgi dispersal during microtubule disruption: Regeneration of Golgi stacks at peripheral endoplasmic reticulum exit sites. Molecular Biology of the Cell 7, 631–650.

Connolly, C.N., Futter, C.E., Gibson, A., Hopkins, C.R., Cutler, D.F., 1994. Transport into and out of the Golgi complex studied by transfecting cells with cDNAs encoding horseradish peroxidase. Journal of Cell Biology 127, 641–652.

Cooper, A.A., Gitler, A.D., Cashikar, A., *et al.*, 2006. Alpha-synuclein blocks ER-Golgi traffic and Rab1 rescues neuron loss in Parkinson's models. Science 313, 324–328.

Cosson, P., Letourneur, F., 1997. Coatomer (COPI)-coated vesicles: Role in intracellular transport and protein sorting. Current Opinion in Cell Biology 9, 484–487.

Dahm, T., White, J., Grill, S., Füllekrug, J., Stelzer, E.H., 2001. Quantitative ER ↔ Golgi transport kinetics and protein separation upon Golgi exit revealed by vesicular integral membrane protein 36 dynamics in live cells. Molecular Biology of the Cell 12, 1481–1498.

Day, K.J., Staehelin, L.A., Glick, B.S., 2013. A three-stage model of Golgi structure and function. Histochemistry and Cell Biology 140, 239–249.

Diao, A., Rahman, D., Pappin, D.J.C., Lucocq, J., Lowe, M., 2003. The coiled-coil membrane protein golgin-84 is a novel Rab effector required for Golgi ribbon formation. Journal of Cell Biology 160, 201–212.

Dittmer, F., von Figura, K., 1999. Phosphorylation of arylsulphatase A occurs through multiple interactions with the UDP-N-acetylglucosamine-1-phosphotransferase proximal and distal to its retrieval site by the KDEL receptor. Biochemical Journal 340, 729–736.

Dominguez, M., Dejgaard, K., Füllekrug, J., *et al.*, 1998. gp25L/emp24/p24 protein family members of the cis-Golgi network bind both COP I and II coatomer. Journal of Cell Biology 140, 751–765.

Duden, R., Griffiths, G., Frank, R., Argos, P., Kreis, T.E., 1991. Beta-COP, a 110 kd protein associated with non-clathrin-coated vesicles and the Golgi complex, shows homology to beta-adaptin. Cell 64.649-565.

Duijsings, D., Lanke, K.H., van Dooren, S.H., *et al.*, 2009. Differential membrane association properties and regulation of class I and class II Arfs. Traffic 10, 316–323.

Ehlers, M.D., 2013. Dendritic trafficking for neuronal growth and plasticity. Biochemical Society Transactions 41, 1365–1382.

Emery, G., Parton, R.G., Rojo, M., Gruenberg, J., 2003. The transmembrane protein p25 forms highly specialized domains that regulate membrane composition and dynamics. Journal of Cell Science 116, 4821–4832.

Fan, J.Y., Roth, J., Zuber, C., 2003. Ultrastructural analysis of transitional endoplasmic reticulum and pre-Golgi intermediates: A highway for cars and trucks. Histochemistry and Cell Biology 120, 455–463.

Farhan, H., Reiterer, V., Kriz, A., *et al.*, 2008. Signal-dependent export of GABA transporter 1 from the ER-Golgi intermediate compartment is specified by a C-terminal motif. Journal of Cell Science 121, 753–761.

Fiedler, K., Simons, K., 1994. A putative novel class of animal lectins in the secretory pathway homologous to leguminous lectins. Cell 77, 625–626.

Friend, D.S., Murray, M.J., 1965. Osmium impregnation of the Golgi apparatus. American Journal of Anatomy 117, 135–149.

Füllekrug, J., Sönnichsen, B., Schäfer, U., *et al.*, 1997. Characterization of brefeldin A-induced vesicular structures containing cycling proteins of the intermediate compartment/*cis*-Golgi network. FEBS Letters 404, 75–81.

Gagnon, E., Duclos, S., Rondeau, C., *et al.*, 2002. Endoplasmic reticulum-mediated phagocytosis is a mechanism of entry into macrophages. Cell 110, 119–131.

Garcia-Mata, R., Szul, T., Alvarez, C., Sztul, E., 2003. ADP-ribosylation factor/COPI-dependent events at the endoplasmic reticulum-Golgi interface are regulated by the guanine nucleotide exchange factor GBF1. Molecular Biology of the Cell 14, 2250–2261.

Ge, L., Melville, D., Zhang, M., Schekman, R., 2013. The ER-Golgi intermediate compartment is a key membrane source for the LC3 lipidation step of autophagosome biogenesis. Elife 2, e00947. doi:10.7554/eLife.00947.

Gee, H.Y., Noh, S.H., Tang, B.L., Kim, K.H., Lee, M.G., 2011. Rescue of ΔF508-CFTR trafficking via a GRASP-dependent unconventional secretion pathway. Cell 146, 746–760.

Ghanem, E., Fritzsche, S., Al-Balushi, M., *et al.*, 2010. The transporter associated with antigen processing (TAP) is active in a post-ER compartment. Journal of Cell Science 123, 4271–4279.

Gilbert, A., Jadot., M., Leontieva, E., *et al.*, 1998. Delta F508 CFTR localizes in the endoplasmic reticulum-Golgi intermediate compartment in cystic fibrosis cells. Experimental Cell Research 242, 144–152.

Gill, D.J., Chia, J., Senewiratne, J., Bard, F., 2010. Regulation of O-glycosylation through Golgi-to-ER relocation of initiation enzymes. Journal of Cell Biology 189, 843–858.

Gommel, D., Orci, L., Emig, E.M., *et al.*, 1999. p24 and p23, the major transmembrane proteins of COPI-coated transport vesicles, form hetero-oligomeric complexes and cycle between the organelles of the early secretory pathway. FEBS Letters 447, 179–185.

Griffiths, G., Ericsson, M., Krijnse-Locker, J., *et al.*, 1994. Localization of the Lys, Asp, Glu, Leu tetrapeptide receptor to the Golgi complex and the intermediate compartment in mammalian cells. Journal of Cell Biology 127, 1557–1574.

Griffiths, G., Pepperkok, R., Locker, J.K., Kreis, T.E., 1995. Immunocytochemical localization of beta-COP to the ER-Golgi boundary and the TGN. Journal of Cell Science 108, 2839–2856.

Hamlin, J.N., Schroeder, L.K., Fotouhi, M., *et al.*, 2014. Scyl1 scaffolds class II Arfs to specific subcomplexes of coatomer through the γ-COP appendage domain. Journal of Cell Science 127, 1454–1463.

Hammond, A.T., Glick, B.S., 2000. Dynamics of transitional endoplasmic reticulum sites in vertebrate cells. Molecular Biology of the Cell 11, 3013–3030.
Hammond, C., Helenius, A., 1994. Quality control in the secretory pathway: Retention of a misfolded viral membrane glycoprotein involves cycling between the ER, intermediate compartment, and Golgi apparatus. Journal of Cell Biology 126, 41–52.
Hatsuzawa, K., Hashimoto, H., Hashimoto, H., *et al.*, 2009. Sec22b is a negative regulator of phagocytosis in macrophages. Molecular Biology of the Cell 20, 4435–4443.
Haubruck, H., Prange, R., Vorgias, C., Gallwitz, D., 1989. The ras-related mouse ypt1 protein can functionally replace the YPT1 gene product in yeast. EMBO Journal 8, 1427–1432.
Hauri, H.P., Kappeler, F., Andersson, H., Appenzeller, C., 2000. ERGIC-53 and traffic in the secretory pathway. Journal of Cell Science 113, 587–596.
Hay, J.C., Klumperman, J., Oorschot, V., *et al.*, 1998. Localization, dynamics, and protein interactions reveal distinct roles for ER and Golgi SNAREs. Journal of Cell Biology 141, 1489–1502.
Heino, S., Lusa, S., Somerharju, P., *et al.*, 2000. Dissecting the role of the Golgi complex and lipid rafts in biosynthetic transport of cholesterol to the cell surface. Proceedings of the National Academy of Sciences of the United States of America 97, 8375–8380.
Hermosilla, R., Oueslati, M., Donalies, U., *et al.*, 2004. Disease-causing V(2) vasopressin receptors are retained in different compartments of the early secretory pathway. Traffic 5, 993–1005.
Horstman, H., Ng, C.P., Tang, B.L., Hong, W., 2002. Ultrastructural characterization of endoplasmic reticulum-Golgi transport containers (EGTC). Journal of Cell Science 115, 4263–4273.
Hosobuchi, M., Kreis, T., Schekman, R., 1992. SEC21 is a gene required for ER to Golgi protein transport that encodes a subunit of a yeast coatomer. Nature 360, 603–605.
Howe, C., Garstka, M., Al-Balushi, M., *et al.*, 2009. Calreticulin-dependent recycling in the early secretory pathway mediates optimal peptide loading of MHC class I molecules. EMBO Journal 28, 3730–3744.
Hsu, N.Y., Ilnytska, O., Belov, G., *et al.*, 2010. Viral reorganization of the secretory pathway generates distinct organelles for RNA replication. Cell 141, 799–811.
Hsu, V.W., Yuan, L.C., Nuchtern, J.G., *et al.*, 1991. A recycling pathway between the endoplasmic reticulum and the Golgi apparatus for retention of unassembled MHC class I molecules. Nature 352, 441–444.
Huang, J., Birmingham, C.L., Shahnazari, S., *et al.*, 2011. Antibacterial autophagy occurs at PI(3)P-enriched domains of the endoplasmic reticulum and requires Rab1 GTPase. Autophagy 7, 17–26.
Isberg, R.R., O'Connor, T.J., Heidtman, M., 2009. The Legionella pneumophila replication vacuole: Making a cosy niche inside host cells. Nature Reviews Microbiology 7, 13–24.
Itin, C., Roche, A.-C., Monsigny, M., Hauri, H.-P., 1996. ERGIC-53 is a functional mannose-selective and calcium-dependent human homologue of leguminous lectins. Molecular Biology of the Cell 7, 483–493.
Jackson, M.R., Nilsson, T., Peterson, P.A., 1990. Identification of a consensus motif for retention of transmembrane proteins in the endoplasmic reticulum. EMBO Journal 9, 3153–3162.
Jackson, M.R., Nilsson, T., Peterson, P.A., 1993. Retrieval of transmembrane proteins to the endoplasmic reticulum. Journal of Cell Biology 121, 317–333.
Jamieson, J.D., Palade, G.E., 1967. Intracellular transport of secretory proteins in the pancreatic exocrine cell. I. Role of the peripheral elements of the Golgi complex. Journal of Cell Biology 34, 577–596.
Jäntti, J., Hildén, P., Rönkä, H., *et al.*, 1997. Immunocytochemical analysis of Uukuniemi virus budding compartments: Role of the intermediate compartment and the Golgi stack in virus maturation. Journal of Virology 71, 1162–1172.
Jarvela, T., Linstedt, A.D., 2012. Irradiation-induced protein inactivation reveals Golgi enzyme cycling to cell periphery. Journal of Cell Science 125, 973–980.
Jönsson, M., Eklund, E., Fransson, L.A., Oldberg, A., 2003. Initiation of the decorin glycosaminoglycan chain in the endoplasmic reticulum-Golgi intermediate compartment. Journal of Biological Chemistry 278, 21415–21420.
Kamena, F., Diefenbacher, M., Kilchert, C., Schwarz, H., Spang, A., 2008. Ypt1p is essential for retrograde Golgi-ER transport and for Golgi maintenance in S. cerevisiae. Journal of Cell Science 121, 1293–1302.
Kano, F., Yamauchi, S., Yoshida, Y., *et al.*, 2009. Yip1A regulates the COPI-independent retrograde transport from the Golgi complex to the ER. Journal of Cell Science 122, 2218–2227.
Kappeler, F., Klopfenstein, D.R., Foguet, M., Paccaud, J.P., Hauri, H.-P., 1997. The recycling of ERGIC-53 in the early secretory pathway. ERGIC-53 carries a cytosolic endoplasmic reticulum-exit determinant interacting with COPII. Journal of Biological Chemistry 272, 31801–31808.
Kawamoto, K., Yoshida, Y., Tamaki, H., *et al.*, 2002. GBF1, a guanine nucleotide exchange factor for ADP-ribosylation factors, is localized to the *cis*-Golgi and involved in membrane association of the COPI coat. Traffic 3, 483–495.
Klaus, J.P., Eisenhauer, P., Russo, J., *et al.*, 2013. The intracellular cargo receptor ERGIC-53 is required for the production of infectious arenavirus, coronavirus, and filovirus particles. Cell Host & Microbe 14, 522–534.
Kleijmeer, M.J., Kelly, A., Geuze, H.J., *et al.*, 1992. Location of MHC-encoded transporters in the endoplasmic reticulum and *cis*-Golgi. Nature 357, 342–344.
Klumperman, J., Locker, J.K., Meijer, A., *et al.*, 1994. Coronavirus M proteins accumulate in the Golgi complex beyond the site of virion budding. Journal of Virology 68, 6523–6534.
Klumperman, J., Schweizer, A., Clausen, H., *et al.*, 1998. The recycling pathway of protein ERGIC-53 and dynamics of the ER-Golgi intermediate compartment. Journal of Cell Science 111, 3411–3425.
Krijnse-Locker, J., Ericsson, M., Rottier, P.J., Griffiths, G., 1994. Characterization of the budding compartment of mouse hepatitis virus: Evidence that transport from the RER to the Golgi complex requires only one vesicular transport step. Journal of Cell Biology 124, 55–70.
Krijnse-Locker, J., Parton, R.G., Fuller, S.D., Griffiths, G., Dotti, C.G., 1995. The organization of the endoplasmic reticulum and the intermediate compartment in cultured rat hippocampal neurons. Molecular Biology of the Cell 6, 1315–1332.
Kuismanen, E., Saraste, J., 1989. Low temperature-induced transport blocks as tools to manipulate membrane traffic. Methods in Cell Biology 32, 257–274.
Ladinsky, M.S., Mastronarde, D.N., McIntosh, J.R., Howell, K.E., Staehelin, L.A., 1999. Golgi structure in three dimensions: Functional insights from the normal rat kidney cell. Journal of Cell Biology 144, 1135–1149.
Lahtinen, U., Dahllöf, B., Saraste, J., 1992. Characterization of a 58 kDa *cis*-Golgi protein in pancreatic exocrine cells. Journal of Cell Science 103, 321–333.
Lahtinen, U., Hellman, U., Wernstedt, C., Saraste, J., Pettersson, R.F., 1996. Molecular cloning and expression of a 58-kDa cis-Golgi and intermediate compartment protein. Journal of Biological Chemistry 271, 4031–4037.
Lavoie, C., Paiement, J., Dominguez, M., *et al.*, 1999. Roles for alpha(2)p24 and COPI in endoplasmic reticulum cargo exit site formation. Journal of Cell Biology 146, 285–299.
Lazzarino, D.A., Gabel, C.A., 1988. Biosynthesis of the mannose-6-phosphate recognition marker in transport-impaired mouse lymphoma cells. Demonstration of a two-step phosphorylation. Journal of Biological Chemistry 263, 10118–10126.
Lee, M.C., Miller, E.A., Goldberg, J., Orci, L., Schekman, R., 2004. Bi-directional protein transport between the ER and Golgi. Annual Review of Cell and Developmental Biology 20, 87–123.
Lewis, M.J., Pelham, H.R., 1990. A human homologue of the yeast HDEL receptor. Nature 348, 162–163.
Lin, C.C., Love, H.D., Gushue, J.N., Bergeron, J.J., Ostermann, J., 1999. ER/Golgi intermediates acquire Golgi enzymes by brefeldin A-sensitive retrograde transport *in vitro*. Journal of Cell Biology 147, 1457–1472.
Lipatova, Z., Belogortseva, N., Zhang, X.Q., *et al.*, 2012. Regulation of selective autophagy onset by a Ypt/Rab GTPase module. Proceedings of the National Academy of Sciences of the United States of America 109, 6981–6986.
Lippincott-Schwartz, J., Cole, N.B., Marotta, A., Conrad, P.A., Bloom, G.S., 1995. Kinesin is the motor for microtubule-mediated Golgi-to-ER membrane traffic. Journal of Cell Biology 128, 293–306.
Lippincott-Schwartz, J., Donaldson, J.G., Schweizer, A., *et al.*, 1990. Microtubule-dependent retrograde transport of proteins into the ER in the presence of brefeldin A suggests an ER recycling pathway. Cell 60, 821–836.
Lotti, L.V., Torrisi, M.R., Pascale, M.C., Bonatti, S., 1992. Immunocytochemical analysis of the transfer of vesicular stomatitis virus G glycoprotein from the intermediate compartment to the Golgi complex. Journal of Cell Biology 118, 43–50.
Luna, A., Matas, O.B., Martínez-Menárguez, J.A., *et al.*, 2002. Regulation of protein transport from the Golgi complex to the endoplasmic reticulum by CDC42 and N-WASP. Molecular Biology of the Cell 13, 866–879.
Lynch-Day, M.A., Bhandari, D., Menon, S., *et al.*, 2010. Trs85 directs a Ypt1 GEF, TRAPPIII, to the phagophore to promote autophagy. Proceedings of the National Academy of Sciences of the United States of America 107, 7811–7816.
Majoul, I., Straub, M., Hell, S.W., Duden, R., Söling, H.D., 2001. KDEL-cargo regulates interactions between proteins involved in COPI vesicle traffic: Measurements in living cells using FRET. Developmental Cell 1, 139–153.
Malsam, J., Satoh, A., Pelletier, L., Warren, G., 2005. Golgin tethers define subpopulations of COPI vesicles. Science 307, 1095–1098.

Manolea, F., Claude, A., Chun, J., Rosas, J., Melancon, P., 2008. Distinct functions for Arf nucleotide exchange factors at the Golgi complex: GBF1 and BIGs are required for assembly and maintenance of the Golgi stack and TGN, respectively. Molecular Biology of the Cell 19, 523–535.

Marie, M., Dale, H.A., Kouprina, N., Saraste, J., 2012. Division of the intermediate compartment at the onset of mitosis provides a mechanism for Golgi inheritance. Journal of Cell Science 125, 5403–5416.

Marie, M., Dale, H.A., Sannerud, R., Saraste, J., 2009. The function of the intermediate compartment in pre-Golgi trafficking involves its stable connection with the centrosome. Molecular Biology of the Cell 20, 4458–4470.

Marie, M., Sannerud, R., Dale, H.A., Saraste, J., 2008. Take the 'A' train: On fast tracks to the cell surface. Cellular and Molecular Life Sciences 65, 2859–2874.

Marra, P., Maffucci, T., Daniele, T., *et al.*, 2001. The GM130 and GRASP65 Golgi proteins cycle through and define a subdomain of the intermediate compartment. Nature Cell Biology 3, 1101–1113.

Marra, P., Salvatore, L., Mironov Jr., A., *et al.*, 2007. The biogenesis of the Golgi ribbon: The roles of membrane input from the ER and of GM130. Molecular Biology of the Cell 18, 1595–1608.

Martínez-Menárguez, J.A., Geuze, H.J., Slot, J.W., Klumperman, J., 1999. Vesicular tubular clusters between the ER and Golgi mediate concentration of soluble secretory proteins by exclusion from COPI-coated vesicles. Cell 98, 81–90.

Martinez-Menárguez, J.A., Prekeris, R., Oorschot, V.M., *et al.*, 2001. Peri-Golgi vesicles contain retrograde but not anterograde proteins consistent with the cisternal progression model of intra-Golgi transport. Journal of Cell Biology 155, 1213–1224.

Miller, S., Krijnse-Locker, J., 2008. Modification of intracellular membrane structures for virus replication. Nature Reviews Microbiology 6, 363–374.

Mironov, A.A., Mironov Jr., A.A., Beznoussenko, G.V., *et al.*, 2003. ER-to-Golgi carriers arise through direct en bloc protrusion and multistage maturation of specialized ER exit domains. Developmental Cell 5, 583–594.

Mochizuki, Y., Ohashi, R., Kawamura, T., *et al.*, 2013. Phosphatidylinositol 3-phosphatase myotubularin-related protein 6 (MTMR6) is regulated by small GTPase Rab1B in the early secretory and autophagic pathways. Journal of Biological Chemistry 288, 1009–1021.

Monetta, P., Slavin, I., Romero, N., Alvarez, C., 2007. Rab1B interacts with GBF1 and modulates both ARF1 dynamics and COPI association. Molecular Biology of the Cell 18, 2400–2410.

Munro, S., 2011. The golgin coiled-coil proteins of the Golgi apparatus. Cold Spring Harbor Perspectives in Biology. doi:10.1101/cshperspect.a005256.

Munro, S., Pelham, H.R., 1987. A *C*-terminal signal prevents secretion of luminal ER proteins. Cell 48, 899–907.

Murshid, A., Presley, J.F., 2004. ER-to-Golgi transport and cytoskeletal interactions in animal cells. Cellular and Molecular Life Sciences 61, 133–145.

Neve, E.P., Lahtinen, U., Pettersson, R.F., 2005. Oligomerization and interacellular localization of the glycoprotein receptor ERGIC-53 is independent of disulfide bonds. Journal of Molecular Biology 354, 556–568.

Nichols, W.C., Seligsohn, U., Zivelin, A., *et al.*, 1998. Mutations in the ER-Golgi intermediate compartment protein ERGIC-53 cause combined deficiency of coagulation factors V and VIII. Cell 93, 61–70.

Nilsson, T., Jackson, M., Peterson, P.A., 1989. Short cytoplasmic sequences serve as retention signals for transmembrane proteins in the endoplasmic reticulum. Cell 58, 707–718.

Niu, T.K., Pfeifer, A.C., Lippincott-Schwartz, J., Jackson, C.L., 2005. Dynamics of GBF1, a brefeldin A-sensitive Arf1 exchange factor at the Golgi. Molecular Biology of the Cell 16, 1213–1222.

Nyfeler, B., Nufer, O., Matsui, T., Mori, K., Hauri, H.-P., 2003. The cargo receptor ERGIC-53 is a target of the unfolded protein response. Biochemical and Biophysical Research Communications 304, 599–604.

Oprins, A., Duden, R., Kreis, T.E., Geuze, H.J., Slot, J.W., 1993. Beta-COP localizes mainly to the *cis*-Golgi side in exocrine pancreas. Journal of Cell Biology 121, 49–59.

Oprins, A., Rabouille, C., Posthuma, G., *et al.*, 2001. The ER-to-Golgi interface is the major concentration site of secretory proteins in the exocrine pancreatic cell. Traffic 2, 831–838.

Orci, L., Stamnes, M., Ravazzola, M., *et al.*, 1997. Bidirectional transport by distinct populations of COPI-coated vesicles. Cell 90, 335–349.

Palmer, K.J., Watson, P., Stephens, D.J., 2005. The role of microtubules in transport between the endoplasmic reticulum and Golgi apparatus in mammalian cells. Biochemical Society Symposium 72, 1–13.

Palokangas, H., Ying, M., Väänänen, K., Saraste, J., 1998. Retrograde transport from the pre-Golgi intermediate compartment and the Golgi complex is affected by the vacuolar H + -ATPase inhibitor bafilomycin A1. Molecular Biology of the Cell 9, 3561–3578.

Paroutis, P., Touret, N., Grinstein, S., 2004. The pH of the secretory pathway: Measurement, determinants, and regulation. Physiology (Bethesda) 19, 207–215.

Pelham, H.R., 1988. Evidence that luminal ER proteins are sorted from secreted proteins in a post-ER compartment. EMBO Journal 7, 913–918.

Pelham, H.R., 1989. Control of protein exit from the endoplasmic reticulum. Annual Review of Cell and Developmental Biology 5, 1–23.

Pepperkok, R., Scheel, J., Horstmann, H., *et al.*, 1993. Beta-COP is essential for biosynthetic membrane transport from the endoplasmic reticulum to the Golgi complex *in vivo*. Cell 74, 71–82.

Peter, F., Plutner, H., Zhu, H., Kreis, T.E., Balch, W.E., 1993. β-COP is essential for transport of protein from the endoplasmic reticulum to the Golgi *in vitro*. Journal of Cell Biology 122, 1155–1167.

Plutner, H., Cox, A.D., Pind, S., *et al.*, 1991. Rab1b regulates vesicular transport between the endoplasmic reticulum and successive Golgi compartments. Journal of Cell Biology 115, 31–43.

Plutner, H., Davidson, H.W., Saraste, J., Balch, W.E., 1992. Morphological analysis of protein transport from the ER to Golgi membranes in digitonin-permeabilized cells: Role of the p58- containing compartment. Journal of Cell Biology 119, 1097–1116.

Popoff, V., Langer, J.D., Reckmann, I., *et al.*, 2011. Several ADP-ribosylation factor (Arf) isoforms support COPI vesicle formation. Journal of Biological Chemistry 286, 35634–35642.

Presley, J., Ward, T.H., Pfeffer, A.C., *et al.*, 2002. Dissection of COPI and Arf1 dynamics *in vivo* and role in Golgi membrane transport. Nature 417, 187–193.

Presley, J.F., Cole, N.B., Schroer, T.A., *et al.*, 1997. ER-to-Golgi transport visualized in living cells. Nature 389, 81–85.

Presley, J.F., Smith, C., Hirschberg, K., *et al.*, 1998. Golgi membrane dynamics. Molecular Biology of the Cell 9, 1617–1626.

Rabouille, C., Klumperman, J., 2005. The maturing role of COPI vesicles in intra-Golgi transport. Nature Reviews Molecular Cell Biology 6, 812–817.

Rambourg, A., Clermont, Y., Marraud, A., 1974. Three-dimensional structure of the osmium-impregnated Golgi apparatus as seen in the high voltage electron microscope. American Journal of Anatomy 140, 27–45.

Raposo, G., van Santen, H.M., Leijendekker, R., Geuze, H.J., Ploegh, H.L., 1995. Misfolded major histocompatibility complex class I molecules accumulate in an expanded ER-Golgi intermediate compartment. Journal of Cell Biology 131, 1403–1419.

Rios, R.M., Sanchis, A., Tassin, A.M., Fedriani, C., Bornens, M., 2004. GMAP-210 recruits γ-tubulin complexes to *cis*-Golgi membranes and is required for Golgi ribbon formation. Cell 118, 323–335.

Risco, C., Rodríguez, J.R., López-Iglesias, C., *et al.*, 2002. Endoplasmic reticulum-Golgi intermediate compartment membranes and vimentin filaments participate in vaccinia virus assembly. Journal of Virology 76, 1839–1855.

Roghi, C., Allan, V.J., 1999. Dynamic association of cytoplasmic dynein heavy chain 1a with the Golgi apparatus and intermediate compartment. Journal of Cell Science 112, 4673–4685.

Rojo, M., Pepperkok, R., Emery, G., *et al.*, 1997. Involvement of the transmembrane protein p23 in biosynthetic protein transport. Journal of Cell Biology 139, 1119–1135.

Sáenz, J.B., Sun, W.J., Chang, J.W., *et al.*, 2009. Golgicide A reveals essential roles for GBF1 in Golgi assembly and function. Nature Chemical Biology 5, 157–165.

Sannerud, R., Marie, M., Nizak, C., *et al.*, 2006. Rab1 defines a novel pathway connecting the pre-Golgi intermediate compartment with the cell periphery. Molecular Biology of the Cell 17, 1514–1526.

Saraste, J., Dale, H.A., Bazzocco, S., Marie., M., 2009. Emerging new roles of the pre-Golgi intermediate compartment in biosynthetic-secretory trafficking. FEBS Letters 583, 3804–3810.

Saraste, J., Goud, B., 2007. Functional symmetry of endomembranes. Molecular Biology of the Cell 18, 1430–1436.

Saraste, J., Kuismanen, E., 1984. Pre- and post-Golgi vacuoles operate in the transport of Semliki Forest virus membrane glycoproteins to the cell surface. Cell 38, 535–549.

Saraste, J., Kuismanen, E., 1992. Pathways of protein sorting and membrane traffic between the rough endoplasmic reticulum and the Golgi complex. Seminars in Cell Biology 3, 343–355.

Saraste, J., Lahtinen, U., Goud, B., 1995. Localization of the small GTP-binding protein Rab1 to early compartments of the secretory pathway. Journal of Cell Science 108, 1541–1552.

Saraste, J., Palade, G.E., Farquhar, M.G., 1986. Temperature-sensitive steps in the transport of secretory proteins through the Golgi complex in exocrine pancreatic

cells. Proceedings of the National Academy of Sciences of the United States of America 83, 6425–6429.
Saraste, J., Palade, G.E., Farquhar, M.G., 1987. Antibodies to rat pancreas Golgi subfractions: Identification of a 58 kDa *cis*-Golgi protein. Journal of Cell Biology 105, 2021–2029.
Saraste, J., Svensson, K., 1991. Distribution of the intermediate elements operating in ER to Golgi transport. Journal of Cell Science 100, 415–430.
Satoh, A., Wang, Y., Malsam, J., Beard, M.B., Warren, G., 2003. Golgin-84 is a Rab1 binding partner involved in Golgi structure. Traffic 4, 153–161.
Satoh, M., Hirayoshi, K., Yokota, S., Hosokawa, N., Nagata, K., 1996. Intracellular interaction of collagen-specific stress protein HSP47 with newly synthesized procollagen. Journal of Cell Biology 133, 469–483.
Scales, S.J., Pepperkok, R., Kreis, T.E., 1997. Visualization of ER-to-Golgi transport in living cells reveals a sequential mode of action for COPII and COPI. Cell 90, 1137–1148.
Schecterson, L.C., Hudson, M.P., Ko, M., *et al.*, 2010. Trk activation in the secretory pathway promotes Golgi fragmentation. Molecular and Cellular Neuroscience 43, 403–413.
Schimmöller, F., Singer-Krüger, B., Schröder, S., *et al.*, 1995. The absence of Emp24p, a component of ER-derived COPII-coated vesicles, causes a defect in transport of selected proteins to the Golgi. EMBO Journal 14, 1329–1339.
Schindler, R., Itin, C., Zerial, M., Lottspeich, F., Hauri, H.-P., 1993. ERGIC-53, a membrane protein of the ER-Golgi intermediate compartment, carries an ER retention motif. European Journal of Cell Biology 61, 1–9.
Schweizer, A., Fransen, J.A., Bächi, T., Ginsel, L., Hauri, H.P., 1988. Identification, by a monoclonal antibody, of a 53-kD protein associated with a tubulo-vesicular compartment at the *cis*-side of the Golgi apparatus. Journal of Cell Biology 107, 1643–1653.
Schweizer, A., Fransen, J.A., Matter, K., *et al.*, 1990. Identification of an intermediate compartment involved in protein transport from endoplasmic reticulum to Golgi apparatus. European Journal of Cell Biology 53, 185–196.
Segev, N., 2001. Ypt and Rab GTPases: Insight into functions through novel interactions. Current Opinion in Cell Biology 13, 500–511.
Sesso, A., de Faria, F.P., Iwamura, E.S., Corrêa, H., 1994. A three-dimensional reconstruction study of the rough ER-Golgi interface in serial thin sections of the pancreatic acinar cell of the rat. Journal of Cell Science 107, 517–528.
Shima, D.T., Scales, S.J., Kreis, T.E., Pepperkok, R., 1999. Segregation of COPI-rich and anterograde-cargo-rich domains in endoplasmic-reticulum-to-Golgi transport complexes. Current Biology 9, 821–824.
Short, B., Preisinger, C., Körner, R., *et al.*, 2001. A GRASP55-rab2 effector complex linking Golgi structure to membrane traffic. Journal of Cell Biology 155, 877–883.
Simpson, J.C., Nilsson, T., Pepperkok, R., 2006. Biogenesis of tubular ER-to-Golgi transport intermediates. Molecular Biology of the Cell 17, 723–737.
Sinka, R., Gillingham, A.K., Kondylis, V., Munro, S., 2008. Golgi coiled-coil proteins contain multiple binding sites for Rab family G proteins. Journal of Cell Biology 183, 607–615.
Stamnes, M.A., Craighead, M.M., Hoe, M.H., *et al.*, 1995. An integral membrane component of coatomer-coated transport vesicles defines a family of proteins involved in budding. Proceedings of the National Academy of Sciences of the United States of America 98, 8011–8015.
Stauber, T., Simpson, J.C., Pepperkok, R., Vernos, I., 2006. A role for kinesin-2 in COPI-dependent recycling between the ER and the Golgi complex. Current Biology 16, 2245–2251.
Stephens, D.J., Lin-Marq, N., Pagano, A., Pepperkok, R., Paccaud, J.P., 2000. COPI-coated ER-to-Golgi transport complexes segregate from COPII in close proximity to ER exit sites. Journal of Cell Science 113, 2177–2185.
Stephens, D.J., Pepperkok, R., 2001. Illuminating the secretory pathway: When do we need vesicles? Journal of Cell Science 114, 1053–1059.
Stephens, D.J., Pepperkok, R., 2002. Imaging of procollagen transport reveals COPI-dependent cargo sorting during ER-to-Golgi transport in mammalian cells. Journal of Cell Science 115, 1149–1160.
Stinchcombe, J.C., Nomoto, H., Cutler, D.F., Hopkins, C.R., 1995. Anterograde and retrograde traffic between the rough endoplasmic reticulum and the Golgi complex. Journal of Cell Biology 131, 1387–1401.
Storrie, B., White, J., Röttger, S., *et al.*, 1998. Recycling of Golgi-resident glycosyltransferases through the ER reveals a novel pathway and provides an explanation for nocodazole-induced Golgi scattering. Journal of Cell Biology 143, 1505–1521.
Strating, J.R., Martens, G.J., 2009. The p24 family and selective transport processes at the ER-Golgi interface. Biology of the Cell 101, 495–509.
Sugawara, T., Nakatsu, D., Kii, H., *et al.*, 2012. PKCδ and ε regulate the morphological integrity of the ER-Golgi intermediate compartment (ERGIC) but not the anterograde and retrograde transports via the Golgi apparatus. Biochimica et Biophysica Acta 1823, 861–875.
Sztul, E., Lupashin, V., 2009. Role of vesicle tethering factors in ER-Golgi membrane traffic. FEBS Letters 583, 3770–3783.
Szul, T., Grabski, R., Lyons, S., *et al.*, 2007. Dissecting the role of the ARF guanine nucleotide exchange factor GBF1 in Golgi biogenesis and protein trafficking. Journal of Cell Science 120, 3929–3940.
Tang, B.L., Low, S.H., Hauri, H.-P., Hong, W., 1995a. Segregation of ERGIC-53 and the mammalian KDEL-receptor upon exit from the 15 °C compartment. European Journal of Cell Biology 68, 398–410.
Tang, B.L., Low, S.H., Hong, W., 1995b. Differential response of resident proteins and cycling proteins of the Golgi to brefeldin A. European Journal of Cell Biology 68, 199–205.
Tang, B.L., Wang, Y., Ong, Y.S., Hong, W., 2005. COPII and exit from the endoplasmic reticulum. Biochimica et Biophysica Acta 1744, 293–303.
Tang, B.L., Wong, S.H., Qi, X.L., Low, S.H., Hong, W., 1993. Molecular cloning, characterization, subcellular localization and dynamics of p23, the mammalian KDEL receptor. Journal of Cell Biology 120, 325–338.
Tisdale, E.J., 1999. A Rab2 mutant with impaired GTPase activity stimulates vesicle formation from pre-Golgi intermediates. Molecular Biology of the Cell 10, 1837–1849.
Tisdale, E.J., Artalejo, C.R., 2006. Src-dependent atypical protein kinase C iota/lambda (aPKCiota/lambda) tyrosine phosphorylation is required for aPKCiota/lambda association with Rab2 and glyceraldehyde-3-phosphate dehydrogenase on pre-Golgi intermediates. Journal of Biological Chemistry 281, 8436–8442.
Tisdale, E.J., Azizi, F., Artalejo, C.R., 2009. Rab2 utilizes glyceraldehyde-3-phosphate dehydrogenase and protein kinase C{iota} to associate with microtubules and to recruit dynein. Journal of Biological Chemistry 284, 5876–5884.
Tisdale, E.J., Bourne, J.R., Khosravi-Far, R., Der, C.J., Balch, W.E., 1992. GTP-binding mutants of Rab1 and Rab2 are potent inhibitors of vesicular transport from the endoplasmic reticulum to the Golgi complex. Journal of Cell Biology 119, 749–761.
Tisdale, E.J., Plutner, H., Matteson, J., Balch, W.E., 1997. p53/58 binds COPI and is required for selective transport through the early secretory pathway. Journal of Cell Biology 137, 581–593.
Tomás, M., Martínez-Alonso, E., Ballesta, J., Martínez-Menárguez, J.A., 2010. Regulation of ER-Golgi intermediate compartment tubulation and mobility by COPI coats, motor proteins and microtubules. Traffic 11, 616–625.
Tooze, J., Tooze, S., Warren, G., 1984. Replication of coronavirus MHV-A59 in sac-cells: Determination of the first site of budding of progeny virions. European Journal of Cell Biology 33, 281–293.
Tooze, S.A., Tooze, J., Warren, G., 1988. Site of addition of N-acetyl-galactosamine to the E1 glycoprotein of mouse hepatitis virus-A59. Journal of Cell Biology 106, 1475–1487.
Trucco, A., Polishchuk, R.S., Martella, O., *et al.*, 2004. Secretory traffic triggers the formation of tubular continuities across Golgi subcompartments. Nature Cell Biology 6, 1071–1081.
Tsvetanova, N.G., Riordan, D.P., Brown, P.O., 2012. The yeast Rab GTPase Ypt1 modulates unfolded protein response dynamics by regulating the stability of HAC1 RNA. PLoS Genetics 8, e1002862.
Urbani, L., Simoni, R.D., 1990. Cholesterol and vesicular stomatitis virus G protein take separate routes from the endoplasmic reticulum to the plasma membrane. Journal of Biological Chemistry 265, 1919–1923.
Vavassori, S., Cortini, M., Masui, S., *et al.*, 2013. A pH-regulated quality control cycle for surveillance of secretory protein assembly. Molecular Cell 50, 783–792.
Velloso, L.M., Svensson, K., Pettersson, R.F., Lindqvist, Y., 2003. The crystal structure of the carbohydrate-recognition domain of the glycoprotein sorting receptor p58/ERGIC-53 reveals an unpredicted metal-binding site and conformational changes associated with calcium ion binding. Journal of Molecular Biology 334, 845–851.
Volchuk, A., Amherdt, M., Ravazzola, M., *et al.*, 2000. Megavesicles implicated in the rapid transport of intracisternal aggregates across the Golgi stack. Cell 102, 335–348.
Volpicelli-Daley, L.A., Li, Y., Zhang, C.J., Kahn, R.A., 2005. Isoform-selective effects of the depletion of ADP-ribosylation factors 1-5 on membrane traffic. Molecular Biology of the Cell 16, 4495–4508.
Wang, C., Yoo, Y., Fan, H., *et al.*, 2010. Regulation of Integrin β1 recycling to lipid rafts by Rab1a to promote cell migration. Journal of Biological Chemistry 285, 29398–29405.

Ward, T.H., Polishchuk, R.S., Caplan, S., Hirschberg, K., Lippincott-Schwartz, J., 2001. Maintenance of Golgi structure and function depends on the integrity of ER export. Journal of Cell Biology 155, 557–570.
Warren, G., 1987. Signals and salvage sequences. Nature 327, 17–18.
Wilson, D.W., Lewis, M.J., Pelham, H.R., 1993. pH-dependent binding of KDEL to its receptor *in vitro*. Journal of Biological Chemistry 268, 7465–7468.
Winslow, A.R., Chen, C.W., Corrochano, S., *et al.*, 2010. α-Synuclein impairs macroautophagy: Implications for Parkinson's disease. Journal of Cell Biology 190, 1023–1037.
Xu, D., Hay, J.C., 2004. Reconstitution of COPII vesicle fusion to generate a pre-Golgi intermediate compartment. Journal of Cell Biology 167, 997–1003.
Xu, D., Joglegar, A.P., Williams, A.L., Hay, J.C., 2000. Subunit structure of a mammalian ER/Golgi SNARE complex. Journal of Biological Chemistry 275, 39631–39639.
Yamamoto, K., Fujii, R., Toyofuku, Y., *et al.*, 2001. The KDEL receptor mediates a retrieval mechanism that contributes to quality control at the endoplasmic reticulum. EMBO Journal 20, 3082–3091.
Ying, M., Flatmark, T., Saraste, J., 2000. The p58-positive pre-Golgi intermediates consist of distinct subpopulations of particles that show differential binding of COPI and COPII coats and contain vacuolar $H^+$-ATPase. Journal of Cell Science 113, 3623–3638.
Ying, M., Sannerud, R., Flatmark, T., Saraste, J., 2002. Colocalization of $Ca^{2+}$-ATPase and GRP94 with p58 and the effects of thapsigargin on protein recycling suggest the participation of the pre-Golgi intermediate compartment in intracellular $Ca^{2+}$-storage. European Journal of Cell Biology 81, 469–483.
Yoo, J.S., Moyer, B.D., Bannykh, S., *et al.*, 2002. Non-conventional trafficking of the cystic fibrosis transmembrane conductance regulator through the early secretory pathway. Journal of Biological Chemistry 277, 11401–11409.
Yu, S., Satoh, A., Pypaert, M., *et al.*, 2006. mBet3p is required for homotypic COPII vesicle tethering in mammalian cells. Journal of Cell Biology 174, 359–368.
Zhang, T., Hong, W., 2001. Ykt6 forms a SNARE complex with syntaxin 5, GS28, and Bet1 and participates in a late stage in endoplasmic reticulum-Golgi transport. Journal of Biological Chemistry 276, 27480–27487.
Zhang, T., Wong, S.H., Tang, B.L., *et al.*, 1997. The mammalian protein (rbet1) homologous to yeast Bet1p is primarily associated with the pre-Golgi intermediate compartment and is involved in vesicular transport from the endoplasmic reticulum to the Golgi apparatus. Journal of Cell Biology 139, 1157–1168.
Zhang, T., Wong, S.H., Tang, B.L., Xu, Y., Hong, W., 1999. Morphological and functional association of Sec22b/ERS-24 with the pre-Golgi intermediate compartment. Molecular Biology of the Cell 10, 435–453.
Zhao, X., Claude, A., Chun, J., *et al.*, 2006. GBF1, a *cis*-Golgi and VTCs-localized ARF-GEF, is implicated in ER-to-Golgi protein traffic. Journal of Cell Science 119, 3743–3753.
Zheng, C., Page, R.C., Das, V., *et al.*, 2013. Structural characterization of carbohydrate binding by LMAN1 protein provides new insight into the endoplasmic reticulum export of factors V (FV) and VIII (FVIII). Journal of Biological Chemistry 288, 20499–20509.
Zheng, C., Zhang, B., 2013. Combined deficiency of coagulation factors V and VIII: An update. Seminars in Thrombosis and Hemostasis 39, 613–620.
Zoppino, F.C., Militello, R.D., Slavin, I., Alvarez, C., Colombo, M.I., 2010. Autophagosome formation depends on the small GTPase Rab1 and functional ER exit sites. Traffic 11, 1246–1261.
Zuber, C., Fan, J., Guhl, B., *et al.*, 2001. Immunolocalization of UDP-glucose: glycoprotein glucosyltransferase indicates involvement of pre-Golgi intermediates in protein quality control. Proceedings of the National Academy of Sciences of the United States of America 98, 10710–10715.
Zuber, C., Fan, J.Y., Guhl, B., Roth, J., 2004. Misfolded proinsulin accumulates in expanded pre-Golgi intermediates and endoplasmic reticulum subdomains in pancreatic beta cells of Akita mice. FASEB Journal 18, 917–919.

## Further Reading

Bannykh, S.I., Nishimura, N., Balch, W.E., 1998. Getting into the Golgi. Trends in Cell Biology 8, 21–25.
Brownhill, K., Wood, L., Allan, V., 2009. Molecular motors and the Golgi complex: Staying put and moving through. Seminars in Cell & Developmental Biology 20, 784–792.
Capitani, M., Sallese, M., 2009. The KDEL-receptor: New functions for an old protein. FEBS Letters 583, 3863–3871.
Dancourt, J., Barlowe, C., 2010. Protein sorting receptors in the early secretory pathway. Annual Review of Biochemistry 79, 777–802.
Farquhar, M.G., Palade, G.E., 1998. The Golgi apparatus: 100 years of progress and controversy. Trends in Cell Biology 8, 2–10.
Klumperman, J., 2011. Architecture of the mammalian Golgi. Cold Spring Harbor Perspectives in Biology 3, a005181.
Lippincott-Schwartz, J., Roberts, T.H., Hirschberg, K., 2000. Secretory protein trafficking and organelle dynamics in living cells. Annual Review of Cell and Developmental Biology 16, 557–589.
Zanetti, G., Pahuja, K.B., Studer, S., Shim, S., Schekman, R., 2012. COPII and the regulation of protein sorting in mammals. Nature Cell Biology 14, 20–28.