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# Morphogenesis and Dynamics of Post-Golgi Transport Carriers

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## 1. Introduction

The identities of many intracellular organelles and of specific domains of the cell surface rely on the delivery of proteins and lipids through biosynthetic or/and endocytic pathways to the sites of their specific activities. The Golgi complex serves as a central station in the biosynthetic pathway, from where proteins are sorted towards their different destinations, such as various domains of the cell surface or the endosomal-lysosomal system. To be delivered from the Golgi complex to their target compartments, cargo proteins are incorporated into dynamic membrane-bound organelles that are generally known as 'post-Golgi carriers'. Given that these post-Golgi carriers have such an important role in the process of intracellular transport their morphology, living dynamics and molecular composition became the subjects of significant interest over the last decade.

Post-Golgi carriers (PGCs) were originally discovered and described as a result of the development of green fluorescent protein (GFP) technology and live-cell imaging (Lippincott-Schwartz et al., 2000). The first few fluorescently tagged cargo proteins observed in living cells revealed a new world of highly dynamic structures traveling from the Golgi complex to the plasma membrane (Hirschberg et al., 1998; Nakata et al., 1998). With time, the list of molecules that could be visualized *in vivo* expanded greatly, to expose the unexpected complexity of the post-Golgi transport pathways. However, in mammalian cells, most of PGSs have several common features that are independent of the pathway(s) to which they belong.

PGCs form from membrane domains of the Golgi complex that lack resident Golgi enzymes, and there are known as 'PGC precursors' (Hirschberg et al., 1998; Keller et al., 2001; Polishchuk et al., 2003; Puertollano et al., 2003). The shapes and sizes of PGCs that can even carry the same cargo vary across a relatively wide range. Most that were seen under light microscopy were clearly larger that plasma membrane (PM)-associated clathrin vesicles and 100-nm-diameter fluorescent beads (Lippincott-Schwartz et al., 2000). Indeed, while the smaller PGSs can usually have an extension of 300 nm to 400 nm, some large carriers can reach dozens of microns in length. Video microscopy has revealed that many of these

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carriers are globular in appearance, although they are frequently stretched into tubular shapes during their translocation through the cytosol. Thus, PGCs have been frequently referred to as 'pleiomorphic' structures. PGCs use microtubules to move towards particular locations within the cell. Although carriers can form and support post-Golgi transport without association with microtubules, the correct targeting of cargo proteins is usually compromised under such conditions (Kreitzer et al., 2003; Rindler et al., 1987)

The life cycle of a PGC consists of three stages: (i) formation; (ii) transition through the cytosol; and (iii) docking and fusion with the target membrane (Fig. 1) (Polishchuk et al., 2000). In this chapter we take you on a journey with newly born PGCs, to follow them through all of the stages of their life cycle.
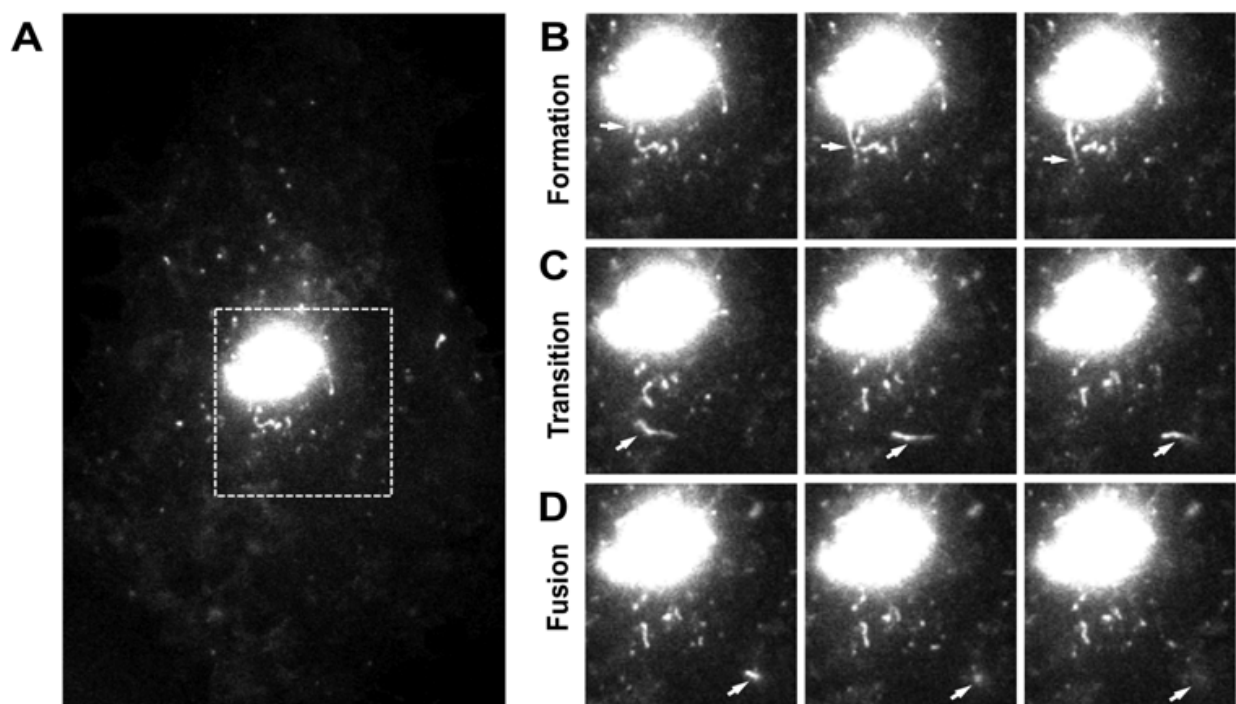


**A.** An example of PGCs dynamics, as taken in the area (dashed box) of a living cell expressing VSVG-GFP upon release of this chimeric protein from the Golgi complex. **B.** Representative images extracted from a time-lapse sequence (corresponding to the area outlined by the dashed box in panel A) show the dynamics of PGC formation from the Golgi complex (arrows). **C.** Transition of the same newly formed PGC through the cytoplasm (arrows). **D.** Fusion of the same PGC (panels B, C) with the target membrane, as shown in these three subsequent time-lapse images by arrows.

Fig. 1. The life cycle of a PGC.

## 2. PGC formation

The process PGC morphogenesis occurs at the level of the most distal Golgi compartment, known as the trans-Golgi network (TGN), and this was characterized in detail using a combination of time-lapse and electron microscopy (EM). This process comprises three main steps: (i) formation of specialized TGN export domain, known as the PGC precursor; (ii) extrusion of this domain along microtubules; and (iii) fission of the export domain to generate a free carrier (Polishchuk et al., 2003).

## 2.1 Morphogenesis of PGC precursors at the TGN

The first step of PGC formation coincides with the segregation of the cargo proteins from the Golgi-resident enzymes. This appears to be a common process for proteins that exit the Golgi complex to head towards different post-Golgi compartments, such as the basolateral PM (Hirschberg et al., 1998; Polishchuk et al., 2003), the apical PM (Keller et al., 2001), and the endosomal-lysosomal system (Puertollano et al., 2003). These enzyme-free Golgi domains usually exhibit a tubular structure and contain *bona-fide* TGN markers (Polishchuk et al., 2003; Puertollano et al., 2003). At the EM level, this segregation of the cargo proteins from the Golgi-resident enzymes corresponds to the transition of the cargo-containing compartments from cisterna-like into a tubular network morphology (Polishchuk et al., 2003).

This thus raises the question of the mechanism by which the originally flat Golgi membranes are converted into highly bent, tubular-reticular TGN structures. A long time ago, Rambourg and Clermont (Rambourg and Clermont, 1990) noted that the cisternae in the middle of a Golgi stack appear quite 'solid' and contain just a few small fenestrae (Fig. 2A). During progression toward the *trans* face of the Golgi complex, both the number and size of these fenestrae increase (Rambourg and Clermont, 1990). Thus the *trans*-most Golgi cisternae generally look like flat tubular webs (Fig. 2A, B), which frequently 'peel off' from the Golgi stack (Rambourg and Clermont, 1990) (Fig. 2C, D), and these can then be easily transformed into TGN membranes by a few fission events (see Fig. 2B). A similar conversion of the Golgi compartments was seen to occur along the Golgi stack in the yeast *Pichia pastoris* (Mogelsvang et al., 2003).

The mechanisms behind this transformation are not yet completely clear. The cisterna-like morphology of the Golgi compartments can be stabilized through the formation of large polymers formed by the Golgi enzymes (Nilsson et al., 1996). Indeed, truncation of the protein domains responsible for enzyme oligomerization results in a loss of the regular Golgi morphology (Nilsson et al., 1996). Thus, a gradual reduction in Golgi enzyme polymers in the *trans*-Golgi compartments would favor transformation of cargo-containing cisternae into networks of tubular membranes. This process can be further assisted by changes in the lipid composition of the *trans*-Golgi membranes, which include the input of material from the endocytic system (Pavelka et al., 1998) and the local activities of TGN-specific lipid-modifying enzymes or lipid-transfer proteins (De Matteis and Luini, 2008). Therefore, the membranes become thicker in the TGN and they thus fail to provide a favorable environment for the short transmembrane domains of the Golgi enzymes (Munro, 1995). As a result, the Golgi enzymes get pushed out of the tubular TGN back towards the core Golgi regions that are composed of the stacked cisternae.

The bending of the flat cisternae membranes into TGN tubules might be also facilitated by the action of specific proteins. Various roles of structural proteins in membrane deformation/ tubulation have been widely recognized (McMahon and Gallop, 2005). These proteins act either by forcing membrane curvature or by sensing and stabilizing it. As an example of the former, coat proteins (such as clathrin) polymerize into curved structures that can bend membrane domains (Antonny, 2006). Alternatively, such TGN proteins as the FAPPs can insert their amphipathic helices into the outer leaflet of a lipid bilayer, thereby increasing the positive membrane curvature (Lenoir et al., 2010). Other rigid curved proteins, or protein modules (such as the BAR domain), can bind to curved membranes and stabilize them by electrostatic interactions (Cullen, 2008; McMahon and Gallop, 2005).
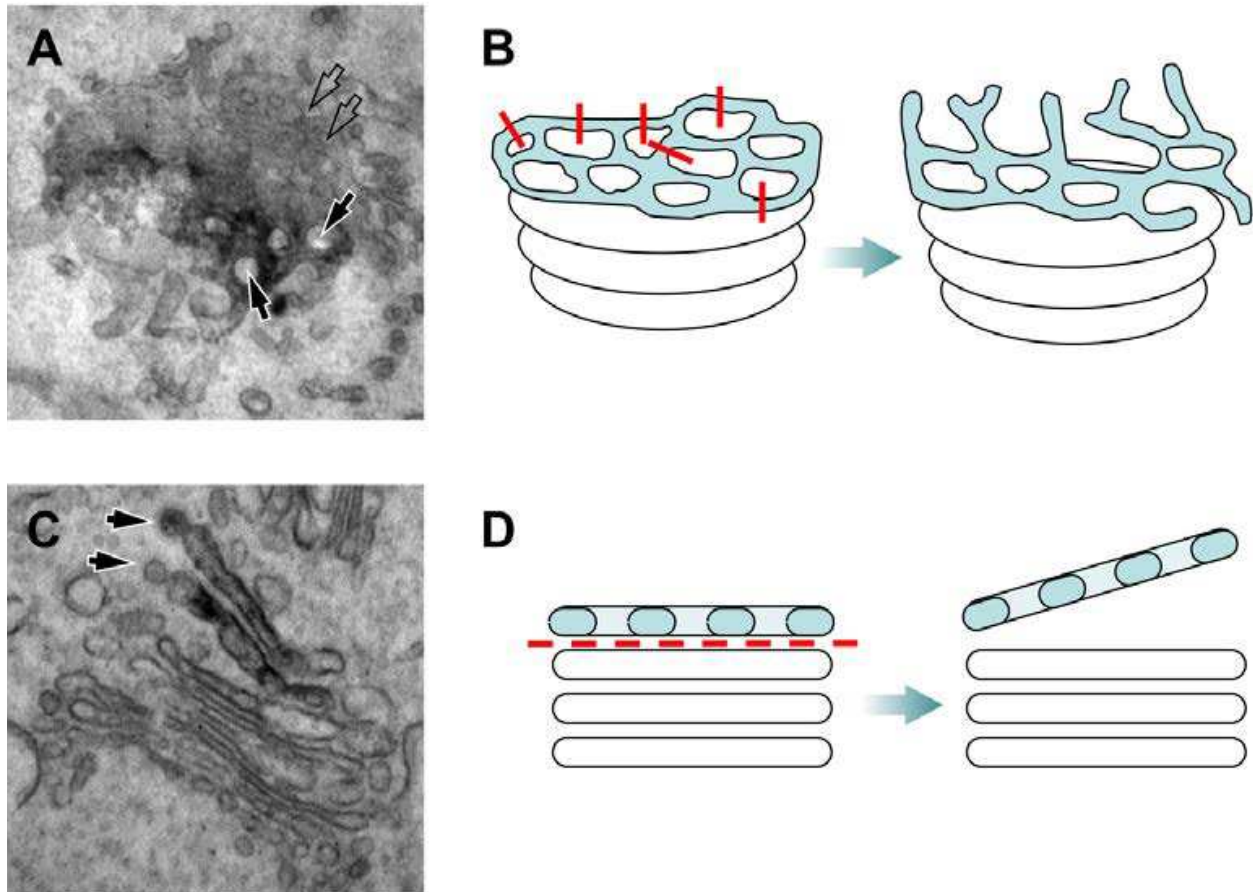
**A.** Thin *en-face* section of the Golgi stack in cells transfected with a TGN38-HRP chimera. The solid arrows show the increase in size of the fenestrae of a TGN38-positive cisterna (dark staining) over the fenestrae of an unstained cisterna (empty arrows). **B.** Scheme showing how highly fenestrated *trans*-most Golgi cisternae can be transformed into tubular network through a few fission events (red lines). **C.** Thin section across the Golgi complex in cells transfected with a TGN38-HRP chimera to show two of the *trans*-most cisternae (arrows) detaching from the rest of the stack. **D.** The process of cisternae peeling off from the rest of the Golgi stack might be determined by a loss of the stacking proteins/ mechanisms (red dashed line) in the *trans*-Golgi compartment.

Fig. 2. Transformation of Golgi cisternae into TGN.

On the other hand, changes in lipid content, and hence membrane curvature, can be modulated via lipid-metabolizing enzymes that reside at the Golgi complex (De Matteis and Luini, 2008). The transmembrane or inter-organelle transfer of lipids can contribute to the generation of particular lipid environments in the membranes of the TGN. In this respect, it is important to note that numerous contact sites between the ER and the trans-cisternae of the Golgi complex have been detected by EM tomography (Ladinsky et al., 1999). Such contact sites can be used for lipid transfer between the ER and the *trans*-Golgi, which can be mediated by specific lipid-transfer proteins, such as CERT and OSBP (De Matteis et al., 2007).

Finally, conversion of the Golgi cisternae into tubular PGC precursors at the TGN can be accompanied by the loss of the stacking mechanisms at the *trans* side of the Golgi complex. The two main proteins that are involved in the maintenance of cisterna stacking, GRASP65 and GRASP55, have been detected mainly in the *cis* and medial Golgi, rather than in the *trans*-Golgi (Barr et al., 1997; Shorter et al., 1999). Therefore, this intercisternal 'glue' migh be

gradually lost as a cisterna progresses towards the *trans* pole of the Golgi complex. This has been confirmed both in mammals and yeast by observations that the *trans*-most cisterna frequently peels off from the main Golgi stack (Clermont et al., 1995; Mogelsvang et al., 2003) (Fig. 2C, D). It is likely that two or more mechanisms act in synergy here, to allow the conversion of flat Golgi cisternae into the tubulo-reticular PGC precursors at the TGN.

In addition to the formation of tubular domains at the exit face of the Golgi complex, this is also the level at which the cargo proteins that are directed to different post-Golgi destinations need to be sorted. The classical view in the membrane transport field implies that this sorting at the TGN (as well as throughout the whole secretory pathway) is driven mainly by the coat-adaptor-protein machinery, which interacts specifically with amino-acid signals of certain transmembrane cargo proteins; this then provides the mechanical force for the budding and fission of transport vesicles (Mellman and Warren, 2000).

This holds true for endo-lysosome-directed carriers, which have been scrupulously characterized. In contrast, PGCs carrying a cargo like the G-protein of vesicular stomatitis virus (VSVG) can be formed in a coat-protein-independent and AP-independent manner. Both VSVG-positive PGCs and their precursors do not exhibit β-COP or γ-, δ- and ε-adaptins at their membranes (Polishchuk et al., 2003). Other clathrin adaptors, such as the GGAs, are excluded from VSVG carriers as well (Polishchuk et al., 2003; Puertollano et al., 2003). Similarly, coats and adaptors have never been detected on PGCs that are carrying proteins to the apical PM surface in polarized cells (Kreitzer et al., 2003). Thus, these carriers should form either by virtue of some still-unknown adaptors that have not yet been visualized by EM, or by their association with specific lipid microdomains that are involved in sorting (Simons and Gerl, 2010). This might be the case for proteins directed to the apical surface of the PM in polarized epithelial cells, the concentration of which at the TGN appears to be through their partition into cholesterol-rich and sphingolipid-rich membrane domains, which are known as 'membrane rafts' (Simons and Gerl, 2010).

Thus, the TGN represents a mosaic of different export domains, which strongly resemble free PGCs in their molecular composition. EM has also revealed structural similarities between carriers and their precursors at the TGN. For example, while the PGCs that carry VSVG mainly have a tubular morphology, several can have a complex structure and even contain clearly visible fenestrae (Polishchuk et al., 2003; Polishchuk et al., 2000), as would be expected of membranes that derive from a protrusion of the TGN. Indeed, PGC precursors visualized using correlative light-electron microscopy (CLEM) comprise tubular segments that consist of complex branching and fenestrated membranes remaining continuous with the parent membranes of the Golgi stack (Polishchuk et al., 2003). Similarly, carriers containing the apical cargo protein HA frequently exhibit tubular morphology, as do the HA-positive domains at the TGN (Puertollano et al., 2001). Thus structural similarities with their precursor at the TGN appear to be a common feature of the different types of PGCs. This strongly suggests that PGCs form by the fission of these precursor domains (or large parts of them) from the rest of the TGN membranes.

## 2.2 Elongation of PGC precursors

After the initial budding from the donor compartment, PGC precursors frequently undergo further extension, which in some cases, can reach to lengths of over a dozen microns. This

process is usually coupled to the loading of the cargo, and it allows the adapting of bulky proteins into budding PGCs; e.g. rigid 300-nm-long procollagen rods (Polishchuk et al., 2003), and long tubular multimers of von Willebrand blood coagulation factor (Zenner et al., 2007).

The process of tubule elongation can be supported via the recruitment of various scaffold proteins. Clathrin has been shown to polymerize into tubular shapes (Zhang et al., 2007). In addition, local activities of lipid-modifying enzymes might be required to maintain the production of specific lipids that favor a tubule-like conformation of membranes, and therefore, to support PGC growth (Brown et al., 2003). Finally, elongation of PGC precursors is frequently assisted by the cytoskeleton. A number of actin-associated and microtubule-associated motor proteins appear to be implicated in the pulling out of tubular structures from donor membranes (Kreitzer et al., 2000; Sahlender et al., 2005). Remarkably, microtubules and kinesin alone appear to be sufficient to trigger the formation of long membrane tubules from liposomes *in vitro* (Roux et al., 2002) and this process has been shown to operate for PGC elongation (Polishchuk et al., 2003).

## 2.3 Fission of PGCs from the TGN

The dynamics of the PGC fission process appears to be fairly complex. Live-cell imaging has revealed PGC fission frequently to coincide with the mechanical pulling out of carrier precursor from the TGN along microtubules (Polishchuk et al., 2003). Apparently, this pulling force that the molecular motors such as kinesin (see below) can apply to the TGN membranes is important in the extension of PGC precursors from the Golgi body and for the later fission of the PGC (Kreitzer et al., 2000; Polishchuk et al., 2003). In cell-free systems, addition of kinesin to Golgi membranes (and even to liposomes) together with microtubules induces the formation of tubule-like membranes that are similar to PGC precursors (Roux et al., 2002), while a block in kinesin function in cells by microinjection of an inhibitory antibody (Kreitzer et al., 2000) or expression of a 'headless' kinesin mutant (Nakata and Hirokawa, 2003) prevents PGC formation from the Golgi complex. Kinesin has been seen to be associated with the tip of PGC precursors, although it can also attach to other points along the membrane of a PGC precursor (Polishchuk et al., 2003). The movement of kinesins along microtubules can then create tension within a PGC precursor that will facilitate the fission process (Shemesh et al., 2003). Indeed, based on *in-vitro* data, membranes under tension have recently been proposed to have an important role in fission (Roux et al., 2006). The extension of tubular PGC precursors might also result in looser lipid packing in its membrane. This exposes the membranes of a budding PGC to easier access by proteins that promote membrane fission at the TGN, such as dynamin and CtBP1-S/BARS (Corda et al., 2006; McNiven et al., 2000). Nonetheless, PGCs can also form when microtubules have been destroyed by nocodazole treatment; in this case, the pulling force to create membrane tension in fission-prone regions might be applied by the actin motors (Miserey-Lenkei et al., 2010; Sahlender et al., 2005).

Live-cell imaging and CLEM have also shown that fission does not take place randomly along the membranes of PGC precursors. Fission frequently takes place at the thinnest and geometrically simplest regions of the elongating PGC membranes (Fig. 3A), which at the EM level correspond to thin tubular segments of membranes (Polishchuk et al., 2003). In contrast, fission does not take place at the TGN regions with a complex morphology (i.e., in
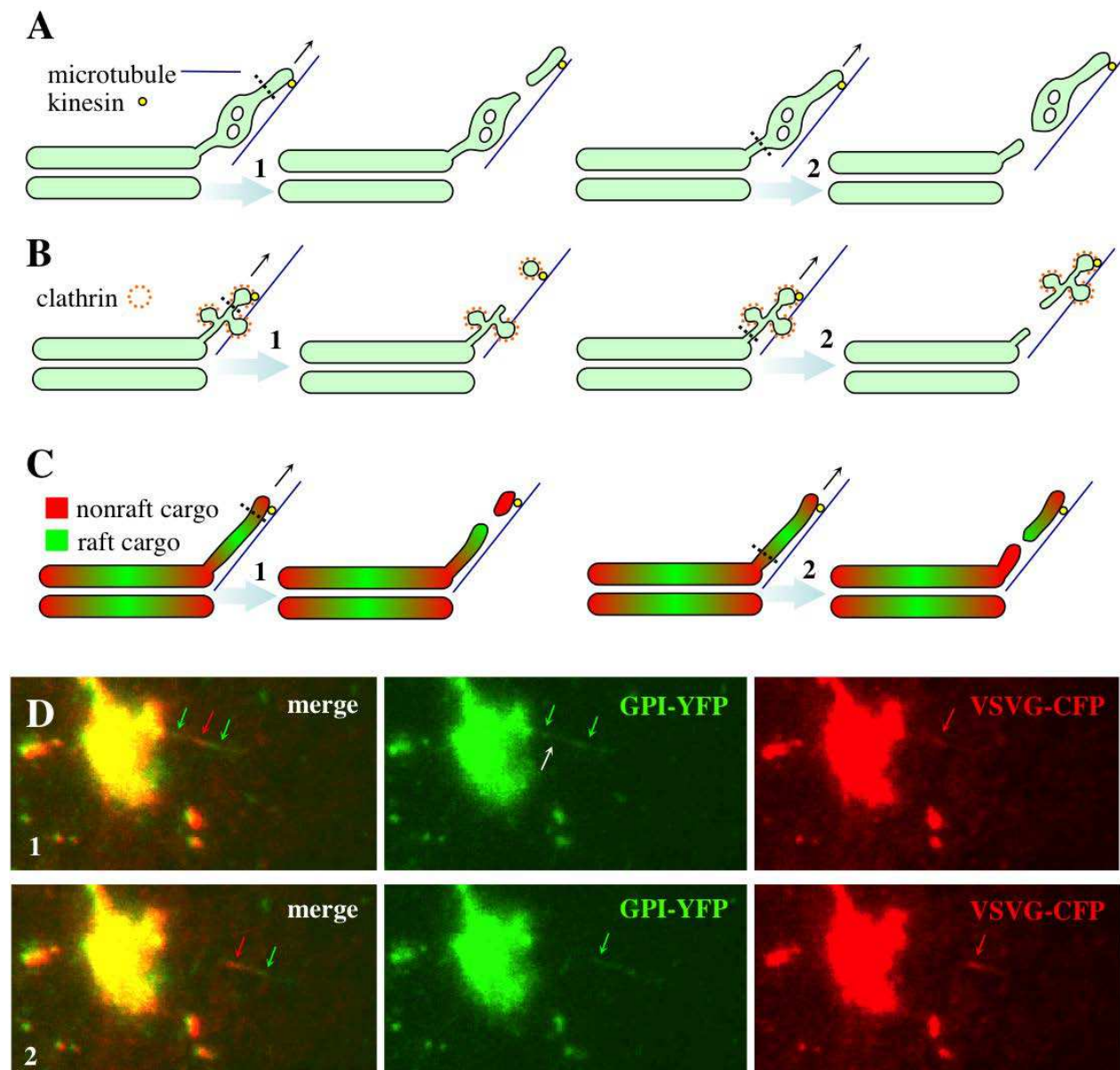
**A**. TGN precursors of post-Golgi carriers are pulled along microtubules by kinesin. The fission of the carriers (dashed line) occurs at the thinnest parts of the PGC precursor, which correspond to thin tubular segments of the TGN membrane at the EM level. In contrast, fission does not take place at TGN regions with a complex morphology (i.e., containing tubular networks and fenestrae, or in thick vacuolar regions). If fission occurs close to the tip of a PGC precursor, the carrier will be smaller in size (panel 1). In contrast, larger PGCs can be formed by cleavage at the base of a PGC precursor (panel 2). **B**. PGCs directed to endosomes can detach from the TGN as simple clathrin-coated vesicles when the fission (dashed line) occurs at the neck of the clathrin-coated bud (1). Alternatively, entire chunks of the TGN membrane containing 2-3 clathrin-coated buds can be cleaved from the Golgi complex (2). **C**. In budding PGCs that contain raft and non-raft proteins and lipids, fission (dashed line) can occur at the fission-prone border between raft and nonraft areas, which leads to the formation of PGCs with either single (1) or several (2) membrane microdomains. **D.** The subsequent frames extracted from the time-lapse sequence to demonstrate budding (1) and fission (2) of PGC that contains different membrane microdomains, which are labeled with either the raft cargo GPI-YFP (green arrow) or with the nonraft cargo VSVG-CFP (red arrows).

Fig. 3. Fission of post-Golgi transport carriers.

those containing tubular networks and branching tubules, or in thick vacuolar regions). Obviously, the precise points of fission will define not only the composition of a PGC carrier, but also its morphology. If fission occurs close to the tip of the TGN tubule, a carrier will be smaller in size. In contrast, larger PGCs can form by cleavage at the base of a PGC precursor (Fig. 3A). Similarly, endosome-directed PGCs can apparently detach from the TGN as simple cargo-containing vesicles if the fission occurs at the neck of the clathrin-coated buds (Fig. 3B). However, many clathrin-positive PGCs have a grape-like morphology (a tubule with several 'buds'), which suggests that entire chunks of TGN membranes that contain 2-3 clathrin-coated buds can be cleaved from the Golgi complex (Polishchuk et al., 2006).

Fission along a budding PGC precursor might also be greatly facilitated by heterogeneous lipid microdomains (Roux et al., 2005). Indeed, liposome tubules have been shown to break into small pieces at the border between phosphatidylcholine-enriched and cholesterol-enriched regions (Roux et al., 2005). Given that multiple lipid microdomains can be seen along a forming PGC, it appears that large post-Golgi carriers might contain several regions that are filled with raft and non-raft proteins (Polishchuk et al., 2004) (Fig. 3C, D).

Finally, fission of PGCs might be regulated by the cargo proteins themselves. According to a recent hypothesis, in some cases fission appears not to occur until the cargo is completely loaded into the budding tubule (Bard and Malhotra, 2006). For example, if a large and rigid procollagen rod is still present in the neck of a budding tubule, this neck can apparently not be constricted sufficiently (by dynamin or coat proteins) to trigger fission.

## 3. PGC transition to the target membrane

After fission from the TGN, PGCs move to their target membranes. Given that budding PGCs are associated with motor proteins, these can dock onto microtubules and use them as the 'highways' to reach their destination. In this context, different members of the kinesin superfamily (Kamal et al., 2000; Nakata and Hirokawa, 2003; Teng et al., 2005), or even other microtubule motors, such as dynein (Tai et al., 1999), have been shown to drive post-Golgi transport of specific cargo towards their acceptor compartment. Such high fidelity of cargo selection by motors at the TGN, and as a consequence, its further delivery to the correct surface or intracellular domain, might be regulated by interactions of motor proteins directly with the cargo (Kamal et al., 2000; Teng et al., 2005) or with components of the TGN sorting machinery (Nakagawa et al., 2000). For instance, transport of HA and annexin 13b to the apical PM surface in epithelial cells relies on the raft-associated motor protein KIFC3 (Noda et al., 2001). KIF13A has been shown to operate in the other post-Golgi route that directs the mannose-6-phosphate receptor from the Golgi complex to the endosomes (Nakagawa et al., 2000). A number of neuronal proteins, such as bAPP, GAP43 and SNAP25, require KIF5 for their correct targeting (Nakata and Hirokawa, 2003). The microtubule minus-end-directed motor dynein has been described as supporting rhodopsin transport in rod photoreceptors (Tai et al., 1999; Yeh et al., 2006).

Of note, the sorting of specific cargo to either axons or dendrites by different kinesins has been demonstrated in a single individual neuron (Nakata and Hirokawa, 2003). KIF5 has been shown to carry VSVG-containing PGCs to the axon, while KIF17A provides the delivery of the Kv2.1 ion channel to dendrites (Nakata and Hirokawa, 2003). In such cases,

how do the different motors know where to deliver these specific cargoes? Apparently motor heads of KIF5 and KIF17A can bind with higher affinity to different subsets of microtubules, which will provide directional cues for polarized axonal transport (Nakata and Hirokawa, 2003). Different populations of microtubules have been found also in other cell types, and therefore, these might serve as highways for polarized cargo delivery (Nakata and Hirokawa, 2003; Spiliotis et al., 2008).

In this context, microtubule architecture appears to play a significant role in targeting of TGN-derived PGCs to their correct acceptor membrane(s). Several molecular players, such as Par-1 (Cohen et al., 2004) and septins (Spiliotis et al., 2008), have been shown to regulate microtubule organization in vertical or horizontal arrays during the polarization of columnar (MDCK cells) or planar (hepatocytes) epithelia, respectively. As a consequence of the divers microtubule architecture, same apical proteins can be delivered from the Golgi complex directly to the correct surface domain of MDCK cells, while in hepatocytes, these apical markers first appear at the basolateral surface, and then transcytosis is used for their apical delivery (Cohen et al., 2004).

The other important issue that needs to be addressed is whether any sorting processes take place in the PGCs *en route* to their acceptor compartment. This occurs, for example, when the mannose-6-phosphate receptor is sorted from the maturing secretory granules by clathrin-coated vesicles (Klumperman et al., 1998). So several approaches have been used to determine whether similar sorting events occur with PGCs. Mature TGN-derived carriers can be arrested before their fusion with the PM, either by microinjection of an anti-NSF antibody or by treatment with tannic acid (Polishchuk et al., 2003; Polishchuk et al., 2004). In contrast to secretory granules, a comparison of mature with newly formed PGCs did not reveal significant variations in either their ultrastructure or their molecular composition (Polishchuk et al., 2003; Polishchuk et al., 2004). Similarly, mature carriers directed from the Golgi complex to endosomes accumulated in cells upon endosome ablation. However, they did not show any significant transformation, except for a slight reduction in the area covered by clathrin (Polishchuk et al., 2006).

Live-cell imaging of MDCK cells has revealed that PCGs that contain both the basolateral marker VSVG-CFP and the apical marker GPI-YFP do not sort out each of these proteins into separate structures, but deliver both of these proteins to the PM (Polishchuk and Mironov, 2004). GPI-YFP is then sorted from the basolateral surface to the apical surface through transcytosis. On the other hand, segregation of proteins from their common PGC into two separate carriers has also been documented (Jacob and Naim, 2001). This suggests that sorting from the PGC does happen, but that it is likely to depend on the nature of the transported cargo proteins.

The complexity of sorting events in the post-Golgi space became more evident with discovery that certain cargoes can pass through an endosomal intermediate before their arrival at the PM. Such indirect 'through-endosome' trafficking of cargo to the cell surface might be significantly facilitated by close association of the TGN membranes with a number of the endocytic compartments in the perinuclear area of a cell (Pavelka et al., 1998). The list of the proteins using this pathway has been updated recently, and it has now been shown that in MDCK epithelial cells, VSVG, the LDL receptor, and E-cadherin can be detected in endosomes before their exit to the PM (Ang et al., 2004; Lock and Stow, 2005). These findings, however, raise a number of further questions. The first is whether this indirect

transport route exists in different cells. The second is whether different cargoes move through the same endosomal compartment on their way to the cell surface in epithelial cells. This appears not to be a case, as a number of proteins (such as VSVG and the LDL receptor) have been reported to use a Rab8-positive sub-population of endosomes as an intermediate station on their way to the basolateral PM in epithelial cells (Ang et al., 2004), while others cargoes (such as E-cadherin, for example) move to the PM through a Rab11-containing endocytic compartment (Lock and Stow, 2005). Furthermore, ablation of the different endocytic compartments through horseradish peroxidase (HRP)-catalyzed crosslinking has revealed a number of apical proteins to take various through-endosome routes to reach the cell surface (Weisz and Rodriguez-Boulan, 2009). It remains to be understood, however, whether any cross-talk between these routes exists. Unfortunately, detailed characterization of PGCs that operate either to or from these intermediate endocytic stations is still missing. Moreover, directionality and selectivity of the post-Golgi routes, and therefore the PGC properties, might change upon cell differentiation. For example, at the early stages in MDCK cells, GPI-anchored proteins are transported to the basolateral surface of the PM, from where that tend to be transcytosed to the apical surface (Polishchuk et al., 2004). Later, however, the cells tend to divert GPI-anchored proteins into a direct Golgi-to-apical surface route (Paladino et al., 2006). Further efforts need to be made to understand this interplay of the different mechanisms that define the PGC path from the TGN to the target compartment.

## 4. Docking and fusion of PGCs with acceptor membranes

To complete their movement across a cell, PGCs need to fuse with their acceptor membrane to deliver their contents. Docking and fusion of PGCs with the cell surface has been studied by total internal reflection (TIRF) microscopy, which allows the visualization of intracellular events within very narrow (50-150 nm) distance from the PM. This TIRF analysis demonstrated that after the docking heterogeneously sized PGCs, these usually fuse completely with the PM (Schmoranzer et al., 2000; Toomre et al., 2000). However, occasionally, larger tubular PGCs can fuse with the PM only partially, at their tips (Schmoranzer et al., 2000; Toomre et al., 2000), using a kind of 'kiss-and-run' mechanism. Interestingly, some PGCs also remain attached to microtubules even as their fusion with the PM initiates (Schmoranzer and Simon, 2003).

PGC fusion with the PM might not be randomly distributed, and might instead be concentrated at several 'hot spots' along the PM (Toomre et al., 2000). In general, the delivery of the PGCs is frequently directed to rapidly growing membrane surfaces. In motile cells, this process is restricted to the leading edge of the cell (Polishchuk et al., 2004; Schmoranzer et al., 2003). Visualization of exocytosis in polarized cells has revealed that the vertical growth of epithelia relies on the directed delivery of PGCs to the lateral surface of the columnar cells, where the tethering factors, such as mammalian exocyst or *Drosophila* DLG, reside (Kreitzer et al., 2003; Lee et al., 2003; Polishchuk et al., 2004). Similarly, the exocyst can define PGC docking sites in neurite growth cones of differentiating neurons (Vega and Hsu, 2001). Interestingly, during tissue biogenesis, a number of cells (e.g. epithelial cells) show an incredible flexibility in terms of their transport routes (Mostov et al., 2003; Rodriguez-Boulan et al., 2005). In epithelia growing on a filter support for 2-3 days, GPI-anchored proteins (which are normally apically targeted) have been found together

with basolateral markers within the same carriers docked onto the lateral membranes of the MDCK cells (Polishchuk et al., 2004). Only after their arrival at the basolateral surface of the PM were these proteins sorted to the apical domain of the PM by transcytosis (Polishchuk et al., 2004). However, after 4 days in culture, MDCK cells apparently start to switch delivery of GPI-anchored proteins from a transcytotic to direct route (Paladino et al., 2006). Similarly, in thyroid epithelial cells, the delivery of dipeptidylpeptidase-IV to the apical PM surface changes from transcytosis to a direct route as the epithelial monolayer matures (Zurzolo et al., 1992). This might happen because the target patch for the PGC fusion forms at the lateral surface of epithelial cells earlier then at the apical targeting patch (Mostov et al., 2003; Nelson, 2003; Rodriguez-Boulan et al., 2005). Indeed, Sec6 undergoes recruitment to the sites of cell-to-cell junctions as soon as subconfluent cells start to contact each other (Yeaman et al., 2004), while expression of the apical sorting machinery components occurs later during the process of cell polarization (Halbleib et al., 2007). Thus, during the early stages of polarization, most of PGCs fuse near junctional complexes at the lateral domain of the PM (Kreitzer et al., 2003; Polishchuk et al., 2004), which contributes to fast vertical elongation of a cell within the epithelial sheet (Mostov et al., 2003; Nelson, 2003; Rodriguez-Boulan et al., 2005).

The precise spatial targeting of PGCs to the correct PM area might be important for processes that contribute to correct tissue development, such as, for example, the parallel alignment of collagen fibers in a tendon (Canty et al., 2004). The cellular mechanism of this alignment is thought to involve the assembly of intracellular collagen fibrils within PGCs. The PGCs carrying procollagen subsequently connect to the extracellular matrix via finger-like projections of the PM, which are known as fibripositors, and which are oriented along the axis of the tendon (Canty et al., 2004). Interestingly, actin filament disassembly results in the rapid loss of fibripositors and in the subsequent disappearance of intracellular fibrils. In this case, a significant proportion of collagen fibrils are found to no longer be orientated with the long axis of the tendon. This suggests an important role for the actin cytoskeleton in the alignment of PGC delivery and in the further organization of the collagenous extracellular matrix in the embryonic tendon (Canty et al., 2006).

In brain tissue, transformation of the contact between an axon and a dendrite into a synapse is accompanied by the accumulation of the synaptic machinery at the site, with these delivered in TGN-derived carriers. In cultured hippocampal neurons, PGCs are linked via spectrin to clusters of the neural cell adhesion molecule (NCAM) in the PM. These complexes are trapped at sites of initial neurite-to-neurite contact within several minutes of the formation of the initial contact. The accumulation of PGCs at contacts with NCAM-deficient neurons is reduced when compared with wild-type cells, which suggests that NCAM mediates the anchoring of intracellular organelles in nascent synapses (Sytnyk et al., 2002).

## 5. Concluding remarks

The extensive characterization of PGC morphology by the combination of live-cell imaging and EM has provided significant advances in our understanding of the mechanisms that operate during PGC morphogenesis and the other steps of the PGC lifecycle. It appears that the type and size of a cargo can strongly impact on the architecture of a PGC, its path to the

target compartment, and way of its fusion with the acceptor membrane. This is achieved through the interplay of the cargo molecules with the components of the sorting and transport machineries at the TGN and in the more distal compartments of the post-Golgi transport routes. Therefore, the integrity of the mechanisms involved in cargo sorting into PGCs, and its further delivery to the correct target destination plays a fundamental role in the maintenance of cell homeostasis, as well as in the organization of specific tissue architecture and function.

## 6. Acknowledgments

## 7. References

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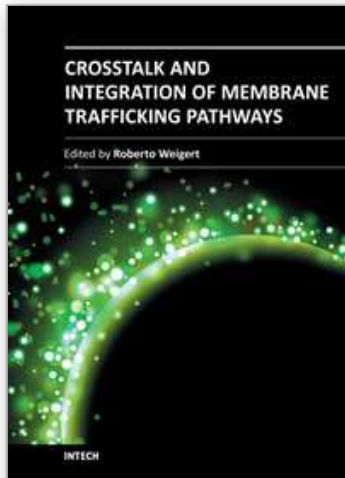
**Crosstalk and Integration of Membrane Trafficking Pathways**

Edited by Dr. Roberto Weigert

Membrane traffic is a broad field that studies the complex exchange of membranes that occurs inside the cell. Protein, lipids and other molecules traffic among intracellular organelles, and are delivered to, or transported from the cell surface by virtue of membranous carriers generally referred as "transport intermediates". These carriers have different shapes and sizes, and their biogenesis, modality of transport, and delivery to the final destination are regulated by a multitude of very complex molecular machineries. A concept that has clearly emerged in the last decade is that each membrane pathway does not represent a close system, but is fully integrated with all the other trafficking pathways. The aim of this book is to provide a general overview of the extent of this crosstalk.

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