The EMBO Journal (2006) 25, 2663–2673 | © 2006 European Molecular Biology Organization | All Rights Reserved 0261-4189/06
www.embojournal.org

THE EMBO JOURNAL

EMBO Member's Review

The physiology of membrane transport and endomembrane-based signalling

Michele Sallese, Teodoro Pulvirenti and Alberto Luini*

Laboratory of Membrane Traffic, Department of Cell Biology and Oncology, Consorzio Mario Negri Sud, Santa Maria Imbaro, Chieti, Italy

Some of the important open questions concerning the physiology of the secretory pathway relate to its homeostasis. Secretion involves a number of separate compartments for which their transport activities should be precisely cross-coordinated to avoid gross imbalances in the trafficking system. Moreover, the membrane fluxes across these compartments should be able to adapt to environmental 'requests' and to respond to extracellular signals. How is this regulation effected? Here, we consider evidence that endomembrane-based signalling cascades that are similar in organization to those used at the plasma membrane coordinate membrane traffic. If this is the case, this would also represent a model for a more general inter-organelle signalling network for functionally interconnecting different intracellular activities, a necessity for the maintenance of cellular homeostasis and to express harmonic global cellular responses.

The EMBO Journal (2006) **25,** 2663–2673. doi:10.1038/sj.emboj.7601172; Published online 8 June 2006
Subject Categories: membranes & transport; signal transduction
Keywords: Golgi; membrane transport; signalling

In vivo physiology of the secretory pathway

The genetics and biochemistry of intracellular membrane trafficking have been progressively elucidated over the last few decades, and some of the molecular machineries underlying elementary transport events, such as membrane bending, budding, fusion and fission, are now understood in great detail (Lee *et al*, 2004). In contrast, there has been much less progress towards a true understanding of supramolecular, or 'global', aspects of the trafficking system that underlie the physiology of membrane transport *in vivo*. Among these are the issues concerning the micro-anatomy and micro-dynamics of the trafficking compartments and the organizational principles of transport *in vivo* (Trucco *et al*, 2004; Luini *et al*, 2005), some aspects of which have seen significant advances only recently.

*Corresponding author. Laboratory of Membrane Traffic, Department of Cell Biology and Oncology, Consorzio Mario, Negri Sud, Santa Maria Imbaro, Chieti 66030, Italy. Tel.: +39 0872 570355; Fax: +39 0872 570412; E-mail: luini@dcbo.negrisud.it

Received: 9 January 2006; accepted: 5 May 2006; published online: 8 June 2006

The reasons for this knowledge gap are multiple, and although these include historical/cultural aspects, the main cause has been the lack of suitable technologies. An understanding of the physiology of trafficking requires the visualization of traffic events *in vivo* with high resolution in time and space—a possibility that has only become (partially) available in the last few years owing to the advent of increasingly powerful video-microscopy and electron tomography approaches, as well as of effective ancillary techniques (e.g. traffic synchronization and correlative electron microscopy) (Polishchuk *et al*, 2000). These advances have spurred a series of studies that have led to new models of the organization of transport *in vivo*—models that, incidentally, have not always been easy to reconcile with previous schemes that were based mostly on genetic and *in vitro* biochemical data (Mellman and Warren, 2000; Pelham and Rothman, 2000; Lee *et al*, 2004; Behnia and Munro, 2005). Moreover, as a natural consequence of these studies, new fundamental issues about the physiology of traffic have come to the fore. One in particular is the issue of the identity of the mechanism underlying the internal coordination and homeostasis of these compartments as well as the regulation of transport organelles by exogenous cues. Here, we will focus on the signalling circuits that might support the coordination of the compartments of the secretory pathway.

Homeostasis of the secretory compartments

Intracellular trafficking pathways comprise a number of anatomically separate compartments that are constantly exchanging their membranes and cargo proteins in an organized sequence of trafficking segments (e.g. endoplasmic reticulum (ER) to Golgi complex, Golgi complex to plasma membrane (PM), etc.). The membrane fluxes along each of these segments should be precisely coordinated under physiological conditions to avoid gross imbalances in the system; moreover, the overall trafficking fluxes (including those regulated and constitutive) should be able to adapt to environmental 'requests' and to react to extracellular signals as part of the global cellular response.

How is this regulation effected? Several mechanisms are in principle conceivable. For instance, it is possible to explain the ability of the secretory system to control traffic fluxes across different compartments and to maintain the size and composition of these compartments simply on the basis of a few parameters, such as the properties and amounts of the coat and SNARE proteins in the system (Bonifacino and Glick, 2004; Heinrich and Rapoport, 2005). Moreover, it is possible that simple constitutive phosphorylation and dephosphorylation events, such as those proposed to participate in

©2006 European Molecular Biology Organization

the clathrin-dependent endocytic cycle, might also have roles in complex trafficking responses (Flett *et al*, 2005). Other considerations, however, suggest that more sophisticated control mechanisms might exist (see below), superimposed onto these basic 'automatic' processes, acting perhaps in the same way in which computerized electronic circuits regulate the performance of mechanical parts of modern cars. Here, we will consider one specific hypothesis; namely, that these regulatory circuits involve endomembrane-based signalling cascades that are similar in their organization and components to those used by the cell at the PM. Moreover, these signalling cascades should be initiated by traffic itself and affect the activity of other organelles (this type of signalling organization could therefore be defined as 'inter-organelle signalling'). This hypothesis is supported by two main arguments. The first is that a variety of 'classical' signalling molecules, including G proteins, kinases and phospholipases, are physically associated with secretory endomembranes, and in particular with the Golgi complex (Stow and de Almeida, 1993; Denker *et al*, 1996; Donaldson and Lippincott-Schwartz, 2000; Bard *et al*, 2002; Nagahama *et al*, 2002; Bivona and Philips, 2003; Wylie *et al*, 2003; Larocca *et al*, 2004; Preisinger *et al*, 2004; Diaz Anel and Malhotra, 2005; Ghanekar and Lowe, 2005; Le Niculescu *et al*, 2005). They are therefore well placed to participate in traffic-initiated signalling (Helms *et al*, 1998; Cabrera *et al*, 2003). The second, more speculative argument is that given the sophisticated spatio-temporal control that has evolved for PM-initiated signal transduction pathways (Pierce *et al*, 2002), it would be uneconomical for cells not to use these mechanisms to generate signals operating across intracellular organelles to mediate inter-compartment coordination of membrane trafficking.

In the following, we will review some of the available evidence pertaining to the presence of signalling molecules on endomembranes and to the role of these molecules in the regulation/coordination of the trafficking pathway. We do not plan to be exhaustive in this survey. Potentially relevant aspects of the relationship between signalling and traffic have been reviewed recently and will not be treated here. These include the well-established links between signalling and endocytosis, whereby signalling complexes generated at the PM can modulate endocytic activity and can themselves be modulated while they reside on endocytic organelles (Di Fiore and De Camilli, 2001; Sorkin and Von Zastrow, 2002; Conner and Schmid, 2003; Miaczynska *et al*, 2004). They also include the links between signalling and autophagy (Codogno and Meijer, 2005). Neither will we discuss the significance and the mechanisms of regulation of overall secretory traffic rates by surface receptors, aspects that have been previously reviewed by us and others (Luini and De Matteis, 1993; Bannykh *et al*, 1995). Rather, we will focus mainly on the secretory pathway and on a selected group of 'traditional' signalling protein families and pathways that have been more extensively characterized in the context of secretory traffic and that provide relevant examples of the issues we are dealing with here. We will also summarize the literature data on a larger number of molecules for which only limited information is at present available, but which might turn out to be relevant to the issue being examined (Table I).

While this evidence will be discussed in the light of an inter-organelle signalling hypothesis for traffic coordination (Figure 1A), it is also necessary to consider other schemes that can explain the presence of signalling molecules on endomembranes. An alternative possibility, for instance, is that Golgi-based transduction proteins might serve to mediate inputs that initiate at the PM but regulate Golgi function (Figure 1C), as shown previously by us and others (De Matteis *et al*, 1993; Luton *et al*, 1999) (a similar regulatory mechanism has been shown to exist also in the case of endocytosis (Sorkin and Von Zastrow, 2002; Miaczynska *et al*, 2004)). Furthermore, it is possible that transduction molecules present on the Golgi complex act as relay devices in signalling networks initiated at the PM that control cell growth and other functions (Figure 1D). This has been shown to be the case for Golgi- and endosome-based signalling proteins (Di Fiore and De Camilli, 2001; Sorkin and Von Zastrow, 2002; Bivona *et al*, 2003; Miaczynska *et al*, 2004). Finally, signalling proteins might simply be in transit through the Golgi to the PM following their synthesis (Michaelson *et al*, 2002) (Figure 1E). It should also to be borne in mind that these models (schematized in Figure 1) are not mutually exclusive.

The cAMP-PKA pathway

The centrepiece of the cAMP-PKA pathway is protein kinase A (PKA), a tetrameric serine/threonine kinase that consists of two regulatory and two catalytic subunits. The kinase becomes active when the catalytic and regulatory subunits dissociate in the presence of cAMP. In addition to PKA, the pathway includes the enzyme adenylyl cylase (AC, which synthesizes cAMP) and AC activators (such as Gαs), pathway inactivating phosphodiesterases (PDEs, which hydrolyze cAMP) and the PKA-anchoring scaffold proteins(s) (AKAPs, see below). Remarkably, all of these components have been shown to be located on endomembranes, and particularly on the Golgi complex (Cheng and Farquhar, 1976a, b; Maier *et al*, 1995; Denker *et al*, 1996; Pooley *et al*, 1997; Martin *et al*, 1999; Birkeli *et al*, 2003; Li *et al*, 2003; Asirvatham *et al*, 2004; Larocca *et al*, 2004); our unpublished observations).

The cAMP-PKA pathway can exert potent effects on many trafficking steps. Transport from the ER to the Golgi complex can be accelerated through stimulation of cAMP synthesis and impaired by the PKA inhibitor H89 (Muniz *et al*, 1996). However, this transport step is not affected by other PKA blockers (i.e. KT5720 and PKI) (Aridor and Balch, 2000; Lee and Linstedt, 2000), suggesting that H89 acts through other kinase(s) and indicating that while the cAMP-PKA cascade can accelerate this step, it is not required for its execution at steady state. PKA also regulates intra-Golgi transport and transport from the *trans* Golgi network (TGN) to the PM (Muniz *et al*, 1996). Velasco and co-workers showed that PKA catalytic activity is required for the budding of constitutive transport vesicles from the TGN *in vitro* (Muniz *et al*, 1997) and that the stimulation of cAMP synthesis *in vivo* accelerates transport from the Golgi to the PM. An increase in cAMP also modifies the structure of the Golgi complex, with induction of the formation of numerous tubules interconnecting non-equivalent Golgi cisternae (Muniz *et al*, 1996). Finally, the activity of PKA appears also to be required for retrograde transport from the endosomal compartment to the Golgi complex and from the Golgi to the ER (Birkeli *et al*, 2003) (Cabrera *et al*, 2003).

 ©2006 European Molecular Biology Organization

© 2006 European Molecular Biology Organization

Table I Signalling proteins and their functions on intracellular organelles

Signalling protein	Organelle localization	Molecular function	Role on endomembranes	References
AKAP350	Centrosome, ERGIC	A-kinase-anchoring protein	Unknown	Shanks *et al* (2002)
ATF6	ER	Transcription factor	Transduces effects of UPR	Rutkowski and Kaufman (2004)
BIG2	Golgi	Nucleotide exchange factor and AKAP	Unknown	Li *et al* (2003)
C3G	Golgi	Rap1 exchange factor	MAP kinase pathway	Radha *et al* (2004)
Cbl	Golgi	E3 ubiquitin ligase	Unknown	Bard *et al* (2002)
Cdc42	Golgi	Small GTP-binding protein	Involved in ER-to-Golgi, TGN-to-PM and Golgi-to-ER transport	Luna *et al* (2002), Matas *et al* (2004), Stamnes (2002)
ERK	Golgi	Serine/threonine kinase	Involved in Golgi disassembly during mitosis	Cha and Shapiro (2001), Acharya *et al* (1998)
Fps	Golgi	Tyrosine kinase	Unknown	Zirngibl *et al* (2001)
GIV	COPI vesicles	Regulator of G-protein signalling	Unknown	Le Niculescu *et al* (2005)
GRP1	Golgi	Ras exchange factor	Necessary for Ras activation on the Golgi	Bivona *et al* (2003)
$G\alpha_i$	Golgi	Heterotrimeric GTP-binding protein	Regulates TGN-to-PM transport	Pimplikar and Simons (1993)
$G\alpha_{i3}$	*Cis*-Golgi	Heterotrimeric GTP-binding protein	Regulates transport of constitutively secreted proteins	Wilson *et al* (1994), Stow and de Almeida (1993)
$G\alpha_q$	Golgi, mitochondria	Heterotrimeric GTP-binding protein	Unknown	Wilson *et al* (1994), Denker *et al* (1996)
$G\alpha s$	TGN	Heterotrimeric GTP-binding protein	TGN-to-PM transport	Pimplikar and Simons (1993)
$G\alpha_z$	Golgi	Heterotrimeric GTP-binding protein	Maintenance of Golgi structure	Nagahama *et al* (2002) Yamaguchi *et al* (2000)
IRE1	ER	Serine/threonine kinase	Transduces effects of UPR	Rutkowski and Kaufman (2004)
JAK-2	Transitional ER	Tyrosine kinase	Coordinates ERES assembly	Lavoie *et al* (2000)
KDELr	ER, Golgi	Receptor protein	Transduces effects of UPR	Yamamoto *et al* (2003)
Lck	Golgi	Tyrosine kinase	Unknown	Bijlmakers *et al* (1997)
LimK	Golgi	Serine/threonine kinase	Kinase-dead mutant produces tubular processes emerging from the Golgi stack	Rosso *et al* (2004)
MEK-1	Golgi	Serine/threonine kinase	Involved in Golgi disassembly during mitosis	Colanzi *et al* (2003)
MINK	Golgi	Serine/threonine kinase	Unknown	Hu *et al* (2004)
MST4	Golgi	Serine/threonine kinase	Depletion by siRNA causes Golgi dispersion	Preisinger *et al* (2004)
MTG	*Cis*-Golgi	Myeloid translocation gene	Unknown	Asirvatham *et al* (2004)
Myomegalin	Golgi, centrosome	Scaffold protein	Unknown	Verde *et al* (2001)
PCTAIRE	ER	Cycin-dependent kinase	Regulator of ER-to-Golgi transport	Palmer *et al* (2005)
PDE4D3	Golgi, centrosome	Phosphodiesterase	Unknown	Jin *et al* (1998)
PDE7A	*Cis*-Golgi	Phosphodiesterase	Unknown	Asirvatham *et al* (2004)
PERK	ER	Serine/threonine kinase	Transduces effects of UPR	Rutkowski and Kaufman (2004)
PI3K	Golgi	Lipid kinase	Involved in Golgi disassembly during mitosis	Domin *et al* (2000)
PKA	Golgi	Serine/threonine kinase	Regulates endosome-to-TGN, Golgi-to-ER, and TGN-to-PM transport	Birkeli *et al* (2003), Muniz *et al* (1997), Cabrera *et al* (2003)
PKC	Golgi	Serine/threonine kinase	Regulates TGN-to-PM transport and apoptosis	Buccione *et al* (1996) Kajimoto *et al* (2004)
PKD	Golgi	Serine/threonine kinase	TGN-to-PM transport	Diaz Anel and Malhotra (2005), Ghanekar and Lowe (2005)
PLK3	Golgi	Serine/threonine kinase	Involved in Golgi disassembly during mitosis	Xie *et al* (2004)
PSKH1	*Cis*-Golgi, centrosome, nuclei	Serine/threonine kinase	Mutant kinase causes Golgi disassembly	Brede *et al* (2003)
PTPH1	ERES	Protein tyrosine phosphatase	Coordinates ERES assembly	Lavoie *et al* (2000)
Rac	TGN	Small GTP-binding protein	TGN sorting	Faucherre *et al* (2003)
Rap1	Golgi	Small GTP-binding protein	Unknown	Wienecke *et al* (1996)
Ras	Golgi, ER	Small GTP-binding protein	Supports cell transformation via MAPK	Bivona and Philips (2003)
RGS2	Golgi	Regulator of G-protein signalling	Unknown	Sullivan *et al* (2000)
RGS4	Golgi	Regulator of G-protein signalling	Regulates Golgi-to-PM transport via interaction with β-COP	Sullivan *et al* (2000)
RGS-GAIP	TGN	Regulator of G-protein signalling	Unknown	De Vries *et al* (1998)
RGSZ	Golgi	Regulator of G-protein signalling	Regulates ER-to-PM transport and Golgi disruption	Chatterjee and Fisher (2000), Nagahama *et al* (2002)
Sef	Golgi	Scaffold protein	Spatial regulator of ERK signalling	Torii *et al* (2004)
$sG\alpha_{i2}$	Golgi	Heterotrimeric GTP-binding protein	Maintenance of Golgi structure	Montmayeur and Borrelli (1994)
Src	Golgi	Tyrosine kinase	Regulates retrograde transport of KDELr	Bard *et al* (2003), Bard *et al* (2002)
TrkA	Golgi	Tyrosine kinase receptor	Neuronal survival	Rajagopal *et al* (2004)
$XLG\alpha_s$	Golgi	Heterotrimeric GTP-binding protein	Unknown	Pasolli *et al* (2000) Ugur and Jones (2000)
YSK1	Golgi	Serine/threonine kinase	Kinase-dead and siRNA causes Golgi dispersion	Preisinger *et al* (2004)
βγ	Unknown	Heterotrimeric GTP-binding protein	TGN-to-PM transport and regulation of Golgi complex organization	Jamora *et al* (1999), Diaz Anel and Malhotra (2005)

AKAP, protein kinase A-anchoring scaffold protein; GTP, guanine 5′-triphosphate; MAP, mitogen-activated protein; PM, plasma membrane; ER, endoplasmic reticulum; ERGIC, ER–Golgi intermediate compartment; TGN, *trans*-Golgi network; ERES, ER exit sites; UPR, unfolded protein response; KDELr, KDEL receptor.

The membranes of the secretory system host several signalling proteins, ranging from the heterotrimeric G-proteins to small G-proteins, scaffold proteins, kinases, phosphodiesterases, GTPase-activating proteins and guanine nucleotide exchange factors. For many of these, the only information available relates to the effects of their perturbation on Golgi morphology and function, with their physiological roles remaining, as yet, unknown.

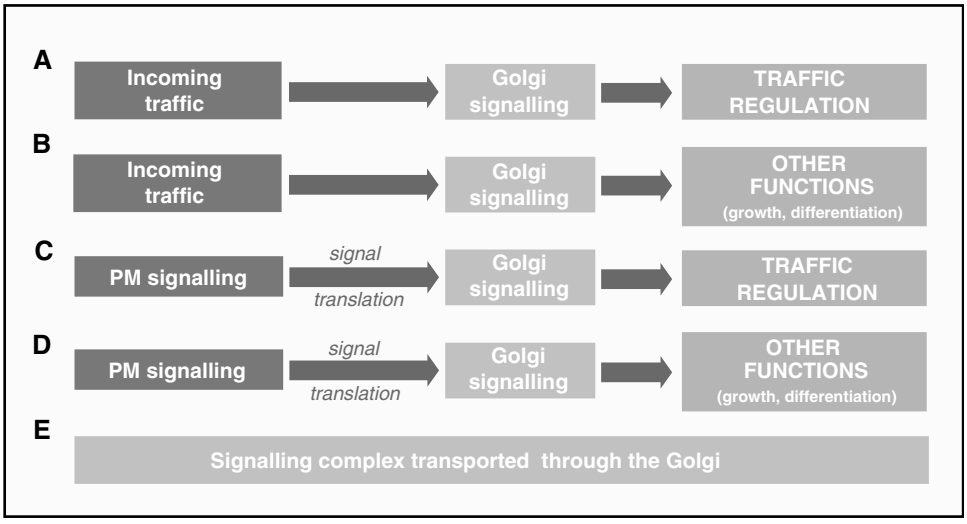

Figure 1 Generic models for the roles of signalling proteins at the Golgi complex. (**A, B**). Direct activation of Golgi signalling by endogenous events (e.g. incoming traffic)—self-regulation of the trafficking pathway (A) and/or regulation of other cellular functions (B); (**C**) activation of PM signals—signal translation—activation of Golgi signaling—regulation of trafficking; (**D**) activation of PM signals—signal translation—activation of Golgi signaling—regulation of other cellular functions (e.g. cell proliferation, motility); (**E**) passive residence of signalling molecules in transit to the PM.

The mechanisms of action through which PKA exerts these effects have been identified only in part. One mechanism relates to the ability of PKA to recruit ADP ribosylation factor-1 (ARF1) to the Golgi complex (Martin *et al*, 2000). ARF1 belongs to the Ras superfamily of small GTP-binding proteins and is the master regulator of many fundamental steps along the secretory pathway, including the assembly of coat proteins, such as COPI and clathrin (Donaldson *et al*, 2005). The recruitment of ARF1 from the cytosol to Golgi membranes has been shown to be increased by the catalytic portion of PKA and by cAMP (Martin *et al*, 2000). Conversely, ARF1 recruitment is impaired by PKI (a selective PKA inhibitor) and by PKA depletion (Martin *et al*, 2000). This has been confirmed *in vivo* by direct stimulation of cAMP formation using forskolin, which led to an increase in the recruitment of ARF1 to the Golgi complex (Martin *et al*, 2000).

Another target of PKA that might contribute to the regulation of membrane transport is the KDEL receptor (KDELr). The KDELr is a seven-transmembrane-domain protein that cycles between the ER and the Golgi complex (Lewis and Pelham, 1992). In doing so, it returns to the ER the ER-resident chaperones that have leaked through to post-ER compartments during membrane transport. PKA phosphorylates a COPI-binding motif on the C-tail of the KDELr, which is necessary for the retrograde transport of the KDELr itself (Cabrera *et al*, 2003). PKA also phosphorylates certain SNAREs (soluble *N*-ethyl-maleimide-sensitive fusion protein attachment protein receptor) and regulates their ability to support membrane fusion in endocytic and exocytic steps in mammals (Hong, 2005), as well as in ER-to-Golgi and intra-Golgi transport in yeast (Hong, 2005; Weinberger *et al*, 2005). Furthermore, PKA is involved in the ER quality control process for a class of proteins that bear the RXR transient-ER-retention motif through which they bind to COPI (Michelsen *et al*, 2005). These include G-protein-coupled receptors (GPCRs) and multimeric ion channels, the transport of which is delayed in the ER through a COPI-mediated retrieval mechanism. When these protein complexes are properly assembled, their RXR motif is masked by an interaction with the partner subunit(s) or by PKA or protein kinase C (PKC)-mediated phosphorylation of a nearby serine or threonine, which itself generates a binding site for the adaptor protein 14-3-3 that prevents the RXR motif from interacting with COPI (Kuwana *et al*, 1998; Michelsen *et al*, 2005).

The presence of the components of the cAMP-PKA pathway (including those responsible for the formation of cAMP) on the Golgi and their regulation of traffic through this organelle is consistent with a scheme (Figure 1A) in which PKA-based Golgi-initiated signalling coordinates the activities of different Golgi subcompartments. However, the complete circuit in which this pathway might be involved, and the circumstances in which this circuit might be activated, remains to be defined, and alternative explanations for the presence of the pathway components on the Golgi cannot be excluded at this time (e.g. schemes in Figure 1B and C).

The DAG-PKC pathway

The protein kinase C (PKC) are a family of at least 10 serine/threonine kinases that can be structurally and functionally subclassified into three main groups: those Ca^{2+}- and diacylglycerol (DAG)-dependent, those calcium-independent and those atypical, calcium- and DAG-independent. Most of these can localize to the Golgi complex via their C1 lipid-binding domains (Schultz *et al*, 2004), which can bind to DAG and/or to ceramides, retinoic acid and archidonic acid. This binding can have important functional consequences. In neuroblastoma and HeLa cells treated with ceramides, PKCδ and PKCε show a C1-domain-dependent translocation to the Golgi complex, and this translocation is necessary for the apoptotic effects promoted by ceramides (Kajimoto *et al*, 2001; Schultz *et al*, 2003; Kajimoto *et al*, 2004). Similar effects are seen with IFN-γ treatment: this acts via ceramide production and the

 ©2006 European Molecular Biology Organization

consequent translocation of PKCδ to the Golgi complex, which results in cell apoptosis. PKCδ is activated only after its Golgi translocation, via direct phosphorylation on tyrosines 311 and 332 by a Golgi-located Src, and this phosphorylation is necessary for the apoptotic effect promoted by PKCδ (Kajimoto *et al*, 2004). Another Golgi targeting mechanism is via the RACK (receptor for activated PKC)-anchoring proteins. PKCε is targeted to the Golgi complex via a direct interaction with the β′ subunit of the coatomer β′-COP, which acts as a RACK through its characteristic WD-40 repeat that is found in other RACKs (Csukai *et al*, 1997).

The recruitment of the PKCs to endomembranes also has regulatory effects on traffic. The intermediate compartment that operates between the ER and the Golgi is an important sorting station where anterograde-directed proteins are segregated from recycling proteins. Here, Rab2, a small GTPase and a key player in the intermediate compartment function, recruits PKCι/λ (Tisdale and Artalejo, 2006), which promotes the binding of COPI to intermediate compartment membranes (Tisdale, 2000, 2003), and phosphorylates glyceraldehyde 3-phosphate dehydrogenase (GAPDH). GAPDH then ultimately influences microtubule dynamics and transport in the early secretory pathway (Tisdale, 2002). ER-to-Golgi transport is impaired by the specific PKC inhibitor calphostin C, whereas it is activated by DAG analogues (e.g. phorbol 12-myristate 13-acetate (PMA)) (Fabbri *et al*, 1994). However, several other PKC inhibitors that act as ATP competitors (e.g. H7, H8, staurosporine) and the downregulation of PKC have no effects on ER-to-Golgi transport, thus casting doubts as to the role of PKC here, and suggesting instead the involvement of another (as yet unidentified) protein that contains a DAG-binding domain (Fabbri *et al*, 1994).

Constitutive intra-Golgi and TGN-to-PM transport can be modulated by PKC (De Matteis *et al*, 1993; Fabbri *et al*, 1994). De Matteis *et al* (1993) have demonstrated that the transport of glucosaminoglycans (GAGs) and the temperature-sensitive variant of the G protein of vesicular stomatitis virus (VSVG) from the Golgi to the PM requires PKC activity (Buccione *et al*, 1996), and that the direct activation of PKC by PMA or via activation of the IgE receptor stimulates GAG secretion. In this context, the action of PKC is likely to involve more than one mechanism, including the regulation of the GTP-dependent binding of ARF1 to Golgi membranes (De Matteis *et al*, 1993). As the IgE receptor has been shown to stimulate traffic from the ER to the Golgi in addition to Golgi-to-PM traffic (Bannykh *et al*, 1995; Buccione *et al*, 1996), these data identify a case of stimulation of the entire constitutive secretory pathway by an extracellular ligand. The significance of these observations has been discussed previously (Luini and De Matteis, 1993). In an analogous study, Fabbri *et al* (1994) showed that the PKC inhibitor calphostin C blocks the transport of VSVG from the TGN to the PM. Using an *in vitro* system to generate post-Golgi vesicles, Sabatini and co-workers showed that PKC is involved in membrane carrier fission from the TGN (however, they proposed that in this case PKC does not act via its kinase activity) (Simon *et al*, 1996). Finally, in a series of studies that is further discussed below, the Malhotra laboratory (see above) showed that PKCη acts as a kinase that activates protein kinase D (PKD) and that this is required for the fission of TGN carriers (Diaz Anel and Malhotra, 2005).

Therefore, PKC on the Golgi complex appears to be involved in at least two main functions: apoptosis-related signalling and the regulation of traffic. One of the PKC-dependent pathways that controls traffic initiates at the cell surface with the activation of IgE receptors, and so this fits well with scheme C in Figure 1. Whether PKC is also activated on the Golgi by traffic itself (scheme A in Figure 1) is unknown, but it is possible that traffic can induce changes in the lipid composition (DAG, ceramides) in specific Golgi domains, which could then result in the regulation of PKC activity (Baron and Malhotra, 2002; Diaz Anel and Malhotra, 2005).

Heterotrimeric G proteins

Heterotrimeric G proteins are a family of GTPases that are primarily involved in the transduction of signals initiated at the PM by seven-transmembrane-domain receptors. However, they have also been proposed to have direct roles in membrane transport based on the evidence that a number of different Gα subunits are located on internal membranes, including the Golgi complex (Denker *et al*, 1996; Helms *et al*, 1998; Qian *et al*, 2006), and that manipulation of the levels or activities of these G proteins markedly affects the functioning of transport pathways.

The distribution of the different Gα subunits on endomembranes has been the target of many studies in different laboratories (Stow *et al*, 1991b; Wilson *et al*, 1994). Stow and co-workers demonstrated that $G\alpha_{i3}$ is present on Golgi membranes in epithelial cells (Stow *et al*, 1991a; Stow and de Almeida, 1993) and Farquhar and co-workers (Wilson *et al*, 1994) showed that $G\alpha_{i3}$ is mostly on the *cis* side of the Golgi in pituitary cells. A partial Golgi distribution was also described for $G\alpha_q$ (Denker *et al*, 1996). Both a short and a long form of $G\alpha_s$ have been identified in the Golgi in liver and adrenal medulla cells (Maier *et al*, 1995), and Huttner and co-workers showed that $G\alpha_s$ colocalizes with the *trans*-Golgi marker protein TGN38 in PC12 cells (Leyte *et al*, 1992). $G\alpha_z$ has also been shown to localize to the Golgi complex in BHK cells, where it overlaps with the Golgi marker mannosidase II and follows the redistribution of Golgi proteins after treatment with brefeldin A (BFA, a fungal toxin that disassembles and redistributes the Golgi into the ER) (Nagahama *et al*, 2002).

From a functional standpoint, several secretory transport steps have been proposed to be regulated by heterotrimeric G proteins on the basis of a variety of lines of evidence. Transport from the ER to the Golgi complex was suggested to be controlled by these G proteins, since mastoparan (an activator of $G\alpha_i$) causes a block in the exit of VSVG from the ER (Schwaninger *et al*, 1992). A similar result was also seen in permeabilized cells treated with purified βγ subunits (which deplete the Gα pool by associating with it), suggesting that both activatory and inhibitory heterotrimeric G proteins are involved in the export of proteins from the ER.

Heterotrimeric G proteins have also been implicated in intra-Golgi and post-Golgi trafficking. Manipulation (overexpression or treatment with pertussis toxin) of $G\alpha_{i3}$ (which is located in the Golgi; see above) indicated that this protein has an overall inhibitory role on anterograde secretory traffic (Stow *et al*, 1991a). COPI (a protein complex crucially involved in intra-Golgi traffic (Rothman, 1994)) has been

©2006 European Molecular Biology Organization

shown to interact with the hetrotrimeric G protein GTPase-activating protein RGS4 (regulator of G protein signalling-4) (Sullivan *et al*, 2000). This interaction impairs COPI binding to Golgi membranes (although ARF1 localization and Golgi integrity are not affected), and apparently results in inhibition of the transport of acquaporin 1 to the PM and the secretion of placental alkaline phosphatase. Thus, it was hypothesized that RGS4 regulates transport through the sequestration of β-COP from the cytosol, with this sequestration inhibiting β-COP recruitment to Golgi membranes. Potentially linked to these observations, Velasco and co-workers showed that the *in vitro* interaction of PKA with Golgi membranes is sensitive to modulators of heterotrimeric G proteins, and that myristoylated $G\alpha_{i3}$ can stimulate this interaction, suggesting a role for G proteins in the recruitment of PKA to Golgi membranes (Martin *et al*, 1999). PKA participates in COPI-dependent retrograde transport of the KDELr by the phosphorylation of the C-tail of the KDELr, which is mandatory for its interaction with β-COP and its inclusion in COPI-coated vesicles (see also below). Collectively, the above data suggest that G-protein-dependent signalling is involved in the regulation of the COPI trafficking machinery, although the precise mechanism of this involvement remains unclear.

Heterotrimeric G proteins also appear to be involved in the regulation of TGN-to-PM transport. In an early study, Pimplikar and Simons (1993) showed that treatment with reagents that influence $G\alpha_s$ specifically impair apical, and not basolateral, transport. On the other hand, a selectivity towards basolateral transport was seen with treatments influencing $G\alpha_i$, leading to the conclusion that $G\alpha_s$ and $G\alpha_i$ could be involved in the regulation of polarized TGN-to-PM transport. In addition to Gα subunits, the βγ subunits appear to have a role in transport from the TGN. In what is the most complete series of studies in this area to date, Malhotra and co-workers showed that PKD, a key regulator of membrane fission at the TGN, is activated by a signalling pathway that involves βγ subunits ($\beta_1\gamma_2/\beta_3\gamma_2$), and that this activation passes through PKCη, in this way regulating transport to the cell surface and organization of the Golgi complex (Diaz Anel and Malhotra, 2005) (Liljedahl *et al*, 2001; Yeaman *et al*, 2004). RGS proteins are also present on the TGN, and it has been shown that RGS-Gα-interacting protein (RGS-GAIP) is localized to clathrin-coated buds and vesicles in the Golgi region. However, at this stage, the function of RGS-GAIP remains to be defined (De Vries *et al*, 1998; Wylie *et al*, 1999, 2003; Chatterjee and Fisher, 2000; Gleeson *et al*, 2004).

$G\alpha_z$ and $G\alpha_{i2}$ also appear to have key roles in the maintenance of the overall structure of the Golgi complex (Yamaguchi *et al*, 2000). Their overexpression results in inhibition of nordihydroguaiaretic acid (NDGA)-induced disassembly of the Golgi complex. Conversely, overexpression of the regulator (repressor) of $G\alpha_z$ signalling (RGS_z) stimulated disruption of the Golgi complex, and delayed ER-to-PM transport of VSVG (Nagahama *et al*, 2002).

Thus, there is abundant (albeit heterogeneous) evidence for a role for heterotrimeric G proteins and of some of their accessory proteins in secretory transport, as well as in the control of the structure of the Golgi. The fact that they are both present and able to exert effects on this organelle suggests that they might operate within local regulatory circuits, which would be consistent with scheme A in Figure 1. However, a coherent picture of the pathway(s) in which they are involved remains to be defined.

The Ras signalling pathway

Ras is the prototype of a large family of small GTPases that are involved in multiple cellular functions, including the transduction of the effects of many extracellular ligands, such as growth factors, via PM receptors. Recently, Ras has been shown to be present on the Golgi and to be activated there as a consequence of PM-based signalling. Different Ras family proteins localize to distinct subdomains of the PM (Prior *et al*, 2001) and/or to various intracellular compartments, including the Golgi, in a palmitoylation-dependent fashion (Choy *et al*, 1999; Michaelson *et al*, 2001; Rocks *et al*, 2005). Moreover, downstream elements of the Ras pathway have also been demonstrated to be located on the Golgi complex (Philips, 2004). Over the last few years, this has prompted a number of studies to address the question of whether these proteins can actually signal from endomembranes. In particular, Philips and co-workers (Chiu *et al*, 2002) investigated the pool of Ras localized on endomembranes using an *in vivo* probe for activated Ras. They concluded that stimulation with mitogens results in the activation of Ras both on the PM and on the Golgi complex, but with different kinetics and through different mechanisms (some of these findings have, however, been disputed by others (Augsten *et al*, 2006). Philips and colleagues have also shown that activation of H-Ras on the Golgi complex, but not on the PM, is dependent on Src-family kinases (Chiu *et al*, 2002). Thus, following Src-dependent activation of phospholipase Cγ (PLCγ), Ras guanine nucleotide releasing protein 1 (RasGRP1) translocates to the Golgi complex, where it activates Ras (Bivona and Philips, 2003). This pathway differs from that at the PM, indicating that distinct modes of activation and deactivation can regulate Ras in different subcellular compartments. This strategy might allow the cell to differentially regulate the activation of Ras in different cellular locations (Bivona and Philips, 2003).

As noted, not only Ras but also other components of the Ras pathway appear to be located on the Golgi complex. Recently, Nishida and co-workers (Torii *et al*, 2004) demonstrated the 'spatial' modulatory activity of a novel scaffold protein, Sef, which is localized to the Golgi. Sef had been previously identified as a negative regulator of FGF signalling, and here it was shown to bind the active MEK/ERK complex, anchoring it to the Golgi complex (Sorkin, 2005). When ERK is trapped within this heterotrimeric complex, it can still phosphorylate one of its cytosolic substrates (RSK2), although it cannot dissociate and translocate to the nucleus to activate the array of nuclear ERK effectors (e.g. Elk-1). Reinforcing the concept that Sef works as a spatial modulator, it was shown that Golgi-entrapped ERK can still phosphorylate the nuclear target Elk-1 if the latter is made to move into the cytosol (Torii *et al*, 2004). What is still unknown is which proteins control the function of Sef and when they promote the switch from nuclear to cytosolic signalling of the ERK pathway. To complete the picture, Sef activation switches off the nuclear ERK pathway while activating the TAK1/JNK apoptotic pathway (Yang *et al*, 2004).

Finally, it has been shown that the nerve growth factor (NGF) receptor (TrkA) can localize to the Golgi complex,

 ©2006 European Molecular Biology Organization

where it can be functionally activated, and thus impinge on the Ras pathway (Sorkin, 2005). Stimulation of the G protein-coupled receptors for adenosine or pituitary adenylate cyclase-activating polypeptide (PACAP) in neuronal cells has been shown to support cell survival via *trans*-activation of the pool of Golgi-based TrkA receptor. TrkA transactivation requires new protein synthesis and engages a population of immature receptors that are localized exclusively at the Golgi complex (Rajagopal *et al*, 2004). A BFA treatment, which results in Golgi disassembly and redistribution into the ER, completely prevented TrkA transactivation, while not affecting its stimulation via NGF from the PM, indicating the requirement of a preserved Golgi structure for this signalling pathway. Moreover, *trans*-activation of TrkA on the Golgi complex depends on Src and calcium ions (Rajagopal *et al*, 2004).

To date, these observations have been interpreted as indicating a role of the Ras signalling pool on the Golgi complex as a relay device for signalling cascades that are initiated at the PM and are involved in growth and proliferation (Figure 1D). There is no evidence so far for a link between Ras and the regulation of traffic. If this is so, the case of Ras might be different from that of the signalling pathways discussed above, which appeared to be involved in traffic regulation.

Physiological significance of endomembrane-based signalling and concluding remarks

In addition to the signalling pathways discussed above, a myriad of other signalling molecules of various kinds have been found to reside on secretory compartments, and in particular in the Golgi complex (Table 1). Most of these are only partially (or not yet) characterized in terms of their function at the Golgi, or of their precise subcompartment locations. This collective body of knowledge strongly suggests the existence of a hugely complex and rich endomembrane-based signalling network, the function and connectivity of which are only beginning to be glimpsed at.

To begin to bring order to this complex body of information, we propose the five generic model pathways illustrated in Figure 1. Evidence is so far available for the sequence in Figure 1C (activation of PM signals—signal translation—activation of Golgi signaling—regulation of traffic (De Matteis *et al*, 1993; Fabbri *et al*, 1994; Buccione *et al*, 1996) and Figure 1D (activation of PM signals—signal translation—activation of Golgi signaling—regulation of other functions (e.g. cell proliferation and motility) (Bivona and Philips, 2003). Moreover, for some G proteins, evidence has been produced that their Golgi location reflects their transit or cycling through the secretory pathway (Michaelson *et al*, 2002; Takida and Wedegaertner, 2004; Rocks *et al*, 2005) (Figure 1E). The formal demonstration of direct activation of a Golgi-based signalling pathway by a traffic event (Figure 1A and B) is instead still lacking, despite a large number of tantalizing observations that have indicated that such pathways exist. However, an example of initiation of signalling cascades on a secretory organelle by endogenous events is provided by the case of the ER stress responses (Patil and Walter, 2001; Zhang and Kaufman, 2004), whereby ER stresses can activate signalling pathways on the cytosolic surface of the ER, which then regulate survival-related and other cellular functions.

The overall picture discussed above defines almost by itself the tasks being called for. Much is known about the endomembrane localization and the effects on traffic of signalling molecules. What is largely lacking, however, is an understanding of how information flows through this maze of signalling molecules that would allow us to define complete specific pathways, along with their physiological significance and context. In particular, the question of the activation mechanisms of signalling complexes at trafficking endomembranes remains open. Let us take, for instance, the case of the heterotrimeric G proteins. How might they be activated at their intracellular sites? GPCR-like molecules that can bind G proteins on their cytosolic tail have been reported to reside on internal endomembranes (Boivin *et al*, 2003; Konger *et al*, 2005). Moreover, more than one type of GPCR-unrelated molecule has been reported that might in principle participate in the activation of G proteins at the Golgi

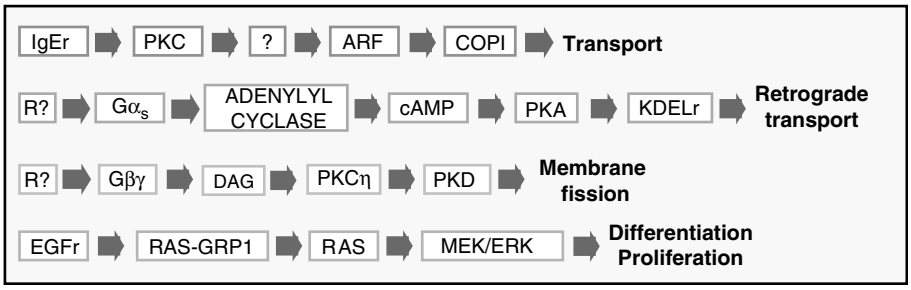

Figure 2 Representative Golgi-based signalling pathways. Several signalling complexes have been shown to be present on intracellular organelles. Here we depict the main elements of four representative pathways: the IgE receptor (IgEr) on the PM activates PKC, which promotes the recruitment of ARF1 and COPI to the Golgi complex via an unknown mediator. This pathway regulates the transport of cargo toward the PM. A hypothetical receptor (R?) with a potential intracellular location activates $G\alpha_s$ that, in turn, activates adenylyl cyclase, to generate the cAMP that is necessary for activation of PKA at the Golgi complex. This active PKA phosphorylates the KDELr, uncovering the COPI-binding motif that is necessary for its retrograde transport. A hypothetical receptor (R?) activates a heterotrimeric G protein to release its $\beta\gamma$ subunit. This generates diacylglycerol (DAG) through an unknown mechanism, which in turn activates PKCη. The subsequent phosphorylation and activation of PKD is required for membrane fission at the TGN. Stimulation of the EGF receptor (EGFr) produces via PLCγ the Ca^{2+} and DAG that are necessary for translocation of the Ras exchange factor Ras-GRP1 to the Golgi complex. Ras-GRP1 activates Ras on the Golgi, and the resulting activation of the MAP kinase (MEK/ERK) pathway supports basic cellular functions, such as cell growth and differentiation.

©2006 European Molecular Biology Organization

complex (Blumer and Lanier, 2003). However, no information is available so far on possible functional links between any of these molecules and the Golgi heterotrimeric G proteins, nor is it known whether the initial activating stimuli are driven by extracellular signals or by endogenous (e.g. traffic initiated) events. Also, the identity of the G protein effectors on endomembranes remains unclear, although there is some evidence for the existence of traffic regulation by 'classical' G-protein-effector pathways (as discussed above, and see Figure 2). Similar questions apply to other signalling pathways. The discovery of endogenous activation mechanisms that can initiate inter-organelle signalling would greatly advance our understanding of the coordination of the many intracellular activities involved in the maintenance of harmonic global cellular behaviour, a critical, yet largely unexplored, aspect of the physiology of traffic and of other cell functions.

Acknowledgements

We thank CP Berrie and C Wilson for editorial assistance, and E Fontana for artwork preparation. We also like to apologise to all of the authors of original studies whom we have not been able to reference and for the general use of review referencing, both of which arise from space limitations. We also acknowledge and thank the Italian Association for Cancer Research (AIRC, Milan, Italy), Telethon Italia and the Ministero dell'Istruzione, dell'Università e della Ricerca (MIUR, Italy) for financial support. TP is the recipient of a fellowship of the Italian Foundation for Cancer Research (FIRC, Milan, Italy) and a fellowship from Fondazioni Bancarie Abruzzesi and Fondazione Negri Sud ONLUS (Progetto Sviluppo Sud).

References

Acharya U, Mallabiabarrena A, Acharya JK, Malhotra V (1998) Signaling via mitogen-activated protein kinase kinase (MEK1) is required for Golgi fragmentation during mitosis. *Cell* **92:** 183–192

Aridor M, Balch WE (2000) Kinase signaling initiates coat complex II (COPII) recruitment and export from the mammalian endoplasmic reticulum. *J Biol Chem* **275:** 35673–35676

Asirvatham AL, Galligan SG, Schillace RV, Davey MP, Vasta V, Beavo JA, Carr DW (2004) A-kinase anchoring proteins interact with phosphodiesterases in T lymphocyte cell lines. *J Immunol* **173:** 4806–4814

Augsten M, Pusch R, Biskup C, Rennert K, Wittig U, Beyer K, Blume A, Wetzker R, Friedrich K, Rubio I (2006) Live-cell imaging of endogenous Ras-GTP illustrates predominant Ras activation at the plasma membrane. *EMBO Rep* **7:** 46–51

Bannykh S, Aridor M, Plutner H, Rowe T, Balch WE (1995) Regulated export of cargo from the endoplasmic reticulum of mammalian cells. *Cold Spring Harb Symp Quant Biol* **60:** 127–137

Bard F, Mazelin L, Pechoux-Longin C, Malhotra V, Jurdic P (2003) Src Regulates Golgi structure and KDEL receptor-dependent retrograde transport to the endoplasmic reticulum. *J Biol Chem* **278:** 46601–46606

Bard F, Patel U, Levy JB, Jurdic P, Horne WC, Baron R (2002) Molecular complexes that contain both c-Cbl and c-Src associate with Golgi membranes. *Eur J Cell Biol* **81:** 26–35

Baron CL, Malhotra V (2002) Role of diacylglycerol in PKD recruitment to the TGN and protein transport to the plasma membrane. *Science* **295:** 325–328

Behnia R, Munro S (2005) Organelle identity and the signposts for membrane traffic. *Nature* **438:** 597–604

Bijlmakers MJ, Isobe-Nakamura M, Ruddock LJ, Marsh M (1997) Intrinsic signals in the unique domain target p56(lck) to the plasma membrane independently of CD4. *J Cell Biol* **137:** 1029–1040

Birkeli KA, Llorente A, Torgersen ML, Keryer G, Tasken K, Sandvig K (2003) Endosome-to-Golgi transport is regulated by protein kinase A type II alpha. *J Biol Chem* **278:** 1991–1997

Bivona TG, Perez DC, I Ahearn IM, Grana TM, Chiu VK, Lockyer PJ, Cullen PJ, Pellicer A, Cox AD, Philips MR (2003) Phospholipase Cgamma activates Ras on the Golgi apparatus by means of RasGRP1. *Nature* **424:** 694–698

Bivona TG, Philips MR (2003) Ras pathway signaling on endomembranes. *Curr Opin Cell Biol* **15:** 136–142

Blumer JB, Lanier SM (2003) Accessory proteins for G protein-signaling systems: activators of G protein signaling and other nonreceptor proteins influencing the activation state of G proteins. *Receptors Channels* **9:** 195–204

Boivin B, Chevalier D, Villeneuve LR, Rousseau E, Allen BG (2003) Functional endothelin receptors are present on nuclei in cardiac ventricular myocytes. *J Biol Chem* **278:** 29153–29163

Bonifacino JS, Glick BS (2004) The mechanisms of vesicle budding and fusion. *Cell* **116:** 153–166

Brede G, Solheim J, Stang E, Prydz H (2003) Mutants of the protein serine kinase PSKH1 disassemble the Golgi apparatus. *Exp Cell Res* **291:** 299–312

Buccione R, Bannykh S, Santone I, Baldassarre M, Facchiano F, Bozzi Y, Di Tullio G, Mironov A, Luini A, De Matteis MA (1996) Regulation of constitutive exocytic transport by membrane receptors. A biochemical and morphometric study. *J Biol Chem* **271:** 3523–3533

Cabrera M, Muniz M, Hidalgo J, Vega L, Martin ME, Velasco A (2003) The retrieval function of the KDEL receptor requires PKA phosphorylation of its C-terminus. *Mol Biol Cell* **14:** 4114–4125

Cha H, Shapiro P (2001) Tyrosine-phosphorylated extracellular signal-regulated kinase associates with the Golgi complex during G2/M phase of the cell cycle: evidence for regulation of Golgi structure. *J Cell Biol* **153:** 1355–1367

Chatterjee TK, Fisher RA (2000) Cytoplasmic, nuclear, and golgi localization of RGS proteins. Evidence for N-terminal and RGS domain sequences as intracellular targeting motifs. *J Biol Chem* **275:** 24013–24021

Cheng H, Farquhar MG (1976a) Presence of adenylate cyclase activity in Golgi and other fractions from rat liver. I. Biochemical determination. *J Cell Biol* **70:** 660–670

Cheng H, Farquhar MG (1976b) Presence of adenylate cyclase activity in Golgi and other fractions from rat liver. II. Cytochemical localization within Golgi and ER membranes. *J Cell Biol* **70:** 671–684

Chiu VK, Bivona T, Hach A, Sajous JB, Silletti J, Wiener H, Johnson II RL, Cox AD, Philips MR (2002) Ras signalling on the endoplasmic reticulum and the Golgi. *Nat Cell Biol* **4:** 343–350

Choy E, Chiu VK, Silletti J, Feoktistov M, Morimoto T, Michaelson D, Ivanov IE, Philips MR (1999) Endomembrane trafficking of ras: the CAAX motif targets proteins to the ER and Golgi. *Cell* **98:** 69–80

Codogno P, Meijer AJ (2005) Autophagy and signaling: their role in cell survival and cell death. *Cell Death Differ* **12** (Suppl 2)**:** 1509–1518

Colanzi A, Sutterlin C, Malhotra V (2003) RAF1-activated MEK1 is found on the Golgi apparatus in late prophase and is required for Golgi complex fragmentation in mitosis. *J Cell Biol* **161:** 27–32

Conner SD, Schmid SL (2003) Regulated portals of entry into the cell. *Nature* **422:** 37–44

Csukai M, Chen CH, De Matteis MA, Mochly-Rosen D (1997) The coatomer protein beta'-COP, a selective binding protein (RACK) for protein kinase Cepsilon. *J Biol Chem* **272:** 29200–29206

De Matteis MA, Santini G, Kahn RA, Di Tullio G, Luini A (1993) Receptor and protein kinase C-mediated regulation of ARF binding to the Golgi complex. *Nature* **364:** 818–821

Denker SP, McCaffery JM, Palade GE, Insel PA, Farquhar MG (1996) Differential distribution of alpha subunits and beta gamma subunits of heterotrimeric G proteins on Golgi membranes of the exocrine pancreas. *J Cell Biol* **133:** 1027–1040

De Vries L, Elenko E, McCaffery JM, Fischer T, Hubler L, McQuistan T, Watson N, Farquhar MG (1998) RGS-GAIP, a GTPase-activating

 ©2006 European Molecular Biology Organization

protein for Galphai heterotrimeric G proteins, is located on clathrin-coated vesicles. *Mol Biol Cell* **9:** 1123–1134

Diaz Anel AM, Malhotra V (2005) PKCeta is required for beta1gamma2/beta3gamma2- and PKD-mediated transport to the cell surface and the organization of the Golgi apparatus. *J Cell Biol* **169:** 83–91

Di Fiore PP, De Camilli P (2001) Endocytosis and signaling an inseparable partnership. *Cell* **106:** 1–4

Domin J, Gaidarov I, Smith ME, Keen JH, Waterfield MD (2000) The class II phosphoinositide 3-kinase PI3K-C2alpha is concentrated in the trans-Golgi network and present in clathrin-coated vesicles. *J Biol Chem* **275:** 11943–11950

Donaldson JG, Honda A, Weigert R (2005) Multiple activities for Arf1 at the Golgi complex. *Biochim Biophys Acta* **1744:** 364–373

Donaldson JG, Lippincott-Schwartz J (2000) Sorting and signaling at the Golgi complex. *Cell* **101:** 693–696

Fabbri M, Bannykh S, Balch WE (1994) Export of protein from the endoplasmic reticulum is regulated by a diacylglycerol/phorbol ester binding protein. *J Biol Chem* **269:** 26848–26857

Faucherre A, Desbois P, Satre V, Lunardi J, Dorseuil O, Gacon G (2003) Lowe syndrome protein OCRL1 interacts with Rac GTPase in the trans-Golgi network. *Hum Mol Genet* **12:** 2449–2456

Flett A, Semerdjieva S, Jackson AP, Smythe E (2005) Regulation of the clathrin-coated vesicle cycle by reversible phosphorylation. *Biochem Soc Symp* **72:** 65–70

Ghanekar Y, Lowe M (2005) Protein kinase D: activation for Golgi carrier formation. *Trends Cell Biol* **15:** 511–514

Gleeson PA, Lock JG, Luke MR, Stow JL (2004) Domains of the TGN: coats, tethers and G proteins. *Traffic* **5:** 315–326

Heinrich R, Rapoport TA (2005) Generation of nonidentical compartments in vesicular transport systems. *J Cell Biol* **168:** 271–280

Helms JB, Helms-Brons D, Brugger B, Gkantiragas I, Eberle H, Nickel W, Nurnberg B, Gerdes HH, Wieland FT (1998) A putative heterotrimeric G protein inhibits the fusion of COPI-coated vesicles. Segregation of heterotrimeric G proteins from COPI-coated vesicles. *J Biol Chem* **273:** 15203–15208

Hong W (2005) SNAREs and traffic. *Biochim Biophys Acta* **1744:** 493–517

Hu Y, Leo C, Yu S, Huang BC, Wang H, Shen M, Luo Y, Daniel-Issakani S, Payan DG, Xu X (2004) Identification and functional characterization of a novel human misshapen/Nck interacting kinase-related kinase, hMINK beta. *J Biol Chem* **279:** 54387–54397

Jamora C, Yamanouye N, Van Lint J, Laudenslager J, Vandenheede JR, Faulkner DJ, Malhotra V (1999) Gbetagamma-mediated regulation of Golgi organization is through the direct activation of protein kinase D. *Cell* **98:** 59–68

Jin SL, Bushnik T, Lan L, Conti M (1998) Subcellular localization of rolipram-sensitive, cAMP-specific phosphodiesterases. Differential targeting and activation of the splicing variants derived from the PDE4D gene. *J Biol Chem* **273:** 19672–19678

Kajimoto T, Ohmori S, Shirai Y, Sakai N, Saito N (2001) Subtype-specific translocation of the delta subtype of protein kinase C and its activation by tyrosine phosphorylation induced by ceramide in HeLa cells. *Mol Cell Biol* **21:** 1769–1783

Kajimoto T, Shirai Y, Sakai N, Yamamoto T, Matsuzaki H, Kikkawa U, Saito N (2004) Ceramide-induced apoptosis by translocation, phosphorylation, and activation of protein kinase Cdelta in the Golgi complex. *J Biol Chem* **279:** 12668–12676

Konger RL, Billings SD, Thompson AB, Morimiya A, Ladenson JH, Landt Y, Pentland AP, Badve S (2005) Immunolocalization of low-affinity prostaglandin E receptors, EP and EP, in adult human epidermis. *J Invest Dermatol* **124:** 965–970

Kuwana T, Peterson PA, Karlsson L (1998) Exit of major histocompatibility complex class II-invariant chain p35 complexes from the endoplasmic reticulum is modulated by phosphorylation. *Proc Natl Acad Sci USA* **95:** 1056–1061

Larocca MC, Shanks RA, Tian L, Nelson DL, Stewart DM, Goldenring JR (2004) AKAP350 interaction with cdc42 interacting protein 4 at the Golgi apparatus. *Mol Biol Cell* **15:** 2771–2781

Lavoie C, Chevet E, Roy L, Tonks NK, Fazel A, Posner BI, Paiement J, Bergeron JJ (2000) Tyrosine phosphorylation of p97 regulates transitional endoplasmic reticulum assembly *in vitro*. *Proc Natl Acad Sci USA* **97:** 13637–13642

Lee MC, Miller EA, Goldberg J, Orci L, Schekman R (2004) Bi-directional protein transport between the ER and Golgi. *Annu Rev Cell Dev Biol* **20:** 87–123

Lee TH, Linstedt AD (2000) Potential role for protein kinases in regulation of bidirectional endoplasmic reticulum-to-Golgi transport revealed by protein kinase inhibitor H89. *Mol Biol Cell* **11:** 2577–2590

Le Niculescu H, Niesman I, Fischer T, DeVries L, Farquhar MG (2005) Identification and characterization of GIV, a novel Galpha i/s-interacting protein found on COPI, endoplasmic reticulum–Golgi transport vesicles. *J Biol Chem* **280:** 22012–22020

Lewis MJ, Pelham HR (1992) Ligand-induced redistribution of a human KDEL receptor from the Golgi complex to the endoplasmic reticulum. *Cell* **68:** 353–364

Leyte A, Barr FA, Kehlenbach RH, Huttner WB (1992) Multiple trimeric G-proteins on the trans-Golgi network exert stimulatory and inhibitory effects on secretory vesicle formation. *EMBO J* **11:** 4795–4804

Li H, Adamik R, Pacheco-Rodriguez G, Moss J, Vaughan M (2003) Protein kinase A-anchoring (AKAP) domains in brefeldin A-inhibited guanine nucleotide-exchange protein 2 (BIG2). *Proc Natl Acad Sci USA* **100:** 1627–1632

Liljedahl M, Maeda Y, Colanzi A, Ayala I, Van Lint J, Malhotra V (2001) Protein kinase D regulates the fission of cell surface destined transport carriers from the trans-Golgi network. *Cell* **104:** 409–420

Luini A, De Matteis MA (1993) Receptor-mediated regulation of constitutive secretion. *Trends Cell Biol* **3:** 290–292

Luini A, Ragnini-Wilson A, Polishchuck RS, De Matteis MA (2005) Large pleiomorphic traffic intermediates in the secretory pathway. *Curr Opin Cell Biol* **17:** 353–361

Luna A, Matas OB, Martinez-Menarguez JA, Mato E, Duran JM, Ballesta J, Way M, Egea G (2002) Regulation of protein transport from the Golgi complex to the endoplasmic reticulum by CDC42 and N-WASP. *Mol Biol Cell* **13:** 866–879

Luton F, Verges M, Vaerman JP, Sudol M, Mostov KE (1999) The SRC family protein tyrosine kinase p62yes controls polymeric IgA transcytosis *in vivo*. *Mol Cell* **4:** 627–632

Maier O, Ehmsen E, Westermann P (1995) Trimeric G protein alpha subunits of the Gs and Gi families localized at the Golgi membrane. *Biochem Biophys Res Commun* **208:** 135–143

Martin ME, Hidalgo J, Rosa JL, Crottet P, Velasco A (2000) Effect of protein kinase A activity on the association of ADP-ribosylation factor 1 to Golgi membranes. *J Biol Chem* **275:** 19050–19059

Martin ME, Hidalgo J, Vega FM, Velasco A (1999) Trimeric G proteins modulate the dynamic interaction of PKAII with the Golgi complex. *J Cell Sci* **112** (Part 22)**:** 3869–3878

Matas OB, Martinez-Menarguez JA, Egea G (2004) Association of Cdc42/N-WASP/Arp2/3 signaling pathway with Golgi membranes. *Traffic* **5:** 838–846

Mellman I, Warren G (2000) The road taken: past and future foundations of membrane traffic. *Cell* **100:** 99–112

Miaczynska M, Pelkmans L, Zerial M (2004) Not just a sink: endosomes in control of signal transduction. *Curr Opin Cell Biol* **16:** 400–406

Michaelson D, Ahearn I, Bergo M, Young S, Philips M (2002) Membrane trafficking of heterotrimeric G proteins via the endoplasmic reticulum and Golgi. *Mol Biol Cell* **13:** 3294–3302

Michaelson D, Silletti J, Murphy G, D'Eustachio P, Rush M, Philips MR (2001) Differential localization of Rho GTPases in live cells: regulation by hypervariable regions and RhoGDI binding. *J Cell Biol* **152:** 111–126

Michelsen K, Yuan H, Schwappach B (2005) Hide and run. Arginine-based endoplasmic-reticulum-sorting motifs in the assembly of heteromultimeric membrane proteins. *EMBO Rep* **6:** 717–722

Montmayeur JP, Borrelli E (1994) Targeting of G alpha i2 to the Golgi by alternative spliced carboxyl-terminal region. *Science* **263:** 95–98

Muniz M, Alonso M, Hidalgo J, Velasco A (1996) A regulatory role for cAMP-dependent protein kinase in protein traffic along the exocytic route. *J Biol Chem* **271:** 30935–30941

Muniz M, Martin ME, Hidalgo J, Velasco A (1997) Protein kinase A activity is required for the budding of constitutive transport vesicles from the trans-Golgi network. *Proc Natl Acad Sci USA* **94:** 14461–14466

Nagahama M, Usui S, Shinohara T, Yamaguchi T, Tani K, Tagaya M (2002) Inactivation of Galpha(z) causes disassembly of the Golgi apparatus. *J Cell Sci* **115:** 4483–4493

©2006 European Molecular Biology Organization

Palmer KJ, Konkel JE, Stephens DJ (2005) PCTAIRE protein kinases interact directly with the COPII complex and modulate secretory cargo transport. *J Cell Sci* **118:** 3839–3847

Pasolli HA, Klemke M, Kehlenbach RH, Wang Y, Huttner WB (2000) Characterization of the extra-large G protein alpha-subunit XLalphas. I. Tissue distribution and subcellular localization. *J Biol Chem* **275:** 33622–33632

Patil C, Walter P (2001) Intracellular signaling from the endoplasmic reticulum to the nucleus: the unfolded protein response in yeast and mammals. *Curr Opin Cell Biol* **13:** 349–355

Pelham HR, Rothman JE (2000) The debate about transport in the Golgi—two sides of the same coin? *Cell* **102:** 713–719

Philips MR (2004) Sef: a MEK/ERK catcher on the Golgi. *Mol Cell* **15:** 168–169

Pierce KL, Premont RT, Lefkowitz RJ (2002) Seven-transmembrane receptors. *Nat Rev Mol Cell Biol* **3:** 639–650

Pimplikar SW, Simons K (1993) Role of heterotrimeric G proteins in polarized membrane transport. *J Cell Sci Suppl* **17:** 27–32

Polishchuk RS, Polishchuk EV, Marra P, Alberti S, Buccione R, Luini A, Mironov AA (2000) Correlative light-electron microscopy reveals the tubular–saccular ultrastructure of carriers operating between Golgi apparatus and plasma membrane. *J Cell Biol* **148:** 45–58

Pooley L, Shakur Y, Rena G, Houslay MD (1997) Intracellular localization of the PDE4A cAMP-specific phosphodiesterase splice variant RD1 (RNPDE4A1A) in stably transfected human thyroid carcinoma FTC cell lines. *Biochem J* **321** (Part 1)**:** 177–185

Preisinger C, Short B, De CV, Bruyneel E, Haas A, Kopajtich R, Gettemans J, Barr FA (2004) YSK1 is activated by the Golgi matrix protein GM130 and plays a role in cell migration through its substrate 14-3-3zeta. *J Cell Biol* **164:** 1009–1020

Prior IA, Harding A, Yan J, Sluimer J, Parton RG, Hancock JF (2001) GTP-dependent segregation of H-ras from lipid rafts is required for biological activity. *Nat Cell Biol* **3:** 368–375

Qian L, Yang T, Chen H, Xie J, Zeng H, Warren DW, MacVeigh M, Meneray MA, Hamm-Alvarez SF, Mircheff AK (2002) Heterotrimeric GTP-binding proteins in the lacrimal acinar cell endomembrane system. *Exp Eye Res* **74:** 7–22

Radha V, Rajanna A, Swarup G (2004) Phosphorylated guanine nucleotide exchange factor C3G, induced by pervanadate and Src family kinases localizes to the Golgi and subcortical actin cytoskeleton. *BMC Cell Biol* **5:** 31

Rajagopal R, Chen ZY, Lee FS, Chao MV (2004) Transactivation of Trk neurotrophin receptors by G-protein-coupled receptor ligands occurs on intracellular membranes. *J Neurosci* **24:** 6650–6658

Rocks O, Peyker A, Kahms M, Verveer PJ, Koerner C, Lumbierres M, Kuhlmann J, Waldmann H, Wittinghofer A, Bastiaens PI (2005) An acylation cycle regulates localization and activity of palmitoylated Ras isoforms. *Science* **307:** 1746–1752

Rosso S, Bollati F, Bisbal M, Peretti D, Sumi T, Nakamura T, Quiroga S, Ferreira A, Caceres A (2004) LIMK1 regulates Golgi dynamics, traffic of Golgi-derived vesicles, and process extension in primary cultured neurons. *Mol Biol Cell* **15:** 3433–3449

Rothman JE (1994) Mechanisms of intracellular protein transport. *Nature* **372:** 55–63

Rutkowski DT, Kaufman RJ (2004) A trip to the ER: coping with stress. *Trends Cell Biol* **14:** 20–28

Schultz A, Jonsson JI, Larsson C (2003) The regulatory domain of protein kinase Ctheta localises to the Golgi complex and induces apoptosis in neuroblastoma and Jurkat cells. *Cell Death Differ* **10:** 662–675

Schultz A, Ling M, Larsson C (2004) Identification of an amino acid residue in the protein kinase C C1b domain crucial for its localization to the Golgi network. *J Biol Chem* **279:** 31750–31760

Schwaninger R, Plutner H, Bokoch GM, Balch WE (1992) Multiple GTP-binding proteins regulate vesicular transport from the ER to Golgi membranes. *J Cell Biol* **119:** 1077–1096

Shanks RA, Steadman BT, Schmidt PH, Goldenring JR (2002) AKAP350 at the Golgi apparatus. I. Identification of a distinct Golgi apparatus targeting motif in AKAP350. *J Biol Chem* **277:** 40967–40972

Simon JP, Ivanov IE, Adesnik M, Sabatini DD (1996) The production of post-Golgi vesicles requires a protein kinase C-like molecules, but not its phosphorylating activity. *J Cell Biol* **135:** 355–370

Sorkin A (2005) TRKing signals through the Golgi *Sci. STKE* **2005:** e1

Sorkin A, Von Zastrow M (2002) Signal transduction and endocytosis: close encounters of many kinds. *Nat Rev Mol Cell Biol* **3:** 600–614

Stamnes M (2002) Regulating the actin cytoskeleton during vesicular transport. *Curr Opin Cell Biol* **14:** 428–433

Stow JL, de Almeida JB (1993) Distribution and role of heterotrimeric G proteins in the secretory pathway of polarized epithelial cells. *J Cell Sci Suppl* **17:** 33–39

Stow JL, de Almeida JB, Narula N, Holtzman EJ, Ercolani L, Ausiello DA (1991a) A heterotrimeric G protein, G alpha i-3, on Golgi membranes regulates the secretion of a heparan sulfate proteoglycan in LLC-PK1 epithelial cells. *J Cell Biol* **114:** 1113–1124

Stow JL, Sabolic I, Brown D (1991b) Heterogeneous localization of G protein alpha-subunits in rat kidney. *Am J Physiol* **261:** F831–F840

Sullivan BM, Harrison-Lavoie KJ, Marshansky V, Lin HY, Kehrl JH, Ausiello DA, Brown D, Druey KM (2000) RGS4 and RGS2 bind coatomer and inhibit COPI association with Golgi membranes and intracellular transport. *Mol Biol Cell* **11:** 3155–3168

Takida S, Wedegaertner PB (2004) Exocytic pathway-independent plasma membrane targeting of heterotrimeric G proteins. *FEBS Lett* **567:** 209–213

Tisdale EJ (2000) Rab2 requires PKC iota/lambda to recruit beta-COP for vesicle formation. *Traffic* **1:** 702–712

Tisdale EJ (2002) Glyceraldehyde-3-phosphate dehydrogenase is phosphorylated by protein kinase Ciota/lambda and plays a role in microtubule dynamics in the early secretory pathway. *J Biol Chem* **277:** 3334–3341

Tisdale EJ (2003) Rab2 interacts directly with atypical protein kinase C (aPKC) iota/lambda and inhibits aPKCiota/lambda-dependent glyceraldehyde-3-phosphate dehydrogenase phosphorylation. *J Biol Chem* **278:** 52524–52530

Tisdale EJ, Artalejo CR (2006) Src-dependent aPKCiota/lambda tyrosine phosphorylation is required for aPKCiota/lambda association with Rab2 and glyceraldehyde-3-phosphate dehydrogenase on Pre-golgi intermediates. *J Biol Chem* **281:** 8436–8442

Torii S, Kusakabe M, Yamamoto T, Maekawa M, Nishida E (2004) Sef is a spatial regulator for Ras/MAP kinase signaling. *Dev Cell* **7:** 33–44

Trucco A, Polishchuk RS, Martella O, Di Pentima A, Fusella A, Di Giandomenico D, San Pietro E, Beznoussenko GV, Polishchuk EV, Baldassarre M, Buccione R, Geerts WJ, Koster AJ, Burger KN, Mironov AA, Luini A (2004) Secretory traffic triggers the formation of tubular continuities across Golgi sub-compartments. *Nat Cell Biol* **6:** 1071–1081

Ugur O, Jones TL (2000) A proline-rich region and nearby cysteine residues target XLalphas to the Golgi complex region. *Mol Biol Cell* **11:** 1421–1432

Verde I, Pahlke G, Salanova M, Zhang G, Wang S, Coletti D, Onuffer J, Jin SL, Conti M (2001) Myomegalin is a novel protein of the golgi/centrosome that interacts with a cyclic nucleotide phosphodiesterase. *J Biol Chem* **276:** 11189–11198

Weinberger A, Kamena F, Kama R, Spang A, Gerst JE (2005) Control of Golgi morphology and function by Sed5 t-SNARE phosphorylation. *Mol Biol Cell* **16:** 4918–4930

Wienecke R, Maize Jr JC, Shoarinejad F, Vass WC, Reed J, Bonifacino JS, Resau JH, de Gunzburg J, Yeung RS, DeClue JE (1996) Co-localization of the TSC2 product tuberin with its target Rap1 in the Golgi apparatus. *Jr Oncogene* **13:** 913–923

Wilson BS, Komuro M, Farquhar MG (1994) Cellular variations in heterotrimeric G protein localization and expression in rat pituitary. *J Endocrinol* **134:** 233–244

Wylie F, Heimann K, Le TL, Brown D, Rabnott G, Stow JL (1999) GAIP, a Galphai-3-binding protein, is associated with Golgi-derived vesicles and protein trafficking. *J Am J Physiol* **276:** C497–C506

Wylie FG, Lock JG, Jamriska L, Khromykh T, Brown DL, Stow JL (2003) GAIP participates in budding of membrane carriers at the *trans*-Golgi network. *Traffic* **4:** 175–189

Xie S, Wang Q, Ruan Q, Liu T, Jhanwar-Uniyal M, Guan K, Dai W (2004) MEK1-induced Golgi dynamics during cell cycle progression is partly mediated by Polo-like kinase-3. *Oncogene* **23:** 3822–3829

Yamaguchi T, Nagahama M, Itoh H, Hatsuzawa K, Tani K, Tagaya M (2000) Regulation of the Golgi structure by the alpha subunits of heterotrimeric G proteins. *FEBS Lett* **470:** 25–28

 ©2006 European Molecular Biology Organization

Yamamoto K, Hamada H, Shinkai H, Kohno Y, Koseki H, Aoe T (2003) The KDEL receptor modulates the endoplasmic reticulum stress response through mitogen-activated protein kinase signaling cascades. *J Biol Chem* **278:** 34525–34532

Yang X, Kovalenko D, Nadeau RJ, Harkins LK, Mitchell J, Zubanova O, Chen PY, Friesel R (2004) Sef interacts with TAK1 and mediates JNK activation and apoptosis. *J Biol Chem* **279:** 38099–38102

Yeaman C, Ayala MI, Wright JR, Bard F, Bossard C, Ang A, Maeda Y, Seufferlein T, Mellman I, Nelson WJ, Malhotra V (2004) Protein kinase D regulates basolateral membrane protein exit from *trans*-Golgi network. *Nat Cell Biol* **6:** 106–112

Zhang K, Kaufman RJ (2004) Signaling the unfolded protein response from the endoplasmic reticulum. *J Biol Chem* **279:** 25935–25938

Zirngibl R, Schulze D, Mirski SE, Cole SP, Greer PA (2001) Subcellular localization analysis of the closely related Fps/Fes and Fer protein-tyrosine kinases suggests a distinct role for Fps/Fes in vesicular trafficking. *Exp Cell Res* **266:** 87–94

©2006 European Molecular Biology Organization