Signalling to and from the secretory pathway

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The e-mail address for Catherine Rabouille was incorrect. The correct address is c.rabouille@hubrecht.eu

In addition, some of the text headings were erroneously styled to give incorrect precedence. The correct format should be as follows:

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We apologise for any confusion caused.

Signalling to and from the secretory pathway

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Summary

For growth, survival, communication and homeostasis, cells transport a large number of proteins to the plasma membrane and the extracellular medium by using the secretory pathway. Consequently, to adapt to the surrounding environment and the different intracellular contexts, the secretory pathway needs to accommodate and respond to a plethora of endogenous and exogenous stimuli. It is now well established that several kinases, known to be activated by environmental stimuli, signal from the plasma membrane to the secretory pathway in order to remodel its architecture and modulate the cellular secretion capacity. By contrast, membranes of the early secretory pathway, similar to the endosomal system, can also initiate and modulate signalling cascades, thereby spatially organising cellular signalling and eliciting a different cellular outcome than when signalling is localised to the plasma membrane. This Commentary highlights recent contributions to our understanding of the mutual regulation of the secretory pathway and cellular signalling.

Key words: Endoplasmic reticulum exit sites, Golgi, Signal transduction

Introduction

The secretory pathway is where synthesis and delivery of soluble proteins occur that have been secreted into the extracellular space – a process called secretion (Box 1). Most of the cellular transmembrane proteins (except those of the mitochondria) use this pathway to reach their final destination. The secretory pathway comprises the rough endoplasmic reticulum (rough ER), ER exit sites (ERESs) the ER-to-Golgi intermediate compartment (ERGIC), the Golgi complex and post-Golgi carriers en route to their final destination. The organelles of the secretory pathway have a precise organisation and structure in order to sustain their function in membrane transport (Box 1), as well as providing the proper environment for protein folding and post-translational modifications (Mellman and Warren, 2000; Spang, 2009).

Signal transduction is a way to convert a mechanical and/or chemical stimulus that has been directed to a cell into a specific cellular response (Box 2). It begins with a signal to a receptor and ends with a change in cell function, proceeding through a series of interactions that activate and inhibit numerous kinases and signalling molecules that engage in much crosstalk (Box 2). It is now well established that the secretory pathway responds to endogenous signalling cascades. The best studied to date is the mitotic kinase cascade, which leads to the disassembly of the Golgi complex at metaphase (Rabouille and Kondylis, 2007; Colanzi and Corda, 2007; Rabouille and Kondylis, 2007), and the fragmentation and/or disappearance of the ERES (Kano et al., 2004; Stephens, 2003; Kondylis et al., 2007).

The secretory pathway also undergoes major changes in response to developmental programs. For instance, the expression of certain genes that encode proteins of the secretory pathway changes at specific stages of development in *Drosophila melanogaster* and zebrafish (Dunne et al., 2002; Schotman et al., 2009; Coutinho et al., 2004). Furthermore, the maturation of professional secretory cells, such as antibody-producing plasma cells (Iwakoshi et al., 2003), leads to an increase in ERES number and size, as well as Golgi volume (Farhan et al., 2008; Guo and Linstedt, 2006; Forster et al., 2006), thus rendering the cell competent to accommodate an increase in cargo load. These changes are due to activation of the unfolded protein response (UPR), which induces the expression of a variety of proteins, including vesicle coat and cargo receptor proteins, as well as enzymes involved in the synthesis of lipid bilayers (Acosta-Alvear et al., 2007).

A trend is now emerging that the secretory pathway also responds to signalling cascades that are induced by exogenous stimuli, such as hormones, nutrients or growth factors. By modifying aspects of its functional organisation, the secretory pathway responds to the qualitative and quantitative secretion demands imposed by cell growth, survival and homeostasis. By contrast, and just as exciting, it is now clear that membranes of the secretory pathway – similar to membranes of the endosomal system (Murphy et al., 2009) – house signalling molecules such as kinases, and initiate and/or propagate signalling cascades. Furthermore, the functional cellular outcomes that are triggered seem to differ depending on which intracellular location (i.e. ER or Golgi versus plasma membrane) they emanate. This illustrates the potential power of the compartmentalisation of signalling and the need for two fields of research to merge and communicate in an unprecedented way.

Here, we discuss recent findings that illustrate the relationship between signalling cascades and the early secretory pathway. This Commentary is divided into two sections. The first, Regulation of the secretory pathway by signalling, describes how the secretory pathway responds to signals that are generated outside the secretory pathway (e.g. on the plasma membrane) and are transmitted to the secretory pathway to modulate its functional organisation. The second section, Signalling from the secretory pathway, presents evidence on how organelles of the secretory pathway contribute to the compartmentalisation of signal transduction to elicit differential cell responses, such as proliferation, transformation and migration (summarised in Fig. 1). This section also includes examples in which signalling is generated on membranes of the secretory

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pathway – rather than on the plasma membrane – to directly modulate its functional organisation.

Regulation of the secretory pathway by signalling

ER export is regulated by signalling

The exit of newly synthesised proteins from the ER depends on these proteins being properly folded, but also on the ability of the export machinery to incorporate them into carriers that are coated into the membrane-coating coat protein II (COPII) and that leave the ER (see Box 1 for details on COPII). Both events are subjected to regulation through signalling. In addition, the lipid composition of the membrane also contributes to how efficiently COPII vesicles form (Matsuoka et al., 1998), so lipid signalling represents a further mode of regulation in ER export (Fig. 1A).

Modification of ER chaperones

Protein folding depends on the chaperoning capacity of the ER, and that can be compromised when the load of proteins to fold and transport increases dramatically. This stress induces the UPR, which leads to increased synthesis of chaperones, thereby potentiating the ER folding capacity as well as the degradation of aberrant and/or unfolded proteins. These changes are quantitative, but they do not affect biochemical properties of chaperones, such as their affinity for misfolded proteins. Such changes could be implemented by post-translational modifications including, for instance, phosphorylation. However, most ER chaperones are luminal and, thus, inaccessible to the direct cytosolic signalling pathways. Therefore, their regulation by cellular signalling has remained largely unexplored. The notable exception is the transmembrane chaperone calnexin, with its short cytosolic C-terminus that is phosphorylated on at least four amino acid residues. Whether any of these phosphorylation events modulates its chaperone activity has been addressed by studying the binding of calnexin with chemically misfolded α1-antitrypsin (Cameron et al., 2009). When the cytosolic tail of calnexin is phosphorylated by the mitogen-activated protein kinase (MAPK) extracellular signal-regulated kinase 1 (ERK1), its association to misfolded α1-antitrypsin is enhanced. By contrast, pharmacological inhibition of ERK activity leads to the release of misfolded α1-antitrypsin from calnexin, suggesting that the ER folding machinery can be a target of cellular signalling.

Modulation of COPII recruitment

The second form of ER export regulation occurs through the recruitment and subsequent formation of the COPII coat. Strikingly, induction of the UPR also leads to an increased synthesis of coat components, as well as ERES regulatory proteins (such as Sec16) (see Box1) (Farhan et al., 2008), thereby increasing the ability to generate COPII vesicles. However, unlike chaperones, components

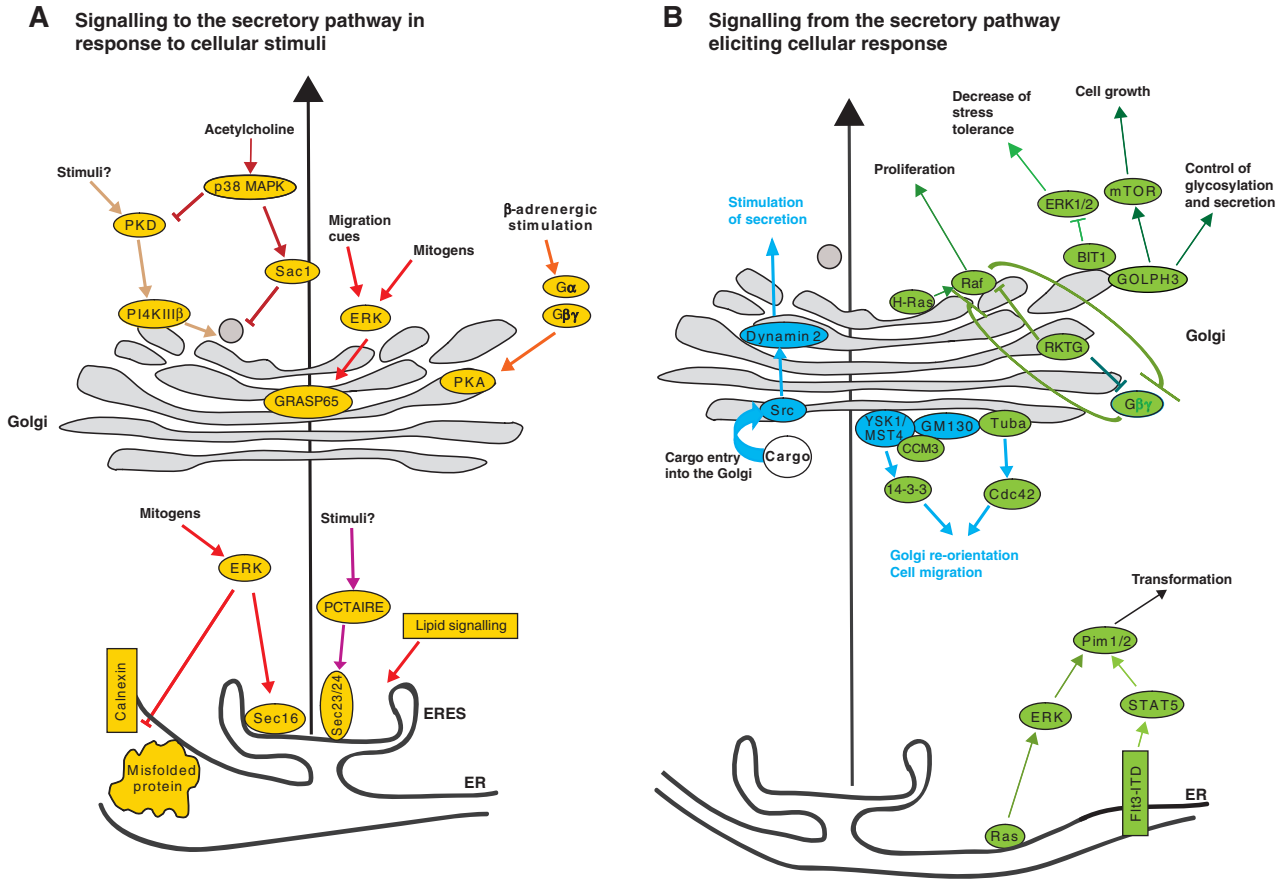


Fig. 1. Signalling to and from the early secretory pathway. (**A**,**B**) ER, ERESs and Golgi complex with the different signalling cascades that are either directed towards these organelles (A, yellow), or emanating from them (B, green). Autochthonous Golgi signalling pathways are shown in blue. Stimuli that trigger signalling to the secretory pathway (A) or the cellular responses elicited by signalling from the secretory pathway (B) are shown in yellow or green, respectively. The long black arrows indicate direction of transport along the secretory pathway.

of the COPII coat, as well as ERES regulatory proteins, are largely cytosolic and, therefore, directly accessible to cytoplasmic signalling pathways. Indeed, evidence that COPII-coat-mediated ER export is regulated by signalling is increasing. More than a decade ago, it was shown that a high concentration of the kinase inhibitor H89 blocks COPII-coated vesicle budding (Aridor and Balch, 2000), and that the phosphatase inhibitor okadaic acid inhibits ER export (Pryde et al., 1998). Alkaline-phosphatase-treated Sec31 decreases the production of COPII vesicles in vitro, indicating that phosphorylation of Sec31 regulates its activity (Salama et al., 1997). Moreover, although the kinase PCTAIRE-1 has not been formally shown to phosphorylate the COPII component Sec23A, it physically interacts with this COPII-coat subunit and regulates both ERES number and ER-to-Golgi anterograde transport (Palmer et al., 2005). Finally, it is interesting to note that the COPII subunit Sec24C is, upon the entry of the cell into mitosisis, phosphorylated on serine and threonine residues that are potentially inaccessible during interphase owing to their O-glycosylation (Dudognon et al., 2004). Whether this phosphorylation is linked to the ERES disassembly that occurs during mitosis remains to be established. Sec24C and Sec24D were also shown to be directly phosphorylated by the serine-threonine kinase Akt, and this phosphorylation modulates the interaction of Sec24 with Sec23 (Sharpe et al., 2010). This finding provides a basis for further investigations into the role of nutrient and growth factor signalling through Akt to the ER export machinery.

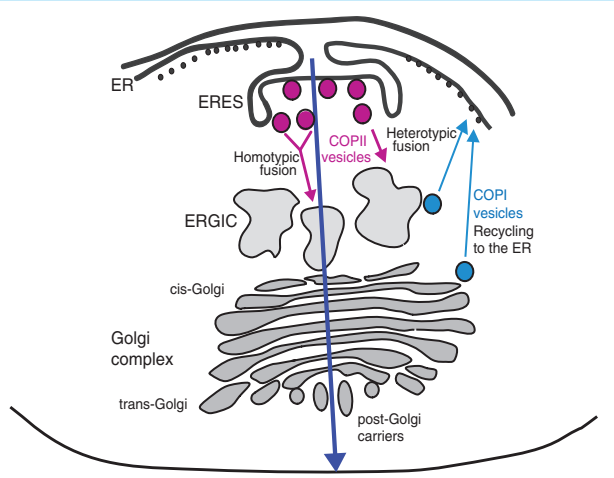
Recently, a screen was performed using small interfering RNA (siRNA) to deplete all 916 human kinases and phosphatases to uncover regulators of the secretory pathway (Farhan et al., 2010). Changes in the distribution of ERGIC-53, a marker protein of the ERGIC (Schweizer et al., 1988), were used as a read-out. This approach led to the identification of 122 kinases and phosphatases that affect the organisation of the secretory pathway. Positive hits were detected more frequently in several signalling pathways, including the MAPK pathway. More specifically, the MAPK protein ERK2 was found to directly phosphorylate Sec16 on threonine residue 415, and this phosphorylation event controls the number of ERESs as well as ER-to-Golgi transport. The functional relevance of this regulation might lie in the fact that signalling by growth factors, such as epithelial growth factor (EGF), induces protein synthesis and, thereby, increases the protein load exiting the ER. Phosphorylation of Sec16 by ERK2 leads to an increase of ERESs, thus enabling the cell to cope with conditions of higher cargo flux.

Lipid modification

ERESs can also be regulated by lipid modification. The formation of phosphatidylinositol (4)-phosphate PtdIns(4)*P* was shown to be induced by recruitment of the small GTPase Sar1, and live cell imaging showed that this lipid is enriched on ERESs during ER export, suggesting that it has a role in the regulation of ER-to-Golgi transport (Blumental-Perry et al., 2006). This finding is controversial because the PH-domain of the four-phosphate-adaptor protein 1 (FAPP1) was used as a reporter for PtdIns(4)*P*, and previous work had shown that FAPP1 is recruited to membranes by coincidentally detecting of Arf1 and PtdIns(4)*P* (Godi et al., 2004). As Arf1 is unlikely to be found on ERESs, the discovery that PtdIns(4)*P* is present on ERESs should be interpreted with caution. However, the involvement of PtdIns signalling in ER export is further supported by the requirement of PtdIns(4)*P* kinase (PI4K) IIIα for the adaptation of ERESs to increased cargo load, and depletion of PtdIns was also shown to inhibit COPII vesicle budding (Shindiapina and Barlowe, 2010). Further work is needed to elucidate the precise role of lipid signalling in export from the ER.

Taken together, these data indicate that ERESs respond to signalling (Farhan et al., 2010) as well as to changes in cargo load

Box 1. The secretory pathway



After their synthesis in the ER, newly synthesized proteins are exported at ER exit sites (ERESs) – ribosome-free regions of the rough ER where COPII components and vesicles concentrate (Barlowe et al., 1994; Lee et al., 2004) (see Figure). COPII coat assembly begins by activation of the small GTPase Sar1 by its exchange factor Sec12. Active Sar1 on the ER recruits the Sec23–Sec24 dimeric complex, thereby forming the pre-budding complex that captures cargo proteins (Lee et al., 2004). Subsequently, the Sec13–Sec31 heterotetrameric complex is recruited to form the outer layer of the COPII coat (Lee et al., 2004). In addition, biogenesis, maintenance and regulation of ERESs and their ability to form COPII vesicles is dependent on the large hydrophilic protein Sec16 (Supek et al., 2002; Connerly et al., 2005; Watson et al., 2006; Farhan et al., 2008), which forms a strong association with the cup-shaped ER membrane that is associated with ERESs (Ivan et al., 2008; Hughes et al., 2009). After formation, COPII vesicles can fuse homotypically to generate pre-Golgi intermediates (Xu and Hay, 2004). Alternatively, COPII vesicles may heterotypically fuse with the pre-formed ERGIC (Appenzeller-Herzog and Hauri, 2006). Subsequently, proteins reach the Golgi complex where they are further modified by glycosylation and/or processing, sorted and dispatched towards their correct final destinations or returned to the ER through COPI-coated vesicles (Jackson, 2009).

The Golgi complex comprises stacks of flattened membrane-bound compartments that are called cisternae. Golgi stacks are flanked on each side by a reticular membrane network that builds the cis-Golgi network (CGN) on the entry side of the Golgi and the trans-Golgi network (TGN) on its exit side (see Figure). In mammalian cells, Golgi stacks are laterally connected by tubulovesicular regions, thereby forming the Golgi ribbon (Jackson, 2009). This ribbon is typically found in the juxtanuclear region, closely associated with the centrosome. In *Drosophila* and plant cells, and in cells of the methylotrophic yeast *Pichia pastoris*, Golgi stacks do not form a ribbon but are found in close proximity to ERESs, forming what are called transitional ER (tER)–Golgi units (Kondylis and Rabouille, 2009).

(Guo and Linstedt, 2006; Farhan et al., 2008). Thus, ERESs represent a site of secretory-pathway regulation, where environmental signals and stimuli from within the cell can be integrated.

Signalling regulates the functional organisation of the Golgi complex

The Golgi complex occupies a central function in the secretory pathway and, as such, also needs to sense and integrate signals from various sources (including neurotransmitters, mitogens, hormones, cytokines, etc.). Accordingly, a number of signalling molecules have been shown to localise to the Golgi membrane and to phosphorylate key Golgi proteins, resulting – at least in tissue-culture cells – in the modulation of either Golgi architecture or its function in protein trafficking (Fig. 1A). However, how these signalling cascades are elicited in vivo remains, in most cases, to be determined. In addition, signalling molecules localised to the early secretory pathway have also been reported to have non-signalling roles (Rismanchi et al., 2009; Burman et al., 2008), but we have not reported on those here.

PKD and PtdIns(4)*P*

Almost two decades ago, it was suggested that protein kinase C (PKC) family members are involved in the regulation of secretion (DeMatteis et al., 1993). One particular PKC isoform, protein kinase D (PKD; also known as PKCμ) localises to the trans-Golgi network (TGN), where it activates PI4K IIIβ (Hausser et al., 2005) and supports formation of post-Golgi transport carriers (Liljedahl et al., 2001; Bossard et al., 2007). The importance of PtdIns(4)*P* synthesis at the Golgi membrane makes this process a good candidate pathway for the regulation of export from this organelle and, unlike in the ER, the role of PtdIns(4)*P* at the Golgi enjoys a much wider acceptance. Serum starvation of cultured mammalian cells (Blagoveshchenskaya et al., 2008), as well nutrient starvation of cultured yeast cells (Faulhammer et al., 2007), have recently been shown to lead to the recruitment of the phosphoinositide phosphatase Sac1 to the Golgi complex, and the consequential local depletion of PtdIns(4)*P* causes a reduction in export from the Golgi (Blagoveshchenskaya et al., 2008). Stimulation of serum-starved cells with mitogens leads to a redistribution of Sac1 from the Golgi membrane back to the ER in a p38/MAPK-dependent manner, thus allowing the generation of PtdIns(4)*P* and increased export from the Golgi complex. This work elegantly demonstrates that nutrient status can be sensed and is transmitted through p38 MAPK signalling to the Golgi complex where it enhances trafficking.

p38

Although these results show p38 MAPK signalling to be a positive modulator of secretion, another study proposed the opposite : insulin-producing pancreatic β-cells that are deficient in the p38δ MAPK isoform exhibit enhanced insulin secretion compared with their wild-type counterparts (Sumara et al., 2009). In this study, p38δ MAPK was shown to inhibit PKD and, thus, has a negative effect on exit from the Golgi. Therefore, the higher concentration of secreted insulin in the p38δ-knockout cells is probably caused by elevated PKD activity. Interestingly, the p38δ–PKD connection was suggested to be part of the signalling pathway elicited by the neurotransmitter acetylcholine to stimulate insulin secretion (Sumara et al., 2009), pointing to its physiological relevance. It is currently unclear how to reconcile these two apparently contradictory roles of p38 MAPK and secretion (one positive and one negative). This could partly be due to the fact that the positive effect of p38 MAPK on Golgi export was detected by assaying general secretion in fibroblasts (Blagoveshchenskaya et al., 2008), whereas the negative effect of p38δ MAPK was only shown for insulin secretion in pancreatic β-cells (Sumara et al., 2009). It is also not clear whether p38δ deficiency affects secretion in other cell types and whether other p38 MAPK isoforms have similar effects on secretion in other cell lines.

PKA

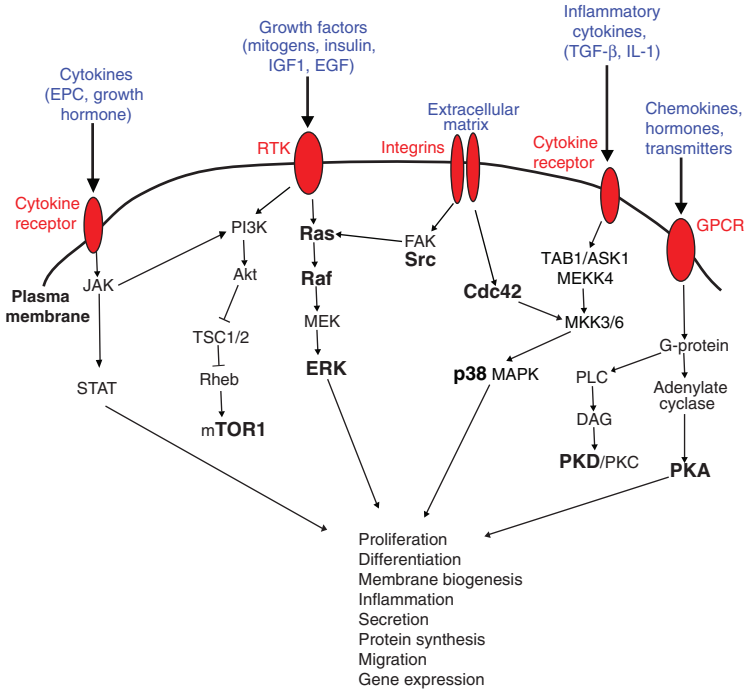
The above described signalling induced by acetylcholine is an example of how a G-protein-coupled receptor (GPCR), in this case the acetylcholine receptor, regulates a highly specialised Golgi function. GPCR signalling has been known for more than a decade to also modulate several aspects of Golgi biology. Gαs-coupled GPCRs (Box 2) are known to activate protein kinase A (PKA), which in turn regulates the budding of vesicles from the TGN (Muniz et al., 1997), the retrograde transport of the KDEL receptor (Cabrera et al., 2003) and the reformation of the Golgi after brefeldin-A-mediated retrieval of the Golgi to the ER (Bejarano et al., 2006). Plasma membrane PKA is typically activated by local cAMP, which is produced upon signals that activate adenylate cyclases. Diffusion of cAMP throughout the cytoplasm, however, is prevented by the activity of phosphodiesterases that degrade cAMP. Therefore, it remains unclear whether and how Golgi-associated PKA is activated by signals that originate at the plasma membrane, and whether cAMP is involved in this activation.

In this respect, Golgi-associated PKA has recently been shown to be activated by cAMP following a signal at the plasma membrane (Mavillard et al., 2010). Activation of the plasma membrane β-adrenergic receptor (which is known to increase cAMP production) led to activation of PKA, in turn resulting in a remodelling of Golgi structure and facilitated post-Golgi trafficking (Mavillard et al., 2010). β-adrenergic stimulation is known to be important in the development of cardiomyocytes, as it exerts a pro-proliferative effect on neonatal cardiomyocytes (Tseng et al., 2001). It is therefore possible that β-adrenergic signalling to the Golgi complex has a role in this process. Enhanced secretory activity of the Golgi might serve to support secretion of factors important for cardiomyocyte development. This, as well as identifying the molecular targets of PKA on the Golgi, is a task for the future.

Signalling during directional cell migration

Contrary to the kinases discussed above, whose physiological activation by external stimuli still remains poorly understood, directional cell migration is a clear example of a cellular process influencing Golgi function for which the external cue is identified. When cells migrate towards a chemoattractant, the reorientation of the Golgi towards the leading edge of the migrating cell and the structural integrity of the Golgi are crucial (Yadav et al., 2009). The signalling cascade dictating Golgi re-orientation is starting to be elucidated: ERK-mediated GRASP65 phosphorylation on serine residue 277 has been shown to have an important role (Bisel et al., 2008). Polarisation of the Golgi in response to growth factors is blocked by pre-treatment with ERK inhibitors, and expression of a phosphorylation-deficient GRASP65 mutant blocks the Golgi re-orientation. Remarkably, Ser277 is also phosphorylated by CDK1 during mitosis, and this modification causes disassembly of the Golgi (Wang et al., 2003). Phosphorylation of GRASP65 on Ser277 by ERK also causes disassembly of the Golgi in vitro, but such a disassembly is not observed in vivo using light microscopy (Bisel

Box 2. Signal transduction



Signal transduction converts stimuli to a specific and adequate cellular response through signalling cascades made up of kinases, phosphatases, GTPases, nucleotides and lipid mediators (see Figure, signalling components displayed in bold are those discussed in greater detail in the main text). Stimuli can arise from membrane-impermeable factors in the extracellular medium (hormones, growth factors), from membrane-permeable hormones or from mechanical cues. Typically, an upstream molecule activates a downstream effector, or de-inhibits a signalling pathway by inactivating an inhibitory molecule.

Among the best-described signal transduction pathways is the mitogen-activated protein (MAP) kinase – Ras–Raf–MEK–ERK – pathway that operates downstream of receptor tyrosine kinases and integrins. Cytokine receptors signal either through activation of signal transducers and activators of transcription (STATs) or by activation of p38 MAP kinases (MAPKs) or Jun kinases (JNKs) (not shown in Figure). G-protein coupled receptors (GPCRs) belong to a family of proteins with seven transmembrane domains that couple through their cytosolic portions to heterotrimeric G-proteins composed of α-, β- and γ-subunits. Upon GPCR activation, the α-subunit dissociates from the βγ-dimer. Most cellular responses are elicited through the α-subunit, and only a few through the βγ-dimer. There are three types of α-subunit: Gαs activates adenylate cyclases, Gαi inhibits adenylate cyclases and Gαq activates phospholipase Cβ. Adenylate cyclases lead to an elevation of cellular cAMP levels, which then activates protein kinase A (PKA) family members. Signalling through Gαq, however, leads to activation of protein kinase C (PKC) family members. Signalling by receptor tyrosine kinases (RTKs) also activates PI3 kinases, which in turn signal through protein kinase B (PKB/Akt) to activate mammalian target of rapamycin 1 (mTOR1), a key regulator of cell growth and differentiation. There is extensive crosstalk between various signalling pathways which – for sake of simplicity – is not depicted in the Figure.

et al., 2008). Nevertheless, undetected local remodelling might modulate its anchoring and/or positioning and promote its orientation, as well as facilitating intra-Golgi transport that is probably needed for efficient membrane delivery towards the leading edge. The presence of such a local remodelling needs to be clarified to understand the mechanism by which GRASP65 phosphorylation regulates the orientation of the Golgi in interphase. Another group of kinases that is associated with the Golgi in directional migration are germinal-center kinases. As the stimulus for their activation does not come from the plasma membrane but is generated at the Golgi, we will discuss those findings it in the next section.

Finally, a recent screen aiming at identifying signalling molecules that are involved in secretory-pathway organisation has identified several kinases and phosphatases that have a role in the regulation of Golgi architecture. Interestingly, the depletion of those kinases, which results in the disruption of the Golgi structure, also inhibits directional cell migration – in line with the role of its structural integrity mentioned above (Farhan et al., 2010). This implies that, in addition to ERK, several signalling pathways regulate the Golgi in cell migration. Future work is needed to unveil and dissect the molecular details of the signalling cascades that regulate Golgi directional polarity.

Signalling from the secretory pathway

The temporal control of signalling networks has always received great attention. However, in recent years, mounting evidence has emphasised the importance of the spatial control of signalling for the quality and intensity of its output. It is well accepted that the endosomal system is involved in generating and modulating signalling (Murphy et al., 2009). A good example is ERK activation by GPCRs, which is transient when the GPCR is signalling from the plasma membrane but sustained when the GPCR is endocytosed and signals from the endosomes (Kholodenko et al., 2010). Analogous

to the endosomal system, organelles of the secretory pathway contribute not only to signalling, but also to the compartmentalisation of signal transduction to elicit differential cell responses, such as proliferation, transformation and migration (Fig. 1B).

A role for the ER in the compartmentalisation of signalling cascades

Ras

Ten years ago, it was discovered that farnesylation of all Ras GTPases occurs in the ER, whereas palmitoylation of H-Ras and N-Ras takes place on the Golgi complex (Choy et al., 1999). Furthermore, the signalling field was surprised to learn (Di Fiore, 2003) that Ras, when localised at the membrane involved with the secretory pathway, can respond to mitogens and initiate signalling from there (Chiu et al., 2002; Bivona et al., 2003). Indeed, a constitutively active form of H-Ras restricted to the ER membrane (through its fusion to the first transmembrane domain of avian bronchitis virus M protein) was able to activate downstream targets such as ERK (Chiu et al., 2002). This opened up the exciting possibility that the membranes involved in the secretory pathway serve as platforms to initiate specific signalling.

Remarkably, Ras localised to the ER seems to follow different rules compared with Ras located on the plasma membrane. First, the exchange factor RasGFR-1 only activates Ras on the ER (Arozarena et al., 2004). Second, Ras at the ER seems to promote survival and proliferation of growth-factor-starved NIH3T3 fibroblasts, whereas control fibroblasts and those expressing active Ras on the Golgi or at the plasma membrane die after 2–3 days (Matallanas et al., 2006). Of course, one possible caveat is that the Ras mutant used in these studies was artificially targeted to the ER membrane, and may not faithfully represent physiological Ras signalling from the ER. Nevertheless, it provides proof of principle that signalling from the ER can occur and is associated with a different outcome than signalling from other cellular locations.

Flt3

The stimulus activating Ras at the ER stems from the activation of receptor tyrosine kinases (RTKs) that are usually localised at the plasma membrane. For a long time, it remained elusive whether RTKs can also signal from the secretory pathway but, recently, a mutant version of the RTK Flt3 was shown to signal from the ER. Wild-type Flt3 localises to the plasma membrane and signals to ERK and PI3 kinase. A mutant form of Flt3, however, that exhibits internal tandem duplication (ITD) in its juxtamembrane region is retained in the ER and activates STAT5, leading to upregulation of its downstream Pim1 and Pim2 kinase targets (Choudhary et al., 2009). Aberrant activation of STAT5 is well known to be important for myeloid transformation (Moriggl et al., 2005) and, in agreement with this, the ITD mutation is the most frequent mutation in acute myeloid leukaemia (Choudhary et al., 2009). Taken together, this example illustrates the role of compartmentalised ER-based signalling in pathological situations with possible links to tumourigenesis.

Signalling from the Golgi membrane

In addition to the ER, the Golgi complex also has a prominent role in modulating signalling pathways, as it has been shown to house a variety of signalling molecules. These molecules may be activated at other locations and then translocate to the Golgi where they propagate a differential signalling outcome. The scaffold proteins that localise to the Golgi are the key molecules to confer spatial control to signalling pathways. Finally, Golgi proteins themselves initiate signalling cascades that modify different aspects of the functional organisation of this organelle. However, because we feel that this represents a special case, we will discuss it in a separate section below. Altogether, the Golgi complex can be considered to be a hub for cellular signalling (Fig. 1B).

Ras

As mentioned above, H-Ras and N-Ras are also found to be, at least transiently, associated with the Golgi membrane, where they are palmitoylated (Hancock, 2003). Similar to Ras at the ER, constitutively active Ras mutants that are artificially targeted to the Golgi membrane can activate downstream signalling molecules (Chiu et al., 2002; Matallanas et al., 2006). Furthermore, probing growth-factor-induced Ras activation in COS-1 cells with fluorescent sensors revealed that Ras was activated both on the Golgi and the plasma membrane (Chiu et al., 2002). In Jurkat cells, Ras is restricted to the Golgi where it is activated by its exchange factor RasGRP1, which itself is specifically activated and localised there in response to the signalling cascade initiated by activation of T-cell receptors (Bivona et al., 2003; Quatela and Philips, 2006). Therefore, Ras signalling from the Golgi complex represents a genuine cellular phenomenon. Further work is needed to clarify whether Golgi-localised Ras activates a specific pool of ERK that is tethered to the Golgi by scaffold proteins (see below).

Signalling cascades are localised to the Golgi complex by scaffold proteins

Immediately downstream of Ras (Box 2), the Raf–MEK–ERK signalling cascade is activated. This is typically organised by scaffold proteins, molecules that bind to two or three components of this cascade and regulate signal propagation (Kolch, 2005). Scaffold proteins not only bring the components of the cascade in close proximity, but can also tether the cascade to defined locations in the cell. As Ras signals from Golgi membrane, it is to be expected that the Raf–MEK–ERK cascade also localises to the Golgi with the help of one or more scaffold proteins. The first scaffold protein that was clearly shown to act on the Golgi is interleukin-17 receptor D (IL17RD, also known and hereafter referred to as Sef). Sef interacts with MEK and ERK and localises them to the Golgi (Torii et al., 2004). This is further illustrated by increased localisation of ERK to the Golgi that is observed upon Sef overexpression, and the increased phosphorylation of cytosolic substrates such as the ribosomal protein kinase RSK2, mirrored by a reduction in phosphorylation of nuclear ERK substrates, such as Elk1 (Torii et al., 2004). In addition, Sef was also shown to be required for Ras signalling from the ER (Casar et al., 2009). Whether Golgi-localised Ras also requires Sef for the activation of downstream targets needs to be shown, and the role of Sef in endomembrane-based signalling remains to be fully investigated.

A second Golgi scaffold protein, peptidyl-tRNA hydrolase 2 (PTRH2, also known and hereafter referred to as BIT1), has recently been discovered in the human liver cancer cell line HuH7 and FR3T3 rat fibroblasts. BIT1 has been shown to localise to membranes involved with the secretory pathway rather than mitochondria (Yi et al., 2010), as was reported previously in HeLa cells (Jan et al., 2004). BIT1 was also shown to interact with ERK1/2 and, therefore, promotes scaffold-modulating ERK activity (Yi et al., 2010). However, only those BIT1 molecules located on the Golgi bind ERK1/2 and negatively regulate its activity (Kairouz-Wahbe et al., 2008). The inhibition of ERK signalling allows BIT1 to act as a pro-

apoptotic factor and reduces stress tolerance by inducing apoptosis. When BIT1 is restricted to the ER, ERK activity is increased and the apoptotic signal induced by stress is reduced. Whether this is only true in the cancer cell lines mentioned above or whether this represents a general mechanism remains to be established.

RKTG

As discussed above, RTKs and GPCRs are capable of activating a plethora of downstream signalling molecules [including PKA, p38 MAPK, Src family kinases (SFKs) and ERKs] that also signal to the Golgi to regulate its morphology and function. However, until recently it was unclear whether one or more Golgi-based proteins can contribute to the modulation of signalling by RTKs or GPCRs. Progestin and adipoQ receptor family member 3 (PAQR3, also known and hereafter referred to as, RKTG) is a member of the progestin and adipoQ receptor family. It localises to the Golgi and seems to have an important modulatory role in signal transduction downstream of both RTKs and GPCRs. It binds to and inhibits Raf, transduces EGF receptor signals from Ras to MEK and, thereby, to ERK (Box 2). As a result, active RKTG inhibits EGF signalling to ERK. The finding that cells from RKTG-knockout mice have higher basal ERK activity supports this notion (Feng et al., 2007). How RKTG is activated, however, is not known.

RKTG can also regulate signalling through GPCRs by binding to the Gβγ subunit of heterotrimeric G proteins and by tethering Gβγ to the Golgi complex (Jiang et al., 2010). This recruitment impedes the interaction between Gβγ and β-adrenergic receptor kinase 1 (ARBK1, also known and hereafter referred to as GRK2), whose activation is crucial for the desensitisation of GPCRs, such as the β2-adrenergic receptor (ADRB2). Accordingly, overexpression of RKTG inhibits desensitisation of this receptor (Jiang et al., 2010). Gβγ also mediates ADRB2-induced activation of Akt, which itself is inhibited by RKTG overexpression (Jiang et al., 2010). Therefore, RKTG represents a Golgi-based modulator of signal transduction pathways that originates from the plasma membrane.

Interestingly, binding of Gβ and Raf to RKTG is mutually exclusive (Jiang et al., 2010). Therefore, the binding of Gβ to RKTG would result in an elevation of the free (non-bound) pool of Raf and, consequently, Gβ would indirectly contribute to a stronger activation of the Raf–MEK–ERK pathway. Whether such an RKTG-mediated switch really takes place remains to be determined. Furthermore, these studies have been performed with overexpressed RKTG. It may be relevant to study the endogenous protein in order to understand how the binding of Raf and Gβ to RKTG is regulated.

A Golgi protein involved in mTOR and PtdIns(4)*P* signalling

The mammalian target of rapamycin (mTOR) has been shown to localise to the ER and the Golgi (Drenan et al., 2004). Although there is no evidence for mTOR signalling at the ER, ARF1 and Rab1 have recently been shown to modulate the phosphorylation of one of the mTOR targets, S6K, which suggests that Golgi components are involved (Li et al., 2010). More strikingly, the Golgi protein GOLPH3 Vps74 was clearly shown to have a positive role in the activation of mTOR (Scott et al., 2009). GOLPH3 was first identified as having a crucial role in Golgi localisation of glycosyltransferases when it was shown to sort them into COPI vesicles (Tu et al., 2008). Accordingly, glycoproteins secreted from yeast cells deficient in Vps74p are underglycosylated (Wood et al., 2009); therefore, it is perhaps surprising to find it involved in mTOR signalling. Whether regulation of mTOR signalling by GOLPH3 requires a physical interaction of the two proteins remains unclear, and we do not know whether mTOR is recruited to the Golgi membrane in a GOLPH3-dependent manner. It has previously been shown that mTOR1 localises to the Golgi by means of the so-called HEAT repeat domains (Liu and Zheng, 2007). Does GOLPH3 act as a recruiter for mTOR1 by interacting with the HEAT repeat domains? If not, what is the exact role of GOLPH3 in mTOR1 signalling? All these questions need to be addressed in the future.

GOLPH3 has an additional role in the functional organisation of the Golgi, as it is involved in maintaining its structure by binding to PtdIns(4)*P* on Golgi membranes and by bridging it to the actin cytoskeleton (Dippold et al., 2009). Because PtdIns(4)*P* has previously been associated with export from the Golgi (see above), the finding that GOLPH3 binds PtdIns(4)*P* potentially links PI4 kinase signalling to retention of Golgi-resident proteins and to the regulation of secretion. It might therefore be interesting in the future to explore whether mTOR1 signalling is controlled by the levels of PtdIns(4)*P* in the Golgi. As described above, prolonged starvation leads to higher levels of the PI4-phosphatase Sac1 in the Golgi (Blagoveshchenskaya et al., 2008). Will these reduced PtdIns(4)*P* levels in the Golgi lead to reduced amounts of GOLPH3 bound to Golgi membranes? If true, is the reduced amount of Golgi-localised GOLPH3 linked to the inhibition of mTOR1 signalling during starvation? These open questions also need answering.

Autochthonous signalling – the Golgi generates signalling to modulate aspects of its functional organisation

So far, we have described examples of signalling molecules that are activated at the plasma membrane and then either translocate to the Golgi (Ras) or are modulated by Golgi-localised proteins (GOLPH3, RKTG). However, there are at least two reports of a signalling cascade that is generated by Golgi components and modulates Golgi function or Golgi organisation (Fig. 1B).

The SFKs, KDEL receptor and cargo load

The arrival of a wave of cargo to the Golgi has recently been shown to activate local SFKs (Pulvirenti et al., 2008), making them potential sensors of changes in cargo flux. The activation of SFKs was found to be mediated by the binding of the KDEL receptor to incoming luminal chaperones that harbour KDEL motifs; the KDEL receptor then enters the Golgi together with the cargo wave (Pulvirenti et al., 2008). This finding suggests that cargo receptors can also be signalling receptors, similar to those found at the plasma membrane. In turn, SFK activation augments secretion. Furthermore, SFKs also regulate steady-state trafficking within and/or from the Golgi, because it was shown in the same study that their pharmacological inhibition blocks trafficking to and through this organelle.

The mechanism behind the SFK activation of secretion might be found in the phosphorylation of dynamin 2, which is required for transport from the Golgi to the plasma membrane (Weller et al., 2010). Hyperactivation of SFKs, however, was also shown to induce fragmentation of the Golgi complex (Weller et al., 2010), perhaps by hyperphosphorylation of dynamin 2. It is therefore possible that weak activation of SFKs by a cargo only causes local remodelling in the Golgi structure that facilitates intra-Golgi transport. In this respect, the arrival of a cargo wave at the Golgi has been shown to lead to activation ofphospholipase A2

(PLA2), which is thought to induce the formation of inter-cisternal tubules that have been proposed to support intra-Golgi trafficking (San Pietro et al., 2009). As both SFKs and PLA2 are activated by the arrival of a cargo wave at the Golgi, it would be interesting to determine whether there is any causal relationship between SFKs and PLA2. In addition, it needs to be determined whether SFKs that are activated on the Golgi can signal to pathways other than the secretory pathway and whether Golgi-localised SFKs are activated by other stimuli. It also remains unclear whether a specific SFK member is predominantly involved in this cargo-load activation. Furthermore, the mechanism by which SFKs are activated by the KDEL receptor is still unclear and needs to be investigated in greater detail.

The Golgi scaffolding molecule GM130 is required for Golgi re-orientation during cell migration

As discussed above, the Golgi complex has a primary role in cell migration in response to specific cues. Signalling from the plasma membrane (via ERK) leads to phosphorylation of the Golgi matrix protein GORASP1 (also known as GRASP65) to modulate the role of the Golgi in cellular signalling. However, the Golgi complex also generates signalling that regulates its own re-orientation. This is achieved by the specific recruitment of kinases by the Golgi matrix protein GOLGA2 (also known as GM130), which can therefore be viewed as a signalling scaffold molecule. Preisinger and co-workers have shown that the Golgi matrix protein GM130 interacts with the kinases MST4 and YSK1, both of which belong to the family of germinal-centre kinases III (GCKIII) (Preisinger et al., 2004). The binding of these kinases to GM130 leads to their activation, a step crucial for the Golgi re-orientation necessary for cell migration, and gives them a previously unappreciated role to these kinases. Thus, unlike GRASP65, GM130 does not seem to be the target of these kinases but rather functions as a scaffold to recruit and activate them at the Golgi. The GM130–GCKIII complex also binds to programmed cell death 10 (PDCD10), which serves to stabilise GCKIII (Fidalgo et al., 2010).

GCKIII also phosphorylates the polypeptide (YWHAZ, also know as 14-3-3ζ) (Preisinger et al., 2004; Fidalgo et al., 2010), and this modification might help to recruit the Par complex to the Golgi. The Par complex is a master regulator of polarity (Hurd et al., 2003), and is activated by Cdc42 at the plasma membrane (Etienne-Manneville, 2004). If the Par complex were to be recruited to the Golgi by the GM130–GCKIII–CCM3 complex, would it also be activated by Cdc42? This hypothetical scenario would necessitate Golgi-based activation of Cdc42, which has recently shown to be the case – with evidence supporting the recruitment of dynamin-binding protein (DBMBP; also known as scaffold protein Tuba), an exchange factor and activator of Cdc42, by GM130 (Kodani et al., 2009). By recruiting polarity regulators to the Golgi complex, GM130 potentially receives a central role in the cell migration. Thus, in analogy to the plasma membrane, the Golgi complex represents a platform where polarity signalling can be initiated.

Conclusions and Perspectives

Work in the past three decades contributed to our understanding of the biogenesis and maintenance of organelles and the mechanism of vesicle formation, tethering and fusion. Although the scientific community is still far from understanding all the mechanistic details, especially those at the atomic level, future work will increasingly and inevitably focus on the regulation of these machineries.

One of the emerging findings is that the Ras–MEK–ERK pathway regulates many aspects of the secretory pathway, both at the ER and the Golgi, and that it has a central role in signalling from or to these organelles. Furthermore, the essential role of the Golgi in cell migration and directional polarity is now established. As directional cell migration underlies processes such as cancer cell metastasis, finding regulators for this process bears the potential to identify new drug targets for future tumour therapies.

We are only beginning to grasp the extent of the regulation of the secretory pathway by cellular signalling. To complement the examples given above, systems biology may provide us with the tools to study the regulation in a systematic genome-wide manner. Pioneering RNA interference (RNAi) screens have been performed to study the regulation of endocytosis (Pelkmans et al., 2005; Collinet et al., 2010), and to identify new factors involved in secretion in cultured *Drosophila* cells (Bard et al., 2006; Wendler et al., 2010). Recently, a screen of the kinome and phosphatome was performed to understand the regulation of the ER–Golgi system by signalling both in *Drosophila* (C.R., unpublished data) and mammalian (Farhan et al., 2010) cells. Although such systems biology approaches are certainly valuable, we have to be careful in interpreting the information gained from large-scale screens. The temptation of data over-interpretation is as large as the number of hits generated. Many (if not most) of the potential regulators from any screen have an indirect role. This is because metabolic and signal transduction pathways are highly interconnected (Polak and Hall, 2009). An example is the identification of ERK2 as a regulator of ERESs (Farhan et al., 2010). The same work has also identified the epidermal growth factor receptor and the kinases A-Raf and Raf1 as regulators in the same hit category (i.e. regulators of ERESs). Because these molecules are upstream activators of ERK2 (Box 2), it is unlikely that their effect on ERESs is direct – rather it might be mediated by ERK2.

Furthermore, the effect of signalling molecules on the secretory pathway has usually been studied by using siRNAs or pharmacologic inhibitors, which are not acute or specific, respectively. Perhaps the best way of defining the role of specific kinases is to identify their direct substrates on membranes of the secretory pathway as well as the spatio-temporal regulation of their activation. In this respect, new tools are becoming available, such as engineered kinases or phosphatases that can be reversibly tethered to defined locations in the cell (Szentpetery et al., 2010). Understanding the interplay between signalling and secretion will allow us to understand how the secretory pathway remains a highly processive, yet accurate system. This will also advance our understanding of how the secretory pathway is integrated into developmental programs, and how its misregulation may lead to pathological conditions.

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