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Distribution and role of heterotrimeric G proteins in the secretory pathway of polarized epithelial cells

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SUMMARY

The movement of newly synthesized proteins in the constitutive secretory pathway, from their site of synthesis in the endoplasmic reticulum to the cell surface or to intracellular destinations, requires an orderly sequence of transport steps between membrane-bound compartments. Until recently, the trafficking and secretion of proteins through this pathway was thought to occur as a relatively automatic, unregulated series of events. Recent studies show that protein trafficking in the constitutive secretory pathway requires GTP hydrolysis by families of GTP-binding proteins (G proteins), which at multiple steps potentially provide regulation and specificity for protein trafficking. Many monomeric G proteins are known to be localized and functional on membrane compartments in the constitutive secretory pathway. Now, members of the heterotrimeric G protein family have also been localized on intracellular membranes and compartments such as the Golgi complex. We have studied the localization and targeting of Gα subunits to distinct membrane domains in polarized epithelial cells. The distribution of different Gα subunits on very specific membrane domains in cultured epithelial cells and in epithelial cells of the kidney cortex, is highly suggestive of roles for these G proteins in intracellular trafficking pathways. One of these G protein subunits, $G\alpha_{i\text{-}3}$, was localized on Golgi membranes. Studies on LLC-PK1 cells overexpressing $G\alpha_{i\text{-}3}$ provided evidence for its functional role in regulating the transport of a constitutively secreted heparan sulfate proteoglycan through the Golgi complex. Inhibition or activation of heterotrimeric G proteins by pertussis toxin or by aluminium fluoride respectively, have provided further evidence for regulation of intracellular transport by pertussis toxin-sensitive G proteins. Although the functions of Golgi-associated G proteins are not yet understood at the molecular level, heterotrimeric G proteins have been implicated in the binding of cytosolic coat proteins and vesicle formation on Golgi membranes. Future studies will elucidate how multiple G proteins, of both the heterotrimeric and monomeric families, are involved in the regulation of Golgi function and protein trafficking in the secretory pathway.

Key words: G proteins, secretion, Golgi, vesicle trafficking

INTRODUCTION

The delivery of newly synthesized proteins to different membrane domains results in a geographic segregation of functions within polarized epithelial and neuronal cells. Similarly, the polarized secretion of newly synthesized proteins from distinct sides of epithelial cells enables differentiation and specialization of separate compartments of the extracellular milieu. Many membrane proteins and soluble proteins synthesized in the rough ER are trafficked via the constitutive secretory pathway, through the Golgi complex, to the cell surface or other destinations (Farquhar, 1985; Kelly, 1985; Palade, 1975; Rothman and Orci, 1992). The trafficking of these proteins is believed to occur as a continuous stream of small quantities of proteins that are shuttled between compartments in small vesicles or other membrane-bound carriers. Although the mechanisms governing the sorting and vesicular transport of proteins through this constitutive pathway have been studied extensively, there is still little understanding of how this dynamic, multi-stage pathway is regulated.

One example of a protein that is secreted in a constitutive and a polarized fashion from many epithelial cells is the heparan sulfate proteoglycan BMHSPG, which is a component of the basement membrane underlying the epithelia. The intracellular processing and secretion of the BMHSPG has been studied in kidney epithelial cell lines (Caplan et al., 1987; de Almeida and Stow, 1991; Stow and Farquhar, 1987; Stow et al., 1989). In polarized monolayers of cultured LLC-PK1 cells and MDCK cells, the BMHSPG is sorted and secreted in a polarized fashion from the basal surface of the cells (Caplan et al., 1987; de Almeida and Stow, 1991). The BMHSPG is secreted relatively abundantly and continuously from LLC-PK1 cells grown on filters and the sequential post-translational modifications of biosynthetically labelled BMHSPG can be used

to follow its intracellular trafficking. Thus, assaying the processing and secretion of BMHSPG in LLC-PK1 cells has provided a useful model in which to study the secretory pathway. Importantly it has enabled us to follow an endogenous protein in intact cells for studies of secretion, particularly with respect to the regulation of secretion by G proteins, as described below.

The use of non-hydrolyzable nucleotide analogs such as GTPγS provided the first clue that GTP-binding proteins were involved in various steps of vesicle trafficking (Balch, 1990; Beckers and Balch, 1989; Melancon et al., 1987; Ruohola et al., 1988; Tooze et al., 1990). Addition of GTPγS to permeabilized cells and or cell-free assays was generally found to block constitutive protein transport between membrane-bound compartments, by inhibiting either fusion or formation of vesicle carriers. The effects of GTPγS were initially attributed to the many examples of monomeric G proteins found throughout the secretory pathway (Balch, 1990). However, it has since become known that members of the heterotrimeric G protein family are also present and functional in the secretory pathway.

DISTRIBUTION OF HETEROTRIMERIC G PROTEINS

Two large gene families of guanine nucleotide-binding (G) proteins have been identified. The ras supergene family consists of several families of small molecular mass, monomeric G proteins, which are found in soluble or membrane-attached forms in yeast and higher cells and are involved in a diverse range of cellular events, including protein trafficking and secretion (Downward, 1990). The other main family of G proteins has long been recognized for its involvement in signal transduction pathways and the regulation of receptors at the cell surface (Gilman, 1987; Stryer and Bourne, 1986). Heterotrimeric G proteins exist as αβγ subunit complexes in which the α subunit is responsible for binding and hydrolysis of GTP. The βγ subunit is complexed to α during the inactive portion of the hydrolysis cycle and more recent studies have shown that βγ may have its own independent signalling functions following dissociation from α (Federman et al., 1992; Tang and Gilman, 1991). The Gα subunits have traditionally been classified by their functional sensitivity to bacterial toxins; $G\alpha_i$ and α_o are ADP-ribosylated by pertussis toxin, whereas $G\alpha_s$ is specifically ADP-ribosylated by cholera toxin. The heterotrimeric G proteins exist largely as membrane-associated proteins and the individual subunits have myristoylation or other fatty acyl modifications for membrane attachment (Fukada et al., 1990; Jones et al., 1990; Mumby et al., 1990).

Multiple gene products of each of the α, β and γ subunits associate in distinct combinations generating heterogeneity amongst these G proteins (Simon et al., 1991). Discrete deployment of these heterogeneous G proteins to different sites on membranes throughout the cell then may allow for the simultaneous regulation of many different signal transduction pathways. The cellular distribution and intracellular localization of heterotrimeric G protein subunits is thus important to elucidate. The localization of different G proteins on membranes in cells and tissues has recently been studied in our laboratory and by others. Detection and localization of different G proteins have been made possible by the availability of specific peptide antibodies that can distinguish between the different G protein subunits (Spiegel et al., 1990).

Distinct membrane targeting of $G\alpha_i$ proteins in LLC-PK1 cells

The polarized epithelial cell line LLC-PK1 expresses only two of the known pertussis toxin-sensitive Gα subunits, $G\alpha_{i\text{-}2}$ and $G\alpha_{i\text{-}3}$. Our studies showed that $G\alpha_{i\text{-}2}$ and $G\alpha_{i\text{-}3}$ are localized on distinct membrane domains and have quite separate functions in these polarized cells (Ercolani et al., 1990; Holtzman et al., 1991; Stow et al., 1991a). $G\alpha_{i\text{-}2}$ was found to be associated with the plasma membrane, while $G\alpha_{i\text{-}3}$ was found in a novel position on intracellular Golgi membranes. The immunofluorescence staining of $G\alpha_{i\text{-}2}$ resembles a cobblestone pattern, typical of basolateral plasma membrane staining, in confluent monolayers of LLC-PK1 cells (Ercolani et al., 1990). The $G\alpha_{i\text{-}2}$ subunit is targeted in a polarized fashion to the basolateral membrane, particularly the lateral membrane, and is not found on the apical membrane of these cells. The localization of $G\alpha_{i\text{-}2}$ on the cell membrane is consistent with its proposed role in regulating adenylyl cyclase in response to hormone receptors (Ercolani et al., 1990). $G\alpha_{i\text{-}2}$ is developmentally expressed in LLC-PK1 cells. The expression of $G\alpha_{i\text{-}2}$ is under transcriptional control and its appearance on the plasma membrane parallels the gradual development of polarity as the cultures progress from a sparse, non-polar morphology to a confluent, polarized morphology (Holtzman et al., 1991). Due to the lateral membrane localization of $G\alpha_{i\text{-}2}$ and its pattern of expression, it is tempting to speculate that this Gα subunit may actually be involved in the acquisition of functional or morphological polarity in these cells.

In contrast to $G\alpha_{i\text{-}2}$, its highly homologous relative, $G\alpha_{i\text{-}3}$, was found to be localized on the perinuclear Golgi complex in LLC-PK1 cells (Ercolani et al., 1990; Stow et al., 1991a). It is not currently known how different Gα subunits are targeted to distinct membrane domains. Our recent studies, using expression of chimeric proteins, have identified regions of the Gα proteins that appear to be responsible for their targeting (J. Stow, L. Ercolani, D. Ausiello et al., unpublished data). The targeting or membrane association of Gα subunits in LLC-PK1 cells appears to be a saturable process. This was also elegantly demonstrated in fibroblasts where the localization of $G\alpha_{i\text{-}3}$ was dependent on its level of overexpression (Hermouet et al., 1992). While LLC-PK1 cells express $G\alpha_{i\text{-}3}$ only on their Golgi membranes, other cells have $G\alpha_{i\text{-}3}$ associated with additional membranes. In the A6 toad kidney cells, $G\alpha_{i\text{-}3}$ is found on the perinuclear Golgi complex and is also present near the apical cell surface where it is intimately associated with, and regulates, Na^+ channel activity (Ausiello et al., 1992; Cantiello et al., 1989). In fibroblasts, $G\alpha_{i\text{-}3}$ is present on Golgi membranes and also in some cases at the plasma membrane where it can regulate adenylyl cyclase (Hermouet et al., 1992). The multiple locations and functions

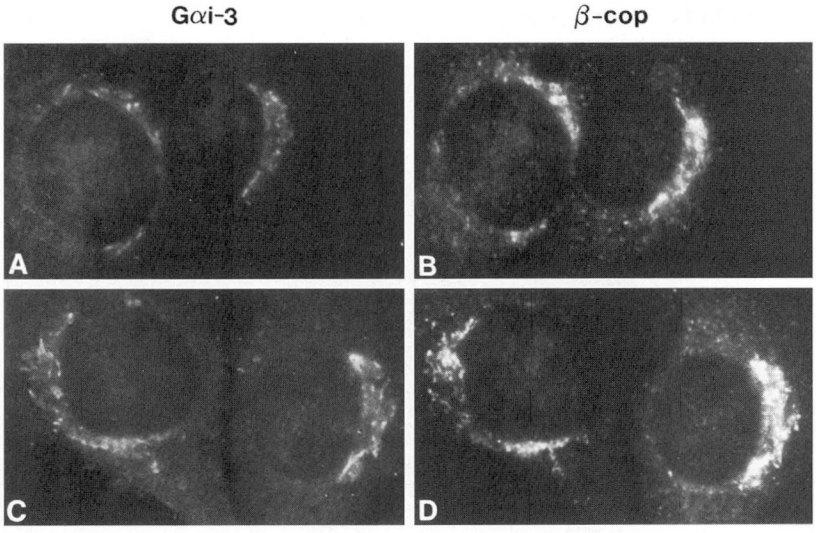

Fig. 1. Immunofluorescence localization of $G\alpha_{i-3}$ on Golgi membranes. NRK cells were double-labelled with antibodies specific for $G\alpha_{i-3}$ and β-COP, which were then reacted with FITC- and rhodamine-labelled conjugates, respectively. (A and C) Localization of $G\alpha_{i-3}$ in two sets of cells; (B and D) β-COP staining in the same two sets of cells. Staining for both proteins is over the perinuclear Golgi complexes in these cells. The staining of $G\alpha_{i-3}$, which is associated with Golgi cisternae, has a more linear appearance. β-COP is believed to be associated with Golgi vesicles, and its staining over the Golgi is more punctate.

for $G\alpha_{i-3}$ in such cells reinforces the concept that a given G protein subunit may be used promiscuously, for different functions in different cells, and for different functions within the same cell.

Heterotrimeric G proteins on Golgi membranes

At a time when heterotrimeric G proteins were thought to function only at the level of the plasma membrane, several studies provided evidence for the association of pertussis toxin-sensitive G proteins on intracellular membranes such as the rough ER (Audigier et al., 1988), the Golgi complex (Ali et al., 1989; Toki et al., 1989) and endosomes (Ali et al., 1989). However, the functions of these intracellular G proteins and their potential involvement in trafficking pathways, such as the secretory pathway, were not known at that time. It is now clear that $G\alpha_{i-3}$ and other G protein subunits are fairly universal components of Golgi membranes. $G\alpha_{i-3}$ has now been specifically detected on the Golgi membranes in different tissues and cells, including different kidney cell lines in our own laboratory (Fig. 1) and in a number of other cell types by other groups (Barr et al., 1991; Hermouet et al., 1992; Ktistakis et al., 1992; Stow et al., 1991a). $G\alpha_{i-3}$ on Golgi membranes is localized on the cytoplasmic face of Golgi cisternae where it is peripherally, but stably, attached to the membrane (Stow et al., 1991a). While weak cytoplasmic staining of $G\alpha_{i-3}$ disappears after cycloheximide treatment of cells, the more intense Golgi staining remains, suggesting that $G\alpha_{i-3}$ is a stable resident of the Golgi membranes and that the cytoplasmic staining may be attributed to a newly synthesized pool of the subunit (Stow et al., 1991a). $G\alpha_{i-3}$ on the Golgi membranes associates with a $\beta\gamma$ complex, as was shown by its ability to be efficiently ADP-ribosylated by pertussis toxin, which requires heterotrimer formation (Sunyer et al., 1989). In isolated rat liver Golgi membranes, $G\alpha_{i-3}$ is the only pertussis toxin-sensitive protein that can be detected by immunoblotting (Fig. 2) or by ^{32}P-ADP-ribosylation with pertussis toxin (Stow et al., 1991a). Other G protein subunits are however present on these membranes. Immunoblotting confirmed the presence of the β subunit and of the cholera toxin-sensitive $G\alpha_s$ subunit on rat liver Golgi membranes (Fig. 2). Golgi membranes from other cells may contain different combinations of Gα subunits. *Trans*-Golgi membranes from PC12 cells contain $G\alpha_{i-3}$ and other pertussis toxin-sensitive subunits; $G\alpha_o$ and $G\alpha_{i-1,2}$, and the $G\alpha_s$ subunit have also been found on these membranes (Leyte et al., 1992).

The localization of $G\alpha_i$ subunits on various intracellular membranes, together with functional studies such as those described below, have now established a role for these G proteins in the secretory pathway.

Polarized distribution of Gα subunits in the kidney

The distinct intracellular and intercellular distributions of G proteins found in cultured cells are also found in epithelial cells in tissues and this is graphically demonstrated by the distribution of Gα subunits in the kidney cortex. The renal nephron of the kidney is composed of a large number of morphologically and functionally distinct polarized epithelial cells. The apical or lumenal membranes and the basolateral membranes of these cells host a variety of ion channels and hormone receptors that are known to be coupled to G protein-mediated signal transduction pathways. The distribution of different Gα subunits on membrane domains throughout kidney epithelial cells is therefore potentially important for the segregation of functions associated with these membranes.

Using specific peptide antibodies, the distribution of different Gα subunits in cells along the nephron has been studied by several groups (Brunskill et al., 1991; Gupta et al., 1991; Stow et al., 1991b). There is a heterogeneous distribution of $G\alpha_i$ and $G\alpha_s$ subunits in cells from functionally distinct areas of the tubules (Brunskill et al., 1991; Stow et al., 1991b) and examples of $G\alpha_i$, $G\alpha_s$ and $G\alpha_o$ have been found in the renal glomerulus (Gupta et al., 1991). Proximal tubule cells express several different Gα subunits, the largest number appearing to be concentrated in this region of the nephron. $G\alpha_{i-2}$, $G\alpha_{i-3}$ and $G\alpha_s$ are all heavily concentrated in the sub-apical vesicles of proximal tubule cells (Fig. 3). $G\alpha_{i-3}$ was also found associated with the basolateral membrane of cells in some segments of the proximal tubules (Stow et al., 1991b). Cells of the cortical collect-

ing duct show a highly polarized distribution of $G\alpha_s$, which is on the basolateral membranes of the principal and intercalated cells (Fig. 3). The principal cells also contain $G\alpha_{i\text{-}2}$, which is restricted to lateral membranes, in a similar localization to that seen in cultured renal LLC-PK_1 cells (Ercolani et al., 1990; Stow et al., 1991a). The distribution of the $G\alpha_{i\text{-}1}$ subunit provides the most dramatic example

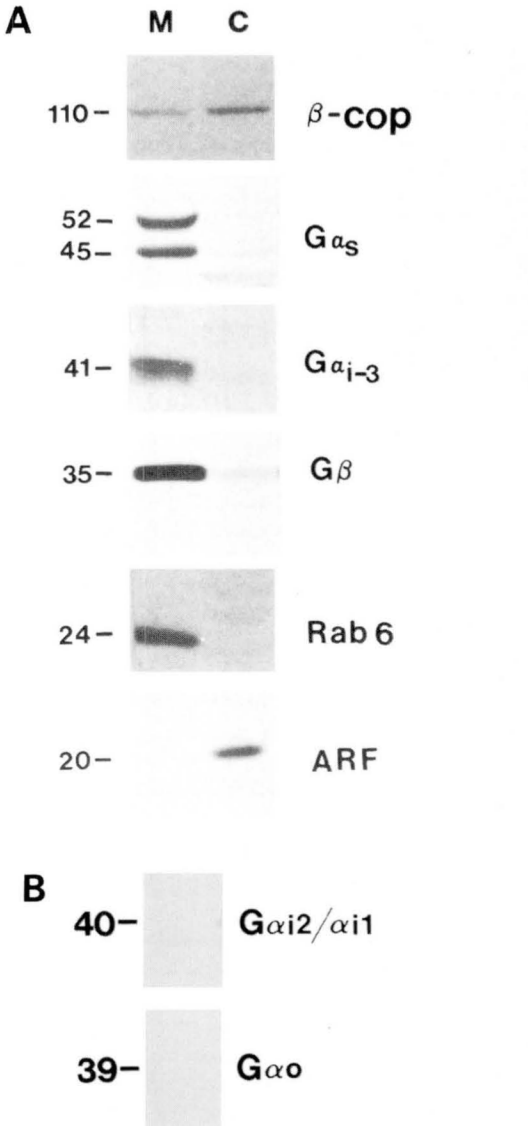

Fig. 2. Immunoblotting of Golgi-associated proteins. (A) Golgi membranes (M) and cytosol (C) were purified from rat liver homogenates and separated by SDS-PAGE on a 5%-15% gradient gel. Proteins were eletrophoretically transferred to membranes and sections of the gels were stained with specific antibodies that were detected by an alkaline phosphatase reaction. Various heterotrimeric G protein subunits, including $G\alpha_{i\text{-}3}$, $G\alpha_s$ and Gβ, were detected as bands of the appropriate molecular masses, as indicated. These G protein subunits were found only on the membranes and not in the cytosol. Monomeric G proteins were also detected, rab 6 was found on the Golgi membranes and ARF was found in the cytosol in freshly prepared fractions. The coatomer protein β-COP was found predominantly in the cytosol but was also detected on Golgi membranes. The positions of marker proteins (kDa) are indicated. (B) Immunoblotting with peptide antibodies specific for different Gα subunits showed that $G\alpha_{i\text{-}3}$ (shown in A) is the only pertussis toxin-sensitive G protein present on these membranes. Antibodies against $G\alpha_{i\text{-}1 \text{ and } 2}$ and $G\alpha_o$ failed to detect any proteins on these membranes.

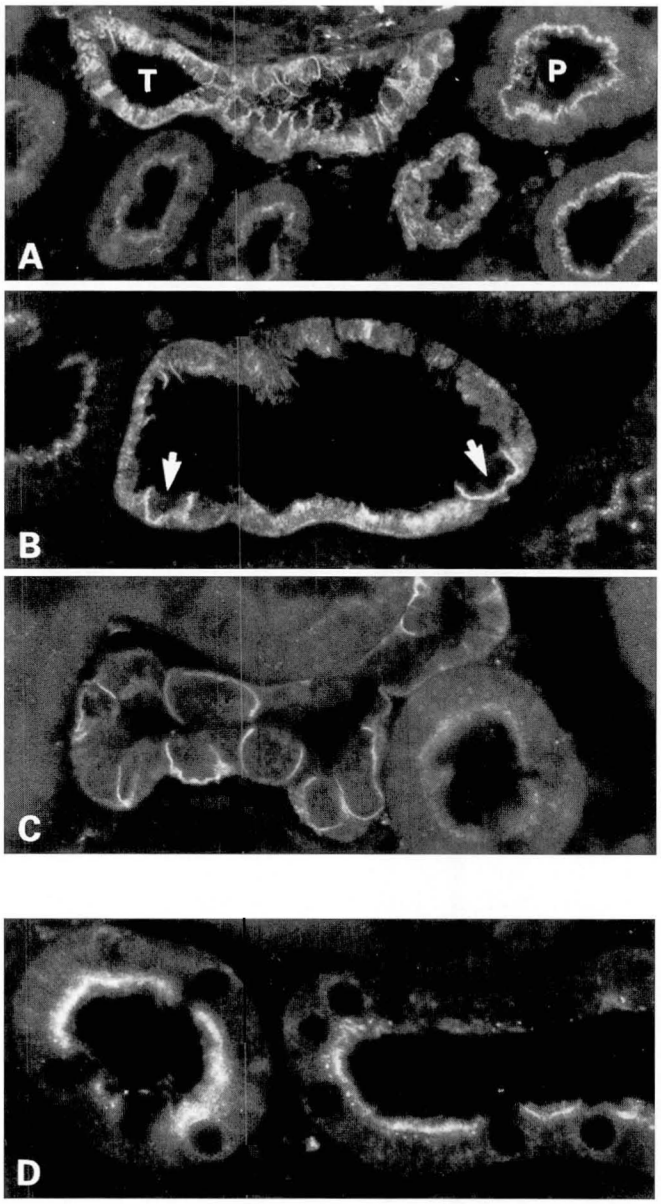

Fig. 3. Immunofluorescence localization of Gα subunits in rat kidney cortex. Rat kidney sections were fixed and stained with specific peptide antibodies to detect different G proteins. The polarized distribution of $G\alpha_s$ in the kidney cortex is shown in (A, B and C). (A) In the thick ascending limb (T) $G\alpha_s$ was found on the highly infolded basolateral membranes of these cells. In contrast, proximal tubule cells (P) had staining of $G\alpha_s$ in the subapical invaginations. (B) Basolateral staining of $G\alpha_s$ was found on the infoldings of the principal cells in the cortical collecting duct and on the basal and lateral domains of the intercalated cells (arrows). (C) An area of cortical collecting duct shows staining of the $G\alpha_s$ in a highly polarized fashion on the basolateral membranes of intercalated cells. (D) Staining of $G\alpha_{i\text{-}3}$ in the proximal tubule cells, where it is concentrated in the subapical invaginations just below the level of the lumenal brush border.

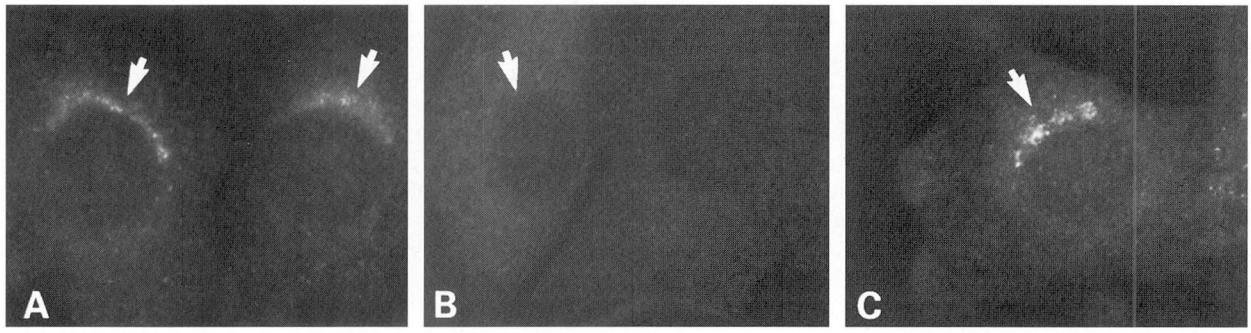

Fig. 4. Effect of BFA and aluminium fluoride on Golgi membrane localization of p200. The p200 protein was localized in NRK cells by immunofluorescence with a specific antibody. In untreated cells (A), there was staining over the perinuclear Golgi complex (arrow) and weaker staining in the cytoplasm. Following treatment with BFA (5 μM for 30 minutes) (B) the Golgi was no longer stained and there was only weak staining of the cytoplasm (arrow). Pretreatment of the cells with 30 mM NaF + 50 μM $AlCl_3$ (C) prior to addition of BFA under the same conditons, resulted in retention of the Golgi staining (arrow).

of a highly selective and polarized localization of G proteins. $G\alpha_{i\text{-}1}$ is found on the apical membrane of cells in the thick ascending limb of the distal nephron; however in a highly specialized group of cells within this tubule, the macula densa, $G\alpha_{i\text{-}1}$ was found at the basal aspect of the cells (Stow et al., 1991b). The inverted polarity of the $G\alpha_{i\text{-}1}$ protein is perhaps related to the altered morphological polarity of the macula densa cells (Rouiller and Orci, 1969), which are uniquely positioned for their highly specialized roles in the control of glomerular filtration rate and renin secretion. The functional relevance of the polarized localization of $G\alpha_{i\text{-}1}$ in thick ascending limb cells or in macula densa cells is not known.

In general, the functions of these G protein subunits on distinct membrane domains in kidney epithelial cells are not known. However their distributions on specific membrane domains, once again, suggests that geographic segregation of G protein subunits may be important for the control of both membrane protein signalling events and membrane trafficking.

FUNCTION OF HETEROTRIMERIC G PROTEINS IN THE SECRETORY PATHWAY

The localization of $G\alpha_{i\text{-}3}$ on the Golgi membranes provided us with the first suggestion that this G protein might be involved in Golgi trafficking, and subsequent studies in polarized LLC-PK_1 cells produced evidence for a functional role of $G\alpha_{i\text{-}3}$ in secretory trafficking (Stow et al., 1991a). The constitutive polarized secretion of BMHSPG in LLC-PK_1 cells was used as a model to determine the effects of overexpression of $G\alpha_{i\text{-}3}$. The overexpression of close-to-physiological amounts of $G\alpha_{i\text{-}3}$ resulted in correct targeting of this protein to Golgi membranes and functionally it produced a significant retardation of the secretion of BMHSPG. The effect of the overexpressed, and presumably activated, $G\alpha_{i\text{-}3}$ was at the level of trafficking through the Golgi complex where precursors of the BMHSPG accumulated. These studies suggested that activated $G\alpha_{i\text{-}3}$ might be imposing a rate-limiting or regulatory step in the transport of proteins, exemplified by BMHSPG, through the Golgi complex. This retardation or block in trafficking could be relieved by pretreating cells with pertussis toxin which increased the rate of intracellular trafficking and secretion of BMHSPG. Pertussis toxin ADP-ribosylates $G\alpha_{i\text{-}3}$, functionally uncoupling it from its receptor or activating protein. Since $G\alpha_{i\text{-}3}$ is the only known pertussis toxin substrate on Golgi membranes in LLC-PK_1 cells, the effects of the toxin can be attributed directly to $G\alpha_{i\text{-}3}$. Furthermore, since pertussis toxin also enhanced secretion in cells expressing only endogenous $G\alpha_{i\text{-}3}$, this G protein can be regarded as a normal, intrinsic regulator in the secretory pathway. Interestingly, either blocking or enhancing the rate of intracellular transport disrupted neither the sequence of post-translational processing of the BMHSPG nor the polarity of its secretion, also supporting the concept that the activation/deactivation of $G\alpha_{i\text{-}3}$ is perhaps a normal regulatory step in this pathway.

The effects of $G\alpha_{i\text{-}3}$ overexpression on constitutive secretion are consistent with the effects of agents that cause a more widespread activation of heterotrimeric G proteins. It has been known for some time that exogenous addition of aluminium fluoride can be used to activate G proteins; in the context of the GDP-binding pocket of Gα, the fluoride ions of this complex mimic the γ phosphate of GTP, prompting activation of the G protein (Higashijima et al., 1991). Recently, aluminium fluoride has been shown to be a more specific activator for heterotrimeric G proteins than for several families of monomeric G proteins (Kahn, 1991) and it has been used specifically to implicate heterotrimeric G proteins in a number of membrane trafficking pathways. For instance, functional assays have shown that heterotrimeric G proteins are involved in regulating several steps of the endocytic pathway (Carter et al., 1993; Columbo et al., 1992). In an earlier study, Melancon and coworkers provided some of the first evidence that heterotrimeric G proteins were required for Golgi trafficking when they showed that both GTPγS and aluminium fluoride blocked in vitro vesicle transport between Golgi compartments (Melancon et al., 1987). Using another cell-free assay, which measures the budding of secretory vesicles and secretory granules off *trans*-Golgi network (TGN) membranes, Barr and coworkers also provided important evidence for the involvement of heterotrimeric G proteins in secretory trafficking (Barr et al., 1991, 1992). Data show-

ing that vesicle budding was blocked by both GTPγS and aluminium fluoride but enhanced by addition of free βγ subunits or by pertussis toxin (Barr et al., 1991), are consistent with similar effects on secretion produced by overexpression of $G\alpha_{i-3}$ and pertussis toxin in intact cells (Stow et al., 1991). Further studies on vesicle budding from TGN membranes have also shown that, in addition to $G\alpha_{i-3}$, there are other pertussis toxin- and cholera toxin-sensitive G proteins on these membranes (Leyte et al., 1992). Cholera toxin, which ADP-ribosylates and activates $G\alpha_s$, also stimulated TGN vesicle budding, suggesting that the $G\alpha_s$ may act in concert with $G\alpha_i$s as an opposing and therefore regulatory partner. Taken together, these studies confirm the requirement for a pertussis toxin-sensitive Gα protein in secretory trafficking, although the molecular mechanisms by which it, together with other G proteins, perform this regulation are still unknown.

Molecular mechanisms of G protein regulation

Due to the presence of multiple G protein subunits on Golgi membranes, it is easy to speculate that there is more than one mode of regulation based on GTP-hydrolysis and perhaps more than a single signal transduction pathway involved. The key proteins to which the heterotrimeric G proteins are functionally connected should be, in traditional terms, a receptor and an effector or their equivalents. Neither of these proteins has yet been identified on Golgi membranes. The receptor on the Golgi could fit into the traditional family of G-protein-coupled, seven membrane-spanning domain receptors, or possibly the family of single membrane-spanning domain receptors involved in sorting, or it could defy presumption and be a completely different class of membrane protein. Studies to date have also not identified a likely effector on the Golgi. We were not able to find effects on vesicle trafficking with intermediates in pathways of common effectors such as phospholipase or adenylyl cylcase (J. Stow, unpublished data).

There are several lines of evidence to connect heterotrimeric G proteins to other Golgi-associated proteins involved in vesicle trafficking. One set of proteins that appears to be intimately involved in trafficking is the coatomer, including the β-COP protein (Duden et al., 1991; Serafini et al., 1991). β-COP is a component of a cytosolic complex, which binds to Golgi membranes where it forms a specialized coat that may be responsible for structuring Golgi transport vesicles or other vehicles. β-COP and other cytosolic proteins such as γ-adaptin and the monomeric G protein ARF (ADP-ribosylating factor) have two features in common: their binding to Golgi membranes is prevented by the fungal metabolite brefeldin A (BFA) and enhanced by activation of G proteins (Donaldson et al., 1991b; Ktistakis et al., 1992; Robinson and Kries, 1992). The BFA-induced dissociation of β-COP is prevented by aluminium fluoride or mastoparan, which in turn is also altered by pertussis toxin, implying that a heterotrimeric G protein is responsible for stabilizing its membrane association (Donaldson et al., 1991b; Ktistakis et al., 1992). The binding of β-COP and ARF to Golgi membranes was measured directly, in vitro, to show that a heterotrimeric G protein was involved in this binding (Donaldson et al., 1991a). We have identified a cytosolic protein, p200, which binds specifically to Golgi membranes (Narula et al., 1992). The binding of p200 to Golgi membranes is regulated in a similar fashion to the coatomer proteins, and its association with the Golgi is influenced by BFA and by activation of G proteins. The dissociation of p200 in the presence of BFA in NRK cells is prevented by activation of heterotrimeric G proteins by aluminium fluoride (Fig. 4). Our recent studies have shown that p200 binding to Golgi membranes is also affected by pertussis toxin (J. Stow, B. de Almeida, unpublished data). These studies have provided evidence that heterotrimeric G proteins, including specifically pertussis toxin-sensitive G proteins, are involved in regulating the dynamic interaction of soluble vesicle coating proteins with Golgi membranes. The effects of G protein activation/inhibition on coat protein binding are not yet reconciled into a ready explanation of how G proteins ultimately regulate secretion. Obviously, other proteins in this molecular network need to be identified in order for us to understand how G proteins are able to provide regulation in constitutive pathways of protein secretion and membrane trafficking.

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