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Essay

Role of Heterotrimeric G Proteins in Membrane Traffic

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GTP-binding proteins or GTPases are versatile cyclic molecular switches (reviewed in Gilman, 1987; Bourne *et al.*, 1990, 1991). In the past few years there has been an explosion of interest in unraveling their role in membrane traffic (reviewed in Bourne, 1988; Balch, 1990). GTP-binding proteins are classified into two broad families: the ras-like monomeric GTP binding proteins and the heterotrimeric G proteins. Evidence from genetics, immunolocalization, and functional assays has established that two subfamilies of monomeric GTP-binding proteins, the rab and ARF subfamilies, are required for membrane traffic (reviewed in Pfeffer, 1992; Rothman and Orci, 1992). More recently, attention has turned to the role of heterotrimeric G proteins in membrane traffic (reviewed in Balch, 1992; Barr *et al.*, 1992). This review will focus solely on the recent data that suggest that heterotrimeric G proteins are involved in membrane traffic.

CLASSICAL CYCLE OF HETEROTRIMERIC G PROTEINS

Heterotrimeric G proteins transduce extracellular signals to intracellular effectors through coupling with transmembrane receptors (Figure 1, Classical Model) (reviewed in Gilman, 1987; Kaziro *et al.*, 1991). In their "inactive" state, heterotrimeric G proteins are a complex of α, β, and γ subunits in which the guanine nucleotide binding site of the α subunit is occupied by GDP. When an extracellular signal in the form of a ligand binds to a receptor (or when light interacts with rhodopsin), the ligand-receptor complex catalytically acts as a guanine nucleotide release factor (GRF), causing the α subunit to release GDP. The G protein with an "empty" guanine nucleotide binding site on its α subunit has an increased affinity for the receptor. However, the complex is transient because the high concentration of intracellular GTP drives binding of GTP onto the α subunit (reviewed in Ross and Gilman, 1980; Hamm, 1990). Binding of GTP causes the G protein to dissociate into α and $\beta\gamma$ subunits. The free GTP-bound α subunit can now activate a downstream effector. There are a broad range of such effectors, including adenylyl cyclases, phospholipases, and ion channels (Kaziro *et al.*, 1991). In the next step, the G_α subunit will spontaneously hydrolyze its bound GTP to GDP. Consequently, the G_α reassociates with the $\beta\gamma$ subunit, returning the system unidirectionally to the beginning of the cycle.

The α subunits are thought to provide the principal specificity to each type of G protein. About 20 varieties of α subunits are known, some having well-defined specificities for particular receptors and effectors (reviewed in Simon *et al.*, 1991). In contrast, there is less heterogeneity in β and γ subunits and only recently have differences in their functions been investigated (reviewed in Lefkowitz, 1992). The classic view is that the role of $\beta\gamma$ is to complex with and inactivate the α subunit.

However, there is now considerable evidence indicating that $\beta\gamma$ also acts on effectors. Jelsema and Axelrod (1987) showed that the $\beta\gamma$ subunit of G_t stimulated phospholipase A_2. Logothetis *et al.* (1987) showed that $\beta\gamma$ activated a K^+ channel. Although this result was originally controversial, it recently has been confirmed and extended (Ito *et al.*, 1992). Perhaps the most revealing case is control of adenylyl cyclase by G_s. Studies using purified recombinant adenylyl cyclases of various isoforms have shown that purified $\beta\gamma$ stimulates types II and IV cyclase, inhibits type I cyclase, and has no effect on other types of cyclase (Tang and Gilman, 1991). $G_{s\alpha}$ in the GTPγS-bound form, i.e., dissociated from $\beta\gamma$, must be present for $\beta\gamma$ to have these effects on types I, II, and IV cyclases. Furthermore, experiments on whole cells show that $\beta\gamma$ can affect adenylyl cyclase in vivo (Federman *et al.*, 1992). In the case of the yeast *Saccharomyces cerevisiae*, $\beta\gamma$ alone apparently carries the signal to the downstream effector (Blumer and Thorner, 1991). Very recently, $\beta\gamma$ has been shown to bind to the β-adrenergic receptor kinase and target this kinase to the membrane (Pitcher *et al.*, 1992). This is, as far as we know, the first demonstration of a physical interaction between $\beta\gamma$ and a protein other than G_α.

A commonly used test to show that a heterotrimeric G protein is involved in a given process, such as membrane traffic, is to add purified $\beta\gamma$ to the system. Often this will antagonize the activation of a G protein, and this is usually interpreted as being due to the $\beta\gamma$ complexing with and inactivating the α subunit (Northup *et al.*, 1983). However, it can be very difficult to sort

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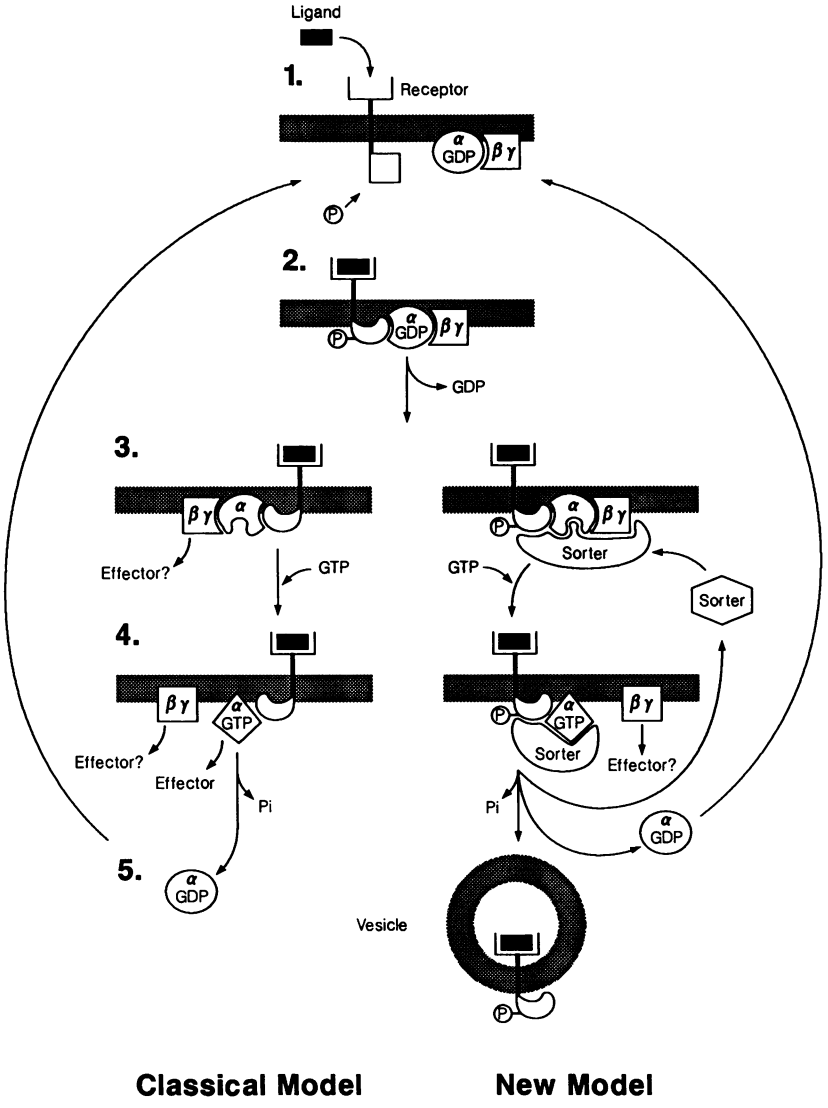

Figure 1. Comparison of classical and new models for G protein function. The first two steps are largely in common for both models. The models diverge significantly at step 3, with the classical model of signal transduction shown at the left and the new model of membrane traffic shown at the right. In step 1, ligand binds to the receptor, causing a conformational change in the receptor. In the case of the pIgR, phosphorylation of a serine on the cytoplasmic domain of the receptor can also cause a change. (Phosphorylation of other receptors has other functions, such as desensitization.) In step 2 the receptor interacts with the $G_{\alpha\beta\gamma}$. The G_α dissociates its GDP. In step 3 the receptor is associated with the G protein in the nucleotide-free state. This complex is thermodynamically stable, but ordinarily transient as the high concentration of GTP in the cytosol drives GTP binding to the G_α. In the new model we speculate that the nucleotide-free G protein may now interact with a sorter molecule. This sorter may also occlude the nucleotide-binding site, maintaining the G protein in the nucleotide-free state, at least temporarily. In step 4 GTP has bound to the G_α subunit, which therefore dissociates from the $\beta\gamma$. The G_α and/or $\beta\gamma$ subunits then interact with downstream effectors. In the classical model, these are typically adenylyl cyclase, phospholipases, ion channels, etc. In the new model, this may be a sorter protein and/or coat protein. Finally in step 5 the GTP is hydrolyzed. The complex dissociates and the G protein return to step 1. In the new model the receptor and ligand are sorted into a vesicle. It is also possible that sorting is not into a discrete carrier vesicle but into a particular part of a complex tubular structure. Although (and unlike the classical model) the new model does not provide a mechanism for the G protein to amplify a signal, the model could easily be modified to provide for amplication. For instance, one active G_α and/or $\beta\gamma$ molecule could cause the binding of multiple coat proteins to the membrane.

out the role of $\beta\gamma$ in crude systems. If addition of $\beta\gamma$ antagonizes the activation of a G protein, then this can be either due to the $\beta\gamma$ complexing with the α subunit or alternatively to a direct inhibitory effect of the $\beta\gamma$ on the downstream effector.

A NEW ROLE FOR HETEROTRIMERIC G PROTEINS AS A REGULATORY ELEMENT IN MEMBRANE TRAFFIC

Our knowledge of the classical G protein cycle can be a very useful guide to understanding how G proteins function in membrane traffic. As we review the data on this new topic, it is worthwhile to keep in mind how well these data fit with or contradict the classical G protein paradigm.

Traditionally G proteins act as signal transducers at the plasma membrane. One clue that they may have another function(s) is the finding that there are intracellular pools of heterotrimeric G proteins, both bound to internal membranes (Codina *et al.*, 1988; Ali *et al.*, 1989), and for $G_{s\alpha}$, also free in the cytoplasm (Ransnäs *et al.*, 1989; Negishi *et al.*, 1992; Bomsel and Mostov, unpublished data). One may speculate that these intracellular pools perform different functions (e.g., acting in membrane trafficking) from the G proteins at the plasma membrane.

One major approach to demonstrate that G proteins are involved in membrane traffic is to show that various agents that alter G protein function have effects on membrane traffic steps. Many toxins and other agents (listed in Table 1) have specific effects on the unidirectional cycling of G proteins. The effects of these agents on membrane traffic are summarized in Table 2.

The original indication that G proteins are involved in membrane traffic was the finding several years ago

Table 1. Probes for G protein function

Nucleotide analogues	
GTPγS	Hydrolysis resistant analogue of GTP. Long-lived activation of heterotrimeric G proteins and monomeric GTP-binding proteins.
GDPβS	Stable analogue of GDP that cannot be converted to GTP. Prevents activation of heterotrimeric G proteins and monomeric GTP-binding proteins. Commercial preparations often contaminated with other nucleotides.
AlF_4^-	Phosphate analogue that binds in pocket where γ-phosphate usually binds. Binds to and activates GDP-bound form of all heterotrimeric G proteins tested. No effect on any monomeric GTP-binding protein tested. Also acts on protein phosphatases, enzymes of phosphoinositol metabolism, ATPases, and other enzymes
Enzymes	
Pertussis toxin	ADP-ribosylates $G_{i\alpha}$ and $G_{o\alpha}$ subunits. Prevents their interaction with receptors, thereby inactivating these G proteins.
Cholera toxin	ADP-ribosylates $G_{s\alpha}$ subunits. Blocks intrinsic GTPase activity of $G_{s\alpha}$, thereby activating G_s.
Other compounds	
Mastoparan	Fourteen residue peptide from wasp venom. Very basic and probably forms amphipathic helix that mimics the part of cytoplasmic domain of receptors that couples to G_i and G_o. Accelerates nucleotide exchange and thereby activates $G_{i\alpha}$ and $G_{o\alpha}$. Much less effect on other GTP-binding proteins. Also acts on phospholipase A_2, calmodulin, nucleotidases.
Mellitin benzakonium chloride, methylbenzetonium	Amphipathic peptide (mellitin) or amines that may work by a similar mechanism to mastoparan. Not very specific.

Table 2. Involvement of heterotrimeric G proteins in membrane traffic

Golgi transport	
Transport from cis to medial Golgi	GTPγS or AlF_4^- block transport and lead to accumulation of nonclathrin-coated vesicles (Melançon *et al.*, 1987; Taylor *et al.*, 1992).
In vivo transport through Golgi	Over-expression of transfected $G_{\alpha i\text{-}3}$ (normally found in Golgi) slows intra-Golgi traffic. Pertussis toxin reverses this effect and accelerates traffic in non-transfected cells (Stow *et al.*, 1991).
Production of constitutive and regulated secretory vesicles from TGN	GTPγS or AlF_4^- block vesicle production. Pertussis toxin partially prevents GTPγS inhibition. Addition of purified βγ stimulates vesicle production (Barr *et al.*, 1991).
Endosome function and polarized sorting	
Endosome-endosome fusion	GTPγS and AlF_4^- stimulates fusion with low cytosol but inhibits fusion with high cytosol. Both effects of GTPγS are reversed by mastoparan and related compounds. Purified βγ inhibits fusion stimulated by GTPγS in low cytosol (Colombo *et al.*, 1992).
Production of transcytotic vesicles containing pIgR	Stimulated by cholera toxin, GTPγS, or AlF_4^- and by binding of ligand to pIgR or phosphorylation of pIgR. Inhibited by depletion of $G_{s\alpha}$ from cytosol and stimulated by addition of recombinant $G_{s\alpha}$ (GDP-bound form only). Addition of βγ stimulates, but only when $G_{s\alpha}$ is also present. (Bomsel and Mostov unpublished data)
Coat protein binding	
β-COP	Binding to membranes blocked by BFA. Binding promoted by GTPγS, AlF_4^-, or mastoparan, which antagonize BFA. Pertussis toxin blocks ability of mastoparan to antagonize BFA. Addition of βγ blocks binding. Binding can be divided into an ARF and GTPγS dependent membrane activation step, which is BFA-sensitive, and a β-COP binding step, which does not require free ARF or GTPγS and is not affected by BFA (Donaldson et al., 1992; Klausner et al., 1992; Ktistakis et al., 1992).
γ-Adaptin	Binding blocked by BFA. Binding promoted by GTPγS or AlF_4^-, which antagonize BFA. In MDCK and PtK_1 cells, γ-adaptin binding is sensitive to BFA, whereas β-COP binding is not. This may reflect presence of different G proteins or receptors in these cells (Robinson and Kreis, 1992).
ARF	Binding blocked by BFA. Binding promoted by GTPγS, which antagonizes BFA. Binding is not affected by AlF_4^-, which does not activate ARF, suggesting that ARF must be in GTP-bound state to bind. Addition of βγ blocks ARF binding (Donaldson et al., 1991b, 1992).

that GTPγS (a nonhydrolyzable analogue of GTP) and complexes of aluminum and fluoride (probably AlF_4^-), which bind to the GDP-bound form of some GTP-binding proteins and mimic the γ phosphate of GTP (reviewed in Chabre, 1990), inhibited various steps in the secretory (Melançon *et al.*, 1987) and endocytotic pathways (Mayorga *et al.*, 1989). It was not clear at the time if these agents were acting on monomeric GTP-binding proteins and/or heterotrimeric G proteins. Very recently it was found that AlF_4^- acts on many (perhaps all) heterotrimeric G proteins but not on any of the six different monomeric GTP-binding proteins tested (Kahn, 1991). This indicates that the AlF_4^- effects were not due to an effect on monomeric GTP-binding proteins (Barr *et al.*, 1992). However, AlF_4^- also affects many other enzymes, such as protein phosphatases, ATPases, and enzymes involved in phosphinositol metabolism (Chabre, 1990). Therefore, one cannot simply equate an effect of AlF_4^- with heterotrimeric G protein involvement.

Further, more direct evidence for the involvement of heterotrimeric G proteins in traffic has come recently from in vivo and in vitro studies of the Golgi, *trans* Golgi network (TGN), early endosomes, and regulated secretory granules.

$G_{i\alpha\text{-}3}$ is normally partially bound to the cytoplasmic surface of membranes of the Golgi apparatus (Ercolani *et al.*, 1990; Stow *et al.*, 1991). Overexpression of $G_{i\alpha\text{-}3}$ by transfection slows traffic of proteoglycans through the Golgi. Pertussis toxin (PTX), which ADP-ribosylates $G_{i\alpha}$ and $G_{o\alpha}$ and thereby prevents their activation by receptors, antagonizes the effect of overexpressing $G_{i\alpha\text{-}3}$ and even accelerates secretion in untransfected cells expressing normal levels of $G_{i\alpha\text{-}3}$. Hence, $G_{i\alpha\text{-}3}$ appears to tonically repress, rather than constitutively promote, traffic through the Golgi.

In vitro budding of both constitutive and regulated secretory vesicles from the TGN is inhibited by GTPγS or AlF_4^- (Barr *et al.*, 1991), suggesting that activation of a G protein inhibits this budding. In contrast, addition of purified βγ stimulated budding of these vesicles. This was interpreted as resulting from βγ complexing with α, thereby driving the formation of the inactive $G_{\alpha\beta\gamma}$ complex and antagonizing the action of GTPγS or AlF_4^-. However, as discussed above, βγ could alternatively act directly on a downstream effector. Treatment with PTX mitigates the inhibitory effect of GTPγS, which suggests that tonic activation of a G_i or G_o causes inhibition of vesicle budding. These results, as well as those described in the preceding paragraphs, are consistent with the classical scheme of the G protein cycle.

Fusion of endosomes in an in vitro system also seems to involve a G protein, although in this case the interpretation of the data is less clear (Colombo *et al.*, 1992). GTPγS stimulates fusion when the endosome fusion assay is performed in a low concentration of cytosol (<0.5 mg/ml) but inhibits fusion when a high concentration of cytosol is used (>1.0 mg/ml). It is not clear why the effect of this agent is biphasic, although in vivo the concentration of cytosolic proteins is far higher than even the highest amount used in these studies. AlF_4^- gave inhibition at high cytosol, but activation at low cytosol was not observed.

Mastoparan was used to provide further insight. It is a peptide toxin from wasp venom that resembles the cytoplasmic loop of G-protein coupled receptors and thereby acts as a GRF for certain G proteins (G_i and G_o, but not G_s) (Higashijima *et al.*, 1988). Mastoparan reversed both the activation and inhibition of fusion produced by GTPγS under low and high cytosol conditions, respectively. It must be pointed out that mastoparan also affects many other proteins, such as phospholipase A_2, calmodulin, and nucleotidases (Argiolas and Pisano, 1983; Barnette *et al.*, 1983; Malencik and Anderson, 1983) and therefore (like AlF_4^-) an effect of mastoparan cannot be equated with involvement of a heterotrimeric G protein. Other amphiphilic peptides and hydrophobic amines that, like mastoparan, can act as GRFs also reversed the GTPγS effect, although these may have the same sort of low specificity as mastoparan.

Addition of purified βγ also inhibited the GTPγS stimulated fusion found at a low cytosol concentration. These results do not easily fit with the conventional view of the G protein cycle and hint that something more complicated may be occurring. Mastoparan would be expected to increase nucleotide exchange and mimic the effect of GTPγS, whereas βγ should have the opposite effect. The authors of this study reconcile these data by proposing that two G proteins are involved. Activation of one G protein by mastoparan would cause the production of free βγ, which would then complex with and inactivate a second G protein.

The notion that two heterotrimeric G proteins can have antagonistic effects through coupling via a common βγ subunit was originally proposed for regulation of adenylyl cyclase, which is stimulated by G_s and inhibited by G_i (for review, see Gilman, 1987). At least in theory, networks of interacting heterotrimeric G proteins can provide for complex patterns of regulation, and it would not be surprising if multiple heterotrimeric G proteins are involved in other membrane traffic events. As above, another possible explanation for the data on the endosome fusion assay is that the free βγ acts directly on a downstream effector.

For some years GTP-binding proteins have been known to be involved in the exocytosis of regulated secretory granules (reviewed in Gomperts, 1990). At least two GTP-binding proteins are thought to be involved. A heterotrimeric G protein acts in a conventional signal transduction role to couple a plasma membrane receptor to a phospholipase that generates intracellular second messengers. A second GTP-binding protein acts downstream of the phospholipase and apparently is involved in the terminal stages of exocytosis. Recent ev-

idence indicates that this latter GTP-binding protein is affected by pertussis toxin and mastoparan and is therefore probably a G_i or G_o (Aridor and Sagi-Eisenberg, 1990).

BINDING OF COAT PROTEINS TO MEMBRANES: A TARGET FOR REGULATION BY HETEROTRIMERIC G PROTEINS

Recent data indicate that G proteins regulate the binding of components of coated vesicles, which transport membrane from one organelle to another. Two types of coated vesicles, clathrin coated and nonclathrin coated, have been characterized. Clathrin-coated vesicles consist of a membraneous vesicle enclosed in a clathrin protein basket. They travel from the plasma membrane to early endosomes and from the TGN to early and/or late endosomes. The clathrin lattice is bound to the membrane by an intermediate layer of so-called adaptor complexes. Two types of adaptor complexes, HA1/AP1 and HA2/AP2, have been described and are found in clathrin-coated vesicles derived from the TGN and plasma membrane, respectively (Pearse and Robinson, 1990).

Nonclathrin-coated vesicles are believed to be involved in transport between stacks of the Golgi and probably other steps in the secretory pathway (Duden *et al.*, 1991; Serafini *et al.*, 1991a; Waters *et al.*, 1991; Rothman and Orci, 1992). Analogous to clathrin-coated vesicles, the major constituent of the coat of these vesicles is the "coatamer," a complex of proteins that cycle between cytosolic and membrane-bound forms. The coatamer has several subunits. The best characterized subunit, β-COP, is homologous to the 100 to 116-kDa adaptin subunits of adaptor complexes, and it has been suggested that other subunits of the coatamer may be homologous to other components of clathrin-coated vesicles. The exact role of the coatamer (and other coat proteins) remains unclear—it may be to form vesicles, pinch off vesicles, or prevent formation of long tubules (Klausner *et al.*, 1992). The fungal metabolite brefeldin A (BFA) prevents the binding to membranes of at least three types of coat proteins: β-COP, γ-adaptin (found in the HA1/AP1 adaptor complex in TGN-derived clathrin-coated vesicles) (Klausner *et al.*, 1992; Robinson and Kreis, 1992; Wong and Brodsky, 1992), and a novel 200-kDa protein recently described by Narula *et al.*, (1992). In contrast, BFA has no effect on binding of the α-adaptin found in the HA2/AP2 adaptor complex from plasma membrane-derived coated vesicles. These results reinforce the idea of functional specificity for each type of adaptor.

HETEROTRIMERIC G PROTEINS AND ARF: TWO INTERACTING CLASSES OF GTP-BINDING PROTEINS THAT CONTROL COAT ASSEMBLY

The monomeric GTP-binding protein ADP-ribosylation factor (ARF) is another component of both nonclathrin-coated (Serafini *et al.*, 1991b) and clathrin-coated vesicles (Lenhard *et al.*, 1992). At least eight ARF-like genes have been cloned (Kahn *et al.*, 1992). It has been speculated that individual species of ARF may be localized to specific organelles and may be involved in targeting vesicles to the correct compartment (reviewed in Rothman and Orci, 1992).

The binding of ARF to membranes is prevented by BFA (Donaldson *et al.*, 1991a), and the binding of ARF, coatamer, and the γ-adaptin to membranes seems to be regulated by a heterotrimeric G protein(s). First, whereas BFA prevents the binding of all three of these proteins to the membrane, GTPγS promotes their binding and prevents the effect of BFA. AlF_4^- promotes binding of β-COP and γ-adaptin to Golgi membranes (Donaldson *et al.*, 1991b; Klausner *et al.*, 1992; Robinson and Kreis, 1992). However, AlF_4^- does not promote the binding of ARF to membranes. As ARF is activated by GTPγS, but not by AlF_4^-, this result suggests that ARF must be in the GTP-bound state to bind to membranes. Second, binding of β-COP and coatomer to Golgi membranes is a two-step process (Donaldson *et al.*, 1992). The first step is an activation step that requires ARF and GTPγS and is inhibited by BFA. The second step is binding of coatomer to the activated membranes; this does not require free ARF or GTPγS and is not inhibited by BFA. Third, at least for β-COP and ARF, preincubation with purified βγ prevents the action of GTPγS (Donaldson *et al.*, 1991a). This may be due to βγ binding to α to drive the formation of inactive $G_{\alpha\beta\gamma}$, or βγ may directly act on a downstream effector. Fourth, mastoparan promotes β-COP binding to Golgi membranes and antagonizes the effect of BFA (Ktistakis *et al.*, 1992). ADP-ribosylation of G_i or G_o by PTX is known to prevent activation of these G proteins by mastoparan. Pretreatment with PTX was found to largely prevent the ability of mastoparan to antagonize the effect of BFA. This further supports the hypothesis that a G_i or G_o is involved in β-COP binding. Fifth, cytosolic ARF confers sensitivity to GTPγS on in vitro intra-Golgi transport and vesicle formation (Taylor *et al.*, 1992). However, AlF_4^- inhibits this transport even in the absence of ARF, suggesting that a heterotrimeric G protein is also involved.

Taken together these data suggest the following scheme (Donaldson *et al.*, 1991a, 1992; Klausner *et al.*, 1992). Ordinarily ARF is in the GDP-bound form, which is soluble. An active G_α and/or βγ acts as a GRF for ARF and causes ARF to release GDP and bind GTP. BFA may interfere with the guanine nucleotide exchange process (see below). The GTP-bound form of ARF binds to membranes. Membrane binding may initially be rather non-specific, but the ARF may subsequently interact with a component on certain membranes, resulting in high affinity specific binding of ARF to a particular membrane. ARF in turn causes other coat proteins, such as β-COP, to assemble onto the membrane.

OTHER POSSIBLE FUNCTIONS FOR ARF

Other data raise the reciprocal possibility that ARF may also be a GRF for a G protein. The N-terminal 17 amino acids of ARF are essential for its function (Kahn *et al.*, 1992). A synthetic peptide corresponding to this sequence antagonizes the effects of ARF and blocks many membrane traffic events. This ARF peptide physically resembles mastoparan and several other compounds that act as GRFs, i.e., it is very basic and potentially forms an amphipathic helix. This peptide may therefore act like mastoparan, both on heterotrimeric G proteins and on other proteins. In the endosome fusion assay, at least, the ARF peptide gives exactly the same profile of complex effects as mastoparan (Lenhard *et al.*, 1992). One possibility, therefore, is that a normal function of ARF is to act as a GRF for certain G proteins and that its basic amphipathic N-terminal region normally interacts with G proteins. Alternatively, the effect of the ARF peptide on membrane traffic events in vitro may simply be due to the basic amphipathic properties of the peptide, which may nonspecifically activate certain G proteins. It should be kept in mind that although ARF was discovered as a cofactor for the cholera toxin-catalyzed ADP-ribosylation of $G_{s\alpha}$ (Kahn and Gilman, 1984), ARF acts on the cholera toxin molecule, not the G_s (Gill and Coburn, 1987; Bobak *et al.*, 1990). Given all of these uncertainties, it is difficult to draw unambiguous conclusions about the role of ARF and the significance of the ARF peptide.

ARF is also involved in assembly of the nuclear envelope (Boman *et al.*, 1992). However, this process is reportedly not sensitive to AlF_4^-. This result raises the possibility that ARF may have a function in membrane traffic that is independent of an AlF_4^- sensitive heterotrimeric G protein. Alternatively, the heterotrimeric G protein involved already may have been activated under the conditions of this assay.

Although ARF is clearly important in Golgi traffic in yeast, we are not aware of a report of the involvement of a heterotrimeric G protein in membrane traffic in yeast. Given the evidence for heterotrimeric G protein involvement in mammalian cells, it is likely that a heterotrimeric G protein(s) is involved in yeast. It is possible that, as in the case of signal transduction, only the $\beta\gamma$ subunit is needed for yeast membrane traffic. Traffic in yeast may involve an unidentified and possibly quite divergent member of the heterotrimeric G protein family (α and/or $\beta\gamma$).

FURTHER LESSONS FROM THE INVOLVEMENT OF G_s IN REGULATION OF POLARIZED MEMBRANE TRAFFIC

Recent work from our laboratory has suggested an additional role for G proteins and has allowed a detailed dissection of the molecular mechanism involved. Polarized epithelial cells have separate apical and basolateral surfaces, which have very different protein and lipid compositions (reviewed in Bomsel and Mostov, 1991; Mostov *et al.*, 1992). These cells must sort plasma membrane proteins to the correct destination. The only branch point for polarized sorting common to all epithelial cells is in early endosomes, to which proteins are delivered after endocytosis from one surface. In early endosomes these proteins are sorted into vesicles for recycling, degradation, or transcytosis to the opposite surface.

As a model system to study this sorting process, we have used the polymeric immunoglobulin receptor (pIgR). This receptor is normally endocytosed at the basolateral surface and sorted in the early endosome into vesicles that are transcytosed to the apical surface. In vivo, sorting of the pIgR is controlled by two independent signals—phosphorylation of Ser^{664} in the cytoplasmic domain of the pIgR and binding of the ligand dimeric IgA (dIgA) (Bomsel *et al.*, unpublished data). The stimulation of transcytosis by ligand binding suggests that pIgR is a signal transducing receptor and may be coupled to a G protein. Supporting this hypothesis, treatment of whole cells with AlF_4^- or cholera toxin, which activate G_s, increases transcytosis. Although cholera toxin is often considered to be specific for G_s, in fact it ADP-ribosylates many other proteins quite extensively, and so an effect of cholera toxin is not definitive evidence that G_s is involved (Gill and Coburn, 1987).

To analyze the potential role of G_s directly, we developed a perforated cell system to study the budding of transcytotic vesicles containing pIgR from early endosomes. To demonstrate that budding requires G_s, we took advantage of the observation that activated $G_{s\alpha}$ dissociates from the $\beta\gamma$ (which is anchored to the membrane by an isoprenyl group) and is at least partially released into the cytosol (Ransnäs *et al.*, 1989; Negishi *et al.*, 1992) of $G_{s\alpha}$. This may be a consequence of the fact that $G_{s\alpha}$ lacks N-terminal myristoylation. Our transcytotic vesicle budding reaction requires addition of crude cytosol. We found that removal of $G_{s\alpha}$ from the added cytosol inhibits budding of transcytotic vesicles, irrespective of stimulation by phosphorylation and/or ligand binding. This indicates that the G_s is required for vesicle budding and also suggests that it acts downstream from these two signals. Recombinant $G_{s\alpha}$ can replace endogenous $G_{s\alpha}$. To our knowledge this is the only membrane traffic system where the involvement of a G_α protein has been definitively demonstrated by addition of the purified G_α. However, we found that this recombinant $G_{s\alpha}$ must be added in the GDP-bound form, which presumably is able to bind $\beta\gamma$ in the membrane. $G_{s\alpha}$ added in the GTPγS bound form could not restore activity to our assay. This is a puzzling observation, as in the classic model only the GTPγS-bound form of $G_{s\alpha}$ is competent to act on the downstream ef-

fector. One possible explanation is that the GDP-bound form of $G_{s\alpha}$ has a direct role in this process. For instance, the GDP-bound form of $G_{s\alpha}$ may have a high affinity for the pIgR, as discussed below. This would be analogous to the recent proposal that GDP-bound G_α has a direct role in other systems (Bourne and Stryer, 1992).

A non-mutually exclusive explanation is that in our system the heterotrimeric $G_{\alpha\beta\gamma}$ form of the G_s is an obligate step in the reaction. This possibility prompted us to examine the involvement of $\beta\gamma$. Indeed we found that both the α and $\beta\gamma$ subunits of G_s are directly involved in transcytotic sorting. In most of the other systems described above, addition of purified $\beta\gamma$ antagonized the effect of agents that activate G proteins, such as GTPγS. As mentioned above, these results were interpreted as indicating that the added $\beta\gamma$ complexed with and inactivated G_α. In contrast, in our assay, addition of purified $\beta\gamma$ stimulates budding. This is the first indication that in a membrane trafficking process, $\beta\gamma$ does not act by simply complexing with the G_α subunit and instead acts on a downstream effector. However, $G_{s\alpha}$ must be present for $\beta\gamma$ to stimulate budding. Our result is analogous to the recent demonstration that both $G_{s\alpha}$ and $\beta\gamma$ synergistically stimulate type II adenylyl cyclase, although in that case the $G_{s\alpha}$ must be in the GTPγS-bound form (Tang and Gilman, 1991). However, in the case of the pIgR we do not know what protein is acted on by $\beta\gamma$. The $\beta\gamma$ may target a protein to the membrane, as has been suggested for β-adrenergic receptor kinase (Pitcher *et al.*, 1992).

G_s appears to be part of the machinery for sorting pIgR specifically into transcytotic vesicles, as G_s is not involved in recycling of transferrin from the same early endosomes back to the basolateral plasma membrane. G_s is thus the first identified component of the machinery involved in polarized sorting in epithelial cells. We have proposed a model for transcytotic sorting of pIgR (Bomsel *et al.*, unpublished data) (Figure 1, New Model). Ligand binding and/or Ser^{664} phosphorylation independently causes the receptor to bind to and activate G_s, which is the first step in this signal transducing pathway. We hypothesize that the pIgR interacts with a "sorter" molecule, which would be the downstream effector of the activated G_s. This sorter may be or may interact with a complex of coat proteins, perhaps including ARF. A 108-kDa protein that is enriched in transcytotic vesicles may be a component of the coat (Sztul *et al.*, 1991). Binding of this protein is apparently controlled by G_s (Sztul, personal communication). Our evidence suggests that both the $G_{s\alpha}$ and $\beta\gamma$ subunits act synergistically, presumably by binding to the sorter. The $\beta\gamma$ has been shown to interact with some receptors, such as rhodopsin (Kelleher and Johnson, 1988). It is possible that $\beta\gamma$ interacts directly with the pIgR. The complex of ligand, pIgR, sorter, and possibly one or both subunits of the G_s are then sorted into transcytotic vesicles. Eventually, hydrolysis of GTP by $G_{s\alpha}$ returns the cycle to the starting point. The net result of activation of G_s may be to increase the concentration of pIgR in transcytotic vesicles and/or to increase production of transcytotic vesicles.

Transcytosis of pIgR bound to its ligand, dIgA, is strongly inhibited by BFA (Hunziker *et al.*, 1991b). However, we found that when the pIgR is not bound to its ligand, transcytosis of pIgR is not blocked by BFA. One possible explanation is that ligand binding causes the pIgR to induce the binding of a BFA-sensitive coat protein. Without ligand, bound transcytosis may not need this coat protein, binding of the coat protein may be insensitive to BFA, or transcytosis may utilize a different pathway. Nevertheless, irrespective of ligand binding, transcytosis (or at least budding of transcytotic vesicles) cannot occur in the absence of $G_{s\alpha}$, indicating that this step is downstream. Identifying the effector of G_s will help resolve this puzzle.

LIGAND-RECEPTOR INTERACTION MAY REGULATE MEMBRANE TRAFFIC THROUGH G PROTEIN ACTIVATION: A WORKING HYPOTHESIS

We further speculate that the pIgR may be a general model for other receptors that transport various ligands to their proper intracellular destination (Figure 1, New Model). These ligands would be particular cargo molecules (soluble or membrane bound) moving through various secretory or endocytotic pathways. Some of these cargo molecules may be newly synthesized or newly endocytosed, whereas others could be recycling. At appropriate locations, such as branch points, these cargo molecules would bind to receptors that would in turn activate G proteins. Much as in the case with the pIgR and G_s, these G proteins may interact with a sorter. This sorter may be or may interact with a coat protein. The net result would be to increase (or in some cases decrease) the concentration of cargo in a vesicle and/or to induce (or in some cases inhibit) the formation of vesicles to carry the cargo to the proper destination. Traffic would thereby respond to the amount of cargo. It is also possible that G protein activation sorts cargo not into carrier vesicles but into specific portions of complex tubular or tubulovesicular structures (see below).

Although the only example of a receptor so far identified that appears to use a mechanism of G protein activation for sorting is the pIgR, a second possible example could be the IGF II/Man-6-P receptor that delivers lysosomal enzymes from the TGN to endosomes. This receptor is also coupled to $G_{i\text{-}2}$ despite the fact that it spans the membrane only once (Okamoto *et al.*, 1990). Binding of one ligand, insulin-like growth factor-II (IGF-II), stimulates $G_{i\text{-}2}$ Binding of Man-6-P, and presumably of Man-6-P containing lysosomal enzymes, modulates the effects of IGF-II and its trafficking (Rogers and

Hammerman, 1989) and may affect sorting of the receptor into vesicles targeted to endosomes (see below).

The third possible example is the low-density lipoprotein receptor. Recent provocative data indicate that ligand binding to this receptor activates phosphatidylinositol turnover. This process is sensitive to PTX and therefore probably involves a G protein (Tkachuk *et al.*, 1992; Voyno-Yasenetskaya, personal communication).

The fourth possible example is erd2. This receptor retrieves resident endoplasmic reticulum (ER) proteins from the Golgi back to the ER. The distribution of erd2 is controlled by ligand binding, suggesting that it is a signal transducing receptor (Lewis and Pelham, 1992). The erd2 protein is very similar to many G protein coupled receptors in that it spans the membrane seven times. Overexpression of a protein that is very closely related to erd2 (erd2-like protein [ELP]) confers a BFA-like effect on the cells (Hsu *et al.*, 1992). This suggests that the function of ELP is somehow related to the machinery that is altered by BFA (see below). The function of erd2 requires that it bind its ligand tightly in one compartment (probably the Golgi) and releases it in another (probably the ER). Coupling erd2 to a G protein might provide one way to accomplish this regulated change in affinity. In the case of plasma membrane receptors coupled to G proteins, the receptor's affinity for ligand is maximal when complexed to a G protein with an empty guanine nucleotide binding site, but this affinity is reduced when GTP is bound (Ross and Gilman, 1980; Gilman, 1987). In some cases GDP binding also reduces stability of the ligand-receptor-G complex, but in other cases (e.g., G_t) the GDP-bound state has high affinity for the activated receptor (Hamm, 1990). By analogy we can imagine that the complex of erd2 and G protein with an empty nucleotide binding site (or perhaps with GDP bound) binds the ligand tightly in the Golgi, but after reaching the ER, GTP binds to the G protein, causing the erd2 to release its ligand. This mechanism requires that the binding of GTP to the G protein (or perhaps release of GDP) be prevented when the G protein is in the Golgi. This might be accomplished by the sorter molecule, which could block the nucleotide binding site, but only when the complex is in the Golgi (see Figure 1, New Model, step 3).

A similar scheme may operate with the pIgR. $G_{s\alpha}$ (empty or perhaps with GDP bound) may have a high affinity for the pIgR, and this binding may be utilized to correctly sort the pIgR into transcytotic vesicles. There is some suggestion that dissociation of $G_{s\alpha}$ from the pIgR is triggered by binding or fusion of the transcytotic vesicles with the apical plasma membrane. Transcytotic vesicles apparently contain $G_{s\alpha}$ and cholera toxin treatment reduces the fusion of these vesicles with the apical plasma membrane (Bomsel *et al.*, unpublished data; Sztul, personal communication). A second GTP-binding protein may be involved in this process (Sztul, personal communication). In cholestatic (bile duct obstructed) rat liver, presumptive transcytotic vesicles containing pIgR accumulate underneath the apical plasma membrane, apparently unable to fuse with that surface (Larkin and Palade, 1991). It was recently reported that in such livers the amount of immunologically detectable $G_{s\alpha}$ is unchanged, but much of this $G_{s\alpha}$ can no longer be modified by cholera toxin (Rodriguez-Henche, N., Guijarro, L., Couvineau, A., Arilla, E., Laburthe, M., and Prieto, J., unpublished observations). We speculate that this $G_{s\alpha}$ accumulates on these arrested transcytotic vesicles in a form unable to react with cholera toxin, thereby accounting for the fusion block.

BFA MAY ACT ON A G PROTEIN-COUPLED RECEPTOR THAT REGULATES VESICLE FORMATION AND LIPID COMPOSITION

BFA also may help to unravel the mechanisms of receptor-G protein coupling (reviewed in Klausner *et al.*, 1992). Studies with BFA have led to the notion that membrane traffic events can often proceed by two alternative mechanisms: vesicular carriers and tubules that connect two compartments. Coat protein binding is thought to favor formation of vesicles. BFA inhibits binding of certain coat proteins to membranes and causes dramatic formation of tubules from the Golgi, TGN, and endosomes (Lippincott-Schwartz *et al.*, 1991; Orci *et al.*, 1991; Wood *et al.*, 1991; Reaves and Banting, 1992). In contrast, activation of G proteins by AlF_4^-, GTPγS, or mastoparan antagonizes BFA and promotes coat protein binding. Furthermore, addition of $\beta\gamma$ prevents binding of β-COP and ARF to membranes. Traffic in the absence of drugs is probably a balance between vesicular and tubular events, and this balance is apparently regulated by a G protein(s). The site of action of BFA is intimately related to this regulation. BFA is active at micromolar concentrations, and several stereoisomers are inactive. This and other data suggest that BFA acts by mimicking an endogenous ligand that normally binds to a receptor that controls intracellular membrane traffic. As a derivative of palmitic acid, BFA may mimic an endogenous fatty acid or derivative such as palmityl-CoA or an eicosanoid. We suggest that this endogenous lipid binds to a G protein-coupled receptor. It is worth noting that some receptors for eicosanoids are seven-membrane spanning G protein-coupled receptors (Sugimura *et al.*, 1992). It also may be relevant that ARF is activated by arachidonic acid (Bobak *et al.*, 1990). This hypothetical receptor may monitor the lipid composition of membranes or rate of lipid synthesis and thereby adjust membrane traffic to maintain correct lipid compositions in various compartments. Properly balanced lipid composition in the Golgi, for instance, has been shown to play a key role in traffic through this organelle (Cleves *et al.*, 1991). In yeast, the SEC 14 gene product binds phosphatidylinositol (a major lipid in yeast) and phosphatidylcholine and has been suggested to regulate phosphatidylcholine biosynthesis (Bankaitis,

personal communication). Perhaps SEC 14 is somehow analogous to the hypothesized BFA receptor. BFA may be a potent agonist or antagonist of the lipid-monitoring receptor. Consequently, addition of BFA would unbalance membrane traffic by overstimulating or inhibiting a normal control process.

The formation of numerous tubules in the presence of BFA has additionally led to the suggestion that some membranes may have the tendency to "spontaneously" form tubules and that the role of coat proteins is to regulate this (Klausner *et al.*, 1992). The distortion of planar membranes leading to tubule and/or vesicle formation may require (and might even be driven by) changing the ratio and/or composition of lipids in the two halves of the lipid bilayer. We might imagine that to shape the growing tubule, extra lipids are increasingly added to the cytoplasmic half of the bilayer at the site of budding. This process is likely to be performed by phospholipid translocators that flip lipids across the bilayer. Phospholipases (which are often effectors for heterotrimeric G proteins) may play a complementary role by removing lipid from the other half of the bilayer or otherwise perturbing the bilayer. ARF binding also may cause budding and/or tubulation. ARF is homologous to phospholipases and can bind to lipid bilayers (Bobak *et al.*, 1990). Perhaps in binding it reshapes the bilayer (e.g., by changing lipid packing density in the cytoplasmic half of the bilayer), thereby providing the membrane curvature needed to form buds and/or tubules. We speculate that this bilayer remodeling machinery is regulated by a heterotrimeric G protein(s). This G protein is coupled to a receptor, which is the target of BFA.

G PROTEINS AND CELLULAR REGULATION: CROSS-TALK BETWEEN SIGNAL TRANSDUCTION AND MEMBRANE TRAFFIC

The notion that G protein activation stimulates budding would seem to contradict the data that activation of $G_{i\text{-}3}$ inhibits traffic through the Golgi (Stow *et al.*, 1991) and that activation of a G_i inhibits budding from the TGN (Barr *et al.*, 1991). However, much as adenylyl cyclase can be stimulated or inhibited by different G proteins, traffic though the Golgi and other compartments might be subject to both positive and negative control by antagonistic G proteins. Slowing traffic through a compartment such as the Golgi might be important, for instance, to control the exposure of a cargo molecule (e.g., proteoglycans) to resident oligosaccharide processing enzymes. Similarly, regulating passage through the endocytotic pathway would control exposure to low pH and proteases. Furthermore, slowing or accelerating traffic through one pathway might lead to increased or decreased diversion of cargo to an alternative pathway. For instance, activation of $G_{i\text{-}3}$ in the Golgi might increase retrograde traffic from the Golgi to the ER (the presumed erd2 pathway) and might thereby appear to slow anterograde export from the Golgi to the cell surface.

More generally, G proteins provide a versatile way for membrane traffic to be regulated. Each step in membrane traffic must be tightly controlled and coordinated with other steps. For instance, to maintain constant organelle size and composition, traffic to the Golgi must equal traffic from the Golgi and similarly traffic to the cell surface should balance traffic from the surface. Moreover, the level of membrane traffic also must be regulated to meet the changing physiologic needs of the cell. For instance, a growing cell would require much more membrane synthesis than a quiescent cell. Membrane traffic also varies in response to various hormones and growth factors that bind to G protein-coupled receptors. We suggest that the machinery for responding to extracellular signals and the machinery for controlling membrane traffic may be much more closely connected than has been appreciated previously.

We have, perhaps artificially, divided the functions of G proteins into two areas: signal transduction and regulation of membrane traffic. This division reflects how these functions were discovered. However, there are numerous possibilities for cross-talk between these two classes, and in some cases a G protein may simultaneously play both roles. Both the α and $\beta\gamma$ subunits may be involved in these processes. For instance, activation of a G protein at the cell surface by a hormone may result in the production of free $\beta\gamma$, which could then act on a membrane trafficking process. Also, once activated, a receptor and/or G protein may be internalized and act in the endosome to affect intracellular membrane traffic. One G protein-coupled receptor with a well-known dual function in growth regulation and membrane traffic is the IGF-II/Man-6-P receptor, and it provides an obvious point for integrating the two processes. This example offers particularly complex possibilities for control, as binding of one ligand IGF-II leads to stimulation of G_{i2} (Okamoto *et al.*, 1990, 1991), whereas binding of the other ligand, Man-6-P (or enzymes containing Man-6-P) can modulate the effects of IGF-II (Rogers and Hammerman, 1989). Furthermore, the postulated receptor that binds BFA also may be involved in growth control, as cells that are less sensitive to BFA (MDCK, PtK_1) tend to be nontransformed cells (Hunziker *et al.*, 1991a; Ktistakis *et al.*, 1991).

Clearly, much remains to be learned about the roles of G proteins. Many G proteins have been cloned recently and for some little is known of their function (Simon *et al.*, 1991). Some may play specific roles in particular membrane traffic events. For instance, G_o is quite abundant in neurons (Kaziro *et al.*, 1991). In preliminary data, $G_{o\alpha}$ can substitute for $G_{s\alpha}$ in our in vitro assay of budding of transcytotic vesicles containing pIgR (Bomsel *et al.*, unpublished data). Although G_o is probably not found in epithelia transporting pIgR, our data

suggest that G_o may be involved in a membrane traffic pathway in neurons that is analogous to transcytosis, such as post-endocytotic sorting of membrane proteins into synaptic vesicles or polarized axonal transport.

CONCLUDING SPECULATION: ORIGIN OF G PROTEINS

Finally, we hypothesize that G proteins originally evolved to control membrane trafficking and that their role in transducing extracellular signals evolved later. Prokaryotes, which apparently lack G proteins, use other mechanisms to respond to extracellular signals. In contrast, a hallmark of eukaryotes is compartmentalization by intracellular membranes and G proteins may have originated to control traffic between the various membranous organelles. Using G proteins to transduce extracellular signals might be a natural extension of a system that responds to intracellular signals borne by cargo molecules. Some of the cargo molecules that act on internal G protein-coupled receptors may reach the surface or be secreted and thereby have access to the corresponding receptors on other cells. These cargo molecules would therefore be primitive extracellular signaling molecules or hormones. A fundamental action of such signaling molecules would be to alter membrane traffic patterns in their target cells, which could lead to global changes in cellular metabolism.

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REFERENCES

Ali, N., Milligan, G., and Evans, W.H. (1989). Distribution of G-proteins in rat liver plasma-membrane domains and endocytic pathways. Biochem. J. *261*, 905–912.

Argiolas, A., and Pisano, J.J. (1983). Facilitation of phospholipase A_2 activity by mastoparans, a new class of mast cell degranulating peptides from wasp venom. J. Biol. Chem. *258*, 13697–13702.

Aridor, M., and Sagi-Eisenberg, R. (1990). Neomycin is a potent secretagogue of mast cells that directly activates a GTP-binding protein involved in exocytosis. J. Cell Biol. *111*, 2885–2891.

Balch, W.E. (1990). Small GTP-binding proteins in vesicular transport. Trends Biochem. Sci. *15*, 473–477.

Balch, W.E. (1992). From G minor to G major. Current Biol. *2*, 157–160.

Barnette, M.S., Daly, R., and Weiss, B. (1983). Inhibition of calmodulin activity by insect venom peptides. Biochem. Pharmacol. *32*, 2929–2933.

Barr, F.A., Leyte, A., and Huttner, W.B. (1992). Trimeric G proteins and vesicle formation. Trends Cell Biol. *2*, 91–94.

Barr, F.A., Leyte, A., Mollner, S., Pfeuffer, T., Tooze, S.A., and Huttner, W.B. (1991). Trimeric G-proteins of the trans-Golgi network are involved in the formation of constitutive secretory vesicles and immature secretory granules. FEBS Lett. *293*, 239–243.

Blumer, K.J., and Thorner, J. (1991). Receptor-G protein signalling in yeast. Annu. Rev. Physiol. *53*, 37–57.

Bobak, D.A., Bliziotes, M.M., Noda, M., Tsai, S.-C., Adamik, R., and Moss, J. (1990). Mechanism of activation of cholera toxin by ADP-robosylation factor (ARF): both low- and high-affinity interactions of ARF with guanine nucleotides promote toxin activation. Biochemistry *29*, 855–861.

Boman, A.L., Taylor, T.C., Melançon, P., and Wilson, K.L. (1992). A role for ADP-ribosylation factor in nuclear vesicle dynamics. Nature *358*, 512–514.

Bomsel, M., and Mostov, K.E. (1991). Sorting of plasma membrane proteins in epithelial cells. Curr. Opin. Cell Biol. *3*, 647–653.

Bourne, H.R. (1988). Do GTPases direct membrane traffic in secretion? Cell *53*, 669–671.

Bourne, H.R., Sanders, D.A., and McCormick, F. (1990). The GTPase superfamily: a conserved switch for diverse cell functions. Nature *348*, 125–132.

Bourne, H.R., Sanders, D.A., and McCormick, F. (1991). The GTPase superfamily: conserved structure and molecular mechanism. Nature *349*, 117–127.

Bourne, H.R., and Stryer, L. (1992). The target sets the tempo. Nature *358*, 541–543.

Chabre, M. (1990). Aluminofluoride and beryllofluoride complexes: new phosphate analogs in enzymology. Trends Biochem. Sci. *15*, 6–10.

Cleves, A.E., McGee, T.P., Whitters, E.A., Champion, K.M., Aitken, J.R., Dowhan, W., Goebl, M., and Bankaitis, V.A. (1991). Mutations in the CDP-choline pathway for phospholipid biosynthesis bypass the requirement for an essential phospholipid transfer protein. Cell *64*, 798–800.

Codina, J., Kimura, S., Kraus-Friedmann, N., and Higham, S. (1988). Demonstration of the presence of G-proteins in hepatic microsomal fraction. Biochem. Biophys. Res. Commun. *150*, 848–852.

Colombo, M.I., Mayorga, L.S., Casey, P.J., and Stahl, P.D. (1992). Evidence of a role for heterotrimeric GTP-binding proteins in endosome fusion. Science *255*, 1695–1697.

Donaldson, J.G., Cassel, D., Kahn, R.A., and Klausner, R.D. (1992). ADP-ribosylation factor, a small GTP-binding protein, is required for binding of the coatomer protein β-COP to Golgi membranes. Proc. Natl. Acad. Sci. USA *89*, 6408–6412.

Donaldson, J.G., Kahn, R.A., Lippincott-Schwartz, J., and Klausner, R.D. (1991a). Binding of ARF and β-COP to Golgi membranes: possible regulation by a trimeric G protein. Science *254*, 1197–1199.

Donaldson, J.G., Lippincott-Schwartz, J., and Klausner, R.D. (1991b). Guanine nucleotides modulate the effects of brefeldin A in semipermeable cells: regulation of the association of a 110-kD peripheral membrane protein with the Golgi apparatus. J. Cell Biol. *112*, 579–588.

Duden, R., Allan, V., and Kreis, T. (1991). Involvement of β-COP in membrane traffic through the Golgi complex. Trends Cell Biol. *1*, 14–19.

Ercolani, L., Stow, J.L., Boyle, J.F., Holtzman, E.J., Lin, H., Grove, R., and Ausiello, D.A. (1990). Membrane localization of the pertussis toxin-sensitive G-protein subunits $\alpha_{i\text{-}2}$ and $\alpha_{i\text{-}3}$ and expression of me-

tallothionein-α_{i-2} fusion gene in LLC-PK$_1$ cells. Proc. Natl. Acad. Sci. USA *87*, 4635–4639.

Federman, A.D., Conklin, B.R., Schrader, K.A., Reed, R.R., and Bourne, H.R. (1992). Hormonal stimulation of adenylyl cyclase through G_i-protein $\beta\gamma$ subunits. Nature *356*, 159–161.

Gill, D.M., and Coburn, J. (1987). ADP-ribosylation by cholera toxin: functional analysis of a cellular system that stimulates the enzymic activity of cholera toxin fragment A_1. Biochemistry *26*, 6364–6371.

Gilman, A.G. (1987). G proteins: transducers of receptor-generated signals. Annu. Rev. Biochem. *56*, 615–619.

Gomperts, B.D. (1990). G_E: A GTP-binding protein mediating exocytosis. Annu. Rev. Physiol. *52*, 591–606.

Hamm, H.E. (1990). Surfaces of interaction between G_t and rhodopsin in the GDP-bound and empty-pocket configurations. In: The Biology and Medicine of Signal Transduction, ed. Y. Nishizuka, New York: Raven Press, 76–81.

Higashijma, T., Burnier, J., and Ross, E. (1990). Regulation of G_1 and G_0 by mastoporan, related amphiphilic peptides, and hydrophobic amines. J. Biol. Chem. *265*, 14176–14186.

Hsu, V.W., Shah, N., and Klausner, R.D. (1992). A brefeldin A-like phenotype is induced by the overexpression of a human ERD-2-like protein, ELP-1. Cell *69*, 625–635.

Hunziker, W., Harter, C., Matter, K., and Mellman, I. (1991a). Basolateral sorting in MDCK cells requires a distinct cytoplasmic domain determinant. Cell *66*, 907–920.

Hunziker, W., Whitney, J.A., and Mellman, I. (1991b). Selective inhibition of transcytosis by Brefeldin A in MDCK cells. Cell *57*, 1–20.

Ito, H., Tung, R.T., Sugimoto, T., Kobayashi, I., Takahashi, K., Katada, T., Ui, M., and Kurachi, Y. (1992). On the mechanism of G protein $\beta\gamma$ subunit activation of the muscarinic K^+ channel in guinea pig atrial cell membrane. J. Gen. Physiol. *99*, 961–983.

Jelsema, C.J., and Axelrod, J. (1987). Stimulation of phospholipase A2 activity in bovine rod outer segments by the $\beta\gamma$ subunits of transduction and its inhibition by the alpha subunit. Proc. Natl. Acad. Sci. USA *84*, 3623–3627.

Kahn, R.A. (1991). Fluoride is not an activator of the smaller (20-25kDa) GTP-binding proteins. J. Biol. Chem. *266*, 15595–15597.

Kahn, R.A., and Gilman, A.G. (1984). Purification of a protein cofactor required for ADP-ribosylation of the stimulatory regulatory component of adenylate cyclase by cholera toxin. J. Biol. Chem. *259*, 6228–6234.

Kahn, R.A., Randazzo, P., Serafini, T., Weiss, O., Rulka, C., Clark, J., Amherdt, M., Roller, P., Orci, L., and Rothman, J.E. (1992). The amino terminus of ADP ribosylation factor (ARF) is a critical determinant of ARF activities and is a potent and specific inhibitor of protein transport. J. Biol. Chem. *267*, 13039–13046.

Kaziro, Y., Itoh, H., Kozasa, T., Nakafuku, M., and Satoh, T. (1991). Structure and function of signal-transducing GTP-binding proteins. Annu. Rev. Biochem. *60*, 349–400.

Kelleher, D.J., and Johnson, G.L. (1988). Transducin inhibition of light-dependent rhodopsin phosphorylation: evidence for $\beta\gamma$ subunit interaction with rhodopsin. Mol. Pharmacol. *34*, 452–460.

Klausner, R.D., Donaldson, J.G., and Lippincott-Schwartz, J. (1992). Brefeldin A: insights into the control of membrane traffic and organelle structure. J. Cell Biol. *116*, 1071–1080.

Ktistakis, N.T., Linder, M.E., and Roth, M.G. (1992). Action of brefeldin A blocked by activation of a pertussis-toxin-sensitive G protein. Nature *356*, 344–346.

Ktistakis, N.T., Roth, M.G., and Bloom, G.S. (1991). PtK1 cells contain a nondiffusible, dominant factor that makes the Golgi apparatus resistant to Brefeldin A. J. Cell Biol. *113*, 1009–1023.

Larkin, J.M., and Palade, G.E. (1991). Transcytotic vesicular carriers for polymeric IgA receptors accumulate in rat hepatocytes after bile duct ligation. J. Cell Sci. *98*, 205–216.

Lefkowitz, R.J. (1992). The subunit story thickens. Nature *358*, 372.

Lenhard, J.M., Kahn, R.A., and Stahl, P.D. (1992). Evidence for ADP-ribosylation factor (ARF) as a regulator of in vitro endosome-endosome fusion. J. Biol. Chem. *267*, 13047–13052.

Lewis, M.J., and Pelham, R.B. (1992). Ligand-induced redistribution of human KDEL receptor from the Golgi complex to the endoplasmic reticulum. Cell *68*, 353–364.

Lippincott-Schwartz, J., Yuan, L.C., Tipper, C., Amherdt, M., Orci, L., and Klausner, R.R. (1991). Brefeldin A's effects on endosomes, lysosomes, and the TGN suggest a general mechanism for regulating organelle structure and membrane traffic. Cell *67*, 601–606.

Logothetis, D.E., Kurachi, Y., Galper, J., Neer, E.J., and Clapham, D.E. (1987). The $\beta\gamma$ subunits of GTP-binding proteins activate the muscarinic K+ channel in heart. Nature *325*, 321–326.

Malencik, D.A., and Anderson, S.R. (1983). High affinity binding of the mastoparans by calmodulin. Biochem. Biophys. Res. Commun. *114*, 50–56.

Mayorga, L.S., Diaz, R., and Stahl, P.D. (1989). Regulatory role for GTP-binding proteins in endocytosis. Science *244*, 1475–1478.

Melançon, P., Glick, B.S., Malhotra, V., Weidman, P.J., Serafini, T., Gleason, M.L., Orci, L., and Rothman, J.E. (1987). Involvement of GTP-binding "G" proteins in transport through the Golgi stack. Cell *51*, 1053–1062.

Mostov, K., Apodaca, G., Aroeti, B., and Okamoto, C. (1992). Plasma membrane protein sorting in polarized epithelial cells. J. Cell Biol. *116*, 577–583.

Narula, N., McMorrow, I., Plopper, G., Doherty, J., Matlin, K.S., Burke, B., and Stow, J.L. (1992). Identification of a 200-kD, Brefeldin-sensitive protein on golgi membranes. J. Cell Biol. *117*, 27–38.

Negishi, M., Hashimoto, H., and Ichikawa, A. (1992). Translocation of α subunits of stimulatory guanine nucleotide-binding proteins through stimulation of the prostacyclin receptor in mouse mastocytoma cells. J. Biol. Chem. *267*, 2364–2369.

Northup, J.K., Sternweis, P.C., and Gilman, A.G. (1983). The subunits of the stimulatory regulatory component of adenylate cyclase. J. Biol. Chem. *258*, 11361–11368.

Okamoto, T., Murayama, Y., Hayashi, Y., Inagaki, M., Ogata, E., and Nishimoto, I. (1991). Identification of the G_s activator region of the β2-adrenergic receptor that is autoregulated via protein kinase A-dependent phosphorylation. Cell *67*, 723–730.

Okamoto, T., Nishimoto, I., Murayama, Y., Ohkuni, Y., and Ogata, E. (1990). Insulin-like growth factor-II/mannose 6-phosphate receptor is incapable of activating GTP-binding proteins in response to mannose 6-phosphate, but capable in response to insulin-like growth factor-II. Biochem. Biophys. Res. Commun. *168*, 1201–1210.

Orci, L., Tagaya, M., Amherdt, M., Perrelet, A., Donaldson, J.G., Lippincott-Schwartz, J., Klausner, R.D., and Rothman, J.E. (1991). Brefeldin A, a drug that blocks secretion, prevents the assembly of non-clathrin-coated buds on Golgi cisternae. Cell *64*, 1183–1195.

Pearse, B., and Robinson, M. (1990). Clathrin, adaptors and sorting. Ann. Rev. Cell Biol. *6*, 151–171.

Pfeffer, S.R. (1992). GTP-binding proteins in intracellular transport. Trends Cell Bio. *2*, 41–46.

Pitcher, J.A., Inglese, J., Higgins, J.B., Arriza, J.L., Casey, P.J., Kim, C., Benovic, J.L., Kwatra, M.M., Caron, M.G., and Lefkowitz, R.J. (1992). Role of $\beta\gamma$ subunits of G proteins in targeting the β-adrenergic receptor kinase to membrane-bound receptors. Science *257*, 1264–1267.

Ransnäs, L.A., Svoboda, P., Jasper, J.R., and Insel, P.A. (1989). Stimulation of β-adrenergic receptors of S49 lumphoma cells redistributes the α subunit of the stimulatory G protein between cytosol and membranes. Proc. Natl. Acad. Sci. USA *86*, 7900–7903.

Reaves, B., and Banting, G. (1992). Perturbation of the morphology of the trans-Golgi network following brefeldin A treatment: redistribution of a TGN-specific integral membrane protein, TGN38. J. Cell Biol. *116*, 85–94.

Robinson, M.S., and Kreis, T.E. (1992). Recruitment of coat proteins onto Golgi membranes in intact and permeabilized cells: effects of brefeldin A and G protein activators. Cell *69*, 129–138.

Rogers, S.A., and Hammerman, M.R. (1989). Mannose 6-phosphate potentiates insulin-like growth factor II-stimulated inositol triphosphate production in proximal tubular basolateral membranes. J. Biol. Chem. *264*, 4273–4276.

Ross, E.M., and Gilman, A.G. (1980). Biochemical properties of hormone-sensitive adenylate cyclase. Annu. Rev. Biochem. *49*, 533–564.

Rothman, J.E., and Orci, L. (1992). Molecular dissection of the secretory pathway. Nature *355*, 409–415.

Serafini, T., Orci, L., Amherdt, M., Brunner, M., Kahn, R.A., and Rothman, J.E. (1991a). ADP-ribosylation factor is a subunit of the coat of Golgi-derived COP-coated vesicles: a novel role for a GTP-binding protein. Cell *67*, 239–253.

Serafini, T., Stenbeck, G., Brecht, A., Lottspeich, F., Orci, L., Rothman, J., and Wieland, F. (1991b). A coat subunit of Golgi-derived non-clathrin-coated vesicles with homology to the clathrin-coated vesicle coat protein beta-adaptin. Nature *349*, 215–220.

Simon, M.I., Strathmann, M.P., and Gautam, N. (1991). Diversity of G proteins in signal transduction. Science *252*, 802–808.

Stow, J.L., de Almeida, J.B., Narula, N., Holtzman, E.J., Ercolani, L., and Ausiello, D.A. (1991). A heterotrimeric G protein, $G\alpha_{i\text{-}3}$, on Golgi membranes regulates the secretion of a heparan sulfate proteoglycan in LLC-PK_1 epithelial cells. J. Cell Biol. *114*, 1113–1124.

Sugimura, Y., Namba, T., Honda, A., Hayashi, Y., Negishi, M., Ichikawa, A., and Narumiya, S. (1992). Cloning and expression of a cDNA for mouse prostaglandin E receptor EP_3 subtype. J. Biol. Chem. *267*, 6463–6466.

Sztul, E., Kaplin, A., Saucan, L., and Palade, G. (1991). Protein traffic between distinct plasma membrane domains: isolation and characterization of vesicular carriers involved in transcytosis. Cell *64*, 81–89.

Tang, W.-J., and Gilman, A.G. (1991). Type-specific regulation of adenylyl cyclase by G protein βγ subunits. Science *254*, 1500–1503.

Taylor, T.C., Kahn, R.A., and Melançon, P. (1992). Two distinct members of the ADP-ribosylation factor family of GTP-binding proteins regulate cell-free intra-Golgi transport. Cell *70*, 69–79.

Tkachuk, V.A., Bochkov, V.N., Voyno-Yasenetskaya, T.A., Avdonin, P.V., and Bobik, A. (1992). Low density lipoprotein activated calcium channels in human platelets: characterization and regulation. Euro. J. Biochem. (*in press*).

Waters, M.G., Serafini, T., and Rothman, J.E. (1991). "Coatomer": a cytosolic protein complex containing subunits of non-clathrin-coated Golgi transport vesicles. Nature *449*, 248–251.

Wong, D.H., and Brodsky, F.M. (1992). 100 kD proteins of Golgi- and trans-Golgi network associated coated vesicles have related but distinct membrane binding properties. J. Cell Biol. *117*, 1171–1179.

Wood, S.A., Park, J.E., and Brown, W.J. (1991). Brefeldin A causes a microtubule-mediated fusion of the trans-Golgi network and early endosomes. Cell *67*, 591–600.