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GTP-Binding Proteins and Formation of Secretory Vesicles

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SIGNAL TRANSDUCTION DURING
BIOMEMBRANE FUSION

I. INTRODUCTION: SORTING IN AND VESICLE FORMATION FROM THE TRANS-GOLGI NETWORK

Protein secretion is a common property of most animal cells. Two pathways of protein secretion are known. In the ubiquitous constitutive secretory pathway, proteins are continuously released into the environment without intracellular storage and in the absence of external stimuli. The regulated secretory pathway, on the other hand, functions only in certain specialized cell types such as exocrine cells, endocrine cells, and neurons. It involves the selective sorting of a subset of secretory proteins into highly specialized storage organelles, the secretory granules, which fuse with the plasma membrane to release their content only in response to an external signal (for reviews, see Burgess and Kelly, 1987; Huttner and Tooze, 1989; Miller and Moore, 1990).

Until constitutive and regulated secretory proteins reach the trans-Golgi network (TGN), their intracellular transport route appears to be the same (reviewed in Griffiths and Simons, 1986). In the TGN, the two pathways of secretion diverge, and two distinct populations of secretory vesicles are formed: constitutive secretory vesicles (CSVs) and immature secretory granules (ISGs) (Orci *et al.*, 1987; Tooze *et al.*, 1987; Tooze and Huttner, 1990). This implies the existence of a mechanism by which regulated secretory proteins destined to ISGs are segregated from constitutive secretory proteins destined to CSVs. Various morphological and biochemical lines of evidence suggest that the selective aggregation of regulated secretory proteins in the TGN is a key step in the sorting process (Burgess and Kelly, 1987; Gerdes *et al.*, 1989; Tooze *et al.*, 1989; Chanat and Huttner, 1991). The dense-cored aggregates formed by regulated secretory proteins exclude constitutive secretory proteins.

Besides the sorting of the secretory protein cargo, the formation of CSVs and ISGs from the TGN involves a series of events that occur whenever a vesicle forms from a donor compartment. These include (1) the segregation of cargo proteins from resident proteins of the donor compartment; (2) the assembly of the membrane components characteristic of a given vesicle (certain lipids, membrane proteins which, e.g., determine the intracellular traffic of the vesicle); (3) the formation of a membrane "bud"; (4) the assembly of a coat on the cytoplasmic surface of the bud; and (5) the pinching off of the vesicle from the donor compartment (scission).

This chapter discusses recent data on post-TGN vesicle biogenesis obtained with a strategy based on the biochemical identification of CSVs and ISGs and the use of a cell-free system to study their formation (Tooze and Huttner, 1990). We focus, in particular, on the role of GTP-binding proteins in this

process, and speculate on the number and nature of events during ISG and CSV biogenesis in which these proteins participate as regulatory components.

A. Biochemical Characterization of Post-trans-Golgi Network Secretory Vesicles

In the TGN, a subset of secretory proteins is post-translationally modified by sulfation on tyrosines (Baeuerle and Huttner, 1987) and on carbohydrate chains (Kimura *et al.*, 1984). The specificity of these two types of sulfation as TGN modifications has been exploited to selectively label marker molecules for both the constitutive and regulated pathway of secretion as they pass through the TGN, and to detect their exit from the TGN (Tooze and Huttner, 1990). Cells of the neuroendocrine line PC12 contain two major tyrosine-sulfated proteins, chromogranin B (secretogranin I) and secretogranin II (SgII) that have been shown to be efficiently targeted to secretory granules (Lee and Huttner, 1983; Rosa *et al.*, 1985; Rosa *et al.*, 1989; Gerdes *et al.*, 1989). These characteristics make chromogranin B and SgII ideal markers for the regulated secretory pathway. PC12 cells also synthesize one major heparan sulfate proteoglycan (hsPg) (Schubert *et al.*, 1988; Gowda *et al.*, 1989), that can be used as a marker for the constitutive pathway of secretion: it is excluded from secretory granules and its secretion is blocked at 20°C (Tooze and Huttner, 1990). The latter property has been shown to be a hallmark for proteins traveling by the constitutive pathway (Matlin and Simons, 1983). Furthermore, sulfate labeling of secretogranins and the hsPg can be used to selectively monitor their post-TGN transport via the regulated and constitutive pathway, respectively. For this purpose, however, it is essential to separate ISGs and CSVs from the TGN and from each other, for instance, by physical separation of these cellular compartments. An outline for a method to achieve this follows.

To biochemically identify ISGs and CSVs, and to separate them from their donor compartment, the TGN, the following method has been designed (Tooze and Huttner, 1990; Tooze and Huttner, 1992). PC12 cells are pulse-labeled for 5 min with [^{35}S]sulfate, with or without subsequent chase, and then homogenized. Given the time needed for sulfate uptake and activation, plus translocation of activated sulfate (approximately 2 min; Baeuerle and Huttner, 1987), this allows for an effective labeling time of about 3 min. With the use of velocity-controlled sucrose gradient centrifugation, organelles in the postnuclear supernatant (PNS) prepared from the [^{35}S]sulfate-labeled cells are then fractionated to separate the TGN from post-TGN secretory vesicles,

and the distribution of the sulfate-labeled marker proteins across the gradient is analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) in conjunction with fluorography.

When no chase has been performed, this procedure yields a single peak of labeled proteins, including SgII and hsPg, in the bottom half of the gradient, coinciding with the peak of the TGN marker enzyme, sialyl transferase (Tooze and Huttner, 1990). [In general, chromogranin B is not analyzed as a marker for the regulated pathway because its migration on SDS-PAGE partly overlaps with that of the constitutively secreted hsPg (Tooze and Huttner, 1990).] This shows that after a 5-min [^{35}S]sulfate pulse, the marker proteins are still present in the TGN. After a 15-min chase, however, most of the labeled SgII and hsPg is found in the top half of the gradient. Apparently, the labeled proteins leave the TGN during this period and are then present in vesicles which, due to their smaller size, sediment more slowly than the TGN. The formation of post-TGN vesicles occurs rapidly, with a half-time of approximately 5 min (Tooze and Huttner, 1990).

B. Separation of Two Classes of Post-trans-Golgi Network Secretory Vesicles

On exit from the TGN, sulfate-labeled SgII and hsPg are packaged directly in two distinct classes of post-TGN vesicles, the ISGs and CSVs (Tooze and Huttner, 1990). These two populations can be separated from one another on the basis of their different buoyant densities in sucrose. For this purpose, vesicle-containing fractions from the velocity gradient just described are subjected to a second, equilibrium sucrose gradient centrifugation. Because ISGs (characterized by dense cores) have a significantly greater density than CSVs, this procedure yields two distinct peaks of labeled proteins: one higher in the gradient, containing the sulfate-labeled hsPg in CSVs, and one lower in the gradient, containing the sulfate-labeled SgII in ISGs (Tooze and Huttner, 1990).

C. Cell-Free Formation of Post-trans-Golgi Network Secretory Vesicles

To allow detailed investigations of the molecular mechanisms underlying post-TGN vesicle formation, a cell-free system has been developed (Tooze and Huttner, 1990). This system is based on the ability to monitor bio-

chemically the transport and sorting of SgII and hsPg from the TGN to ISGs and CSVs, respectively. Protein sorting to the regulated and constitutive secretory pathways is reconstituted *in vitro* when a PNS, derived from PC12 cells pulse-labeled with [^{35}S]sulfate, is prepared in an iso-osmotic, low-ionic strength, sucrose-containing buffer at neutral pH and then supplemented with ATP and an ATP-regenerating system (Tooze and Huttner, 1990). The two types of post-TGN vesicles formed in the cell-free system were found to have similar properties to CSVs and ISGs formed *in vivo*. Unlike most existing cell-free transport assays (for review, see Goda and Pfeffer, 1989), this system measures only the formation of vesicles and not the transfer of a defined marker protein from a donor to an acceptor compartment, which is a multistep process involving both vesicle formation and vesicle fusion.

II. CURRENT RESEARCH: GTP-BINDING PROTEINS AND VESICLE FORMATION

Proteins that bind GTP fulfill a wide range of regulatory functions in all cell types (Bourne *et al.*, 1990). A feature that many of these proteins share is the ability to undergo a cycle of GTP binding and hydrolysis, during which they switch from an inactive (GDP-bound) to an active (GTP-bound) conformational state (Wittinghofer and Pai, 1991). In the case of the small ras-like GTP-binding proteins of the mammalian rab family, which have been implicated in vesicle fusion, it has been postulated that they form part of a proofreading machinery that ensures the correct targeting of vesicles to the appropriate acceptor membrane (for reviews, see Bourne, 1988; Balch, 1990; Goud and McCaffrey, 1991; Pfeffer, 1992). As will be discussed in detail, recent work indicates that GTP-binding proteins are also involved in vesicle formation, and that, in fact, several distinct classes of such proteins participate in this process.

A. Nonhydrolyzable Analogs of GTP Inhibit Cell-Free Vesicle Formation

An important finding obtained with the cell-free system derived from PC12 cells described in the previous section was that the formation of secretory vesicles from the TGN was inhibited by nonhydrolyzable analogs of GTP (Tooze *et al.*, 1990), implying that GTP hydrolysis is required for this pro-

cess. It was shown that the formation of both ISGs and CSVs is inhibited by GTPγS. Another nonhydrolyzable analog of GTP, GMP-PMP, also inhibited the formation of post-Golgi vesicles, albeit much less effectively than GTPγS on a molar basis. This is in agreement with previous findings on the relative effects of GMP-PNP and GTPγS on cell-free intra-Golgi vesicle traffic (Melançon *et al.*, 1987). The inhibition of post-TGN vesicle formation by nonhydrolyzable GTP analogs was largely prevented by the addition of excess GTP and was specific with respect to the guanine moiety, as a nonhydrolyzable ATP analog, ATPγS did not affect the formation of either class of post-TGN vesicles (Tooze *et al.*, 1990).

With regard to the kinetics of cell-free vesicle formation in the presence of GTPγS, the following observations were made. After 60 min at 37°C in the presence of GTPγS, formation of ISGs was inhibited by approximately 40% and that of CSVs by approximately 80%. After 120 min, the inhibition of formation of both vesicle classes was about 50% (Tooze *et al.*, 1990). For CSVs, this is in agreement with data on the kinetics of inhibition by GTPγS of vesicle formation from other donor membranes (see Section II,C).

B. One Target for GTPγS in Post-trans-Golgi Network Vesicle Formation Is a Heterotrimeric G Protein

Trimeric G proteins, unlike the small ras-like GTPases (Kahn, 1991), are known to be affected by $[AlF_4]^-$ (Higashijima *et al.*, 1991). In the presence of GDP, this compound activates both inhibitory and stimulatory G proteins by mimicking the γ-phosphate group of GTP (Higashijima *et al.*, 1991). Using the cell-free system derived from PC12 cells, it was found that 40 μ*M* $[AlF_4]^-$, but not $AlCl_3$ or KF, inhibited the formation of ISGs and CSVs to the same extent as 10 μ*M* GTPγS (Barr *et al.*, 1991). Given the specificity of $[AlF_4]^-$, this was interpreted as being indicative of a role for trimeric G proteins in the regulation of post-TGN vesicle formation. Moreover, as both GTPγS and $[AlF_4]^-$ activate trimeric G proteins, it was inferred that such proteins exert, directly or indirectly, an inhibitory effect on vesicle formation.

To test this putative role of trimeric G proteins, the effect of purified G protein βγ subunits on cell-free vesicle formation has been investigated (Barr *et al.*, 1991). The rationale for these experiments was based on the observations that βγ subunits exert the opposite effect to nonhydrolyzable GTP analogues on the activation state of G protein α subunits (for reviews, see Gilman, 1987; Taylor, 1990; Birnbaumer *et al.*, 1990). This is presumably the result of a shift in the association equilibrium between the α and βγ subunits

toward the trimeric, inactive form. Indeed, it was found that βγ subunits stimulated the exit of the constitutive and regulated secretory marker proteins from the TGN in a manner that was directly proportional to the amount of subunits added (Barr *et al.*, 1991). Interestingly, the fold stimulation of exit from the TGN was greater for the hsPg, the constitutive secretory marker (4-fold at 400 n*M* βγ), than for SgII, the regulated secretory marker (2.5-fold at 400 n*M* βγ). In addition, it was found that the sorting of SgII into ISGs is perturbed by βγ subunits (400 n*M*), resulting in the packaging of approximately 40% of SgII into vesicles with a buoyant density characteristic of CSVs. The packaging of hsPg into CSVs was not altered under these conditions. These findings may reflect differences in the mechanisms by which these two classes of vesicles form, or in the way in which their formation is regulated by trimeric G proteins.

The ADP-ribosylating toxins of *Vibrio cholerae* and *Bordetella pertussis* have been extensively used to characterize and distinguish trimeric G proteins. Cholera toxin is known to specifically ribosylate the α subunit of G_s (Cassel and Pfeuffer, 1978), while pertussis toxin acts on the α subunit of G_i and G_o (for review, see Kaziro *et al.*, 1991). Accordingly, these toxins were employed to identify the G protein(s) regulating post-TGN vesicle formation. The approach used was to carry out toxin-dependent ADP ribosylation in a PC12-derived PNS under the conditions of cell-free vesicle formation, and then to analyse the distribution of ADP-ribosylated proteins using velocity sucrose gradient centrifugation (Barr *et al.*, 1991). In the presence of pertussis toxin, an approximately 40 kDa protein was ADP-ribosylated, which on velocity sucrose gradient centrifugation was detected in two peaks; one in the top three fractions of the gradient, which are known to contain various subcellular organelles including post-TGN vesicles (Tooze and Huttner, 1990) and plasma membrane (Régnier-Vigouroux *et al.*, 1991), and the other in the lower part of the gradient known to contain TGN membranes (Tooze and Huttner, 1990). The latter material was found to colocalize with the TGN on subsequent equilibrium sucrose gradient centrifugation. No ADP-ribosylated α subunit comigrating with the TGN was observed when cholera toxin was used (Barr *et al.*, 1991). The conclusion drawn from these data was that a $G\alpha_i$ or $G\alpha_o$, or both, is present on TGN membranes of PC12 cells. In line with previous reports by other investigators (Ercolani *et al.*, 1990; Stow *et al.*, 1991), the pertussis toxin-sensitive G protein on PC12 cell TGN membranes is likely to be G_{i3}.

If pertussis toxin-sensitive G proteins regulate vesicle formation, one might expect that pertussis toxin-catalyzed ADP ribosylation prior to cell-free vesicle formation reduces the inhibition by GTPγS of this process. It has been shown that after ADP ribosylation by pertussis toxin, G protein α subunits are

unable to interact with receptors, and thus are able to only undergo the basal, but not the receptor-mediated, GDP/GTP exchange (Birnbaumer *et al.*, 1990). ADP ribosylation by pertussis toxin therefore reduces the activation of α subunits. Preliminary experiments indeed showed that pertussis toxin-catalyzed ADP ribosylation partially prevents the inhibition of post-TGN vesicle formation by GTPγS (Barr *et al.*, 1991).

Work by other investigators also shows that heterotrimeric G proteins regulate secretory protein traffic through the Golgi complex and is consistent with the notion that this regulation occurs, at least in part, at the level of secretory vesicle formation. Thus, the G protein α subunit α_{i3}, which is present in virtually all cell types (Kaziro *et al.*, 1991), has been localized to the Golgi complex (Ercolani *et al.*, 1990; Stow *et al.*, 1991). Overexpression of this α subunit was found to inhibit the constitutive secretion of an hsPg in LLC-PK1 cells (Stow *et al.*, 1991). In addition, a stimulation of hsPg secretion was observed after exposure of the cells to pertussis toxin, the conclusion being that G_{i3} had become inactivated (Stow *et al.*, 1991). Taken together, it appears that pertussis toxin-sensitive G proteins function as part of a signal transducing machinery in the Golgi, mediating an inhibition of vesicle formation.

Trimeric G protein-mediated signal transduction pathways need not be restricted to Golgi membranes, but may operate on other endomembranes as well, and may involve other G proteins in addition to the pertussis toxin-sensitive ones.[1] Possibly, endomembrane signal transduction, in analogy to signal transduction at the plasma membrane (for review, see Gilman, 1987), involves a receptor of the seven transmembrane domain type. At present, we can only speculate on the function of signal transduction processes in vesicle formation; they could, for example, pass information about the lumenal content of an organelle to a cytoplasmic effector system, or they could function in the retention of organelle-specific resident proteins (Machamer, 1991).

C. Other GTP-Binding Proteins Involved in Vesicle Formation

The conclusion that GTP hydrolysis is required for vesicle formation (Tooze *et al.*, 1990) has been confirmed and extended by a more recent report that indicates that GTP hydrolysis plays a role in the formation of transport vesi-

[1]Since the submission of this manuscript, we have found (Leyte *et al.*, 1992) that multiple αi/αo and αs G-protein subunits are associated with the TGN. These G-protein subunits exert inhibitory and stimulatory effects on the formation of CSVs and ISGs.

cles from the endoplasmic reticulum (ER). In a cell-free system derived from yeast, this process has been found to be inhibited by nonhydrolyzable GTP analogues (Rexach and Schekman, 1991). The kinetics of inhibition by GTPγS of post-ER vesicle formation in the yeast cell-free system were similar to those observed for the formation of CVSs (Tooze *et al.*, 1990). In the yeast system, too, the degree of inhibition decreased at longer times of incubation. The similarity between the effects of GTPγS in the two cell-free systems supports the idea that GTP-binding proteins carry out a similar role in vesicle formation throughout the secretory pathway.

1. Small Ras-like GTP-Binding Proteins

Further work in the yeast cell-free system revealed that at least part of the requirement for GTP hydrolysis reflected the involvement of the small ras-like GTP-binding protein Sar1 (d'Enfert *et al.*, 1991). Moreover, small ras-like GTP-binding proteins of the mammalian ADP ribosylation factor (ARF) family (Kahn *et al.*, 1991) have been found to be among the components of the nonclathrin coat of intra-Golgi transport vesicles, referred to as coat proteins (Serafini *et al.*, 1991). Although no direct link between nonclathrin coat assembly and vesicle formation has yet to be reported, the interaction of two coat proteins, ARF and β-COP (Duden *et al.*, 1991), with Golgi membranes is affected under conditions known to inhibit vesicle formation, such as the presence of GTPγS (Donaldson *et al.*, 1991a,b; Serafini *et al.*, 1991). This points toward an involvement of ARF in vesicle formation, a suggestion consistent with the observation that a yeast mutant for ARF is defective in invertase secretion (Stearns *et al.*, 1990). Since the yeast GTP-binding protein Sar1 is homologous to mammalian ARF (Nakano and Muramatsu, 1989), it is tempting to speculate that in general, small ras-like GTP-binding proteins of the ARF/Sar1 family are involved in vesicle formation by regulating coat assembly at the level of the donor membrane, and that this regulation is mediated via GTP binding and hydrolysis.

2. GTP-Binding Motor Proteins

Studies on the *shibire* mutant of *Drosophila melanogaster* led to the identification of another class of GTP-binding proteins that may be involved in vesicle formation. The *shibire* mutant was originally identified as a mutant with alterations in the structure of the neuromuscular junction leading to paralysis (Poodry and Edgar, 1979). In this mutant the primary defect is at the level of endocytosis via clathrin-coated pits, which are unable to bud from the plasma membrane (Kosaka and Ikeda, 1983). The *shibire* gene has recently been cloned and sequenced (van der Bliek and Meyerowitz, 1991). The corre-

sponding protein was found to be a homologue of rat dynamin (Chen *et al.*, 1991), a 100 kDa microtubule-binding protein with GTPase activity able to induce microtubule mobility *in vitro* (Shpetner and Vallee, 1989). Together, these findings raise the possibility that GTP-binding motor proteins such as dynamin may be involved in the scission of endocytotic vesicles from the plasma membrane.

Several observations suggest that such a role for these proteins need not be restricted to vesicle formation from the plasma membrane. Dynamin as well as the *shibire* gene product show homology to the yeast Vps1 protein, which is involved in protein sorting from the TGN to the vacuole and is a putative GTPase (Rothman *et al.*, 1990). Clathrin is thought to be involved not only in endocytosis, but also in the formation of vesicles from the TGN, which deliver lysosomal enzymes bound to mannose-6-phosphate receptors to endosomes (Kornfeld, 1987). In addition, patches of clathrin have been found on ISGs (Tooze and Tooze, 1986), and a species of clathrin light chain (LC_b) is enriched in cells and tissues with the regulated secretory pathway (Acton and Brodsky, 1990). If there indeed exists a clathrin-dependent mechanism for vesicle formation from the TGN, it is conceivable that this mechanism would employ a motor protein like dynamin, and therefore, GTP hydrolysis in the final scission event. By analogy, one may speculate on the existence of a similar GTP-binding protein as part of the "scission machinery" that operates in the nonclathrin-dependent mechanism of vesicle formation.

III. FINAL COMMENTS

As mentioned in the Introduction, the formation of vesicles from a donor compartment is a complex cascade of events. Given the distinct classes of GTP-binding proteins that appear to be involved in the overall process of vesicle formation, it is likely, but not proven, that these proteins exert functions in several of the individual steps in vesicle formation. In which step and how do these proteins act? In the following sections, various possibilities are discussed concerning secretory vesicle formation from the TGN.

A. The Role of Heterotrimeric G Proteins in Vesicle Formation

From the data previously described (Barr *et al.*, 1991; Stow *et al.*, 1991; see also footnote 1 and Leyte *et al.*, 1992), it appears that secretory vesicle formation from Golgi membranes is under negative control mediated by G_{i3}.

An inhibitory mechanism for the exit of constitutive and regulated secretory proteins from the TGN might be related to the obvious requirement for a regulatory system that prevents vesicles forming from the TGN unless appropriate cargo is available. Such a system might regulate the rate of vesicle formation to ensure, for example, (1) that secretory proteins destined to undergo post-translational modifications [e.g., sialylation (Roth *et al.*, 1985) and tyrosine sulfation (Baeuerle and Huttner, 1987)] only leave the TGN after having acquired these modifications, which can be crucial for the fate of a protein (e.g., Leyte *et al.*, 1991); or (2) that regulated secretory proteins only leave the TGN after having been segregated via aggregation (Chanat and Huttner, 1991) from constitutive secretory proteins. The lack of completion of these process might somehow be monitored by a transmembrane receptor, for instance by recognition of unmodified sites for posttranslational modification or of nonaggregated regulated secretory proteins. Such a putative transmembrane receptor might then interact with a trimeric G protein, stimulating GDP–GTP exchange on the G protein α subunit and hence promoting its activation. The activated α subunit in turn presumably interacts with an effector on the cytoplasmic side of the TGN membrane (see Fig. 1). Until the activated α subunit hydrolyzes its bound GTP to GDP, this effector will either reduce vesicle formation below the normal rate, or be prevented from maintaining vesicle formation at the normal rate. In this regard, it is interesting to

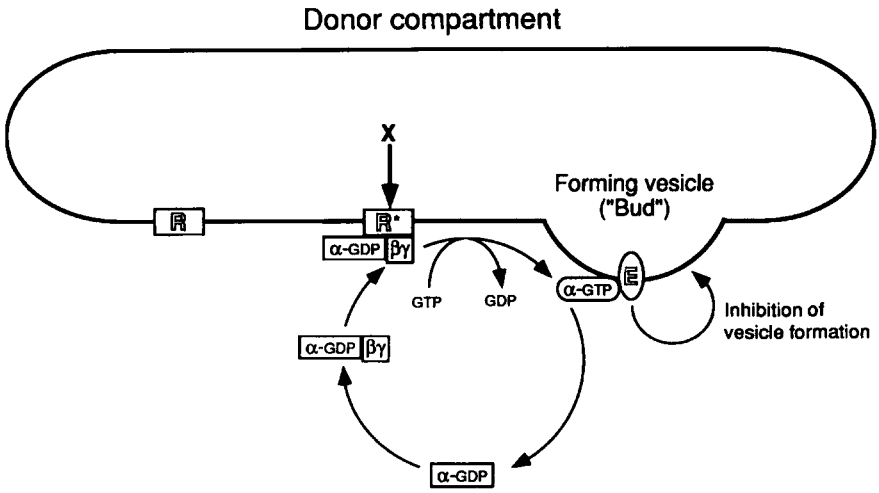

Fig. 1. Schematic representation of a signal transduction machinery in the membrane of the *trans*-Golgi network involved in the regulation of vesicle formation. X, ligand; R, receptor; R*, activated receptor; α, β, and γ, trimeric G protein subunits; E, effector. For detail, see text.

note that inhibition of tyrosine sulfation has been shown to retard the exit of a constitutive secretory protein from the TGN (Friederich *et al.*, 1988).

So far, there are no clues as to the identity of the effector system through which activated α subunits exert an inhibitory effect on post-TGN vesicle formation. However, if one extrapolates from recent observations made with intra-Golgi transport vesicles (Serafini *et al.*, 1991), the effector system may include small ras-like GTP-binding proteins such as ARF. An activated G protein could, for instance, directly or indirectly prevent ARF from recruiting coat proteins to the membrane of a nascent bud, as proposed by Serafini *et al.* (1991). An interaction between trimeric G proteins, ARF, and coat proteins is suggested from the observation that addition of βγ subunits inhibits the association of both ARF and β-COP with Golgi membranes (Donaldson *et al.*, 1991b).

B. Regulation of Coat Assembly

This leads to the regulation of vesicle formation via the control of coat assembly on the cytoplasmic surface of the forming vesicle. As mentioned before, small GTP-binding proteins have been shown to be part of the non-clathrin coat of intra-Golgi transport vesicles (Serafini *et al.*, 1991). This may also be true for post-TGN secretory vesicles. Although the nature of the coats of CSVs and ISGs is not precisely known (Tooze, 1991), the transient presence of these coats implies that their assembly and disassembly before and after scission, respectively, are somehow regulated. Both assembly and disassembly may involve the same small GTP-binding protein that could promote either coating or uncoating, depending on its GTP/GDP-bound state. Free coat subunits of the small GTP-binding protein family may only associate with the membrane when they are in the GTP-bound form. GTP hydrolysis might be linked to a proofreading step related to either coat polymerization or the proper assembly of the various components of the vesicle membrane. Such a "conditional" GTP-hydrolysis could, for instance, ensure the production of vesicles that contain all transmembrane and peripheral molecules required to define its intracellular destination.

C. Promotion of Scission

If scission, the final step in vesicle formation, is promoted by a motorlike protein of the dynamin family (see Section II,C,2), this step, too, might be a GTP-dependent process. Extrapolating from the apparent lack of scission of endocytotic vesicles in the *shibire* mutant of *Drosophila melanogaster*, a

similar, though possibly CSV- or ISG-specific motor protein may recognize a completed bud on the TGN and link it to microtubules (Vallee and Shpetner, 1990). Subsequent GTP hydrolysis could then promote movement along these microtubules, and thereby the shearing off of the bud from the donor membrane.

D. Conclusion

It is becoming apparent that GTP-binding proteins (heterotrimeric G proteins, small ras-like proteins, and possibly motor proteins) are key components of the regulatory machinery involved in vesicle formation. Future work is needed to characterize these GTP-binding proteins, and a major goal is to identify the various components acting upstream and downstream of them, in particular, the receptor and effector systems.

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