Inactivation of Gα_z causes disassembly of the Golgi apparatus

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Summary

We showed previously that overexpression of the α subunit of G_z or G_{i2} suppresses nordihydroguaiaretic acid-induced Golgi disassembly. To determine whether the active form of Gα is required to maintain the structure of the Golgi apparatus, we examined the effects of a series of Gα GAPs, regulators of G protein signaling (RGS) proteins, on the Golgi structure. Expression of RGSZ1 or RGSZ2, both of which exhibit high selectivity for Gα_z, markedly induced dispersal of the Golgi apparatus, whereas expression of RGS proteins that are rather selective for Gα_q or other Gα_i species did not. A mutated RGSZ1, which is deficient in the interaction with Gα_z, did not induce Golgi disassembly. These results suggest that the active form of Gα_z, but not Gα_{i2}, is crucial for maintenance of the structure of the Golgi apparatus. Consistent with this idea, Golgi disruption also took place in cells transfected with a dominant-negative Gα_z mutant. Although previous studies showed that the expression of Gα_z is confined to neuronal cells and platelets, immunofluorescence and mRNA expression analyses revealed that it is also expressed, albeit at low levels, in non-neuronal cells, and is located in the Golgi apparatus. These results taken together suggest a general regulatory role for Gα_z in the control of the Golgi structure.

Key words: Golgi apparatus, Heterotrimeric G protein, Regulator of G protein signaling

Introduction

Heterotrimeric G proteins are classically known to function as signal transducers on the plasma membrane (Kaziro et al., 1991; Neer, 1995; Hamm, 1998). Each G protein consists of an α subunit that binds a guanine nucleotide, and a βγ complex. The interaction of a G protein with an activated receptor triggers guanine nucleotide exchange on the α subunit, leading to its dissociation from the βγ dimer. The activated α-subunit and βγ dimer act on appropriate downstream effectors. Individual subunits of G proteins consist of multiple species with different tissue distributions. Gα family members are classified into four subfamilies, α_s, α_i, α_q and $\alpha_{12/13}$, and each member is coupled with different receptors to mediate different signals (Simon et al., 1991).

Recently, the functions of heterotrimeric G proteins in various endomembrane systems have also been recognized (Lang, 1999). These include protein transport from the endoplasmic reticulum (ER) to the Golgi apparatus (Schwaninger et al., 1992), intra-Golgi transport (Helms et al., 1998), cargo sorting and vesicle budding from the *trans*-Golgi network (Leyte et al., 1992; Pimplikar and Simons, 1993), control of exocytosis (Ohara-Imaizumi et al., 1992; Aridor et al., 1993; Vitale et al., 1993; Ohnishi et al., 1997), and autophagic sequestration (Ogier-Denis et al., 1996).

In mammalian cells the Golgi apparatus is of fundamental importance for the secretion and posttranslational modification of secretory and membrane proteins. The Golgi apparatus consists of a set of highly dynamic membrane compartments that are maintained through the continuous anterograde and retrograde flow of proteins and lipids. The Golgi apparatus undergoes disassembly and reassembly during mitosis, and there is a cell cycle-dependent mechanism that controls the Golgi structure and thus ensures the fidelity and reliability on the partitioning of this organelle into daughter cells (Warren and Malhotra, 1998). Golgi disassembly at the onset of mitosis may be controlled by signaling proteins at the periphery of Golgi membranes (Nelson, 2000).

We and others have previously shown the involvement of heterotrimeric G proteins in regulation of the Golgi structure (Hidalgo et al., 1995; Jamora et al., 1997; Jamora et al., 1999; Yamaguchi et al., 1997; Yamaguchi et al., 2000). However, it is currently unclear as to which subunit of heterotrimeric G proteins is involved in this regulation, and little is known about the mechanism by which G proteins exert their actions. A study involving a semi-intact cell system demonstrated that Gβγ causes vesiculation of the Golgi apparatus through direct activation of protein kinase D (Jamora et al., 1999). On the other hand, we showed that the activation of G proteins by GTPγS or AlF_4^- protects the Golgi apparatus from disassembly caused by nordihydroguaiaretic acid (NDGA). Consistent with the involvement of Gα-mediated signaling in Golgi organization, the addition of Gβγ can reverse the effect of GTPγS on NDGA-induced Golgi disassembly through reformation of the heterotrimer (Yamaguchi et al., 1997). Furthermore, overexpression of Gα_{i2} or Gα_z, both of which are α_i family members, results in attenuation of the NDGA effect

(Yamaguchi et al., 2000). Although our data comprise circumstantial evidence that $G\alpha_{i2}$ and/or $G\alpha_z$ are involved in Golgi organization, it is not clear whether these G proteins actually regulate the Golgi structure in normal living cells.

Recently, a novel family of G protein regulators, i.e. regulators of G protein signaling (RGS) proteins, has emerged (Berman and Gilman, 1998; Hepler, 1999; De Vries et al., 2000). RGS proteins stimulate the intrinsic GTPase activity of heterotrimeric G proteins, thereby decreasing the concentration of active Gα in cells. In addition to this effect, RGS proteins prevent the binding of Gα to their effectors. Therefore, RGS proteins serve as negative regulators of G protein-mediated signaling pathways (Berman and Gilman, 1998; De Vries et al., 2000).

RGS proteins appear to be ideal tools for demonstrating the physiological relevance of our finding that active Gα subunits prevent NDGA-induced Golgi disassembly. In this study, we overexpressed various RGS proteins that preferentially inactivate a different set of G proteins, and examined the Golgi morphology. The results suggest that, indeed, active $G\alpha_z$, but not $G\alpha_{i2}$, is necessary for maintenance of the integrity of the Golgi structure.

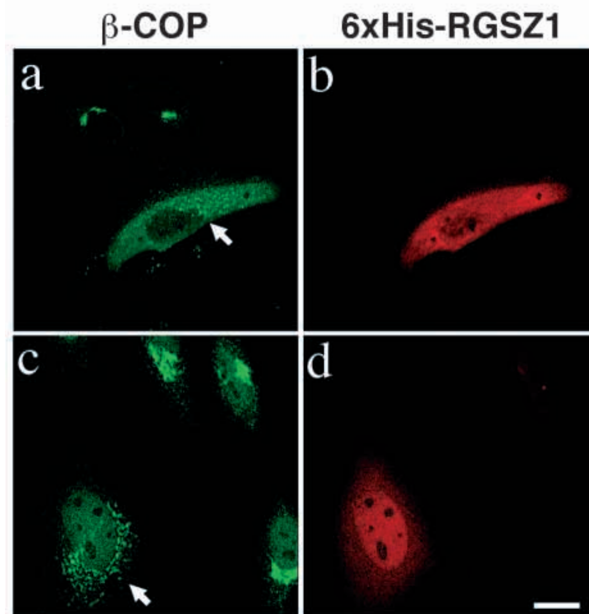


Fig. 1. Dispersal of β-COP in cells expressing RGSZ1. HeLa cells were transfected with a plasmid for 6×His-RGSZ1. The cells were allowed to express RGS proteins for 14 hours, fixed, and then processed for β-COP staining (a,c). Expressed RGSZ1 was detected with the monoclonal anti-tetra-His antibody (b,d). Arrows indicate RGSZ1-expressing cells with dispersed β-COP staining. Bar, 20 μm.

Materials and Methods

Antibodies

The mouse monoclonal anti-tetra-His antibody was from Qiagen. The mouse monoclonal anti-FLAG antibody (M2) and rabbit polyclonal anti-FLAG antibody were obtained from Sigma. The mouse monoclonal anti-mannosidase II (Man II) antibody (53FC3) was purchased from BabCo. The mouse monoclonal anti-GM130, anti-p115, and anti–membrin antibodies were purchased from Transduction Laboratories. The rabbit polyclonal anti-βCOP and anti-syntaxin 5 antibodies were raised in this laboratory. The rabbit polyclonal anti-transferrin antibody was from DAKO. The rabbit anti-NADH-cytochrome P-450 reductase antibody and mouse monoclonal anti-ERGIC-53 antibody were generous gifts from Dr Akitsugu Yamamoto (Kansai Medical University) and Dr Hans-Peter Hauri (University of Basel), respectively. The FITC-conjugated anti-mouse and anti-rabbit IgG antibodies were from Rockland and Chemicon International, respectively. The Texas red-conjugated anti-mouse and anti-rabbit IgG antibodies were from Kirkegaard & Perry Laboratories and Southern Biotechnology Associates, respectively.

Plasmids

The cDNAs for RGS4, GAIP, RGSZ1 and RGSZ2 were kindly donated by Drs. Elliott Ross and Tohru Kozasa (University of Texas Southwestern Medical Center), and subcloned into mammalian expression vectors pcDNA3 (Invitrogen) and pFLAG-CMV-6 (Sigma). The full-length cDNA encoding human RGS2 was amplified from a human leukocyte cDNA library (Clontech) by means of the polymerase chain reaction (PCR) with primers corresponding to the initiation and termination sites of RGS2. To obtain the full-length cDNA encoding RGS3, nucleotides corresponding to the N-terminal and C-terminal regions were independently amplified from the human cDNA library by PCR, and then cloned into pFLAG-CMV-6. The expression plasmid for the vesicular stomatitis virus ts045 G protein fused to the green fluorescent protein (VSVG-GFP) was a generous gift from Dr Jennifer Lippincott-Schwartz (National Institutes of Health, USA).

Site-directed mutagenesis

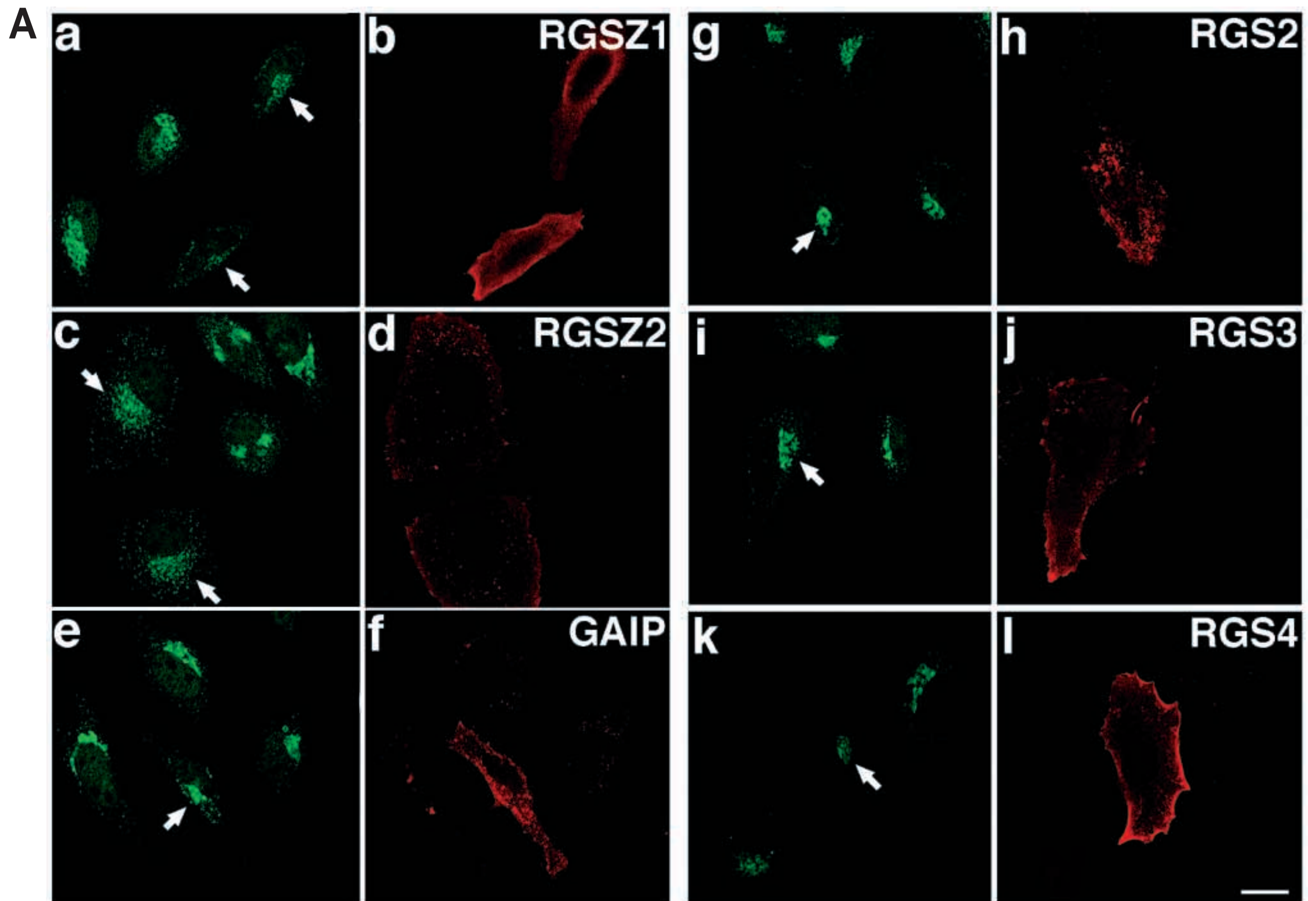
A rat cDNA containing the entire coding sequence of $G\alpha_z$ was cloned into a eukaryotic expression vector, pALTER-MAX (Promega). $G\alpha_z$(G204A/E246A/A327S), in which Gly-204, Glu246, and Ala-327 of $G\alpha_z$ were replaced with Ala, Ala, and Ser, respectively, was generated by oligonucleotide-directed mutagenesis using an Altered Sites II Mammalian Mutagenesis System (Promega). A Gα binding deficient mutant of RGSZ1, RGSZ1(E116A/N117A), in which Glu-116 and Asn-117 were individually substituted by Ala, was prepared by PCR-mediated mutagenesis.

Cell culture and transient transfection

HeLa, BHK, and Clone9 cells were cultured at 37°C in α-minimum essential medium supplemented with 10% fetal bovine serum, 50 IU/ml penicillin, and 50 μg/ml streptomycin, under humidified air containing 5% CO_2. PC12 cells were maintained in Dulbecco's modified Eagle's medium supplemented with 50 IU/ml penicillin, 50 μg/ml streptomycin, 10% fetal bovine serum, and 5% horse serum. Cells were transfected by lipofection using LipofectAMINE PLUS according to the manufacturer's instructions (Life Technologies).

Indirect immunofluorescence analysis

Indirect immunofluorescence microscopy was performed essentially as described previously (Tagaya et al., 1996). Cells were grown on 15-mm diameter glass coverslips in 12-well tissue culture plates. At the indicated times after transfection, cells were subjected to fixation in 4% paraformaldehyde in phosphate-buffered saline (PBS) for 20 minutes at room temperature, followed by permeabilization with 0.2% Triton X-100 in PBS for 20 minutes. They were then incubated for 30 minutes in PBS containing 2% bovine serum albumin (BSA), and incubated for 1 hour at 37°C with appropriate primary antibodies in PBS-2% BSA. The cells were washed three times with PBS, and then stained with FITC- and/or Texas red-conjugated secondary antibodies. An Olympus BX50 fluorescence microscope was used for routine immunofluorescence analysis. Confocal images were obtained with



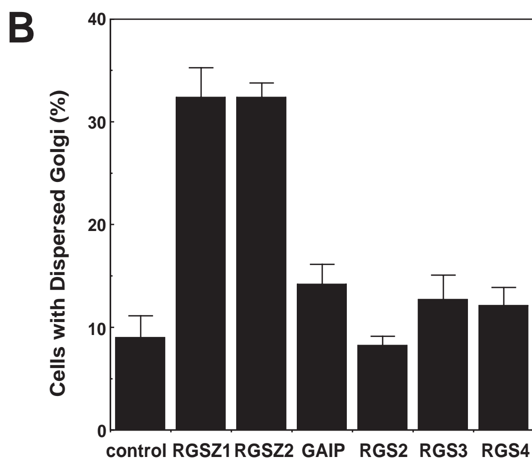


Fig. 2. Disassembly of the Golgi apparatus is caused by Gα$_z$-selective RGSZ1 and RGSZ2, but not by other RGS proteins. (A) HeLa cells were transfected with a plasmid encoding N-terminal FLAG-tagged RGSZ1 (a,b), RGSZ2 (c,d), GAIP (e,f), RGS2 (g,h), RGS3 (i,j), or RGS4 (k,l). At 20 hours after transfection, the cells were fixed, and then double-stained with the anti-β-COP antibody (a,c,e,g,i,k) and anti-FLAG antibody (b,d,f,h,j,l). Arrows indicate cells expressing RGS proteins. Bar, 20 μm. (B) The percentage of transfected HeLa cells with a dispersed β-COP staining was determined. The results are expressed as the percentage of the number of cells with a dispersed Golgi apparatus and as the mean±s.e.m. for at least three separate experiments.

an Olympus Fluoview 300 laser confocal microscope. For the transferrin internalization assay, transfected cells were incubated in the presence of 25 μg/ml of FITC-conjugated transferrin (Sigma) for 1 hour at 37°C. After extensive washing in PBS, they were fixed with 4% paraformaldehyde in PBS. Since the fluorescence intensity of FITC-conjugated transferrin was weak, the fixed cells were further stained with the anti-transferrin rabbit polyclonal antibody and the FITC-conjugated secondary antibody.

Semi-quantitative reverse transcription-PCR (RT-PCR)

Total RNA was isolated from cells using isogen reagent (Nippon Gene) and then used as the template for RT-PCR analysis. RT-PCR reactions were performed with a BcaBEST RNA PCR kit (Takara). PCR was performed for 45 and 20 cycles for Gα$_z$ and glyceraldehyde-3-phosphate dehydrogenase (GAPDH), respectively, using the following sense and antisense primers: Gα$_z$, 5′-CACCTGGA-GGACAACGCCGCT-3′ and 5′-TTTCGGTTTAGGTCCTCGAACT-GA-3′ (500-bp product); GAPDH, 5′-CATGGAGAAGGCTGGGG-CTC-3′ and 5′-CTCAGTGTAGCCCAGGATGC-3′ (523-bp product), respectively. The number of cycles was pre-determined to fall within the linear range of amplification of each PCR product. The PCR products were electrophoresed on 1.6% agarose gels, stained with ethidium bromide, and visualized by UV irradiation.

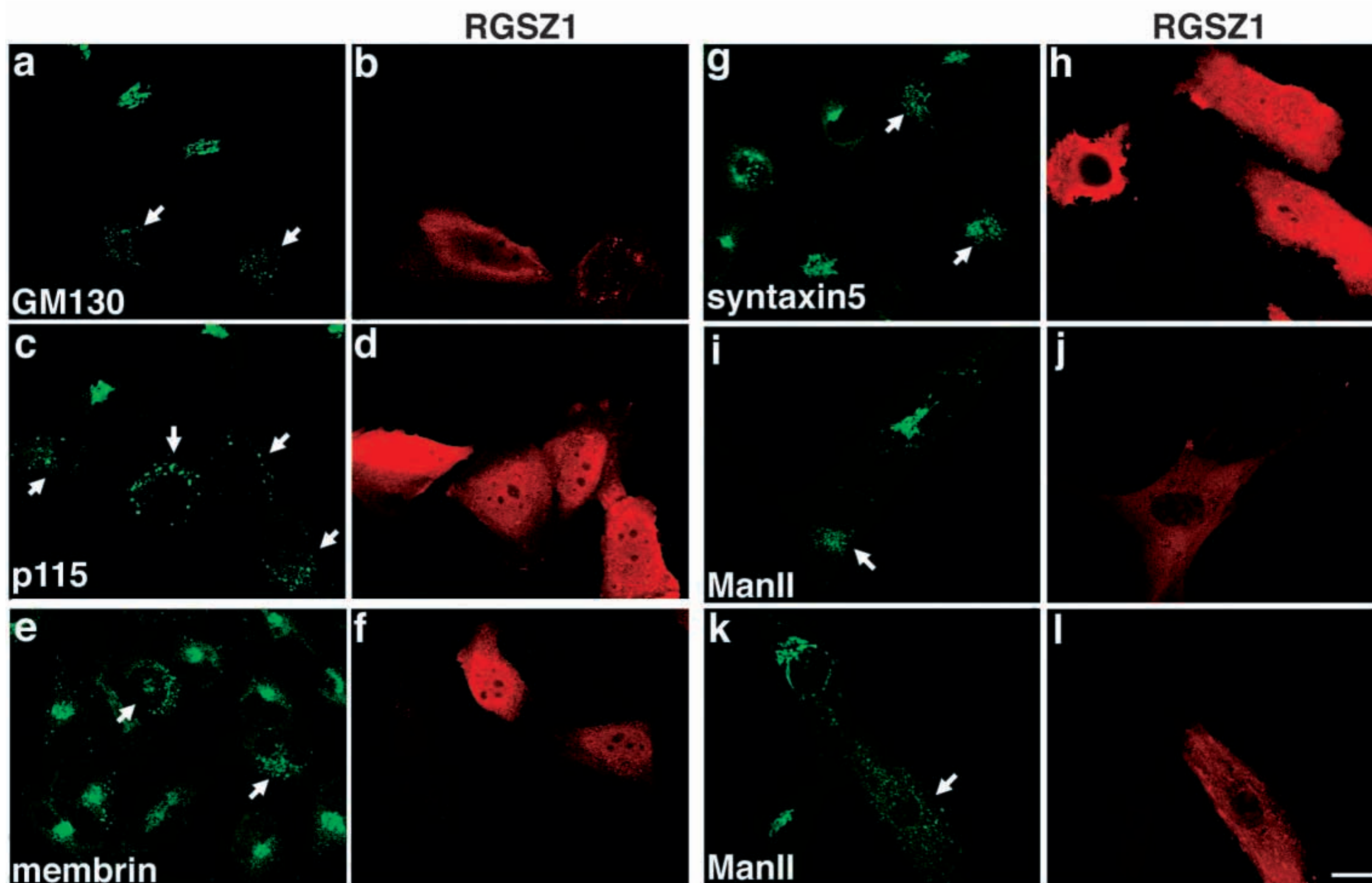


Fig. 3. Disassembly of the Golgi apparatus in cells expressing RGSZ1. HeLa cells (a-h) and BHK cells (i-l) were transfected with a plasmid encoding N-terminal FLAG-tagged RGSZ1. At 20 hours after transfection, the cells were fixed, and then double-stained with the anti-FLAG antibody (b,d,f,h,j,l) and antibodies to the various Golgi proteins indicated (a,c,e,g,i,k). Arrows indicate RGSZ1-expressing cells with a dispersed Golgi apparatus. Bar, 20 μm.

Results

Overexpression of $G\alpha_z$-selective GAP proteins causes dispersal of β-COP

We previously demonstrated that overexpression of $G\alpha_z$ or $G\alpha_{i2}$ prevents NDGA-induced Golgi disassembly (Yamaguchi et al., 2000). To address the physiological significance of this finding, we examined the effect of overexpression of RGS proteins on the structure of the Golgi apparatus.

RGS proteins can attenuate G-protein mediated signaling pathways by acting as GAPs for the $G\alpha_i$, $G\alpha_q$, and $G\alpha_{12/13}$ families, but not the $G\alpha_s$ one. To date, more than 20 RGS proteins in mammalian tissues have been identified. Certain RGS proteins exhibit high selectivity for a single or a few Gα species (Berman and Gilman, 1998; De Vries et al., 2000). RGSZ1 (Glick et al., 1998; Wang et al., 1998) and RGSZ2 show high selectivity for $G\alpha_z$.

By immunofluorescence microscopy, we first examined the localization of a Golgi marker β-COP in HeLa cells expressing N-terminal hexahistidine-tagged (6×His-) RGSZ1. Although nontransfected cells showed a typical ribbon-like Golgi structure, a dispersed or punctate β-COP staining pattern was frequently observed in cells overexpressing 6×His-RGSZ1 (Fig. 1). The degree of β-COP dispersal was dependent on the level of RGSZ1 expression. When RGSZ1 was expressed at high levels, β-COP was almost completely dispersed (Fig. 1a). On the other hand, expression of RGSZ1 at moderate levels yielded a punctate or large dot-like β-COP staining pattern (Fig. 1c), which might represent fragmented Golgi. Approximately 50% of the cells expressing a detectable level of RGSZ1 showed a completely or partially disrupted β-COP staining pattern, whereas less than 7% of the nontransfected ones did. The latter value may represent the percentage of cells in the late G_2 and M phases. It is known that the Golgi ribbon-like structure is reorganized into a punctate structure and exhibits a more perinuclear localization at the G_2/M transition, and then is completely dispersed (Shima et al., 1998) or transported back to the ER (Zaal et al., 1999) during mitosis. The effect of the RGS proteins on the Golgi structure is not peculiar to HeLa cells, similar results being obtained for BHK and Clone9 cells (data not shown).

Selective attenuation of the $G\alpha_z$ function induces Golgi disassembly

We next examined whether or not other RGS proteins exert similar effects on the Golgi apparatus. RGSZ2 is another $G\alpha_z$-specific RGS that exhibits 62.7% amino acid identity with RGSZ1. GAIP, which is most similar to RGSZ1 (62.6% amino acid identity) and RGSZ2 (57.6% amino acid identity), exhibits

GAP activity toward all $G\alpha_i$ family members (De Vries et al., 1995; Berman et al., 1996). RGS2 exhibits high selectivity for $G\alpha_q$ (Heximer et al., 1997). RGS3 is highly selective for the $G\alpha_i$ family except for $G\alpha_z$ (Scheschonka et al., 2000). RGS4 stimulates the GTPase activity of both $G\alpha_i$ and $G\alpha_q$ (Berman et al., 1996; Watson et al., 1996). These RGS proteins were expressed as FLAG-tagged proteins in HeLa cells, and then the Golgi morphology was examined. As shown in Fig. 2A, expression of RGSZ2 as well as that of RGSZ1 induced the dispersal of β-COP, whereas expression of GAIP, RGS2, RGS3, or RGS4 had essentially no effects on β-COP staining. The number of cells expressing a detectable level of RGS proteins was determined, and the percentage of cells with a disrupted β-COP staining pattern was determined (Fig. 2B). More than 30% of the FLAG-RGSZ1- or FLAG-RGSZ2-expressing cells showed a dispersed β-COP staining pattern, whereas no significant dispersal of β-COP was observed for other RGS-expressing cells compared to control cells. These results suggest that the dispersal of β-COP by RGSZ1 and RGSZ2 is specific.

RGSZ1 stimulates Golgi disruption without markedly affecting other cellular structures or functions

Since β-COP is a peripheral membrane protein that undergoes association and disassociation with the Golgi apparatus in an ARF1-dependent manner (Donaldson et al., 1992; Palmer et al., 1993), the results shown in Figs 1 and 2 may imply that the expression of RGSZ1 merely induces the release of β-COP from Golgi membranes. To test this possibility, we examined the localization of several Golgi-associated peripheral and integral membrane proteins in RGSZ1-expressing cells. As shown in Fig. 3a-d, peripheral Golgi proteins GM130 and p115 showed scattered staining patterns and were distributed as distinct dots around the nucleus in RGSZ1-expressing cells. As shown in Fig. 3e-h, cis-Golgi integral membrane proteins membrin and syntaxin 5 exhibited similar fragmented patterns in RGSZ1-expressing cells. Man II, a Golgi-resident integral membrane protein, also showed a scattered distribution (Fig. 3i-l). These results demonstrate that RGSZ1 does not merely induce the release of peripheral proteins from the Golgi membrane, but also causes the fragmentation of entire Golgi stacks.

We wondered whether or not the fragmentation of the Golgi apparatus is a consequence of the disassembly of microtubules, which are known to play an important role in maintaining the Golgi structure (Lippincott-Schwartz, 1998; Thyberg and Moskalewski, 1999). As shown in Fig. 4a,b, expression of RGSZ1 had no effect on the structure of microtubules. In addition, expression of RGSZ1 did not conspicuously affect the ER structure in most cells (Fig. 4c,d), although the formation of punctate aggregations was observed in some cells (data not shown). RGSZ1 expression also did not affect the uptake of FITC-transferrin (Fig. 4e,f). These results demonstrate that overexpression of RGSZ1 affects the Golgi structure without marked effects on other cellular structures or functions.

Effect of RGSZ1 expression on vesicular transport

We next examined whether or not RGSZ1 expression perturbs anterograde vesicular transport. For this purpose, a

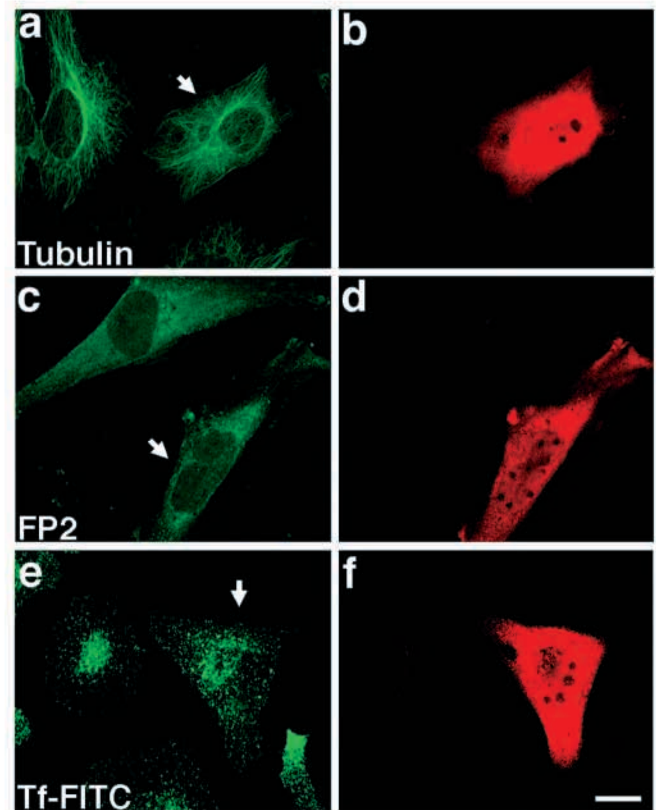


Fig. 4. Cellular structures and functions other than the Golgi apparatus are not markedly affected by the expression of RGSZ1. A plasmid for 6×His-RGSZ1 was transfected into HeLa cells. At 14 hours after transfection, the cells were fixed, and then processed for indirect immunofluorescence. The panels show the localization of tubulin (a), NADH-cytochrome P-450 reductase (FP2; c), and FITC-transferrin (Tf-FITC; e). For the FITC-transferrin uptake experiment, HeLa cells transfected with 6×His-RGSZ1 were incubated with FITC-transferrin at 37°C for 1 hour. The cells were then processed for immunofluorescence. 6×His-RGSZ1-expressing cells were detected with the anti-tetra-His antibody (b,d,f). Arrows indicate RGSZ1-expressing cells. Bar, 20 μm.

temperature-sensitive VSVG mutant protein fused to GFP (VSVG-GFP) (Presley et al., 1997) was used. HeLa cells were co-transfected with plasmids for VSVG-GFP and FLAG-RGSZ1 and incubated at 40°C for 20 hours, and then the temperature was changed to 32°C to initiate transport from the ER. At 1 hour after the temperature shift, VSVG-GFP had moved from the ER to the plasma membrane through the Golgi in 80% of the control cells (Fig. 5). In RGSZ1-expressing cells, the transport of VSVG-GFP was significantly delayed. At 1 hour after the temperature shift, VSVG-GFP had reached the plasma membrane in 40% of the RGSZ1-expressing cells. In the other RGSZ1-expressing cells, VSVG-GFP was detected in dot-like structures that might represent fragmented Golgi membranes. Essentially the same results were obtained for BHK and Vero cells.

The inhibitory effect of RGSZ1 on the transport of VSVG-GFP might be underestimated. We noticed that the extent of Golgi dispersion was lower in cells expressing both RGSZ1 and VSVG-GFP than in those expressing RGSZ1 alone. In many cells, fragmented, large dot-like Golgi structures rather

than completely dispersed ones were observed. It seemed likely that VSVG-GFP was transported to the plasma membrane through the perinuclear dot-like structures marked by Man II (Fig. 6a-c), β-COP (Fig. 6d-f), and ERGIC-53 (Fig. 6g-i). We speculate that the transport of VSVG-GFP would be severely inhibited if the Golgi apparatus were completely dispersed by high expression of RGSZ1.

Association of RGSZ1 with $G\alpha_z$ is required for RGSZ1-mediated Golgi dispersal

The RGS family is defined by the RGS-box that binds to Gα subunits and is responsible for the GAP function. A point mutation in the RGS-box of RGS4 abrogates its ability to bind to Gα and therefore is not able to inactivate Gα (Druey and Kehrl, 1997; Srinivasa et al., 1998). To determine whether or not the GAP activity of RGSZ1 is required for Golgi dispersion, RGSZ1(E116A/N117A), in which Glu-116 and Asn-117 substituted individually by Ala, was constructed and assessed as to its Golgi disassembling activity. This mutation was designed in analogy to the RGS4 mutation (Srinivasa et al., 1998). A similar mutation has been successfully used for assessment of the function of RGS3 (Scheschonka et al., 2000).

We first tested whether RGSZ1(E116A/N117A), as expected, cannot bind to $G\alpha_z$. The wild-type or mutated FLAG-RGSZ1 was co-expressed in cells with an active mutant of $G\alpha_z$, $G\alpha_z$(QL), in which Gln-205 was replaced with Leu (Fig. 7A). The wild-type RGSZ1, which mainly remains in the cytosol (Fig. 3), was efficiently recruited to the plasma and internal membranes, and co-localized with expressed $G\alpha_z$(QL), indicating the interaction of RGSZ1 with $G\alpha_z$ in cells. In contrast, the mutated RGSZ1 remained in the cytosol and was not co-localized with expressed $G\alpha_z$(QL). This indicates that the mutated RGSZ1 we constructed has the expected functional property in cells.

As shown in Fig. 7B, cells expressing wild-type RGSZ1 showed a dispersed β-COP staining pattern (Fig. 7Ba,b). In contrast, RGSZ1(E116A/N117A) had no detectable effect on the distribution of β-COP (Fig. 7Bc,d). The level of expression of the RGSZ1 mutant was comparable to that of the wild-type one (Fig. 7C). Similar results were obtained when Clone9 cells were used (data not shown).

Expression of a dominant-negative $G\alpha_z$ subunit causes Golgi disruption

As an alternative approach for investigating the effect of attenuation of the $G\alpha_z$ function, we employed a dominant-negative mutant strategy. Several mutants possessing mutations in the conserved nucleotide-binding region of GTPases including the α-subunits of heterotrimeric G proteins have been characterized. The mutant we constructed was a triple one (G204A/E246A/A327S), in which Gly-204, Glu-246, and Ala-327 were replaced with Ala, Ala, and Ser, respectively. This mutant is equivalent to a triple mutant of $G\alpha_s$, which has decreased guanine nucleotide-binding ability, and dominantly inhibits receptor-mediated hormonal activation of $G\alpha_s$ by sequestering Gβγ and activated receptors (Iiri et al., 1999).

The triple $G\alpha_z$ mutant was not co-localized with RGSZ1 when co-expressed, suggesting that the $G\alpha_z$ mutant is not in a GTP-bound active state in cells (data not shown). The triple $G\alpha_z$ mutant plasmid was transfected into HeLa cells, and after

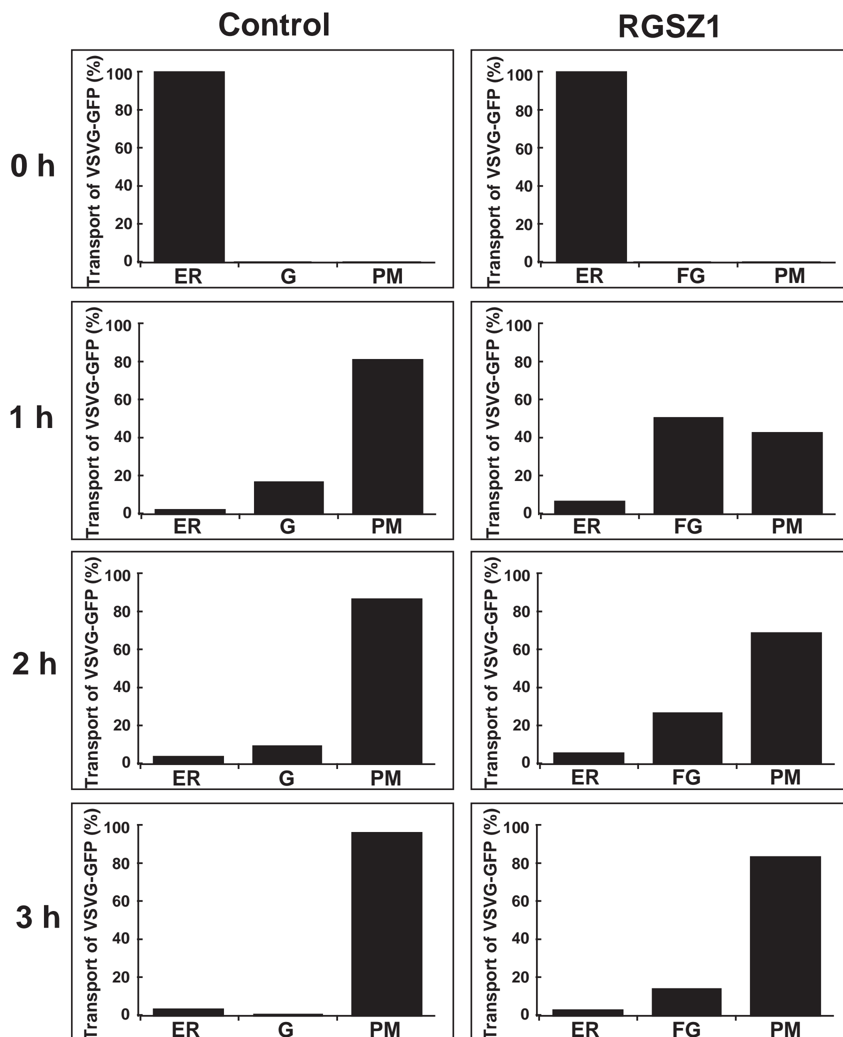


Fig. 5. Effect of RGSZ1 expression on intracellular trafficking of VSVG-GFP. HeLa cells were transfected with an expression plasmid for VSVG-GFP with a control vector or a FLAG-RGSZ1 expression plasmid. The cells were incubated for 20 hours at 40°C, and then the temperature was changed to 32°C. At the indicated times the cells were fixed for immunofluorescence analysis. RGSZ1-expressing cells were identified by using the anti-FLAG antibody. The compartment to which VSVG-GFP had been transported at the indicated times after the temperature shift was determined. ER, G, FG and PM denote ER, Golgi, fragmented Golgi and plasma membrane, respectively. Values are expressed as a percentage of the total number of cells expressing both VSVG-GFP and RGSZ1. More than 100 cells were scored for each time point.

20 hours the cells were processed for immunofluorescence (Fig. 8). The triple $G\alpha_z$ mutant was mainly distributed in intracellular membranous structures including those in the perinuclear region. Golgi protein membrin was dispersed in 39% of the cells expressing the triple $G\alpha_z$ mutant, whereas it was not dispersed in wild-type $G\alpha_z$-expressing cells. Taken together, the effects of overexpression of RGS proteins and the dominant-negative $G\alpha_z$ mutant strongly suggest a critical role of active $G\alpha_z$ in maintenance of the Golgi structure.

Association of $G\alpha_z$ with the Golgi apparatus

Previous studies showed that the expression of $G\alpha_z$ is limited primarily to platelets, neurons and chromaffin cells, suggesting specific roles in these tissues (Matsuoka et al., 1988; Casey et al., 1990; Hinton et al., 1990). If $G\alpha_z$ is involved in the organization of the Golgi apparatus, it should be expressed ubiquitously. To demonstrate the expression of $G\alpha_z$ and to determine its localization in non-neuronal cultured cells, we performed indirect immunofluorescence microscopic analysis. Immunoreactivity to the anti-$G\alpha_z$ antibody was mainly observed in the perinuclear region, with some in the plasma membranes of BHK and Clone9 cells (Fig. 9a,c). The perinuclear structure positive for the anti-$G\alpha_z$ antibody most likely corresponds to the Golgi apparatus, because it was also stained by medial Golgi marker Man II (Fig. 9b,d). The staining with the anti-$G\alpha_z$ antibody is specific because it was totally abolished when the antibody was preincubated with the peptide used for immunization (Fig. 9e,g). Similar Golgi labeling patterns for $G\alpha_z$ were detected for other cells including PC12, Chinese hamster ovary, and NRK cells (data not shown). To confirm that the perinuclear $G\alpha_z$ staining reflects the Golgi structure, we treated cells with brefeldin A (BFA). BFA is known to cause the redistribution of Golgi-resident proteins to the ER (Klausner et al., 1992). The perinuclear $G\alpha_z$ staining as well as the Golgi marker Man II staining was dispersed upon the treatment of cells with BFA (Fig. 9i-l), suggesting that endogenous $G\alpha_z$ is located in the Golgi apparatus.

Expression of $G\alpha_z$ was also assessed by semi-quantitative RT-PCR analysis (Fig. 10). mRNA for $G\alpha_z$ was expressed in non-neuronal Clone9 and NRK cells, although the neuron-like PC12 cells showed much higher expression. The control GAPDH mRNA level was approximately the same in these samples. The ubiquitous expression of $G\alpha_z$ is consistent with the recent observation that $G\alpha_z$ is detectable in various tissues of mouse (Hendry et al., 2000).

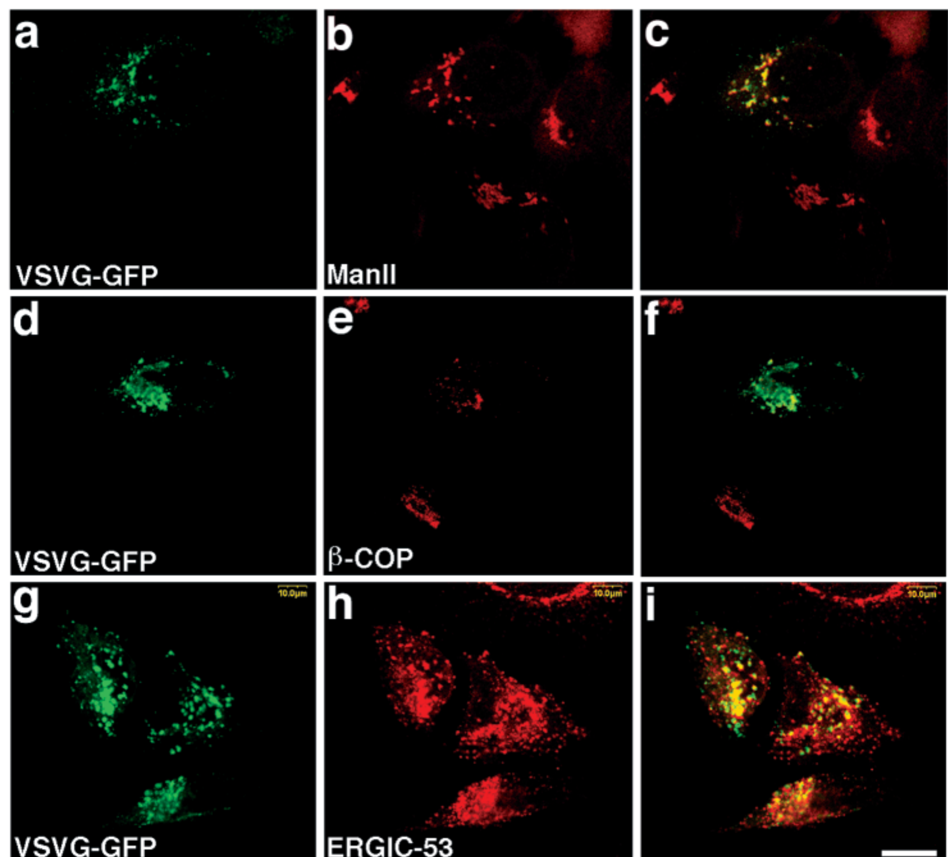


Fig. 6. Comparison of the distribution of VSVG-GFP with Golgi-resident proteins. BHK cells (a-c) or HeLa cells (d-i) were co-transfected with expression plasmids for VSVG-GFP and FLAG-RGSZ1. The cells were incubated for 20 hours at 40°C, and then the temperature was changed to 32°C. After 1 hour at 32°C, the cells were fixed and then stained with antibodies to Man II (b), β-COP (e), or ERGIC-53 (h). Merged images are presented on the right (c,f,i). Bar, 10 μm.

Discussion

We and others previously demonstrated that the Golgi apparatus is disassembled by NDGA (Yamaguchi et al., 1997; Fujiwara et al., 1998; Drecktrah et al., 1998), and that this process is blocked by the addition of various G protein activators (Yamaguchi et al., 1997). Furthermore, overexpression of $G\alpha_{i2}$ or $G\alpha_z$ attenuates NDGA-induced Golgi dispersal (Yamaguchi et al., 2000). However, these results were obtained under artificial conditions in which the Golgi apparatus was perturbed by NDGA. Therefore, the physiological implication of these findings remains to be examined. Here, we addressed the possibility that $G\alpha_z$ is involved in the organization of the Golgi apparatus by examining the effects of RGS proteins and a dominant-negative $G\alpha_z$ mutant on the morphology of the Golgi apparatus.

When $G\alpha_z$-selective GAPs such as RGSZ1 (Glick et al., 1998; Wang et al., 1998) and RGSZ2 were expressed, various Golgi-resident proteins including β-COP and Man II showed dispersed distribution patterns in several types of cells. In contrast, the structures of the ER and microtubules were not markedly affected by the expression of these proteins. No significant Golgi disassembly was observed when RGS

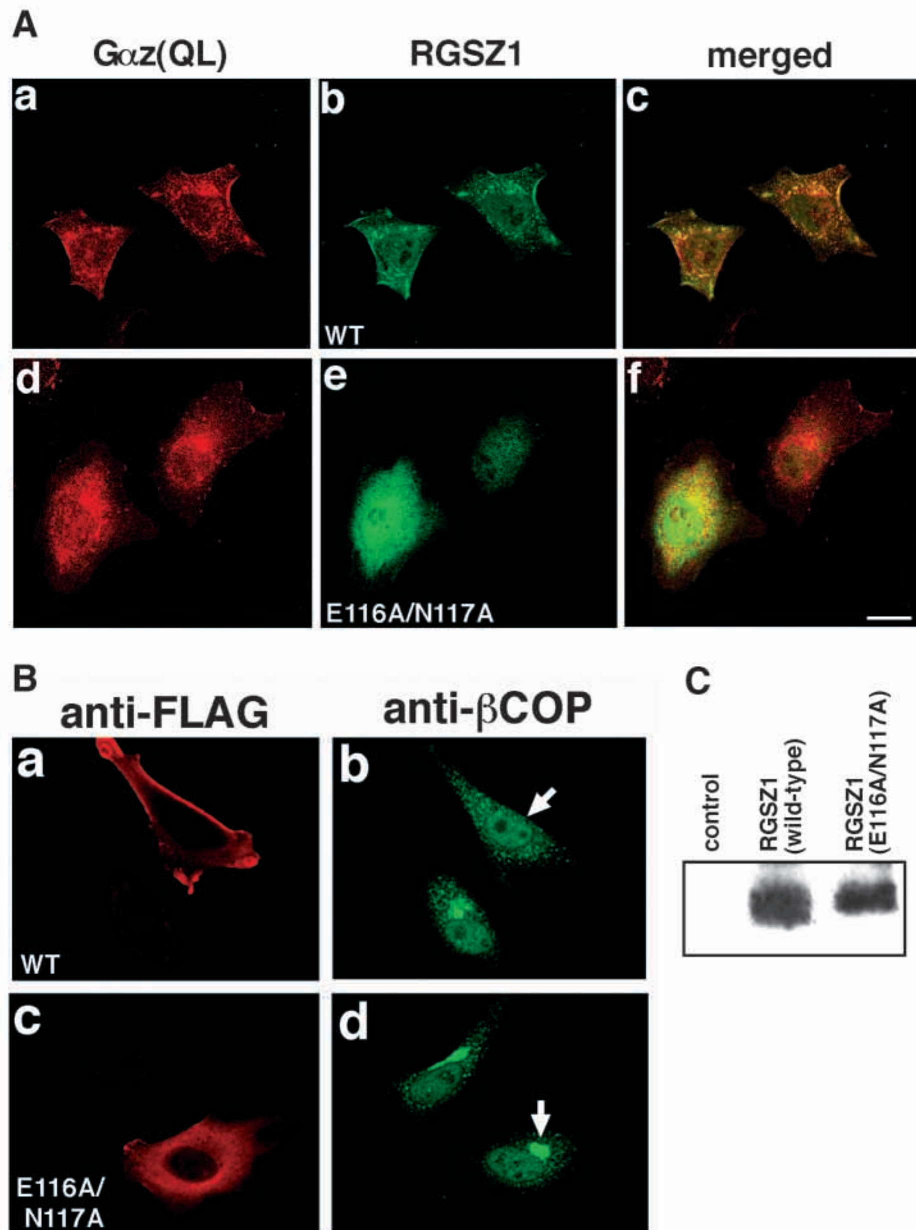


Fig. 7. Disruption of the Golgi apparatus by RGSZ1 depends on its $G\alpha_z$ binding and GAP function. (A) Lack of the interaction of the mutated RGSZ1 with $G\alpha_z$(QL). The wild-type FLAG-RGSZ1 or E116A/N117A mutant was co-expressed with $G\alpha_z$(QL) in HeLa cells. At 15 hours after transfection, the cells were fixed, and then double-stained with the anti-$G\alpha_z$ antibody (a,d) and anti-FLAG antibody (b,e). Merged images are presented on the right (c,f). Bar, 20 μm. (B) HeLa cells transfected with wild-type RGSZ1 (WT; a,b) or mutant RGSZ1 cDNA (E116A/N117A; c,d) were double-stained for FLAG (a,c) and β-COP (b,d). Arrows indicate RGSZ1-expressing cells. (C) Western blot of extracts of cells expressing the wild-type FLAG-RGSZ1 or E116A/N117A mutant. The cell extracts were subjected to SDS-polyacrylamide gel electrophoresis followed by western blotting using the anti-FLAG antibody. Bands were detected using enhanced chemiluminescence reagents.

proteins other than RGSZ1 and RGSZ2 were expressed. These results suggest that the inactivation of $G\alpha_z$ but not other Gα proteins, such as $G\alpha_{i2}$, induces Golgi disassembly. Chatterjee and Fisher demonstrated that RGSZ1, when expressed in COS-7 cells, is localized in the Golgi apparatus (Chatterjee and Fisher, 2000). In our experiments, expressed RGSZ1 was mainly distributed throughout the cytoplasm and nucleus. This discrepancy can be partly explained by the use of different cell lines. We also observed Golgi-like perinuclear localization of exogenously expressed RGSZ1 in COS-7 cells. However, similar to in other cell lines, a dispersed pattern of a Golgi marker β-COP was observed in cells expressing RGSZ1 at a high level (data not shown).

The idea that inactivation of $G\alpha_z$ induces disassembly of the Golgi apparatus was supported by another finding with the use of a dominant-negative mutant, $G\alpha_z$(G204A/E246A/A327S). This mutant was designed in analogy with a dominant-negative $G\alpha_s$ mutant in which three conserved residues are simultaneously mutated (Iiri et al., 1999). Since this type of mutant remains in a guanine nucleotide-free form, it can inhibit Gα-mediated signaling, probably by occupying activated receptors. As expected, overexpression of the triple $G\alpha_z$ mutant induced Golgi disassembly.

Although $G\alpha_z$ is formally a member of the G_i family, it possesses several unique biochemical properties distinct from those of other $G\alpha_i$ members (Fields and Casey, 1997). One unique character of $G\alpha_z$ is its insensitivity to pertussis toxin-mediated ADP-ribosylation (Casey et al., 1990), a modification that inactivates other members of the G_i family. In addition to general G-protein activators such as GTPγS and AlF_4^-, mastparan, a peptide that selectively activates the G_i family, also blocked the NDGA-induced Golgi disassembly (data not shown). In fact, the overexpression of either $G\alpha_z$ or $G\alpha_{i2}$, both of which belong to the G_i family, has a similar protective effect

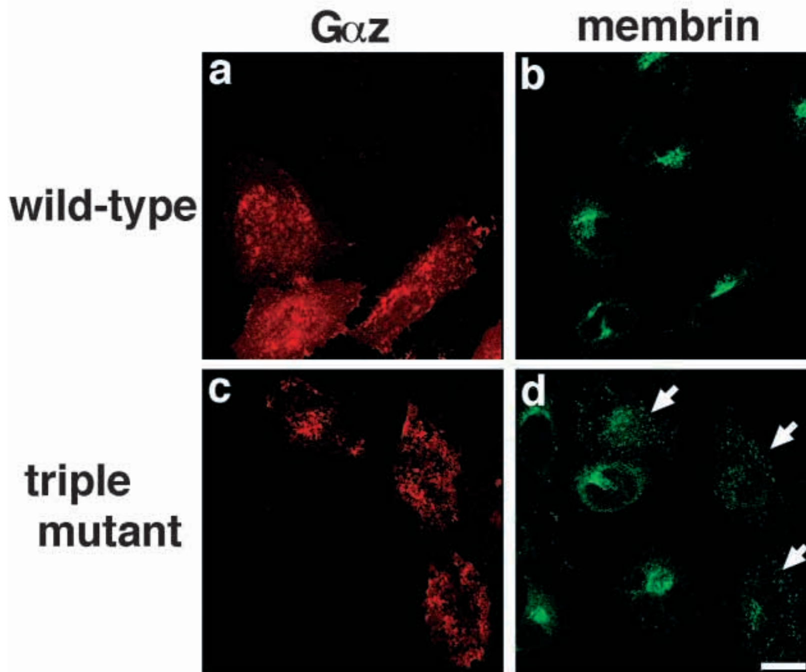


Fig. 8. Expression of a dominant-negative $G\alpha_z$ results in Golgi disruption. HeLa cells were transfected with wild-type $G\alpha_z$ (a,b) or a triple mutant (G204A/E246A/A327S) (c,d). The cells were allowed to express $G\alpha_z$ for 20 hours, fixed, and then double-stained with the anti-$G\alpha_z$ antibody (a,c) and anti-membrin antibody (b,d). Arrows indicate cells overexpressing the mutated $G\alpha_z$ with a dispersed Golgi apparatus. Bar, 20 μm.

against NDGA-induced Golgi disassembly (Yamaguchi et al., 2000). However, treatment of cells with pertussis toxin did not affect the Golgi morphology (data not shown), suggesting the involvement of a pertussis toxin-insensitive Gα, i.e. $G\alpha_z$, in the maintenance of the Golgi structure. The fact that RGS3 and RGS4, both of which can attenuate $G\alpha_i$ activity, lack Golgi disassembly activity is consistent with this idea. Although overexpression of both $G\alpha_{i2}$ and $G\alpha_z$ can block NDGA-induced Golgi disassembly, $G\alpha_z$ may be involved in the maintenance of Golgi structure under physiological conditions.

Based on the results reported here, we envisage a mechanism by which $G\alpha_z$ controls the structure of the Golgi apparatus. When $G\alpha_z$ binds GTP, it activates a signaling cascade that is required for maintenance of the Golgi structure. When $G\alpha_z$ is inactivated upon the hydrolysis of bound GTP, the Golgi structure undergoes disassembly as a consequence of loss of the signaling. Thus, $G\alpha_z$ functions as a molecular switch that organizes the Golgi structure. This hypothesis predicts that $G\alpha_z$ preferentially binds GTP, thereby being constitutively active, in interphase cells. From this point of view, $G\alpha_z$ seems to be a favorable G protein because it exhibits a very slow intrinsic rate of GTP hydrolysis. Its *k* cat value is 200-fold lower than those of $G\alpha_s$ and $G\alpha_i$ (Casey et al., 1990).

Investigation of the mechanism by which $G\alpha_z$ maintains the Golgi structure is a future challenge. The Golgi disassembly induced by inactivation of $G\alpha_z$ does not appear to involve fast release of coat proteins (data not shown), as seen in cells treated with BFA. In addition, expression of RGSZ1 does not affect microtubule organization. $G\alpha_z$ may act on Golgi stacking proteins or factors that can link this organelle to the cytoskeletal elements. However, our results do not exclude the possibility that $G\alpha_z$ may control the export of secretory and Golgi-resident proteins from the ER. The transport of VSVG-

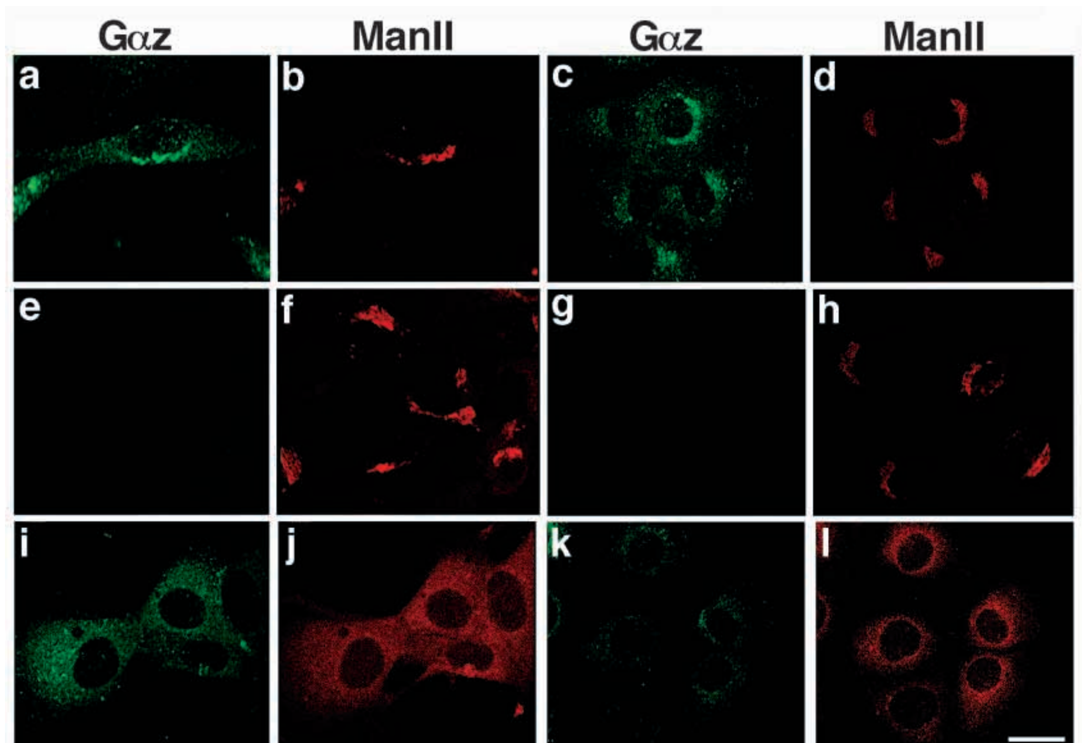


Fig. 9. Golgi localization of $G\alpha_z$. BHK cells (a-j) and Clone9 cells (c-l) were fixed and then subjected to fluorescence microscopic analysis. The rabbit polyclonal anti-Gαz antibody and mouse monoclonal anti-Man II antibody were used to visualize $G\alpha_z$ (a,c,e,g,i,k) and Man II (b,d,f,h,j,l), respectively. In panels e-h, the anti-$G\alpha_z$ antibody was preincubated with the peptide used for immunization. In panels i to l, cells were preincubated with 10 μM BFA for 10 minutes (i,j) or 30 minutes (k,l) before fixation.

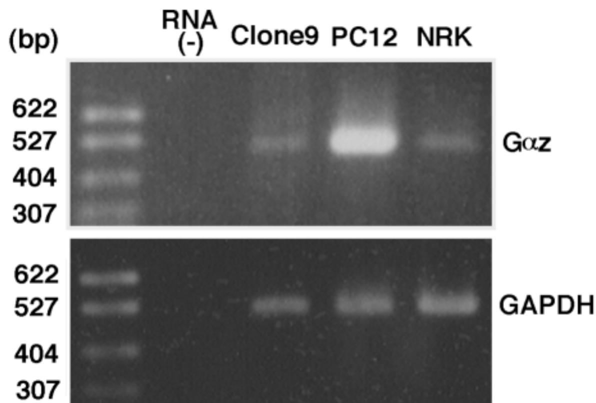


Fig. 10. Semi-quantitative RT-PCR analysis of $G\alpha_z$ mRNA from cultured cells. Total cellular RNA (1 μg) was subjected to the RT-PCR reaction, representative ethidium bromide-stained gels of the $G\alpha_z$ and GAPDH RT-PCR products being shown. Size standards are indicated at the left.

GFP was partly inhibited in RGSZ1-expressing cells. Although our antibody failed to detect it, a portion of $G\alpha_z$ may exist in the ER.

Our present view that active $G\alpha_z$ is required for maintenance of the Golgi apparatus does not necessarily contradict the finding by Malhotra and colleagues that the βγ dimer causes Golgi disassembly (Jamora et al., 1997; Jamora et al., 1999). A similar situation was observed for trafficking from the ER to the Golgi apparatus. The addition of βγ dimer, which leads to inactivation of active Gα by shifting the equilibrium toward the formation of the trimeric complex, inhibits the formation of vesicles from the ER in semi-intact cells. Mastparan, an activator for heterotrimeric G proteins, also blocks vesicle formation (Schwaninger et al., 1992). The presence of multispecies of G proteins in organelles may account for these intricate and apparently inconsistent observations. It should be noted that the expression of $G\alpha_z$ in non-neuronal cells is not high. The amount of the βγ dimer released as a consequence of the activation of $G\alpha_z$ must be very small, and therefore may not be enough to cause Golgi disassembly. On the other hand, activation of major G proteins may yield a large amount of the βγ dimer, which results in disassembly of the Golgi apparatus. G_i family proteins may be more widely involved in the regulation of organelle structures in early secretory pathways. Wang et al. reported that treatment of rat hepatocytes with pertussis toxin caused the redistribution and fragmentation of the ER (Wang et al., 2000). Thus, different G proteins may be involved in more intricate cellular functions than presently assumed.

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References

Aridor, M., Rajmilevich, G., Beaven, M. A. and Sagi-Eisenberg, R. (1993). Activation of exocytosis by the heterotrimeric G protein G_{i3}. *Science* **262**, 1569-1572.

Berman, D. M. and Gilman, A. G. (1998). Mammalian RGS proteins: barbarians at the gate. *J. Biol. Chem.* **273**, 1269-1272.

Berman, D. M., Wilkie, T. M. and Gilman, A. G. (1996). GAIP and RGS4 are GTPase-activating proteins for the G_i subfamily of G protein α subunits. *Cell* **86**, 445-452.

Casey, P. J., Fong, H. K. W., Simon, M. I. and Gilman, A. G. (1990). G_z, a guanine nucleotide-binding protein with unique biochemical properties. *J. Biol. Chem.* **265**, 2383-2390.

Chatterjee, T. K. and Fisher, R. A. (2000). Cytoplasmic, nuclear, and Golgi localization of RGS proteins. Evidence for N-terminal and RGS domain sequences as intracellular targeting motifs. *J. Biol. Chem.* **275**, 24013-24021.

De Vries, L., Mousli, M., Wurmser, A. and Farquhar, M. G. (1995). GAIP, a protein that specifically interacts with the trimeric G protein G α_{i3}, is a member of a protein family with a highly conserved core domain. *Proc. Natl. Acad. Sci. USA* **92**, 11916-11920.

De Vries, L., Zheng, B., Fischer, T., Elenko, E. and Farquhar, M. G. (2000). The regulator of G protein signaling family. *Annu. Rev. Pharmacol. Toxicol.* **40**, 235-271.

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.

Drecktrah, D., de Figueiredo, P., Mason, R. M. and Brown, W. J. (1998). Retrograde trafficking of both Golgi complex and TGN markers to the ER induced by nordihydroguaiaretic acid and cyclofenil diphenol. *J. Cell Sci.* **111**, 951-965.

Druey, K. M. and Kehrl, J. H. (1997). Inhibition of regulator of G protein signaling function by two mutant RGS4 proteins. *Proc. Natl. Acad. Sci. USA* **94**, 12851-12856.

Fields, T. A. and Casey, P. J. (1997). Signalling functions and biochemical properties of pertussis toxin-resistant G-proteins. *Biochem. J.* **321**, 561-571.

Fujiwara, T., Takami, N., Misumi, Y. and Ikehara, Y. (1998). Nordihydroguaiaretic acid blocks protein transport in the secretory pathway causing redistribution of Golgi proteins into the endoplasmic reticulum. *J. Biol. Chem.* **273**, 3068-3075.

Glick, J. L., Meigs, T. E., Miron, A. and Casey, P. J. (1998). RGSZ1, a G_z-selective regulator of G protein signaling whose action is sensitive to the phosphorylation state of $G_z\alpha$. *J. Biol. Chem.* **273**, 26008-26013.

Hamm, H. E. (1998). The many faces of G protein signaling. *J. Biol. Chem.* **273**, 669-672.

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

Hendry, I. A., Kelleher, K. L., Bartlett, S. E., Leck, K. J., Reynolds, A. J., Heydon, K., Mellick, A., Megirian, D. and Matthaei, K. I. (2000). Hypertolerance to morphine in $G_{z\alpha}$-deficient mice. *Brain Res.* **870**, 10-19.

Hepler, J. R. (1999). Emerging roles for RGS proteins in cell signalling. *Trends Pharmacol. Sci*. **20**, 376-382.

Heximer, S. P., Watson, N., Linder, M. E., Blumer, K. J. and Hepler, J. R. (1997). RGS2/G0S8 is a selective inhibitor of $G_q\alpha$ function. *Proc. Natl. Acad. Sci. USA* **94**, 14389-14393.

Hidalgo, J., Muniz, M. and Velasco, A. (1995). Trimeric G proteins regulate the cytosol-induced redistribution of Golgi enzymes into the endoplasmic reticulum. *J. Cell Sci.* **108**, 1805-1815.

Hinton, D. R., Blanks, J. C., Fong, H. K., Casey, P. J., Hildebrandt, E. and Simons, M. I. (1990). Novel localization of a G protein, Gz-alpha, in neurons of brain and retina. *J. Neurosci*. **10**, 2763-2770.

Iiri, T., Bell, S. M., Baranski, T. J., Fujita, T. and Bourne, H. R. (1999). A $G_{s\alpha}$ mutant designed to inhibit receptor signaling through G_s. *Proc. Natl. Acad. Sci. USA* **96**, 499-504.

Jamora, C., Takizawa, P. A., Zaarour, R. F., Denesvre, C., Faulkner, D. J. and Malhotra, V. (1997). Regulation of Golgi structure through heterotrimeric G proteins. *Cell* **91**, 617-626.

Jamora, C., Yamanouye, N., Lint, J. V., Laudenslager, J., Vandenheede, J. R., Faulkner, D. J. and Malhotra, V. (1999). Gβγ-mediated regulation of Golgi organization is through the direct activation of protein kinase D. *Cell* **98**, 59-68.

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.

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.

Lang, J. (1999). Molecular mechanism and regulation of insulin exocytosis as a paradigm of endocrine secretion. *Eur. J. Biochem*. **259**, 3-17.

Leyte, A., Barr, F. A., Kehlenbach, R. H. and Huttner, W. B. (1992). Multiple trimeric G-proteins on the trans-Golgi network exert stimulatory and inhibitory effects on secretory vesicle formation. *EMBO J*. **11**, 4795-4804.

Lippincott-Schwartz, J. (1998). Cytoskeletal proteins and Golgi dynamics. *Curr. Opin. Cell Biol.* **10**, 52-59.

Matsuoka, M., Itoh, H., Kozasa, T. and Kaziro, Y. (1988). Sequence analysis of cDNA and genomic DNA for a putative pertussis toxin-insensitive guanine nucleotide-binding regulatory protein α subunit. *Proc. Natl. Acad. Sci. USA* **85**, 5384-5388.

Neer, E. J. (1995). Heterotrimeric G proteins: organizers of transmembrane signals. *Cell* **80**, 249-257.

Nelson, W. J. (2000). W(h)ither the Golgi during mitosis? *J. Cell Biol*. **149**, 243-248.

Ogier-Denis, E., Houri, J.-J., Bauvy, C. and Codogno, P. (1996). Guanine nucleotide exchange on heterotrimeric G_{i3} protein controls autophagic sequestration in HT-29 cells. *J. Biol. Chem*. **271**, 28593-28600.

Ohara-Imaizumi, M., Kameyama, K., Kawae, N., Takeda, K., Muramatsu, S. and Kumakura, K. (1992). Regulatory role of the GTP-binding protein, G_o, in the mechanism of exocytosis in adrenal chromaffin cells. *J. Neurochem*. **58**, 2275-2284.

Ohnishi, H., Ernst, S. A., Yule, D. I., Baker, C. W. and Williams, J. A. (1997). Heterotrimeric G-protein $G_{q/11}$ localized on pancreatic zymogen granules is involved in calcium-regulated amylase secretion. *J. Biol. Chem*. **272**, 16056-16061.

Palmer, D. J., Helms, J. B., Beckers, C. J., Orci, L. and Rothman, J. E. (1993). Binding of coatomer to Golgi membranes requires ADP-ribosylation factor. *J. Biol. Chem.* **268**, 12083-12089.

Pimplikar, S. W. and Simons, K. (1993). Regulation of apical transport in epithelial cells by a G_s class of heterotrimeric G protein. *Nature* **362**, 456-458.

Presley, J. F., Cole, N. B., Schroer, T. A., Hirschberg, K., Zaal, K. J. and Lippincott-Schwartz, J. (1997). ER-to-Golgi transport visualized in living cells. *Nature* **389**, 81-85.

Scheschonka, A., Dessauer, C. W., Sinnarajah, S., Chidiac, P., Shi, C. S. and Kehrl, J. H. (2000). RGS3 is a GTPase-activating protein for $G_{i\alpha}$ and $G_{q\alpha}$ and a potent inhibitor of signaling by GTPase-deficient forms of $G_{q\alpha}$ and $G_{11\alpha}$. *Mol. Pharmacol*. **58**, 719-728.

Schwaninger, R., Plutner, H., Bokoch, G. M. and Balch, W. E. (1992). Multiple GTP-binding proteins regulate vesicular transport from the ER to Golgi membranes. *J. Cell Biol*. **119**, 1077-1096.

Shima, D. T., Cabrera-Poch, N., Pepperkok, R. and Warren, G. (1998). An ordered inheritance strategy for the Golgi apparatus: visualization of mitotic disassembly reveals a role for the mitotic spindle. *J. Cell Biol*. **141**, 955-966.

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

Srinivasa, S. P., Watson, N., Overton, M. C. and Blumer, K. J. (1998). Mechanism of RGS4, a GTPase-activating protein for G protein α subunits. *J. Biol. Chem*. **273**, 1529-1533.

Tagaya, M., Furuno, A. and Mizushima, S. (1996). SNAP prevents Mg^{2+}-ATP-induced release of *N*-ethylmaleimide-sensitive factor from the Golgi apparatus in digitonin-permeabilized PC12 cells. *J. Biol. Chem*. **271**, 466-470.

Thyberg, J. and Moskalewski, S. (1999). Role of microtubules in the organization of the Golgi complex. *Exp. Cell Res.* **246**, 263-279.

Vitale, N., Mukai, H., Rouot, B., Thierse, D., Aunis, D. and Bader, M.-F. (1993). Exocytosis in chromaffin cells. Possible involvement of the heterotrimeric GTP-binding protein G_o. *J. Biol. Chem*. **268**, 14715-14723.

Wang, J., Ducret, A., Tu, Y., Kozasa, T., Aebersold, R. and Ross. E. M. (1998). RGSZ1, a G_z-selective RGS protein in brain. Structure, membrane association, regulation by $G\alpha_z$ phosphorylation, and relationship to a G_z GTPase-activating protein subfamily. *J. Biol. Chem*. **273**, 26014-26025.

Wang, Y.-J., Gregory, R. B. and Barritt, G. J. (2000). Regulation of F-actin and endoplasmic reticulum organization by the trimeric G-protein G_{i2} in rat hepatocytes. Implication for the activation of store-operated Ca^{2+} inflow. *J. Biol. Chem*. **275**, 22229-22237.

Warren, G. and Malhotra. V. (1998). The organisation of the Golgi apparatus. *Curr. Opin. Cell Biol*. **10**, 493-498.

Watson, N., Linder, M. E., Druey, K. M., Kehrl, J. H. and Blumer, K. J. (1996). RGS family members: GTPase-activating proteins for heterotrimeric G-protein α-subunits. *Nature* **383**, 172-175.

Yamaguchi, T., Yamamoto, A., Furuno, A., Hatsuzawa, K., Tani, K., Himeno, M. and Tagaya, M. (1997). Possible involvement of heterotrimeric G proteins in the organization of the Golgi apparatus. *J. Biol. Chem*. **272**, 25260-25266.

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

Zaal, K. J. M., Smith, C. L., Polishchuk, R. S., Altan, N., Cole, N. B., Ellenberg, J., Hirschberg, K., Presley, J. F., Roberts, T. H., Siggia, E., Phair, R. D. and Lippincott-Schwartz, J. (1999). Golgi membranes are absorbed into and reemerge from the ER during mitosis. *Cell* **99**, 589-601.