Exocytic pathway-independent plasma membrane targeting of heterotrimeric G proteins

Satoshi Takida, Philip B. Wedegaertner*

Department of Microbiology and Immunology, Kimmel Cancer Center, Thomas Jefferson University, 233 S. 10th St., 839 BLSB, Philadelphia, PA 19107, USA

Received 19 March 2004; revised 21 April 2004; accepted 22 April 2004

Available online 4 May 2004

Edited by Felix Wieland

Abstract **Heterotrimeric G proteins are lipid-modified, peripheral membrane proteins that function at the inner surface of the plasma membrane (PM) to relay signals from cell-surface receptors to downstream effectors. Cellular trafficking pathways that direct nascent G proteins to the PM are poorly defined. In this report, we test the proposal that G proteins utilize the classical exocytic pathway for PM targeting. PM localization of the G protein heterotrimers $\alpha_s\beta_1\gamma_2$ and $\alpha_q\beta_1\gamma_2$ occurred independently of treatment of cells with Brefeldin A, which disrupts the Golgi, or expression of Sar1 mutants, which prevent the formation of endoplasmic reticulum to Golgi transport vesicles. Moreover, the palmitoylation of α_q was unaffected by Brefeldin A treatment, even though the palmitoylation of SNAP25 was blocked by Brefeldin A. Non-palmitoylated mutants of α_s and α_q failed to stably bind to $\beta\gamma$ and displayed a dispersed cytoplasmic localization when co-expressed with $\beta\gamma$. These findings support a refined model of the PM trafficking pathway of G proteins, involving assembly of the heterotrimer at the endoplasmic reticulum and transport to the PM independently of the Golgi.**

Keywords: Heterotrimeric G protein; Palmitoylation; Exocytic pathway; Golgi; Membrane localization; Trafficking

1. Introduction

Heterotrimeric G proteins ($\alpha\beta\gamma$) need to be tethered to the inner leaflet of the plasma membrane (PM) where they are coupled to receptors and relay signals to downstream effectors. Numerous studies have identified requirements for PM localization of the α and $\beta\gamma$ subunits. Lipid modification, namely myristoylation and/or palmitoylation for α and isoprenylation for $\beta\gamma$, is an essential membrane targeting signal [1]. In addition, both α and $\beta\gamma$ require interaction with each other for efficient PM localization [2–4]. Much less well understood is a cellular trafficking pathway by which the G protein subunits reach the PM after synthesis. The $\beta\gamma$ complex localizes at the endoplasmic reticulum (ER) when expressed without α, while co-expression of α leads to strong PM localization of $\alpha\beta\gamma$ [2,5]. These results and others have suggested a model whereby α and $\beta\gamma$ interact to form a heterotrimer at an endomembrane location before reaching the PM, with the ER as an essential component of the trafficking pathway. An unresolved question, however, is whether heterotrimeric G proteins traffic to the PM via the Golgi apparatus using the classical secretory/exocytic pathway.

The Golgi apparatus is a highly polarized compartment where proteins from the ER are further processed, sorted, packed, and released to a specific subcellular location. Although most integral membrane and secreted proteins require the Golgi for proper transport, both Golgi-dependent and Golgi-independent trafficking has been described for lipid-modified, peripheral membrane proteins. Sensitivity to brefeldin A (BFA) has implicated the Golgi complex in PM targeting and/or palmitoylation of some peripheral membrane-bound proteins [6]. For instance, SNAP25, a synaptic SNARE protein, requires a functional exocytic pathway, and its transport to the PM and palmitoylation are blocked by BFA [7]. On the other hand, PM targeting but not palmitoylation of the tyrosine kinase Lck was inhibited by BFA treatment [8]. Other proteins bypass Golgi as exemplified by the tyrosine kinase Fyn whose PM targeting and palmitoylation are unaffected by BFA [9]. Thus, it appears that multiple cellular pathways exist for targeting peripheral membrane proteins to the PM. For G protein α subunits of the α_i family, which undergo both myristoylation and palmitoylation, the effect of BFA has been studied to some extent. PM localization of transfected α_z was not blocked by BFA [3], and neither was palmitoylation of endogenous α_i and α_o [7]. Relatively little is known about the requirement of a functional Golgi complex in PM targeting of $\beta\gamma$, and for PM targeting and palmitoylation of α subunits, such as α_s and α_q, that are palmitoylated but not myristoylated.

In the present study, we investigated a trafficking pathway for the G protein heterotrimers $\alpha_s\beta_1\gamma_2$ and $\alpha_q\beta_1\gamma_2$, focusing on the potential involvement of the Golgi. PM targeting of both the α and $\beta\gamma$ subunits was insensitive to BFA. We also demonstrate that dominant negative mutants of Sar1, a GTPase required for the formation of vesicles that move from the ER to Golgi, blocked PM localization of a G protein-coupled receptor but failed to prevent $\alpha_s\beta_1\gamma_2$ and $\alpha_q\beta_1\gamma_2$ from traveling to the PM. Furthermore, palmitoylation of α_q occurs even in the presence of BFA treatment, while palmitoylation of SNAP25 requires an intact Golgi in HEK293 cells. Lastly, palmitoylation-deficient mutants of α_s and α_q failed to form a

* Corresponding author. Fax: +1-215-923-2117.
E-mail address: P_Wedegaertner@mail.jci.tju.edu (P.B. Wedegaertner).

Abbreviations: G protein, guanine nucleotide binding protein; BFA, Brefeldin A; ER, endoplasmic reticulum; PM, plasma membrane

0014-5793/$22.00
doi:10.1016/j.febslet.2004.04.062

stable complex with βγ consistent with the proposal that palmitoylation of α occurs early in the trafficking pathway and is required for heterotrimer formation.

2. Materials and methods

2.1. Cell culture

HEK293 and COS7 cells were obtained from the American Type Culture Collection (Manassas, VA) and grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum and maintained at 37 °C in a 95% air, 5% CO_2-humidified atmosphere.

2.2. Constructs

Wild type α_s (HA-tagged) or β_1 (myc- and His-tagged) in pcDNA3.1 and non-tagged γ_2 in pcDNA3 were described previously. The expression vector for vsvg-tagged Sar1T39N was provided by Stephen J. Gould (Johns Hopkins University). Sar1H79G (vsvg-tagged) was created from Sar1T39N using QuickChange site-directed mutagenesis kit (Stratagene, La Jolla, CA). pEGFP-C1 containing p230-C98 was given by Paul A. Gleeson (Monash University). The SNAP-25/GFP/myc construct was given by Maurine E. Linder (Washington University). The β-AR construct was provided by Jeffery L. Benovic (Kimmel Cancer Center).

2.3. Transfection

Unless otherwise noted, cells were seeded 1 day before transfection. An indicated amount of DNA constructs was transfected into cells using FuGene 6 (Roche, Indianapolis, IN) according to the manufacturer's protocol.

2.4. Antibodies

The anti-HA mouse monoclonal antibody 12CA5 was from Roche and the anti-Myc mouse monoclonal antibody 9E10 was from Covance (Berkeley, CA). The anti-β_2-AR polyclonal antibody was provided by Mark von Zastrow (University of California, San Francisco). Anti-vsvg polyclonal and monoclonal P5D4 antibodies were from Sigma (St. Louise, MO). Goat anti-rabbit or goat anti-mouse antibodies conjugated with either Alexa 488 or Alexa 594 was from Molecular Probes (Eugene, OR).

2.5. Immunofluorescence microscopy

Cells were fixed with 3.7% formaldehyde in phosphate-buffered saline (PBS) for 15 min and permeabilized by incubation in blocking buffer (2.5% non-fat milk and 1% Triton X-100 in TBS) for 20 min. Cells were then incubated with primary antibodies indicated in blocking buffer for 1 h. The cells were washed with blocking buffer and incubated in a 1:250 dilution of a goat anti-mouse or a goat anti-rabbit antibody conjugated with either Alexa 488 or Alexa 594 for 30 min. The coverslips were washed with 1% Triton X-100 in TBS, rinsed in distilled water, and mounted on glass slides with Prolong Antifade reagent (Molecular Probes, Eugene, OR). Microscopy was performed with an Olympus BX60 microscope and a Sony DKC-5000 digital camera, or using an Olympus BX61 microscope and an ORCA-ER (Hamamatsu, Bridgewater, NJ) cooled CCD camera controlled by Slidebook version 4.0 (Intelligent Imaging Innovations, Denver, CO). Images were transferred to Adobe Photoshop for digital processing.

2.6. BFA treatment

In order to determine the appropriate time to treat transfected cells with BFA, time-course fixation and immunostaining experiments were performed. We found that β_2-AR could be detected at the PM 16–18 h after transfection. However, when $\alpha_s\beta_1\gamma_2$ was expressed, PM localization of α_s and $\beta_1\gamma_2$ was observed 31–33 h after transfection. Based on these experiments, we added BFA to cells 12 or 27 h after transfection for β_2-AR or $\alpha_s\beta_1\gamma_2$, respectively. Cells were treated with BFA for 5 h before formaldehyde fixation.

2.7. Ni–NTA pull down of β1(γ2)

Taking advantage of the hexahistidine tagged amino terminus of β_1, Ni–NTA pull down of $\beta_1\gamma_2$ and associated α was carried out as described previously [2].

2.8. Palmitoylation assay

α_q in conjunction with $\beta_1\gamma_2$, or SNAP-25, was transfected into COS7 cells. 36 h after transfection, the cells were metabolically labeled with [^{3}H]palmitate for 3 h in the absence or presence of BFA (10 μg/ml) and then lysed. The cell lysate was subject to immunoprecipitation using 9E10 antibody for SNAP25 or Ni–NTA pull down for α_q. The samples were separated by SDS–PAGE and transferred onto polyvinylidene difluoride membrane. The membrane was sprayed with EnHance (Perkin–Elmer Life Science) and exposed to Hyperfilm MP (Amersham Biosciences) at −80 °C for 7–10 days. After fluorography, the protein was detected by immunoblotting using an antibody indicated.

3. Results

3.1. PM targeting of $\alpha_s\beta_1\gamma_2$ is insensitive to BFA treatment

To examine the involvement of the ER-Golgi pathway, we examined the effects of BFA on localization of the G protein heterotrimer $\alpha_s\beta_1\gamma_2$ in HEK293 cells. BFA, a metabolite of the fungus *Eupenicillium brefeldianum*, specifically causes a rapid disassembly and redistribution of the Golgi complex and has been used widely as a tool to demonstrate the involvement of the Golgi in a particular pathway or process. Fluorescence microscopy was utilized to locate the subcellular distribution of transfected proteins. A Golgi marker (pEGFP-p230-C98) displayed characteristic Golgi localization in the absence of BFA (Fig. 1a), while treatment of cells with BFA led to scattered distribution of the markers (Fig. 1b), indicating that BFA caused complete disassembly of the Golgi apparatus in HEK293 cells. We utilized the β_2-AR receptor as a control for

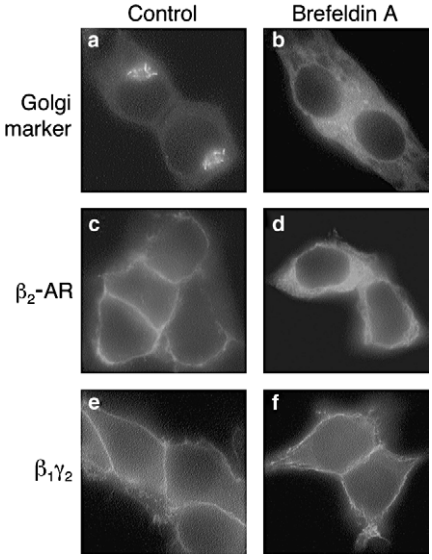

Fig. 1. BFA prevents PM localization of β_2-AR but not $\beta_1\gamma_2$. (a) and (b) HEK293 cells were transfected with 100 ng of a plasmid containing a GFP-tagged Golgi marker protein, p230-C98. 16 h after transfection, cells were incubated in the absence (a) and presence (b) of 10 μg/ml of BFA for 3 h and then fixed. (c) and (d) HEK293 cells transiently transfected (12 h) with an expression vector encoding β_2-AR (300 ng) were incubated in the absence (c) or presence (d) of 10 μg/ml of BFA for 5 h and then fixed. (e) and (f) Plasmids encoding β_1 (300 ng) and γ_2 (100 ng) were transfected into HEK293 cells in conjunction with pcDNA3 containing α_s (600 ng). 27 h after transfection, cells were incubated in the absence (e) or presence (f) of 10 μg/ml of BFA for 5 h and then fixed. Transfected proteins were visualized by immunofluorescence staining as described under Section 2 using a rabbit polyclonal anti-β_2-AR antibody for β_2-AR and a mouse monoclonal anti-myc 9E10 antibody for βγ.

the effect of BFA. As a transmembrane protein, β_2-AR should be targeted to the PM through the exocytic pathway, and thus its PM localization should be blocked by BFA. As expected, BFA efficiently blocked PM trafficking of β_2-AR (Figs. 1c and d). In contrast, BFA did not prevent PM localization of a G protein heterotrimer in HEK293 cells. $\beta_1\gamma_2$ transiently expressed in conjunction with α_s exhibited strong PM localization in the absence or presence of BFA (Figs. 1e and f). Likewise, PM localization of α_s was unaffected by BFA treatment (not shown).

3.2. Sar1-independent PM targeting of heterotrimeric G proteins

Multiple GTPases are known to regulate vesicular traffic in the exocytic pathway, and one of these, Sar1, plays a pivotal role in the formation of vesicles that ferry between the ER and Golgi. Both a GDP-restricted mutant, Sar1T39N, and a GTP-restricted mutant, Sar1H79G, function as dominant negatives to prevent vesicle trafficking between the ER and Golgi [10–12]. We tested the effects of these mutants in HEK293 cells transiently expressing the G protein heterotrimers $\alpha_s\beta_1\gamma_2$ or $\alpha_q\beta_1\gamma_2$. As a positive control, β_2-AR showed little or no PM localization when co-expressed with Sar1T39N or Sar1H79G (Figs. 2A, a–c). On the other hand, co-expression of Sar1T39N or Sar1H79G with $\alpha_s\beta_1\gamma_2$ or $\alpha_q\beta_1\gamma_2$ did not prevent PM localization of the heterotrimer. $\beta_1\gamma_2$ (Fig. 2A, f–h), α_s (Fig. 2B, a and b) and α_q (Fig. 2B, d and e) all retained efficient PM targeting in the presence of Sar1T39N or Sar1H79G. Likewise, in COS7 cells, SarT39N did not block PM localization of $\beta_1\gamma_2$, α_s and α_q (data not shown). Thus, the results in Figs. 1 and 2 suggest that at least some heterotrimeric G proteins utilize a PM trafficking pathway that is independent of the ER to Golgi portion of the classical exocytic pathway.

3.3. Palmitoylation of non-myristoylated α is insensitive to BFA

We next examined whether palmitoylation of α_q requires a functional Golgi. It has been shown that palmitoylation of both GAP-43 and SNAP25, which like α_q are modified only with palmitate, is sensitive to BFA [7]. Transfected cells were metabolically labeled with [^{3}H]palmitate in the absence or presence of BFA and the level of incorporated palmitate was visualized by fluorography. Consistent with earlier reports [7,13], palmitoylation of SNAP25 was greatly impaired by BFA treatment (Fig. 3, lane 2) and, as a matter of fact, lack of the modification resulted in a change of apparent molecular weight as previously described [7]. However, virtually no difference in incorporation of [^{3}H]palmitate into α_q (Fig. 3, lanes 3 and 4) was seen in the absence and presence of BFA, indicating palmitoylation of the non-myristoylated α subunits occurs independently of the ER-Golgi pathway.

3.4. Palmitoylation defective non-myristoylated α subunits are not targeted to the Golgi complex

To further examine a potential role for the Golgi in palmitoylation of G proteins, localization of a palmitoylation defective mutant of α_s and α_q was examined. It has been reported

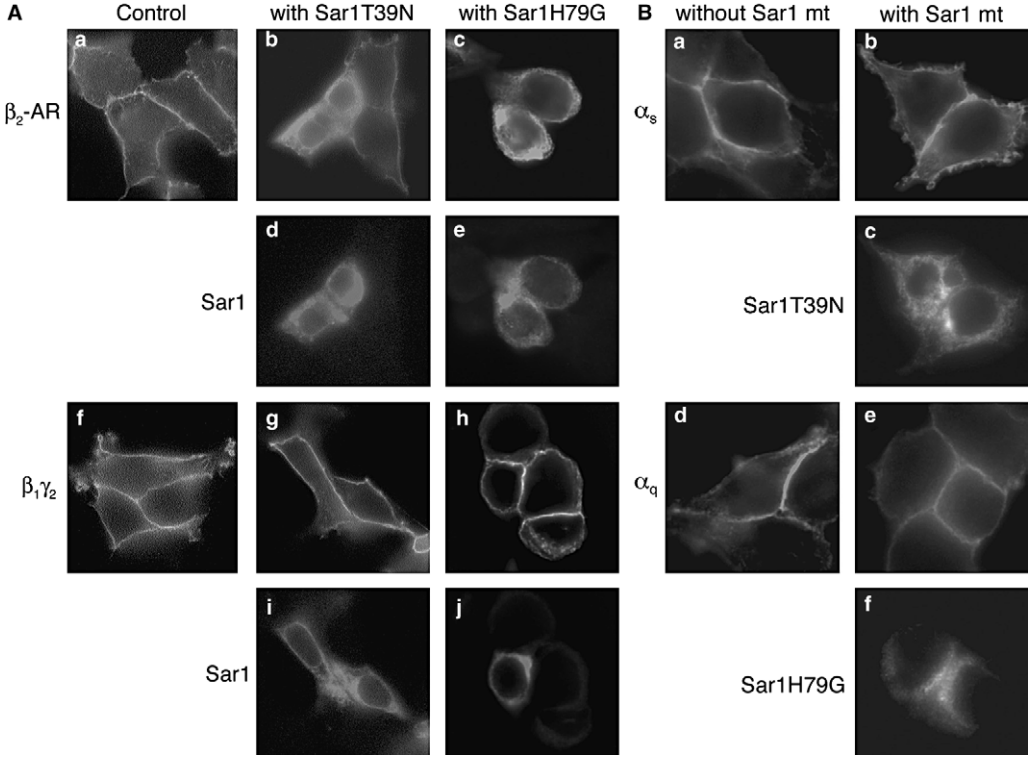

Fig. 2. Sar1 mutants prevent PM localization of β_2-AR but not heterotrimeric G proteins. (A) Expression vectors for β_2-AR (300 ng) (a–e) or α_s (600 ng), β_1 (300 ng), and γ_2 (100 ng) (f–j) were transfected into HEK293 cells in the absence (a and f) or presence of either pcDNA3 containing Sar1T39N (250 ng) (b, d, g, and i) or Sar1H79G (250 ng) (c, e, h, and j). (B) Expression vectors for α_s (600 ng), β_1 (300 ng), and γ_2 (100 ng) (a–c) or for α_q (600 ng), β_1 (300 ng), and γ_2 (100 ng) (d–f) were transfected into HEK293 cells in the absence (a and d) or presence of either pcDNA3 containing Sar1T39N (250 ng) (b and c) or Sar1H79G (250 ng) (e and f). 36 h after transfection, immunofluorescence staining was performed to visualize transiently expressed proteins using a rabbit polyclonal anti-β_2-AR antibody for β_2-AR, a monoclonal anti-myc 9E10 antibody for βγ, a monoclonal anti-HA 12CA5 antibody for α_s and α_q, and either a polyclonal or monoclonal anti-vsvg antibody for Sar1 mutants.

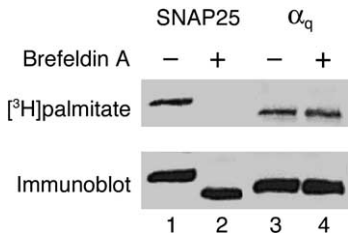

Fig. 3. BFA inhibits palmitoylation of SNAP25 but not α_q. COS7 cells were transfected with constructs for SNAP25 (600 ng) (lanes 1 and 2), or α_q (600 ng), β_1 (300 ng), and γ_2 (100 ng) (lanes 3 and 4). 30 h after transfection, cells were labeled for 3 h with 1 mCi/ml of [^{3}H]palmitate in the absence (lanes 1 and 3) or presence (lanes 2 and 4) of BFA (10 μg/ml) and lysed. SNAP25 was immunoprecipitated using the 9E10 monoclonal antibody and α_q bound to $\beta_1\gamma_2$ was pulled down with Ni–NTA magnetic agarose beads. The samples were separated by SDS–PAGE and transferred onto a polyvinylidene difluoride membrane. The PVDF membrane was exposed to a film at −80 °C (top panel). Subsequently, the membrane was subjected to Western blotting for SNAP25 and α_q (bottom panel).

that a palmitoylation deficient α_{i1} subunit, which retains incorporation of myristate, is localized at the Golgi when expressed with βγ. We tested whether the non-myristoylated α subunits are targeted to Golgi when their cysteine sites of palmitoylation are mutated. α_sC3S or α_qC9,10S, β_1 and γ_2 were transfected into HEK293 cells in conjunction with a Golgi marker and immunofluorescence staining was performed. In contrast to wild type α_s or α_q, which displayed prominent PM localization (Fig. 2B, a and d), α_sC3S or

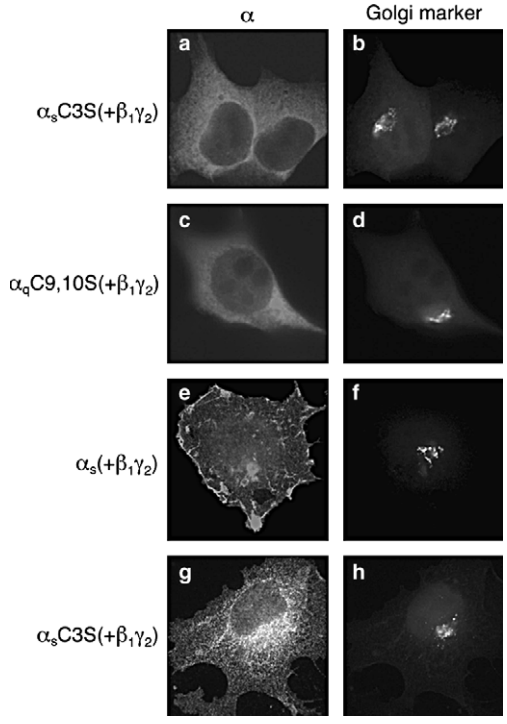

Fig. 4. Palmitoylation-deficient α subunits are not found at the Golgi. Expression vectors for α_sC3S (600 ng), β_1 (300 ng), and γ_2 (100 ng) (a, b, g, and h), α_qC9,10S (600 ng), β_1 (300 ng), and γ_2 (100 ng) (c and d), or α_s (600 ng), β_1 (300 ng), and γ_2 (100 ng) (e and f) were transfected into HEK293 cells (a–d) or COS7 cells (e–h) in conjunction with a Golgi marker, pEGFP-C1-p230-C98 (100 ng). Cells were fixed and the α subunits were visualized by immunofluorescence staining as described under Section 2. The 12CA5 anti-HA monoclonal antibody was used to probe for α_s or α_q.

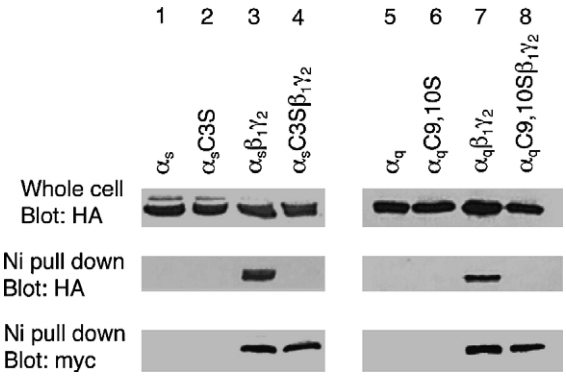

Fig. 5. βγ pull down of wild type and palmitoylation-deficient α subunits. Plasmids containing α_s (lane 1), α_sC3S (lane 2), α_s, β_1, and γ_2 (lane 3), α_sC3S, β_1, and γ_2 (lane 4), α_q (lane 5), α_qC9,10S (lane 6), α_q, β_1, and γ_2 (lane 7), or α_qC9,10S, β_1, and γ_2 (lane 8) were transfected into HEK293 cells. Transfection was carried out in 6-cm plates using 3 μg of total plasmid DNA at a 6:3:1 ratio of α:β:γ. 36 h after transfection, the cells were lysed and subjected to a Ni–NTA pull down assay. Note that the wild type or mutant of α_s or α_q was HA-tagged, while the β_1 subunit contained a myc and His tag. Whole cell lysates (top panel) and eluates were analyzed by SDS–PAGE and Western blotting for the α subunit (middle panel) using 12CA5 anti-HA monoclonal antibody or for the β subunit (bottom panel) using 9E10 anti-myc monoclonal antibody.

α_qC9,10S exhibited dispersed distribution throughout the cytoplasm and, moreover, neither of them was colocalized with the Golgi marker (Figs. 4a and c). Similarly, in COS7 cells, wild type α_s (Fig. 4e) or α_q (data not shown) was targeted to the PM, while palmitoylation defective α_s (Fig. 4g) or α_q (data not shown) exhibited a cytoplasmic and perinuclear distribution with no colocalization with the Golgi marker (Fig. 4h). Thus, the results of Figs. 3 and 4 suggest that the Golgi is not the site of palmitoylation of α_s or α_q.

3.5. βγ did not pull down non-palmitoylated α

Lastly, we looked at whether palmitoylation of α is required for assembly of a stable αβγ trimer. $\beta_1\gamma_2$, in which β_1 contains a hexahistidine tag, was expressed in conjunction with wild type or palmitoylation site mutants of α_s and α_q, and then $\beta_1\gamma_2$ was pulled down from cell lysates with Ni–NTA magnetic agarose beads. Immunoblotting of the pull down for the presence of α_s or α_q reveals the ability of the α subunit to stably bind $\beta_1\gamma_2$. Using this assay, wild type α_s (Fig. 5, lane 3) and wild type α_q (Fig. 5, lane 7) effectively interacted with $\beta_1\gamma_2$; however, neither α_sC3S (Fig. 5, lane 4) nor α_qC9,10S (Fig. 5, lane 8) could be detected in the pull down, indicating that the palmitoylation deficient mutants do not form a stable complex with $\beta_1\gamma_2$. In order to assure no non-specific binding to the beads, we carried out parallel experiments on HEK293 cells transiently expressing wild type or mutant α in the absence of βγ. No bands corresponding to α subunits were detected in these controls (Fig. 5, middle panel, lanes 1, 2, 5, and 6), thus verifying the assay. In addition, equivalent amounts of $\beta_1\gamma_2$ were pulled down in the different samples (Fig. 5, bottom panel, lanes 3, 4, 7, and 8).

4. Discussion

Data presented herein demonstrate that the expressed G protein heterotrimers $\alpha_s\beta_1\gamma_2$ and $\alpha_q\beta_1\gamma_2$ arrive at the PM independently of the classical exocytic pathway. Perturbants of traffic via this pathway had no effect on PM localization of the

α and βγ subunits and on palmitoylation of α. BFA did not prevent PM localization of βγ or α_s when expressed together, whereas it completely impeded that of β_2-AR (Fig. 1). Our result is in close agreement with the previous finding that BFA treatment did not prevent accumulation of newly synthesized βγ in a detergent insoluble fraction [14,15]. We here further demonstrated that dominant negative Sar1 mutants did not affect PM targeting of αβγ (Fig. 2). Collectively, the data with BFA and Sar1 mutants suggest that at least some G protein heterotrimers travel to the PM in a Golgi-independent fashion. The existence of a BFA-insensitive, Sar1-independent trafficking pathway in mammalian cells has been recently reported. One of the palmitoylated proteins in lipid rafts, flotillin-1/reggie-2, was found to traverse to the PM through such a Golgi-independent pathway [10]. In addition, several other palmitoylated proteins, including *Saccharomyces cerevisiae* Ras2p, which utilizes palmitate and a farnesyl group as membrane targeting signals, have been shown to reach the PM by a route other than the classical secretory pathway [6,16].

Consistent with Golgi-independent αβγ trafficking, we found that BFA treatment did not impede palmitoylation of non-myristoylated α, as exemplified by α_q (Fig. 3). This result agrees with a recent report indicating that palmitoylation of α_i, a subunit that undergoes both myristoylation and palmitoylation, is insensitive to BFA treatment [7]. The subcellular location(s) of palmitate attachment to α subunits has not been well defined. Identification of a relevant palmitoyltransferase will be a key component to further define the trafficking pathway of G proteins. One study defined a Gα palmitoyltransferase activity that was enriched in a PM preparation and demonstrated that the presence of βγ enhanced enzymatic palmitoylation of α [17]. These results led to proposals that heterotrimer assembly and palmitoylation occur at the PM. On the other hand, a palmitoyltransferase for yeast Ras2p has been recently localized to ER membranes [18], and a palmitoyltransferase for yeast casein kinase is found at the Golgi [19]. Clearly, palmitoylation of at least some peripheral membrane proteins occurs at intracellular locations, and different proteins utilize different pathways to reach their proper membrane destination [6,20,21].

Does palmitoylation of α occur prior to αβγ trimer formation or after? Our results indicate that $\beta_1\gamma_2$ is unable to form a stable complex with palmitoylation defective α_sC3S and α_qC9,10S (Fig. 4). These results are consistent with previous in vitro studies using purified proteins showing that palmitoylated α_s was able to bind the βγ dimer substantially more tightly than non-palmitoylated α_s [22]. These data suggest the possibility that the βγ and α subunits form a complex after palmitoylation of α. We and others previously reported that βγ transits to the ER after undergoing prenylation and, in fact, is localized there when expressed alone [2,5]. Moreover, co-expression of wild type α resulted in efficient PM localization of βγ, whereas palmitoylation defective α mutants, α_sC3S and α_qC9,10S, failed to do so, with βγ remaining at the ER [2]. Taken together, and given the ER localization of a palmitoyltransferase for Ras2p, it is tempting to speculate that palmitoylation of the α subunit may take place at ER. However, molecular mechanisms by which α travels to ER need to be clarified. In this regard, we did not observe clear localization of the palmitoylation defective cysteine-to-serine α mutants at the ER.

A recent study speculated that G protein heterotrimer assembly and α subunit palmitoylation occur at Golgi membranes, based on colocalization at the Golgi of βγ and a palmitoylation defective α_{i2} [5]. However, in our hands, Golgi localization was not seen when βγ was co-expressed with α_sC3S or α_qC9,10S (Fig. 4), and BFA failed to block palmitoylation of α_q and PM trafficking of α_s and βγ. These contrasting results may suggest that different G protein heterotrimers can take different routes to the PM. G proteins containing α subunits that are both myristoylated and palmitoylated (e.g., α_i) may transit to the PM via the Golgi, in contrast to Golgi-independent PM targeting of heterotrimers containing non-myristoylated α subunits (e.g., α_s and α_q). Arguing against this possibility, however, are demonstrations that palmitoylation of endogenous α_i and PM targeting of α_z are insensitive to BFA treatment [3,7].

The precise nature of the Golgi-independent G protein trafficking remains to be further investigated. Also, still unknown is the existence of accessory molecules which help shuttle the subunits to the PM, particularly after formation of the αβγ trimer. Our results do not rule out the involvement of such proteins. In conclusion, we demonstrated that both PM localization of the G protein subunits and palmitoylation of the non-myristoylated α subunit occur independently of the conventional exocytic pathway. The refined model of PM trafficking of the G protein subunits is consistent with formation of an αβγ trimer at ER and trafficking to the PM without transit to the Golgi apparatus.

References

[1] Wedegaertner, P.B. (1998) Biol. Signals Recept. 7, 125–135.
[2] Takida, S. and Wedegaertner, P.B. (2003) J. Biol. Chem. 278, 17284–17290.
[3] Fishburn, C.S., Herzmark, P., Morales, J. and Bourne, H.R. (1999) J. Biol. Chem. 274, 18793–18800.
[4] Evanko, D.S., Thiyagarajan, M.M. and Wedegaertner, P.B. (2000) J. Biol. Chem. 275, 1327–1336.
[5] Michaelson, D., Ahearn, I., Bergo, M., Young, S. and Philips, M. (2002) Mol. Biol. Cell. 13, 3294–3302.
[6] Bijlmakers, M.J. and Marsh, M. (2003) Trends Cell. Biol. 13, 32–42.
[7] Gonzalo, S. and Linder, M.E. (1998) Mol. Biol. Cell 9, 585–597.
[8] Bijlmakers, M.J. and Marsh, M. (1999) J. Cell. Biol. 145, 457–468.
[9] van't Hof, W. and Resh, M.D. (1997) J. Cell. Biol. 136, 1023–1035.
[10] Morrow, I.C., Rea, S., Martin, S., Prior, I.A., Prohaska, R., Hancock, J.F., James, D.E. and Parton, R.G. (2002) J. Biol. Chem. 277, 48834–48841.
[11] South, S.T., Sacksteder, K.A., Li, X., Liu, Y. and Gould, S.J. (2000) J. Cell. Biol. 149, 1345–1360.
[12] Aridor, M., Bannykh, S.I., Rowe, T. and Balch, W.E. (1995) J. Cell. Biol. 131, 875–893.
[13] Gonzalo, S., Greentree, W.K. and Linder, M.E. (1999) J. Biol. Chem. 274, 21313–21318.
[14] Rehm, A. and Ploegh, H.L. (1997) J. Cell. Biol. 137, 305–317.
[15] Rehm, A. and Ploegh, H.L. (1997) FEBS Lett. 416, 39–44.
[16] Dong, X., Mitchell, D.A., Lobo, S., Zhao, L., Bartels, D.J. and Deschenes, R.J. (2003) Mol. Cell. Biol. 23, 6574–6584.
[17] Dunphy, J.T., Greentree, W.K., Manahan, C.L. and Linder, M.E. (1996) J. Biol. Chem. 271, 7154–7159.
[18] Lobo, S., Greentree, W.K., Linder, M.E. and Deschenes, R.J. (2002) J. Biol. Chem. 277, 41268–41273.
[19] Roth, A.F., Feng, Y., Chen, L. and Davis, N.G. (2002) J. Cell. Biol. 159, 23–28.
[20] Linder, M.E. and Deschenes, R.J. (2004) J. Cell. Sci. 117, 521–526.
[21] Linder, M.E. and Deschenes, R.J. (2003) Biochemistry 42, 4311–4320.
[22] Iiri, T., Backlund Jr., P.S., Jones, T.L., Wedegaertner, P.B. and Bourne, H.R. (1996) Proc. Natl. Acad. Sci. USA 93, 14592–14597.