Receptor-mediated Reversible Translocation of the G Protein βγ Complex from the Plasma Membrane to the Golgi Complex*

Received for publication, September 15, 2004, and in revised form, September 21, 2004 Published, JBC Papers in Press, September 23, 2004, DOI 10.1074/jbc.M410639200

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Heterotrimeric G proteins have been thought to function on the plasma membrane after activation by transmembrane receptors. Here we show that, after activation by receptors, the G protein βγ complex selectively translocates to the Golgi. Receptor inactivation results in Gβγ translocating back to the plasma membrane. Both translocation processes occur rapidly within seconds. The efficiency of translocation is influenced by the type of γ subunit present in the G protein. Distinctly different receptor types are capable of inducing the translocation. Receptor-mediated translocation of Gβγ can spatially segregate G protein signaling activity.

Heterotrimeric (αβγ) G proteins are localized to the plasma membrane of mammalian cells, facilitating interaction and activation by transmembrane G protein-coupled receptors (1–3). Extensive characterization of the effectors on which the G proteins act has suggested that the activated G protein α and βγ subunits function on the plasma membrane (3–6). It has been thought that the post-translational addition of a lipid moiety to the γ subunit aids in the localization of Ga and Gβγ complex to the plasma membrane, where they act on the effector molecules (7). However, there is little information about the properties of these proteins or the signaling they mediate in intact live mammalian cells, because studies attempting to observe G protein function in living mammalian cells have been limited.

To visualize the impact of receptor activation and inactivation on the spatial distribution of G protein subunits, we tagged G protein subunits with the yellow and cyan mutant forms of the green fluorescent protein, YFP1 and CFP, respectively. The fusion proteins were expressed in mammalian cell lines and observed after activating overexpressed or endogenous receptors using fluorescence-based imaging methods. Although we have previously obtained evidence indicating the direct involvement of the G protein γ subunit in receptor interaction (8, 9), we examined the effect of receptor activation on αo-CFP, β1, and different γ subunit types tagged with YFP in Chinese hamster ovary (CHO) cells overexpressing M2 muscarinic receptors. We discovered that γ-YFP translocated from the plasma membrane to the cell interior on receptor activation and translocated back to the plasma membrane on inactivation. The β subunit co-translocated with the γ subunit. The rapidity of the translocation process and proportion of βγ complex that translocated were dependent on the γ subunit type present in the expressed G protein. Experiments using a marker for the Golgi complex and a Golgi disrupting agent, brefeldin A, indicated that the βγ complex translocates to the Golgi complex. The translocation was sensitive to Gαo- and Gγ-coupled receptor stimulation. Endogenous receptors also stimulated Gβγ translocation. The translocation of the βγ complex is selective, because αo-CFP or a chimeric αo-q-CFP that couples to Gγ-coupling receptors do not translocate from the plasma membrane in response to receptor stimulation. The rapidity of the Gβγ complex translocation process suggests that it is diffusion-mediated.

EXPERIMENTAL PROCEDURES

Chemicals and Expression Constructs—All chemicals were purchased from Sigma unless otherwise indicated. CHO cells stably expressing the M2 muscarinic receptor (10), M3 muscarinic receptor (11), and 5HT1A receptor (from Dr. D. Manning, University of Pennsylvania) have been described previously (12). CHO cells were grown in CHO IIIa medium (Inovirgen) containing dialyzed fetal bovine serum (M2-CHO, M3-CHO, CHO) or charcoal-stripped fetal bovine serum (5HT1A-CHO, CHO) (Atlanta Biologicals), methotrexate (M2-CHO, M3-CHO), penicillin, streptomycin, glutamine, and fungizone. G418 and hygloxanthine/thymidine were added to 5HT1A-CHO medium. HT-1080 cells were grown in ATCC-2003 medium (American Type Culture Collection, Manassas, VA) containing dialyzed fetal bovine serum, penicillin, streptomycin, and fungizone. αo-CFP, αo-q-CFP, and β1-YFP constructs have been described previously (13). The citrine mutant of YFP and the non-oligomerizing forms of both CFP and YFP (14, 15) were engineered by mutagenesis and used in the experiments. The γ5 and γ11 cDNAs were introduced downstream of YFP (γ11 was also introduced downstream of CFP) in the pENTRY vectors (Invitrogen). The αo, β1, and γ11 cDNAs were also cloned into pENTRY vectors. All constructs were checked by determining the nucleotide sequence and transferred to pDEST (Invitrogen) for mammalian expression. YFP-tagged galactosyl transferase (galT) was obtained from M. Philips, New York University Medical School.

Imaging—Cells were cultured on glass coverslips and transiently transfected with appropriate combinations of different G protein subunits as described in the text and figure legends. The coverslips were washed with Hanks’ buffer saline solution supplemented with 10 mM Hepes, pH 7.4, and 1 mg/ml glucose and mounted on an imaging chamber with an internal volume of 25 μl (Warner Instruments). A fluid delivery system, including a programmable valve controller and Teflon valves (10 μl open/closure time), was used to deliver the buffer with or without agonist or antagonist through the chamber at a rate of 1 ml/min with a pressure-regulated flow controller (Automate Scientific). The cells were visualized with a Zeiss Axioscope fluorescent microscope using a 63× oil immersion objective (1.4 numerical aperture) and 100-W mercury lamp with a Hamamatsu CCD Orca-ER camera. The shutter, emission, and excitation filter wheels were con-

* This work was supported by National Institutes of Health Grants GM46963 and GM69027 and American Heart Association Postdoctoral Fellowship 0225378Z (to M. A.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

§ The on-line version of this article (available at http://www.jbc.org) contains Supplemental Fig. 1, Movies 1–3.

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§ The abbreviations used are: YFP, yellow fluorescent protein; CFP, cyan fluorescent protein; CHO, Chinese hamster ovary; galT, galactosyl transferase.

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trolled by a Sutter Lambda 10–2 optical filter changer (Sutter Instrument Company) run by MetaVue (Universal Imaging) software. The filter and beam splitter combinations (from Chroma Technology) were as follows: CFP (CC), D436/20 excitation (x), D480/40 emission (m); for YFP (YY), D500/20 (x), D535/30 (m); a polychroic beam splitter (Chroma 86002BS); and 3 or 10% neutral density filters. In those cases where both CFP and YFP fusions were co-expressed in cells, the cells expressing relatively similar levels of the two fusions were selected based on emission intensities. Images were acquired at 20-s intervals. The exposure times were between 0.6 and 1.4 s. The cells were treated with pertussis toxin at a final concentration of 100 ng/ml 6–15 h prior to the analysis. To disrupt Golgi in the cells, 10 μM brefeldin A was used in the culture medium 6–10 h before cell analysis.

RESULTS AND DISCUSSION

CHO cells stably expressing M2 muscarinic receptors were transiently transfected with cDNAs encoding G protein αo, β1 and γ11 subunits tagged with YFP. γ11 is a farnesylated γ subunit that is present in several mammalian tissues (16). YFP was attached to the N terminus of γ11, because we had earlier discovered that the tag did not affect receptor activation of the G protein and it does not affect the farnesylation that occurs at the C terminus of the subunit (13). The cells were imaged as described above. Images of individual cells before and after sequential exposure to an agonist, carbachol, and an antagonist, atropine, are shown in Fig. 1A and Supplemental Fig. 1 (Movie 1). The emission signal from γ11-YFP is initially localized to the plasma membrane. After M2 muscarinic receptor activation, the γ11-YFP translocates to the cell interior. Subsequent inactivation of the M2 receptor leads to the translocation of γ11-YFP back to the plasma membrane. Plots of the time courses of changes in the YFP emission intensity on the plasma membrane and the intracellular region where the translocated YFP signal localizes are shown in Fig. 1B (left and right panels). The t for the translocation of γ11-YFP protein in both directions is 20 s (Fig. 1B). When M2-CHO cells expressing αo-CFP, β1, and γ11-YFP were exposed to different concentrations of agonist and the translocation response measured, the translocation of Gβγ was exquisitely sensitive to the extent of receptor activation (Supplemental Fig. 1). To examine the impact of this translocation on the G protein α subunit, we co-expressed αo-CFP with β1 and γ11-YFP into the same cells. CFP was inserted downstream of Gly-92 of αo (13). αo-CFP did not affect the translocation properties of γ11-YFP compared with native αo (Fig. 1C). As anticipated, based on our previous findings (13), the CFP emission increases with receptor activation and decreases with receptor inactivation due to the corresponding changes in the fluorescence resonance energy transfer occurring from CFP to YFP (Fig. 1C). This result indicates that the γ subunit translocation results from the activation of the α subunit in the G protein heterotrimer. Consistent with this result, pertussis toxin-treated M2-CHO cells expressing β1 and γ11-YFP do not show receptor-mediated translocation of γ11-YFP, demonstrating a requirement for both the endogenous αi subunit and receptor activation of a G protein for translocation to occur (data not shown). Although it is required for translocation of γ11-YFP, the α subunit itself does not translocate in response to receptor activation (Supplemental Fig. 1 (Movie 2)). To examine whether this selective translocation of the γ subunit included the β subunit, we co-expressed β1-YFP along with γ11-CFP and observed the response of β1-YFP to receptor stimulation. Fig. 1D shows that the β subunit co-translocates with the γ subunit consistent with extensive previous evidence that the β subunit is in a tight complex with the γ subunit (1–3). When the β1-YFP protein was co-expressed with untagged γ11, it also translocated, indicating that the fluorescent protein tag of γ11-YFP did not affect translocation (Supplemental Fig. 1). In a control experiment, when αo-CFP and β1-YFP were expressed in M2-CHO cells without γ11, very little receptor-dependent translocation of β1-YFP was detected, indicating that translocation was dependent on the presence of the γ11 subunit (data not shown). It is likely that γ11 subunit dependence of β subunit translocation is linked to the role of the γ subunit in receptor interaction. We then examined
whether a distinctly different γ subunit type, γδ (which has 33% identity at the primary structure level with γ11), translocates similarly. M2-CHO cells were transiently transfected with αo-CFP, β1, and γ5-YFP. Unlike γ11, which is farnesylated, γ5 is geranylgeranylated. The results of imaging the cells in the presence of agonist and antagonist are shown in Fig. 1E and Supplemental Fig. 1 (Movie 3). γ5-YFP translocates in response to the receptor state, although the proportion of γ5 translocated from the plasma membrane at steady state is distinctly less (Supplemental Fig. 1, compare Movie 2 with Movie 3), and the rate at which translocation occurs is also slower with a t 1/2 of 1–2 min.

To identify the organelle to which the G protein βγ complex was translocated, we co-expressed a trans Golgi complex marker, galT tagged with YFP (17, 18), with γ11-CFP in M2-CHO cells and imaged the cells after M2 activation. Overlaying the images of CFP and YFP emission showed that γ11-CFP was localized in the Golgi complex (Fig. 2A). To confirm this localization, we treated the transfected M2-CHO cells with brefeldin A, which is known to disrupt the Golgi complex (19). When these cells were examined after agonist treatment, translocation of γ11-YFP to the cell interior could no longer be detected (Fig. 2B). Control cells expressing γ11-CFP did not show γ11 localization, indicating that the Golgi complex was indeed disrupted in these cells (Fig. 2B).

We then tested the generality of the receptor-mediated Gβγ translocation process in the following systems. To eliminate the possibility that translocation was peculiar to M2 receptors, we tested the ability of a distinctly different isofrom of the acetylcholine receptor, M3, to induce translocation of βγ11. M3 couples to the Gq class of α subunits, whereas M2 couples to the Gi/o class (11). In CHO cells stably expressing M3 receptors, we co-expressed β1 and γ11-YFP with a chimeric αo subunit containing the C-terminal tail of αq (Fig. 3A). This chimeric Gaαo-q is activated by the M3 receptor (but not M2) as we have shown before (13). The cells were treated with pertussis toxin to prevent cross-activation of G, proteins in CHO cells by M3 (CHO cells do not express Gq (13)). Fig. 3A shows that γ11-YFP translocated to the Golgi complex when M3 receptors were activated and translocated back to the plasma membrane when they were inactivated. To examine whether receptors other than muscarinic receptors can induce translocation, we performed similar experiments with CHO cells stably expressing 5HT1A receptors. γ11-YFP translocated as in the case of M2 and M3, depending on the activation state of the 5HT1A receptor (Fig. 3B). Translocation was not dependent on overexpression of receptors because an endogenous receptor in CHO cells, 5HT1B also induced translocation (Fig. 3C). To test whether a cell line from a different species could induce translocation, we transfected a human lung fibrosarcoma cell line (HT1080) with cDNAs for M2 receptors, αo-CFP, β1, and γ11-YFP and imaged the cells in the presence of agonist and antagonist as above. Translocation was induced, indicating that the process is not peculiar to CHO cells (Fig. 3D).

Based on a variety of in vitro experiments, it has been thought that G proteins, which are peripherally associated with the plasma membrane, are activated by transmembrane receptors, and after subunit dissociation, act on effector molecules on the plasma membrane (1–3, 7). Consistent with this proposal, known effectors of G proteins (such as adenyl cyclase and ion-conducting channels) are membrane proteins, and others (such as phospholipase C isozymes) act on substrates localized to the plasma membrane (3–6). The rapid translocation of the G protein βγ complex from plasma membrane to the Golgi complex in response to receptor stimulation is therefore very surprising. This hitherto unanticipated translocation suggests that the βγ complex may act on an effector molecule located in the Golgi complex. Goβ3 has previously been shown to be present in the Golgi, although receptor-dependent effects on this localization were not identified (17). The
G protein βγ complex has been shown earlier to act on protein kinase D in the Golgi complex to regulate Golgi disassembly (20). However, that work did not identify the signaling pathway or its mechanistic basis that allowed the G protein βγ complex to act on the Golgi. The relationship between the translocation seen here and the previously reported action of Gβγ on protein kinase D in Golgi is not clear. It is possible that the function of an unidentified effector in Golgi is modulated by receptor-regulated translocation of Gβγ. There is now evidence that, in addition to its role in the plasma membrane, Ras acts on effectors in the Golgi complex (21). A recent report shows that a protein resident in the Golgi complex binds MEK when signaling is activated by a tyrosine kinase receptor (22). These evidences for the Golgi complex acting as a distinct site for downstream elements of the tyrosine kinase signaling pathways makes the cytoplasmic surface of this organelle an attractive locale for integrating regulated cross-talk with G protein pathways through the translocation of the βγ complex seen here. Overall, the ability of one signaling arm of the G protein to spatially isolate its activity from the plasma membrane to an internal organelle demonstrates a novel mode by which G protein-mediated signaling can be spatially segregated.

Another role for this translocation may be the rapid decrease in the concentration of the βγ complex in the plasma membrane resulting in a dampening of signaling activity because of the reduced concentration of heterotrimer available for activation. This effect can be further modulated by differential magnitudes and rates of translocation as seen in the case of γ11 and γ5. The rate of translocation of Gβγ back to the plasma membrane on receptor inactivation is as rapid as the translocation to the Golgi on activation of the receptor. Both processes occur within a time frame that suggests that translocation is diffusion-mediated rather than actively controlled. The rapid reversibility of the translocation suggests that the receptor can exert tight control over the signaling effects of the spatial segregation of Gβγ.

The results here demonstrate that by imaging signaling activity in a live cell, previously unanticipated novel mechanisms underlying signaling can be identified. The ability to image the translocation of Gβγ in a living cell in response to a receptor agonist, as well as an antagonist, can be of value in the rapid, non-invasive, high throughput and high content screening of potential therapeutic compounds directed at G protein-coupled receptors. These receptors form the most important target of commercially available therapeutic drugs at present.

Acknowledgments—We thank Dr. I. Azpiazu for help with image processing and valuable discussions. We thank Dr. D. Manning for the cell line and Dr. M. Philips for the Golgi marker.

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