Regulator of G Protein Signaling 8 (RGS8) Requires Its NH₂ Terminus for Subcellular Localization and Acute Desensitization of G Protein-gated K⁺ Channels*

Osamu Saitoh‡§, Ikuo Masuho‡, Ion Terakawa‡, Satoshi Nomoto|, Tomiko Asano**; and Yoshihiro Kubo***

From the ‡Department of Molecular and Cellular Neurobiology, Tokyo Metropolitan Institute for Neuroscience, 2-6 Musashidai, Fuchu-shi, Tokyo 183-8526, Japan, the §Hamamatsu University School of Medicine, Hamamatsu, Aichi 431-3192, Japan, the ¶Department of Health Science, Jichi Medical School and CREST, 3311-1 Yakushiji Minamikawachi, Tochigi 329-0498, Japan, the ¶¶Department of Biochemistry, Institute for Developmental Research, Aichi Human Service Center, Kasugai, Aichi 480-03, Japan, the §§Department of Neurophysiology, Tokyo Metropolitan Institute for Neuroscience, 2-6 Musashidai, Fuchu-shi, Tokyo 183-8526, Japan, and the §§§Department of Physiology, Tokyo Medical and Dental University, Graduate School and Faculty of Medicine, Yushima, Bunkyo-ku, Tokyo 113-8519, Japan

The abbreviations used are: RGS, regulators of G protein signaling; RFP, red fluorescent protein; GIRK, G protein-gated inwardly rectifying K⁺ channel; NRGS8, non-regulatory G protein subunit of RGS8; Ga, G protein subunits of heterotrimeric G proteins; GFP, green fluorescent protein; RGS1, RGS3, RGS4, and GAIP, members of RGS protein family; DDT1MF2, DDT1 MF2 cells; DOPN, Doupnik.

Functional roles of the NH₂-terminal region of RGS (regulators of G protein signaling) 8 in G protein signaling were studied. The deletion of the NH₂-terminal region of RGS8 (ΔNRGS8) resulted in a partial loss of the inhibitory function in pheromone response of yeasts, although Ga binding was not affected. To examine roles in subcellular distribution, we coexpressed two fusion proteins of RGS8-RFP and ΔNRGS8-GFP in DDT1MF2 cells. RGS8-RFP was highly concentrated in nuclei of unstimulated cells. Coexpression of constitutively active Ga, resulted in translocation of RGS8 protein to the plasma membrane. In contrast, ΔNRGS8-GFP was distributed diffusely through the cytoplasm in the presence or absence of active Ga. When coexpressed with Ga protein-gated inwardly rectifying K⁺ channels, NRGS8 accelerated both turning on and off similar to RGS8. Acute desensitization of G protein-gated inwardly rectifying K⁺ current observed in the presence of RGS8, however, was not induced by ΔNRGS8. Thus, we, for the first time, showed that the NH₂ terminus of RGS8 contributes to the subcellular localization and to the desensitization of the G protein-coupled response.

RGS1 (regulators of G protein signaling) proteins comprise a large family of more than 20 members that modulate heterotrimeric G protein signaling (1, 2). This protein family was originally identified as a pheromone desensitization factor in yeast (3). Many members of RGS protein family were subsequently identified by virtue of a common stretch of 120 amino acids termed the RGS domain in organisms ranging from yeast to human (1, 2, 4, 5). It was shown that several RGS proteins (RGS1, RGS3, RGS4, and GAIP) attenuate G protein signaling in cultures (4, 6, 7). Biochemical studies demonstrated that RGS members function as a GTPase-activating protein for α subunits of heterotrimeric G proteins (8, 9, 10). Therefore, RGS proteins are proposed to down-regulate G protein signaling in vivo by enhancing the rate of Ga GTP hydrolysis.

We previously searched for RGS proteins specifically expressed in neural cells using a culture system of neurally differentiating P19 cells. We isolated cDNA of RGS8 and identified it as a RGS protein induced in differentiated P19 cells (11). In addition, since RGST had been reported to be expressed predominantly in the brain (5), we also isolated a full-length cDNA of RGST (12). Biochemical studies indicated that RGS8 binds to Ga, and Ga3, and that RGST binds to Ga, Ga3, and Ga,3. To examine effects of each RGS protein on G protein signaling, we coexpressed a G protein-coupled receptor and a G protein-coupled inwardly rectifying K⁺ channel (GIRK1/2) (13–15) in Xenopus oocytes and analyzed the activation and deactivation kinetics. We observed that RGS8 significantly speeds up both activation and deactivation of GIRK current (11). Doupnik et al. (16) reported the similar accelerated kinetics of GIRK current by RGS1, RGS3, or RGS4. We further observed that RGS8 induces acute desensitization of receptor-activated GIRK current in the presence of ligands (11). In the case of RGST, activation of GIRK current was clearly accelerated as with RGS8, but the acceleration effect on deactivation was significantly weaker than that of RGS8. The acute desensitization of receptor-activated GIRK observed with RGS8 was not apparent with RGST. Thus, RGST and RGS8 were shown to accelerate G protein-mediated modulation of GIRK current differentially (12). What is the structural basis for the weaker off acceleration and reduced desensitization in the case of RGST? One possibility is that a difference in the NH₂-terminal domain contributes to the differential modulation of GIRK current. The conserved NH₂-terminal domains of RGS4 and RGS16 were recently shown to be important for membrane association and biological function by the analysis of the ability to inhibit pheromone signaling in the budding yeast (17–19). A homologous domain was also found in the NH₂ terminus of RGS8. Therefore, we investigated functional roles of the NH₂-terminal domain of RGS8 in G protein signalings. We examined effects of deletion of this NH₂-terminal domain on the pheromone signaling of yeasts, the Ga binding, the subcellular...
distribution, and the modulation of the receptor-activated GIRQ current.

**EXPERIMENTAL PROCEDURES**

**Yeast Pheromone Response Assay**—A bioassay was used to measure the sensitivity of the pheromone response in yeast that expresses GPRO proteins as described (20). A DNA fragment containing the Myc tag (MEQKLISEEDLRSQG) was introduced into pTS210 yeast expression vector under the control of a galactose-inducible promoter. By polymerase chain reaction amplification, cdNA fragments containing the coding sequence of RS8 or ΔNRGS8 (mutant RS8 which lacks NH2-terminal 35 amino acids) were isolated. After confirmation by sequencing analysis, they were fused in-frame immediately downstream of the Myc tag in pTS210. The st2 deletion mutant yeast SNNY86 (21) was transformed with each cdNA in pTS210 and selected on ura dropout plates. Independent colonies of each yeast transformant were grown and a halo bioassay was performed.

**Western Blotting of Epitope-tagged GPRO Proteins**—Single colonies of yeasts transformed with Myc-tagged GPRO constructs were inoculated into ura− dropout medium supplemented with 2% galactose or glucose and were grown to an identical density (A600 = 1). Yeast cells were precipitated and lysed in SDS sample buffer. Proteins were extracted by sonication, centrifuged on SDS-polyacrylamide gels, and transferred to nitrocellulose membranes. Expression levels of Myc-tagged GPRO proteins were then examined by Western blotting using anti-Myc tag monoclonal antibody (9E10, BabCO). Signals were detected with an ECL system (Amersham Pharmacia Biotech).

**Immunoprecipitation**—By polymerase chain reaction amplification, cdNA fragments for RS8 and ΔNRGS8 were isolated. For ΔNRGS8, a forward primer that included the ATG start codon was used. After confirmation of their nucleotide sequences, they were cloned into pCXN2 expression vector provided by Professor Miyazaki (22). Biotinylated proteins were generated by in vitro transcription/translation of the resultant plasmid cdNAs using the TNT-coupled reticulocyte system (Promega) and biotinylated lysine-tRNA complex (TranscendTM tRNA, CLONTECH). Each biotinylated protein was mixed with purified bovine serum albumin and precipitated and lysed in SDS sample buffer. Proteins were extracted by sonication, centrifuged on SDS-polyacrylamide gels, and transferred to nitrocellulose membranes. Expression levels of Myc-tagged GPRO proteins were then examined by Western blotting using anti-Myc tag monoclonal antibody (9E10, BabCO). Signals were detected with an ECL system (Amersham Pharmacia Biotech).

**Cell Fractionation**—A Syrian hamster leiomysarcoma cell line, DDT1MF2, was grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum. RS8 and ΔNRGS8 expression constructs were described in the previous section on immunoprecipitation. The plasmid cdNAs of these expression constructs were transfected into DDT1MF2 cells by the CaPO4 methods (23). After selection in the presence of G418 (0.8 mg/ml, Life Technologies, Inc.), stable lines were established. The cultured cells were sonicated in 50 mM Tris acetate buffer, pH 7.5, containing 10 mM MgCl2, 1 mM dithiothreitol, 1 mM EDTA, and 0.1 mM phenylmethylsulfon fluoride and the homogenate was centrifuged (44,000 × g, 20 min, 4 °C). The resultant supernatant and precipitate were mixed with SDS sample buffer and used as cytosolic and particulate fractions.

**Chimeric cdNAs of RS8 and Fluorescent Protein**—We expressed RS8 and ΔNRGS8 as a chimeric protein with fluorescent protein at the carboxyl terminus. By polymerase chain reaction amplification, cdNA fragments for RS8 and ΔNRGS8 were isolated. For ΔNRGS8, a forward primer that included the ATG start codon was used. After confirmation by sequencing analysis, RS8 cdDNA was fused in-frame to red fluorescent protein (RFP) in pPRed1-N1 (CLONTECH) in one particular experiment to green fluorescent protein (GFP) in pEGFP-N1. Resultant plasmid cdDNAs were transiently transfected into DDT1MF2 cells using FuGENE 6 (Roche Molecular Biochemicals). At 48 h after the transfection, transfected cells were fixed by treatment with 4% paraformaldehyde for 30 min. Confocal microscopy was performed with a Zeiss microscope connected with an LSM410 Laser Scan-
anti-Myc antibody. Expression of RGS8 was detected by Western blotting with pTS210, and determination whether the NH2-terminal domain of RGS8 influences its intracellular distribution or not, wild type RGS8 or ΔNRGS8 cDNA was transfected to DDT1MF2 cells, which do not express endogenous RGS8, and their cell homogenates were fractionated. Cytoplasmic and particulate fractions were further confirmed by an immunofluorescence method. Surprisingly, RGS8-ΔNRGS8 was diffusely distributed through the cytoplasm within identical cells (Fig. 5, upper). In the other hand, ΔNRGS8-GFP was diffusely distributed through the cytoplasm within identical cells (Fig. 5, middle). These observations were consistent with the analysis using the cell fractionation method (Fig. 4), since the particulate fraction contains nuclei and cell membranes. These results clearly demonstrated that RGS8 is localized in nuclei in DDT1MF2 cells, and that the NH2-terminal domain of RGS8 is required for this unique nuclear localization. Sequence comparison revealed that the NH2 terminus of RGS8 is similar to the conserved NH2-terminal sequences of RGS4 and RGS16, but that the first 7 amino acids of RGS8 are unique (Fig. 1). A search for the subcellular localization sites of protein identified a putative nuclear localization signal sequence, PRRNK at amino acids 7–11. This nuclear localization signal sequence was further confirmed by an immunofluorescence method. DDT1MF2, NIH3T3, and HEK293 cells were transfected with cDNA of wild type RGS8 without fluorescent protein and were immunostained with anti-RGS8 antibody. In all cases, similar was present in the particulate fraction. In contrast, ΔNRGS8 was abundantly detected in the cytoplasm. In both transfected cells, Gαq11 was exclusively present in the particulate fraction. These results clearly demonstrated that the NH2-terminal domain of RGS8 is required for its subcellular distribution.

NH2-terminal Deletion Shifts the Distribution of RGS8 from the Particulate to Cytosolic Fraction—How can the NH2-terminal domain modulate the function of RGS8 without changing the properties of interaction with Gα? It has been demonstrated that the NH2-terminal domains of RGS4 and RGS16 serve as a membrane targeting sequence in yeasts (18, 19). To further investigate the cellular distribution of RGS8 in cultured cells and also to examine roles of its NH2 terminus, two different fluorescent proteins were fused to RGS8 and ΔNRGS8, respectively, and they were coexpressed in DDT1MF2 cells. RGS8 was expressed as a chimeric protein with RFP at the carboxyl terminus and ΔNRGS8 was coexpressed as a chimeric protein with GFP. Surprisingly, RGS8-RFP was highly concentrated in nuclei of most transfected cells (Fig. 5, upper). On the other hand, ΔNRGS8-GFP was diffusely distributed through the cytoplasm within identical cells (Fig. 5, middle). In the lower panel (Fig. 5, lower), the distribution of ΔNRGS8-GFP was examined by confocal microscopy. It was clearly demonstrated that ΔNRGS8-GFP was diffusely distributed through the cytoplasm, whereas ΔNRGS8-RFP was highly concentrated in nuclei (Fig. 5, upper). These observations were consistent with the analysis using the cell fractionation method (Fig. 4), since the particulate fraction contains nuclei and cell membranes. These results clearly demonstrated that RGS8 is localized in nuclei in DDT1MF2 cells, and that the NH2-terminal domain of RGS8 is required for this unique nuclear localization. Sequence comparison revealed that the NH2 terminus of RGS8 is similar to the conserved NH2-terminal sequences of RGS4 and RGS16, but that the first 7 amino acids of RGS8 are unique (Fig. 1). A search for the subcellular localization sites of protein identified a putative nuclear localization signal sequence, PRRNK at amino acids 7–11. This nuclear localization signal sequence was thought to function in the distribution of RGS8 in nonactivated DDT1MF2 cells. The nuclear localization of RGS8 was further confirmed by an immunofluorescence method. DDT1MF2, NIH3T3, and HEK293 cells were transfected with cDNA of wild type RGS8 without fluorescent protein and were immunostained with anti-RGS8 antibody. In all cases, similar...
nuclear localization of RGS8 was observed (data not shown). RGS proteins interact directly with Go in its active state (9, 10) and the heterotrimeric G protein complex is mainly associated with cell membranes. Since RGS8 was found to be localized to nuclei in DDT1MF2 cells, it is considered to be unable to efficiently interact with Go in the cells activated by stimulation of G proteins. To investigate the subcellular localization of RGS8 after G protein activation, a constitutively active Go was further coexpressed in DDT1MF2 cells. We previously showed that RGS8 binds preferentially to Go and Go (11). When Go, Q205L was further coexpressed for 48 h, a marked translocation of RGS8 to the plasma membrane was observed (Fig. 6, upper). In many cells, RGS8-RFP was concentrated to unique membrane structures that may correspond to membrane ruffles or microprojections and to the cell periphery. The nuclear localization of RGS8-RFP, which was intense in unstimulated cells, tended to decrease or disappear in Go, Q205L-expressing cells. The distribution of ΔNRGS8-GFP, however, was not significantly influenced by expression of active Go and ΔNRGS8-GFP showed a relatively uniform pattern of distribution (Fig. 6, middle). In control cells transfected with RFP cDNA, a diffuse and sometimes punctate pattern was observed and the distribution was clearly different from that of RGS8-RFP. Thus, RGS8 was shown to be translocated to plasma membrane by G protein-activated signalings. It is clear that the NH2-terminal domain of RGS8 is required for this translocation. ΔNRGS8 could bind to Go, but it could not translocate to the membrane on coexpression of Go, Q205L. Therefore, it was thought that the membrane recruitment of RGS8 was not a direct result of the physical association with Go. We further investigated the morphological structure of the region to which RGS8 was translocated on coexpression of Go, Q205L by confocal microscopy. Three-dimensional reconstitution of confocal slice images revealed that the structures where RGS8 accumulated as indicated in Fig. 6, left, were fine projections or microspikes present on the surface of DDT1MF2 cells (Fig. 7).

RGS8-induced Desensitization of the Receptor-activated GIRK Current Was Abolished by Deletion of Its NH2-terminal Domain—GIRK channels are known to be activated directly by Gbg subunits released by the Gi family. They are activated by G protein-coupled receptors such as m2 muscarinic and D2 dopamine receptors. We previously coexpressed GIRK1/GIRK2 heteromultimer and m2 muscarinic receptor with or without RGS8 in Xenopus oocytes and analyzed the effects of RGS8 on G protein-mediated modulation of K+ currents. We reported...
that RGS8 speeds up the activation and deactivation kinetics of GIRK upon receptor stimulation. We also reported the RGS8-induced acute desensitization of the receptor-activated GIRK current (11, 12). Here, we examined the functional role of the NH2-terminal domain, which determines subcellular localization, in the RGS8-mediated modulation of on-off kinetics of GIRK current. We coexpressed RGS8 or ΔNRGS8 with GIRK1/2 heteromultimer and m2 muscarinic receptor in *Xenopus* oocytes. ΔNRGS8 coexpression accelerated both the turning on and off to a similar extent as the wild type RGS8. The acute desensitization of receptor-activated GIRK current, however, was much less clear in oocytes expressing ΔNRGS8 (Fig. 8A). These observations were confirmed quantitatively by comparing the time constants of the exponential functions used for the fitting of the activation (τon) and deactivation (τoff) phases (detail in the legends of Fig. 8B) and the extent of desensitization (Fig. 8B). Thus, we identified a novel function of the NH2-terminal domain of RGS8 to cause acute desensitization of GIRK current activated by receptor stimulation.

**DISCUSSION**

In this study, we demonstrated the functional significance of the NH2-terminal domain of RGS8. First, we compared this region of RGS8 with those of RGS4, RGS5, and RGS16 and identified a conserved sequence in the NH2 terminus. By using a yeast halo assay, we showed that the deletion of the NH2-terminal domain of RGS8 results in a partial loss of function to inhibit the pheromone response pathway. We used pTS210, a single-copy expression vector under the control of a galactose-inducible promoter. When a higher concentration of galactose or multicopy vector was used, we could not detect a clear difference in the sensitivity to the mating pheromone of yeasts carrying RGS8 and ΔNRGS8. Under these conditions of overexpression, a proper subcellular distribution might not be necessary for sufficient function of RGS8 because of high amount within the cells.

We unexpectedly found that RGS8 is predominantly localized in the nuclei of DDT1MF2 cells. This nuclear localization of RGS8 was shown by its fusion protein with a fluorescent protein (GFP or RFP) and further confirmed immunocytochemically with anti-RGS8 antibody. Similar nuclear localization of RGS8 was observed in 3T3 and HEK293 cells transfected with RGS8 cDNA. We, moreover, showed that the NH2-terminal domain of RGS8 is required for its nuclear localization within cells. In the case of RGS4, despite having a similar conserved sequence in the NH2 terminus (Fig. 1), a predominant localization in the cytoplasm has been reported using HEK293 cells transfected with RGS4 cDNA (24). Considering the subcellular distributions of RGS8 and RGS4, it is possible that the first 7 amino acids in the NH2 terminus of RGS8 are responsible for the unique nuclear localization. Indeed, we found a potential NLS sequence at amino acids 7–11. Quite recently, a truncated isoform of RGS3, termed RGS3T, has been reported to be localized to the nucleus and induce apoptosis in RGS3T-transfected Chinese hamster ovary cells (25). Two potential nuclear localization signal sequences were found in the NH2 terminus of RGS3T and truncation of the NH2 terminus resulted in a reduction of nuclear localization. RGS8 in nucleus might also function in cellular processes such as apoptosis.

We showed that RGS8 is translocated from the nucleus to the plasma membrane structures by coexpression of GoQ205L in DDT1MF2 cells. The NH2-terminal domain of RGS8 was shown to play critical roles in this membrane recruitment, since mutant RGS8 lacking the NH2-terminal sequence of 35 amino acids could not translocate to the membrane within identical cells. This ΔNRGS8 contains an intact RGS domain, which is sufficient for the interaction with GoQ. Therefore, the mechanism of the membrane translocation was considered to be independent of RGS8-G protein interaction. RGS4 was also reported to be recruited from the cytoplasm to the plasma membrane on the expression of activated, GTPase-deficient GoQ205L (24). It was shown that a non-GoQ binding mutant of RGS4 could translocate like wild type RGS4, indicating that this translocation was not a direct result of physical association with an activated GoQ. It is possible that G protein activation triggers binding of unidentified cellular factors to the subcellular localization signals of the NH2 terminus of RGS proteins for translocation to the membrane.

When coexpressed in *Xenopus* oocytes with a G protein-coupled receptor and GIRK1/2, ΔNRGS8 accelerated the turning on and off of GIRK current upon receptor stimulation similar to wild type RGS8. Wild type RGS8 induced significant levels of acute desensitization of the response during receptor activation, but the desensitization in oocytes expressing ΔNRGS8 was similar to that in control oocytes. These results demonstrated that the NH2-terminal domain of RGS8 is required to cause acute desensitization of GIRK. Indeed, we previously reported that RGS7, which contains the characteristic long NH2 terminus different from that of RGS8, showed slower desensitization. Kovoor et al. (26) also made similar recordings of receptor-activated GIRK current in *Xenopus* oocytes expressing RGS proteins. Their data indicated that RGS4 induces acute desensitization, but that RGS7 and RGS9 do not cause significant desensitization. RGS4 has a similar NH2-terminal sequence to RGS8, and both RGS7 and RGS9 possess the long NH2 terminus containing DEP (Dishevelled/EGL10/pleckstrin homology) and GGL (G protein γ subunit-like) domains instead of the short conserved NH2 terminus of RGS8.

What is the mechanism behind the desensitization in the presence of RGS8? If we assume that there are only two forms...
of G protein (nonactive and active) and two rate constants (\( \alpha \), on-rate; \( \beta \), off-rate), the current amplitude is expected to increase single exponentially with a time constant of \( 1/(\alpha + \beta) \). In this case, no desensitization is expected to occur. To explain the presence of desensitization in the two-state model, it is necessary to assume a gradual decrease in the on-rate or a gradual increase in the off-rate in the course of the receptor stimulation. As the turn-on speed, determined dominantly by \( \alpha \), was not significantly decreased by the second ligand application immediately after the initial trial (data not shown), it appears

![Three-dimensional reconstructed image of RGS8 translocated by G protein activation.](image)

**FIG. 7.** Three-dimensional reconstructed image of RGS8 translocated by G protein activation. RGS8 was expressed as a chimeric protein with GFP in DDT1MF2 cells. A constitutively active Ga cDNA (RGS8 + Ga, Q205L) or control vector (RGS8 + vector) was co-transfected. At 48 h after transfection, confocal images were collected and a three-dimensional reconstructed image was obtained. The obtained image was rotated.

![Effects of RGS8 and ΔNRGS8 on turning on and turning off kinetics, and on the desensitization of GIRK current upon stimulation of m2 muscarinic receptor.](image)

**FIG. 8.** Effects of RGS8 and ΔNRGS8 on turning on and turning off kinetics, and on the desensitization of GIRK current upon stimulation of m2 muscarinic receptor. A, GIRKI/2 and m2 muscarinic receptor without (upper trace) or with RGS8 (middle trace) or with ΔNRGS8 (lower trace) were coexpressed in Xenopus oocytes. Current traces at a holding potential of \(-80\) mV are shown. 10 μM ACh was applied at the time indicated by the bars. B, Comparison of the time constants \( \tau_{on} \) (top) and \( \tau_{off} \) (middle), and the desensitization level (bottom) of the GIRK 1/2 current upon stimulation of m2 muscarinic receptor in the absence (left) or presence of RGS8 (middle) or ΔNRGS8 (right). The turning on phases of three groups were fitted with a single-exponential function, and the time constants of them were compared (\( \tau_{on} \)). The turning off phases of RGS(−) data were fitted with a single exponential function. In contrast, a two-exponential function was used to fit those of the data with RGS8 and ΔNRGS8 satisfactorily. The time constants of the fast (and major) component of them were compared with that of the single time constant of RGS(−) data (\( \tau_{off} \)). \( \tau_{on} \) and \( \tau_{off} \) values of RGS8 and ΔNRGS8 were significantly different from those without RGS protein (average and S.D. are shown, \( n = 9 \) or \( 10 \), \( p \) value < 0.05) by Student’s unpaired \( t \) test. The level of desensitization was measured as a percentage of the reduction of the induced current after 1 min of ligand application. Values of RGS8 were significantly different from those with ΔNRGS8 or from those without RGS (average and S.D. are shown, \( n = 9 \) or \( 10 \), \( p \) value < 0.05) by Student’s unpaired \( t \) test.
that the on-rate was not decreased during the ligand stimulation. If the off-rate is increased during the response, the turning off after ligand washout should be faster with RGS8, which showed a more intense desensitization. As the turning off speed after ligand washout was similar between RGS8 and ΔNRGS8, the off-rate was not increased during the response. Thus, the desensitization could not be explained by a gradual change of the on- or off-rate. What then is the mechanism of desensitization in the presence of RGS8? With the assumption that there are three states, a nonactive nonready state, a nonactive ready state, and an active state, the desensitization could be explained by the depletion of the ready pool as discussed by Chuang et al. (27). The presence of a large ready pool enables the acceleration of the turning on, but at the same time the depletion of the ready pool during the response could result in the acute desensitization even if the on-rate and off-rate are not changed. If this is the case, the NH₂-terminal region of RGS8 is thought to be necessary for the formation of the ready pool and for its depletion during the response. It is speculated that the NH₂-terminus of RGS8 controls the G protein pool by controlling its subcellular distribution, although further study is required to understand how this enlarged G protein pool is regulated by RGS proteins.

Acknowledgments—We are grateful to Professor M. Lazdunski for GIRK2 cDNA, Dr. A. Connolly for m2 muscarinic receptor cDNA, and Dr. J. D. Jordan for GαQ205L cDNA. We thank Dr. H. Nakata for helpful discussion, Dr. K. Nishi for use of a confocal microscope, M. Odagiri for technical assistance, and M. Kato for advice and help with confocal images.

REFERENCES

1. Dohlman, H. G., and Thorner, J. (1997) J. Biol. Chem. 272, 3871–3874
2. Berman, D. M., and Gilman, A. G. (1998) J. Biol. Chem. 273, 1269–1272
3. Chan, B. K., and Otte, C. A. (1982) Mol. Cell. Biol. 2, 11–20
4. Druey, K. M., Blumer, K. J., Kang, V. H., and Kehrl, J. H. (1996) Nature 379, 742–746
5. Koelle, M. R., and Horvitz, H. R. (1996) Cell 84, 115–125
6. Yan, Y., Chi, P. P., and Bourne, B. (1997) J. Biol. Chem. 272, 11924–11927
7. Huang, C., Hepler, J. R., Gilman, A. G., and Mummy, S. M. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 6159–6163
8. Berman, D. M., Wilkie, T. M., and Gilman, A. G. (1996) Cell 86, 445–452
9. Watson, N., Linder, M. E., Druey, K. M., Kehrl, J. H., and Blumer, K. J. (1996) Nature 383, 172–175
10. Hunt, T. W., Fields, T. A., Casey, P. J., and Peralta, E. G. (1996) Nature 383, 175–177
11. Saitoh, O., Kubo, Y., Miyatani, Y., Asano, T., and Nakata, H. (1997) Nature 390, 525–529
12. Saitoh, O., Kubo, Y., Odagiri, M., Ichikawa, M., Yamagata, K., and Sekine, T. (1999) J. Biol. Chem. 274, 9899–9904
13. Kubo, Y., Reuveny, E., Slesinger, P. A., Jan, Y. N., and Jan, L. Y. (1993) Nature 364, 802–806
14. Lesage, F., Guillermare, E., Fink, M., Duprat, F., Heurteloux, C., Fosset, M., Roney, G., Barhanin, J., and Lazdunski, M. (1995) J. Biol. Chem. 270, 28660–28667
15. Veilimirovich, B. M., Gordon, E. A., Lim, N. F., Navarro, B., and Clapham, D. E. (1996) FEBS Lett. 379, 31–37
16. Doupnik, C. A., Davidson, N., Lester, H. A., and Kofuji, P. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 10461–10466
17. Chen, C., and Lin, S.-C. (1998) FEBS Lett. 422, 359–362
18. Srinivasa, S. P., Bernstein, L. S., Blumer, K. J., and Linder, M. E. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 5584–5589
19. Chen, C., Seow, K. T., Guo, K., Yaw, L. P., and Lin, S.-C. (1999) J. Biol. Chem. 274, 18799–18806
20. Saitoh, O., Odagiri, M., Masuho, I., Nomoto, S., and Kinoshita, N. (2000) Biochem. Biophys. Res. Commun. 270, 34–39
21. Nomoto, S., Adachi, K., Yang, L.-X., Hirata, Y., Muraguchi, S., and Kizuki, K. (1997) Biochem. Biophys. Res. Commun. 241, 281–287
22. Niwa, H., Yamamura, K., and Miyazaki, J. (1991) Gene (Amst.) 108, 193–200
23. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) Molecular Cloning: A LaboratoryManual, Cold Spring Harbor Lab., Cold Spring Harbor, NY
24. Druey, K. M., Sullivan B. M., Brown, D., Fischer, E. R., Watson, N., Blumer, K. J., Gerfen, C. R., Scheschenka, A., and Kehrl, J. H. (1998) J. Biol. Chem. 273, 18405–18410
25. Dulin, N. O., Pratt, P., Tiruppathi, C., Niu, J., Voyno-Yasenetskaya, T., and Dunn, M. J. (2000) J. Biol. Chem. 275, 21317–21323
26. Kovoor, A., Chen, C.-K., He, W., Wensel, T. G., Simon, M. L., and Lester, H. A. (1999) J. Biol. Chem. 275, 3397–3402
27. Chuang, H.-H., Yu, M., Jan, Y. N., and Jan, L. Y. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 11727–11732
28. Chen, C., Zheng, B., Han, J., and Lin, S.-C. (1997) J. Biol. Chem. 272, 8679–8685