Nuclear Localization of G Protein β5 and Regulator of G Protein Signaling 7 in Neurons and Brain*

Received for publication, October 10, 2000, and in revised form, December 20, 2000
Published, JBC Papers in Press, January 4, 2001, DOI 10.1074/jbc.M009247200

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The role that Gβ5 regulator of G protein signaling (RGS) complexes play in signal transduction in brain remains unknown. The subcellular localization of Gβ5 and RGS7 was examined in rat PC12 pheochromocytoma cells and mouse brain. Both nuclear and cytosolic localization of Gβ5 and RGS7 was evident in PC12 cells by immunocytochemical staining. Subcellular fractionation of PC12 cells demonstrated Gβ5 immunoreactivity in the membrane, cytosolic, and nuclear fractions. Analysis by limited proteolysis confirmed the identity of Gβ5 in the nuclear fraction. Subcellular fractionation of mouse brain demonstrated Gβ5 and RGS7 but not Gγ2/3 immunoreactivity in the nuclear fraction. RGS7 and Gβ5 were tightly complexed in the brain nuclear extract as evidenced by their communoprecipitation with anti-RGS7 antibodies. Chimeric protein constructs containing green fluorescent protein fused to wild-type Gβ5 but not green fluorescent fusion proteins with Gβ1β or a mutant Gβ5 impaired in its ability to bind to RGS7 demonstrated nuclear localization in transfected PC12 cells. These findings suggest that Gβ5 undergoes nuclear translocation in neurons via an RGS-dependent mechanism. The novel intracellular distribution of Gβ5-RGS complex suggests a potential role in neurons communicating between classical heterotrimeric G protein subunits and/or their effectors at the plasma membrane and the cell nucleus.

Seven transmembrane-spanning receptors respond to extracellular signals and in turn regulate intracellular processes through their interaction with signal-transducing heterotrimeric guanine nucleotide-binding regulatory proteins (G proteins) in eukaryotic cells (1). Complementary DNAs from five G protein β subunit genes (Gβ1–5) have been identified by molecular cloning. Whereas the Gβ1–4 isoforms are highly homologous (80–90%) and widely expressed (2), the Gβ5 isoform exhibits much less homology with other isoforms (~50%) and is preferentially expressed in brain (3). A splice variant of Gβ5, Gβ5-long (Gβ5L), is present in retina, which contains a 42-amino acid N-terminal extension (4).

Although Gβ5 can be shown to interact with classical components of heterotrimeric G proteins signaling pathways such as Gγ2 (3, 5, 6), Gαq (6), and phospholipase C-β (3, 5, 7, 8) when tested in vitro, no such interactions have been demonstrated in native tissues. Instead, Gβ5 has been purified from retinal cytosol bound to regulator of G protein signaling (RGS)1 protein-7 (RGS7) (9, 10) and from brain bound to RGS6 (11) and RGS7 (10, 11). Additionally, a tight native complex between Gβ5L and RGS9 was isolated from retinal rod outer segment membrane extracts (12). Studies of recombinant proteins in vitro show that a tight interaction with RGS proteins 6, 7, and 11 is demonstrable for Gβ5 and Gβ5L but not for the other Gβ isoforms, mediated by a Gγ-like (GGL) domain present in a subfamily of RGS proteins (13–16). These recent novel observations underscore the view of Gβ5 as a unique and highly specialized G protein subunit but leave open the question of its function within the brain.

To this end this work was undertaken to examine the intracellular distribution of Gβ5. The results demonstrate that Gβ5, along with RGS7, is expressed prominently in the neuronal nucleus, as well as the cell membrane and cytosol. This distribution pattern suggests that Gβ5-RGS complexes may shuttle information between classical G protein-signaling elements at the plasma membrane and the cell nucleus.

EXPERIMENTAL PROCEDURES

Cell Culture—For maintenance, rat pheochromocytoma PC12 cells were grown in 75-cm² flasks grown at 37 °C and 5% CO2 containing DMEM supplemented with 10% horse serum, 5% fetal bovine serum, 4 mM l-glutamine, 1 × penicillin/streptomycin (Biofluids, Rockville, MD) (supplemented DMEM) without the addition of nerve growth factor (NGF).

Immunocytochemistry and Confocal Laser Microscopy—Cells were processed for immunofluorescent staining as described previously (17). Briefly, PC12 cells were plated onto poly-l-lysine-precocated covered chamber slides (Lab-Tek II, Nalge) and grown in supplemented DMEM containing 50 ng/ml NGF at 37 °C for 16 h. The medium was discarded, and the cells were washed and then fixed in 2% (v/v) formalin in phosphate-buffered saline. The slides were then incubated with one or more primary antibodies in phosphate-buffered saline, 10% fetal calf serum (v/v), and 0.075% (w/v) saponin for 1.5 h, washed, and then incubated with appropriate labeled secondary antibody (fluorescein isothiocyanate-conjugated donkey anti-rabbit IgG and rhodamine red-conjugated donkey anti-goat IgG (Jackson ImmunoResearch Labs, West Grove, PA) in the same buffer for 45 min. For imaging of nuclei,
cells were treated with the DNA-binding cyanine dye YOYO (18). After staining, 1–2 drops of PermMount (Vector Labs, Burlingame, CA) were added to the sample surface. For epifluorescent imaging, cells were viewed in a Zeiss Axioshot inverted microscope (Carl Zeiss Inc., Thornwood, NY), and images were captured with a PentaMax camera (Princeton Consultants, Princeton, NJ) and cooled 12-bit frame grabs digitized at 1,000 x 1,000 image size using the Metamorph software. Z-stacks were acquired at 0.5 μm increments in the z-axis and deconvolved using a fully automated 3D deconvolution algorithm. Images were analyzed with NIH Image (Wayne Rasband, National Institutes of Health, Bethesda, MD) and Adobe Photoshop (Adobe Systems Inc., Mountain View, CA) software.

**Subcellular Fractionation of Mouse Brain and PC12 Cells—**CD1 mouse brain (1 g, wet weight) or a PC12 cell pellet (2 × 10⁶) was homogenized (30 strokes) in Buffer A (with a Dounce homogenizer with a pestle in 5 ml of Buffer A (50 mM triethanolamine HCl, pH 7.5, 25 mM KCl, 5 mM MgCl₂, 0.5 mM dithiothreitol, 17 μg/ml AEBFF, 2 μg/ml each aprotinin, leupeptin, and pepstatin; and 1 μg/ml soybean trypsin inhibitor) containing 0.25 m sucrose on ice. The homogenate was centrifuged at 1,000 × g for 5 min at 4 °C to remove tissue debris (first pellet). The first supernatant was then centrifuged at 2,000 × g for 15 min at 4 °C. The second pellet was saved, and the second supernatant was further centrifuged at 100,000 × g for 1 h at 4 °C. The third pellet was then resuspended in 1 ml of Buffer A containing 50% sucrose and labeled as nuclear extract. For independent experimental replication, the membrane, nuclear, and cytosol fractions of both mouse brain and PC12 cells were also obtained commercially (Geneza Biotechnology, Quebec, Canada). Proteins from different fractions were quantified by both the Bradford method (20) and Coomassie Blue staining of SDS-polyacrylamide gels. Limited Proteolysis of PC12 Nuclear Extract—PC12 nuclear extract prepared as above was dialyzed against 20 mM Tris-HCl and 5 mM dithiothreitol overnight at 4 °C, then loaded onto a Mono Q anion exchange column (Amersham Pharmacia Biotech) equilibrated in the same buffer. The column was eluted in a gradient from 0 to 0.6 m NaCl, and the fractions containing a broad peak of G₁β₅ (C-terminal antibody reactive GRAV (hydroimmune eluting at 7.8 m NaCl) were collected, and the first equilibrated fraction was then dialyzed against 20 mM Tris-HCl and 5 mM dithiothreitol. Subcellular Localization of Gβ₅ in PC12 Cells—Biochemical analysis of fractions prepared from mouse (4, 11) and rat (10, 22) brain homogenates demonstrated previously Gβ₅ association with both membrane and cytosolic fractions. To resolve and analyze the subcellular distribution of Gβ₅ better, a homogenous mixture of neuronal cells in continuous culture such as PC12 cells, which upon differentiation assume a neuron-like phenotype, offers many advantages over brain. The expression of Gβ₅ mRNA and protein in rat pheochromocytoma PC12 cells as well as several other cell lines of neuroendocrine origin was recently documented by ribonuclease protection and immunoblotting, respectively (29). The levels of Gβ₅ expression in PC12 cells were not altered significantly by NGF treatment and differentiation, respectively (29). We therefore studied the intracellular distribution of Gβ₅ in NGF-differentiated PC12 cells by immunocytochemical methods (Fig. 1A and B). Unexpectedly, an epifluorescent signal over the nucleus, in addition to diffuse cytoplasmic staining, was evident with ATDG antibody directed against the N terminus of Gβ₅ (11) (Fig. 1A). Cells processed in parallel with preimmune antibodies produced a weaker background signal (Fig. 1B). To

**RESULTS**

**Subcellular Localization of Gβ₅ in PC12 Cells—**Biochemical analysis of fractions prepared from mouse (4, 11) and rat (10, 22) brain homogenates demonstrated previously Gβ₅ association with both membrane and cytosolic fractions. To resolve and analyze the subcellular distribution of Gβ₅ better, a homogenous mixture of neuronal cells in continuous culture such as PC12 cells, which upon differentiation assume a neuron-like phenotype, offers many advantages over brain. The expression of Gβ₅ mRNA and protein in rat pheochromocytoma PC12 cells as well as several other cell lines of neuroendocrine origin was recently documented by ribonuclease protection and immunoblotting, respectively (29). The levels of Gβ₅ expression in PC12 cells were not altered significantly by NGF treatment and differentiation, respectively (29). We therefore studied the intracellular distribution of Gβ₅ in NGF-differentiated PC12 cells by immunocytochemical methods (Fig. 1A and B). Unexpectedly, an epifluorescent signal over the nucleus, in addition to diffuse cytoplasmic staining, was evident with ATDG antibody directed against the N terminus of Gβ₅ (11) (Fig. 1A). Cells processed in parallel with preimmune antibodies produced a weaker background signal (Fig. 1B). To

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... centrifugation and resuspended in 0.4 ml of DMEM without fetal bovine serum. The cell suspension was transferred to an electrophoresis cuvette and mixed with 15 μg of pTRE-HAGβ₅ plasmid or pTRE vector and 1 μg of pTK-HG plasmid (encoding the hygromycin resistance gene). After the mixture was incubated in the hood for 10 min, the cells were electrotransfected at 1,000 V and 37°C in a vacuum chamber. Cells were immediately transferred to 10 ml of fresh DMEM containing 10% FBS and G418 in a 100-mm dish (biocompatible) and incubated at 37 °C. After a 24-h incubation, hygromycin was added to a final concentration of 200 μg/ml, and the culture was placed back in the incubator until individual colonies were visible. Individual colonies were expanded, and the level of HA-Gβ₅ gene expression was determined by Western blot analysis. Peak protein expression upon doxycycline induction (2.5 μg/ml) occurred in 6–12 h.

**Preparation of cDNAs Encoding GFP Fusion Proteins and RG75 for Transient Transfection—**For construction of a GFP-Gβ₅ fusion expression plasmid, wild-type Gβ₅ cDNA was cloned in-frame downstream of a red-shifted GFP variant in the pEFGP-C2 vector (CLONTECH) using the Rapid Ligation Kit (Roche). The starting methionine of Gβ₅ in this construct was eliminated by mutagenesis into alanine using the Quick-Change site-directed mutagenesis kit (Stratagene, La Jolla, CA). The resultant construct was named pEFP-Gβ₅. For construction of the pEFP-Gβ₅-L22P,L26P mutant, codons corresponding to leucine residues 22 and 26 of the wild-type Gβ₅ sequence within the pEFP-Gβ₅-G12 fusion protein was altered to threonine using the Quick-Change kit. For construction of the Gβ₅ fusion protein, the Gβ₅ cDNA was polymerase chain reaction amplified using a 5′-primer that converted the starting methionine to alanine, and the resulting polymerase chain reaction product was ligated in-frame into the pEGFP-C2 vector. The resultant construct was named pEFP-Gβ₅. For transient expression of RG75, codons 2–269 (full-length except for the starting methionine) of bovine RGS7 (kindly provided by Dr. Vladlen Slepak) were amplified by polymerase chain reaction employing the Pwo polymerase (Boehringer Mannheim). Primers encoding 5′- and 3′- BamHI linkers were employed, and after digestion the resulting construct was ligated in-frame into the BamHI site of pcDNA/HisMax-C vector (Invitrogen), which adds N-terminal His6 and Xpress epitope tags. The DNA sequence of all inserts was verified by diodeoxy sequencing. Transient Expression of GFP Fusion Proteins in PC12 Cells—1 day prior to transient transfection of GFP fusion plasmids, PC12 cells at 90% confluence were harvested and plated in 2 ml of supplemented DMEM containing 50 ng/ml NGF into the chambers of polyp-n-glycine coated chamber slides as detailed above. The number of cells was adjusted to 70% of their density at harvest. After overnight incubation of the cells, 2 μg of plasmid DNA and 8 μl of Lipofectamine 2000 reagent (Life Technologies), diluted in 200 μl of Opti-MEMI medium, were mixed, incubated at room temperature for 20 min, and added directly to each chamber. After mixing gently, the culture was incubated for 24–48 h. The expression of each fusion construct was then verified by immunoblotting, and the fluorescence of the GFP fusion proteins was determined by fluorescence microscopy as detailed above.
confirm that the Gβ5 immunofluorescent signal over the nucleus seen by light microscopy was indeed associated with the nucleus, subcellular fractionation of PC12 cells and immunoblotting analysis with two different antibodies to Gβ5 were performed: N-terminally directed ATDG and the C-terminally directed SGS antibody (5) (Fig. 1, E and F). In the resulting immunoblots both antibodies demonstrated a ~39-kDa immunoreactive band in the nuclear fraction of PC12 cells of identical mobility to the Gβ5 present in the membrane and cytosolic fractions (Fig. 1E, upper panel, and 1F). In contrast, Gβ1 immunoreactivity was confined to the membrane fraction (Fig. 1E, middle panel). To verify the identity of the ~39-kDa immunoreactive band in the PC12 cell nuclear fraction as Gβ5, immunoblots with antibody SGS directed against the C terminus of Gβ5(5) were performed on control and experimental samples subjected to limited proteolytic digestion (5) (Fig. 1G). Partial digestion with endoproteinase Lys-C resulted in major C-terminal fragments of ~22 kDa and 12 kDa, whereas treatment with V8 protease resulted in a major ~35-kDa C-terminal immunoreactive fragment (Fig. 1G). The C-terminal fragments were identical in mobility to those generated in parallel from mouse brain membranes (Fig. 1G) and to those generated from recombinant Gβ5 as described previously (5).

**Dual Immunofluorescence Analysis of Gβ5 and RGS7 in Stably Transfected PC12 Cells by Confocal Microscopy**—To increase the signal to noise ratio for further immunofluorescence studies, PC12 cells were stably transfected with an N-terminally HA epitope-tagged Gβ5 cDNA under the control of an inducible promoter. When induced by doxycycline in NGF-differentiated PC12 cells, the pattern of Gβ5 immunoreactivity was the same as that seen by epifluorescence in the naïve PC12 cells, with strong cytoplasmic and nuclear expression whether analyzed with ATDG antibody (Fig. 2, A and C) or anti-HA antibody (not shown). The nuclear localization was confirmed by confocal microscopy and dual immunofluorescence analysis with the DNA-binding cyanine dye YOYO (18) (Fig. 2, D–F).

Three groups have independently isolated tight native complexes of Gβ5 bound to RGS7 from rodent brain (10, 11, 24). We...
Fig. 2. Confocal dual immunofluorescence analysis of stably transfected, NGF-differentiated PC12 cells. Cells stably transfected with pTRE-HA\(\beta_5\) encoding HA epitope-tagged \(\beta_5\) were induced with doxycycline and analyzed by laser confocal microscopy after dual staining with affinity-purified ATDG anti-\(\beta_5\) antibody (11) (red signal) and anti-RGS7 (C-19) antibody (green signal) (panels A–C), or ATDG antibody and the nuclear dye YOYO (18) (green signal) (panels D–F) as described under “Experimental Procedures.” Immunofluorescence was monitored singly or in combination (merge) as indicated. The yellow-orange signal indicates colocalization of probes.

Fig. 3. Immunoblot analysis of mouse brain subcellular fractions and immunoprecipitation analysis of the brain nuclear fraction. Panel A, mouse brain homogenate was fractionated into crude membrane (M), cytoplasmic (C), and nuclear (N) fractions and analyzed by SDS-polyacrylamide gel electrophoresis and immunoblotting with the antibodies shown as described under “Experimental Procedures.” Anti-\(\beta_5\) blots employed the N-terminally directed antibody ATDG (11). The RGS7 immunoblots employed either antibody C-19 or R-20 (Santa Cruz) as shown. In the final panel the R-20 antibody was preadsorbed with the cognate peptide. The relative mobility of the major immunoreactive bands is given on the right in kDa. Panel B, mouse brain nuclear extract was incubated with either goat anti-RGS7 C-terminal antibody C-19 or normal goat IgG, and after precipitation the washed immunoprecipitates were analyzed for RGS7 and \(\beta_5\) immunoreactivity by immunoblotting as described under “Experimental Procedures.” The relative mobility of the specific immunoreactive bands (in kDa) and the goat immunoglobulin heavy chain (HC) is indicated on the right.

Therefore examined the cells for evidence of nuclear expression of RGS7. Confocal microscopic analysis of cells probed with antibody to the C terminus of RGS7 revealed strong cytoplasmic and a weaker, patchy nuclear staining pattern (Fig. 2B). A similar distribution of RGS7 was evident in naive PC12 cells analyzed with the same antibody (Fig. 1C; compare with 1D). Dual immunofluorescence analysis with both \(\beta_5\) and RGS7 antibodies revealed colocalization of proteins as evidenced by the faint orange coloration of nuclei staining with both antibodies (Fig. 2, B and C).

Subcellular Localization of \(\beta_5\) and RGS7 in Brain—Mouse brain was studied to probe the generality of the \(\beta_5\) expression pattern determined in naive and transfected sympathetic neuron-like PC12 cells. Analysis of mouse brain subcellular fractions was performed by immunoblotting (Fig. 3A). This approach was preferable over immunocytochemical analysis for as a recent confocal dual immunofluorescence study with antibodies to \(\beta_5\) and RGS7 documented their colocalization in many regions of rat brain but reached no conclusion about their subcellular distribution (24). As in PC12 cells, \(\beta_5\) immunoreactivity at 39 kDa was evident in the membrane, cytosolic, and nuclear fractions of mouse brain, whereas the \(\beta_1\) 36-kDa immunoreactive band was confined to the membrane fraction (Fig. 3A). Antibodies to G\(\gamma_2/\gamma_3\) reacted strongly only with a ~6.5-kDa band in the membrane fraction (Fig. 3A). Immunoblotting with two different antibodies to RGS7, C-19 and R-20, yielded bands in all three subcellular fractions. In the membrane and cytosolic fractions the immunoreactivity of the major band at ~55 kDa, but not the reactivity of a faint upper band in the cytosol, could be blocked by preincubation with the cognate peptide (Fig. 3A). In the nuclear fraction a doublet of immunoreactive bands migrating slightly slower than the ~55kDa membrane and cytosolic bands disappeared with peptide preincubation (Fig. 3A), confirming their identity as RGS7-related proteins. Interestingly, a recent analysis of the intracellular distribution of epitope-tagged RGS4 in transfected COS-7 cells also found preferential nuclear expression of a slower migrating species (25). The nature and significance of the slower migrating forms of RGS7 in brain nuclei are unclear.

Tight complexes between native \(\beta_5\) and RGS6 and RGS7 present in brain membranes and cytosol have been demonstrated in previous studies (10, 11, 24), and the presence of both \(\beta_5\) and RGS7 in the brain nuclear fraction raises the question of their possible interaction in that compartment. To check for possible complex formation between the RGS7 and \(\beta_5\) in the brain nuclear fraction, immunoprecipitation with C-terminally directed RGS7 antibody was performed (Fig. 3B). Both an
of GFP-G<sub>b</sub> and 26 were mutated to prolines (GFP-G<sub>b</sub> (13) comprising the putative RGS dimerization interface (13, 14), as evidenced by the greatly diminished ability of the GFP-G<sub>b</sub> containing RGS proteins (13, 14), as evidenced by the greatly diminished ability of the GFP-G<sub>b</sub> mutant to coimmunoprecipitate but not in control immunoprecipitates employing normal goat IgG. These results are consistent with a tight association between G<sub>b</sub> and RGS7 in the mouse brain nuclear fraction.

Subcellular Localization of GFP Fusions with Wild-type and Mutant G<sub>b</sub>—The colocalization of G<sub>b</sub> with RGS7 in the nuclei of PC12 cells (Fig. 2) taken together with the results above demonstrating coimmunoprecipitation of G<sub>b</sub> with RGS7 from brain nuclear extract (Fig. 3B) suggest that nuclear G<sub>b</sub> is present as a heterodimer with one or more GGL domain-containing RGS proteins. To check if such heterodimerization might be required for proper nuclear localization of G<sub>b</sub>, three chimeric protein constructs containing GFP were prepared and studied in transiently transfected PC12 cells. These chimeras contained GFP fused in frame to (a) wild-type G<sub>b</sub> (GFP-G<sub>b</sub>), (b) a double point mutant G<sub>b</sub> in which leucines at positions 22 and 26 were mutated to prolines (GFP-G<sub>b</sub>-L22P,L26P), or (c) G<sub>b</sub> (GFP-G<sub>b</sub>), the last serving as a negative control. The leucine residues mutated in GFP-G<sub>b</sub>-L22P,L26P were chosen so as to disrupt the postulated N-terminal coiled-coil region of G<sub>b</sub>, comprising the putative RGS dimerization interface (13, 14) (Fig. 4A).

All three GFP fusion proteins were expressed in transiently transfected PC12 cells and had the expected immunoreactivity according to their chimeric composition as evidenced by immunoblots (Fig. 4C). Furthermore, the effect of the L22P,L26P mutations in G<sub>b</sub> was to disrupt interaction with GGL domain-containing RGS proteins (13, 14), as evidenced by the greatly diminished ability of the GFP-G<sub>b</sub> mutant to coimmunoprecipitate with RGS7 in transfected PC12 cells under conditions permissive for the wild-type GFP-G<sub>b</sub> fusion (Fig. 4D).

Like G<sub>b</sub> in naïve and stably transfected PC12 cells analyzed immunocytochemically, the fluorescence of GFP-G<sub>b</sub> was strong in the nuclei of the majority of transiently transfected PC12 cells (Fig. 4B, left panel). In contrast the pattern of signal in GFP-G<sub>b</sub>-L22P,L26P and GFP-G<sub>b</sub> transfected cells showed only faint fluorescence in the cell nuclei of most labeled cells (Fig. 4B, right and middle panels). Such a faint background nuclear signal is often seen with GFP fusion proteins targeted predominantly to the cytosol and may be related to the ability of the GFP moiety to enter the nucleus passively (26). The anomalous cytosolic expression of the GFP-G<sub>b</sub> fusion compared with endogenous G<sub>b</sub> (compare Figs. 1E and 3A) is likely caused by impaired G<sub>b</sub> nuclear expression of GFP-G<sub>b</sub> fusion proteins has been noted previously by other laboratories (27, 28).

DISCUSSION

The nuclear localization documented here illustrates another novel property of G<sub>b</sub> and provides the first example of a heterotrimeric G<sub>b</sub> subunit expressed in the neuronal cell nucleus. G<sub>b</sub> was found in the nucleus of growth factor-stimulated Swiss 3T3 cells as part of a heterotrimer with G<sub>a</sub>, although it was absent from the nucleus fraction in quiescent cells (29). In neurons several Ga isoforms have been found to undergo retrograde axonal transport and localize in the cell nucleus (for review, see Ref. 30). Whether any functional interactions might exist between G<sub>b</sub>-RGS complexes or perhaps other RGS proteins (25, 31) and Ga subunits within the neuronal nuclear compartment remains to be shown.

The molecular basis for the nuclear targeting of G<sub>b</sub> in neurons and brain is unclear. The primary sequence of G<sub>b</sub> lacks an obvious consensus nuclear localization signal (NLS) (32). Furthermore, its sequence is wholly contained within the retinal splice variant G<sub>b</sub>L, which is strongly membrane-anchored (4, 12) and is therefore unlikely to contain a functional NLS.

The demonstration here that mutational perturbation of the N-terminal region of G<sub>b</sub> blocks its ability to form tight complexes with the GGL domain-containing RGS7, also prevents nuclear expression of the GFP fusion protein suggests that RGS7 (or other G<sub>b</sub>-binding RGS proteins (14)) may con-
tain a NLS. Recent evidence that other RGS proteins including RGS2, RGS10 (25), and a truncated version of RGS3 (RGS3T) (31) exhibit nuclear localization and contain putative NLS sequences makes this possibility more likely. Neither RGS6 nor RGS7 contains the Arg-Lys-Arg-Lys and Arg-Arg-Arg NLS sequence postulated for RGS3 (31) nor the Lys-Lys-Aax-Axx/Lys putative NLS motif present in RGS2, RGS4, and RGS16 (25). Nevertheless, RGS6 and RGS7 contain other sequence patterns suggestive of NLS (32) including Lys-Ely-Elys-Pro and Lys-Elys/Elys-Pro-Pro-Pro-Pro in the long splice variant of RGS6 (15) and RGS7 (33), respectively. Whether these or other candidate sequences in RGS6 and RGS7 actually function as NLS remains to be tested experimentally, however.

In PC12 cells the nuclear staining with RGS7 antibody was consistently fainter and less uniform than the Gβ5 staining. This result might be expected because RGS7 is only tested heterotrimeric G protein subunits and/or their effectors when containing RGS proteins such as RGS9 and 11 expressed in brain with anti-RGS6 antibody demonstrates nuclear expression level (not shown). Nevertheless the question of possible cell compartmen cons likely other RGS proteins (25, 31) among membrane, cytoplasmic, and nuclear compartments might allow for information transfer between signaling elements at the plasma membrane and protein targets in the cell nucleus. Thus as has been shown for RGS proteins such as p115 RhoGEF (37, 38) and PDZ-RhoGEF (39) perhaps Gβ5-RGS heterodimers function as intracellular signal transducers.

Acknowledgments—We thank George Poy for oligonucleotide synthesis and DNA sequencing, Dr. Carolyn Smith for sharing her expertise in confocal imaging, Dr. Vladlen Slepak for the RGS7 cDNA, and Dr. Allen Spiegel for continued support and encouragement.

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J. Biol. Chem. 2001, 276:10284-10289.
doi: 10.1074/jbc.M009247200 originally published online January 4, 2001

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