Phosphorylation and Nuclear Translocation of a Regulator of G Protein Signaling (RGS10)*

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Heterotrimeric G proteins are involved in the transduction of hormonal and sensory signals across plasma membranes of eukaryotic cells. Hence, they are a critical point of control for a variety of agents that modulate cellular function. Activation of these proteins is dependent on GTP binding to their α (Gα) subunits. Regulators of G protein signaling (RGS) bind specifically to activated Gα proteins, potentiating the intrinsic GTPase activity of the Gα proteins and thus expediting the termination of Gα signaling. Although there are several points in most G protein controlled signaling pathways that are affected by reversible covalent modification, little evidence has been shown addressing whether or not the functions of RGS proteins are themselves regulated by such modifications. We report in this study the acute functional regulation of RGS10 through the specific and inducible phosphorylation of RGS10 protein at serine 168 by cAMP-dependent kinase A. This phosphorylation nullifies the RGS10 activity at the plasma membrane, which controls the G protein-dependent activation of the inwardly rectifying potassium channel. Surprisingly, the phosphorylation-mediated attenuation of RGS10 activity was not manifested in an alteration of its ability to accelerate GTPase activity of Gα. Rather, the phosphorylation event correlates with translocation of RGS10 from the plasma membrane and cytosol into the nucleus.

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FIG. 1. Phosphorylation of serine 168 of RGS10 by cAMP-dependent kinase A. A, primary amino acid sequence of human RGS10. The putative cAMP-dependent kinase A motif (165KRAS168) is indicated by the black box. B, phosphorylation of RGS10 by PKA in vitro. Purified His-tagged RGS10 and RGS10-S168A were incubated with the catalytic subunit of PKA and [γ-32P]ATP. The proteins were resolved by SDS-PAGE and detected by autoradiograph (upper panel) and by staining with Coomassie Brilliant Blue (lower panel). Lane 1, wild-type RGS10; lane 2, protein size marker; lane 3, no RGS10; lane 4, RGS10-S168A. C, PKA-mediated phosphorylation of RGS10 in intact cells. HEK 293 cells transiently transfected with RGS10 or RGS10-S168A were metabolically labeled with [32P]orthophosphate and stimulated with 25 μM forskolin for 15 min at 37 °C. Whole cell lysates were then immunoprecipitated with an anti-RGS10 antibody resolved by SDS-PAGE, and analyzed by autoradiography (upper panel) and immunoblotting (lower panel) with an anti-RGS10 antibody. Lane 1, mock transfection; lane 2, RGS10; lane 3, RGS10, dominant-negative PKA and forskolin; lane 4, RGS10 and forskolin; lane 5, RGS10-S168A; lane 6, RGS10-S168A and forskolin. wt, wild type; SA, S168A. D, effect of PKA-mediated phosphorylation on RGS10 binding to activated Goαi3. HEK 293 cells transiently transfected with Myc-tagged RGS10 and RGS10-S168A. The indicated whole cell lysates were incubated with GDP (−) or GDP and AlF4−. The whole cell lysates were then immunoprecipitated with an anti-Myc monoclonal antibody and analyzed by immunoblotting with polyclonal antisera specific for the endogenous Goαi3 (upper panel) and a polyclonal antibody specific for RGS10 (lower panel). wt, wild type; SA, S168A.

manifested as a reduction in its ability to accelerate GTPase activity of Go proteins but, instead, correlates with a translocation of the RGS into the nucleus.

EXPERIMENTAL PROCEDURES

Clones—The RGS10-S168A clone was generated by site-directed mutagenesis (16). The mammalian expression vector for PKI was kindly provided by G. S. McKnight. All clones expressed in mammalian cells were subcloned into the mammalian expression vector pcDNA3 (Invitrogen).

Protein Expression and Purification—Bacterial expression plasmids for RGS10 proteins were constructed by subcloning RGS10 cDNA into the His tag fusion vector pRSETA (Invitrogen). Plasmids were transformed into the bacterial strain BL21 DE3pLysS and induced with 250 μM isopropyl-1-thio-β-D-galactopyranoside for 2 h during exponential growth phase. Cells were lysed in 50 mM Tris-HCl (pH 7.5), 500 mM NaCl, 25 mM imidazole (IMAC-25 buffer), and 0.1% Triton X-100 in the presence of a mixture of protease (Sigma) inhibitors, and then centrifuged at 100,000 × g for 45 min. The supernatant was loaded on to a Ni2+-nitrilotriacetic acid column (Amersham Pharmacia Biotech), washed with IMAC-25 buffer, and eluted with a linear gradient from 25 to 250 mM imidazole. The fractions containing the hexahistidine-tagged RGS10 proteins were then dialyzed against 50 mM Tris-HCl, pH 7.5, and 5 mM DTT. The dialyzed protein was then loaded on to an anion exchange column (MonoQ, Amersham Pharmacia Biotech) and eluted with a linear gradient from 0 to 300 mM NaCl in 50 mM Tris-HCl (pH 7.5) and 5 mM DTT. The hexa-His-RGS10 purity was >95%, as determined by Coomassie Blue-stained protein gels.

In Vitro PKA Phosphorylation—Hexahistidine-tagged RGS10 (0.1 mg/ml) was incubated with 180 units/ml catalytic subunit of PKA (New England Biolabs) at 30 °C for 15–120 min in 50 mM Tris-HCl (pH 7.5), 10 mM MgCl2, and 200 μM ATP. For the autoradiographic studies, the reaction mixture was supplemented with γ-labeled ATP to a final specific activity of 200 μCi/μmol. RGS10 phosphorylation was assayed by gel-shift on a SDS-PAGE (12.5% gel) in the presence of reducing agents. The phospho-RGS10 was then separated from the PKA and nucleotides gel-shift on a SDS-PAGE (12.5% gel) in the presence of reducing agents.

Cell Culture, Immunoprecipitation, and Metabolic Labeling Studies—All tissue culture cells were grown in DMEM supplemented with 10% fetal calf serum and antibiotics unless otherwise indicated at 37 °C in 5% CO2. A Myc epitope tag (MEQKLISEEDL) was fused to the 5’end of a cDNA fragment encoding RGS10 by site-directed mutagenesis (5, 16) and confirmed by DNA sequencing. The Myc-RGS10 was inserted into the mammalian expression vector pcDNA3 (Invitrogen) and used to transiently transfect human HEK 293 cells (5). Transfected cells were lysed in buffer (PBS, 6 mM MgCl2, 1% Nonidet P-40, 0.25% sodium deoxycholate, and protease inhibitor mixture (Sigma) as described previously (5). Binding of RGS10 to endogenous Goαi3 loaded with GDP or GDP and AlF4− required the lysates to be incubated for 30 min at 20 °C in the presence of GDP (10 μM) or GDP (10 mM) and AlF4− (30 μM).

Electrophysiology—Two-electrode voltage clamp was used to measure the currents from individual Xenopus oocytes immersed in Barth’s solution at room temperature as described previously (17). Xenopus laevis oocytes were injected with a 50-nl solution containing cRNA of m2 muscarinic receptor, G protein-coupled inwardly rectifying K+ (GIRK) 1, and GIRK 4. Twelve hours later, cRNA encoding RGS10wt or RGS10-S168A were also injected. Electrophysiological recordings were made 3 days after injection under two-electrode voltage clamp in a
continuous flow chamber perfused with Barth’s solution at room temperature. Oocytes were clamped at −60 mV. During each recording carbachol (2 μM) was applied to each oocyte for 20 s and then washed out with Barth’s solution.

Protein kinase A inhibitory peptide (PKI; Calbiochem; 50 nl of 0.2 μM dissolved in water) was injected into oocytes co-expressing the GIRK 1/4 channel combination, m2 muscarinic receptor, and, when indicated, with RGS10 30 min prior to recording. For oocytes stimulated with forskolin (Calbiochem; 25 μM dissolved in OR2, 82.5 mM NaCl, 2 mM KCl, 1.8 mM CaCl₂, 1 mM MgCl₂, 5 mM HEPES, pH 7.5), they were incubated in the forskolin-containing solution for 30 min prior to recording. All current activation and deactivation time constants were determined by fitting current trace to a Boltzman equation.

**GTPase Studies**—Purified bovine brain Gαs (40 nM) (18) was loaded with GTP upon incubation with 1 μM [γ-32P]GTP (5–50,000 cpm/pmol) containing 50 mM HEPES (pH 7.7), 10 mM EDTA, 1 mM DTT, and 0.025% Lubrol; the stoichiometry of loading was 15–20%. Magnesium was excluded from the loading reactions to slow intrinsic GTP hydrolysis. Unbound GTP was removed by centrifuging (2000 × g for 3 min at 4 °C) the reaction through 1 ml of G50 Sephadex (Amersham Pharmacia Biotech) equilibrated in 50 mM Hepes (pH 7.7), 1 mM EDTA, 1 mM DTT. GTP-loaded Gαs (13 nM) was incubated for 3 min with or without RGS10 (50 nM), as indicated in the figure legend, in a total volume of 30 μl containing 50 mM Hepes (pH 7.7), 1 mM EDTA, 1 mM DTT, 1.7 mM MgCl₂, and 330 μM GTP. GTP hydrolysis was evaluated by quantitating the release of phosphate (19).

**Immunocytochemistry**—HEK 293 cells transiently expressing RGS10 were trypsinized and plated on sterile polylysine-coated cover-slips and incubated overnight. Two hours prior to stimulation, the cells were placed in serum-free DMEM and then stimulated with forskolin (Calbiochem; 25 μM) as indicated. Cells were then incubated in anti-RGS10 (1 μg/ml, Santa Cruz Biotechnology) with or without PKA (1 μg/ml, Santa Cruz Biotechnology) in 1.5% normal donkey serum. Coverslips were then washed three times in PBS, mounted on glass slides, and examined with a fluorescence confocal microscope.

**RESULTS AND DISCUSSION**

**Specific and Inducible Phosphorylation of RGS10**—Regulation of signaling pathways by post-translational modifications that result in conformational change/or changes in localization of the signaling components is an almost universal phenomenon. To investigate whether human RGS10 might be regulated by post-translational modifications, the primary sequence of RGS10 was analyzed for potential sites of phosphorylation. RGS10 was found to contain one consensus PKA phosphorylation motif (Ser168) (Fig. 1A).

To explore whether PKA could directly phosphorylate RGS10, in vitro kinase assays were performed. A RGS10 derivative with an amino-terminal hexahistidine tag, expressed in Escherichia coli and purified by immobilized metal affinity chromatography (IMAC), was incubated in the presence of a recombinant catalytic subunit of PKA and [γ-32P]ATP. This study revealed that RGS10 was indeed phosphorylated by PKA (Fig. 1B, top panel, lane 1). To determine whether the Ser168 identified by primary amino acid sequence analysis was a unique PKA phosphorylation site, this residue was mutated to alanine by site-directed mutagenesis. RGS10-S168A was not phosphorylated when incubated with the catalytic subunit of PKA (Fig. 1B, top panel, lane 4), indicating that this residue was necessary for PKA-mediated phosphorylation of RGS10.

**Modulation of RGS10 Function by PKA**

**FIG. 2.** Effect of the deletion of the PKA-target serine (Ser168) of RGS10 on m2 muscarinic receptor-mediated regulation of GIRK 1/4. A, representative current traces of GIRK 1/4 and m2 muscarinic receptor with or without addition of carbachol (2 μM) dissolved in OR2, 82.5 mM NaCl, 2 mM KCl, 1.8 mM CaCl₂, 1 mM MgCl₂, 5 mM HEPES, pH 7.5. B, comparisons of the activation (tau on, upper panel) and deactivation (tau off, lower panel) time constants of GIRK 1/4 upon stimulation of the m2 muscarinic receptor in the absence or presence of RGS10 or RGS10-S168A. All bars are the means ± S.E. of the mean with the number (n) above each bar. Significance at p < 0.05 (*) for control versus RGS10wt and at p < 0.05 (**) for RGS10 wild type versus RGS10-S168A.
was in fact the sole site of phosphorylation by PKA.

Having found that RGS10 could serve as a substrate for PKA, we then assessed whether this specific phosphorylation could be detected in intact cells. Human embryonic kidney 293 (HEK293) cells were transfected with RGS10 cDNA and subjected to $^{32}$P metabolic labeling. Phosphorylation of RGS10 was observed under these conditions (Fig. 1C). Phosphorylation of RGS10 was induced by incubation of cells expressing RGS10 with forskolin (Fig. 1C, lane 4), providing evidence that the observed phosphorylation was a PKA-dependent event. Furthermore, co-expression of a dominant-negative PKA with RGS10 inhibited the forskolin-induced phosphorylation of RGS10 (Fig. 1C, lane 3). No PKA-inducible phosphorylation of the RGS10-S168A protein was observed, although there was some apparent background phosphorylation of RGS10 at site(s) other than the Ser$^{168}$ (Fig. 1C, lanes 5 and 6).

RGS proteins are thought to function primarily as GTPase-activating proteins for heterotrimeric $\alpha$ subunits by specifically binding to the activated GTP-bound $\alpha$ and stabilizing the transition state for GTP hydrolysis (7, 20). We therefore assessed whether RGS10-S168A could physically associate with activated $\alpha$ by performing co-immunoprecipitation experiments using cells transfected with RGS10 or RGS10-S168A. To facilitate immunoprecipitation in these experiments, a 10-residue epitope tag derived from human Myc protein was inserted immediately after the initiation methionine of RGS10. Myc-RGS10 or Myc-RGS10-S168A was expressed by transient transfection of HEK293 cells. Proteins were recovered by immunoprecipitation of whole cell lysates treated with GDP or GDP and AlF$_4^-$ with an anti-Myc monoclonal antibody, and analyzed by immunoblotting with a polyclonal antibody specific for the endogenous $\alpha_\alpha$. The Myc-RGS10 protein co-immunoprecipitated the $\alpha_{i3}$ protein from the whole cell lysates treated with GDP and AlF$_4^-$ (Fig. 1D, lane 2), but failed to interact with wild-type $\alpha_{i3}$ from whole lysates treated with GDP (Fig. 1D, lane 1). Similarly, Myc-RGS10-S168A co-immunoprecipitated only activated $\alpha_{i3}$ protein (Fig. 1D, lane 4). This finding confirmed that the S168A variant was produced as a functional protein and thus that its inability to serve as a substrate for PKA was not due to misfolding.

Another possible post-translation modification that may impinge on RGS10 function is palmitoylation of Cys$^6$ of RGS10, as has been reported previously for other RGS proteins, GAIP, RGS4, and RGS16 (12–14). RGS10 was transiently expressed in HEK 293 cells metabolically labeled with $^{3}$H]palmitic acid. There was no evidence that immunoprecipitated RGS10 was palmitoylated (data not shown).

RGS10 Modulates GIRK Activation and Deactivation by m2 Muscarinic Receptor—GIRK channels are activated directly by $\beta$ subunits released from $\alpha$ proteins of the $\alpha_i$ family (21, 22). However, discrepancies between native and recombinant GIRK channel kinetics activated by G protein-coupled receptors have also been reported (23, 24). Recent studies have resolved this discrepancy by the demonstration of an acceleration of both activation and deactivation of GIRK 1/2 and GIRK 1/4 currents by several RGS proteins (25–28).

We utilized GIRK channels to determine whether RGS10 was also capable of accelerating G protein-mediated modulation.

![Image](http://www.jbc.org/)

**Fig. 3.** Effect of modulating of PKA activity on the kinetics of m2 muscarinic receptor-evoked GIRK 1/4 current recorded from X. laevis oocytes expressing RGS10. A, representative current traces of GIRK 1/4 and m2 muscarinic receptor with RGS10 after a 30-min stimulation with forskolin (25 $\mu$M, middle trace) or 30 min after PKI being injected (0.2 $\mu$M, bottom trace) in X. laevis oocytes. Carbachol (2 $\mu$M) was applied at the time indicated by the shading. B and C, comparisons of the activation (tau on, B) and deactivation (tau off, C) time constants of GIRK 1/4 channels upon stimulation of the m2 muscarinic receptor in the absence or presence of RGS10 or RGS10-S168A before or after stimulation with forskolin (25 $\mu$M for 30 min) or PKI (0.2 $\mu$M for 120 min). All bars are the means ± S.E. of the mean with the number (n) above each bar. C, control; WT, wild type; SA, S168A. Significance (*) at p < 0.05.
Modulation of RGS10 Function by PKA

RG10 observed here. To investigate the functional effect of preventing PKA-mediated phosphorylation of RGS10 on agonist-induced GIRK current kinetics, electrophysiological recordings were taken from oocytes co-expressing RGS10-S168A with m2 muscarinic receptor and GIRK 1/4 (Fig. 2A, bottom panel). When compared with control and RGS10wt, RGS10-S168A further accelerated GIRK 1/4 activation (3-fold, \( p < 0.05 \)) and deactivation (14-fold, \( p < 0.05 \)) kinetics following application of a m2 muscarinic receptor agonist (carbachol, 2 \( \mu M \)) to the bath for 20 s (Fig. 2). When compared with cells expressing wild-type RGS10, a significant \( (p < 0.05) \) increase in the rate of GIRK channel activation and deactivation was observed in oocytes expressing RGS10-S168A (Fig. 2B). Western blot analysis of oocyte lysates showed no difference in the expression levels of wild-type RGS10 and the RGS10-S168A species (data not shown). The difference observed between wild-type RGS10 and RGS10-S168A on GIRK channel activation and deactivation kinetics is likely due to the high basal levels of PKA in X. laevis oocytes (29). These data suggest that the phosphorylation status of Ser\(^{168}\) of RGS10 may modulate RGS10's molecular interaction with activated Go \(_i\) proteins.

**Modulation of PKA Activity Affects the Kinetics of m2 Muscarinic Receptor-evoked GIRK Current Recorded from X. laevis Oocytes Expressing RGS10**—We next examined more directly whether PKA phosphorylation has a functional consequence on RGS10 action on GIRK channel kinetics by modulating PKA activity in oocytes with an activator (forskolin) and an inhibitor (PKI). Stimulation of PKA activity, by preincubation with forskolin of Xenopus oocytes co-expressing m2 muscarinic receptor, GIRK 1/4 channels, and RGS10 resulted in an inhibition of the apparent activity of the RGS as observed by the GIRK current trace (Fig. 3A, middle trace). Inhibition of PKA activity with PKI led to an apparent enhancement of RGS10 activity toward agonist-mediated GIRK current (Fig. 3A, bottom trace). Furthermore, forskolin treatment inhibited the ability of RGS10 to accelerate the deactivation of GIRK channel currents in response to carbachol (Fig. 3C, bar 2 versus bar 5). In contrast, introduction of PKI resulted in an apparent increase in RGS10’s activity of accelerating both the activation (tau on) and deactivation (tau off) rates of GIRK current (Fig. 3C, bars 1 and C, bar 2 versus bar 8). These changes in GIRK channel kinetics were only significantly observed when wild-type RGS10 was expressed in the oocyte. The tau off of GIRK current for oocytes co-expressing m2 muscarinic receptor, GIRK channel, and RGS10-S168A was increased in the presence of forskolin compared with the absence of forskolin (Fig. 3C, bars 3 versus bar 6). However, the rate of deactivation (tau off) was still significantly more rapid (>3-fold) than in oocytes not expressing RGS10 in the presence if forskolin (Fig. 3C, bars 1 and 4 versus bar 6). Additionally, there was no significant change in the GIRK channel kinetics of oocytes expressing RGS10-S168A when PKI was introduced into the cell when compared with control cells lacking RGS10 (Fig. 3C, bars B and C, bar 3 versus bar 9). No significant difference was observed between control and RGS10 groups (Fig. 3B, bar 1 versus bar 2). We believe this lack of significance for tau on between control and RGS10 groups in Fig. 3B is reflective of experimental variation between days, frogs, and oocyte injections. This variation may be a result of differences in oocyte endogenous PKA expression. The lack of significance, however, does not detract from the major observation of the effect of modulating of PKA activity on the tau off of m2 muscarinic receptor-evoked GIRK 1/4 current recorded from oocytes expressing wild-type RGS10. These data suggest that modulation of PKA activity directly affects the functional activity of RGS10 proteins on GIRK channel kinetics and that this modulation is mediated through phosphorylation of Ser\(^{168}\).

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**Fig. 4. Effect of in vitro phosphorylation of RGS10 by PKA on GAP activity of RGS10.** A, purified hexahistidine-tagged RGS10 was phosphorylated with PKA. Phosphorylation results in a gel shift on SDS-PAGE as viewed by Coomassie Blue staining. This material was assayed by a GTPase activity assay. Lane 1, control; lane 2, RGS10 + PKA; lane 3, RGS10 + inactive PKA. B, purified Go\(_i\) subunit (13 \( \mu M \)) was loaded with \( \gamma\)-\(^{32}\)P-GTP and incubated without or with 50 \( \mu M \) RGS10, PKA-phosphorylated RGS10, and RGS10 incubated with heat-inactivated PKA. GTPase assays were performed at 4 °C for 3 min. C, as in B, except that portions were withdrawn at times indicated, and GTP hydrolysis was determined by measuring the release of phosphate. Data shown represent the mean and standard deviation of three separate experiments.

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Phosphorylation by PKA Does Not Alter GAP Activity of RGS10—To investigate whether phosphorylation of RGS10 by PKA modifies the ability of RGS10 to accelerate GTP hydrolysis by Gα proteins, we assayed the ability of phosphorylated RGS10 (in vitro by PKA) to potentiate the GTP hydrolysis rate of a Gα subunit in vitro (5). Purified RGS10 was incubated with either the catalytic subunit of PKA or heat-inactivated catalytic subunit. Following each reaction, the RGS10 was separated from the PKA by anion-exchange chromatography. Phosphorylation of RGS10 by PKA resulted in a gel-shift on SDS-PAGE (Fig. 4A, lane 2) that was not observed for RGS10 incubated with inactivated PKA (Fig. 4A, lane 3), suggesting that the phosphorylation was stoichiometric. This phosphorylation had essentially no effect on RGS10’s ability to accelerate the GTP hydrolysis rate of Gα (Fig. 4B). To explore further if the ability of RGS10 to accelerate GTP hydrolysis of Gα was modified by phosphorylation of Ser168 by PKA, a time-course analysis was conducted (Fig. 4C). Addition of 50 nM RGS10 caused a >10-fold enhancement of the rate GTP hydrolysis by Gα, and again this activity was unaffected by PKA phosphorylation of RGS10. Evidently, the effects of PKA-dependent phosphorylation of RGS10 on GIRK channel deactivation are not the result of a change in GAP activity of RGS10.

PKA Activation Induces Translocation of RGS10 to the Nucleus—The subcellular localization of various RGS proteins has been reported recently. RGS3, RGS7, and GAIP have been found to be cytosolic (30–33), and sst2, GAIP, RGS3, and RGS16 are all reportedly plasma membrane-associated (3, 12, 14, 31). Dulin et al. (34) recently demonstrated that RGS3 translocates from the cytosol to the plasma membrane upon activation of Gα proteins and that a truncated variant of RGS3 (RGS3T) is localized to the nucleus, which coincides with an induction of apoptosis. Additionally, Chatterjee and Fisher (35) recently reported the accumulation of RGS2 and RGS10 in the nucleus of COS-7 cells transfected with green fluorescent protein constructs of these proteins.

We examined the subcellular localization of RGS10 by immunocytochemistry and confocal microscopy after stable transfection into HEK293 cells. RGS10 was predominately localized in a diffuse manner in the cytosol (Fig. 5). However, treatment of cells with forskolin (25 μM for 2 h) resulted in a dramatic translocation of RGS10 from the cytosol to the nucleus (Fig. 5). The time points utilized for each approach were validated through carefully controlled experiments. For both the co-immunoprecipitation and electrophysiology studies, there was no observed difference between the 15- and 30-min time points (data not shown). Variability was observed with the immunolocalization studies, although we did always observe a discernible difference between the control and the 15-min forskolin stimulation time points (data not shown). However, the 2-h time points provided consistent results, and thus these data were chosen for presentation (Fig. 5).

Investigation of the subcellular distribution of RGS10-S168A required transient transfection of HEK293, since numerous attempts to generate a cell line that stably expresses RGS10-S168A were all unsuccessful. Immunostaining for RGS10-S168A in transfected HEK293 cells revealed both cytoplasmic and nuclear staining (Fig. 5); however, no difference in the localization of RGS10-S168A was observed when the cells were stimulated with forskolin (Fig. 5).

Having found that RGS10 could translocate to the nucleus, we then assessed whether this localization could be detected in cells that endogenously express RGS10. To date endogenous RGS proteins had not been detected by immunostaining of cells, which suggests the endogenous expression of RGS proteins is very low. In H4 cells, there is predominately nuclear staining with some cytosolic staining, suggesting that most of the endogenous RGS10 protein is localized in the nucleus (data not shown; Ref. 35). These data demonstrate that nuclear localization of RGS10 is not a product of overexpression and is further supportive evidence of a functional role for RGS10 in the nucleus.

These results, coupled with the PKA-mediated modulation of GIRK channel kinetics through RGS10 with minimal effect on its GAP activity, suggest that activation of PKA results in the translocation of RGS10 from the cytoplasm into the nucleus where it can no longer participate in regulating Gα at the plasma membrane. Hence, we propose the model, as supported by our findings, that the phosphorylation-mediated attenuation of RGS10 activity (Figs. 2 and 3) was not manifest in an alteration of its ability to activate the GTPase of Gα (Fig. 4). Instead, the phosphorylation event triggered translocation of
RGS10 from the plasma membrane and cytosol into the nucleus (Fig. 5). An alternative model, that PKA phosphorylation blocks export of RGS10 analogous to the nuclear shuttling of the yeast scaffold Ste5, was recently reported by Mahanty et al. (36). However, it still remains unclear as to whether the direct phosphorylation of RGS10 mediates this translocation or if an accessory protein is activated through PKA phosphorylation that is necessary for RGS10 translocation away from the plasma membrane.

The discovery of RGS proteins provided a novel mechanism for the regulation of signaling through heterotrimeric G proteins, and has raised many questions. For example, knowledge of how RGS proteins are themselves regulated is likely to be crucial to understanding their intrinsic functions. The goal of this work was to explore the potential regulation of RGS10 by covalent modification. We found that at least one such modification does indeed occur on RGS10, specifically phosphorylation by PKA. Even though the phosphorylation of RGS10 had a profound effect on its ability to modulate ion channel activation by G proteins, it did not have any effect on the ability of RGS10 to accelerate the GTPase activity of Gα subunits. Instead, the data indicate that PKA phosphorylation of RGS10 results in translocation of the proteins from the cytosol into the nucleus, which would result in the sequestration of RGS10 interactions with activated Gα subunits at the plasma membrane. Although the experiments described here do not address whether RGS10 has specific functions in the nucleus, the inability to generate stable cell lines expressing RGS10-S168A suggests that the phosphorylation of Ser168 may be critical in the functioning of RGS10. Although exploring potential functions of RGS10 in the nucleus is beyond the scope of this current study, our finding of the inducible translocation of RGS10 from the cytosol into the nucleus does suggest that the modulation of GAP activities alone does not constitute the only important target for biological regulation of RGS proteins.

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