Regulator of G Protein Signaling RGS3T Is Localized to the Nucleus and Induces Apoptosis*

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Nickolai O. Dulin‡§, Phillip Pratt¶, Chinnaswamy Tiruppathi‡, Jiaxin Niu‡, Tatyana Voyno-Yasenetskaya‡, and Michael J. Dunn||

From the ‡Department of Pharmacology, University of Illinois at Chicago College of Medicine, Chicago, Illinois 60612-7343 and the ¶Department of Medicine and Cardiovascular Research Center, Medical College of Wisconsin, Milwaukee, Wisconsin 53226

RGS3 belongs to a family of the regulators of G protein signaling (RGS). We previously demonstrated that cytosolic RGS3 translocates to the membrane to inhibit G_{q/11} signaling (Dulin, N. O., Sorokin, A., Reed, E., Elliott, S., Kehrl, J., and Dunn, M. J. (1999) Mol. Cell. Biol. 19, 714–723). This study examines the properties of a recently identified truncated variant termed RGS3T. Both RGS3 and RGS3T bound to endogenous G_{q/11} and inhibited endothelin-1-stimulated calcium mobilization and mitogen-activated protein kinase activity to a similar extent. However, unlike cytosolically localized RGS3, RGS3T was found predominantly in the nucleus and partially in the plasma membrane. Furthermore, RGS3T, but not RGS3, caused cell rounding and membrane blebbing. Finally, 44% of RGS3T-transfected cells underwent apoptosis after serum withdrawal, which was significantly higher than that of RGS3-transfected cells (7%). Peptide sequence analysis revealed two potential nuclear localization signal (NLS) sequences in RGS3T. Further truncation of the RGS3T N terminus containing putative NLSs resulted in a significant reduction of nuclear versus cytoplasmic staining of the protein. Moreover, this truncated RGS3T no longer induced apoptosis. In summary, RGS3 and its truncated variant RGS3T are similar in their ability to inhibit G_{q/11} signaling but are different in their intracellular distribution. These data suggest that, in addition to being a GTPase-activating protein, RGS3T has other distinct functions in the nucleus of the cell.

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† To whom correspondence may be addressed: Dept. of Pharmacology (M/C 868), University of Illinois, Medical Sciences Bldg., E-407, 835 South Wolcott Ave., Chicago, IL 60612. Tel.: 312-355-2568; Fax: 312-996-1225; E-mail: dulin@uiuc.edu.

‡ To whom correspondence may be addressed: Dept. of Medicine and Cardiovascular Research Center, Medical College of Wisconsin, 8701 Watertown Plank Rd., Milwaukee, WI 53226. Tel.: 414-456-8213; Fax: 414-456-6560; E-mail: mdunn@mcw.edu.

§ To whom correspondence may be addressed: Dept. of Pharmacology, University of Illinois, Chicago College of Medicine, Chicago, Illinois 60612-7343.

¶ The abbreviations used are: RGS, regulator of G protein signaling; GAP, GTPase-activating protein; CHO, Chinese hamster ovary; ET-1, endothelin-1; ET_{A}, endothelin-1 receptor type A; GAIP, Ga interacting protein; IP_{3}, inositol trisphosphate; MAP kinase, mitogen-activated protein kinase; NLS, nuclear localization signal; PBS, phosphate-buffered saline; FBS, fetal bovine serum; PCR, polymerase chain reaction.

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Core RGS domain and by function as GTPase-activating proteins (GAP) for the α subunit of heterotrimeric G proteins (2–6). We and others have previously shown that RGS3 is a potent inhibitor of G_{q/11}-mediated signaling, such as gonadotropin-releasing hormone-induced production of inositol trisphosphate (IP_{3}) (7), and endothelin-1-induced calcium mobilization and activation of mitogen-activated protein (MAP) kinase (1). One of the unique features of RGS3 is that is it normally a cytosolic protein, which translocates to the membrane and binds to G_{q/11} upon agonist stimulation. Translocation of RGS3 involves the recruitment of its N-terminal non-RGS domain, whereas C-terminal RGS domain provides interaction with G proteins (1).

Recently, a truncated isoform of RGS3, termed RGS3T, has been identified by polymerase chain reaction (PCR) analysis (8). RGS3T is a much smaller molecule, which lacks a large portion of the N-terminal domain of RGS3 but retains a core RGS domain and a smaller (about 70 amino acids) N-terminal tail. RGS3T is expressed ubiquitously in various tissues, whereas full length RGS3 is more selectively expressed in heart, lung, testis, skeletal, and smooth muscle (3, 8), suggesting the possibility of distinct function(s) of these proteins. Because RGS3T lacks a domain which is implicated in cytosolic localization of RGS3 and its agonist-induced recruitment to the membrane (1), we sought to determine whether this deletion affects intracellular localization of RGS3T as well as its ability to bind G_{q/11} and to inhibit G_{q/11}-mediated signaling.

Here we demonstrate that RGS3 and RGS3T are similar in their ability to bind G_{q/11} and inhibit G_{q/11}-mediated signaling but are different in their intracellular distribution, wherein full length RGS3 is localized in the cytoplasm, whereas RGS3T is localized predominantly in the nucleus and partially in the plasma membrane. Moreover, nuclear localization parallels with the unique ability of RGS3T to induce apoptosis, suggesting that RGS3T is not just a regulator of G protein signaling, but has other function(s) in the nucleus.

**EXPERIMENTAL PROCEDURES**

**Materials**—Human RGS3 cDNA, previously cloned by Druey et al. (3), was kindly provided by Dr. John Kehrl. The cDNAs for the full length RGS3 (RGS3-1519), RGS3T (RGS3-314–519), and RGS3-79–519, termed here as RGS3C, were amplified by PCR from the original RGS3 cDNA template and subcloned into pCMV-tag3 vector (Stratagene, La Jolla, CA) to introduce a myc tag at the 5’ end of each insert (see Fig. 5). The identity of PCR products relative to the original plasmid was confirmed by sequencing. As was shown previously, addition of tags to other RGS proteins (RGS10, RGS4, and GAIP) did not affect their RGS function (4, 9, 10). The cDNA for type A endothelin receptor (ET_{A}) (11) was kindly provided by Dr. Masashi Yanagisawa. Anti-α_{1C}, antibodies were from Santa Cruz Biotechnology (Santa Cruz, CA). Anti-Myc antibodies and protease inhibitors were from Roche Molecular Biochemicals (Indianapolis, IN). Endothelin-1 was...
from Calbiochem (Cambridge, MA).

**Cell Culture and Transient Transfection of cDNA—**Chinese hamster ovary (CHO) cells were maintained in Ham's F-12 medium supplemented with 2 mM glutamine, 100 units/ml streptomycin, 100 units/ml penicillin, and 10% fetal bovine serum. For transient expression of proteins in CHO cells, plasmids were transfected with DNA using LipofectAMINE Plus or LipofectAMINE-2000 reagents (Life Technologies, Inc., Gaithersburg, MD) for 24–48 h, following the manufacturer's protocol.

**Immunoprecipitation and Western Blot Analysis—**Approximately 10° CHO cells grown on 10-cm² dishes were harvested by scraping, centrifuged, and resuspended in 1 mL of buffer containing 50 mM HEPES, pH 7.4, 0.5 mM NaCl, 0.5% Lubrolasure, 5 mM MgCl₂, 1 mM dithiothreitol, 10 mM NaF, 30 μM AICl₃, and protease inhibitors (1 μg/ml leupeptin, 1 μg/ml aprotonin, 1 mM phenylmethylsulfonyl fluoride). The lysates were cleared from insoluble material by centrifuging at 20,000 × g for 15 min following incubation with 5 μl of agarose-conjugated anti-Myc antibodies (2 μg/μl beads) for 2 h at 4°C on a rotator, and washing three times with 1 ml of the same buffer. Under the lysis conditions described above, the nuclei remain intact, resulting in a significant reduction in the amount of extracted RGS3T compared to RGS3, similar to their relative amounts in postnuclear supernatants after preparation of intact nuclei (see below, Fig. 4). Therefore, a limiting amount of anti-Myc antibodies was adjusted to provide an incomplete but even immunoprecipitation of microsomes. The samples were boiled in the same buffer for 5 min, subjected to electrophoresis, and analyzed by Western blotting with anti-Myc and anti-α,α,α,α,α antibodies, respectively.

**Cytosolic Ca²⁺ Measurement—**Cytosolic Ca²⁺ concentration ([Ca²⁺]ₗ) in single CHO cells was measured using the fura-2 fluorescence imaging method (12). Subconfluent cells grown on 25-mm diameter glass coverslips were transfected with 0.5 μg DNA using LipofectAMINE Plus or LipofectAMINE-2000 reagents (Life Technologies, Inc., Gaithersburg, MD) for 24–48 h, followed by overnight serum starvation in 0.2% fetal bovine serum (FBS). Cells were then washed twice with Hank's balanced salt solution and were loaded with 5 μM fura-2/AM for 1 h at 25°C. Cells were then washed again and imaging was performed using an Attofluor RatioVision digital fluorescence microscope equipped with a Zeiss Axiovert S100 inverted microscope and a Fluor 40×, 1.3-NA oil immersion objective as described (12). Regions of interest on individual cells were marked and excited at 334 and 380 nm with emission at 520 nm. The values of 334/380 excitation ratio (R₃₃₄/₃₈₀) as a function of [Ca²⁺]ₗ, were captured at 6-s intervals. After drug treatment, the cells were imaged in the microscope and analyzed independently by two investigators and expressed in percentage of apoptotic cells within the population of myc-positive cells.

**RESULTS**

**Binding of RGS3 and RGS3T to Gq/11—**Using a coimmunoprecipitation technique with antibodies generated against the N-terminal peptide of RGS3, we have previously demonstrated that an interaction exists between RGS3 and AlF₄⁻-activated Gq/11 (1). In the present study, we used myc-tagged RGS3 and RGS3T, which enable specific and relatively equal immunoprecipitation of both RGS3 isoforms with anti-Myc antibodies (Fig. 1A). This permits a side by side comparison of the binding properties of these two proteins. Consistent with previous studies (1), no binding of RGS3 and RGS3T to Gq/11 was detected in the absence of AlF₄⁻ (data not shown). Activation of G proteins with AlF₄⁻ resulted in the binding of both RGS3 and RGS3T to endogenous Gq/11 as detected by immunoblotting of corresponding anti-Myc immunoprecipitates with Gq/11 antibodies (Fig. 1B). No Gq/11 was detected in the anti-Myc immunoprecipitates from the cells transfected with the vector alone (Fig. 1B), whereas the amounts of Gq/11 in the total lysates were equal under all transfection conditions (Fig. 1C). In addition, both RGS3 and RGS3T bound to coexpressed GT-Pase-deficient mutant Gα₃Q11 in the absence of AlF₄⁻ (data not shown). There was no consistent and significant difference between RGS3 and RGS3T in the magnitude of binding to Gq/11 using this coimmunoprecipitation technique.

**Effect of RGS3 and RGS3T on Gq/11 Signaling—**To confirm the functional significance of interaction between Gq/11 and RGS3 isoforms, we next examined the effect of RGS3 and RGS3T on endothelin-1-induced calcium responses and MAP kinase activity as examples of Gq/11-mediated signaling. After transfection of Gq/11 coupled type A endothelin receptor (ET₁) cDNA into CHO cells, endothelin (10⁻⁷ M) induced a typical transient increase in cytosolic [Ca²⁺]ₗ, determined as the fluorescent ratio (R₃₃₄/₃₈₀) equal to 0.38 ± 0.04 (n = 35) (Fig. 2). After overexpression of RGS3 and RGS3T, the response of [Ca²⁺]ₗ to ET₁ was significantly attenuated, having an initial rise in the R₃₃₄/₃₈₀ value equal to 0.13 ± 0.02 (n = 35; p < 0.001) and 0.12 ± 0.03 (n = 35; p < 0.001), respectively. Interestingly,
RGS3 or RGS3T cDNA resulted in a marked decrease of the response to endothelin-1, with a striking similarity in the "dose dependence" of the inhibitory effect of RGS3 and RGS3T (Fig. 3). These data indicate that RGS3 and RGS3T are similar in their ability to bind Goq11 and to inhibit Gq11-mediated calcium mobilization and activation of the MAP kinase pathway.

Intracellular Localization of RGS3 and RGS3T—As shown in Fig. 4, immunostaining of RGS3-transfected CHO cells with anti-Myc antibodies revealed the cytoplasmic localization of RGS3, which is in agreement with previously published results (1). Surprisingly, after the same immunofluorescent procedure, RGS3T showed an intense nuclear staining in all transfected CHO cells. A similar pattern of expression was also observed overexpression of RGS3 and RGS3T in COS7 cells (data not shown), suggesting that nuclear localization of RGS3T is not cell type-specific. In addition, overexpression of RGS3T significantly altered the morphology of the cells, which resulted in cell rounding and membrane blebbing (Fig. 4).

Sequence analysis of RGS3T revealed the presence of two stretches of positively charged amino acids in its N terminus (Arg341-Lys-Arg-Lys, and Arg360-Arg-Arg of the full length RGS3, Fig. 5A). These sites are similar to the nuclear localization signal (NLS) sequences (13, 14), which target proteins to the nucleus of the cell. To examine whether these putative NLSs provide a mechanism of RGS3T nuclear localization, we further truncated RGS3T from its N terminus to generate a fragment (RGS3C-379–519), which we termed RGS3C (C terminus) and which lacked the putative NLSs and contained only the RGS domain of RGS3 (Fig. 5). Immunofluorescent microscopy of RGS3C-transfected cells revealed a significant reduction of nuclear staining of RGS3C and its accumulation in the cytoplasm (Fig. 4).

We further confirmed the immunofluorescent data by Western blotting of in vitro prepared nuclear (N) and postnuclear (S, supernatant) fractions from cells transfected with RGS3 isoforms. Consistently, RGS3 was detected only in the supernatant; RGS3T was predominantly in the nuclear fraction; and RGS3C was in both fractions with slightly lower amounts in
the nuclei (Fig. 5B). Goq11 was mainly in the postnuclear fraction, and its distribution was not affected by the presence of RGS3 isoforms (Fig. 5C). However, a small amount of Goq11 was also detected in the nuclear fraction, the significance of which is not clear.

The nuclear localization of RGS3T was also detected at lower levels of expression (Fig. 6). Reduction of RGS3T cDNA 100-fold during transfection (1 μg/10⁶ cells (Fig. 4) versus 10 ng/10⁶ cells balanced with 0.99 μg of empty vector (Fig. 6)) led to a low, close to the limit of detection, level of RGS3T expression but still revealed the nuclear localization of RGS3T. However, in the latter case RGS3T was not diffusely distributed within the nucleus, but showed a distinct punctate staining, the meaning of which will be studied in the future. No significant cytoplasmic staining was detected at low doses of RGS3T cDNA, suggesting that nuclear binding sites are the first to be occupied by RGS3T. Finally, we did not observe significant changes in morphology of the cells after expression of RGS3T at these low levels.

We also examined whether RGS3 and RGS3T are differentially distributed between the membrane and cytosol in the cytoplasm (Fig. 7). The membrane/cytosol preparation of postnuclear fraction revealed that, unlike RGS3, which was exclusively cytosolic as expected, RGS3T was present both in the cytosol and the membrane fraction, although cytosolic localization was predominant (Fig. 7A). Detection of RGS3T in the membrane fraction was consistent with our immunofluorescence data (Fig. 4) and was not a result of impurity of the membrane preparation, because under identical conditions, full length RGS3 gave a much stronger signal in the cytosol but was not detected in the membrane. Interestingly, RGS3C, similarly to RGS3, was found only in the cytosol and its membrane localization was barely detectable. This suggests that the 65-amino acid long N-terminal region of RGS3T, which is truncated in RGS3C, is also responsible for the membrane binding of RGS3T. As expected, Goq11 was detected only in the membrane fraction, and its distribution was not affected by the presence of RGS3 isoforms (Fig. 7B).

RGS3T-induced Apoptosis of CHO Cells—During this study, we consistently observed that a large number of RGS3T-transfected cells underwent a significant change in morphology resulting in cell rounding and membrane blebbing, which are common attributes of apoptosis. In addition, although having successfully developed two stable cell lines overexpressing full length RGS3 (1), we failed to do so with RGS3T. These factors encouraged us to examine whether overexpression of RGS3T would result in apoptosis of CHO cells. After transient transfection with myc-tagged cDNA for RGS3, RGS3T, or RGS3C at a ratio of 1 μg of cDNA/10⁶ cells followed by serum withdrawal, the extent of cell death was estimated by measuring the number of cells with fragmented DNA within the population of myc-positive cells. As shown in Fig. 8, overexpression of RGS3T,
functions in the nucleus. At high expression levels, and at low amounts, RGS3T may serve other roles. These data suggest that RGS3T-induced apoptosis is a function of RGS3T expression levels, and at low amounts, RGS3T may serve other functions in the nucleus.

but not RGS3, resulted in apoptosis of 44.9 ± 11.9% of CHO cells. By contrast, the percentage of apoptotic cells within the population of RGS3- or RGS3C-overexpressing cells was 7.1 ± 1.4% and 8.6 ± 1.1%, respectively, which was similar to myc-negative cells. The effect of RGS3T was not just a result of its accumulation in the nucleus, because nuclear-targeted enhanced green fluorescent protein transfected under the same conditions had no significant effect on apoptosis even though it was concentrated in the nucleus (data not shown). This demonstrates the unique ability of RGS3T to induce apoptosis in CHO cells. Dilution of RGS3T cDNA 10-fold with the vector DNA during transfection resulted in a reduced percentage of apoptotic cells (26.0 ± 4.7%, data not shown). With further reduction of RGS3T cDNA to 10 ng of cDNA/10⁶ cells, RGS3T still showed nuclear staining (Fig. 6), but the cells did not undergo significant apoptosis (data not shown). These data suggest that RGS3T-induced apoptosis is a function of RGS3T expression levels, and at low amounts, RGS3T may serve other functions in the nucleus.

FIG. 6. Nuclear localization of RGS3T at low levels of expression. CHO cells (10⁶) grown on glass chamber slides were cotransfected with RGS3T cDNA together with empty vector at the ratio of 10 ng of RGS3T cDNA/1 µg of vector/10⁶ cells as described under “Experimental Procedures.” The cells were then analyzed by immunofluorescence with anti-Myc/rhodamine-conjugated anti-mouse antibodies (A) and counterstained with DAPI (B). A, an image of the whole cell with the weakest detectable rhodamine fluorescence. B, DAPI staining of the same field showing nuclei of the cells. The arrow indicates RGS3T-transfected cell. Bar, 10 µm.

FIG. 7. Membrane/cytosol distribution of RGS3, RGS3T, and RGS3C. CHO cells transfected with the cDNAs for myc-tagged RGS3, RGS3T, or RGS3C, were subjected to membrane/cytosol fractionation as described under “Experimental Procedures.” The membrane (M) and cytosol (C) fractions were analyzed with anti-Myc (A) or anti-αq/α₁₁ (B) antibodies. Note that the total amount of RGS3T in the membrane and cytosol fractions from the postnuclear supernatant was significantly less than that of RGS3 or RGS3C, because the main RGS3T immunoreactivity was in the nuclear fraction (Fig. 4). The total expression levels of RGS3, RGS3T, and RGS3C were similar (data not shown).

DISCUSSION

It is now established that the main function of RGS proteins is to stimulate GTPase activity of heterotrimeric G proteins, and therefore, to inhibit G protein signaling. RGS3 is a potent inhibitor of Gq₁₁-mediated signaling such as gonadotropin-releasing hormone-induced IP₃ production (7) and endothelin-1-induced calcium mobilization and MAP kinase activation (1). Discovery of an endogenously expressed truncated isoform of RGS3, namely RGS3T (8), raised several important questions about RGS3 function, two of which are addressed in the present study. First, does the N-terminal region regulate the ability of RGS3 to inhibit G protein signaling? Second, what is the difference between these two products of the same RGS3 gene (15), besides their differential tissue distribution (8)?

The N-terminal Domain of RGS3 Is Not Required for G Protein Inhibitory Function—Our coimmunoprecipitation experiments (Fig. 1) and signaling studies (Figs. 2 and 3) convincingly demonstrate that there is no difference between RGS3 and RGS3T in the binding to Goq₁₁, and the ability to inhibit Goq₁₁-coupled endothelin signaling, suggesting that the N-terminal domain of RGS3 does not regulate its GAP function toward Goq₁₁. This is different from previously published data (8) demonstrating that RGS3T, but not full length RGS3, inhibited platelet-activating factor-induced IP₃ production, which was presumably mediated by Goq₁₁. This discrepancy, however, may be explained by the recently published observation of receptor selectivity of RGS proteins, whereas RGS1, RGS4, and RGS16 were more potent to inhibit carbachol-dependent signaling than cholecystokinin, even though both agonists were coupled to Goα (16).

Regarding the importance of the regions outside the RGS domain, several reports document their requirement for the GAP function of RGS proteins. Thus, deletion of the non-RGS N-terminal region of RGS4 significantly reduced its potency in inhibition of carbachol-induced signaling (17). Furthermore, although the core RGS domain of RGS16 by itself retained G protein binding and GAP activity in vitro, it was not functional in vivo without its N-terminal region, indicating the essential role of the N terminus in the function of RGS16 (18). Finally, RGS9-2, a splice variant of retinal RGS9-1, being about 200
amino acids longer than its retinal isoform, dampened the Gi/o-coupled μ-opioid receptor signaling, whereas RGS3-1 did not (19). These examples demonstrate the importance of the regions outside of RGS domain in the GAP function of RGS proteins. In this respect, RGS3 seems to be different from RGS4 (17), RGS16 (18), and RGS9 (19), because its N-terminal region does not regulate the ability of RGS3 to bind G proteins (Fig. 1) and to inhibit G protein signaling (Figs. 2 and 3).

**RGS3T is Localized to the Nucleus and Induces Apoptosis**—The most striking difference between RGS3 and RGS3T revealed in the present work is in their intracellular localization (Figs. 4, 5B, and 6). Consistent with the previous study (1), full length RGS3 was found in the cytoplasm of the intact cells. By contrast, RGS3T was localized predominantly in the nucleus and partially in the membrane.

The membrane localization of RGS3T may provide a mechanism by which RGS3T regulates G protein-mediated signaling from seven transmembrane receptors in the plasma membrane. Compared with full length RGS3, which translocates to the membrane from the cytosol upon agonist stimulation (1), RGS3T seems to associate with the membrane under basal conditions. This would suggest a higher potency of RGS3T in the regulation of G protein signaling. However, this hypothesis did not appear to be true, as evidenced from the present study (Figs. 2 and 3). Unfortunately, because of the high variability in the shape and size of transiently transfected cells, we were not able to assess the possibility of agonist-induced translocation of RGS3T as we previously described for full length RGS3 in stably transfected cells (1). Stable expression of RGS3T appeared to be unattainable probably due to the ability of RGS3T to induce apoptosis (Fig. 8). Therefore, this issue will be addressed in future studies using inducible expression systems.

The question also remains regarding the mechanism by which RGS3T is targeted to the membrane. For some RGS proteins, such as GAIP (20), RGSZ1 (21), RGS4, and RGS10 (22), the membrane binding is proposed to be mediated by palmitoylation. Sequence analysis of RGS3T did not reveal the presence of putative sites for palmitoylation and myristoylation at its N terminus. However, RGS3T contains conserved cystein (Cys427 of full length RGS3) inside its RGS domain. In RGS4 and RGS10, analogous cysteins have been recently shown to be palmitoylated, which provided the membrane binding of these proteins (22). Thus, the possibility of RGS3T palmitoylation still exists. Interestingly, palmitoylation of RGS16 is not necessary for its membrane localization and GAP activity in vitro but is somehow required for its function in vivo (23). The membrane binding of RGS16 is provided by the non-RGS, N-terminal “membrane association domain,” which is also present in RGS4 and RGS5, and possibly contributes to the membrane localization of these proteins as well (24). Based on our fractionation experiments, the N-terminal 65-amino acid region of RGS3T, outside the RGS domain, is responsible for membrane targeting of RGS3T, because its truncation eliminates membrane binding (Fig. 5A). The active component of this region will be studied in the future.

The nuclear localization of RGS3T is a new finding. Importantly, the cells expressing low levels of RGS3T, close to the limit of detection, still retained nuclear staining and revealed a punctate pattern of RGS3T localization within the nucleus (Fig. 6). This strongly suggests the nuclear localization of endogenous RGS3T. Moreover, the present study also provides a possible mechanism of nuclear localization of RGS3T by recruitment of its putative nuclear localization signal (NLS) sequences, truncation of which decreases nuclear distribution of RGS3T (Figs. 3 and 4). What is unclear is why full length RGS3, which obviously contains the same NLSs, is not targeted to the nucleus. One possibility is that the N-terminal tail of RGS3 somehow prevents nuclear localization, for example, by masking NLSs as a result of the three-dimensional structure. Alternatively, the N-terminal domain, which by itself contains structurally distinct regions (3), may keep RGS3 in the cytoplasm and antagonize the influence of NLSs. In support of this, RGS3-(1–379), which lacks the RGS domain but still retains putative NLSs at its C terminus, behaves similar to the full length RGS3 in terms of localization in the cytoplasm (1). Another important question is the function of RGS3T in the nucleus. Some data supports the idea that an RGS3T nuclear function could be related to heterotrimeric G proteins, which have been also found to localize to the nucleus. Thus, an ADP-ribosylated 40-kDa protein recognized by the antibodies against Gαi1 and Gαi2 has been found in the nuclei prepared from the rat liver (25). In 3T3 fibroblasts, Gαi has been shown to translocate to the nucleus upon stimulation with epidermal growth factor, insulin, or thrombin, where it may regulate mitosis (26). Finally, Gαs has been reported to translocate to the nucleus of neuronal cells after being activated at the nerve terminal (27). There are also examples of nuclear localization of G protein-coupled receptors. Functional prostaglandin E2 receptors, EP3 and EP4, have been demonstrated in the nuclear envelope, where they affect intranuclear calcium transients and transcription of genes such as inducible nitric-oxide synthase (28). Furthermore, type 1 angiotensin II receptors, generally coupled to Gαq11, have been shown to translocate to the nucleus upon stimulation of neuronal cells with angiotensin II.

**FIG. 8.** RGS3T-induced apoptosis of CHO cells. CHO cells were transfected with the cDNAs for myc-tagged RGS3 (A, B), RGS3T (C, D), or RGS3C (E, F) at a ratio of 1 μg of cDNA/1 ml of cells for 24 h, followed by serum deprivation for an additional 24 h. The cells were then fixed, permeabilized, washed, and subjected to digoxigenin nucleotide labeling of the DNA fragments, followed by double immunostaining with anti-myc (A, C, and E) and anti-digoxigenin (B, D, and F) antibodies, respectively. The apoptotic cells overexpressing myc-tagged RGS3 isoforms are indicated by arrows. Shown are the representative images from at least three independent experiments.
The presence of a small amount of Goq11 in the nuclei of CHO cells (Fig. 4C). However, the significance of this is not clear.

Alternatively, the nuclear function of RGS3T may be unrelated to G proteins. There is an increasing number of examples where RGS proteins recruiting their non-RGS domains are shown to bind targets distinct from heterotrimeric G proteins. Thus, protein kinase A-anchoring protein D-AKAP2 binds to the regulatory subunit of protein kinase A (30). A number of RGS proteins, such as RGS6, RGS7, RGS9, RGS11, and EGL-10, directly bind the β2 subunit of heterotrimeric G proteins (31, 32). GAIP interacts with the PDZ domain of GIPC (GAIP-Interacting Protein C terminus) (33). Finally, but not lastly, a guanine nucleotide exchange factor for a small GTPase RhoA, p115RhoGEF, is also an RGS which binds to Goq12 and Goα13 (34) as well as a transducer of G13 signaling on RhoA (35). Although alternative targets of RGS3T have not been described, the possibility exists and will be examined in future.

In the present work, the nuclear localization of RGS3T was functionally linked to its ability to induce apoptosis (Fig. 6). This was based on our observations that RGS3T-transfected cells underwent cell rounding, membrane blebbing, and significant reduction in cell number and was confirmed by digoxigenin nucleotide labeling of DNA fragments in RGS3T-overexpressing cells. Importantly, this effect was specific for RGS3T, because RGS3 and RGS3C failed to induce apoptosis, although they were expressed to a similar extent. Inability of RGS3C to induce apoptosis, even though it was present in the nucleus in significant amounts, suggests the importance of the N terminus of RGS3T in this effect. The fact that RGS3T failed to induce apoptosis at low levels of expression suggests that it serves other function(s) in the nucleus, whereas apoptosis may reflect pathological conditions or certain developmental stages where RGS3 expression is up-regulated. This hypothesis will be examined in the future when detection of RGS3T with specific antibodies becomes possible. At present, the reported RGS3 antibodies have been generated against the N-terminal peptide of RGS3 (1) or against purified RGS3 fusion proteins (7, 36), and their specificity to RGS3T was not examined. The significance of the present study is that it demonstrates and provides a mechanism for the nuclear localization of RGS3T and links it to apoptosis as one of its nuclear effects.

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