Molecular Cloning and Characterization of a Novel Regulator of G-protein Signaling from Mouse Hematopoietic Stem Cells*

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A novel regulator of G-protein signaling (RGS) has been isolated from a highly purified population of mouse long-term hematopoietic stem cells, and designated RGS18. It has 234 amino acids consisting of a central RGS box and short divergent NH₂ and COOH termini. The calculated molecular weight of RGS18 is 27,610 and the isoelectric point is 8.63. Mouse RGS18 is expressed from a single gene and shows tissue specific distribution. It is most highly expressed in bone marrow followed by fetal liver, spleen, and then lung. In bone marrow, RGS18 level is highest in long-term and short-term hematopoietic stem cells, and is decreased as they differentiate into more committed multiple progenitors. The human RGS18 ortholog has a tissue-specific expression pattern similar to that of mouse RGS18. Purified RGS18 interacts with the α subunit of both G and Gq subfamilies. The results of in vitro GTPase single-turnover assays using Goi indicated that RGS18 accelerates the intrinsic GTPase activity of Goi. Transient overexpression of RGS18 attenuated inositol phosphates production via angiotensin receptor and transcriptional activation through cAMP-responsive element via M1 muscarinic receptor. This suggests RGS18 can act on Gq-mediated signaling pathways in vivo.

A large number of extracellular stimuli act via cell surface receptors coupled to G-proteins (1). Inactive G-proteins are heterotrimeric proteins consisting of α, β, and γ subunits. Upon binding of a specific ligand to the G-protein-coupled receptor, the receptor promotes the exchange of GDP to GTP in the α subunit, resulting in dissociation of the α subunit from the βγ subunits. The βγ subunits are tightly associated and do not dissociate under physiological conditions. The free α subunit and βγ subunits then transmit signals through various signal transduction pathways. The activated α subunit has slow intrinsic GTPase activity. When the α subunit is in the GDP-bound form, it re-associates with the βγ subunits, leading to an inactive form. The duration of the G-protein signal depends on the rate of GTP hydrolysis and the rate of subunit re-association. For small GTP-binding proteins such as ras, there are GAPs proteins (GTPase activating protein), which increase the GTP hydrolysis rate. Recently, functional homologs of the ras-GAP have been identified for the heterotrimeric G-protein. These are called RGS (regulator of G-protein signaling) proteins. The first RGS identified, Sst2 (super sensitivity to pheromone) in yeast, is a negative regulator of pheromone signaling (2). Later, the SST2 gene product was shown to function as a GAP for Gpa1, a molecule involved in pheromone desensitization (3). So far ~20 RGS have been identified (4–12), and more could be anticipated. All RGS proteins have a highly conserved domain consisting of 120 amino acid residues, the RGS box, with varying lengths of NH₂ and COOH termini. RGS4 can be expressed in bacteria, and it has been co-crystallized with Goα1 as the GAP-AIF4⋅bound form (13). It was shown that RGS binds to Goα through the switch region, and that site-directed mutagenesis of the contact residues lead to loss of interaction (14).

In vitro most purified native or recombinant RGS proteins can bind Goα and/or Goi via the RGS box (4, 6, 17–19). Overexpression by transient transfection of a RGS into mammalian cells can attenuate signaling from Gi and/or Gq-linked receptors (20–22). The very recently discovered RGS protein, p115RhoGEF, can act as a GTPase activator for Gα12 and Gα13 (23). No RGS that can act on Goα in mammals has been found so far. However, in yeast, Rgs2 was shown to function as a negative regulator of glucose-induced cAMP signaling through direct GTPase activation of the Goα protein Gpa2 (24).

There seems to be tissue specific distribution of RGS. For example, RGS1 is predominantly expressed in B-lymphocytes (8) and monocytes (25, 26), and RGS4 is expressed in neural tissue (27). RGS1, RGS2, RGS3, RGS4, and RGS16 are present in lymphocytes (28, 29), RGS1 (30), RGS7 (31), RGS8 (32), and RGS9 (33) are abundant in brain, and RGS9 in rods (34). RGS3 seems to be ubiquitous.

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank724/EBI Data Bank with accession number(s) AF302685.

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Note added in proof

1 The abbreviations used are: GAP, GTPase activating protein; HSC, hematopoietic stem cell; RGS, regulator of G-protein signaling; SDF-1α, stromal-derived factor-1α; TLCK, Nα-tosyl-l-lysine chloromethyl ketone; GTPγS, [35S]guanosine 5′-O-(thiotriphosphate); HEK, human embryonic kidney; RT-PCR, reverse transcriptase-polymerase chain reaction; kb, kilobase pair(s); TPCK, t-1-tosylamido-2-phenylthyl chloromethyl ketone.
In this paper, we describe cloning of a novel RGS from a long-term hematopoietic stem cell cDNA library. The new RGS, designated as RGS18, is highly expressed in long-term as well as short-term hematopoietic stem cells, and less in more committed hematopoietic populations. RGS18 can bind both Go1 and Goa, in vitro, enhance GTPase activity of Goa, and attenuate signals from Gα coupled receptors.

**EXPERIMENTAL PROCEDURES**

**Cell Culture—**Human embryonic kidney (HEK) 293T cells were maintained in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum, 100 units/ml penicillin, and 100 μg/ml streptomycin. Jurkat human leukemia T lymphocyte (clone E6–1), chronic myelogenous leukemia K-562, acute lymphoblastic leukemia MOLT-3, Burkitt lymphoma Ramos (RA1), and histiocytic lymphoma U-937 cells were obtained from American Type Culture Collections and maintained in RPMI 1640 supplemented with 10% fetal bovine serum.

**Materials—**Dulbecco’s modified Eagle’s medium, glucose, penicillin, streptomycin, and Trizol were obtained from Life Technologies, Inc., and RPMI 1640 was from BioWhittaker. Fetal bovine serum was from HyClone. Leupeptin and glycogen were purchased from Roche Molecular Biochemicals, peptatin A, benzamidine, TLCK, phenylmethylsulfonflyl fluoride, and pepstatin A from Sigma. Avidin, Streptavidin, and horseradish peroxidase were from Pierce. Bovine serum albumin and Niprotect were from Alfa-Aesar. Anti-FLAG M2-agarose, and Saralasin were from Sigma. GR/B filter was from Whatman. C57 Bl/Ka-Thy 1.1 strain of mice was bred in the animal facility at Stanford University. Myristylated Goa was expressed in Escherichia coli (JM109) and purified to homogeneity by anion exchange and hydrophobic interaction chromatography as described (35). Specific activity was 16 nmol/mg of protein as determined by [35S]GTPγS binding.

**Sorting of Long-term Hematopoietic Stem Cells (HSCs)—**Bone marrow cells were obtained by flushing the tibias and femurs of the C57 Bl/Ka-Thy1.1 strain of mice. Cells were stained with a mixture of rat anti-Thy-1.1, rat anti-Mac-1, rat anti-Gr-1, Ter-119 (anti-erythroid-specified antigen), KT3.1 (anti-CD3), 53-7.3 (anti-CD5), GK1.5 (anti-CD4), and 53-6.7 (anti-CD8), washed and then incubated with goat anti-rat antibody conjugated with phycoerythrin. After washing, nonspecific sites were blocked with 1 mg of normal rat serum or rat IgG (m) of phosphate-buffered saline (PBS) and goat anti-mouse (1:200), mouse anti-goat (1:200). Cells were obtained by flushing the tibias and femurs of C57 Bl/Ka-Thy1.1 mice—

**Constructions of a cDNA Library from Long-term HSCs of C57 Bl/Ka-Thy1.1 Mice—**Twenty-eight thousand and two randomly selected long-term HSCs were resuspended in 100 μl of Trizol reagent containing 20 μg of glycogen. Total RNA was isolated as described by manufacturer’s instructions except the sample was re-extracted with 100 μl of Trizol. Total RNA was precipitated with isopropyl alcohol followed by ethanol and then resuspended in water. cDNAs were synthesized using CapFinder cDNA synthesis kit (CLONTECH) with modifications. There were 7.5 million clones in the original library. To test the quality of the library, plasmid DNA from 150 random clones were isolated and sequenced using ABI 3700 sequencer.

**Northern Analysis—**Total RNA was isolated from various mouse tissues and human cell lines using Trizol reagent according to the manufacturer’s instructions. Poly(A)+ RNA was then isolated from total RNA using oligo(dT) paramagnetic beads (Dynal). Two micrograms of poly(A)+ RNA were electroporated into 293T cells, and harvested 24 h later. Poly(A)+ RNA was electroporated into 293T cells, and harvested 24 h later. Poly(A)+ RNA was electroporated into 293T cells, and harvested 24 h later. Poly(A)+ RNA was electroporated into 293T cells, and harvested 24 h later. Poly(A)+ RNA was electroporated into 293T cells, and harvested 24 h later. Poly(A)+ RNA was electroporated into 293T cells, and harvested 24 h later.

**RT-PCR Analysis of RGS18 in Hematopoietic Progenitor Cells—**Long-term and short-term HSCs, common lymphoid progenitors, common myelocyte progenitors, granulocyte macrophage progenitors, and megakaryocyte erythroid progenitors were isolated as described previously (36, 40, 41). Five thousand cells of each of the above populations were double-sorted on a Vantage fluorescence-activated cell sorter. To ensure the correct populations were isolated to sufficient purity, day 12 spleen colony assays were performed on 100 long-term and short-term HSCs from the above sort. Similarly, common myelocyte progenitors, granulocyte macrophage progenitors, and megakaryocyte erythroid progenitors were functionally assayed in methylcellulose cultures according to Ref. 41. Total RNA was prepared from these fluorescence-activated cell sorter-purified cells using the Qiagen RNeasy miniprep kit. RNA was treated with DNase I to eliminate residual DNA contamination prior to reverse transcription reaction. cDNA was obtained using Superscript II (Life Technologies, Inc.) according the manufacturer’s recommendations. PCR was performed using [3P]Labeled primers and KlenTaq-1 (CLONTECH). Hypoxanthine-guanine phosphoribosyltransferase control PCR was performed to normalize the amount of RNA used in each reaction. Generally, 50 cells worth of cDNA was used to long-term and short-term HSCs, 30 cells worth of cDNA for common myelocyte progenitors and granulocyte macrophage progenitors, and 20 cells worth for megakaryocyte erythroid progenitors. After 28 cycles of PCR, one-fifth of the products were run on polyacrylamide gels. Gels were dried and exposed to x-ray films or a PhosphorImager for data acquisition and analyses. RT-PCR of RGS18 from 10 μg of total RNA isolated from thymus was negative (data not shown).

**Binding of RGS to Goa—**Three and a half million 293T cells were transfected with 10 μg of various RGS constructs by the calcium-phosphate method (42). Twenty-four hours after transfection, medium was changed, and cells were further grown for another 24 h. Cells were rinsed once with cold phosphate-buffered saline, and resuspended in Buffer A containing 50 mM HEPES, pH 8.0, 150 mM NaCl, 3% Triton X-100, 0.5 mM phenylmethylsulfonyl fluoride, 2 mM benzamidine, 5 μg of peptatin A, TLCK, and TPCK, and 10 μg of leupeptin and soybean trypsin inhibitor per ml. After 15 min on ice, cell lysates were centrifuged in a microcentrifuge for 15 min at 4 °C. The RGS proteins were immunoprecipitated from the supernatant with 20 μl of anti-FLAG M2 antibody, washed three times with Buffer A, Jurkat cell extracts (50 million cells per point) were precipitated as described (28) using Buffer B (Buffer A plus 1 mM MgCl2 and 0.3 mM NaCl). The extracts were treated with 30 μM GDP alone or 30 μM GDP, 30 μM GTPγS, and 0.1 mM NaF for 30 min at 30 °C and then incubated with immunoprecipitated RGS proteins for 1 h at 4 °C. Bound proteins were washed once with Buffer B without Triton X-100 and then resuspended in 30 of SDS sample buffer containing 12% SDS-polyacrylamide gel. Proteins were transferred electrophoretically to nitrocellulose membrane (Schleicher & Schuell). Western blot was performed using polyclonal antibodies against the α subunits of G12/13 (AS7 from PerkinElmer Life Sciences), G13G13, G12G12, and Gα2 or FLAG (Santa Cruz).

**Single Turnover GTP Hydrolysis Assay—**[3P]GTP (1 μM) was al-
lowed to bind to 50 nM myristylated Go13 for 15 min at 30 °C in Buffer E consisting of 50 mM HEPES, pH 8.5, 5 mM EDTA, 100 mM NaCl, 0.1% Lubrol, and 1 mM dithiothreitol. After lowering the temperature to 4 °C, single turnover GTP hydrolysis was initiated by mixing equal volumes of Go13 preloaded with γ-[35S]GTP and Buffer E plus 30 mM MgSO4, 4 μM unlabeled GTP, and FLAG-tagged RGS proteins bound to M2-agarose beads. The hydrolysis reaction was terminated by adding 1 ml of 15% (w/v) charcoal solution containing 50 mM NaH2PO4, pH 2.3, and placing samples on ice at the indicated time points. The charcoal was removed by centrifugation for 20 min at 4,000 g, and γ-[35S]P, release was assessed by liquid scintillation counting of a 250-μl aliquot of the supernatant in 4 ml of ScintiVerse.

RESULTs

Cloning of a Novel Regulator of G-Protein Signaling from Mouse Hematopoietic Stem Cells—The hematopoietic cells are constantly replenished by a self-replicating common precursor called the HSC. A large body of data on the biology of these cells has been accumulated. However, due to their rarity (less than 0.01% of the bone marrow cells; Ref 36) and the inability to grow these cells in vitro, there is little information regarding the molecular mechanisms that regulate stem cell functions. To better understand long-term self-renewing hematopoietic stem cells on the molecular level, a cDNA library was prepared from small numbers of highly purified long-term self-renewing hematopoietic stem cells. Approximately 150 clones were randomly chosen for sequencing to evaluate the quality of this library. The results of DNA sequencing indicated that the library contained ~50% previously unknown genes that are not present in the expressed sequence tag or GenBank™ data base (data not shown). One of the unknown clones showed limited homology to RGS (regulator of G-protein signaling), and this clone was further analyzed. The novel RGS will be referred to as RGS18. Complete sequencing and translation of the cDNA clone indicated that the clone contained the entire coding sequence (Fig. 1). The first ATG codon in the sequence is at nucleotide position 187, and conforms to the consensus sequence of Kozak (45). The base composition of the entire 1399 base pairs is 65.9% A + T. In the 3'-untranslated region, a polyadenylation signal sequence, AATAAA, is present at nucleotide position 1122, and three ATTATA or ATTATTA sequence motifs (46) are indicated (Fig. 1). In addition, a TTATTGAT sequence motif followed by an AT-rich sequence is present in the 3'-untranslated region. This motif is present in immediate early genes and suggested to play a role in transcriptional activation (47, 48). Translation of cDNA showed that RGS18 has 234 amino acids containing a central RGS box. A data base search using NCBI BLAST generated many nonredundant clones. The homology lies mostly within the RGS box (data not shown). Among the clones, RGS2 and RGS5 are most closely related to RGS18 (Fig. 2A). RGS2 has 51% identity and 67% homology, and RGS5, 49% identity and 66% homology. By searching the expressed sequence tag data base, we have found a human fetal lung expressed sequence tag clone (GenBank™ accession number N894410), showing 85% identity spanning from nucleotide position 203 to 501. IMAGE clone 297800, from which the sequence was derived, was obtained and completely sequenced. In the coding sequence, the human clone has 86% identity at the nucleotide level and 82% identity and 90% homology at the protein level (Fig. 2B), strongly suggesting that the human clone is a RGS18 ortholog. The human ortholog had a longer 3'-untranslated region than its mouse counterpart (Fig. 1), and there are two polyadenylation signal sequences and three ATTATA or ATTATTA sequences in the 3'-untranslated region. RGS18 proteins from both species contain putative phosphorylation sites for casein kinase II, protein kinase C, and protein kinase A (Fig. 2B).

Expression of RGS18 mRNA in Tissues and Cells—Poly(A)+ RNAs isolated from different mouse tissues were analyzed by Northern using the RGS18 cDNA. The NotI-EcoRI fragment containing the 5'-untranslated region and the partial coding region detected a 2.4-kb transcript (Fig. 3A). The highest level of RGS18 expression was observed in bone marrow followed by spleen, fetal liver, and then lung. RGS18 was undetectable in brain, thymus, liver, kidney, and skeletal muscle. A very faint signal was seen in heart. The expression pattern of human RGS18 was also analyzed. In tissues, human RGS18 is highest in peripheral leukocytes followed by bone marrow, spleen, and fetal liver (Fig. 3B). Thymus, as well as lymph nodes, did not express human RGS18, similar to the mouse RGS18 expression pattern. No signal was detected in other tissues tested. In cultured cell lines, RGS18 was expressed only in the monocytic line U937, but not in Molt3 (acute lymphoblastic T-cell leuke-
mic line), K562 (chronic myelogenous leukemia line), and Ramos (B-lymphocytes).

A rabbit antibody against recombinant RGS18 containing the first 202 amino acids was generated and used to test RGS18 expression in mouse tissue extracts (Fig. 3C). Anti-RGS18 recognized a specific protein with an apparent molecular mass of 26 kDa on a SDS-polyacrylamide gel (Fig. 3C, 2), but not when the antibody was preincubated with the recombinant RGS18 polypeptide (Fig. 3C, 1). As predicted from the Northern blot, RGS18 was most highly expressed in the bone marrow.

To confirm expression of RGS18 in long-term self-renewing hematopoietic stem cells, cells at various stages of hematopoiesis were purified from bone marrow by fluorescence-activated cell sorter, and RT-PCR was performed (Fig. 4). Compared with hypoxanthine-guanine phosphoribosyltransferase control, RGS18 signal was highest in long-term and short-term HSCs, and the level was lower in common lymphoid progenitors, common myeloid progenitors, granulocyte macrophage progenitors, and megakaryocyte erythroid progenitors. This indicates that RGS18 is expressed more in the primitive cells, and is downregulated as cells differentiate to more committed lineages.

Southern Analysis of RGS18—Mouse genomic DNA was digested with $\text{Bam}^\text{HI}$, $\text{Eco}^\text{RI}$, or $\text{Hin}^\text{dIII}$, and transferred to the membrane, and hybridized with the 0.5 kb of 5' end of RGS18 cDNA (Fig. 5). $\text{Bam}^\text{HI}$, $\text{Eco}^\text{RI}$, and $\text{Hin}^\text{dIII}$ generated single bands of 9, 2.8, and 6 kb, respectively, suggesting that there is a single copy for RGS18.

Binding of RGS18 to $\text{G}_i$ and $\text{G}_q$ from Jurkat T Leukemic Cell Extracts—From sequence comparison, RGS18 showed the most homology to RGS2 and RGS5. RGS2 has been shown to selectively bind and inhibit $\text{G}_q$ function (20). RGS5 can bind both $\text{G}_i$ and $\text{G}_q$ (9). To determine which G-protein signaling pathway RGS18 might act on, binding of RGS18 to endogenous Go protein was analyzed. HEK293T cells were transfected with the plasmids carrying FLAG-tagged RGS2, RGS4, and RGS18 cDNA. RGS proteins were immunoprecipitated with anti-FLAG M2 antibody coupled to agarose beads, and incubated with Jurkat cell extracts to facilitate binding to endogenous Go protein.
proteins (Fig. 6). In has been shown that RGS binds Ga with high affinity when Ga is complexed with GDP-AlF₄, which mimics the transition state during GTP hydrolysis. As shown in Fig. 6, the RGS proteins bound Ga only in the transition state (Fig. 6, 1AlF₄). No binding was observed in the GDP-bound state (Fig. 6, −AlF₄). The amount of different RGS proteins used in the reaction was similar (Fig. 6, FLAG). No bound Ga protein was seen with the immunoprecipitates prepared from cells transfected with control plasmid (Fig. 6, pc-FLAG). As previously shown (20), RGS2 did interact with Gaᵢ but not with Ga₉, and RGS4 was able to bind both Gaᵢ and Ga₉. RGS18 was also able to interact with Gaᵢ and Ga₉. However, RGS18 did not bind Ga₁₂, Ga₁₃, or Gaₛ (data not shown).

GAP Activity of RGS18—The ability of RGS18 to stimulate GTPase activity of Gaᵢ₁ was compared with other RGS proteins. To obtain large amounts of RGS18 protein, His-tagged RGS18 was expressed in bacteria. However, recombinant protein was insoluble. Therefore, HEK293T cells were transfected with FLAG-tagged RGS plasmids, and RGS proteins were immunoprecipitated as described before. To normalize the amount of RGS proteins in the assays, the immunoprecipitates were resolved on a SDS-polyacrylamide gel and stained with Coomassie Blue. RGS proteins were scanned with a densitometer, and the RGS was normalized with FLAG-agarose beads. Immunoprecipitates prepared from HEK293T cells transfected with pcFLAG showed no stimulation of GTPase activity (Fig. 7, Vector). As shown before, RGS2 showed no GTPase activity toward Gaᵢ₁. RGS4 dramatically enhanced endogenous GTPase activity of Gaᵢ₁. RGS18 also stimulated GTPase activity but not as much as RGS4. RGS4 reduced the calculated t₁⁄₂ for Pᵢ release of Gaᵢ₁ from 1.04 to 0.19 min and RGS18 reduced t₁⁄₂ to 0.56 min.

Inhibition of Ga mediated Signaling by RGS18—Since RGS18 was able to bind the Gaᵢ subunit, biological assays were used to determine whether this interaction has functional significance. If RGS18 can modulate a signal from Ga-coupled receptors, it will be indicative of a functional interaction with Gaᵢ. HEK293T cells were co-transfected with angiotensin 1a receptor plasmid and a FLAG-RGS or control plasmid. Angiotensin 1a receptor has shown to be coupled to the Gaᵢ signaling.
pathway and activation of phospholipase C, which generates inositol 3-phosphate (49). Transfected cells were labeled with myo-[3H]inositol and stimulated with angiotensin II peptide. Both RGS2 and RGS4 inhibited [3H]inositol phosphates release (Fig. 8A). RGS18 was also able to attenuate Gq signaling mediated by angiotensin II. There was no difference in the amount of [125I]angiotensin binding to the cells transfected with the RGS constructs or the empty vector (data not shown). Next, we tested whether RGS18 could attenuate Gq-mediated transcriptional activity. HEK293T cells were transfected with RGS or control plasmid, and M1 muscarinic receptor and pCRE/β-gal. It has been shown that activation of M1 muscarinic receptor, which couples Gq protein, resulted in transcriptional activation through binding of cAMP responsive element-binding protein to cAMP responsive element (50). Carbachol treatment of cells transfected with control plasmid showed ~20-fold activation of transcription of the reporter gene (Fig. 8B). All RGS constructs inhibited transcriptional activation. RGS2 inhibited activation by 75%, RGS4 by 71%, and RGS18 by 77.5% of the control.

**DISCUSSION**

In this paper, we report the cloning of a novel RGS from mouse long-term self-renewing hematopoietic stem cells. The sequence surrounding the third ATG located at nucleotide position 187 was in agreement with the Kozak’s consensus se-
quency for eukaryotic initiation codons (45). The 702-nucleotide open reading frame encodes a polypeptide of 223 residues. This new RGS protein was designated as RGS18. The entire RGS18 cDNA is A + T-rich, and the 3′-untranslated region contains three ATTAA motifs. These features have been linked to mRNA stability (46) and translational control (51). The presence of these structures suggests that expression of RGS18 could be highly regulated. Both mouse and human RGS18 were expressed as a 2.4-kb transcript as determined by Northern hybridization, and showed a hematopoietic tissue-specific expression pattern, with the highest levels in peripheral leukocytes and bone marrow followed by fetal liver and spleen (Fig. 4). There was no RGS18 message detected in thymus and lymph nodes. In cultured cells, only monocytic U937 but not B, T, and myelocyte-derived cell lines expressed RGS18. RT-PCR of RGS18 from cells at the early stages of hematopoiesis indicated that RGS18 is highly expressed in both long-term and short-term HSCs, and less so in cells with more committed lineages (Fig. 4). RGS18 protein can bind Goq and Gaiq (Fig. 6). In vitro GTPase assays, RGS18 enhanced the intrinsic GTPase activity of Goq to a lower extent compared with RGS4 (Fig. 7). RGS2, which acts only on Gai, was not able to stimulate the GTPase activity. Furthermore, RGS18 inhibited the inositol phosphates production mediated by angiotensin 1a receptor and transcriptional activation mediated by M1 muscarinic receptor in HEK293T cells (Fig. 8). Even though the RGS18 sequence is more homologous to RGS2 than RGS4, it clearly interacts with Goq, as well as Gai. Effects of RGS18 on the Gi pathway are contradictory. Therefore, it is necessary to study gain of function and/or loss of function mice to verify the role of RGS18 in Gi pathway in vivo.

There are over 20 RGS genes cloned so far, but their in vivo regulation is not well understood. There are several ways in which cells can regulate RGS functions. First, many RGS proteins are expressed in tissue and cell-type specific manners. For example, some RGS proteins are abundant in lymphocytes...
and monocytes (8, 25, 26), brain (30–33), and rods (34). Furthermore, the level of RGS can be modulated under certain conditions. For example, in antigen-activated B cells, RGS1 and RGS2 are up-regulated and RGS3 and RGS14 are down-regulated (29). RGS1 and RGS2 are also up-regulated in phorbol ester-stimulated B cells and ConA- and cyclohexamide-treated human blood mononuclear cells (25). In vascular smooth muscle, RGS2 message was rapidly increased upon angiotensin stimulation (52). RGS16 expression is induced in human T cells by IL-2 and the induction was diminished by cAMP. RGS2 expression, however, was reciprocated (28). RGS18 also showed a tissue and cell-type specific expression pattern. It is expressed highly in long-term and short-term HSCs, and its level is decreased as these cells are more committed to differentiated pathways. In mature cells, RGS18 appears to be most highly expressed in peripheral blood leukocytes of myelomonocytic lineage.

Another way to regulate the RGS activity is by regulating specific interaction between the Go. The RGS boxes interact with the switch regions of Ga and these interactions are required for the GAP activity. Therefore, the specific interaction with Ga would be determined by divergent sequences outside of the RGS box. The first evidence that the RGS box alone might not be enough to function normally in vivo comes from the Sst2 complementation assay in yeast (53). The full-length RGS16 protein could bind and function as a GAP for Gaq and Gao, in vitro, and attenuated pheromone signaling. The RGS16 core domain was also able to bind Ga and enhance GTPase activity in vitro; however, the mutants lacking the NH2-terminal region were unable to attenuate pheromone signaling (53). Further evidence for the requirement of the non-RGS box is that deletion of the NH2-terminal domain of RGS4 diminished its biological potency by 10,000-fold (54). It has been demonstrated that different RGS can differentially inhibit Ca2+ signaling pathways induced by isopreterenol/somatostatin and carbachol, respectively. Cysteine mutation did not significantly affect the cellular localization of RGS16 and in vitro GAP activity, suggesting that reversible palmitoylation of the protein might be important for biological activity of RGS16. This would also be true for RGS4 and RGS5 whose sequences are conserved at the NH2 terminus. In RGS18 there is no amphipathic structure at the NH2 terminus, and no possible palmitoylation site, suggesting a different mode of regulation for RGS18.

The fact that RGS18 is highly expressed in HSC and mature myelomonocyte compartment and that many other RGS proteins are lymphoid specific suggest that RGS proteins may have functions in regulation of hematolymphoid systems. For example, lymphocyte migration during inflammatory response is induced by a number of chemokines, whose receptors are coupled to Go, RGS1, RGS3, RGS4, and RGS14, which are expressed by the lymphoid system, can inhibit chemotaxis induced by various chemokines including proinflammatory factors, stromal cell-derived factor-1α, and Epstein-Barr virus-induced molecule 1 ligand (26, 29, 63, 64). RGS2, which acts on Goq, showed no effect on chemotaxis. SDF-1α is the ligand for CXCR4 (65), which is expressed by various cells including HSCs (66). During the fetal development, hematopoiesis occurs in the fetal liver. As the fetus develops, hematopoiesis moves to the bone marrow. In adult, bone marrow is the primary site for hematopoiesis. It has been shown that in mice lacking SDF-1α, bone marrow hematopoiesis was absent, even though fetal liver hematopoiesis was normal (67). This suggests that SDF-1α, expressed by bone marrow stromal cells, is responsible for migration of HSCs from fetal liver to bone marrow. Even though many reports show that multiple RGS proteins could modulate SDF-1α-induced chemotaxis, it is possible that there might be specificity of RGS to regulate different chemokine receptors. Since RGS18 can interact with Goq, it would be of interest to test the possible role of RGS18 in inflammatory response and HSC migration.

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Molecular Cloning and Characterization of a Novel Regulator of G-protein Signaling from Mouse Hematopoietic Stem Cells
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