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Role of regulator of G protein signaling proteins in bone

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Abstract

Regulators of G protein signaling (RGS) proteins are a family with more than 30 proteins that all contain an RGS domain. In the past decade, increasing evidence has indicated that RGS proteins play crucial roles in the regulation of G protein coupled receptors (GPCR), G proteins, and calcium signaling during cell proliferation, migration, and differentiation in a variety of tissues. In bone, those proteins modulate bone development and remodeling by influencing various signaling pathways such as GPCR-G protein signaling, Wnt, calcium oscillations and PTH. This review summarizes the recent advances in the understanding of the regulation of RGS genes expression, as well as the functions and mechanisms of RGS proteins, especially in regulating GPCR-G protein signaling, Wnt signaling, calcium oscillations signaling and PTH signaling during bone development and remodeling. This review also highlights the regulation of different RGS proteins in osteoblasts, chondrocytes and osteoclasts. The knowledge from the recent advances of RGS study summarized in the review would provide the insights into new therapies for bone diseases.

Keywords

Osteoblasts; Osteoclasts; Bone; Heterotrimeric G protein; G protein coupled receptor; PTH; PTHrP; Calcium Sensing Receptor; Regulator of G Protein Signaling; RGS2; RGS5; RGS10; RGS12; RGS18; Wnt; bone remodeling; bone development

2. INTRODUCTION

Bone is a mineralized connective tissue consisting of 33% organic matrix. Bone remodeling, cycles of bone apposition and resorption, continues throughout life (1). Osteoblasts are mononuclear cells, which arise from osteoprogenitor cells located in the deep layer of periosteum and the bone marrow and are responsible for bone formation. In contrast,

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osteoclasts are highly specialized multinucleated cells derived from the hematopoietic cell precursors in the bone marrow and peripheral blood, and are capable of breaking down both the inorganic and organic matrix of bone to resorb the bone. Both osteoblasts and osteoclasts participate in bone remodeling and are affected by a variety of signals during their differentiation and function (1). Signal transduction from the extracellular environment across the plasma membrane and into the intracellular milieu is a fundamental aspect of cellular regulation, and coordinates essential aspects of cell and organ homeostasis. G Protein Coupled Receptors (GPCRs), also known as seven trans-membrane domain receptors, constitute a large protein family of receptors. Those receptors perceive many extracellular signals (*e.g.* neurotransmitters, hormones, phospholipids and growth factors) and transduce them to heterotrimeric G proteins, which further transduce those signals intracellularly to appropriate downstream effectors and thereby play an important role in various signaling pathways (2). GPCRs represent important specific targets for a variety of physiological functions and therapeutic approaches, such as the control of blood pressure, allergic response, kidney function, hormonal disorders, neurological diseases, bone development, and bone remodeling (3, 4). By binding with specific agonists/ligands, GPCRs leads to the rapid activation of heterotrimeric G-proteins (binding of GTP) and the regulation of intracellular second messengers (*e.g.* cAMP and intracellular Ca^{2+} levels). The expressions of multiple GPCRs have been found in osteoblasts, osteocytes, and osteoclasts which regulate local and systemic signaling (3).

G proteins are a family of intracellular proteins that act as mediators of proliferation, differentiation, and apoptosis in multiple cell types (5). G proteins are composed of three non-identical subunits, $\text{G}\alpha$ (33–35 kDa), $\text{G}\beta$ (~35 kDa) and $\text{G}\gamma$ (~15 kDa) (6). The kinetics of G-protein signaling are regulated by regulators of G protein signaling (RGS) proteins that act as scaffolds to help assemble signaling complexes (7) and as GTPase activating proteins (GAPs) on $\text{G}\alpha$ subunits. RGS proteins regulate heterotrimeric G proteins by increasing the rates at which their subunits hydrolyze bound GTP and return to the inactive state (8). Hence, the G-protein activity cycle is sequentially regulated by RGS proteins, and is critical for the rapid activation and inactivation of cellular responses (8).

The RGS protein family has more than 30 family members (9). Sequence and structure analysis suggests that mammalian RGS proteins are divided into nine subfamilies including RZ/A, R4/B, R7/C, R12/E, GEF/F, GRK/G, SNX/H and D-AKAp2/I (8, 10) as shown in Table 1 (11). Those proteins specifically and selectively regulate G protein mediated receptors, ion channels, and other signaling events (11). Many studies have demonstrated that RGS proteins serve as key modulators in neurons (12, 13), the cardiovascular system (14, 15), inflammatory disorders (16, 17), and cancer (18). The importance of RGS proteins in bone has been addressed in recent years. Here, we reviewed current findings outlining the regulation of RGS gene expression, and RGS proteins in the regulation of G protein and GPCRs. We further summarized the various signaling pathways that are either dependent upon or related to RGS function in bone development and remodeling, and the role of RGS proteins in the regulation of bone cell differentiation and function.

3. REGULATION OF RGS GENE EXPRESSION

Increasing evidence has demonstrated that RGS proteins are regulated through multiple mechanisms, including the regulation of GPCR signaling, bacterial LPS, proinflammatory cytokines, and cell stress conditions. Additionally, the detrimental effects of cell stress under certain conditions such as hyperoxia, hypoxia/ischemia, mechanical stress, and drug treatment also influence the RGS expression (19).

3.1. G-protein-mediated pheromone signaling

Some studies showed that RGS gene expression is negatively and/or positively regulated by G-protein-mediated pheromone signaling. For example, Gq/Gi-coupled agonists can increase the expression of several RGS genes, such as RGS2, RGS4, and RGS16 (19). Interestingly, angiotensin II (Gq/Gi-coupled agonists) has a dual effect on RGS2 expression. Short-term angiotensin II (ANG II) stimulation for one day in fibroblasts increases RGS2 levels. In contrast, prolonged ANG II stimulation (3–14 days) markedly decreases RGS2 (20). Similarly, stimulation of opioid receptors can induce a rapid and persistent (8 hours) increase in RGS4 mRNA (21). While Gs/cAMP-coupled agonists increase RGS2 expression, but inhibit the expression of RGS3 (22), RGS4 (22, 23), RGS5 (24), and RGS16 (25) in various cell types. Additionally, cAMP reduces mRNA levels of RGS13, and its PKA-mediated phosphorylation and subsequent stabilization maintains high protein levels of the RGS13 protein, likely due to protection from proteasomal degradation (26).

3.2. Bacterial LPS and proinflammatory cytokines

In addition to above mentioned proteins, bacterial LPS and proinflammatory cytokines (important mediators of the immune response and inflammation) also regulate RGS gene expression (19). In immune cells, LPS variably affects the expression of RGS proteins, depending on the RGS and the cell type (19). For example, LPS down-regulates RGS2 mRNA in macrophages (27, 28), but up-regulates RGS1 expression in dendritic cells and macrophages (27, 29). IFN- β induces the expression of RGS1 in peripheral blood mononuclear cells, monocytes, T cells, and B cells (30), and stimulates the expression of RGS2 and RGS16 in mononuclear leukocytes (31, 32).

3.3. PTH and TSH

The expression of RGS proteins is also regulated by PTH signaling (33, 34). For example, RGS2 expression is increased by PTH stimulation in osteoblasts. Moreover, the expression of RGS2 mRNA is rapidly and transiently increased 2-to 5-fold by injection of PTH in femoral metaphyseal spongiosa of young male rats (33). Thyrotropin (TSH) signaling is also involved in the RGS regulation (35). The expression of RGS2 mRNA is significantly up-regulated within 1 hour after TSH stimulation in human thyroid primary cultures and reaches a maximum after 4 hours (35). Co-expression of RGS2 and TSH receptor in COS-7 cells inhibits TSH-G(q) α signaling, implying that RGS 2 is involved in TSHR-induced G(q) signal transduction (35).

4. RGS PROTEINS IN THE REGULATION OF SIGNALING PATHWAYS

4.1. RGS proteins and GPCR-G protein signaling

GPCRs transduce signals by coupling to heterotrimeric (α -, β -, γ -subunit-containing) GTP-binding (G) proteins that regulate effector molecules (36). RGS proteins have a conserved RGS core domain of approximately 120 amino acids that is necessary and sufficient for binding to G α subunits (9). There are four principal G α proteins (G $_s$, G $_i$, G $_{q/11}$, G $_{12/13}$). The structures of G subunit reveal a conserved protein fold composed of a helical domain and a GTPase domain that undergoes conformational changes when binding GTP and GDP (37). One critical regulatory point in the G-protein cycle is the deactivation of G-proteins by GTP hydrolysis that is enhanced by GTPase activating proteins (GAP) - RGS proteins (8). RGS proteins are capable of accelerating GTPase activity up to 1000-fold to promote GTP hydrolysis by α subunit of heterotrimeric G proteins, thereby inactivating G protein and rapidly switching off GPCR signaling (38). In addition to functioning as a deactivator for G proteins, Zhong *et al.* found that high concentrations of receptors lead to saturation of GDP-GTP exchange making GTP hydrolysis rate-limiting and local depletion of inactive

heterotrimeric G-GDP, which is reversed by RGS GAP activity. RGS enhances receptor-mediated Gi protein activation even as it deactivates the Gi protein (39). Therefore, the authors proposed that RGS can constrict the spatial range of active G protein around some receptors to limit the spill-over of G protein signals to more distant effector molecules, thus enhancing the specificity of Gi protein signals (39, 40). Beside the GAP role of RGS proteins, RGS12 was recently found to function as a Ras/Raf/MEK scaffold to regulate nerve growth factor (NGF)-mediated neurite outgrowth in PC12 cells by facilitating the coordination of H-Ras, B-Raf, and MEK2 and prolonging extracellular regulated kinases (ERK) activation (24). Additionally, RGS4 (41) and RGS3/5(42) were also found to regulate activation of ERK as well as Gq-mediated PLC β . G $\beta\gamma$ released by Gi is also able to activate PLC β -PKC signaling and can be distinguished from Gq-activated PLC β by Gi specific inhibitor PTX (pertussis toxin) (43) and Gq specific inhibitor (44). PLC β seems to have paradoxical roles, being both an effector and a GAP for G $\alpha_q/11$ (2, 45).

Some studies have shown that mutation of upstream genes of RGS proteins such as G protein or GPCR have severe skeleton phenotypes (36). Deficiency of Gas in osteoblasts impairs bone formation due to the reduced differentiation of osteoblasts through inhibiting adenylyl cyclase in cAMP-PKA pathway (46), suggesting that Gas stimulates adenylyl cyclase. In contrast, Gai inhibits the activity of adenylyl cyclase by antagonizing Gas-mediated increases in adenylyl cyclase activity in osteoblasts(47). Transgenic expression of a constitutively active Gi-coupled GPCR in osteoblasts leads to marked trabecular osteopenia (48), consistent with the concept of Gi-coupled signaling and opposing Gs-mediated trabecular bone formation. Millard *et al.* (49) investigated the skeletal effects of blocking Gi-coupled signaling in osteoblasts *in vivo* by transgenic expression of the catalytic subunit of pertussis toxin (PTX) under control of the collagen I α 2.3-kb promoter. The mice show increased periosteal bone formation and cortical thickness which correlates with associated with expanded mineralizing surfaces.

4.2. RGS proteins and Wnt signaling

Mounting studies have shown that RGS proteins such as Axin play critical roles in the regulation of Wnt signaling pathway (50–53). Wnt signaling cascade regulates cell proliferation, differentiation, and motility, and plays a critical role in development (54). Wnts/ β -catenin pathway is commonly called the canonical Wnt pathway. In bone, the canonical pathway plays a substantial role in the control of bone formation and bone remodeling (55). This pathway is mediated by β -catenin which goes into phosphorylation and ubiquitination when there is no Wnt binding. Inhibition of β -catenin phosphorylation prevents its degradation, and thus results in an increase in its cytoplasmic concentration (50). Wnts/ β -catenin subsequently contribute to the proliferation and survival of osteoblasts (56).

Axin2, an atypical RGS protein that serves as a molecular scaffold for a β -catenin destruction complex, has been shown to be a key negative regulator of bone remodeling (50–53). Yan *et al.* reported that deletion of axin2 causes a significant increase in bone mass that results from a significant increase in osteoblast proliferation and differentiation, and a decrease in osteoclast differentiation (50). They further found that axin2 negatively regulates osteoblast differentiation and bone remodeling through the beta-catenin-BMP2/4-Osx signaling pathway in osteoblasts. Hey *et al.* recently found that axin interacts with N-cadherin to negatively regulate Wnt signaling through β -catenin degradation, resulting in impaired osteoblast differentiation. By analyzing the N-cadherin transgenic mice using a Col12.3-N-cadherin-construct, they further found that increased β -catenin degradation induced by N-cadherin-LRP5 interaction inhibits osteoblast function and bone formation and delays bone mass acquisition *in vivo* (57). Additionally, Regard *et al.* reported that activating G α mutations can interact with axin to potentiate Wnt/ β -catenin signaling.

Furthermore, they found that removal of Gαs led to reduced Wnt/β-catenin signaling and decreased bone formation (58). It was also found that Wnt signaling is negatively influenced by GRK2 activity in bone-forming osteoblasts and therefore may be suggested for increasing bone formation (59).

4.3. RGS proteins and PTH signaling

Of several systemic hormones and local factors affecting bone remodeling during adult life, PTH may be one of the most important factors in bone homeostasis (60, 61). RGS proteins are instrumental in the regulation of PTH. It is well known that PTH regulates cell differentiation and function through GPCRs in two principal target organs: kidney and bone (62, 63). By binding with its GPCR, PTH can modulate both Gα_s-regulated adenylate cyclase and Gα_q-regulated phospholipase C, resulting in the activation of cAMP/protein kinase A (PKA) and protein kinase C (PKC)/Ca²⁺ dependent cellular processes and transcriptional responses (64, 65). The relative activation of adenylate cyclase and phospholipase C depends mainly on the duration and magnitude of activation of the Gαs and Gαq protein subunits. RGS proteins, as GAP proteins, have been shown to accelerate the inactivation of G proteins by enhancing their GTPase activity (10), and therefore play critical role in PTH signaling.

Accumulating evidence has shown that PTH-induced RGS2 expression in osteoblasts involves PTH signal transduction via its GPCRs (33, 66, 67). PTH binds to its PTH/PTHrP receptor (68) to activate cAMP/PKA- and PKC/Ca²⁺- dependent signal transduction via Gs- and G_{q/11}- proteins (67). Pulsatile PTH treatment causes a 4- to 10-fold lower RGS2 abundance, compared to a continuous PTH exposure (69). Moreover, functional analysis with RGS2 overexpression suggests that RGS2 down-regulates PTH- and forskolin-induced cAMP production in osteoblasts (34) and limits Gs signaling (70). Further studies showed that the basal levels of endogenous RGS2 do not appear to regulate Gs or Gq signaling in osteoblasts (70). Using Forskolin treatment of wild-type osteoblasts to activate Gs signaling, suppresses Gq-dependent accumulation of inositol phosphates and nucleotide-stimulated calcium release that does not occur in RGS2 (−/−) osteoblasts. Conversely, activation of Gq signaling suppresses PTHrP-dependent cAMP accumulation in wild type but not in rRGS2 (−/−) osteoblasts. Those findings demonstrate that up-regulation of RGS2 contributes to cross-desensitization of Gs- and Gq-coupled signals (70). Additionally, Miles *et al.* (33) confirmed that the expression of RGS2 mRNA is rapidly and transiently increased by human PTH in both metaphyseal (4-to 5-fold) and diaphyseal (2- to 3-fold) bone, as well as in cultured osteoblast cultures (2- to 37-fold). *In vitro*, forskolin and dibutyryl cAMP can similarly elevate RGS2 mRNA. They further found, *in vivo*, different PTH analogs affect RGS2 gene expression. PTH analogs, such as 1–31 and 1–38 (which stimulate intracellular cAMP accumulation, PTHrP, and prostaglandin E2) induce RGS2 mRNA expression; whereas PTH analogs, such as 3–34 and 7–74 (which do not stimulate cAMP production) have no effect on RGS2 expression. In tissue distribution analysis, RGS2 is widely expressed in all examined tissues including heart, spleen, liver, skeletal muscle, kidney, and testis, and significantly expressed in two nonclassical PTH-sensitive tissues: brain and heart. After PTH injection, RGS2 mRNA expression is up-regulated in rat bone but not in any of the other examined tissues. Those findings demonstrate that RGS2 is regulated by PTH, prostaglandin E2, and PTHrP, suggesting increased RGS2 expression in osteoblasts may be one of the early events influencing PTH signaling(33).

Besides PTH signaling, some studies have shown that other factors also regulate PTH-mediated RGS2 expression and function. Ueno *et al.* (31) reported that RGS2 gene expression markedly increases with PTH infusion and its expression is dose-dependently suppressed by treatment of 1,25(OH)₂ D3. Their results suggest that 1,25 (OH)₂ D3 inhibits

PTH-mediated G protein signaling. Homme *et al.* (71) further studied the influence of vitamin D and dexamethasone on PTH-induced RGS2 expression in osteoblast-like cells. By determining RGS2 gene transcription rate, they found that the transcription rate is increased by 35% with 1, 25-(OH)₂D₃ and decreased by 63% with dexamethasone pretreatment, demonstrating that glucocorticoids and vitamin D inversely regulate PTH-induced RGS2 expression via a transcriptional mechanism. Additionally, PTH secretion by parathyroid cells responds to changes in extracellular calcium through signaling by the calcium-sensing receptor (CaR) (72, 73). Koh *et al.* (74) found that RGS5 is selectively up-regulated in parathyroid adenomas relative to normal glands. Transient expression of RGS5 in cells stably expressing CaR results in dose-dependent abrogation of calcium-stimulated inositol trisphosphate production and ERK1/2 phosphorylation. Furthermore, they found that RGS5 knockout mice display significantly reduced plasma PTH levels, which is consistent with attenuated opposition to CaR activity. Collectively, their data suggest that RGS5 can act as a physiological negative regulator of CaR in the parathyroid gland. Similarly, PTH-related peptide (PTHrP) was found to increase osteogenic proliferation through activation of Ras and MAPK signaling pathway, showing that Gq is likely involved in that process (75).

4.4. RGS proteins and calcium (Ca²⁺) oscillations

How Ca²⁺ oscillations regulate tissue specific gene expression and cell differentiation has been an unsolved question in cell biology. Several lines of evidence have shown that RGS proteins play pivotal roles in controlling Ca²⁺ oscillations and cell differentiation in T lymphocytes, neurons, and cardiac myocytes (76–79). Interestingly, different RGS proteins have different receptor preferences in the regulation of calcium signaling and cell differentiation and function (80). In the pancreas, for example, the amino-terminal residues allow selective interaction between RGS4 and the muscarinic acetylcholine receptor (76). Many RGS proteins, including RGS1, RGS2, RGS4 and GAIP, as terminators of the active state of G proteins, can accelerate the GAP activity of Gαq (8, 38) to inactive Ca²⁺ reuptake and delay Ca²⁺ spikes for several seconds or even minutes. Additionally, the amplitude and frequency of changes in cytoplasmic Ca²⁺ concentrations influence the nature of the cellular response. Dolmetsch *et al.* (81, 82) reported that Ca²⁺ oscillations regulate gene expression and cell differentiation in T cells. Rapid oscillations stimulate three transcriptional factors (NFAT, Oct/OAP, NF-κB), whereas infrequent oscillations activate only NF-κB. By differentially controlling the activation of distinct sets of transcription factors and the expression of different genes, oscillation frequency may direct cells along specific developmental pathways (83, 84). These findings highlighted that RGS proteins play critical role in the regulation of Ca²⁺ oscillations.

Some studies have showed that the components of the Ca²⁺ signal, including IP₃ production and Ca²⁺ fluxes across the ER and plasma membranes, regulate the frequency and amplitude of Ca²⁺ oscillations (85). The activation of Gα by agonist activates PLCβ to generate IP₃. IP₃ releases Ca²⁺ from the endoplasmic reticulum (ER), which is followed by the activation of store-operated Ca²⁺ channels in the plasma membrane and Ca²⁺ influx. The increase in Ca²⁺ leads to activation of the plasma membrane Ca²⁺ ATPase (PMCA) and sarco/endoplasmic reticulum Ca²⁺ ATPase (SERCA) pumps to remove Ca²⁺ from the cytosol (86). During this process, RGS proteins, as the negative regulators of GPCRs signaling, play a central role in determining the duration of the stimulated state and controlling Ca²⁺ oscillations by regulating GTPase activity of G protein (87). The RGS domain accelerates GTP hydrolysis on the G α subunit to uncouple receptor stimulation from inositol 1,4,5-trisphosphate (IP₃) production. The C-terminus mediates the interaction with accessory proteins in the complex and the N-terminus acts in a receptor-selective manner to confer regulatory specificity (88). Hence, RGS proteins have both catalytic and scaffolding function in regulating Ca²⁺ signaling. Further study from Wang *et al.* (86) showed that deletion of

RGS2 regulates the kinetics of IP₃ production without affecting the peak level of IP₃, but increases the steady-state level of IP₃ at all agonist concentrations. Deletion of RGS2 decreases the expression of IP₃ receptor 1 and IP₃ receptor 3, and then reduces the sensitivity for IP₃ to release Ca²⁺ from the ER to the cytosol. Thus, Sarco/endoplasmic reticulum Ca²⁺ ATPase 2b is up-regulated to more rapidly remove Ca²⁺ from the cytosol of RGS2-deleted cells. Furthermore, they found that deletion of RGS2 reduced the response of the cells to changes in the ER Ca²⁺ load and to an increase in extracellular Ca²⁺. These findings concentrate the central role of RGS proteins in Ca²⁺ oscillations.

Ca²⁺ oscillations were first found playing critical roles in NFATc1 activation and osteoclast differentiation (89) in 2002. Takayanagi *et al* found that RANKL evokes Ca²⁺ oscillations that lead to calcineurin-mediated activation of NFATc1 and therefore triggers a sustained NFATc1-dependent transcriptional program during osteoclast differentiation. Inhibiting NFATc1 activity using dominant negative alleles blocks osteoclastogenesis, whereas overexpression of the wild-type protein stimulates osteoclast development from embryonic stem cells in a RANKL-independent manner. Both the transient initial release of Ca²⁺ from intracellular stores and the influx through specialized Ca²⁺ channels control the dephosphorylation of the cytoplasmic components (NFATc1 proteins) and lead to their nuclear localization to activate osteoclast specific genes(90). These results indicate that NFATc1 may represent a master switch for regulating terminal differentiation of osteoclasts and functioning downstream of RANKL. Further study (91) showed that mice lacking immunoreceptor tyrosine-based activation motif (ITAM)-harboring adaptors, Fc receptor common γ subunit (FcR γ) and DNAX-activating protein (DAP) 12, exhibit severe osteopetrosis owing to impaired osteoclast differentiation. In osteoclast precursor cells, FcR γ and DAP12 associate with multiple immunoreceptors and activate calcium signaling through phospholipase C γ (PLC γ).

Based on those studies, we proposed that some regulators such as RGS proteins might exist to regulate Ca²⁺ oscillations during osteoclast differentiation. By using differential screening, we found that RGS10 and RGS12 are both prominently expressed in osteoclast-like cells. Regulator of G-protein signaling 10A (RGS10A), but not the RGS10B isoform, is specifically expressed in human osteoclasts. The expression of RGS10A is also induced by RANKL in osteoclast precursors and prominently expressed in mouse osteoclast-like cells. RGS10A silencing by RNA interference blocks intracellular Ca²⁺ oscillations, the expression of NFATc1, and osteoclast terminal differentiation in both bone marrow cells and osteoclast precursor cell lines. Reintroduction of RGS10A rescues the impaired osteoclast differentiation. RGS10A silencing also results in premature osteoclast apoptosis (92). By generating and characterizing the RGS10 knockout model, we found that RGS10-deficient (RGS10^{-/-}) mice exhibit severe osteopetrosis and impaired osteoclast differentiation. The deficiency of RGS10 results in the absence of Ca²⁺ oscillations and loss of NFATc1. Ectopic expression of RGS10 increases the sensitivity of osteoclast differentiation to RANKL signaling. Additionally, ectopic expression of NFATc1 rescues the impaired osteoclast differentiation from deletion of RGS10 (93). Our results further reveal a mechanism that RGS10 competitively interacts with Ca²⁺/calmodulin and phosphatidylinositol 3,4,5-trisphosphate (PIP3) in a Ca²⁺-dependent manner to mediate PLC activation and Ca²⁺ oscillations, and that RGS10 specifically regulates the RANKL-Ca²⁺ oscillation-NFATc1 signaling pathway during osteoclast differentiation.

Compared to RGS10, we found that knockdown of RGS12, the largest protein in the RGS family, could also block Ca²⁺ oscillations, NFATc1 expression and osteoclast differentiation (93). Furthermore, we found that calcium sensing receptor (CaR) is expressed in preosteoclasts and osteoclasts and that RGS12 interacts with N type Ca²⁺ channels and CaR during osteoclast differentiation (94). Interestingly, we found RGS10 knockout mice exhibit

a severe osteopetrosis phenotype, impaired Ca^{2+} oscillation, and osteoclast differentiation, which cannot be rescued by RGS12. Unlike RGS10 (93), RGS12 does not bind with Ca^{2+} /calmodulin (CaM) and PIP3 in a Ca^{2+} -dependent manner during osteoclast differentiation. It acts on N-type Ca^{2+} channels and CaR. Most recently, by specific deletion of RGS12 in osteoclast lineage also caused osteopetrosis phenotype. The further study is going on. Those results demonstrated that RGS proteins may play different roles in regulating Ca^{2+} oscillations and osteoclast differentiation.

5. REGULATION OF DIFFERENTIATION AND FUNCTION OF BONE CELL BY RGS PROTEINS

5.1. Osteoblasts

Osteoblasts are the mononucleate bone forming cells, which derive from the bone marrow. Osteoblasts produce new bone called “osteoid” made of bone collagen and other protein, and are responsible for mineralization of this matrix. Many studies have shown that numerous GPCRs express in osteoblasts, including frizzled, the parathyroid hormone (PTH)/parathyroid hormone-related peptide (PTHrP) receptor type 1 (PTH1R), CaR, calcitonin gene-related peptide receptor, and relaxin family peptide 2 (36). Those proteins are important for the differentiation and function of osteoblasts (36). RGS proteins selectively interact with G proteins and their corresponding GPCR (95). Transgenic mice with a constitutively active mutant CaR (Act-CaR), which target to mature osteoblasts by the 3.5 kb osteocalcin promoter, show reduced bone volume and density of cancellous bone, accompanied by a diminished trabecular network. Constitutive signaling of the CaR in mature osteoblasts increases expression of receptor activator of nuclear factor-kappaB ligand (RANKL), the major stimulator of osteoclast differentiation and activation, which is the likely underlying mechanism for the bone loss (96). Moreover, it is proven that CaR promotes osteoblast proliferation (97, 98) likely through coupling to Gq and Gi signaling to stimulate IP3 production, calcium influx, and ERK phosphorylation (99), suggesting the involvement of RGS proteins. Recently, a newly CaR, GPRC6A, is found to be widely expressed in bone, calvaria, and osteoblastic cell line MC3T3-E1. Overexpression of RGS2 or RGS4 and pretreatment with pertussis toxin (PTX) inhibits activation of GPRC6A by extracellular cations (100).

RGS2 has been implicated in the regulation of G protein signaling in bone. RGS2 is expressed in rat metaphyseal and diaphyseal bone, mouse calvarial organ cultures, and cultured osteoblasts. The levels of RGS2 mRNA are up-regulated in response to PTH, PTHrP, and prostaglandin E2 or by the promoters of protein kinase A activity, such as forskolin, cholera toxin, and cell-permeant cAMP analogues in osteoblasts (18, 19). Some studies have showed that the basal levels of endogenous RGS2 do not regulate Gs or Gq signaling in osteoblasts (70). Functionally, RGS2 increases the rate of GTP hydrolysis on the Gq subunit (9, 23) and also inhibits Gq-stimulated activation of PLC (24). In contrast, RGS2 has no effect on the rate of GTP hydrolysis by the Gs subunit (8, 23), but inhibits intracellular cAMP accumulation (8,13–16). Both Gq and Gs recruit RGS2 to the plasma membrane in mammalian cells (8), and bind directly to RGS2 (16, 20, 21, 25). Further study found that forskolin-activated Gs signaling up-regulates RGS2 expression and suppresses Gq-dependent accumulation of inositol phosphates and nucleotide-stimulated calcium release in wild-type osteoblasts, but not in RGS2 deficient osteoblasts. Conversely, pretreatment with ATP to activate Gq signaling suppresses PTHrP-dependent cAMP accumulation in wild type but not in RGS2 deficient osteoblasts. Further studies showed that endogenously expressed RGS2 can limit Gs signaling. Thus, these studies imply that up-regulation of RGS2 by Gq signaling desensitizes Gs signals. Noticeably, up-regulation of RGS2 contributes to cross-desensitization of Gs- and Gq-coupled signals (70). For example,

up-regulation of RGS2 by agents that activate the Gs signaling pathway, inhibits P2Y and endothelin receptor-stimulated Gq signals; conversely, Gq - mediated up-regulation of RGS2 inhibits PTH1R-stimulated Gs signals, revealing cross-talk between these pathways. Those studies demonstrate that RGS2 expression is regulated by both Gs and Gq signaling in osteoblasts.

Besides these studies, overexpression of osteoblast-specific transcription factor Runx2 directly leads to a stimulation of RGS2 promoter activity to promote RGS2 expression (34). Additionally, PGE2 rapidly and transiently increases the level of RGS2 mRNA in bone cells *in vivo* through the activation of cAMP signal transduction (33). The effect of RGS2 on osteoblasts is primarily mediated by PTH, resulting in increased osteoblast activity and inhibition of the osteoclast activation (31, 101, 102). Runx2 sensitizes cAMP-related GPCR signaling by activating Gpr30 and repressing RGS2 gene expression in osteoblasts to increase responsiveness to mitogenic signals (103). This is also in accordance with the findings from Tsingotjidou *et al.* (101). They demonstrate that RGS2 is a PTH-induced primary response gene in murine osteoblasts, and that RGS2 is induced primarily through the cAMP-PKA pathway and specifically inhibits Gq-coupled receptors.

5.2. Chondrocytes

Chondrocytes are only cells found in cartilage which produce and maintain the cartilaginous matrix, made mainly of collagen and proteoglycans. James *et al.* found that RGS2 can induce the increased growth and matrix apposition of chondrocytes (104). Additionally, overexpression of RGS2 in the chondrogenic cell line ATDC5 can accelerate chondrocytic hypertrophic differentiation (104), as well as advance production of glycosaminoglycans and ALP, and induce parallel increases in the expression of the chondrogenic marker genes *Fgfr3* and *Ibsp* (104), indicating RGS proteins are also involved in chondrocytic differentiation and function. It has been known that RGS proteins are closely involved in CaR regulation. Some studies have shown that deletion of CaR in chondrocytes (cartilage-producing cells) results in mouse lethality before embryonic day 13 (E13). Mice in which chondrocyte-specific deletion of CaR is induced between E16 and E18, are viable, but show the delayed growth plate development (105). This study raises further question about whether RGS proteins are involved in this CaR regulation during chondrocytic differentiation and function.

5.3. Osteoclasts

Osteoclasts are bone-resorbing cells by removing bone mineralized matrix and breaking up the organic bone. Those cells exert an important impact on skeletal metabolism. Disorders of skeletal insufficiency, such as osteoporosis, are characterized by enhanced osteoclastic bone resorption relative to bone formation. In recent years, significant progress in the mechanisms of osteoclast formation and activation, have been made, however, how GPCR/G proteins and RGS proteins regulate osteoclasts is largely unknown. Additionally, although numerous kinds of G-protein-coupled receptors and effectors such as calcitonin receptor, CaR, relaxin family peptide 1, GPR55 and cannabinoid receptor type 1 (36) have been identified in osteoclasts, the regulation of RGS protein has been relatively neglected. Calcium is an important signal for osteoclast motility downstream of tyrosine kinase signals (106). Osteoclasts, as calcium-mobilizing cells, have an active calcium regulatory system, including a membrane Ca^{2+} -ATPase (107) and an endoplasmic Ca^{2+} -ATPase (108). The basolateral membranes of osteoclasts are quite sensitive to elevated Ca^{2+} ; cytosolic Ca^{2+} rises, with cell-matrix detachment and cessation of bone resorption (109). Takayanagi *et al.* (89) found that NFATc1, a member of the NFAT (nuclear factor of activated T cells) family of transcription factor genes, is the most strongly induced transcription factor gene following RANKL stimulation, and that RANKL stimulation results in the induction of Ca^{2+} .

oscillation and contributes to the sustained activation of NFATc1 (89). The NFATc1 expression is dependent on both the TRAF6 and c-Fos pathways (89). Our studies (92, 110) as described previously, revealed that RGS10 and RGS12 are both essential for the terminal differentiation of osteoclasts induced by RANKL. Interestingly, recent study showed that RGS18 also play an important role in osteoclast differentiation (111). Here, we review their function and regulatory mechanism in following sections.

5.3.1. RGS10—Human RGS10 was first characterized by Hunt *et al.* (112), as a member of the RGS protein family. They found that RGS10 associates with the activated forms of two related G-protein subunits, G_{i3} , and G_{az} , but fails to interact with G_{as} subunit. The mRGS10 gene is expressed predominantly in brain and testis (113, 114). It also expressed in atrial myocytes (115), B lymphocytes (114, 116), and human monocyte-derived dendritic cells (29). By differential screening of a human osteoclastoma cDNA library, we found that the RGS10A isoform, but not the RGS10B isoform, is specifically expressed in human osteoclasts. The expression of RGS10A is also induced by RANKL in osteoclast precursors and is prominently expressed in mouse osteoclast-like cells. *RGS10A* silencing by RNA interference blocks intracellular Ca^{2+} oscillations and osteoclast differentiation. Reintroduction of *RGS10A* rescues the impaired osteoclast differentiation. *RGS10A* silencing also causes premature osteoclast apoptosis. *RGS10A* silencing affected the RANKL- Ca^{2+} oscillation-NFATc1 signaling pathway (92). To further understand the function of RGS10 *in vivo*, we generated RGS10-deficient (RGS10^{-/-}) mice. Our result showed that RGS10^{-/-} mice exhibit severe osteopetrosis and impaired osteoclast differentiation, which cannot be rescued by RGS12 (93). Ectopic expression of RGS10 dramatically increases the sensitivity of osteoclast differentiation to RANKL signaling. Deficiency of RGS10 results in the absence of Ca^{2+} oscillations and NFATc1. Ectopic NFATc1 expression rescues impaired osteoclast differentiation from deletion of RGS10. We further found that RGS10 competitively interacts with Ca^{2+} /calmodulin and PIP3 in a Ca^{2+} -dependent manner to regulate Ca^{2+} oscillations. Thus, our results reveal a mechanism by which RGS10 specifically regulates the RANKL-evoked RGS10/calmodulin- Ca^{2+} oscillation-calcineurin-NFATc1 signaling pathway in osteoclast differentiation.

5.3.2. RGS12—RGS12 is the largest protein in the RGS protein family based on its protein molecular weight. It was first identified by Snow *et al* (117). *RGS12* mRNA is expressed in rat spleen, lung, prostate, testis, ovary, kidney and brain (117). The expression of RGS12 is also detected at different embryonic stages during mouse development (118). Due to its multi-domain architecture, RGS12 protein has the potential to regulate multiple signaling pathway components. It contains a RGS domain, which is responsible for GAP activity (119), and another $G\alpha$ -interaction region, the GoLoco motif, which has guanine nucleotide dissociation inhibitor (GDI) activity toward G_{i1} subunits (120, 121). RGS12 also has a pair of Ras-binding domains (RBDs) (122), suggesting that RGS12 may integrate signaling pathways involving both heterotrimeric and monomeric G-proteins. The long RGS12 splice variant has an N-terminal PDZ (PSD-95/Dlg/ZO-1) domain capable of binding the interleukin-8 receptor B (CXCR2) or its own C-terminal (119) and a phosphotyrosine-binding (PTB) domain that associates with tyrosine-phosphorylated N-type calcium channel (123). Several lines of evidences showed that cytosolic Ca^{2+} oscillations are generated mainly by influx of extracellular Ca^{2+} through multiple channels, which include L- and N-type channels, and Ca^{2+} influx is necessary for maintenance of oscillations (123–126). Additionally RGS12 is capable of direct interaction with the N-type calcium channel through its PTB domain and modulates channel activity directly (123, 125, 126). Interestingly, by analyzing RGS12 gene expression pattern, we found that RGS12 is also prominently expressed in RANKL induced osteoclasts. Silence of RGS12 expression using RNA interference impairs phosphorylation of PLC γ and blocks Ca^{2+} oscillations, NFATc1

expression, and osteoclast differentiation. We further found that N-type calcium channels are expressed in RANKL induced osteoclasts and that RGS12 directly interacts with the N-type calcium channels. Compared to RGS10 (93), RGS12 does not bind with Ca^{2+} /calmodulin and PIP3 in a Ca^{2+} -dependent manner during osteoclast differentiation. Instead of these bindings, it acts on N-type Ca^{2+} channels and CaR. Most recently, by specific deletion of RGS12 in osteoclast lineage by breeding RGS12^{lox/lox} mice with CD11b-cre and Mx1-cre also cause osteopetrosis phenotype and impaired Ca^{2+} oscillations, which cannot be rescued by RGS10 (Manuscripts submitted). We also found that overexpression of RGS12 PTB domain dramatically promotes osteoclast differentiation. Those results demonstrate that RGS10 and RGS12 proteins play different roles in regulating Ca^{2+} oscillations and osteoclast differentiation. Our data support that RGS12 regulates osteoclast differentiation through calcium channel- Ca^{2+} oscillation-NFATc1 pathway.

5.3.3. RGS18—Another RGS protein, RGS18, has been reported to play an important role in RANKL-induced osteoclast differentiation as a negative regulator (127). Iwai *et al.* analyzed RGS18 expression in the macrophage/monocyte lineage cell line and the primary osteoclast precursor monocytes derived from mouse bone marrow, and found that both cell lines express mRNA for 10 different mammalian RGS, including RGS18. Interestingly, they found that expression of RGS18 is down-regulated by RANKL. Silence of RGS18 using RNA interference prominently promotes RANKL induced osteoclastogenesis. Without RANKL stimulation, treatment with RGS18 siRNA does not induce osteoclast formation, suggesting that RGS18 absence is not sufficient to stimulate osteoclastogenesis. Blocking of an ovarian cancer G-protein-coupled receptor (OGR1), they found, can reverse the effect of RGS18 inhibition for osteoclastogenesis. Meanwhile, RGS18 is not involved in control of Gi signaling. Hence, they suggest that RGS18-targeted inhibition is dependent on Gq-protein signaling pathway via OGR1. Further study showed that overexpression of exogenous RGS18 inhibits the acidosis-induced NFATc1 activation (127). Thus, the authors conclude that RGS18 acts as a negative regulator of the acidosis-induced osteoclastogenic OGR1/NFAT signaling pathway, and RANKL stimulates osteoclastogenesis by inhibiting RGS18 expression.

6. SUMMARY AND PERSPECTIVES

The importance of GPCR and G protein signaling for determining bone cell growth and differentiation has been recognized for many years (129). After their discovery in the mid-1990s, RGSs were quickly appreciated as key players in the regulation of GPCR/G protein signaling. Many studies have been performed in osteoblasts, osteoclasts, as well as chondrocytes, each focusing on one or a few RGS proteins (Table 2). They have provided a wealth of information regarding the function and mechanisms of RGSs as modulators and integrators for GPCR and G-protein signaling as we described above and in Figure 1. Those studies not only enhance the understanding of RGS proteins in bone biology; but also open up a new approach to targeting RGS proteins in drug discovery. As more reports are being published on the function of this group of proteins, increasing attention would focus on RGS proteins as exciting new candidates for therapeutic intervention and drug development.

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Abbreviations

CaM	calmodulin
CaR	calcium-sensing receptor
CB1	cannabinoid receptor type1
CT	calcitonin receptor
ERK	extracellular regulated kinases
GPCR	G protein coupling receptors
GAPs	GTPase activating proteins
IFN β	interferon- β
IP₃	inositol trisphosphate
M-CSF	macrophage colony stimulating factor
NFAT	nuclear factor of activated T-cells
OGR1	ovarian cancer G-protein–coupled receptor 1
PGE₂	prostaglandin E ₂
PKA	protein kinase A
PKC	protein kinase C
PLC	phospholipase C
PMCA	plasma membrane Ca ²⁺ ATPase
PTH	parathyroid hormone
PTHrP	parathyroid hormone receptor
PTHrP	parathyroid hormone-related peptide
RANKL	receptor activator of nuclear factor kappa-B ligand
RGS	regulators of G protein signaling
PTX	pertussis toxin
SERCA	sarco/endoplasmic reticulum Ca ²⁺ ATPase
TSH	thyrotropin

REFERENCES

1. Boyce BF, Rosenberg E, de Papp AE, Duong le T. The osteoclast, bone remodelling and treatment of metabolic bone disease. *Eur J Clin Invest.* 2012; 42(12):1332–1341. [PubMed: 22998735]
2. Kimple AJ, Bosch DE, Giguere PM, Siderovski DP. Regulators of G-protein signaling and their G α substrates: promises and challenges in their use as drug discovery targets. *Pharmacol Rev.* 2011; 63(3):728–749. [PubMed: 21737532]
3. Gensure RC, Gardella TJ, Juppner H. Parathyroid hormone and parathyroid hormone-related peptide, and their receptors. *Biochem Biophys Res Commun.* 2005; 328(3):666–678. [PubMed: 15694400]
4. Kamenetsky M, Middelhaufe S, Bank EM, Levin LR, Buck J, Steegborn C. Molecular details of cAMP generation in mammalian cells: a tale of two systems. *J Mol Biol.* 2006; 362(4):623–639. [PubMed: 16934836]
5. McCudden CR, Hains MD, Kimple RJ, Siderovski DP, Willard FS. G-protein signaling: back to the future. *Cell Mol Life Sci.* 2005; 62(5):551–577. [PubMed: 15747061]

6. Morris AJ, Malbon CC. Physiological regulation of G protein-linked signaling. *Physiol Rev.* 1999; 79(4):1373–1430. [PubMed: 10508237]
7. Doupnik CA, Davidson N, Lester HA, Kofuji P. RGS proteins reconstitute the rapid gating kinetics of gbetagamma-activated inwardly rectifying K⁺ channels. *Proc Natl Acad Sci U S A.* 1997; 94(19):10461–10466. [PubMed: 9294233]
8. Ross EM, Wilkie TM. GTPase-activating proteins for heterotrimeric G proteins: regulators of G protein signaling (RGS) and RGS-like proteins. *Annu Rev Biochem.* 2000; 69:795–827. [PubMed: 10966476]
9. Hollinger S, Hepler JR. Cellular regulation of RGS proteins: modulators and integrators of G protein signaling. *Pharmacol Rev.* 2002; 54(3):527–559. [PubMed: 12223533]
10. Zheng B, De Vries L, Gist Farquhar M. Divergence of RGS proteins: evidence for the existence of six mammalian RGS subfamilies. *Trends Biochem Sci.* 1999; 24(11):411–414. [PubMed: 10542401]
11. Xie GX, Palmer PP. How regulators of G protein signaling achieve selective regulation. *J Mol Biol.* 2007; 366(2):349–365. [PubMed: 17173929]
12. Terzi D, Stergiou E, King SL, Zachariou V. Regulators of G protein signaling in neuropsychiatric disorders. *Prog Mol Biol Transl Sci.* 2009; 86:299–333. [PubMed: 20374720]
13. Traynor J. mu-Opioid receptors and regulators of G protein signaling (RGS) proteins: from a symposium on new concepts in mu-opioid pharmacology. *Drug Alcohol Depend.* 2012; 121(3):173–180. [PubMed: 22129844]
14. Zhang P, Mende U. Regulators of G-protein signaling in the heart and their potential as therapeutic targets. *Circ Res.* 2011; 109(3):320–333. [PubMed: 21778436]
15. Wieland T, Lutz S, Chidiac P. Regulators of G protein signalling: a spotlight on emerging functions in the cardiovascular system. *Curr Opin Pharmacol.* 2007; 7(2):201–207. [PubMed: 17276730]
16. Cho H, Kamenyeva O, Yung S, Gao JL, Hwang IY, Park C, Murphy PM, Neubig RR, Kehrl JH. The loss of RGS protein-Galpha(i2) interactions results in markedly impaired mouse neutrophil trafficking to inflammatory sites. *Mol Cell Biol.* 2012; 32(22):4561–4571. [PubMed: 22966200]
17. Druey KM. Regulation of G-protein-coupled signaling pathways in allergic inflammation. *Immunol Res.* 2009; 43(1–3):62–76. [PubMed: 18810336]
18. Hurst JH, Hooks SB. Regulator of G-protein signaling (RGS) proteins in cancer biology. *Biochem Pharmacol.* 2009; 78(10):1289–1297. [PubMed: 19559677]
19. Kach J, Sethakorn N, Dulin NO. A finer tuning of G-protein signaling through regulated control of RGS proteins. *Am J Physiol Heart Circ Physiol.* 2012; 303(1):H19–H35. [PubMed: 22542620]
20. Zhang P, Su J, King ME, Maldonado AE, Park C, Mende U. Regulator of G protein signaling 2 is a functionally important negative regulator of angiotensin II-induced cardiac fibroblast responses. *Am J Physiol Heart Circ Physiol.* 2011; 301(1):H147–H156. [PubMed: 21498776]
21. Wang Q, Traynor JR. Opioid-induced down-regulation of RGS4: role of ubiquitination and implications for receptor cross-talk. *J Biol Chem.* 2011; 286(10):7854–7864. [PubMed: 21209077]
22. Cho H, Harrison K, Schwartz O, Kehrl JH. The aorta and heart differentially express RGS (regulators of G-protein signalling) proteins that selectively regulate sphingosine 1-phosphate, angiotensin II and endothelin-1 signalling. *Biochem J.* 2003; 371(Pt 3):973–980. [PubMed: 12564955]
23. Nakagawa T, Minami M, Satoh M. Up-regulation of RGS4 mRNA by opioid receptor agonists in PC12 cells expressing cloned mu- or kappa-opioid receptors. *Eur J Pharmacol.* 2001; 433(1):29–36. [PubMed: 11755131]
24. Yang Z, Cooper PR, Damera G, Mukhopadhyay I, Cho H, Kehrl JH, Panettieri RA Jr, Druey KM. Beta-agonist-associated reduction in RGS5 expression promotes airway smooth muscle hyper-responsiveness. *J Biol Chem.* 2011; 286(13):11444–11455. [PubMed: 21278382]
25. Beadling C, Druey KM, Richter G, Kehrl JH, Smith KA. Regulators of G protein signaling exhibit distinct patterns of gene expression and target G protein specificity in human lymphocytes. *J Immunol.* 1999; 162(5):2677–2682. [PubMed: 10072511]
26. Xie Z, Yang Z, Druey KM. Phosphorylation of RGS13 by the cyclic AMP-dependent protein kinase inhibits RGS13 degradation. *J Mol Cell Biol.* 2010; 2(6):357–365. [PubMed: 20974683]

27. Riekenberg S, Farhat K, Debarry J, Heine H, Jung G, Wiesmuller KH, Ulmer AJ. Regulators of G-protein signalling are modulated by bacterial lipopeptides and lipopolysaccharide. *FEBS J.* 2009; 276(3):649–659. [PubMed: 19120454]
28. Lee HK, Yeo S, Kim JS, Lee JG, Bae YS, Lee C, Baek SH. Protein kinase C-eta and phospholipase D2 pathway regulates foam cell formation via regulator of G protein signaling 2. *Mol Pharmacol.* 2010; 78(3):478–485. [PubMed: 20558593]
29. Shi GX, Harrison K, Han SB, Moratz C, Kehrl JH. Toll-like receptor signaling alters the expression of regulator of G protein signaling proteins in dendritic cells: implications for G protein-coupled receptor signaling. *J Immunol.* 2004; 172(9):5175–5184. [PubMed: 15100254]
30. Tran T, Paz P, Velichko S, Cifrese J, Belur P, Yamaguchi KD, Ku K, Mirshahpanah P, Reder AT, Croze E. Interferonbeta-1b Induces the Expression of RGS1 a Negative Regulator of G-Protein Signaling. *Int J Cell Biol.* 2010; 2010 529376.
31. Ueno Y, Shinki T, Nagai Y, Murayama H, Fujii K, Suda T. In vivo administration of 1,25-dihydroxyvitamin D3 suppresses the expression of RANKL mRNA in bone of thyroparathyroidectomized rats constantly infused with PTH. *J Cell Biochem.* 2003; 90(2):267–277. [PubMed: 14505343]
32. Giorelli M, Livrea P, Defazio G, Iacovelli L, Capobianco L, Picascia A, Salles M, Martino D, Aniello MS, Trojano M, De Blasi A. Interferon beta-1a counteracts effects of activation on the expression of G-protein-coupled receptor kinases 2 and 3, beta-arrestin-1 and regulators of G-protein signalling 2 and 16 in human mononuclear leukocytes. *Cell Signal.* 2002; 14(8):673–678. [PubMed: 12020767]
33. Miles RR, Sluka JP, Santerre RF, Hale LV, Bloem L, Boguslawski G, Thirunavukkarasu K, Hock JM, Onyia JE. Dynamic regulation of RGS2 in bone: potential new insights into parathyroid hormone signaling mechanisms. *Endocrinology.* 2000; 141(1):28–36. [PubMed: 10614620]
34. Thirunavukkarasu K, Halladay DL, Miles RR, Geringer CD, Onyia JE. Analysis of regulator of G-protein signaling-2 (RGS-2) expression and function in osteoblastic cells. *J Cell Biochem.* 2002; 85(4):837–850. [PubMed: 11968023]
35. Eszlinger M, Holzapfel HP, Voigt C, Arkenau C, Paschke R. RGS 2 expression is regulated by TSH and inhibits TSH receptor signaling. *Eur J Endocrinol.* 2004; 151(3):383–390. [PubMed: 15362969]
36. Wu M, Deng L, Zhu G, Li YP. G Protein and its signaling pathway in bone development and disease. *Front Biosci.* 2010; 15:957–985.
37. Oldham WM, Hamm HE. Heterotrimeric G protein activation by G-protein-coupled receptors. *Nat Rev Mol Cell Biol.* 2008; 9(1):60–71. [PubMed: 18043707]
38. De Vries L, Zheng B, Fischer T, Elenko E, Farquhar MG. The regulator of G protein signaling family. *Annu Rev Pharmacol Toxicol.* 2000; 40:235–271. [PubMed: 10836135]
39. Willard MD, Willard FS, Li X, Cappell SD, Snider WD, Siderovski DP. Selective role for RGS12 as a Ras/Raf/MEK scaffold in nerve growth factor-mediated differentiation. *EMBO J.* 2007; 26(8):2029–2040. [PubMed: 17380122]
40. Zhong H, Wade SM, Woolf PJ, Linderman JJ, Traynor JR, Neubig RR. A spatial focusing model for G protein signals. Regulator of G protein signaling (RGS) protein-mediated kinetic scaffolding. *J Biol Chem.* 2003; 278(9):7278–7284. [PubMed: 12446706]
41. Leone AM, Errico M, Lin SL, Cowen DS. Activation of extracellular signal-regulated kinase (ERK) and Akt by human serotonin 5-HT(1B) receptors in transfected BE(2)-C neuroblastoma cells is inhibited by RGS4. *J Neurochem.* 2000; 75(3):934–938. [PubMed: 10936173]
42. Anger T, Klintworth N, Stumpf C, Daniel WG, Mende U, Garlich CD. RGS protein specificity towards Gq-, Gi/o-mediated ERK 1/2 Akt activation, in vitro. *J Biochem Mol Biol.* 2007; 40(6):899–910. [PubMed: 18047785]
43. Klein RF, Nissenson RA, Stewler GJ. Pertussis toxin inhibits hormonal stimulation of bone resorption in fetal rat limb bones. *J Pharmacol Exp Ther.* 1991; 258(3):877–881. [PubMed: 1653845]
44. Roman-Roman S, Shi DL, Stiot V, Hay E, Vayssiere B, Garcia T, Baron R, Rawadi G. Murine Frizzled-1 behaves as an antagonist of the canonical Wnt/beta-catenin signaling. *J Biol Chem.* 2004; 279(7):5725–5733. [PubMed: 14627707]

45. Berstein G, Blank JL, Jhon DY, Exton JH, Rhee SG, Ross EM. Phospholipase C-beta 1 is a GTPase-activating protein for Gq/11, its physiologic regulator. *Cell*. 1992; 70(3):411–418. [PubMed: 1322796]
46. Sakamoto A, Chen M, Nakamura T, Xie T, Karsenty G, Weinstein LS. Deficiency of the G-protein alpha-subunit G(s)alpha in osteoblasts leads to differential effects on trabecular and cortical bone. *J Biol Chem*. 2005; 280(22):21369–21375. [PubMed: 15797856]
47. Luttrell LM. Reviews in molecular biology and biotechnology: transmembrane signaling by G protein-coupled receptors. *Mol Biotechnol*. 2008; 39(3):239–264. [PubMed: 18240029]
48. Peng J, Bencsik M, Louie A, Lu W, Millard S, Nguyen P, Burghardt A, Majumdar S, Wronski TJ, Halloran B, Conklin BR, Nissenson RA. Conditional expression of a Gi-coupled receptor in osteoblasts results in trabecular osteopenia. *Endocrinology*. 2008; 149(3):1329–1337. [PubMed: 18048501]
49. Millard SM, Louie AM, Wattanachanya L, Wronski TJ, Conklin BR, Nissenson RA. Blockade of receptor-activated G(i) signaling in osteoblasts in vivo leads to site-specific increases in cortical and cancellous bone formation. *J Bone Miner Res*. 2011; 26(4):822–832. [PubMed: 20939063]
50. Yan Y, Tang D, Chen M, Huang J, Xie R, Jonason JH, Tan X, Hou W, Reynolds D, Hsu W, Harris SE, Puzas JE, Awad H, O'Keefe RJ, Boyce BF, Chen D. Axin2 controls bone remodeling through the beta-catenin-BMP signaling pathway in adult mice. *J Cell Sci*. 2009; 122(Pt 19):3566–3578. [PubMed: 19737815]
51. Gwak J, Hwang SG, Park HS, Choi SR, Park SH, Kim H, Ha NC, Bae SJ, Han JK, Kim DE, Cho JW, Oh S. Small molecule-based disruption of the Axin/beta-catenin protein complex regulates mesenchymal stem cell differentiation. *Cell Res*. 2012; 22(1):237–247. [PubMed: 21826110]
52. Minear S, Leucht P, Jiang J, Liu B, Zeng A, Fuerer C, Nusse R, Helms JA. Wnt proteins promote bone regeneration. *Sci Transl Med*. 2010; 2(29) 29ra30.
53. Yu HM, Jerchow B, Sheu TJ, Liu B, Costantini F, Puzas JE, Birchmeier W, Hsu W. The role of Axin2 in calvarial morphogenesis and craniosynostosis. *Development*. 2005; 132(8):1995–2005. [PubMed: 15790973]
54. Salahshor S, Woodgett JR. The links between axin and carcinogenesis. *J Clin Pathol*. 2005; 58(3): 225–236. [PubMed: 15735151]
55. Lh H, Li JY, Zhu B, Zhou XD. [Study of antibacterial effect of *Galla Chinensis* on the bacteria biofilm in chemostat]. *Zhongguo Zhong Yao Za Zhi*. 2005; 30(21):1685–1688. [PubMed: 16400949]
56. Westendorf JJ, Kahler RA, Schroeder TM. Wnt signaling in osteoblasts and bone diseases. *Gene*. 2004; 341:19–39. [PubMed: 15474285]
57. Hay E, Laplantine E, Geoffroy V, Frain M, Kohler T, Muller R, Marie PJ. N-cadherin interacts with axin and LRP5 to negatively regulate Wnt/beta-catenin signaling, osteoblast function, and bone formation. *Mol Cell Biol*. 2009; 29(4):953–964. [PubMed: 19075000]
58. Regard JB, Cherman N, Palmer D, Kuznetsov SA, Celi FS, Guettier JM, Chen M, Bhattacharyya N, Wess J, Coughlin SR, Weinstein LS, Collins MT, Robey PG, Yang Y. Wnt/beta-catenin signaling is differentially regulated by Galpha proteins and contributes to fibrous dysplasia. *Proc Natl Acad Sci U S A*. 2011; 108(50):20101–20106. [PubMed: 22106277]
59. Wang L, Gesty-Palmer D, Fields TA, Spurney RF. Inhibition of WNT signaling by G protein-coupled receptor (GPCR) kinase 2 (GRK2). *Mol Endocrinol*. 2009; 23(9):1455–1465. [PubMed: 19556343]
60. Uzawa T, Hori M, Ejiri S, Ozawa H. Comparison of the effects of intermittent and continuous administration of human parathyroid hormone(1–34) on rat bone. *Bone*. 1995; 16(4):477–484. [PubMed: 7605709]
61. Tam CS, Heersche JN, Murray TM, Parsons JA. Parathyroid hormone stimulates the bone apposition rate independently of its resorptive action: differential effects of intermittent and continuous administration. *Endocrinology*. 1982; 110(2):506–512. [PubMed: 7056211]
62. Lee K, Deeds JD, Segre GV. Expression of parathyroid hormone-related peptide and its receptor messenger ribonucleic acids during fetal development of rats. *Endocrinology*. 1995; 136(2):453–463. [PubMed: 7835276]

63. Segre GV, Deeds JD, Lee K. Expression of parathyroid hormone-related peptide and its receptor mRNAs during fetal development of rats. *Miner Electrolyte Metab.* 1995; 21(1–3):129–132. [PubMed: 7565433]
64. Clapham DE, Neer EJ. New roles for G-protein beta gamma-dimers in transmembrane signalling. *Nature.* 1993; 365(6445):403–406. [PubMed: 8413584]
65. Hamm HE. The many faces of G protein signaling. *J Biol Chem.* 1998; 273(2):669–672. [PubMed: 9422713]
66. Ko JK, Choi KH, Kim IS, Jung EK, Park DH. Inducible RGS2 is a cross-talk regulator for parathyroid hormone signaling in rat osteoblast-like UMR106 cells. *Biochem Biophys Res Commun.* 2001; 287(4):1025–1033. [PubMed: 11573967]
67. Abou-Samra AB, Juppner H, Force T, Freeman MW, Kong XF, Schipani E, Urena P, Richards J, Bonventre JV, Potts JT Jr. et al. Expression cloning of a common receptor for parathyroid hormone and parathyroid hormone-related peptide from rat osteoblast-like cells: a single receptor stimulates intracellular accumulation of both cAMP and inositol trisphosphates and increases intracellular free calcium. *Proc Natl Acad Sci U S A.* 1992; 89(7):2732–2736. [PubMed: 1313566]
68. Silve CM, Hradek GT, Jones AL, Arnaud CD. Parathyroid hormone receptor in intact embryonic chicken bone: characterization and cellular localization. *J Cell Biol.* 1982; 94(2):379–386. [PubMed: 6286691]
69. Homme M, Schaefer F, Mehls O, Schmitt CP. Differential regulation of RGS-2 by constant and oscillating PTH concentrations. *Calcif Tissue Int.* 2009; 84(4):305–312. [PubMed: 19225708]
70. Roy AA, Nunn C, Ming H, Zou MX, Penninger J, Kirshenbaum LA, Dixon SJ, Chidiac P. Up-regulation of endogenous RGS2 mediates cross-desensitization between Gs and Gq signaling in osteoblasts. *J Biol Chem.* 2006; 281(43):32684–32693. [PubMed: 16950788]
71. Homme M, Schmitt CP, Himmele R, Hoffmann GF, Mehls O, Schaefer F. Vitamin D and dexamethasone inversely regulate parathyroid hormone-induced regulator of G protein signaling-2 expression in osteoblast-like cells. *Endocrinology.* 2003; 144(6):2496–2504. [PubMed: 12746312]
72. Brown EM. Four-parameter model of the sigmoidal relationship between parathyroid hormone release and extracellular calcium concentration in normal and abnormal parathyroid tissue. *J Clin Endocrinol Metab.* 1983; 56(3):572–581. [PubMed: 6822654]
73. Brown EM. Mechanisms underlying the regulation of parathyroid hormone secretion in vivo and in vitro. *Curr Opin Nephrol Hypertens.* 1993; 2(4):541–551. [PubMed: 7859016]
74. Koh J, Dar M, Untch BR, Dixit D, Shi Y, Yang Z, Adam MA, Dressman H, Wang X, Gesty-Palmer D, Marks JR, Spurney R, Druey KM, Olson JA Jr. Regulator of G protein signaling 5 is highly expressed in parathyroid tumors and inhibits signaling by the calcium-sensing receptor. *Mol Endocrinol.* 2011; 25(5):867–876. [PubMed: 21393447]
75. Miao D, Tong XK, Chan GK, Panda D, McPherson PS, Goltzman D. Parathyroid hormone-related peptide stimulates osteogenic cell proliferation through protein kinase C activation of the Ras/mitogen-activated protein kinase signaling pathway. *J Biol Chem.* 2001; 276(34):32204–32213. [PubMed: 11402023]
76. Zeng W, Xu X, Popov S, Mukhopadhyay S, Chidiac P, Swistok J, Danho W, Yagaloff KA, Fisher SL, Ross EM, Muallem S, Wilkie TM. The N-terminal domain of RGS4 confers receptor-selective inhibition of G protein signaling. *J Biol Chem.* 1998; 273(52):34687–34690. [PubMed: 9856989]
77. Taylor CW, Thorn P. Calcium signalling: IP3 rises again...and again. *Curr Biol.* 2001; 11(9):R352–R355. [PubMed: 11369246]
78. Ishii M, Kurachi Y. Physiological actions of regulators of G-protein signaling (RGS) proteins. *Life Sci.* 2003; 74(2–3):163–171. [PubMed: 14607243]
79. Ishii M, Fujita S, Yamada M, Hosaka Y, Kurachi Y. Phosphatidylinositol 3,4,5-trisphosphate and Ca²⁺/calmodulin competitively bind to the regulators of G-protein-signalling (RGS) domain of RGS4 and reciprocally regulate its action. *Biochem J.* 2005; 385(Pt 1):65–73. [PubMed: 15324308]
80. Xu X, Zeng W, Popov S, Berman DM, Davignon I, Yu K, Yowe D, Offermanns S, Muallem S, Wilkie TM. RGS proteins determine signaling specificity of Gq-coupled receptors. *J Biol Chem.* 1999; 274(6):3549–3556. [PubMed: 9920901]

81. Dolmetsch RE, Lewis RS. Signaling between intracellular Ca^{2+} stores and depletion-activated Ca^{2+} channels generates $[\text{Ca}^{2+}]_i$ oscillations in T lymphocytes. *J Gen Physiol.* 1994; 103(3):365–388. [PubMed: 8195779]
82. Dolmetsch RE, Xu K, Lewis RS. Calcium oscillations increase the efficiency and specificity of gene expression. *Nature.* 1998; 392(6679):933–936. [PubMed: 9582075]
83. Fields RD, Eshete F, Stevens B, Itoh K. Action potential-dependent regulation of gene expression: temporal specificity in Ca^{2+} , cAMP-responsive element binding proteins, and mitogen-activated protein kinase signaling. *J Neurosci.* 1997; 17(19):7252–7266. [PubMed: 9295372]
84. Gu X, Spitzer NC. Distinct aspects of neuronal differentiation encoded by frequency of spontaneous Ca^{2+} transients. *Nature.* 1995; 375(6534):784–787. [PubMed: 7596410]
85. Kiselyov K, Shin DM, Muallem S. Signalling specificity in GPCR-dependent Ca^{2+} signalling. *Cell Signal.* 2003; 15(3):243–253. [PubMed: 12531423]
86. Wang X, Huang G, Luo X, Penninger JM, Muallem S. Role of regulator of G protein signaling 2 (RGS2) in Ca^{2+} oscillations and adaptation of Ca^{2+} signaling to reduce excitability of RGS2–/– cells. *J Biol Chem.* 2004; 279(40):41642–41649. [PubMed: 15292238]
87. Luo X, Popov S, Bera AK, Wilkie TM, Muallem S. RGS proteins provide biochemical control of agonist-evoked $[\text{Ca}^{2+}]_i$ oscillations. *Mol Cell.* 2001; 7(3):651–660. [PubMed: 11463389]
88. Muallem S, Wilkie TM. G protein-dependent Ca^{2+} signaling complexes in polarized cells. *Cell Calcium.* 1999; 26(5):173–180. [PubMed: 10643555]
89. Takayanagi H, Kim S, Koga T, Nishina H, Isshiki M, Yoshida H, Saiura A, Isobe M, Yokochi T, Inoue J, Wagner EF, Mak TW, Kodama T, Taniguchi T. Induction and activation of the transcription factor NFATc1 (NFAT2) integrate RANKL signaling in terminal differentiation of osteoclasts. *Dev Cell.* 2002; 3(6):889–901. [PubMed: 12479813]
90. Crabtree GR, Olson EN. NFAT signaling: choreographing the social lives of cells. *Cell.* 2002; (109 Suppl):S67–S79. [PubMed: 11983154]
91. Koga T, Inui M, Inoue K, Kim S, Suematsu A, Kobayashi E, Iwata T, Ohnishi H, Matozaki T, Kodama T, Taniguchi T, Takayanagi H, Takai T. Costimulatory signals mediated by the ITAM motif cooperate with RANKL for bone homeostasis. *Nature.* 2004; 428(6984):758–763. [PubMed: 15085135]
92. Yang S, Chen W, Stashenko P, Li YP. Specificity of RGS10A as a key component in the RANKL signaling mechanism for osteoclast differentiation. *J Cell Sci.* 2007; 120(Pt 19):3362–3371. [PubMed: 17881498]
93. Yang S, Li YP. RGS10-null mutation impairs osteoclast differentiation resulting from the loss of $[\text{Ca}^{2+}]_i$ oscillation regulation. *Genes Dev.* 2007; 21(14):1803–1816. [PubMed: 17626792]
94. Yang S, Li YP. RGS12 is essential for RANKL-evoked signaling for terminal differentiation of osteoclasts in vitro. *J Bone Miner Res.* 2007; 22(1):45–54. [PubMed: 17042716]
95. Roy AA, Lemberg KE, Chidiac P. Recruitment of RGS2 and RGS4 to the plasma membrane by G proteins and receptors reflects functional interactions. *Mol Pharmacol.* 2003; 64(3):587–593. [PubMed: 12920194]
96. Dvorak MM, Chen TH, Orwoll B, Garvey C, Chang W, Bikle DD, Shoback DM. Constitutive activity of the osteoblast Ca^{2+} -sensing receptor promotes loss of cancellous bone. *Endocrinology.* 2007; 148(7):3156–3163. [PubMed: 17412806]
97. Kameda T, Mano H, Yamada Y, Takai H, Amizuka N, Kobori M, Izumi N, Kawashima H, Ozawa H, Ikeda K, Kameda A, Hakeda Y, Kumegawa M. Calcium-sensing receptor in mature osteoclasts, which are bone resorbing cells. *Biochem Biophys Res Commun.* 1998; 245(2):419–422. [PubMed: 9571166]
98. Yamaguchi T, Chattopadhyay N, Kifor O, Brown EM. Extracellular calcium ($\text{Ca}^{2+}(\text{o})$)-sensing receptor in a murine bone marrow-derived stromal cell line (ST2): potential mediator of the actions of $\text{Ca}^{2+}(\text{o})$ on the function of ST2 cells. *Endocrinology.* 1998; 139(8):3561–3568. [PubMed: 9681508]
99. Chattopadhyay N, Quinn SJ, Kifor O, Ye C, Brown EM. The calcium-sensing receptor (CaR) is involved in strontium ranelate-induced osteoblast proliferation. *Biochem Pharmacol.* 2007; 74(3):438–447. [PubMed: 17531955]

100. Pi M, Faber P, Ekema G, Jackson PD, Ting A, Wang N, Fontilla-Poole M, Mays RW, Brunden KR, Harrington JJ, Quarles LD. Identification of a novel extracellular cation-sensing G-protein-coupled receptor. *J Biol Chem.* 2005; 280(48):40201–40209. [PubMed: 16199532]
101. Tsingotjidou A, Nervina JM, Pham L, Bezouglaia O, Tetradis S. Parathyroid hormone induces RGS-2 expression by a cyclic adenosine 3',5'-monophosphate-mediated pathway in primary neonatal murine osteoblasts. *Bone.* 2002; 30(5):677–684. [PubMed: 11996904]
102. Guo J, Chung UI, Kondo H, Bringham FR, Kronenberg HM. The PTH/PTHrP receptor can delay chondrocyte hypertrophy in vivo without activating phospholipase C. *Dev Cell.* 2002; 3(2):183–194. [PubMed: 12194850]
103. Teplyuk NM, Galindo M, Teplyuk VI, Pratap J, Young DW, Lapointe D, Javed A, Stein JL, Lian JB, Stein GS, van Wijnen AJ. Runx2 regulates G protein-coupled signaling pathways to control growth of osteoblast progenitors. *J Biol Chem.* 2008; 283(41):27585–27597. [PubMed: 18625716]
104. James CG, Appleton CT, Ulici V, Underhill TM, Beier F. Microarray analyses of gene expression during chondrocyte differentiation identifies novel regulators of hypertrophy. *Mol Biol Cell.* 2005; 16(11):5316–5333. [PubMed: 16135533]
105. Chang W, Tu C, Chen TH, Bikle D, Shoback D. The extracellular calcium-sensing receptor (CaSR) is a critical modulator of skeletal development. *Sci Signal.* 2008; 1(35) ra1.
106. Sanjay A, Houghton A, Neff L, DiDomenico E, Bardelay C, Antoine E, Levy J, Gailit J, Bowtell D, Horne WC, Baron R. Cbl associates with Pyk2 and Src to regulate Src kinase activity, alpha(v)beta(3) integrin-mediated signaling, cell adhesion, and osteoclast motility. *J Cell Biol.* 2001; 152(1):181–195. [PubMed: 11149930]
107. Bekker PJ, Gay CV. Characterization of a Ca2(+)-ATPase in osteoclast plasma membrane. *J Bone Miner Res.* 1990; 5(6):557–567. [PubMed: 2143347]
108. Machuca I, Domenget C, Jurdic P. Identification of avian sarcoplasmic reticulum Ca(2+)-ATPase (SERCA3) as a novel 1,25(OH)(2)D(3) target gene in the monocytic lineage. *Exp Cell Res.* 1999; 250(2):364–375. [PubMed: 10413590]
109. Zaidi M, Datta HK, Patchell A, Moonga B, MacIntyre I. 'Calcium-activated' intracellular calcium elevation: a novel mechanism of osteoclast regulation. *Biochem Biophys Res Commun.* 1989; 163(3):1461–1465. [PubMed: 2783143]
110. Yang S, Li YP. RGS12 is essential for RANKL-evoked signaling for terminal differentiation of osteoclasts in vitro. *Journal of Bone and Mineral Research.* 2007; 22(1):45–54. [PubMed: 17042716]
111. Iwai K, Koike M, Ohshima S, Miyatake K, Uchiyama Y, Saeki Y, Ishii M. RGS18 acts as a negative regulator of osteoclastogenesis by modulating the acid-sensing OGR1/NFAT signaling pathway. *Journal of Bone and Mineral Research.* 2007; 22(10):1612–1620. [PubMed: 17576169]
112. Hunt TW, Fields TA, Casey PJ, Peralta EG. RGS10 is a selective activator of G alpha i GTPase activity. *Nature.* 1996; 383(6596):175–177. [PubMed: 8774883]
113. Gold SJ, Ni YG, Dohlman HG, Nestler EJ. Regulators of G-protein signaling (RGS) proteins: region-specific expression of nine subtypes in rat brain. *J Neurosci.* 1997; 17(20):8024–8037. [PubMed: 9315921]
114. Haller C, Fillatreau S, Hoffmann R, Agenes F. Structure, chromosomal localization and expression of the mouse regulator of G-protein signaling10 gene (mRGS10). *Gene.* 2002; 297(1–2):39–49. [PubMed: 12384284]
115. Doupnik CA, Xu T, Shinaman JM. Profile of RGS expression in single rat atrial myocytes. *Biochim Biophys Acta.* 2001; 1522(2):97–107. [PubMed: 11750060]
116. Moratz C, Harrison K, Kehrl JH. Regulation of chemokine-induced lymphocyte migration by RGS proteins. *Methods Enzymol.* 2004; 389:15–32. [PubMed: 15313557]
117. Snow BE, Antonio L, Suggs S, Gutstein HB, Siderovski DP. Molecular cloning and expression analysis of rat Rgs12 and Rgs14. *Biochem Biophys Res Commun.* 1997; 233(3):770–777. [PubMed: 9168931]
118. Martin-McCaffrey L, Hains MD, Pritchard GA, Pajak A, Dagnino L, Siderovski DP, D'Souza SJ. Differential expression of regulator of G-protein signaling R12 subfamily members during mouse development. *Dev Dyn.* 2005; 234(2):438–444. [PubMed: 16145674]

119. Snow BE, Hall RA, Krumins AM, Brothers GM, Bouchard D, Brothers CA, Chung S, Mangion J, Gilman AG, Lefkowitz RJ, Siderovski DP. GTPase activating specificity of RGS12 and binding specificity of an alternatively spliced PDZ (PSD-95/Dlg/ZO-1) domain. *J Biol Chem.* 1998; 273(28):17749–17755. [PubMed: 9651375]
120. Kimple RJ, Kimple ME, Betts L, Sondek J, Siderovski DP. Structural determinants for GoLoco-induced inhibition of nucleotide release by Gα subunits. *Nature.* 2002; 416(6883):878–881. [PubMed: 11976690]
121. Willard FS, Kimple RJ, Kimple AJ, Johnston CA, Siderovski DP. Fluorescence-based assays for RGS box function. *Methods Enzymol.* 2004; 389:56–71. [PubMed: 15313559]
122. Ponting CP. Raf-like Ras/Rap-binding domains in RGS12- and still-life-like signalling proteins. *J Mol Med.* 1999; 77(10):695–698. [PubMed: 10606204]
123. Schiff ML, Siderovski DP, Jordan JD, Brothers G, Snow B, De Vries L, Ortiz DF, Diverse-Pierluissi M. Tyrosine-kinase-dependent recruitment of RGS12 to the N-type calcium channel. *Nature.* 2000; 408(6813):723–727. [PubMed: 11130074]
124. Anantharam A, Diverse-Pierluissi MA. Biochemical approaches to study interaction of calcium channels with RGS12 in primary neuronal cultures. *Methods Enzymol.* 2002; 345:60–70. [PubMed: 11665642]
125. Richman RW, Diverse-Pierluissi MA. Mapping of RGS12-Cav2.2 channel interaction. *Methods Enzymol.* 2004; 390:224–239. [PubMed: 15488181]
126. Richman RW, Strock J, Hains MD, Cabanilla NJ, Lau KK, Siderovski DP, Diverse-Pierluissi M. RGS12 interacts with the SNARE-binding region of the Cav2.2 calcium channel. *J Biol Chem.* 2005; 280(2):1521–1528. [PubMed: 15536086]
127. Iwai K, Koike M, Ohshima S, Miyatake K, Uchiyama Y, Saeki Y, Ishii M. RGS18 acts as a negative regulator of osteoclastogenesis by modulating the acid-sensing OGR1/NFAT signaling pathway. *J Bone Miner Res.* 2007; 22(10):1612–1620. [PubMed: 17576169]
128. Park IK, Klug CA, Li K, Jerabek L, Li L, Nanamori M, Neubig RR, Hood L, Weissman IL, Clarke MF. Molecular cloning and characterization of a novel regulator of G-protein signaling from mouse hematopoietic stem cells. *J Biol Chem.* 2001; 276(2):915–923. [PubMed: 11042171]
129. Bowler WB, Gallagher JA, Bilbe G. G-protein coupled receptors in bone. *Front Biosci.* 1998; 3:d769–d780. [PubMed: 9682032]
130. Oliveira-Dos-Santos AJ, Matsumoto G, Snow BE, Bai D, Houston FP, Whishaw IQ, Mariathasan S, Sasaki T, Wakeham A, Ohashi PS, Roder JC, Barnes CA, Siderovski DP, Penninger JM. Regulation of T cell activation, anxiety, and male aggression by RGS2. *Proc Natl Acad Sci U S A.* 2000; 97(22):12272–12277. [PubMed: 11027316]

Figure 1. RGS proteins in the regulation of GPCR, PTH, and Wnt signaling pathways during bone remodeling. Binding of Wnt to the FZD receptor induces β -catenin accumulation, which translocates to the nucleus to activate target gene transcription, which is important for osteoblast differentiation. Axin2, a member of the RGS family, is found to form a stable complex with β -catenin to prevent its activation without Wnt. In osteoblasts, binding of PTH to its G-protein-coupled receptor (PTHR/PTHrPR) induces the expression of RGS proteins such as RGS2 and RGS5 via activation of the cAMP/PKA- pathway. RGS2 predominately binds to the Gq-subunit of the G-protein complex and inhibits activation of the PKC/ Ca^{2+} dependent signaling cascade, which is involved in osteoblast differentiation. RGS5 likely regulates osteoblast differentiation through the regulation of PTH levels. In osteoclasts, PKC/ Ca^{2+} -induced NFATc1 is crucial for osteoclast differentiation. PLC is suggested to regulate the NFATc1 expression through IP3/DAG. RGS10 and RGS12 regulate the activity of G α_q and G α_i/o to activate PLC, which is critical for calcium oscillation and the activation of NFATc1. NFATc1 activation leads to osteoclast differentiation. RGS18 negatively

regulates osteoclast differentiation through acidosis-induced osteoclastogenic OGR1/NFAT signaling pathway.

Table 1

Classification of RGS protein subfamilies and their members (11)

Subfamily	Members
RZ	RGS17,RGS19,RGS20,RET-RGS1
R4	RGS1,RGS2,RGS3,RGS4,RGS5,RGS8,RGS13,RGS16,RGS18,RGS21
R7	RGS6,RGS7,RGS9,RGS11
R12	RGS10,RGS12,RGS14
RA	Axin, Conductin
GEF	P115-RhoGEF, PDZ-RhoGEF-LARG
GRK	GRK1,GRK2,GRK3,GRK4,GRK4,GRK5,GRK6,GRK8
SNX	RGS-PX1.SMX14,SNX25
D-AKAP2	D-AKAP2

Table 2

RGS proteins in the regulation of bone

Gene name	function	Mechanism	References
RGS2	Increased osteoblastic activity	Regulation of GPCR, PTH levels and Ca ²⁺ oscillations	(31, 33, 34, 66, 67, 69–71, 86, 101–104, 130)
Axin2	A key negative regulator of bone remodeling	Regulation of Wnt signaling	(50–53)
RGS5	Osteoblast differentiation	Regulation of PTH levels	(74)
RGS10	Osteoclast differentiation	Regulation of Ca ²⁺ oscillations and NFATc1	(92)
RGS12	Osteoclast differentiation	Regulation of calcium channel, Ca ²⁺ oscillations and NFATc1	(93, 94)
RGS18	Inhibiting osteoclastogenesis	Regulation of acid-sensing OGR1/NFAT signaling pathway	(127)