Published in final edited form as:

Prog Mol Biol Transl Sci. 2015 ; 133: 47–75. doi:10.1016/bs.pmbts.2015.02.002.

Role of Regulators of G Protein Signaling Proteins in Bone Physiology and Pathophysiology

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Abstract

Regulators of G protein signaling (RGS) proteins enhance the intrinsic GTPase activity of α subunits of the heterotrimeric G protein complex of G protein-coupled receptors (GPCRs) and thereby inactivate signal transduction initiated by GPCRs. The RGS family consists of nearly 37 members with a conserved RGS homology domain which is critical for their GTPase accelerating activity. RGS proteins are expressed in most tissues, including heart, lung, brain, kidney, and bone and play essential roles in many physiological and pathological processes. In skeletal development and bone homeostasis as well as in many bone disorders, RGS proteins control the functions of various GPCRs, including the parathyroid hormone receptor type 1 and calcium-sensing receptor and also regulate various critical signaling pathways, such as Wnt and calcium oscillations. This chapter will discuss the current findings on the roles of RGS proteins in regulating signaling of key GPCRs in skeletal development and bone homeostasis. We also will examine the current updates of RGS proteins' regulation of calcium oscillations in bone physiology and highlight the roles of RGS proteins in selected bone pathological disorders. Despite the recent advances in bone and mineral research, RGS proteins remain understudied in the skeletal system. Further understanding of the roles of RGS proteins in bone should not only provide great insights into the molecular basis of various bone diseases but also generate great therapeutic drug targets for many bone diseases.

1. INTRODUCTION TO BONE

Bone is a connective tissue that plays crucial roles in mineral storage and homeostasis, organ support, and locomotion.^{1,2} Bone is composed of organic and inorganic materials. The inorganic component of bone is primarily constituted of hydroxyapatite, $[\text{Ca}_3(\text{PO}_4)_2]_3 \text{Ca}(\text{OH})_2$. The organic portion of bone consists mainly of type I collagen, a triple-helical molecule containing three polypeptide chains of amino acids that are each cross-linked by hydrogen bonds. The remaining organic constituent of bone contains various noncollagenous

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proteins, including hormones, growth factors, and cytokines.^{3–5} Bone formation occurs through two key mechanisms: intramembranous and endochondral ossification.^{4,6} Intramembranous mineralization gives rise to the cranial vault, some facial bones, parts of the mandible and clavicles, whereas endochondral ossification is responsible for the other bones of the skeleton. During intramembranous bone formation, undifferentiated mesenchymal cells give rise to osteoprogenitor cells which then differentiate into mature osteoblasts.³ During endochondral ossification, mesenchymal stem cells are first condensed to form a template of cartilage that is ultimately replaced by bone. Throughout adult life, bone is constantly remodeling through the synchronized and balanced activities of the osteoclasts, which derive from the hematopoietic stem cell lineage and are responsible for resorbing bone, and the osteoblasts, the bone-forming cells of the mesenchymal stem cell origin.^{7–9} Disruption which shifts the balance in the favor of osteoclasts can affect bone mass and causes many pathological bone disorders, including osteoporosis, periodontitis, endodontitis, and rheumatoid arthritis.^{10–14} Likewise, enhanced function of osteoblasts over osteoclasts can cause osteopetrosis.

2. THE GPCR–G PROTEIN–RGS SIGNALING PATHWAY

The activities of osteoclasts and osteoblasts are highly controlled by autocrine, paracrine, and endocrine factors from the external environment to ensure the systemic balance of calcium–phosphate metabolism while maintaining bone homeostasis.⁵ External stimuli can affect bone cells by binding to their receptors on the cell membranes and thereby trigger signals within the cells. G protein-coupled receptors (GPCRs) are an example of such receptors.^{15,16} GPCRs, also called seven-transmembrane domain receptors, are a large family of protein receptors. These receptors sense a variety of extracellular stimuli from growth factors, cytokines, hormones, neurotransmitters, light, to phospholipids to affect various cellular processes, such as cell proliferation, differentiation, activity, and apoptosis.¹⁷ Specifically, GPCR activation transduces intracellular signals through a heterotrimeric G protein complex which can then direct the signals to downstream effectors for specific outcomes (Fig. 1). GPCRs regulate many physiological events and, consequently, have been exploited therapeutically in many disease states, including diabetes, various cancers as well as bone, neurological, diseases, blood, heart, and kidney diseases.^{18–21} Nearly 40% of the current drugs on the market target GPCRs.

2.1 The G Protein Complex

GPCRs are linked to a heterotrimeric G protein complex at its cytoplasmic domain²² (Fig. 1). The G protein heterotrimeric complexes consist of three different subunits: G α (33–55 kDa), G β (~35 kDa), and G γ (~15 kDa).^{21,22} In humans, the G α subunits are encoded by 16 genes and are divided into 4 subgroups (G α_s , G $\alpha_{o/i}$, G $\alpha_{q/11}$, and G $\alpha_{12/13}$) which are further divided into other subgroups. The G β subunits are encoded by 5 genes and are divided into 6 subgroups. There are 12 different G γ subunits. Given that signaling transduced by numerous combinations of $\beta\gamma$ subunits is not significantly different from one another, the G protein heterotrimeric complexes are mostly defined according to their G α constituents. Upon its activation, a GPCR undergoes a conformational change in its cytoplasmic domain which leads to the activation of the G α subunit through the exchange of its bound GDP for GTP by

the guanine nucleotide exchange factor (GEF) activity of GPCRs¹⁶ (Fig. 1). The activated α subunit ($G\alpha$ -GTP) then dissociates from the $\beta\gamma$ subunits to form two different G protein units which then transduce signaling through activation or inhibition of specific effectors to induce cellular responses.

Whereas $G\alpha_s$ can stimulate the adenylate cyclase (AC) pathway to activate the cyclic adenosine monophosphate (cAMP), activation of $G\alpha_i$ results in the inhibition of the cAMP pathway. $G\alpha_q$ activates the phospholipase C- β (PLC β) pathway which cleaves phosphatidylinositol 4,5-bisphosphate (PIP2) into inositol (1,4,5) triphosphate (IP3) and diacylglycerol (DAG), which can trigger an increase in intracellular Ca^{2+} levels. $G\alpha_{12/13}$ activates the Rho pathway which is involved in cell cytoskeleton biology. The $G\beta\gamma$ subunits can activate many ion channels. Notably, GPCR signaling is terminated when the $G\alpha$ subunits hydrolyse their bound GTP to GDP, via their intrinsic GTPase activity, to reunite with the $G\beta\gamma$ subunits to reform an inactive G protein heterodimer complex (Fig. 1). The kinetics of G protein signaling are controlled by a family of proteins, named regulators of G protein signaling (RGS), which bind to activated $G\alpha$ and accelerate their intrinsic GTPase activities.^{23,24} Therefore, RGS proteins are crucial for the rapid activation and inactivation of cellular responses that are initiated by GPCR stimulation.²⁵

2.2 Introduction to RGS Proteins

The RGS protein family consists of at least 37 members in mammals and is divided into nine subfamilies, including RZ/A, R4/B, R7/C, R12/E, GEF/F, GRK/G, SNX/H, and D-AKAP2/I based on structural analysis¹⁸ (Fig. 2). Nevertheless, all RGS proteins contain a highly conserved 120-amino acid RGS domain, which can enhance the GTPase activity of the $G\alpha$ proteins.²⁶ The GTPase accelerating protein (GAP) of RGS proteins is mainly limited to $G\alpha_q$, $G\alpha_{i/o}$, and $G\alpha_{12/13}$ subunits. The functions of RGS proteins are essential for coordinating signaling output elicited by GPCRs. Indeed, RGS proteins can regulate G protein-mediated receptors, ion channels, and other signaling pathways and can also serve as key modulators in many physiological processes, including cardiovascular, respiratory, nervous and immune functions as well as many disease states, such as diabetes, cardiovascular diseases, cancers, inflammatory diseases, and neurological diseases.^{27–31} RGS proteins are also critical for the skeletal system by regulating various signaling pathways, including Wnt, parathyroid hormone (PTH), and calcium-sensing receptor (CaSR) pathways and Ca^{2+} signaling.^{32,33} Consistent with their critical roles in bone, it was shown that the expression of RGS proteins can be regulated by many bone factors, including proinflammatory cytokines, lipopolysaccharide (LPS), PTH and PTH-related peptide (PTHrP), and thyroid-stimulating hormone (TSH).^{33–35} LPS can inhibit the expression of RGS2 in macrophages but increases the expression of RGS1 in dendritic cells and macrophages.^{36–38} Likewise, interferon β can upregulate the expression of RGS1 in monocytes, B cells, and T cells and induce the expression of RGS2 and RGS16 in mononuclear leukocytes.^{38,39} Moreover, PTH and PTHrP can induce RGS2 in osteoblasts. Injection of PTH in femoral metaphyseal spongiosa of young male rats can increase the expression of RGS2 in a rapid but transient fashion. Moreover, the expression of RGS2 can also be induced by TSH stimulation.³⁵ As such, targeting RGS protein functions can attenuate the symptoms associated with many bone pathological disorders. Here, we

summarized the current knowledge on the roles of RGS proteins in the bone-forming osteoblasts and bone-resorbing osteoclasts. We also reviewed the roles of RGS proteins in some key-skeletal signaling pathways under normal physiological and pathological states.

3. RGS PROTEINS IN OSTEObLASTS

Osteoblasts are mononucleated cells that derive from the mesenchymal stem cell lineage in the bone marrow and are responsible for bone formation.⁶ Osteoblasts induce bone mineralization by producing new bone called “osteoid.” Bone formation by osteoblasts are influenced by many GPCRs, including PTH1R, frizzled (Fz), and CaSR,²¹ which are critical for osteoblast differentiation and function. Transgenic mice bearing a constitutively active form of CaSR in mature osteoblasts via the osteocalcin promoter cause a decrease in bone volume and density in cancellous bone.^{21,40} These effects result from enhanced osteoclast formation and activity triggered by an increase in the expression of receptor activator of nuclear factor κ β ligand (RANKL). Furthermore, CaSR can also promote osteoblast proliferation in part through the activation of the G_{α_q} -PLC β pathway leading to increased Ca^{2+} oscillations.¹⁶ Collectively, these studies demonstrate a role for GPCRs in osteoblast differentiation and suggest a role for RGS proteins in osteoblast biology. Further studies reported that overexpression of RGS2 or RGS4 and pretreatment with pertussis toxin can inhibit the activation of GPRC6A by extracellular cations.⁴¹ However, RGS proteins have not been highly studied in osteoblasts. Here, we discussed the current understanding of the known roles of RGS2, RGS5, and axin in osteoblasts.

3.1 RGS2 in Osteoblasts

RGS2 regulates G protein-mediated functions in various tissues, including lung, brain, heart, and bone.¹⁸ RGS2 is expressed in the rat metaphyseal and diaphyseal, mouse calvarial culture, and cultured osteoblasts and plays a critical role in the skeletal system. Nevertheless, RGS2 function in osteoblasts is a bit complex. RGS2 expression is upregulated by forskolin, PTH, and PTHrP in osteoblasts through the stimulation of the AC-cAMP pathway by G_{α_s} activation,^{42–45} which is critical for osteoblast differentiation. It was reported that a high level of RGS2 is required to attenuate signaling by GPCRs that promote Ca^{2+} mobilization via the activation of the G_{α_q} -PLC β pathway β .^{16,18} RGS2 can also be induced by ATP-induced activation of G_{α_q} to attenuate PTH-induced activation of cAMP. Overall, the finding indicates that, at basal levels RGS2 does not regulate G_{α_q} or G_{α_s} signaling, but at higher levels, RGS2 may cross-desensitize both G_{α_s} and G_{α_q} in osteoblasts.

3.2 RGS5 in Osteoblasts

Recent findings have suggested that PTH-induced RGS5 expression may play a crucial role in cellular responses to extracellular calcium level through activation of CaSR.⁴⁶ RGS5 was reported to be markedly elevated in parathyroid adenomas, and mice deficient in RGS5 showed a reduced level of PTH plasma levels.^{46–48} Notably, forced expression of RGS5 in cells expressing CaSR abrogates the Ca^{2+} -induced IP3 induction by CaSR. These results indicate that RGS5 can act as a physiological negative regulator of CaSR in the parathyroid gland. Unfortunately, our understanding of the role of RGS5 in organ physiology is mostly unknown because of a lack of RGS5-targeted mouse models.¹⁸ Nonetheless, RGS5 is also

expressed in smooth muscle and heart. A novel splice variant of RGS5, RGS5S, which lacks 104 amino acids has recently been cloned and reported to be expressed in the human brain, skeletal system, eye, and small intestine.⁴⁹

3.3 Axin in Osteoblasts

Axin, a member of E/RA subfamily of the RGS protein family, is an important regulator of skeletal homeostasis (Fig. 3).^{50–53} Axin serves as molecular scaffold for the β -catenin destruction complex by interacting with other proteins, such as protein phosphatase 2A (PP2A), adenomatous polyposis coli (APC), glycogen synthase kinase 3 (GSK3), and casein kinase 1 (CK1), thus functioning as a negative regulator of the canonical Wnt– β -catenin signaling pathway.³² Mice deficient in the axin gene display a significant increase in bone density stemming from an increase in osteoblast differentiation and a decrease in osteoclast differentiation.⁵⁰ Axin can also negatively regulate bone mass through the β -catenin–bone morphogenetic proteins 2 and 4 (BMP2/4)–osterix (Osx) signaling pathway in osteoblasts.¹⁶ Axin has also been shown to interact with N-cadherin to mediate β -catenin degradation leading to decrease osteoblast differentiation.⁵⁴ Consistent with that posture, transgenic mice expressing N-cadherin under the control of the Col1 2.3 promoter display an increase in β -catenin degradation leading to a decrease in osteoblasts and bone mass.^{54,55} Notably, axin may also control the Wnt– β -catenin signaling pathway through actions on G α proteins. Whereas G α_s proteins bearing activating mutations can interact with axin to enhance β -catenin activation, a lack of G α_s causes an increase in β -catenin degradation which affects bone mass. All together, these results demonstrate a crucial role for axin as an important negative regulator of bone formation through inhibition of the Wnt– β -catenin pathway.

4. RGS PROTEINS IN OSTEOCLASTS

Osteoclasts play essential roles in skeletal development and bone homeostasis.⁷ Enhanced osteoclast formation and/or activity are responsible for many disorders of skeletal deficiency.¹⁰ Osteoclastogenesis requires two key cytokines: RANKL and macrophage colony-stimulation factor (M-CSF). Whereas M-CSF maintains the proliferation of osteoclast precursors, RANKL is responsible for the differentiation of these precursors into mature osteoclasts. RANKL functions by activating its receptor, RANK, to induce the expression of osteoclast marker genes, including the nuclear factor of activated T cells, c1 (NFATc1), a master regulator of osteoclast differentiation.^{56,57} Osteoclastogenesis also requires the activation of costimulatory signals from immunoreceptor tyrosine-based activation motif-containing (ITAM-containing) receptors, mainly the DNAX-activating protein 12 (DAP12) and Fc receptor common γ (FcR γ) subunits, in osteoclast precursors.⁵⁸ Activation of DAP12 and FcR γ triggers the activation of PLC which can maintain intracellular calcium [Ca²⁺]_i oscillations, which is critical for the activation of NFATc1.^{58–60} Consequently, recombinant recognition sequence binding protein at the J κ site (RBP-J), one of the key inhibitors of osteoclastogenesis, functions by blocking PLC-induced [Ca²⁺]_i oscillations in osteoclast precursors.^{61,62}

In line with the requirement for Ca²⁺, osteoclasts have an active Ca²⁺ regulatory system, including a membrane Ca²⁺-ATPase and endoplasmic Ca²⁺-ATPase as well as receptors, such as ovarian cancer G protein-coupled receptor (ORG1) and N-type Ca²⁺ channel, to

regulate $[Ca^{2+}]_i$ levels.¹⁶ Also, the basomembrane of osteoclasts is very sensitive to elevated Ca^{2+} levels.^{16,21,63} Despite the essential requirement for calcium, which can be activated by the GPCR- G_{α_q} -PLC signaling and the findings that osteoclasts expressed many GPCRs, such as calcitonin receptor, ORG1, and CaSR, the roles of GPCR-G proteins-RGS proteins in regulating osteoclast formation and/or activity remain understudied.^{3,21,64,65} Below, we reviewed the reported roles of RGS10, RGS12, and RGS18 in osteoclasts.

4.1 RGS10 in Osteoclasts

RGS10 is expressed by many tissues and cells, including brain, testis, atrial monocytes, B lymphocytes, dendritic cells, and osteoclasts.¹⁶ RGS10 has two key isoforms: RGS10A and RGS10B. We found that, through a differential screening of a human osteosarcoma cDNA library, RGS10A, but not RGS10B, is specifically expressed by human osteoclasts.⁶⁶ Consequently, we demonstrated that RGS10A is upregulated by RANKL in osteoclast precursors and highly expressed in murine osteoclast-like cells. RGS10A silencing by RNA interference blocks $[Ca^{2+}]_i$ oscillations, the expression of NFATc1, and osteoclast differentiation in bone marrow cells and the osteoclast cell lines. Most importantly, we also showed that mice deficient in the *RGS10* gene (*RGS10*^{-/-} mice) display a severe osteopetrotic phenotype as a result of defective osteoclast formation.⁶⁷ RGS10 overexpression markedly enhances the sensitivity of osteoclast differentiation to RANKL stimulation, restores $[Ca^{2+}]_i$ oscillations, and induces NFATc1 expression. Interestingly, forced expression of NFATc1 could also rescue osteoclastogenesis in *RGS10*^{-/-} bone marrow cells, indicating that RGS10 functions upstream of NFATc1. Hence, we revealed that RGS10 competitively interacts with Ca^{2+} /calmodulin (CaM) and IP3 in a calcium-dependent manner to mediate PLC activation and $[Ca^{2+}]_i$ oscillations for the ultimate activation of NFATc1.³ Hence, our studies have established RGS10 as a crucial regulator of osteoclast differentiation and bone homeostasis.

4.2 RGS12 in Osteoclasts

RGS12 is expressed in many tissues and cells, including lung, spleen, ovary, kidney, prostate, brain, testis, and osteoclasts.¹⁶ RGS12 is the largest member of the RGS protein family (Fig. 2). It contains many domains, including the RGS domain, a GoLoco motif which bears guanine nucleotide dissociation inhibitor (GDI) activity toward G_{α_i} subunits, and a Ras-binding domain which provides additional functions to RGS12. Also, RGS12 possesses a PDZ and PTB domains which bind to the C terminus of GPCRs and interacts with GPCR chemokine receptors, respectively.¹⁶ Given its multiple domains, RGS12 may regulate numerous signaling pathways. In fact, it has been shown that RGS12 can interact with certain types of calcium channels to control calcium oscillations in many cell types. In line with this finding, we found that RGS12 is highly induced by RANKL in osteoclast precursors and plays a crucial role in osteoclastogenesis.⁶⁸ RGS12 knockdown by RNA interference impairs osteoclastogenesis by affecting PLC activation which in turn impacts Ca^{2+} oscillations and the subsequent NFATc1 activation. In addressing this issue, we reported that RANKL can activate the N-type calcium channels to increase $[Ca^{2+}]_i$ levels.⁶⁹⁻⁷¹ Notably, we revealed that RGS12 can interact with N-type calcium channel to regulate its activity. Consistently, targeted deletion of the RGS12 in the hematopoietic cell lineage results in a severe osteopetrotic phenotype from impaired osteoclastogenesis

stemming from defective Ca^{2+} signaling. Whereas the defect in osteoclastogenesis from $\text{RGS12}^{-/-}$ osteoclast precursor cells could be rescued by overexpressing either RGS10 or RGS12, those in $\text{RGS10}^{-/-}$ bone marrow cells can only be rescued by RGS10. Given that RGS10 can activate calcium oscillations through induction of the IP3 pathway while RGS12 can promote calcium influxes through a specific calcium channel, these findings suggest that $[\text{Ca}^{2+}]_i$ oscillations may be the most effective way to maintain $[\text{Ca}^{2+}]_i$ levels in osteoclasts. Hence, our studies reveal critical roles for RGS10 and RGS12 in regulating calcium levels for osteoclast differentiation.

4.3 RGS18 in Osteoclasts

RGS18 is predominantly expressed by selected bone marrow cells, such as granulocytes, monocytes, and platelets but not by lymphocytes and erythrocytes.¹⁸ Investigation of the role of RGS18 in osteoclasts revealed that RANKL attenuates RGS18 expression in the osteoclast precursor cell line and in the primary bone marrow-derived osteoclast precursor monocytes during osteoclastogenesis.⁷² And, RGS18 depletion by RNA interference can promote osteoclastogenesis by RANKL stimulation. However, deletion of RGS18 is unable to promote osteoclast differentiation in the absence of RANKL stimulation, indicating that the RGS18 deficiency requires RANKL to stimulate osteoclastogenesis. Previous reports indicated a role for ORG1 in osteoclastogenesis which stimulates the $\text{G}\alpha_q$ -PLC pathway leading to the activation of NFATc1.^{73–76} Interestingly, RGS18 inhibits osteoclastogenesis by acidosis-induced ORG1 activation by affecting PLC-induced NFATc1 activation because the enhanced osteoclastogenesis from $\text{RGS18}^{-/-}$ cells can be reversed by treatment with anti-ORG1 blocking antibody. Moreover, whereas overexpression of RGS18 can inhibit the NFATc1 activation triggered by ORG1 activation, RGS18 deletion can enhance NFATc1 activation in these conditions. These findings indicate that RGS18 can negatively regulate osteoclastogenesis induced by extracellular acidosis via activation of ORG1.

5. GPCR–RGS PROTEINS SIGNALING IN SKELETAL PHYSIOLOGY

5.1 RGS Proteins and PTH/PTHrP Signaling in Bone

PTH and PTHrP play crucial roles in bone homeostasis through their actions on osteoblasts via the PTH1R, a member of the GPCR family (Fig. 4).^{77–80} Ligand binding on PTH1R triggers the activation of the $\text{G}\alpha_q$ -PLC and $\text{G}\alpha_s$ -AC signaling pathways leading to $[\text{Ca}^{2+}]_i$ mobilizations from IP3 and increases in cAMP levels, respectively.³² Increased $[\text{Ca}^{2+}]_i$ and cAMP levels are critical for osteoblast differentiation and function. Notably, the inactivation of PTH1R signaling, mediated by specific RGS proteins, is vital to maintain the rapid turnover of cellular responses. Many studies have demonstrated a role for RGS2 in inactivating PTH1R signaling through the evidence that forced expression of RGS2 can attenuate PTH-induced cAMP production in osteoblasts.^{16,81} Consistent with the role of RGS2 in inactivating PTH1R signaling, PTH has been shown to be able to upregulate RGS2 expression both *in vivo* and *in vitro*.^{81,82} Notably, PTH-induced RGS2 expression can also be inhibited by 1,25-(OH)₂-D₃ in a dose-dependent manner.⁸¹ Moreover, while pretreatment with vitamin D can promote PTH-induced RGS2 expression, pretreatment with glucocorticoids such as dexamethasone can inhibit upregulation of RGS2 induced by PTH

stimulation.⁸² Hence, PTH-induced RGS2 upregulation may play an important mechanism by which PTH controls its activation.

5.2 RGS Proteins and Wnt Signaling in Bone

The canonical Wnt- β -catenin pathway is critical for skeletal development and bone homeostasis by its central function in osteoblast differentiation.³² Wnt signaling is induced by the binding of Wnt ligands to the dual receptor complex consisted of Fz, a member of the GPCR family, and either lipoprotein receptor-related protein 5 (LRP5) or LRP6 (Fig. 3). In the absence of Wnt signaling, β -catenin is found in complex in the cytoplasm with many proteins, including axin, PP2A, APC, GSK3, and CK1, which results in the phosphorylation of β -catenin by GSK-3 β . Phosphorylated β -catenin is subsequently targeted for ubiquitination and proteosomal degradation. Axin plays a key role in assembling the destruction complex to reduce the β -catenin levels in the cytoplasm³² as demonstrated by many studies.

Wnt signaling is initiated upon the binding of canonical Wnt ligands to their coreceptors Fz and LRP5/6 to stimulate the Wnt- β -catenin signaling pathway by destroying the β -catenin destruction complex (Fig. 3). Axin moves to the plasma membrane at the tail of LRP5/6 via interaction with dishevelled, which is bound on the tail of Fz. They dissociate the β -catenin destruction complex to form a complex which also includes Csk-3 β and FRAT1 (not shown) that prevents the phosphorylation of β -catenin by Csk-3 β .^{32,50} This leads to the accumulation of β -catenin in the cytoplasm and then its migration in the nucleus to activate osteoblast genes and thus promote bone formation.^{83,84} Finally, Wnt can also regulate bone mass through a noncanonical pathway, which does not involve the utilization of LRP5/6 and activation of β -catenin.⁸⁵ Furthermore, Wnt can induce $[Ca^{2+}]_i$ oscillations independently of the canonical and noncanonical pathways.⁸⁶ Hence, axin is an important regulator of Wnt signaling for osteoclast differentiation and bone formation.

5.3 RGS Proteins and Ca^{2+} Oscillations

Ca^{2+} is an important intracellular messenger that is critical for many cellular processes including cell differentiation.⁴⁰ Calcium levels can be maintained or regulated via cellular Ca^{2+} influxes and/or the stimulation-specific calcium channel. CaSR, a member of the GPCR family, acts as a Ca^{2+} sensor in the parathyroid and kidney to detect extracellular Ca^{2+} concentration and thus, helps control systemic calcium homeostasis by activating the $G_{\alpha_{q/11}}$ pathway.⁴⁰ Activation of PLC then induces the formation of DAG and IP3, which then boost $[Ca^{2+}]_i$ levels through activation of store-operated Ca^{2+} channel in the plasma membrane and Ca^{2+} release from the endoplasmic reticulum (ER). The effects of Ca^{2+} on cellular functions are mainly mediated through the Ca^{2+} -binding protein, CaM, which activates the phosphatase calcineurin,^{87–89} which in turn dephosphorylates target genes for activation.^{90,91} Conversely, an increase in intracellular Ca^{2+} levels can activate the plasma membrane Ca^{2+} ATPase and sarco/endoplasmic reticulum Ca^{2+} ATPase pumps to remove Ca^{2+} ions from the cytosol.⁶³

While RGS proteins can regulate the duration of high Ca^{2+} states and control Ca^{2+} oscillations in different cell types by desensitizing the associated GPCRs through

inactivation of $G\alpha_q$, the influences of Ca^{2+} oscillations on the regulation of many tissue functions and cell differentiation have not been reported.⁹² RGS proteins, such as RGS1, RGS2, and RGS4, can bind to $G\alpha_q$ to enhance its GAP activity, leading to the inactivation of GPCR signaling and a decrease in $[Ca^{2+}]_i$ in a cell-specific manner.²³ Consistent with that notion, deletion of RGS2 can reduce cell sensitivity to enhance Ca^{2+} oscillations from ER Ca^{2+} release into the cytosol.⁶³ Indeed, the amplitude and frequency of $[Ca^{2+}]_i$ changes can influence cellular responses.^{93,94} This is underscored by the findings that components of the Ca^{2+} signaling, including IP3 production and Ca^{2+} influxes across the ER and plasma membranes, can regulate the frequency and amplitude of Ca^{2+} oscillations.¹⁶ Importantly, Ca^{2+} oscillation frequency may target cells along specific developmental pathways by differentially controlling the activation of distinct sets of genes.^{95,96} Whereas rapid $[Ca^{2+}]_i$ oscillations can stimulate the activation of NFAT, Oct/OAP, and NF- κ B, infrequent $[Ca^{2+}]_i$ oscillations can activate only NF- κ B.

Similar to many cell types, Ca^{2+} is also critical for osteoblast and osteoclast differentiation.^{40,67} CaSR is expressed in osteoblast precursors and can promote osteoblast differentiation in response to the elevated extracellular Ca^{2+} levels.¹⁶ Also, recent reports have demonstrated that CaM kinase (CaMK) is expressed in osteoblasts, and pharmacological inhibition of CaMK can inhibit osteoblast differentiation both *in vivo* and *in vitro*.⁴⁰ Notably, PTH can influence Ca^{2+} oscillations and promote the expression of several genes in osteoblasts, including the matrix metalloproteinase 13 (Mmp13). Interestingly, CaMKII inhibition significantly decreases Mmp13 expression in osteoblasts in response to PTH stimulation, suggesting that some of the osteoblast responses to PTH are mediated by CaMKII. Moreover, another key osteoblastogenic agent, vitamin D3, can also activate CaMKII in response to Ca^{2+} influxes.⁴⁰ Collectively, these studies demonstrate that Ca^{2+} is a critical factor for osteoblast differentiation.

In osteoclast differentiation, RANKL can induce Ca^{2+} oscillations via the activation of PLC in osteoclast precursors through a crosstalk between RANK and the ITAM-based receptors Dap12 and FcR γ , leading to the activation of calcineurin which dephosphorylates and activates NFATc1.^{58,61} Hence, a threshold level of calcium is required to mediate a sustained level of NFATc1 for osteoclast differentiation.⁶¹ Notably, the role of Ca^{2+} oscillations in osteoclast differentiation is buttressed by the reports showing that mice deficient in both Dap12 and FcR γ display a severe osteopetrosis phenotype due to impaired Ca^{2+} oscillations and impaired NFATc1 activation.^{58,60} Consistently, RBP-J has been identified as an inhibitor of osteoclastogenesis by its ability to interfere with RANKL-induced Ca^{2+} oscillations.⁶¹ Moreover, pharmacological inhibition of CaM or calcineurin and silencing NFATc1, downstream of Ca^{2+} signaling, can all block osteoclast differentiation. Collectively, these studies support a critical role for Ca^{2+} oscillations in osteoclastogenesis.

Given the critical influence of $[Ca^{2+}]_i$ in osteoclast differentiation, we initially proposed that RGS proteins might play a key role in osteoclastogenesis by regulating Ca^{2+} oscillations. We found that RGS10 and RGS12 are specifically induced by RANKL during osteoclastogenesis.^{66,68} RGS10 silencing inhibits osteoclastogenesis by blocking $[Ca^{2+}]_i$ levels *in vitro*, and deletion of RGS10 lead to a severe osteopetrosis.^{66,67} Mechanistically, we revealed that RGS10 competitively interacts with Ca^{2+} /CaM and IP3 in a Ca^{2+} -

dependent manner to activate PLC to evoke Ca^{2+} oscillations for NFATc1 activation and thereby promotes osteoclast differentiation. Similar to RGS10, we also found that RGS12 deficiency could also inhibit osteoclast differentiation by interfering with Ca^{2+} oscillations.⁶⁸ However, unlike RGS10, RGS12 does not bind to $\text{Ca}^{2+}/\text{CaM}$ and IP3 in a Ca^{2+} -dependent manner during osteoclastogenesis, RGS12 can induce calcium influxes via activation of CaSR. These results support the notion that RGS proteins may regulate Ca^{2+} during osteoclast precursors in different ways to maintain a threshold level of Ca^{2+} which is required to osteoclastogenesis (Table 1).

6. GPCR/RGS SIGNALING IN SKELETAL DISORDERS

A variety of GPCRs, including Fz, GPR68, PTH1R, and CaSR, have been reported to play essential roles in skeletal development and/or bone homeostasis.^{16,21} Hence, deletions or mutations of certain GPCRs have resulted in severe skeletal defects in mice. Also, many human diseases have been linked to defective GPCR signaling. As such, many drugs have been designed to target GPCRs for treating certain bone diseases. Nonetheless, whereas the roles of GPCRs in skeletal diseases have been highly studied, our understanding of RGS proteins in skeletal disorders is limited. Given the established roles of RGS proteins in regulating GPCR signaling, the mechanisms by which GPCRs cause skeletal diseases are likely to involve RGS proteins. Below, we provided some examples of skeletal diseases of defective GPCR signaling with emphasis into the aspects of signaling that are or may be regulated by RGS proteins.

6.1 PTH1R and GPCR 48 in Skeletal Development and Diseases

The roles of PTH and PTHrP, through activation of PTH1R, in bone are very complex. Whereas continuous administration of PTH or PTHrP results in bone loss from increased osteoclast activity, their intermittent administration triggers an increase in bone density from enhanced bone formation.^{79,80} PTH1R is expressed in bone and kidney and plays a critical role in Ca^{2+} homeostasis by activating the $\text{G}\alpha_q$ -PLC signaling pathway. We have already discussed that Ca^{2+} is critical for both osteoblast and osteoclast differentiation. PTH1R can also activate the $\text{G}\alpha_s$ -AC-cAMP signaling pathway. Notably, PTH and PTHrP can stimulate PTH1R on osteoblasts to increase the ratio of RANKL/OPG (osteoprotegerin), which in turn promotes osteoclastogenesis. OPG is a natural inhibitor of osteoclastogenesis which functions by competing with RANKL for RANK binding. Mice deficient in OPG display an osteoporosis phenotype from enhanced osteoclast formation and function, whereas transgenic mice with OPG overexpression have resulted in osteopetrosis from defective osteoclastogenesis.^{97,98} The roles of PTH1R signaling in bone development and homeostasis have been established via numerous mice models in which this pathway is perturbed which results in severe skeletal defects.²¹ Hence, the loss-of-function of PTH1R has been linked to Blomstrand osteochondrodysplasia type, a severe form of dwarfism, in humans.⁹⁹ Moreover, ligand-independent activation of PTH1R has been reported to cause Jansen's metaphyseal chondrodysplasia, a disease that is characterized mainly by short stature, normal epiphyseal plates but disorganized metaphyseal regions, hypercalcemia, and hypophosphatemia. Also, defects in PTH1R can cause enchondromatosis and primary failure of tooth eruption in humans.¹⁰⁰

Similar to PTH1R, but in a less complex manner, deletion of GPCR 48, a newly discovered glycoprotein hormone receptor subfamily of GPCRs, have been shown to affect skeletal development by inhibiting osteoblast differentiation while promoting osteoclast differentiation.¹⁰¹ Interestingly, GPCR 48 can also activate the cAMP signaling pathway to reduce the expression level of Atf4 which leads to downregulation of Atf4 target genes in osteoblasts. Given that both PTH1R and GPCR48 activate the cAMP pathway which can be regulated by certain RGS proteins through attenuation of G_{α_s} -AC signaling, RGS proteins are likely to play a key role in regulating PTH1R and GPCR48 in both skeletal physiology and disease. This notion is underscored by a recent report demonstrating that generation of an engineered GPCR with constitutive active Gs in mice can induce a dramatic anabolic skeletal response by 9 weeks of age, which is associated with an increase in bone density, bone volume, and osteoblast markers.¹⁰² So, the inability of the engineered GPCR to be inactivated by specific RGS proteins may be responsible for these bone effects under the constitutive activation of the Gs. Interestingly, this anabolic skeletal response was not observed during the first 4-week postnatal lives, suggesting that RGS protein may regulate bone formation in a temporal fashion. Further studies are needed to address this issue.

6.2 CaSR in Skeletal Development and Disease

As discussed above, CaSR can sense and respond to extracellular Ca^{2+} concentrations to regulate calcium homeostasis. Under elevated levels of plasma calcium, CaSR is activated, which in turn inhibit the release of PTH from the parathyroid gland. Moreover, CaSR can also function as a sensor for other cations such as lanthanum (La^{3+}), gadolinium (Gd^{3+}), barium (Ba^{2+}), strontium (Sr^{2+}), beryllium (Be^{2+}), and magnesium (Mg^{2+}).^{103,104} Mice deficient in CaSR ($CaSR^{-/-}$) display severe rickets, characterized by defective hypertrophic chondrocytes, impaired growth plate calcification, disordered mineral deposition, excessive osteoid accumulation, and prolonged mineralization lag time in metaphyseal bone.¹⁰⁵ Also, the $CaSR^{-/-}$ mice developed hyperparathyroidism, hypercalcemia, and dwarfism as well as increased bone mineralization and resorption. To elucidate the mechanism of CaSR functions in bone, Dvorak and colleagues generated a transgenic mouse with constitutively active CaSR in mature osteoblasts driven by the osteocalcin promoter.¹⁰⁶ These mice show an increase in RANKL expression by osteoblasts which trigger an increase in osteoclast formation and bone resorption. These transgenic mice also show an increase in bone formation. Consistently, CaSR can upregulate PTHrP in osteoblasts to promote osteoblast and osteoclast differentiation in part via an increase in extracellular Ca^{2+} levels and RANKL upregulation.¹⁶

In line with the mouse studies, inactivating mutations of the CaSR gene have been reported to cause many diseases in humans, including the familial hypocalciuric hypercalcemia (FHH) and neonatal severe hyperparathyroidism (NSHPT).^{107,108} NSHPT can cause a life-threatening hypercalcemia and defectively mineralized skeleton, whereas FHH can show mild-to-moderate hypercalcemia. Moreover, mutations that activate CaSR can cause autosomal dominant hypocalcemia (ADH) in humans. ADH patients display low-to-normal levels of PTH and hypercalciuria with hypocalcemia but no overt skeletal deformities. Ligand binding to CaSR can activate the PLC signaling pathway in both osteoblasts and osteoclasts, presumably via the activation of G_{α_q} , to increase intracellular calcium levels. A

role for RGS proteins in regulating the functions of CaSR in the skeletal system was demonstrated by our reports that RGS10 and RGS12 can cause skeletal defects by interfering with Ca^{2+} oscillations via the PLC pathways and Ca^{2+} influxes through the calcium channel, respectively.^{66–68} More studies are needed to explicitly address the roles of other RGS in regulating Ca^{2+} signaling in skeletal physiology and bone disorders.

6.3 The Canonical Wnt Signaling Pathway in Bone Disease

Wnt signaling pathway is a critical regulator of many development processes, including skeletal development.³² In addition, Wnt signaling has also been associated with many bone diseases. Wnt signaling is a very complicated pathway that includes the canonical Wnt- β -catenin, the noncanonical Wnt-planar cell polarity, and the Wnt-calcium (Wnt- Ca^{2+}) pathways. Of the three Wnt signaling components, however, the canonical Wnt- Ca^{2+} pathway has been shown to be the major component of the Wnt signaling that regulates bone cells. So, activation of Wnt signaling prevents β -catenin degradation through obliteration of the multiprotein β -catenin destruction complex and thus increases the β -catenin level in the cytoplasm, from where it is translocated in the nucleus to induce gene expression for cell differentiation and function (Fig. 3).

6.3.1 Axin in Bone Development and Bone Disease—Axin is a key component of the β -catenin destruction complex.^{109,110} The role of axin in suppressing the canonical Wnt- β -catenin signaling pathway has been addressed above (Fig. 4). Consistent with these findings, targeted deletion of axin in mice causes abnormal skull development, a phenotype resembling craniosynostosis in humans, resulting from an increase in calvarial osteoblast development.^{50–52} These mutant mice also display defective bone ossification from enhanced osteoblast proliferation and differentiation. The skeletal anomalies of the axin-deficient mice were shown to be mediated through enhanced activation of β -catenin. This finding is in agreement with that of Yan *et al.* who demonstrated that axin also functions as a negative regulator of bone remodeling in adult mice and promotes osteoblastogenesis through the β -catenin-BMP2/4-Osx signaling pathway.⁵⁰ Taken together, these findings demonstrate a crucial role for this RGS protein in regulating the canonical Wnt- β -catenin signaling pathway in skeletal development and bone disease. Given the central of axin in the regulating Wnt signaling, we further examined the role of Wnt in skeletal development and bone disease.

6.3.2 Mouse Models of Wnt Signaling in Skeletal Homeostasis—The role of the canonical Wnt- β -catenin in bone development and homeostasis is underscored by the findings from many mouse models in which this signaling pathway is disturbed which lead to skeletal deformities.³² Whereas mutations that enhance the Wnt- β -catenin signaling pathway in osteoblasts can trigger an increase in bone mineralization, its inactivation in the osteoblastic lineage cells can result in a decrease in bone mass. For instance, alteration of the Wnt10b gene, one type of Wnt ligand, has established it as a positive regulator of bone homeostasis by enhancing osteoblast differentiation in adult bone.^{111–113} Conversely, deletion of Wls, a chaperone required for the secretion of Wnt proteins, causes severe osteoporosis in adult mice through impairment of osteoblast differentiation and enhancement of osteoclast differentiation.¹¹⁴ Furthermore, mice with mutations in the LRP5 and/or LRP6

have been shown to recapitulate human diseases of defective Wnt signaling. Hence, global LRP5 deficiency or its targeted deletion in osteocytes has recapitulated the osteoporotic low-bone formation phenotype of humans with loss-of-function mutations in LRP5 or LRP6.^{115–117} Conversely, introduction of the human high-bone mass gain-of-function mutations in LRP5 β -propeller 1 has induced a similar phenotype in mice when expressed ubiquitously or selectively in the limbs or in the cells of the osteoblastic lineage.³² Further studies have identified many endogenous enhancers of Wnt signaling, such as four R-spondin (RSPO) proteins which bind to Fz and possibly LRP6 to induce β -catenin activation and endogenous inhibitors of Wnt signaling such as sclerostin and dickkopf (DKK1) which can bind to LRP5 or LRP6 to inhibit Wnt signaling.³² The Wnt signaling in bone is complex, and our discussion here only highlights some aspects of the canonical Wnt- β -catenin signaling pathway and its relevance to bone disease.

6.3.3 Bone Diseases of Defective Wnt Signaling—Many human diseases have been reported to be associated with abnormal Wnt signaling leading to skeletal deformities.³² Some of these diseases result from LRP5 mutations. Loss-of-function mutations in LRP5 can lead to osteoporosis-pseudoglioma syndrome, an autosomal recessive disorder characterized by severe juvenile-onset osteoporosis from decreased bone formation and congenital or juvenile-onset blindness, and other diseases with similar phenotypes.^{118–120} Gain-of-function mutations in LRP5 can cause high bone-mass syndrome from increased bone formation. Notably, gain-of-function mutations of the LRP5 gene can decrease LRP5 binding with sclerostin and DKK1, which in turn promote the activation of the Wnt- β -catenin signaling pathway. Other mutations have shown to affect the Wnt signaling by targeting sclerostin.^{121,122} Indeed, sclerostin deficiency can cause sclerosteosis and Van Buchen disease, two autosomal recessive disorders characterized by bone overgrowth from excessive bone formation mostly in skull and mandible.¹²³ These human reports provide strong evidence that the Wnt- β -catenin signaling pathway is critical for skeletal homeostasis and bone development. Also, inactivating mutations that affect the Wnt signaling cause severe skeletal deformities.

6.4 RGS Proteins in Inflammatory Bone Disease

Excessive osteoclast formation and/or activity are responsible for the tooth loss associated with many oral diseases including periodontitis. Many RGS proteins including RGS10 can promote osteoclasts differentiation, and proinflammatory factors, such as tumor necrosis factor alpha (TNF α), interleukin 1 (IL-1), and IL-6, can potentially influence osteoclast differentiation and function.^{124–126} In fact, the trio of RANKL, TNF α , and IL-1 are known to be strongly associated with bone loss in many oral diseases.^{126–129} We reasoned that given its role in osteoclasts, RGS10 might also play a role in bone loss induced by inflammatory conditions like periodontitis. Toward this end, we employed the adeno-associated virus (AAV)-mediated gene silencing strategy to deplete RGS10 (AAV-shRNA-RGS10) in an established mouse model of bacteria-induced periodontal disease. Our data revealed that RGS10 silencing can protect mice from both the inflammation and bone loss associated with the periodontal disease. We showed that the number of dendritic cells, T cells, and osteoclasts were all decreased in the disease mice-treated with AAV-shRNA-RGS10 as compared to control littermates. Notably, the expression of osteoclast markers and

proinflammatory markers were also reduced in the periodontal lesions. These results indicate that RGS10 can regulate both osteoclast differentiation and inflammatory bone diseases.

Our finding is in agreement with that of a previous report which showed that human monocyte-derived dendritic cells constitutively express high levels of RGS2, RGS10, RGS14, RGS18, and RGS19, but low levels of RGS3 and RGS13.³⁸ Activation of toll-like receptor 3 (TLR3) or TLR4 on these dendritic cells can stimulate the expression of RGS1, RGS16, and RGS20 and attenuate the expression levels of RGS18 and RGS14 without modifying other RGS proteins. These results indicate that TLR signaling can affect GPCR signaling by altering the expression of RGS proteins. The role of RGS in macrophages was addressed by Lee and colleagues who demonstrated that macrophages deficient in RGS10 (RGS10^{-/-}) generate higher levels of TNF, IL-1 β , and IL-12p70 in response to LPS treatment and also exhibited higher cytotoxicity on dopaminergic MN9D neuroblastoma cells.¹³⁰ Moreover, the authors found that Rgs10^{-/-} macrophages display dysregulated M1 responses along with blunted M2 alternative activation responses. While our finding on the role of RGS10 in periodontitis may seem in disagreement with that of this study, these discrepancies may stem from the complex nature of RGS protein functions which can regulate GPCR signaling in a temporal- and cell-specific manner. Nonetheless, more studies are needed to fully investigate the role RGS10 in inflammatory conditions. We anticipate that RGS10, aside from its reported role in periodontitis, may also carry critical roles in other inflammatory bone diseases like rheumatoid arthritis.

7. CONCLUSION AND PERSPECTIVES

Our understanding of the roles of RGS proteins in the skeletal system remains mostly unexplored aside from a few studies that have demonstrated essential roles for some RGS proteins in bone formation and/or homeostasis as well as bone diseases through their regulatory effects on osteoblasts, chondrocytes, and/or osteoclasts. Whereas these limited numbers of studies have helped improve our understanding of GPCR–G proteins–RGS signaling in bone biology, much work remains to be done in this area. Besides, GPCR signaling controls many physiological processes, so impacting the functions of RGS proteins in the skeletal system may undesirably have impacts on other organ systems or vice versa. Consequently, it will be critical to address this issue as we further our understanding of RGS proteins on bone biology with the hope of developing better therapy for bone loss by specifically and efficiently targeting GPCR signaling. Indeed, GPCR signaling has been and is likely to remain a valuable target for many disease states, including allergy, ulcers and reflux, pain, high blood pressure, migraine headache, nausea, schizophrenia, and depression. Notably, therapy targeting sclerostin is being examined for treating osteoporosis.³² Most importantly, through their regulatory roles on GPCR signaling and their cell-specific and -selectivity patterns of functions, targeting RGS proteins may also evolve as an effective and potent therapeutic option for bone loss associated with bone disorders of either excessive osteoclast function or attenuated osteoblast activity.

Acknowledgments

Research reported in this publication was supported by the National Institute of Arthritis and Musculoskeletal and Skin Diseases, part of the National Institutes of Health, under AR-055307 and R01-AR-44741 to Y.P.L.

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Figure 1.

Schematic of GPCR–G protein–RGS activation and inactivation cycle. (A) GPCR inactivation in the absence of ligands. In absence of ligands, $G\alpha$ is linked to GDP ($G\alpha$ -GDP) and forms a heterotrimeric complex with the $G\gamma$ and $G\beta$ subunits, preventing their liberation to activate effectors to induce cellular response. (B) Ligand-induced GPCR activation. In the presence of ligands, GPCR undergoes a conformational change leading to the activation of $G\alpha$ through the exchange of GDP for GTP by the receptor GEF activity. $G\alpha$ -GTP and $G\beta\gamma$ subunits dissociate to modulate the activity of specific effectors to mediate cellular responses. (C) GPCR signaling inactivation by RGS proteins. RGS proteins enhance the GTPase activity of $G\alpha$ to hydrolyze GTP to GDP leading to the inactivation of the $G\alpha$ protein and its subsequent reunification with $G\beta\gamma$ subunits to reform the inactive G protein heterotrimeric complex. This prevents the G proteins from continuing activating effectors for cellular response and thus terminates the cellular response. GDP, guanosine diphosphate; GTP, guanosine triphosphate; GEF, guanine nucleotide exchange factor.

Figure 2. Subclassification of RGS family members. All members of the RGS family possess a common RGS domain but are subdivided into different subfamilies based on protein structures. β -Cat, β -catenin-binding; D-AKAP, dual-specificity A-kinase anchoring protein; DEP, dishevelled/EGL-10/pleckstrin; DH, double homology; DIX, dishevelled homology domain; GAIP, G α interacting protein; GEF, guanine nucleotide exchange factor; GGL, G γ -like; Goloco, G $\alpha_{i/o}$ -Loco; GRK, G protein-coupled receptor kinase; GSK, glycogen synthase kinase 3 β -binding; PDZ, PSD95/dlg/ZO-1/2; PEST, proline, glutamine, serine, threonine-rich; PH, pleckstrin homology; PP2A, protein phosphatase 2A; PTB, phosphotyrosine-binding; PX, phosphatidylinositol-binding; PXA, PX-associated; RBD, Ras-binding domain; SNX, sorting nexin.

Figure 3.

Role of axin in Wnt signaling in bone. (A) In the absence of Wnt ligands, β -catenin is constructively targeted for degradation via a multiprotein destruction complex. β -Catenin is phosphorylated by GSK-3 β and then targeted for ubiquitination (Ub) and proteosomal degradation. Axin plays a key role in this process of β -catenin degradation and thus functions as an inhibitor of the canonical Wnt- β pathway. (B) Binding of canonical Wnt ligands to their co-receptors Fz and LRP5/6 prevent β -catenin phosphorylation by GSK-3 β , which in turn prevent its degradation and allows it to move to the nucleus to mediate gene expression.

Figure 4.

Role of RGS proteins in selected GPCR signaling in bone. In osteoblasts, PTH1R activation upon binding of PTH or PTHrP triggers the activation of the G α_s –AC–cAMP signaling pathway which ultimately promotes osteoblast differentiation. Activation of PTH1R can induce the expression of many RGS proteins, including RGS2 and RGS5. RGS2 can target the cAMP signaling to regulate PTH1R signaling, and RGS5 is likely to regulate osteoblast differentiation by controlling PTH levels. In osteoclasts, activation of GPCRs, such as CasR and OGR1, can activate G α_q or G $\alpha_{i/o}$ to activate the PLC–Ca²⁺ signaling pathway for NFATc1 activation or G α_s to activate the Ac–cAMP pathway to promote osteoblast differentiation and function. RGS10, RGS12, and RGS18 can regulate osteoclast differentiation.

Table 1**RGS Proteins in the Regulation of Osteoblasts and Osteoclasts**

RGS Family Member	Gene Function in Bone Cells	Mechanism of Function in Bone Cells
RGS2	Promote osteoblast differentiation and function in bone formation	Regulation of PTH and Ca ²⁺ signaling
Axin	Negatively regulate osteoblast differentiation and bone formation	Regulation of canonical Wnt signaling
RGS5	Promote osteoclast differentiation	Regulation of PTH levels
RGS10	Regulate osteoclast differentiation	Regulation of Ca ²⁺ signaling and NFATc1 activation
RGS12	Regulate osteoclast differentiation	Regulation of Ca ²⁺ oscillation via calcium channel and NFATc1 activation
RGS8	Inhibit osteoclast differentiation	Regulation of acid-sensing OGR1/NFATc1 signaling pathway