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Effects of PTH on osteocyte function

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1. Introduction

Few crucial advances in the field in the past several years have markedly increased our understanding of the function of osteocytes. The evidence that sclerostin, the product of the Sost gene, is expressed and secreated primarily by osteocytes and inhibits bone formation by osteoblasts, fueled research attempting to identify regulators of this gene as well as other osteocyte products that impact the function of osteoblasts and osteoclasts. The discovery that parathyroid hormone (PTH), a central regulator of bone homeostasis, inhibits sclerostin expression generated a cascade of studies that revealed that osteocytes are crucial target cells of the actions of PTH. This review highlights these investigations and discusses their significance for advancing our understanding of the mechanisms by which osteocytes regulate bone homeostasis and for developing therapies for bone diseases targeting osteocytes.

2. Osteocytes

Osteocytes, former osteoblasts regularly distributed throughout the mineralized bone matrix, are the most abundant cells in bone comprising more than 90% of cells within the matrix or on bone surfaces [1, 2]. Entombed during the process of bone deposition, osteocytes remain highly connected with cells on the bone surface and among themselves, via cytoplasmic processes that radiate from their bodies and travel along canaliculi excavated in the mineralized matrix. Osteocyte projections reach other bone cells, cells in the bone marrow, and endothelial cells of the bone blood vessels, establishing a functional syncytium named the osteocyte network. The osteocytic lacunar-canalicular system allows the transport of proteins produced and secreted by osteocytes that exert their action on cells on other cells or tissues. An example of such molecules is sclerostin, the product of the Sost gene, highly expressed in osteocytes [3–5]. Sclerostin binds to LRP5, LPR6 and LRP4 preventing activation of Wnt signaling and also antagonizes the actions of several members of the bone morphogenetic protein (BMP) family of proteins [6–8]. Both Wnts and BMPs are critical for osteoblastogenesis and also regulate the activity of mature osteoblasts. Loss of sclerostin in humans causes the high bone mass disorders Van Buchem's disease and sclerosteosis [9,

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10]. Moreover, mice with targeted deletion of the Sost gene exhibit high bone mass and, conversely, transgenic mice overexpressing Sost exhibit low bone mass, demonstrating conservation throughout the species of the inhibitory effect of sclerostin on bone formation [3, 11–13]. In addition, an anti-sclerostin antibody has been developed to induce bone anabolism validating the high potential of targeting osteocytes for increasing bone mass and strength [14, 15].

Osteocytes are also a critical source of molecules that influence the development and activity of osteoclasts. Osteocytes directly isolated from mouse bone express higher levels of RANKL than osteoblasts and bone marrow stromal cells [16]. Moreover, mice with deletion of the RANKL gene in osteocytes exhibit an osteopetrotic phenotype and are resistant to bone loss induced by tail suspension, strongly suggesting that osteocytes are a major source of RANKL in vivo [16, 17]. Furthermore, osteocytes secrete osteoprotegerin (OPG) [18], which competes with RANKL for its receptor on osteoclast precursors and mature osteoclasts. In osteocytes, as in osteoblasts, OPG secretion is regulated by the Wnt/β-catenin pathway and mice lacking β-catenin in osteocytes are osteoporotic due to increased osteoclast numbers, whereas osteoblast function is normal. Emerging evidence also points to osteocytes as an additional source of secreted M-CSF in bone [19]. Thus, osteocytes control the bone remodeling process through direct and indirect regulation of osteoclast and osteoblast differentiation and function.

Osteocytes also express and secrete fibroblast growth factor 23 (FGF23), a hormone that regulates phosphate reabsorption in the kidney and that, by changing circulating levels of phosphate, also affects bone mineralization [20–24]. FGF23 also directly activates intracellular signaling in osteocytes and osteoblasts, mediated through binding to the FGFR1/KLOTHO receptor complex recently reported to be expressed in these bone cells [25]. FGF23 has been shown to suppress osteoblast differentiation and matrix mineralization in vitro [26, 27], suggesting a role for FGF23 not only in the regulation of systemic phosphate levels, but also in the local control of bone mineralization.

In conclusion, osteocytes produce and secrete factors (such as sclerostin, RANKL, OPG) that affect other bone cells by paracrine or autocrine mechanisms, and hormones (such as FGF23) that affect other tissues by endocrine mechanisms.

3. PTH and its receptor

PTH is an 84-amino acid polypeptide synthesized and secreted by the parathyroid glands in a calcium-regulated manner. The hormone maintains serum calcium homeostasis, controls renal phosphate reabsorption and vitamin D_3 1α-hydroxylation and modulates bone turnover, through actions mediated by the PTH/PTH-related peptide (PTHrP) receptor (PPR). The receptor is expressed in bone and kidney, but is also found in a variety of other tissues not regarded as classical PTH targets.

Among bone cells, osteoblasts and chondrocytes have been regarded as the main target cells of hormonal effect, but increasing evidence identifies osteocytes as critical effectors of PTH action. Direct action of PTH upon osteocytes “in vivo” was first suggested by early experiments in which cellular retraction, mitochondrial swelling and cell death were observed in osteocytes by light electron microscopy upon administration of PTH extract [28, 29]. Increased proteolytic activity associated with enlarged osteocytic lacunae was also evident in bones of animals receiving the PTH extract for several days. The first evidence that osteocytes indeed expressed PPRs derives from autoradiography studies demonstrating binding of radiolabeled PTH in osteocytes of growing rats [30]. More recently, conclusive and definitive demonstrations that PTH indeed acts on osteocytes were provided from several in vitro and in vivo studies with osteocytic cell lines, primary osteoblastic cell

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preparations and genetically modified mouse models, as will be described below [5, 13, 31–33].

5. Bone re/modeling

a. Osteocytes and the bone remodeling compartment (BRC)

The ability of osteocytes to reach bone surfaces together with the recent evidence demonstrating that osteocytes express molecules that regulate osteoclast and osteoblast function, provides the basis for the long hypothesized role of the osteocyte network of coordinating the remodeling of bone. osteocyte apoptosis has been shown to precede osteoclast accumulation and resorption [34], raising the possibility that osteocytes release molecules that induce lining cell retraction facilitating the access of osteoclast precursors to bone surfaces and creating a canopy over osteoclasts and osteoblasts in the bone multicellular unit (BMU) [35]. M-CSF [19], which stimulates pre-osteoclast proliferation, and RANKL [16, 17], the master cytokine inducer of osteoclast differentiation, would then accumulate in the BRC and stimulate osteoclastogenesis. It is still uncertain whether membrane-bound RANKL in the osteocyte dendritic processes or soluble RANKL released from osteocytes through the canalicular circulation is responsible for osteoclast differentiation. Factors released from the bone matrix upon resorption, in turn, would stimulate proliferation of pre-osteoblasts and their differentiation to mature osteoblasts. It is also likely that osteocyte-derived sclerostin, reaching the BRC through the canalicular system, influences the rate of bone formation independently of resorption, providing an additional level of control of osteoblast activity. Based on these lines of evidence, the BRC might provide a supportive environment for differentiation of osteoclast and osteoblast progenitors. Within this context, as it will be discussed below, PPR actions might regulate bone remodeling by controlling the balance between resorption and formation within the BRC through the regulation of sclerostin, RANKL and OPG in osteocytes.

b. Regulation of Sost/sclerostin by PTH

PTH has profound effects on the skeleton. Elevation of the hormone in the circulation can generate both catabolic and anabolic effects on bone depending on the temporal profile of its increase. Chronic excess of PTH, as in primary hyperparathyroidism or secondary to calcium deficiency, increases the rate of bone remodeling, and can result in loss of bone. In contrast to continuous PTH elevation, intermittent increases of PTH in the circulation, as achieved by daily injections, cause bone gain, and it is the only current bone anabolic therapy. High bone remodeling rates and bone loss with chronic PTH elevation is associated with excessive production of osteoclasts coupled to increased osteoblasts, with a negative balance between bone formation and resorption within each BMU. Instead, the primary effect of intermittent PTH elevation is a rapid increase in osteoblasts and bone formation, attributed to the ability of the hormone to promote proliferation of osteoblast precursors, to inhibit osteoblast apoptosis, to reactivate lining cells to become matrix synthesizing osteoblasts, or to a combination of those effects [36, 37]. The net gain in bone obtained with intermittent PTH administration is due to enhanced osteoblast activity and bone formation. In humans, PTH stimulates bone formation by increasing the bone remodeling rate and the amount of bone formed by each remodeling unit, named “remodeling-based formation” [38]. PTH also stimulates bone formation not coupled to prior resorption, referred to as “modeling-based formation”. The latter mechanism appears to be more evident in rodents.

Recent investigations have markedly advanced our understanding of the cellular and molecular mechanisms behind the contrasting effects of the two PTH regimens on bone. Studies in mice indicate that chronic and intermittent PTH increase osteoblast number by distinct mechanisms. At least in cancellous bone in the mouse, the anabolic effect of

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intermittent PTH can be accounted for by attenuation of osteoblast apoptosis, whereas chronic elevation of PTH had no effect on osteoblast survival [36, 39]. Instead, the osteoblastogenic action of continuous elevation of PTH results from direct actions of the hormone on osteocytes inhibiting the expression of the Sost gene [5] (Figure 1). Continuous treatment with PTH markedly suppressed Sost mRNA and sclerostin protein expression in rodent models [5, 40]. This effect was reproduced in vitro in osteocytic and osteoblastic cell lines and in primary cultures of calvaria cells containing osteocytes, demonstrating that it results from direct effect of PTH on its receptor expressed in osteocytes, rather than arising from hormonal actions on other cells or tissues. Intermittent PTH administration also reduces Sost expression, but to a lesser extent and only transiently after each daily injection [5, 40]. However, after long-term daily PTH injection Sost levels most likely remain suppressed. These findings strongly suggest that sustained downregulation of Sost/sclerostin is not required for bone anabolism induced by intermittent PTH; however, it is likely that repetitive reductions in sclerostin could be part of the increase in bone formation induced by the hormone. These initial findings in rodents have been independently confirmed using several animal models and also validated in humans [40–46].

PTH exerts its inhibitory effect on Sost expression via the cAMP signaling pathway downstream of the PPR, as demonstrated by the fact that PTHrP, the other ligand of this receptor, and stable analogs of cAMP mimic the effects of PTH [25, 47]. However, Sost downregulation appears to not depend on transcription factors of the cAMP responsive element binding protein (CREB) family. Instead, transcription factors of the myocyte enhancer factor (MEF2) family mediate the effect of PTH on Sost expression [41] (Figure 1). Nevertheless, the exact molecular mechanism of this regulation remains unknown. Moreover, it is important to acknowledge that other mechanisms besides downregulation of Sost expression and osteoblast survival are likely to contribute to the profound skeletal effects of PTH.

c. Bone formation

Sost and Wnt signaling—In vivo activation of PPR signaling in osteocytes is sufficient for Sost inhibition as evidenced by reduced expression of Sost and sclerostin in transgenic mice expressing a constitutively active PPR under the control of an 8kb fragment of the dentin matrix protein 1 promoter (named DMP1-8kb-caPTHR1 mice) [32] (Figure 2). Bones from these transgenic mice exhibit elevated expression of Wnt target genes and high levels of beta-galactosidase activity when crossed with Wnt responsive reporter mice, indicating the activation of Wnt signaling by PPR signaling in osteocytes in vivo. DMP1-caPTHR1 mice display a remarkable increase in BMD in both the axial and appendicular skeleton, which is blunted in compound mice lacking the Wnt coreceptor LRP5 or completely abrogated in double transgenic mice overexpressing Sost in osteocytes (DMP1-8kb-caPTHR1; DMP1-8kb-Sost mice) [13, 32]. These findings demonstrate that activation of PPR signaling in osteocytes is sufficient for inhibiting Sost/sclerostin expression and for activating Wnt signaling, with the resulting concomitant increase in bone mass.

Consistent with this gain-of-function of the PPR leading to constitutive decreased in Sost and sclerostin expression, conversely, mice lacking PPR expression in osteocytes, accomplished by conditional deletion of the receptor by crossing PPR^{flox} mice with DMP1-10kb-Cre mouse, displayed tonic elevation of Sost and sclerostin [33] (Figure. 2).

Osteocytic PTH receptor and periosteal bone formation—PTH has profound effects in cortical bone, stimulating periosteal expansion and at the same time accelerating intra-cortical bone remodeling. DMP1-8kb-caPTHR mice exhibit increased cortical bone area and elevated rate of periosteal and endocortical bone formation [13]. In addition,

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DMP1-8kb-caPTHR1 mice display a marked increase in intra-cortical remodeling and cortical porosity. Crossing DMP1-8kb-caPTHR1 mice with LRP5 null mice or with DMP1-Sost mice reduced or completely abolished, respectively, the increased cortical bone area, periosteal bone formation rate, and expression of osteoblast markers and Wnt target genes exhibited by the DMP1-8kb-caPTHR1 mice. In contrast, deletion of LRP5 or Sost overexpression did not decrease bone resorption induced by the DMP1-8kb-caPTHR1 transgene [13]. In fact, DMP1-8kb-caPTHR1 mice lacking LRP5 or double transgenic DMP1-caPTHR1; DMP1-Sost mice exhibit exacerbated intra-cortical remodeling and osteoclast numbers compared to the single DMP1-8kb-caPTHR1 mice, due to decreased expression of the Wnt target gene OPG (see below). These findings demonstrate that the actions of PPR signaling in osteocytes govern periosteal bone formation and cortical bone resorption. In addition, they demonstrate that Sost downregulation and the consequent Wnt activation is required for the stimulatory effect of PTH receptor signaling on periosteal bone formation. Moreover, decreased sclerostin and increased Wnt signaling restrains osteoclast development and activity.

Bone formation in PPR deletion—Conditional PPR deletion obtained by crossing the PPRflox mouse with the tamoxifen inducible 10Kb-DMP1-Cre mouse developed osteopenia, as evidenced by decreased trabecular bone compared with control animals [33]. Importantly, as described above, these mice displayed higher Sost and sclerostin expression as well as the inability to regulate this gene in response to PTH. These observations suggested a role for PPR signaling in osteocytes in trabecular bone formation.

In contrast to mice with tamoxifen-inducible deletion of the PPR, mice with constitutive loss of PPR expression achieved by using the 8 or 10Kb-DMP1-Cre mice developed higher BMD as compared to control littermates. The timing of the skeletal phenotype was dependent on the promoter used. In mice where the receptor was ablated using the 8Kb-DMP1-promoter, an increase in cancellous bone in the spine was detected at 8 weeks of age [48] whereas in mice where the 10Kb-DMP1 promoter was used, the skeletal phenotype was evident at 12 weeks of age, and micro-CT analysis at 14 weeks revealed increased trabecular BV/TV%, trabecular number, cortical thickness and bone area. Histomorphometric analysis revealed a marked reduction in osteoblast number and bone formation rate (unpublished data), indicating an important role of PPR in osteocytes in controlling bone formation (Figure 2). Future studies are required to determine whether this decrease in bone formation is secondary to reduced resorption in the absence of PPR in osteocytes (as will be discussed below). The opposite skeletal phenotype observed when the receptor was ablated with the tamoxifen-inducible model (DMP1-10kb-Cre-ERT2) versus the non-inducible DMP1 promoter (the 8kb and the 10 kb fragments) might be due to recombinase activity during development in the latter models. Alternatively, the phenotype of the inducible PPR deletion using tamoxifen could reflect a potential interaction between the PPR and the estrogen receptor in osteocytes.

Osteoblastic expansion and the hematopoietic stem cell niche—Accumulating evidence demonstrates that cells of the osteoblastic lineage create the essential niche required for self-renewal and differentiation of hematopoietic stem cells (HSC) [49]. Thus, it has been assumed that expansion of the osteoblastic cell pool would increase the number or function of HSC. Consistent with this notion, the increase in osteoblasts induced by PTH administration or by activating its receptor in osteoblastic cells (preosteoblasts, osteoblasts and osteocytes) increases the HSC niche [50]. Remarkably, however, activation of PTH receptor signaling exclusively in osteocytes in DMP1-8kb-caPTHR mice does not increase the HSC niche, despite of the striking high number of osteoblasts and increased bone formation rate exhibited by these mice [51]. These findings establish that osteocytic activation of PTH receptor signaling is not sufficient to augment bone marrow HSC and

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exclude osteocytes as the osteoblastic population required to initiate PTH-dependent HSC expansion. This evidence also conclusively separates the bone anabolic actions of PTH from the ability of the hormone to support HSC development and function, and strongly suggest that therapies directed to increase osteoblast number and function might not necessarily achieve HSC expansion.

d. Bone resorption

Activation of the PTH receptor in osteocytes and bone resorption—In vitro studies have shown that PTH increases osteoclast formation by upregulating RANKL and elevating RANKL/OPG ratio. Although recognized that osteoblastic lineage cells mediate osteoclastogenesis by PTH, the precise stage of differentiation of the PTH target cell is unknown. Recent evidence demonstrates that osteocytes are a major source of RANKL, as well as OPG; and mice lacking RANKL in osteocytes exhibit osteopetrosis.

As discussed earlier, activation of PPR signaling in osteocytes in DMP1-caPTHR1 mice is sufficient to increase bone formation and resorption (Figure 2). DMP1-caPTHR1 mice exhibit increased circulating CTX and osteoclasts number in cancellous and cortical bone [13, 32]; and although Sost overexpression abolishes the increase in bone formation, double TG DMP1-caPTHR1; DMP1-Sost mice still exhibit high resorption. Moreover, whereas increased expression of osteoblast markers is abolished, expression of osteoclast markers, as well as RANKL and M-CSF remains elevated in the double transgenic mice. Sost overexpression markedly decreases Wnt target genes, including OPG resulting in even higher RANKL/OPG in the double TG mice. Thus, osteocytic PPR signaling controls formation and resorption by separate mechanisms. These findings raise the possibility that PTH receptor signaling directly upregulates RANKL gene expression in osteocytes. Alternatively, activation of the PTH receptor could induce in osteocytes the production and secretion of factors, such as IL-6 type cytokines, which in turn would stimulate RANKL expression in other cells, such as stromal-osteoblastic cells. Future investigations to address this question are warranted.

Conditional deletion of the PTH receptor in osteocytes and bone resorption—The physiological role of the PTH receptor in osteocytes has been addressed by deletion of the PPR in the mouse (cKO). Mice in which the PPR was ablated constitutively, using the 8kb or the 10Kb fragments of the DMP1 promoter, or conditionally, using a tamoxifen inducible 10Kb-DMP1 promoter to drive a Cre-recombinase, have been generated in our laboratories. In these cKO mouse models, receptor expression was dramatically reduced, as assessed by semiquantitative real-time PCR in vertebra and long bones. Moreover, purified osteocytes isolated from the cKO mice expressed lower levels of PPR compared to control littermates, whereas PPR expression or function in osteoblasts remained unchanged [33, 48], further validating the DMP1-Cre models to delete genes from osteocytes. Using the 8Kb-DMP1 promoter to delete the PPR [48], RANKL and OPG expression, in tibial diaphysis (composed of cortical bone), was lower in cKO-PPR mice versus control littermates (Figure 2). In contrast, in lumbar vertebra (mostly composed of cancellous bone), only RANKL is decreased resulting in reduced RANKL/OPG ratio. These findings are consistent with osteocytes being the main source of RANKL whereas OPG is also contributed by other cells relatively more abundant in cancellous versus cortical bone. Thus, removal of the PPR from osteocytes using the DMP1-8Kb promoter leads to a site specific reduction in the RANKL/OPG ratio resulting in increased cancellous but not cortical bone. Thus, in this model, PPR actions in osteocytes are required to maintain basal levels of RANKL and OPG, and in the absence of osteocytic PPR the primary effect is a reduction in osteoclast activity in cancellous bone. Interestingly, when receptor ablation was achieved using the DMP1-10Kb promoter, mice also developed a high bone mass phenotype characterized by a significant

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decrease in both osteoblasts and osteoclasts number and by an increase in both cancellous and cortical bone [52] (Figure 2). Detailed analysis of the phenotypes is needed to determine the basis of these subtle differences among the different cKO mouse models and to fully elucidate the role of the receptor in osteocytes.

6. Mineral homeostasis

a. Regulation of FGF23 by PTH and its consequences for systemic and local FGF23 signaling

Osteocytes are richer than osteoblasts in genes related to mineralization and phosphate metabolism, including phosphate-regulating neutral endopeptidase (Phex), DMP1, and matrix extracellular phosphoglycoprotein (MEPE). Osteocytes also produce FGF23, a hormone produced in bone that plays a crucial role in phosphate (Pi) homeostasis by inhibiting its renal reabsorption [20–24].

High dietary Pi and the active form of Vitamin D_3, 1,25-dihydroxy-vitamin D_3 (1,25-D_3) are major stimulators of FGF23 secretion [53–56]. FGF23 is a marker of altered phosphate metabolism that increases before detectable changes in PTH in early CKD [57]. Recent evidence suggests that PTH also regulates FGF23 production. Thus, the hyperphosphatemia exhibited by chronic kidney disease (CKD) patients is accompanied by elevation in both FGF23 and PTH [58, 59]; and parathyroidectomy prevented the increase in FGF23 in a rat model of kidney failure [60].

FGF23 is also elevated in patients and in a mouse model of primary hyperparathyroidism [61, 62]. A patient with Jansen's metaphyseal chondrodysplasia expressing a constitutively active PTHR1 receptor mutant exhibit high FGF23 concentrations in the circulation, despite low Pi and normal 1,25-D_3 levels [63]. Moreover, FGF23 production is increased in in bones and in osteocytes, but not osteoblasts, from DMP1-8kb-caPTHR1 transgenic mice, which express the same mutated PTH receptor than the patient. Circulating FGF23 is also elevated in DMP1-8kb-caPTHR1 mice; however, plasma Pi or renal Pi reabsorption is not altered. Furthermore, the FGF23 receptor complex comprising FGFR1 and KLOTHO is expressed in osteoblastic cells; and FGFR1, GALNT3, as well as downstream targets of FGF23 signaling, are increased in osteocytes but not in osteoblasts from DMP1-caPTHR1 mice. These findings demonstrate that PTH receptor signaling has the potential to modulate the endocrine and auto/paracrine functions of osteocytes by regulating FGF23 through cAMP- and Wnt-dependent mechanisms (Figure 3).

b. Calcium

Osteocytes are also involved in calcium homeostasis. Mice in which the PPR was deleted with tamoxifen-induced DMP1-10kb-Cre demonstrated hypocalcemia when challenged with a low calcium diet [33], suggesting an important role of osteocytes in regulating calcium ion homeostasis. Under physiological conditions, the low calcium diet is easily corrected by increased PTH production and increased remodeling to bring the calcium back to normal. However, when osteocytes lack a functional PPR, this does not happen, suggesting that osteocytes are important regulators of calcium homeostasis via a PTH-dependent mechanism. The role of osteocytes in ion homeostasis however has been, and still is, quite controversial. Early studies suggested a role for osteocytes in controlling mineral homeostasis and the term "osteocytic osteolysis" was created. This concept was quickly abandoned because in vitro studies were unable to demonstrate that isolated osteocytes reabsorb dentin slices. Osteocytic osteolysis was recently revisited when new evidence was generated to support this concept. As described below, during lactation osteocytes can quickly and reversibly remodel the perilacunar space. Hyperparathyroidism [64] and lack of

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mechanical stimuli, such as during immobilization or space flights [65], are additional conditions in which the resorptive capability of osteocytes is evident.

7. Cross talk between the PTH receptor and mechanotransduction in osteocytes

Osteocytes are ideally positioned to integrate the responses of bone to mechanical and hormonal stimuli. Both mechanical loading and PTH promote new bone formation by downregulating the expression of sclerostin; and mice lacking the Wnt coreceptor LRP5 [66] or overexpressing Sost [67] exhibit a defective response to loading. These findings suggest a potential crosstalk between mechanotransduction and PTHR1 signaling.

Earlier studies by Chow et al. demonstrated a blunted osteogenic response to mechanical stimulation by parathyroidectomized rats compared with normal rats, which was restored after a single injection of PTH [68] Consistent with these findings, recent studies demonstrate that expression of the PTH receptor in osteocytes is indispensable for the anabolic response to mechanical loading in mice [69]. Axial loading of the ulnae caused the expected strain-dependent increase in bone formation in control mice, resulting from an increase in both mineralizing surface covered by osteoblasts (MS/BS) as well as activity of individual osteoblasts (MAR). In contrast, loading-induced bone formation was markedly reduced in mice in which the PPR was deleted from osteocytes with the DMP1-8kb-Cre, resulting mainly from lack of stimulation of MAR by loading at any strain magnitude. Loading-induced MS/BS was also reduced in the cKO mice, with significant increases induced only by medium and high strains. These findings indicate that signaling downstream of the PTHR1 in osteocytes is required for the osteogenic response induced by mechanical force, and strongly suggest that the osteocytic PTH receptor is involved in the integration of mechanical and hormonal signals leading to coordinated regulation of bone formation.

On the other hand, the role of PTH signaling in osteocytes during unloading has not been completely elucidated. Studies in unloaded rats and mice have generated conflicting evidence. Turner et al. reported that, in rats, intermittent low doses of PTH (1μg/kg/d) could prevent the reduction of bone formation and the increase in trabecular thinning induced by hindlimb unloading in the proximal tibial methaphysis, while the hormone had no effect on the cortical bone [70]. They suggested that, in rats, PTH treatment and mechanical unloading have synergistic or additive interactions depending on the dose and the bone compartment (cortical versus cancellous) analyzed. Recently, Ono et al. [71] reported that the mice expressing the constitutive active PPR in osteoblasts (Col1a1-caPPR) [72] were resistant to unloading-induced bone loss, suggesting an important role of PPR signaling in preventing bone loss due to disuse or unloading. The importance of PTH in disuse induced bone loss is further supported by the increase in serum calcium levels and suppression of PTH after 2 and 5 days of flight in Spacelab Life Science-1 crew members [73]. Taken together these results indicate PTH can prevent bone loss induced by unloading but the cellular target of this hormonal effects still awaits to be elucidated. Unloading studies using genetically modified animals in which the receptor is ablated from osteocytes will help understand the interaction between mechanical forces and PTH signaling under unloading or disuse conditions.

8. Osteocytic PTHR and lacunae/canalicular remodeling during lactation

The role of PPR signaling in osteocytes during lactation has been recently elucidated [74]. It is known that lactation, both in humans and in rodents, is associated with a rapid and reversible loss of bone mineral density, but the molecular and cellular mechanisms of this skeletal loss were still unknown. To investigate the role of the PPR and PTHrP, known to be

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elevated during lactation [75], Qing and colleagues studied skeletal responses to lactation in control and osteocyte-specific PTHR-KO mice. They reported that, during lactation, osteocytes express osteoclasts-specific genes such as TRAP, Cathepsin K and carbonic anidrase 2, suggesting that these cells are capable of removing, and possibly redepositing, the mineral surrounding the lacunae. Moreover, when the PTHR is ablated from osteocytes, the size of the lacunae does not increase, and TRAP and cathepsin K are not elevated, indicating that PTHrP is an important mediator of this effect. These results are in agreement with previous studies that demonstrated enlarged lacunae in animals treated with exogenous PTH, and suggest an important role for the receptor on osteocytes in controlling skeletal and mineral homeostasis (Figure 4). Additional studies are needed to better comprehend the functions of osteocytes on mineral homeostasis during lactation.

9. Closing remarks and future directions

Only few years have passed since the first reports demonstrating the regulation of sclerostin expression by PTH and it is now known that actions of the PPR in osteocytes regulate several genes greatly impacting bone homeostasis. The number of publications on osteocytes and PTH has triplicate in the last ten years, in part due to the development of in vivo tools for the analysis of these remarkable cells (addressed in the article of Kalajzic et al in this issue). Development of new and improved models that target osteocytes will increase our understanding of the complex regulation of osteocyte biology by the PPR under physiological and pathological conditions. Still remains to be determined which of the skeletal effects of PTH are mediated by direct hormonal actions on osteocytes, and to define the role of other osteocytic genes regulated by PTH in bone homeostasis. It is envisioned that these future investigations will render new candidate genes and pathways, enhancing, beyond the anti-sclerostin antibody, the therapeutic potential of targeting osteocytes to regulate bone mass and strength.

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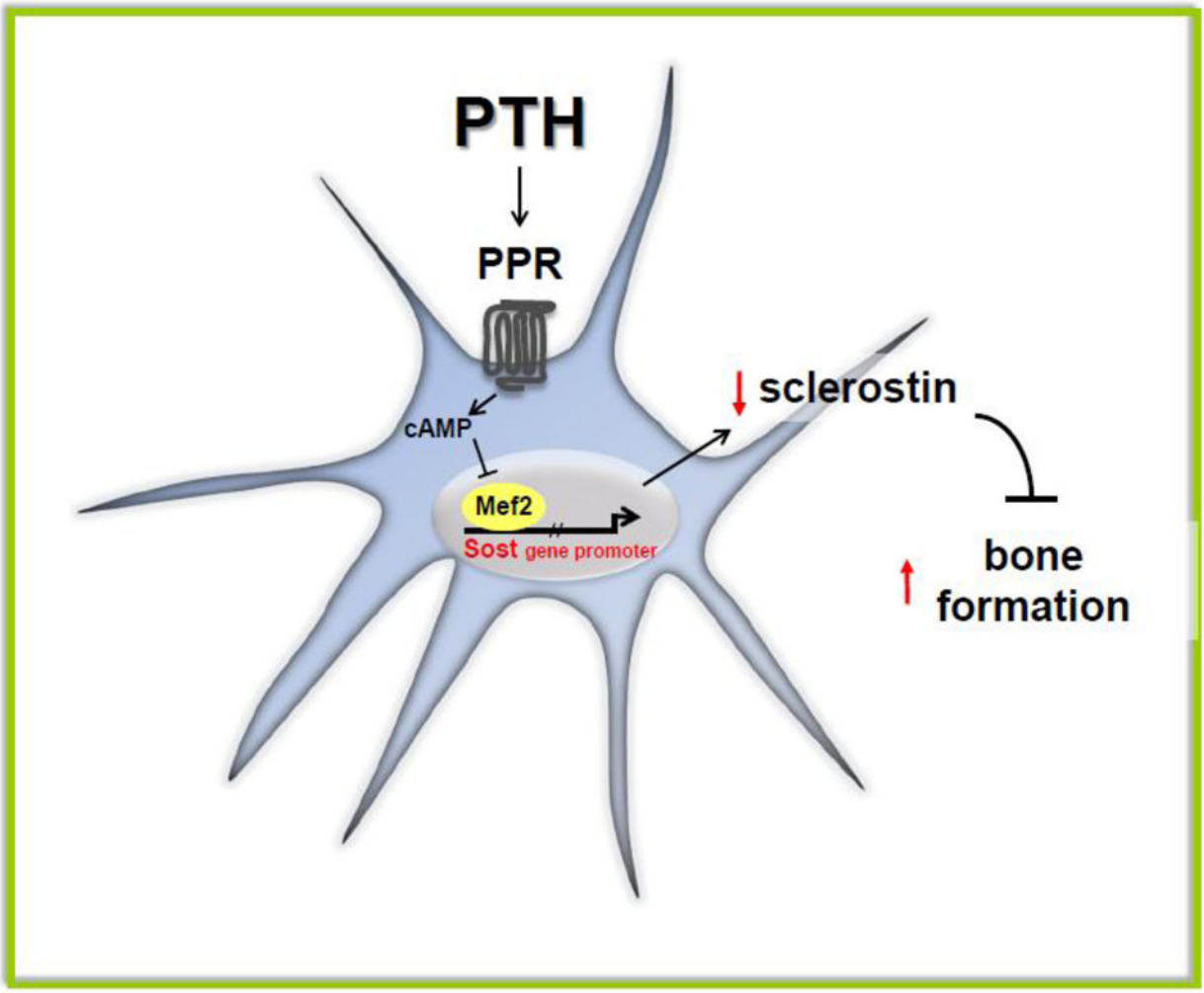

Figure 1. PTH downregulates Sost/sclerostin expression in osteocytes

PPR activation by PTH elevates cAMP levels and inhibits Mef2-stimulated Sost promoter activity leading to decreased expression of the inhibitor of bone formation sclerostin, and elevated bone formation rate.

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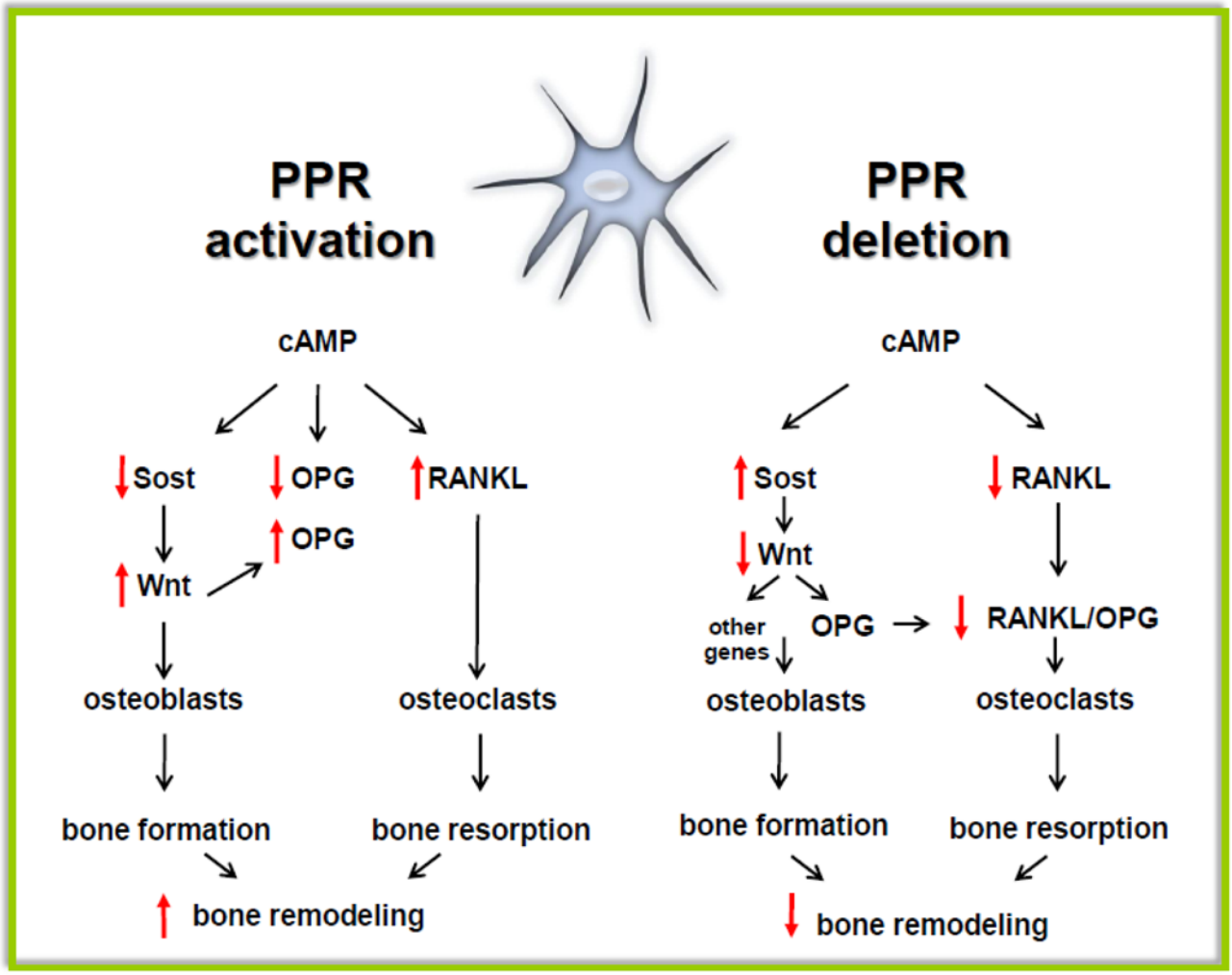

Figure 2. Converse effects of PPR activation and PPR deletion in osteocytes on bone formation and resorption

Osteocyte activation of the PPR leads to cAMP-dependent Sost downregulation and increased Wnt signaling, which in turn increases osteoblasts and stimulates bone formation. PPR activation increases RANKL expression, osteoclasts and resorption. However, OPG expression is not affected, likely resulting from opposing effects on the expression of the gene by cAMP (reduction) and Wnts (elevation). The main effect of deletion of the PPR in osteocytes is increased SOST expression, decreased RANKL expression, reduced osteoclast and osteoblast number with decreased bone resorption and bone formation, depending on the cKO model used (see text for details).

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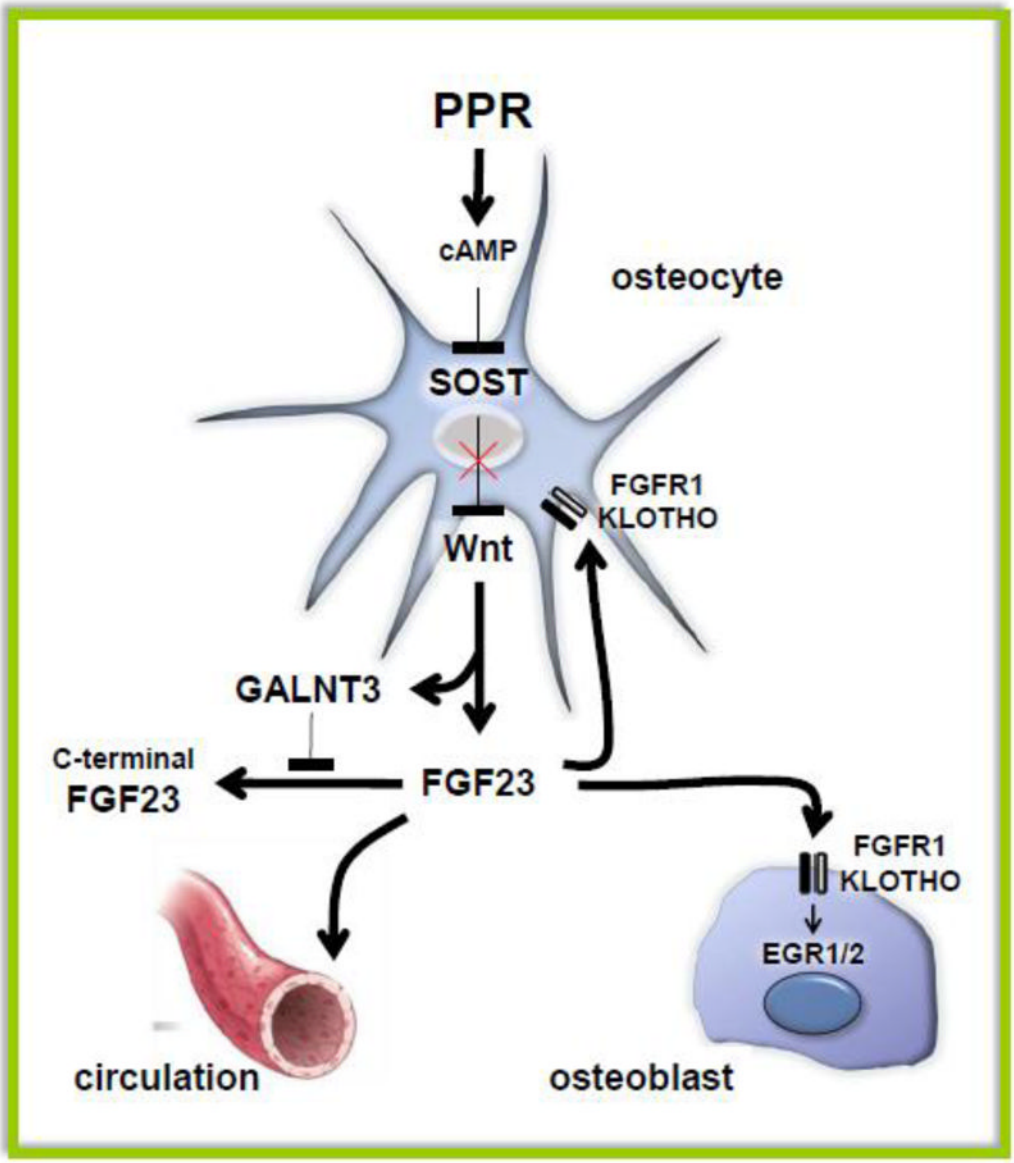

Figure 3. PPR signaling modulates the endocrine and auto/paracrine function of osteocytes by regulating FGF23 expression

cAMP elevation in vivo by a constitutively active PPR or in vitro by the PPR ligands PTH and PTHrP increases FGF23 in a Wnt-dependent manner. A simultaneous increase in GALNT3 stabilizes FGF23, leading to increased intact FGF23 in the circulation as well as locally in bone. Binding of FGF23 to the FGFR1/KLOTHO receptor complex expressed in osteocytes and osteoblasts activates intracellular signaling.

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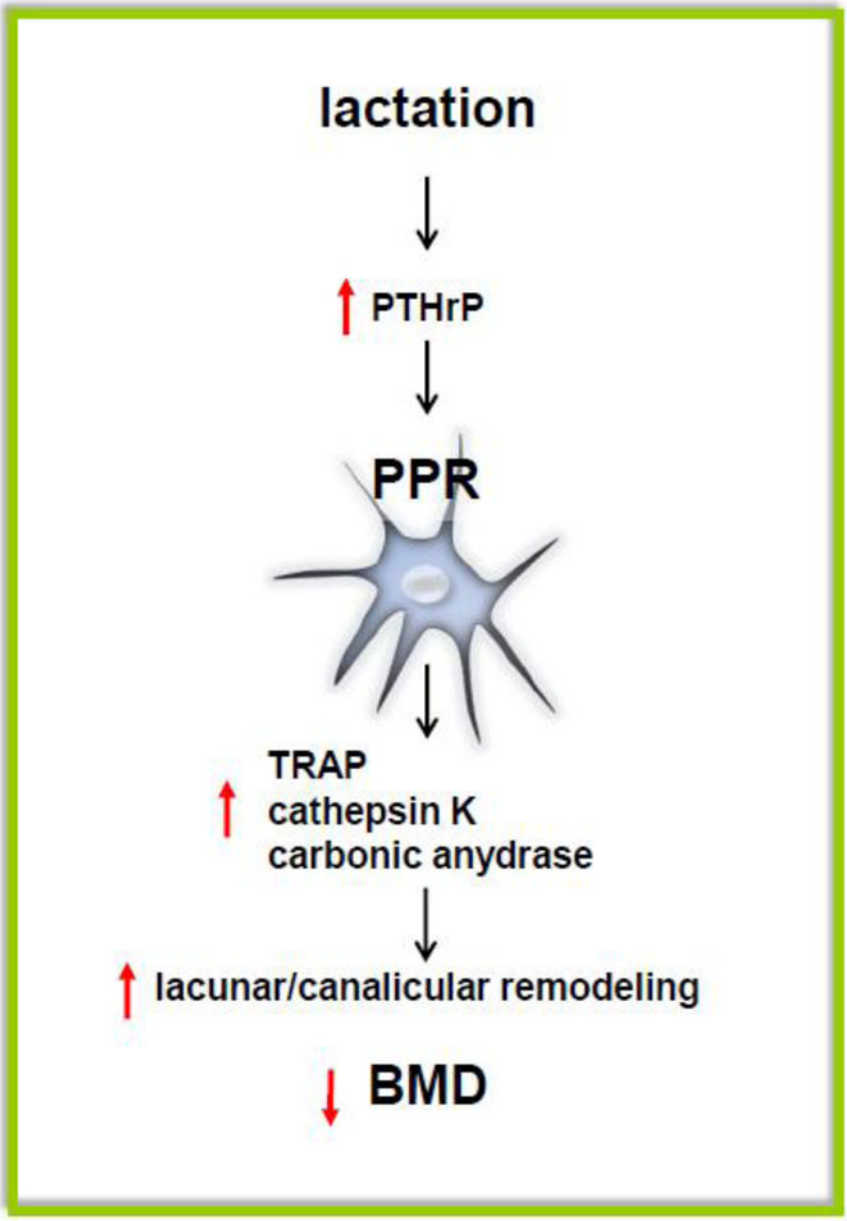

Figure 4. PTH receptor and osteocytic lacunae/canalicular remodeling during lactation

During lactation, increased circulating levels of PTHrP activate PPR expressed in osteocytes and induce increase in lacuna/canalicular remodeling and consequent bone loss. Genes such as TRAP, Cathepsin K and carbonic anydrase 2 are highly expressed in osteocytes from lactating mice and their regulation is dependent on PPR expression.