Regulation of Bone Remodeling by Parathyroid Hormone

Marc N. Wein and Henry M. Kronenberg

Endocrine Unit, Massachusetts General Hospital, Harvard Medical School, Boston, Massachusetts 02114

Correspondence: mnwein@mgh.harvard.edu

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Parathyroid hormone (PTH) exerts profound effects on skeletal homeostasis through multiple cellular and molecular mechanisms. Continuous hyperparathyroidism causes net loss of bone mass, despite accelerating bone formation by osteoblasts. Intermittent treatment with PTH analogs represents the only Food and Drug Administration (FDA)-approved bone anabolic osteoporosis treatment strategy. Functional PTH receptors are present on cells of the osteoblast lineage, ranging from early skeletal stem cells to matrix-embedded osteocytes. In addition, bone remodeling by osteoclasts liberates latent growth factors present within bone matrix. Here, we will provide an overview of the multiple cellular and molecular mechanisms through which PTH influences bone homeostasis. Notably, net skeletal effects of continuous versus intermittent can differ significantly. Where possible, we will highlight mechanisms through which continuous hyperparathyroidism leads to bone loss, and through which intermittent hyperparathyroidism boosts bone mass. Given the therapeutic usage of intermittent PTH (iPTH) treatment for osteoporosis, particular attention will be paid toward mechanisms underlying the bone anabolic effects of once daily PTH administration.

Parathyroid hormone (PTH) is a major endocrine regulator of extracellular calcium and phosphate levels (Gensure et al. 2005). Chronic hyperparathyroidism causes net loss of bone mass caused by excessive stimulation of bone resorption, but also increases osteoblast numbers and bone formation. In contrast, when injected once daily, intermittent PTH (iPTH) amino acids 1–34 (teriparatide) treatment boosts bone mass, increases bone formation, and reduces fractures in at-risk individuals (Silva et al. 2011). PTH and parathyroid hormone related peptide (PTHrP) both signal through the same G protein–coupled receptor (Juppner et al. 1991). Recently, the PTHrP analog, abaloparatide, has also been shown to boost bone mass and reduce fractures in patients with osteoporosis when given by once daily subcutaneous injection (Miller et al. 2016). Currently, iPTH/PTHrP treatments represent the only Food and Drug Administration (FDA)-approved osteoporosis medications that stimulate new bone formation.

It is well documented that iPTH treatment stimulates new bone formation; however, the cellular and molecular mechanisms through which this occurs are incompletely understood. In addition, two phenomena may significantly limit the bone-forming efficacy that these medications achieve. First, iPTH concomitantly stimulates bone formation by osteoblasts and

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bone resorption by osteoclasts (Silva et al. 2011). Therefore, concurrent osteoclast stimulation might mitigate some of the gains in bone mass that are achieved. Second, the ability of iPTH to stimulate new bone formation wanes with time and repeated dosing (Finkelstein et al. 2009). Progressive blunting of the bone anabolic effect of iPTH may also limit efficacy of prolonged treatment.

These mysteries and limitations associated with iPTH therapy highlight the need to achieve a precise understanding of the molecular and cellular mechanisms underlying its effects in bone (Jilka 2007). Over the past three decades, several cellular mechanisms have been proposed to explain how iPTH treatment increases bone formation (Table 1). PTH treatment increases osteoblast cell number among postmitotic cells of the lineage in two ways: PTH significantly decreases osteoblast apoptosis (Jilka et al. 1999) and activates bone lining cells (Kim et al. 2012). Further, PTH acts directly on early cells in the osteoblast lineage. Studies on cultured bone marrow–derived stromal cells (e.g., Nishida et al. 1994), and, more recently, in vivo lineage tracing studies (Balani et al. 2017) have shown these effects. PTH also inhibits adipocyte differentiation of early skeletal stem cells in the osteoblast lineage (Fan et al. 2017). Non-cell-autonomous effects of PTH on osteoblast activity may also occur through increases in actions of growth factors. For example, PTH-induced osteoclastic bone resorption may liberate matrix growth factors that in turn recruit osteoblast progenitors to bone surfaces and stimulate their differentiation (Wu et al. 2010), and PTH increases the production of a variety of growth factors by osteoblasts. T lymphocytes in the bone marrow microenvironment respond to iPTH by producing cytokines that stimulate osteoblast differentiation (Pacifici 2013). Osteoclasts may generate factors important in increasing osteoblast numbers (Charles and Aliprantis 2014). Finally, through effects on osteocytes, PTH reduces levels of the antiosteoblastogenic WNT inhibitor sclerostin (Keller and Kneissel 2005), thus providing another paracrine mechanism through which PTH might stimulate osteoblast differentiation. The examples listed here represent just the tip of the iceberg of how PTH uses multiple, complementary mechanisms to exert its profound influence on bone. Here, we will dive deeper into these cellular mechanisms, highlighting current knowledge of the intracellular signaling pathways involved, and major unresolved questions.

Table 1. Overview of the cellular mechanisms through which PTH promotes bone formation in PTH receptor-expressing cells

Target cell	Effects of parathyroid hormone
Skeletal stem cells	Increased numbers of Sox9-labeled stem cells owing to reduced apoptosis
	Increased Runx2 expression and differentiation of leptin receptor-expressing stem cells
Bone marrow stromal cells	Inhibits adipocyte differentiation
Osteoblasts	Reduced osteoblast apoptosis
	Increased WNT signaling in osteoblasts
Bone lining cells	Direct conversion into bone-forming osteoblasts
T lymphocytes	Increased WNT10B expression, which promotes osteoblast activity
	Increased CD40L expression, which increases OPG expression and promotes marrow stromal cells to become osteoblasts
	Increased IL-17 expression, which stimulates RANKL expression
Osteocytes	Reduced SOST expression, which enhances osteoblast activity
	Increased RANKL expression
	Increased perilacunar/canalicular remodeling

See text for details and references. In addition, PTH used several indirect mechanisms to enhance bone formation and promote skeletal remodeling, as summarized in the text.

OPG, Osteoprotegerin; IL, interleukin.

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OSTEOBLASTS AS PTH TARGETS

Studies in animal models and humans have shown that PTH administration leads to increased bone formation through an increase in osteoblast number and surface, as well as an increase in mineralized matrix deposition through effects on proliferation of precursors, suppression of apoptosis, and activation of lining cells (Jilka 2007). However, the predominant direct effect of iPTH on osteoblasts in vivo involves reductions in osteoblast apoptosis rather than increased proliferation of preosteoblasts. Seminal studies (Jilka et al. 1999) in which osteoblast apoptosis was carefully quantified using TUNEL staining on cancellous bone surfaces revealed that iPTH treatment reduces osteoblast apoptosis in vivo. Interestingly, in vivo, antiapoptotic effects of iPTH are not necessarily confined to bone-forming osteoblasts; we (Balani et al. 2017) recently showed that iPTH treatment also reduces apoptosis of Sox9-labeled early skeletal stem cells. As described below, multiple additional non-cell-autonomous mechanisms exist to account for how iPTH increases osteoblast numbers and activity.

A vast literature exists documenting effects of in vitro treatment of osteoblasts (cell lines and primary cultures) with PTH. In these studies, mixed results predominate with respect to outcomes such as proliferation, apoptosis, overall cell number, and extracellular matrix synthesis, depending largely on the cell-cycle stage and density of cultured cells, dose of PTH used, and mode of PTH treatment (continuous vs. pulsatile in vitro administration) (Swarthout et al. 2002). Nonetheless, some interesting themes have emerged from detailed studies of PTH-induced prosurvival signaling in osteoblasts over the past several decades.

Although iPTH treatment reduces osteoblast apoptosis, this does not occur in the setting of continuous hyperparathyroidism. In cultured osteoblasts, PTH-dependent antiapoptotic effects require protein kinase A–mediated phosphorylation/inactivation of Bad, a proapoptotic Bcl-2 family member (Bellido et al. 2003). In addition, PTH signaling in osteoblasts impinges on Runx2, a transcription factor that is crucial for osteoblast differentiation and function (Ducy et al. 1997, 1999). Runx2 protein levels in vitro and in vivo are tightly controlled by Nedd4 family HECT ubiquitin ligases (Ingham et al. 2004), including Smurf1 and WWP1 (Zhao et al. 2003; Jones et al. 2006; Shu et al. 2013; Wei et al. 2015; Shimazu et al. 2016). In osteoblasts, PTH signaling promotes Smurf1-driven Runx2 ubiquitin-dependent degradation (Bellido et al. 2003). Therefore, PTH simultaneously inhibits osteoblast apoptosis and drives Runx2 degradation; these opposing actions may explain why iPTH administration is needed to elicit net effects that favor osteoblast activity.

In addition to Bad phosphorylation and regulation of Runx2 stability, multiple additional target genes in osteoblasts have been proposed to contribute to the antiapoptotic effects of iPTH therapy. Transcriptomic profiling of osteoblasts treated with PTH or PTHrP identified ephrinB2 as a prominently up-regulated gene (Allan et al. 2008). Bidirectional ephrin/Eph signaling regulates contact-dependent cellular differentiation and survival (Pasquale 2008). Studies in mice lacking ephrinB2 in osteoblasts revealed that ephrinB2/EphB4 signaling is required for iPTH to boost bone mass and reduce osteoblast apoptosis (Takyar et al. 2013; Tonna et al. 2014). Beyond ephrinB2, PTH stimulates the synthesis of additional autocrine/paracrine factors that regulate osteoblast survival. PTH stimulates synthesis of growth factors, including insulin-like growth factor (IGF)-1 and fibroblast growth factor (FGF)2, which are both required for the full anabolic effects of iPTH treatment in mice (Bikle et al. 2002; Hurley et al. 2006; Wang et al. 2007). PTH-induced IGF-1 up-regulation profoundly affects osteoblast energy metabolism, stimulating aerobic glycolysis. Pharmacologic perturbation of glycolysis blunts the bone anabolic effects of iPTH, underscoring the importance of PTH/IGF-1-dependent metabolic reprogramming of osteoblasts (Esen et al. 2015). Interestingly, iPTH treatment also induces the local production of PTHrP by osteoblasts. Mice in which PTHrP is deleted only in osteoblasts show dramatic increases in osteoblast apoptosis and osteopenia; therefore, autocrine/paracrine PTHrP may contribute to skeletal responses to

iPTH therapy (Miao et al. 2005). An extremely well-studied PTH target gene in osteoblasts is the matrix metalloproteinase MMP13. Through an intricate signaling pathway involving protein kinase A (PKA), Runx2, HDAC4, and sirtuin-1 (Shimizu et al. 2010, 2014; Fei et al. 2015), PTH-induced MMP13 up-regulation plays an important role in how osteoblasts remodel old bone matrix as they synthesize new type I collagen. The target genes listed here (ephrinB2, IGF-1, FGF2, PTHrP, and MMP13) likely represent the tip of the iceberg through which PTH directly controls osteoblast apoptosis and function. Future studies will be needed to synthesize an integrated view of how these distinct target genes work together to influence osteoblast biology.

Beyond apoptosis, several additional aspects of how iPTH may influence osteoblast biology deserve special mention. The WNT signaling pathway plays a crucial role in osteoblast differentiation and function (Baron and Kneissel 2013). Cross talk between PTH and WNT signaling in osteoblasts occurs through several cell-intrinsic and cell-extrinsic mechanisms. Here, it is important to note that WNT signaling exerts distinct influences on osteoblasts, depending on their stage of differentiation (Krishnan et al. 2006). In preosteoblasts, canonical WNT signaling stimulates replication and promotes osteoblast differentiation (Kato et al. 2002). In contrast, in mature osteoblasts, canonical WNT signaling drives expression of osteoprotegerin, a soluble decoy receptor for RANKL, which thereby blocks osteoclastic bone resorption (Glass et al. 2005). Cell-extrinsic mechanisms regarding WNT/PTH signaling will be reviewed below in the sections discussing the effects of PTH on osteocytes and T lymphocytes. Within osteoblasts, PTH stimulates the formation of a ternary complex at the plasma membrane of PTH, the PTH/PTHrP receptor, and the WNT coreceptor LRP6 (Wan et al. 2008). When the activated PTH receptor binds to LRP6, this directly activates WNT signaling within cultured osteoblasts. Highlighting the importance of this mechanism, mice lacking LRP6 in osteoblasts fail to respond to iPTH treatment (Li et al. 2013a). In addition to LRP6, the activated PTH receptor may also interact with the WNT proximal signaling protein dishevelled in osteoblasts (Romero et al. 2010). In vivo and in vitro PTH treatment leads to robust induction of canonical WNT signaling and WNT target genes (Kulkarni et al. 2005), an effect potentially mediated in conjunction with the transforming growth factor (TGF)-β signaling protein Smad3 (Tobimatsu et al. 2006). Of course, much of PTH action has little to do with activation of the WNT pathway and, in fact, may oppose some actions linked to canonical WNT signaling. For example, acute PTH treatment inhibits osteoblastic expression of osteoprotegerin (Fu et al. 2002), indicating that PTH can repress a gene that is activated by canonical WNT signaling. In MC3T3-E1 cells, PTH-induced PKA leads to carboxy-terminal β-catenin phosphorylation (Guo et al. 2010). Cyclic adenosine monophosphate (cAMP) signaling has also been shown to impinge on the β-catenin destruction complex at the level of axin (Castellone et al. 2005; Goessling et al. 2009). Therefore, multiple mechanisms exist to link PTH-stimulated increases in intracellular cAMP levels and activation of the canonical WNT signaling pathway. Knowledge regarding how these crucial signaling pathways are integrated to modulate physiologic effects of PTH remains an open area for investigation.

BONE LINING CELLS AS PTH TARGETS

Most of the surface of bones is covered by thin cells with properties that suggest that they are in the osteoblast lineage. These cells, often called bone lining cells, cover most of the bone surface (Miller et al. 1980). Under the electron microscope (EM), they are seen to have few organelles, compared with the plump osteoblasts on active bone-forming surfaces (determined by tetracycline deposition). In contrast to lining cells, osteoblasts contain a well-developed Golgi apparatus and abundant rough endoplasmic reticulum and vesicles. Nevertheless, laser capture microscopy suggests that bone lining cells express many of the genes expressed by osteoblasts, although in a distinctive pattern (Nioi et al. 2015). For decades, there has been much speculation about the possible functions of lin-

ing cells. Early ideas that these cells control a barrier for calcium and potassium fluxes into and out of bone (Canas et al. 1969; Talmage 1970) have not gained traction, partly because of the frequent gaps between lining cells (Miller et al. 1980). Lining cells do contain metalloproteinases that plausibly, along with macrophages, digest collagen as part of the bone remodeling process (Everts et al. 2002).

Another possible role for bone lining cells is that they might serve as reserve cells that, under proper conditions, could become active osteoblasts. It has long been thought that lining cells derive from osteoblasts through careful microscopic observations of the function of the bone-remodeling process at differing stages of activity. Recently, lineage-tracing strategies show more directly that lining cells derive from osteoblasts. When promoters of genes such as those encoding dentin matrix protein-1 (Kim et al. 2012) or osteocalcin (Kim et al. 2016) are used to drive the expression of Cre recombinase that only works after tamoxifen administration, the cells that are marked through the expression of a Cre-dependent reporter gene include large numbers of osteoblasts. Over time, marked osteoblasts are not observed and, instead, the smaller number of cells that continue to be marked include only osteocytes and lining cells. This sort of experiment supports the idea that many osteoblasts die (deduced from the small number of cells that remain marked) or become either osteocytes or osteoblasts.

The idea that the conversion of osteoblasts to lining cells might be a reversible process has been suggested by experiments over many years. Dobnig and Turner (1995) continuously labeled rats with [^{3}H]thymidine, thereby labeling marrow stromal cells. After iPTH administration, the number and activity of osteoblasts rapidly increased, before any [^{3}H]thymidine-marked osteoblasts were detected. The investigators suggested that one possible explanation for this finding was that postmitotic cells such as bone lining cells might have been the source of the increased number of active osteoblasts observed. A second group of investigators in the same year (Leaffer et al. 1995) noted that, after administration of either PTH (1–34) or a PTH/PTHrP analog, the cells on the bone surface increased in thickness and apparent activity through observations with the EM without much change in cell number and that these cells reverted to having the thickness and activity of lining cells several days after cessation of therapy. More recently, lineage-tracking experiments performed after administration of PTH (1–34), using an intermittent administration protocol in mice, showed that previously labeled lining cells increased their thickness and characteristic EM osteoblastic appearance along with an increased expression of messenger RNA (mRNA) for osteocalcin and collagen I(α1) (Kim et al. 2012). Other stimuli can activate lining cells to become functional osteoblasts as well, including administration of antisclerostin antibody (Kim et al. 2016) and genetic ablation of osteoblasts (Matic et al. 2016).

The quantitative importance of the activation of bone lining cells in response to PTH is uncertain. Using histomorphometric criteria, it has been estimated that 20%–30% of the new bone formation after use of PTH (1–34) is based on modeling (new bone formation on surfaces not previously resorbed by osteoclasts) rather than remodeling (reviewed in Langdahl et al. 2016). Plausibly, some of that new bone formation may reflect activation of bone lining cells.

Recently, similar lineage tracing studies were used to investigate whether PTH might regulate the transition of osteoblasts into quiescent bone lining cells. In these studies, PTH treatment appeared to delay this differentiation event. Therefore, it is possible that iPTH both increases conversion of lining cells to osteoblasts and delays osteoblast to lining cell differentiation (Jang et al. 2016).

How iPTH activates bone lining cells is not known. These cells may respond directly to PTH, but it is also possible that PTH action on osteocytes, osteoblasts, or adjacent T cells may be important as well. The similar activation of lining cells by both PTH and antibody to sclerostin noted above suggests that part of the action of PTH may be mediated by both cell-autonomous and cell non-cell-autonomous activation of canonical WNT signaling. Thus, PTH is known to activate WNT signaling di-

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rectly in osteoblasts (Guo et al. 2010), but also suppresses antagonists of WNT signaling, such as sclerostin in osteocytes (Keller and Kneissel 2005) and DKK1 in osteoblasts (Guo et al. 2010). An additional unanswered question is whether continuous hyperparathyroidism affects lining cells in a manner similar to that of intermittent (pharmacologic) PTH treatment.

OSTEOPROGENITORS AS PTH TARGETS

Progenitors of osteoblasts have primarily been defined, although the isolation of cells from marrow is capable of forming colonies in vitro (Owen and Friedenstein 1988) or bone when introduced into the subcutaneous space (Sacchetti et al. 2007) or under the renal capsule (Chan et al. 2015). The cells in marrow capable of forming colonies in vitro (colony-forming units-fibroblast [CFU-F]) are rare and not well characterized. Some of them are capable of forming colonies with cells that can, under appropriate culture conditions, become osteoblasts, chondrocytes, adipocytes, or more cells capable of forming CFU-Fs (and are therefore said to be self-renewing). Such cells in humans express CD146 on their surface and can, after being implanted subcutaneously into immunocompromised hosts, form bone that supports hematopoiesis as well as cells that can be serially transplanted as bone-generating cells (Sacchetti et al. 2007).

Cells capable of forming CFU-Fs have been of great interest in the regenerative medicine community. A distinct question about such cells is what their normal function is. Although it is possible that CFU-F-forming cells are the source of osteoblasts, chondrocytes, and adipocytes in vivo during normal bone formation, this hypothesis is difficult to test. One approach is to use a lineage-tracing strategy to identify marrow stromal cells capable of becoming osteoblasts, chondrocytes, and adipocytes over time and to relate such cells to cells capable of forming CFU-Fs. Several promoters drive the expression of Cre transgenes that appear to mark such progenitor cells. Collagen II-Cre marks cells that, early in development, lead to expression of a reporter gene in mesenchymal condensations and, subsequently, in chondrocytes, osteoblasts, and, postnatally, all the cells of bone, as well as most CFU-F colonies in vitro (Ono et al. 2014). To more clearly identify precursor–product relationships, the investigators used a collagen II-CreER construct to mark reporter cells after tamoxifen administration at various times. When tamoxifen was administered at P3, osteoblasts, chondrocytes, and adipocytes were marked and their descendants continue to be marked for the life of the mouse, but, interestingly, CFU-F-forming colonies were not marked. One possible explanation for the latter finding could be that the cells capable of forming CFU-Fs express the collagen II promoter in fetal life, but no longer do so in CFU-F-forming cells postnatally. Cells marked by the expression of Sox9-CreER have properties similar to those of cells marked with collagen II-CreER, but more clearly mark precursor cells near the endosteal surface postnatally than do collagen II-CreER cells (Ono et al. 2014; Balani et al. 2017). Analogous cells expressing CreER driven by the gremlin promoter are self-renewing and generate osteoblast, chondrocytes, and marrow stromal cells, but not adipocytes in vivo or CFU-Fs in vitro (Worthley et al. 2015). A different pattern of expression was revealed through the use the leptin receptor-Cre transgene to mark mesenchymal cells in bone (Zhou et al. 2014). This transgene marked marrow stromal cells postnatally and then marked osteoblasts and adipocytes in adult bone progressively, starting at 2 months of age. This transgene failed to mark growth plate chondrocytes, although it could mark chondrocytes in fracture callus. Leptin receptor-Cre cells express the cytokine, CXCL12, and therefore mark cells called CXCL12-abundant reticular (CAR) cells (Omatsu et al. 2010). These cells most likely are adipo-osteogenic progenitors and also form a crucial part of the niche for hematopoietic stem cells in marrow.

Thus, a variety of promoters can mark CFU-Fs and also mark cells that, in vivo, differentiate into multiple mesenchymal lineages. It seems likely that multiple cell types can become osteoblasts over time; whether these cells represent multiple pathways to the osteoblast or various

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stages of differentiation along one common pathway remains to be determined. The effect of PTH, administered using protocols of intermittent administration, has been studied using a number of the assays summarized above. Nishida and colleagues showed that after 1 week of iPTH (1–34) administration to rats, the number of CFU-Fs roughly doubled after 2 weeks in culture, when compared to CFU-Fs from vehicle-treated rats (Nishida et al. 1994). By counting cells expressing nestin promoter-driven green fluorescent protein, Mendez-Ferrer et al. (2010) showed that iPTH (1–34) treatment increased the numbers of these cells in marrow. Balani et al. (2017) administered PTH (1–34) intermittently and noted that the number of cells marked by Sox9-CreER increased in number in marrow and, over time, became osteoblasts more quickly than cells from vehicle-treated mice. The increase in Sox9-CreER-marked cells did not occur when the PTH receptor was ablated from Sox9-CreER-expressing cells, suggesting that the effect of PTH on the number of osteoblast precursors requires the expression of PTH receptors in those cells. The increase in the number of cells descended from Sox9-CreER-marked cells was associated with a decrease in the frequency of these cells showing a marker of apoptosis; no change in the proliferation of these precursors was noted. PTH acts also on a subset of cells expressing leptin receptor-Cre to increase their low expression of Runx2 to a higher level and drive these cells near to the bone surface where they differentiate further into osteoblasts (Yang et al. 2017). PTH action also steers early cells of the osteoblast lineage away from the adipocyte lineage, as shown by the dramatic increase in adipocytes in the marrow of mice in which the PTH receptor was deleted early in the lineage through the use of the Prx1-Cre transgene (Fan et al. 2017). This action of the PTH receptor was probably mediated by activation of $G_s\alpha$, because ablation of $G_s\alpha$ in osterix-expressing cells similarly leads to an abundance of marrow fat (Sinha et al. 2014).

Thus, it appears that PTH increases the numbers of osteoblast precursors in marrow, at least in part through direct action on these cells. How much these precursors contribute to the increase in the number of osteoblasts in bones of rodents or people treated with PTH (1–34) is uncertain and undoubtedly differs early after the start of administration of PTH (1–34) and later. Much about this particular mechanism for increasing the number of osteoblasts remains to be discovered. In addition, future lineage tracing studies are needed to understand whether continuous hyperparathyroidism regulates skeletal stem cells in a manner analogous to their regulation by iPTH treatment.

OSTEOCLASTS AS INDIRECT TARGETS OF CONTINUOUS AND INTERMITTENT PTH ACTION

A major skeletal action of hyperparathyroidism (both continuous and intermittent) is increased bone resorption by osteoclasts. Functional PTH receptors are not expressed by osteoclasts. Therefore, non-cell-autonomous mechanisms are responsible for PTH-induced increases in osteoclast numbers and activity. M-CSF and RANKL are the two major cytokines that drive osteoclast differentiation and function (Feng and Teitelbaum 2013). The expression of both of these cytokines has been shown to be increased by PTH. Multiple cellular sources of both of these cytokines exist in the bone microenvironment, including hypertrophic chondrocytes, marrow stromal cells, osteoblasts, resident marrow lymphocytes, and osteocytes (O'Brien et al. 2013). RANKL is a well-studied PTH target gene in multiple PTH receptor-expressing cell types (Fu et al. 2002, 2006; Kim et al. 2006, 2007; Galli et al. 2008). Increases in osteoclasts and bone loss caused by secondary hyperparathyroidism require osteocyte-derived RANKL (Xiong et al. 2014). In this section, we will review the skeletal consequences of RANKL-driven PTH-induced increases in bone resorption by osteoclasts.

Because iPTH treatment stimulates both osteoblasts and osteoclasts, an obvious hypothesis emerged many years ago that treating with both iPTH and antiresorptive agents might enhance the therapeutic effects of teriparatide. However, clinical trial data clearly indicates that the ability of PTH to stimulate bone formation is blunted

by bisphosphonate cotreatment (Black et al. 2003; Finkelstein et al. 2003, 2006, 2010).

Several possibilities exist to account for why bisphosphonates blunt the ability of teriparatide to stimulate bone formation. One interesting model that has emerged is that, by stimulating osteoclastic bone resorption, PTH treatment promotes the liberation of growth factors from bone matrix that stimulate osteoblast migration, differentiation, and function. TGF-β is an abundant growth factor present in a latent form at very high levels in bone matrix. Bone acidification and destruction by osteoclasts leads to activation and liberation of TGF-β1, a process that stimulates the migration of Sca-1^+ skeletal stem cells to bone remodeling sites (Tang et al. 2009). In mice, iPTH leads to increased osteoclastic release of TGF-β1, a process that is blocked by alendronate cotreatment (Wu et al. 2010). This pathway is responsible for recruiting stem cells marked by the Sca-1^+CD29^+CD45^-CD$11b^-$ immunophenotype (Wu et al. 2010). Signaling cross talk between the TGF-β1 that is released by iPTH-induced bone remodeling and PTH itself may exist in osteoblasts. TGF-β1 signaling leads to activation of the intracellular kinase domain of the type II TGF-β1 receptor (TβRII). One TβRII substrate is the intracellular domain of the PTH receptor. PTH receptor phosphorylation induced by TGF-β1 down-regulates PTH receptor signaling by promoting endocytosis of the receptor. Mice in which TGF-β1 signaling cannot occur in osteoblasts/osteocytes show a hyperparathyroidism-like phenotype, which is abrogated by blocking PTH receptor signaling (Qiu et al. 2010). Therefore, intricate inter- and intracellular mechanisms exist to link PTH-induced osteoclast activation to TGF-β1-dependent recruitment of preosteoblasts to remodeling bone surfaces (Atfi and Baron 2010; Crane and Cao 2014a).

Beyond TGF-β1, additional growth factors are released during osteoclastic bone destruction that may enhance osteoblast differentiation and function. Of these, IGF-1 deserves special mention as a well-studied paracrine factor necessary for iPTH action (Bikle and Wang 2012). Although this gene's expression is transcriptionally regulated by PTH signaling in bone, IGF-1 protein levels increase in response to PTH out of proportion to the observed changes in mRNA (Pfeilschifter et al. 1995). IGF-1 is maintained in bone matrix in complex with binding proteins (IGFBPs), and osteoclastic bone remodeling leads to IGFBP cleavage and subsequent IGF-1 release (Crane and Cao 2014b). By activating the mechanistic target of rapamycin (mTOR) pathway, IGF-1 stimulates differentiation of skeletal stem cells into osteoblasts (Crane and Cao 2014b). Taken together, actions of locally generated TGF-β1 and IGF-1 exert complementary effects to stimulate preosteoblast recruitment and differentiation in response to PTH-induced osteoclastic bone resorption. Further studies are needed to address the role of TGF-β1 and IGF-1 in continuous hyperparathyroidism. An appealing (and untested) hypothesis is that chronic elevations in bone resorption consume matrix pools of latent growth factors, thus contributing to net bone loss in chronic hyperparathyroidism over time. This same hypothesis also might apply to the setting of iPTH treatment, an intervention in which bone-forming efficacy is known to wane over time.

It is also possible that osteoclast-derived factors participate in PTH-induced bone formation. Indeed, multiple osteoclast-derived factors that regulate osteoblast activity have been described (Charles and Aliprantis 2014). Whether "clastokines," such as CTHRC1 (Takeshita et al. 2013), S1P (Lotinun et al. 2013), C3a (Matsuoka et al. 2014), Sema4D (Negishi-Koga et al. 2011), cardiotrophin-1 (Walker et al. 2008), and PDGF-BB (Xie et al. 2014) (to name a few), might participate in skeletal responses to iPTH remains to be determined. Finally, it is important to note that although bisphosphonate cotreatment blunts the efficacy of teriparatide, combined teriparatide and denosumab therapy leads to additive effects on bone density (Tsai et al. 2013). A precise explanation for differences between denosumab and bisphosphonates in the setting of iPTH therapy remains to be determined. Denosumab (an anti-RANKL monoclonal antibody) and bisphosphonates exert their antiresorptive effects through distinct mechanisms, so therefore might possess different effects on osteoblast/osteoclast cross talk.

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T LYMPHOCYTES/MACROPHAGES

Of the many hematopoietic cells in the bone marrow microenvironment, T lymphocytes have recently emerged as key participants in bone remodeling. Central memory major histocompatibility complex (MHC) class I–restricted $CD8^+$ T cells are the predominant T-cell subset found in the bone marrow, but additional MHC class II–restricted $CD4^+$ T-cell subsets are also present (Pacifici 2016). "Osteoimmunology" investigators have extensively characterized the osteoclastogenic capacity of T-cell subpopulations, and it is now accepted that $CD4^+$ T helper (Th)17 cells are the predominant T-cell source of RANKL (Sato et al. 2006) and are especially important in bone loss caused by inflammatory arthritis (Schett and Gravallese 2012). In addition to expressing RANKL, T lymphocytes use multiple mechanisms to modulate bone remodeling. CD40L (also known as CD154) is another membrane-bound tumor necrosis factor (TNF) family cytokine that activates nuclear factor (NF)-κB signaling in target cells expressing the CD40 receptor (Croft and Siegel 2017). CD40L expressed by activated T cells induces expression of the antiresorptive factor osteoprotegerin (OPG) by B lymphocytes (Li et al. 2007). Bone marrow stromal cells are another important cellular target of CD40L signaling. In these osteoblast precursors, CD40 signaling drives proliferation, survival, and osteoblast differentiation (Gao et al. 2008; Li et al. 2013b). A final mechanism used by T cells to promote osteoblast activity is production of WNT10B (Terauchi et al. 2009).

Multiple lines of evidence support an important role for T lymphocytes in skeletal responses to PTH. Mice lacking canonical αβ T cells fail to optimally respond to iPTH treatment; importantly, this defect is rescued by adoptive transfer of CD4 and $CD8^+$ T cells (Terauchi et al. 2009). Functional PTH/PTHrP receptors are expressed by T lymphocytes. When the PTH receptor is deleted in T cells using Lck-Cre (Tawfeek et al. 2010), mice fail to increase cancellous bone mass in response to iPTH treatment (Bedi et al. 2012). $CD8^+$ T cells up-regulate WNT10B in response to iPTH, and T-cell-derived WNT10B is necessary for iPTH-induced increases cancellous bone mass (Terauchi et al. 2009). Whereas trabecular responses to PTH require T cells, cortical responses to PTH do not. Bone compartment-selective requirements for T cells in iPTH responses poses an important problem for future studies. An intriguing model has recently emerged to integrate trabecular and cortical responses to iPTH in which PTH-dependent sclerostin down-regulation in osteocytes (see below) mainly drives cortical responses and T-cell-derived WNT10B promotes trabecular bone gains (Li et al. 2014). Underscoring the importance of these preclinical studies is the observation that bone marrow levels of WNT10B are increased in humans treated with teriparatide (D'Amelio et al. 2015). The molecular mechanisms through which PTH stimulates WNT10B gene expression in cytotoxic T cells remain to be determined. Furthermore, the antigen specificity and role of dendritic cell "help" in T-cell responses to iPTH are also incompletely understood at the present time.

Multiple other hematopoietic cell types in the bone marrow microenvironment might participate in skeletal responses to PTH through indirect mechanisms. iPTH treatment increases the numbers of F4/80-positive macrophages on bone surfaces. When macrophage precursors are depleted using the "MAFIA" model (Burnett et al. 2004), osteopenia results associated with impaired anabolic responses to iPTH (Cho et al. 2014). However, when mature macrophages are depleted using clondronate treatment, enhanced skeletal responses to iPTH are observed associated with increased expression of TGF-β1 and WNT10B (Cho et al. 2014). It has been proposed that macrophage efferocytosis (the process of removing dead cell bodies) is the mechanism through which mature osteal macrophages participate in skeletal responses to iPTH (Sinder et al. 2017). Future studies are needed to determine the mechanisms through which macrophage efferocytosis is directly regulated by PTH.

T lymphocytes also participate in the bone resorptive effects of continuous hyperparathyroidism. By producing the inflammatory cytokine TNF-α, $CD8^+$ bone marrow T cells potentiate RANKL-driven osteoclastogenesis (Tawfeek et al. 2010). In addition to TNF-α,

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primary hyperparathyroidism promotes production of interleukin (IL)-17A by Th17 cells. IL-17 levels are increased in mice and humans with primary hyperparathyroidism, and IL-17 blockade blocks trabecular bone loss in mice with continuous hyperparathyroidism (Li et al. 2015b). IL-17 stimulates RANKL production in bone (Kotake et al. 1999), thus explaining how the PTH/T-cell/IL-17 pathway ultimately leads to bone resorption in chronic hyperparathyroidism. As is the case for PTH-mediated WNT10B production in iPTH treatment, future studies are needed to clarify the intracellular signaling mechanism through which chronic hyperparathyroidism drives IL-17 production in T cells.

OSTEOCYTES

The most abundant cell type in bone (Bonewald 2011), osteocytes are former osteoblasts ensconced deeply within bone matrix. Osteocytes are strategically positioned to sense and respond to mechanical and hormonal cues. In doing so, osteocytes relay signals to osteoblasts and osteoclasts on bone surfaces that regulate skeletal modeling and remodeling. Intensive research in the past 10 years has shown that osteocytes are central to understanding skeletal responses to PTH.

Before the recent explosion in knowledge regarding osteocytes and their role in skeletal responses to PTH, there were several clues suggesting that osteocytes respond to PTH. Osteocyte morphology is directly regulated by treatment with crude parathyroid extract. This treatment causes dramatic changes in osteocyte appearance, including cellular retraction, mitochondrial engorgement, and cell death (Heller et al. 1950; Cameron et al. 1967). Ideally poised to mobilize pools of calcium stored in bone, the concept of "osteocytic osteolysis" has long been proposed as a mechanism through which PTH (and PTHrP) rapidly increases blood calcium levels (Weisbrode et al. 1974). More recently, additional morphologic evidence supporting this intriguing model in which bone mineral resorption occurs independently of osteoclasts has surfaced (Tazawa et al. 2004; Nango et al. 2016). This phenomenon may be central to understanding changes associated with lactation, a physiologic state in which massive amounts of skeletal calcium must be liberated (Wysolmerski 2013). Molecular mechanisms dictating physiologic PTHrP-driven osteocytic osteolysis are reviewed below.

A critical observation that moved osteocyte biology forward came from human genetics. Individuals with sclerosteosis display very high bone mass and resistance to fractures. This rare Mendelian skeletal dyscrasia is caused by mutations in SOST, which encodes the protein sclerostin, an osteocyte-specific secretion that may act as a paracrine inhibitor of WNT signaling (Brunkow et al. 2001). Sclerostin is a tonic inhibitor of bone formation; therefore, an important mechanism through which bone anabolic signals may trigger new osteoblast activity is by reducing SOST expression in osteocytes. Indeed, both skeletal loading (Robling et al. 2008) and PTH (Bellido et al. 2005; Keller and Kneissel 2005) rapidly lead to reduced SOST levels in bone. This simple observation propelled osteocytes to the forefront in our thinking about how bone responds to PTH. In addition to SOST, osteocytes also are a major source of RANKL in bone (Nakashima et al. 2011; Xiong et al. 2011). Osteocytic RANKL is up-regulated by PTH, and plays a vital role in PTH-induced increases in bone resorption (Ben-awadh et al. 2014; Xiong et al. 2014). Therefore, one attractive mechanism through which PTH signaling in osteocytes influences skeletal remodeling is by coordinated transcriptional regulation of paracrine mediators, including SOST and RANKL.

Multiple lines of mouse genetic evidence have highlighted the importance of PTH receptor signaling in osteocytes. First, artificially increasing PTH receptor signaling in osteocytes (achieved via transgenic expression of a constitutively active PTH receptor complementary DNA (cDNA) under the control of the 8-kb osteocyte-enriched DMP1 promoter, DMP1-caPTHR1 mice) leads to massive increases in bone mass associated with high turnover (O'Brien et al. 2008; Rhee et al. 2011). Notably, multiple groups have recently shown that the DMP1 promoter fragments used in these studies show activity in mature osteoblasts, marrow

stromal cells, skeletal muscle fibers, cells in the brain (cerebellum and hindbrain), and in gastric and mesenchymal cells (Zhang and Link 2016; Lim et al. 2017). These findings underscore that caution is needed when ascribing "osteocyte-specific" phenotypes observed using this strain. In these animals, constitutive PTH receptor signaling leads to reduced SOST expression and concomitant increases in WNT transcriptional output in osteocytes and osteoblasts. Accordingly, transgenic SOST overexpression or LRP5 deletion significantly blunts the phenotype in DMP1-caPTHR1 animals. These results have been proposed as proof for the involvement of the WNT pathway, although possible distinctions between the role of SOST/LRP5 and canonical WNT signaling should be kept in mind in interpreting these data. The contribution of increased osteoclast activity to this phenotype was addressed by treating DMP1-caPTHR1 mice with alendronate. Interestingly, this pharmacologic manipulation illuminates distinct, compartment-specific effects of PTHR1 signaling in osteocytes: alendronate reduces endocortical bone formation, has no effect on periosteal bone formation, and enhances cancellous bone mass in DMP1-caPTHR1 animals (Rhee et al. 2013). In contrast, mice in which the PTH receptor has been deleted using the best-available osteocyte-"specific" Cre lines reveal the physiologic role of PTH signaling in osteocytes during normal bone remodeling. Using the 9.6-kb DMP1-Cre deleter strain (Lu et al. 2007), PTH receptor deletion causes mild increases in bone mass associated with reduced bone resorption (Saini et al. 2013), a phenotype reminiscent of what is observed in humans with hypoparathyroidism (Bilezikian et al. 2011). Importantly, a similar low-bone turnover phenotype in 8-kb DMP1-Cre PTH receptor null mice is observed (Delgado-Calle et al. 2016). Mice with osteocytes lacking PTH receptors have been used to ascertain the role of the osteocytic PTH receptor signaling in skeletal responses to iPTH treatment. Significantly blunted/absent responses to iPTH are observed when PTH receptors are not present in DMP1-expressing cells (Saini et al. 2013; Delgado-Calle et al. 2016). Canonical PTH receptor signaling via $G_s\alpha$/cAMP in osteoblast lineage cells is required for iPTH-induced gains in bone mass (Sinha et al. 2016).

Because SOST is down-regulated by iPTH treatment and intact WNT signaling is required for mice to respond to iPTH (Kedlaya et al. 2016), it has been of significant interest to determine whether PTH-induced SOST down-regulation is required for iPTH-induced bone anabolism. iPTH effects have been tested in two distinct SOST transgenic overexpressing strains. When a human SOST bacterial artificial chromosome is used to overexpress sclerostin in bone, iPTH responses are significantly blunted (Kramer et al. 2010). In contrast, when similar experiments are performed using a SOST transgene driven by the DMP1 promoter, iPTH treatment boosts bone mass in a normal manner (Delgado-Calle et al. 2016). It is likely that differences between these two transgenic models account for discordant results. However, the fact that SOST-deficient mice still boost bone mass in response to iPTH (Kramer et al. 2010) provides definitive evidence that SOST down-regulation is just one of many mechanisms used by PTH to stimulate bone formation.

Recently, significant progress has been made toward understanding the molecular mechanisms within osteocytes through which PTH regulates target gene expression. Again, insights from human genetics have proved incredibly important in this area. Individuals with Van Buchem disease have high bone mass, resistance to fractures, and low levels of sclerostin. This rare monogenic disorder is caused by an intergenic deletion near the SOST gene that includes a key downstream enhancer region (Balemans et al. 2002). Early, pioneering work toward understanding the function of this downstream enhancer-containing region (Loots et al. 2005) ultimately identified a key binding site for the transcription factor MEF2C located 45 kb downstream from the SOST gene's transcription start site (Leupin et al. 2007). Indeed, deletion of MEF2C in osteocytes (Kramer et al. 2012) or this MEF2-binding enhancer (Collette et al. 2012) leads to low SOST expression and high bone mass.

Having identified that MEF2C is a crucial determinant of osteocytic SOST expression, an

obvious question that emerges is whether PTH blocks MEF2C-driven SOST expression. Early studies using heterologous reporter systems suggested that cAMP signaling might regulate MEF2C activity in the setting of the +45 kb SOST enhancer (Leupin et al. 2007; St John et al. 2015a). In many biologic systems, upstream signals regulate MEF2 transcriptional activity via nucleocytoplasmic shuttling of class IIa HDAC proteins, which serve as potent inhibitors of MEF2-driven gene expression (Haberland et al. 2009). PTHrP suppresses MEF2-driven chondrocyte hypertrophy (Arnold et al. 2007) by driving HDAC4 from the cytoplasm to the nucleus (Kozhemyakina et al. 2009). In UMR106 osteocytic cells, PTH-induced SOST suppression is associated with nuclear accumulation of HDAC5 (Baertschi et al. 2014).

Loss-of-function studies in conditionally immortalized Ocy454 cells (a PTH-responsive murine osteocyte-like cell line [Spatz et al. 2015; Wein et al. 2015]) and in mice reveal that deletion of both HDAC4 and HDAC5 is required to block PTH-dependent SOST down-regulation (Wein et al. 2016). Detailed studies into the signaling mechanisms upstream of PTH-induced HDAC4/5 nuclear translocation have identified salt-inducible kinase 2 (SIK2) as crucial mediators of PTH signaling in osteocytes. SIK2 is a PKA-regulated phosphoprotein; PKA-mediated SIK2 phosphorylation reduces SIK2 cellular activity (Henriksson et al. 2012). Absent PKA phosphorylation, SIK2 tonically phosphorylates class IIa HDACs and promotes their cytoplasmic sequestration. As predicted by this model, small-molecule SIK inhibitors (Clark et al. 2012; Sundberg et al. 2014, 2016) such as YKL-05-099 reduce HDAC4/5 phosphorylation, promote their nuclear translocation, and reduce SOST expression in vitro and in vivo without increasing intracellular cAMP levels (Wein et al. 2016). Surprisingly, small-molecule SIK inhibitors mimic effects of PTH beyond SOST regulation. By reducing CRTC2 phosphorylation, these agents induce RANKL expression. At the transcriptomic level, ~32% of PTH-regulated genes are coregulated by SIK inhibitor treatment. Although HDAC4/5-deficient mice show normal bone anabolic responses to iPTH, YKL-05-099 treatment boosts bone formation and bone mass in vivo (Wein et al. 2016). These studies highlight the importance of SIK2-regulated phosphoproteins (such as HDAC4/5 and CRTC2) in mediating the intracellular effects of PTH in osteocytes, and identify SIK inhibition as a novel strategy to mimic skeletal effects of PTH (Fig. 1).

Interestingly, the role of class IIa HDACs have also been studied in osteoblasts in response to two key inputs that regulate RANKL expression through distinct mechanisms: PTH and the sympathetic nervous system (SNS). Although both of these inputs induce RANKL in a cAMP-dependent manner in osteoblastic cells, SNS-induced RANKL up-regulation requires ATF4, whereas PTH-induced RANKL does not (Elefteriou et al. 2005). HDAC4 may provide a molecular explanation for this intriguing phenomenon. PTH signaling in osteoblasts reduces HDAC4 protein levels as a result of Smurf2-dependent ubiquitination. This signaling event frees MEF2C to transactivate the RANKL gene promoter. In contrast, sympathetic signaling, through unknown intracellular mechanisms, favors the accumulation of HDAC4 and drives its association with ATF4. In this setting, HDAC4 acts as an ATF4 coactivator and promotes ATF4-driven RANKL expression (Obri et al. 2014). Studies in UMR106 cells have also shown that PTH regulates HDAC4 phosphorylation and subcellular localization (Shimizu et al. 2014). Collectively, these studies provide strong support for future studies into class IIa HDACs as key signaling molecules downstream from PTH and other physiologically important inputs in bone cells.

Many PTH-regulated genes in osteocytes are not regulated in an SIK2-dependent manner in osteocytes. Therefore, additional intracellular signaling nodes downstream from the PTH receptor must exist. Nascent polypeptide-associated complex and coregulator α (αNAC) is another PKA substrate that shuttles from the cytoplasm to the nucleus on phosphorylation (Pellicelli et al. 2014). In the nucleus, αNAC associates with bZIP family transcription factors and enhances their activity (Akhouayri et al. 2005). LRP6 is one such PTH-induced aNAC target gene (Hariri and St-Arnaud 2017); a

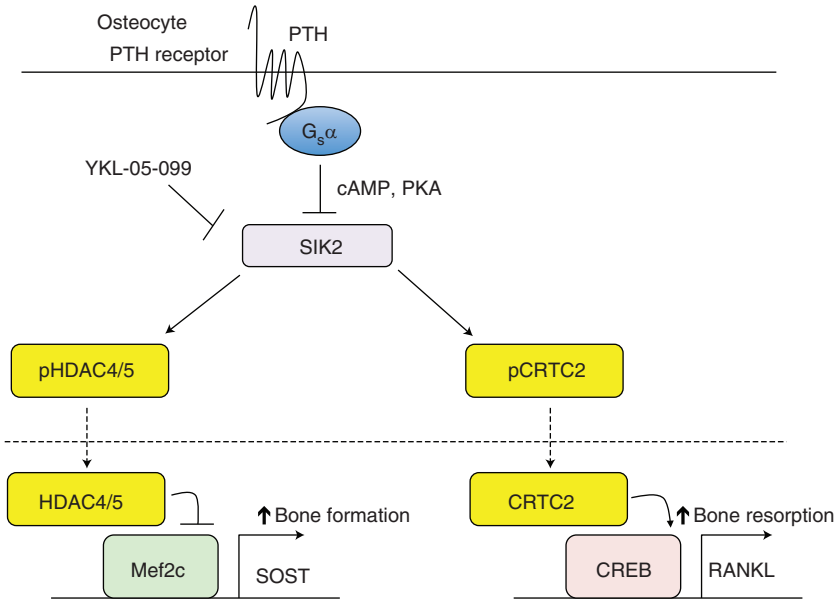

Figure 1. Model showing the intracellular signaling mechanisms used in osteocytes to regulate SOST and RANKL expression downstream from parathyroid hormone (PTH) receptor signaling. Protein kinase–mediated SIK2 phosphorylation inhibits SIK2 cellular activity, which leads to reduced phosphorylation of SIK2 substrates, including HDAC4 and CRTC2. When dephosphorylated, these proteins translocate from the cytoplasm to the nucleus where they regulate gene expression. Because PTH signaling inhibits SIK2 cellular activity, small-molecule SIK inhibitors (such as YKL-05-099) mimic many of the cellular effects of PTH. As detailed in the text, SIK2-independent protein kinase A (PKA)-dependent nodes of PTH receptor signaling are also present in osteocytes. cAMP, Cyclic adenosine monophosphate.

WNT coreceptor, LRP6, is required for iPTH-induced bone anabolism (Li et al. 2013a, 2015a). Therefore, it is likely that PTH uses complementary intra- and intercellular mechanisms in osteocytes to stimulate WNT signaling.

Beyond these focused studies on candidate signaling molecules, several groups have recently performed unbiased approaches to delineate genes regulated by PTH in osteocytes. Like Ocy454 cells, IDG-SW3 cells are a conditionally immortalized murine osteocyte-like cell line (Woo et al. 2011). RNA-seq analysis of these cells over the course of their differentiation and in response to PTH has been performed (St John et al. 2014, 2015b). Interestingly, transcriptional effects of PTH in this cell type are largely similar to those of vitamin D. PTH treatment of IDG-SW3 cells appears to cause them to revert to a less mature, more osteoblast-like phenotype. When mature IDG-SW3 cells are treated with PTH, striking morphologic changes are observed, including fewer dendritic extensions and increased motility (Prideaux et al. 2015). Although the mechanistic basis for these phenomena remain incompletely understood, effects on calcium channel gene expression may contribute. PTH increases expression of L-type (osteoblastic) calcium channels, and reduces expression of T-type (osteocytic) channels. L-type calcium channels are partially responsible for PTH-induced changes in osteocyte morphology, as evidenced by pharmacologic experiments with diltiazem (Prideaux et al. 2015).

Rapid PTH-induced changes in osteocyte morphology may provide an important mechanistic clue into how PTH and PTHrP rapidly liberate skeletal calcium stores during normal physiologic stresses such as calcium deficiency and lactation. Osteocytes can remove bone matrix during lactation by reversible remodeling of the perilacunar/canalicular network. Surprisingly, osteocytes from lactating mice express

cathepsin K and TRAP, genes traditionally thought of as osteoclast specific. Infusion of PTHrP, whose levels are normally high during lactation (Kovacs and Kronenberg 1997), mimics many of these changes. Furthermore, osteocytes lacking PTH receptors fail to undergo perilacunar remodeling during lactation (Qing et al. 2012). In addition to up-regulating cathepsin K and TRAP, PTHrP promotes osteocytic expression of ATP6V0D2, a vacuolar ATPase associated with osteoclastic bone resorption. Indeed, lactating calcium-deficient mice show reduced perilacunar pH, as assessed using a novel green fluorescent protein (GFP)-based reporter system (Jahn et al. 2017). PTH/PTHrP-dependent regulation of perilacunar pH may represent a rapid mechanism for osteocytes to liberate readily accessible pools of calcium. Future studies will be needed to assess the relative contribution of this pathway versus osteoclastic bone resorption. Based on recent clinical data indicating beneficial effects of the PTHrP analog abaloparatide at predominantly cortical sites (Miller et al. 2016), it will be important to study differences between PTH and PTHrP in inducing osteocytic gene expression and perilacunar remodeling.

SUMMARY

Basic, translational, and clinical research over the past three decades has identified PTH and its analogs as important bone anabolic drugs for osteoporosis, and illuminated many of the cellular and molecular mechanisms through which these agents regulate bone remodeling. As we have discussed, there is no one single mechanism to explain how iPTH treatment increases bone formation and bone mass. Instead, multiple complementary mechanisms coordinately explain the potent effects of this hormone, which evolved as the central regulator of calcium metabolism, on skeletal physiology. Of course, PTH did not evolve to be exploited as an osteoporosis treatment agent. Rather, its key physiologic role is to regulate mineral ion homeostasis. The action of continuously administered PTH to increase bone formation may be useful to preserve bone in the face of an increase in bone resorption, or may be an "accidental" reflection of a normal action of paracrine PTHrP on the PTH receptor. Although research-intensive efforts have focused on the cellular and molecular mechanisms through which iPTH boosts bone mass, bone loss caused by continuous hyperparathyroidism remains a major problem for afflicted patients. We have highlighted areas in which knowledge is lacking regarding molecular effects of continuous hyperparathyroidism. In addition, a thorough understanding of how iPTH therapy affects bone will be necessary to design new and improved future osteoporosis treatments.

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Marc N. Wein and Henry M. Kronenberg

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