Calcium and bone homeostasis are intimately related. On the one hand, bone relies on a sufficient supply of calcium to maintain its structural and mechanical properties and thus largely depends on calcium absorption in the intestine and calcium reabsorption in the kidney. On the other hand, bone serves as a calcium reserve from which calcium is mobilized to maintain normal calcium levels in blood. A negative external calcium balance will therefore at all times impair skeletal integrity. In addition to the external calcium balance, skeletal homeostasis also depends on the proper differentiation and functioning of bone cells, which relies for a large part on intracellular Ca$^{2+}$ signaling. Members of the transient receptor potential (TRP) family of ion channels affect skeletal homeostasis by mediating processes involved in the extracellular as well as intracellular Ca$^{2+}$ balance, including intestinal calcium absorption (TRPV6), renal calcium reabsorption (TRPV5), and differentiation of osteoclasts (TRPV1, TRPV2, TRPV4, TRPV5), chondrocytes (TRPV4), and possibly osteoblasts (TRPV1). In this review, we will give a brief overview of the systemic calcium homeostasis and the intracellular Ca$^{2+}$ signaling in bone cells with special focus on the TRP channels involved in these processes.

Keywords: bone, TRP channels, calcium, calcium channels, osteoblast, osteoclast, intestine, kidney

INTRODUCTION
Calcium is crucial for bone homeostasis, as the skeleton is influenced both by the external Ca$^{2+}$ balance as well as by the intracellular Ca$^{2+}$ signaling in bone cells. First of all, the proper functioning of the skeleton relies on normal serum calcium levels, but bone also plays an important role in the maintenance of systemic calcium homeostasis. Indeed, 99% of the bodily calcium is stored in bone where it contributes to its mechanical and structural properties. Consequently, bone requires a sufficient supply of calcium to maintain skeletal integrity. This calcium supply mainly depends on calcium absorption in the intestine and calcium reabsorption in the kidney. A failure of one of these processes, may occur during insufficient dietary calcium intake, aging, and gastrointestinal/renal disorders, therefore greatly increases the risk of a calcium imbalance and (osteoporotic) bone loss (Bullamore et al., 1970; Bikle, 2007). These disorders will not only reduce the calcium supply to bone but may also actively reduce the calcium content in bone as the safeguarding of normal serum Ca$^{2+}$ levels will always predominate over the calcium storage in bone. Consequently, bone will contribute significantly to the external calcium balance by displacing calcium to serum. Although this system is crucial to maintain normal serum Ca$^{2+}$ levels and thus to guarantee the optimal functioning of multiple vital processes, the effect on bone strength is devastating with an increased fracture risk as a consequence.

Secondly, intracellular Ca$^{2+}$ is also an important second messenger in bone cells. Intracellular Ca$^{2+}$ signaling in osteoblasts, osteoclasts, chondrocytes, and nerve endings has been shown to regulate numerous functions, including differentiation, signal transduction, and sensing of mechanical, osmotic, and pain stimuli. The fine-tuning of the intracellular Ca$^{2+}$ levels is thus crucial for normal bone homeostasis, and abnormalities in the transporters involved in Ca$^{2+}$ signaling will unambiguously lead to diseases that also affect bone structure or function (Blair et al., 2007).

The maintenance of extra- and intracellular Ca$^{2+}$ homeostasis is thus crucial for bone biology, and depends for a large part on Ca$^{2+}$ channels. Several types of Ca$^{2+}$ channels exist including (i) ryanodine receptors (RyR) and inositol-1,4,5-trisphosphate receptors (IP$_{3}$R), which mediate the release of Ca$^{2+}$ from the endoplasmic reticulum (ER), (ii) store-operated calcium channels (SOC), which include ORAI1 and STIM1 and which mediate the flux of extracellular Ca$^{2+}$ into the ER via ORAI1 upon intracellular store depletion sensed by STIM1, (iii) voltage-gated Ca$^{2+}$ channels (VGCC) that allow Ca$^{2+}$ influx upon cell depolarization, (iv) stretch-activated Ca$^{2+}$ channels that mediate Ca$^{2+}$ influx after mechanical stimulation, and (v) the transient receptor potential (TRP) family of cation channels (Robinson et al., 2010). In this review, we will focus on the involvement of the TRP channels. The TRP family consists of 28 members which have diverse physiological functions. They are subdivided based on their sequence into six subfamilies, i.e., TRPC (canonical), TRPV (vanilloid), TRPP (polycystin), TRPM (melastatin), TRPA (ankyrin), and TRPML (mucolipin). Especially the members of the TRPV family of ion channels are involved in the extracellular calcium homeostasis and intracellular Ca$^{2+}$ signaling in bone cells. The TRPV family consists of six members, which are all membrane proteins composed of six transmembrane domains that form a cation-permeable pore region. TRPV1–4 are non-selective cation channels, whereas TRPV5 and six are highly Ca$^{2+}$ selective. TRPV channels are activated by a variety of stimuli. TRPV1 is activated by heat, noxious stimuli, a low pH, and numerous chemicals. TRPV2–4 share
their activation by heat albeit at different thresholds. Moreover, TRPV2 and 4 are activated by hypotonicity. TRPV1–4 are therefore involved in nociception, thermo-, and mechanosensing. TRPV5 and 6 are different from the other TRPV channels in that they are highly selective to Ca\(^{2+}\) and that they are not activated by heat but by low intracellular Ca\(^{2+}\) levels. An extensive overview of the structural and functional aspects of the different members of the TRP family is beyond the scope of this manuscript, but can be found in the following articles (Hoenderop et al., 2005; Pedersen et al., 2005; Nilius et al., 2007). We will focus on the TRP channels that are involved in the maintenance of the external calcium balance (TRPV5, TRPV6) and contribute to intracellular Ca\(^{2+}\) signaling in bone cells (TRPV5, TRPV4, TRPV2, TRPV1; Figure 1). We will first give a general overview of calcium homeostasis (see Regulation of the External Calcium Balance and its Repercussion on Bone Homeostasis) followed by a synopsis on intracellular Ca\(^{2+}\) signaling in bone cells (see Intracellular Ca\(^{2+}\) Signaling in Bone Cells). In each of these two parts we mention the most important Ca\(^{2+}\) channels and briefly describe the TRPV channels involved. Finally, we will discuss in more detail the functions of each of the specific TRPV channels in the different tissues and cell types that have a role in calcium homeostasis and bone biology (see Involvement of TRP Channels in Bone Biology).

**REGULATION OF THE EXTERNAL CALCIUM BALANCE AND ITS REPERCUSSION ON BONE HOMEOSTASIS**

The regulation of systemic calcium homeostasis is directed at maintaining serum Ca\(^{2+}\) levels within a very narrow range. Normocalcemia is achieved by a complex endocrine system that modulates calcium absorption in the intestine, net calcium secretion in the kidney, and calcium deposition/mobilization from bone. Briefly, small changes in blood Ca\(^{2+}\) levels trigger the secretion of parathyroid hormone (PTH) from the parathyroid gland. PTH, in turn, promotes the renal formation of active vitamin D, 1,25(OH)\(_2\)D, from its inactive precursor. 1,25(OH)\(_2\)D action is key to ensure normocalcemia by regulating processes in the different calcemic target tissues. Indeed, 1,25(OH)\(_2\)D enhances intestinal calcium transport and renal calcium reabsorption. When these adaptations are insufficient to restore normocalcemia, 1,25(OH)\(_2\)D triggers a shift of calcium from bone to serum. Further activation of this endocrine control system is suppressed by the normalization of serum Ca\(^{2+}\) levels and by the several negative feedback loops (Figure 2; Bouillon et al., 2008; Lieben et al., 2011).

Since the diet is the only source of calcium, calcium absorption in the intestine is a crucial first step in maintaining systemic calcium homeostasis. Intestinal calcium transport consists of two pathways: a passive, paracellular mechanism that depends on high dietary calcium levels, and an active pathway that predominates when calcium intake is normal/low and in particular this mechanism is stimulated by 1,25(OH)\(_2\)D. Active calcium transport is considered to involve a transcellular mechanism with calcium entry through an apical Ca\(^{2+}\) channel, followed by intracellular calcium binding to members of the Calbindin family (Calbindin-D\(_{9k}\)) and extrusion to the extracellular milieu by the Ca\(^{2+}\)-ATPase, PMCA\(_{1b}\) (Figure 2; Van Cromphaut et al., 2001). One member of the TRP family, namely TRPV6, has long been considered to be the rate-limiting step in the active transport of calcium across the enterocyte by mediating apical Ca\(^{2+}\) transfer.

**FIGURE 1 | Involvement of TRP channels in the external calcium balance and intracellular Ca\(^{2+}\) signaling in bone cells.** Bone homeostasis depends on the external calcium balance which determines whether calcium is stored in or released from bone, and consequently affects skeletal strength. In addition, bone homeostasis is also influenced by intracellular Ca\(^{2+}\) signaling in bone cells, which regulates their differentiation and functioning. Several TRP channels contribute to both types of processes and more specifically modulate intestinal calcium transport (TRPV6), renal calcium reabsorption (TRPV5), and differentiation and functioning of osteoblasts (TRPV1), osteoclasts (TRPV5, TRPV4, TRPV2, TRPV1), and chondrocytes (TRPV4). Moreover, TRPV1 expressed in the neurons that innervate bone, plays a crucial role in the sensing of bone pain.
A reduction in serum Ca\(^{2+}\) levels triggers the release of PTH and the production of 1,25(OH)\(_{2}\)D, the active hormone. 1,25(OH)\(_{2}\)D in turn modulates several processes that will ultimately lead to normalization of serum Ca\(^{2+}\) levels: it will increase intestinal and renal calcium transport, and when these adaptations are insufficient, 1,25(OH)\(_{2}\)D will mobilize calcium from bone by stimulating osteoclastic bone resorption and by inhibiting bone mineralization.

**Figure 2** | The regulation of the external calcium balance. A reduction in serum Ca\(^{2+}\) levels triggers the release of PTH and the production of 1,25(OH)\(_{2}\)D, the active hormone. 1,25(OH)\(_{2}\)D in turn modulates several processes that will ultimately lead to normalization of serum Ca\(^{2+}\) levels: it will increase intestinal and renal calcium transport, and when these adaptations are insufficient, 1,25(OH)\(_{2}\)D will mobilize calcium from bone by stimulating osteoclastic bone resorption and by inhibiting bone mineralization.

(Hoenderop et al., 2005). Recent genetic studies confirm that TRPV6 contributes to active intestinal calcium transport, but they also indicate that this channel is not essential for this process (Benn et al., 2008). A detailed overview of the role of TRPV6 in intestinal calcium transport and bone homeostasis will be given in Section “TRPV6.”

Fine-tuning of renal calcium reabsorption also contributes to the maintenance of normocalcemia. Half of the unbound serum Ca\(^{2+}\) is intrinsically filtered in the glomerulus, the bulk of this filtered Ca\(^{2+}\) is passively reabsorbed in the nephron. Reabsorption of the remaining 15% involves an active transport pathway, that resembles intestinal calcium transport but relies on other mediators, i.e., calcium entry via TRPV5, cytosolic binding to Calbindin-D\(_{28k}\) and extrusion by the Na\(^{+}\)/Ca\(^{2+}\) exchanger (NCX1) and PMCA\(_{1b}\) (Figure 2; Bouillon et al., 2008). In contrast to the limited role of TRPV6 in intestinal calcium transport, genetic evidence has confirmed that TRPV5 is the essential and rate-limiting step in renal calcium reabsorption (Hoenderop et al., 2003), which will be discussed in more detail in Section “TRPV5.”

Calcium stored in bone has two important functions, i.e., providing mechanical strength and acting as a calcium reserve. The inherent consequence of this dual role is that it will be difficult, or even impossible to fulfill these two functions when the external calcium balance is negative and experimental evidence indicates that it will be secured by all means. Consequently, a negative calcium balance will lead to bone loss, which hampers the mechanical function of the skeleton and may result in an increased fracture risk. We recently showed that in mice, increased 1,25(OH)\(_{2}\)D levels play a crucial role in this shift of calcium from bone to serum that occurs during a negative calcium balance. Mechanistically, increased 1,25(OH)\(_{2}\)D levels enhance osteoclastic bone resorption and suppress bone matrix mineralization by increasing the osteoblastic expression of mineralization inhibitors including Osteopontin and pyrophosphates (Figure 2; Lieben et al., 2012). The preservation of normal calcium levels in blood and bone is thus closely interconnected, and this situation also implies that the skeleton not only depends on, but moreover participates in the external calcium balance. It is therefore evident that abnormalities in the external calcium balance due to loss of TRPV5 (and TRPV6) will also affect bone homeostasis (see TRPV6 and TRPV5).

**Intracellular Ca\(^{2+}\) Signaling in Bone Cells**

Normal bone homeostasis does not only depend on extracellular calcium, but also on intracellular Ca\(^{2+}\) signaling cascades that regulate the differentiation and functioning of multiple bone cells. In the next section, we will give a concise overview of the molecules involved in intracellular Ca\(^{2+}\) signaling in the bone resorbing osteoclasts (see Ca\(^{2+}\) Signaling in Osteoclasts), the bone forming osteoblasts (see Ca\(^{2+}\) Signaling in Osteoblasts), and the chondrocytes which contribute to endochondral bone development and form the articular cartilage (see Ca\(^{2+}\) Signaling in Chondrocytes).

**Ca\(^{2+}\) Signaling in Osteoclasts**

Recent genetic studies have provided substantial insight in the molecular mechanisms that are involved in and regulate osteoclastic Ca\(^{2+}\) signaling and differentiation. In short, binding of macrophage-colony stimulating factor (M-CSF) to its receptor on osteoclasts precursors promotes their proliferation and survival and induces receptor activator of nuclear factor-κB (RANK) expression (Negishi-Koga and Takayanagi, 2009). Osteoclast differentiation is initiated by the simultaneous activation of RANK after binding to RANK-ligand (RANKL), and of the immunoreceptor tyrosine-based activation motif (ITAM)-associated immunoglobulin-like receptor (IgLR; Takayanagi et al., 2002; Koga et al., 2004). These signaling pathways induce the activation of phospholipase C\(_{γ}\) (PLC\(_{γ}\)). PLC\(_{γ}\) produces IP\(_{3}\), which...
evokes Ca\(^{2+}\) release from the ER via IP\(_3\) receptors, that will subsequently lead to typical Ca\(^{2+}\) oscillations (Kuroda et al., 2008). In vitro findings suggest that TRP channels (TRPV2, see TRPV2; Kajiya et al., 2010) are likely one of the Ca\(^{2+}\) entry pathways that contribute to the Ca\(^{2+}\) oscillations (Kajiya et al., 2010; Hwang and Putney, 2011). With respect to the role of the SOCE, the reported findings are still limited. In this regard, in vivo inactivation of Orai1 in mice is reported to impair the formation of multinucleated osteoclasts and to reduce bone resorption. Consistent herewith, in vitro inhibition of Orai1 in a murine monocyte/macrophage cell line (chemical and siRNA) decreased osteoclastogenesis (Hwang and Putney, 2011; Robinson et al., 2012). Yet conclusive genetic confirmation based on the generation of osteoclast-specific Orai1 null mice is still lacking and the contribution of ORAI1 to Ca\(^{2+}\) signaling during osteoclast differentiation remains elusive. No VGCC’s have been detected thus far in osteoclasts, implying that it is unlikely that they play a role in osteoclast differentiation (Blair et al., 2007). The Ca\(^{2+}\) oscillations turn on a number of Ca\(^{2+}\)/calmodulin-dependent proteins including calcineurin and calmodulin-dependent protein kinases (CaMK). Upon activation of the phosphatase calcineurin, the transcription factor NFATC1 (the nuclear factor of activated T cells 1) becomes phosphorylated, translocates to the nucleus, and increases osteoclast-specific gene transcription. NFATC1 is the master regulator of osteoclast differentiation. For more in-depth information, we refer to the review article by Negishi-Koga and Takayanagi (2009).

FIGURE 3 | Intracellular Ca\(^{2+}\) signaling in osteoclasts. (A) Early during osteoclast differentiation, activation of the RANK, and IgLR receptors induces intracellular Ca\(^{2+}\) oscillations by intracellular Ca\(^{2+}\) release via the IP\(_3\)-R, and possibly via Ca\(^{2+}\) influx through store-operated Ca\(^{2+}\) channels or TRP (TRPV2) channels. The Ca\(^{2+}\) oscillations activate Ca\(^{2+}\)/calmodulin-dependent kinases (CaMK, calcineurin) which induce CREB expression. In addition, CREB induces cFOS in the AP (activator protein) 1 complex, which contributes to the autoamplification of Nfatc1 (Sato et al., 2006). The RANK- and IgLR-induced Ca\(^{2+}\) oscillations are thus crucial in the initiation of osteoclastogenesis by promoting NFATC1 and CREB activity (Figure 3A).

Ca\(^{2+}\) oscillations disappear during osteoclast differentiation and are replaced by a sustained Ca\(^{2+}\) influx via members of the TRP family, including TRPV4 (see TRPV4; Masuyama et al., 2008) and possibly TRPV5 (see TRPV5; Chamoux et al., 2010; Figure 3B). The Ca\(^{2+}\) oscillations followed by the sustained Ca\(^{2+}\) influx are both needed for NFATC1 activation and proper osteoclast differentiation. For more in debt information, we refer to the review article by Negishi-Koga and Takayanagi (2009).

Ca\(^{2+}\) SIGNALING IN OSTEOBLASTS

In contrast to the osteoclasts, little is known about the molecular mechanisms mediating osteoblastic Ca\(^{2+}\) signaling (Blair et al., 2007; Figure 4). Osteoblasts express different families of Ca\(^{2+}\) channels, including members of the store-operated (ORAI1), the stretch-activated, the voltage-gated, and the TRP family (TRPV6, see TRPV6) of calcium channels. VGCC’s are important for proper osteoblast functioning and more specifically for the propagation of calcium waves across neighboring osteoblasts upon mechanical stimulation. Recent in vitro studies have demonstrated that the sensitivity and the dynamics of the calcium waves are even greater in finally differentiated osteoblasts, i.e., osteocytes, which are believed to be the true mechanosensors of bone. The changes in the calcium waves with differentiation are attributed to the presence of a different subset of VGCC’s,
i.e., L-type VGCC’s in osteoblasts versus the T-type VGCC’s in osteocytes (Lu et al., 2012).

In addition, Ca$$^{2+}$$ signaling is very plausibly involved in osteoblast differentiation. Chemical inhibition of calcineurin activity causes a decrease in bone mass and acts by impairing bone osteoblast differentiation. Chemical inhibition of calcineurin subunit stimulates bone formation (Koga et al., 2005). Similar results are obtained when the calcineurin A$$\alpha$$ isoform is genetically inactivated (Sun et al., 2005). In contrast, osteoblast-specific inactivation of the calcineurin B subunit stimulates bone formation (Yeo et al., 2007), indicating that the role of calcineurin in osteoblast differentiation requires further investigation. The results regarding the role of NFAT signaling in osteoblasts, on the other hand, are more consistent, i.e., genetic inactivation of NFATc1 and NFATc2 in osteoblasts impairs signaling in osteoblasts. The results regarding the role of NFAT-mediated gene transcription by Ca$$^{2+}$/calcineurin. The Ca$$^{2+}$$ entry pathways are incompletely characterized but ORAI1 is likely involved.

Ca$$^{2+}$$ SIGNALING IN CHONDROCYTES

Chondrocytes express several Ca$$^{2+}$$ channels, including VGCC’s, stretch-activated Ca$$^{2+}$$ channels, and TRP channels (TRPV4), which are involved in chondrocyte differentiation and osmosensing. First of all, calcium influx via the L-type VGCC’s and TRPV4 is required to activate a Ca$$^{2+}$/calmodulin signaling cascade that promotes chondrocyte differentiation. Consequently, suppression of L-type VGCC’s (Mancilla et al., 2007), TRPV4 (Muramatsu et al., 2007), and Ca$$^{2+}$/calmodulin (Taschner et al., 2008) signaling hampers chondrocyte differentiation in rat metatarsal cultures, chondrogenic cell lines, and chicken chondrocytes respectively. Thus, Ca$$^{2+}$$ influx via VGCC’s and TRPV4 (see TRPV4) is required for proper chondrogenesis (Figure 5A).

Secondly, Ca$$^{2+}$$ signaling is involved in the osmosensing properties of chondrocytes, which are essential to maintain joint health. The articular chondrocytes in particular are exposed to large variations in their osmotic environment due to changes in the water content of the cartilage matrix upon loading and unloading of the joint. Chondrocytes respond directly to this osmotic stress by promoting Ca$$^{2+}$$ influx and increasing intracellular Ca$$^{2+}$$ levels which leads to volumetric changes. Abnormalities in this system result in or fasten the progression of rheumatoid arthritis. Recent studies have provided compelling evidence that TRPV4 is critically involved in the sensing of hypo-osmotic stimuli (Guilak et al., 2010), which we will further discuss in Section “TRPV4” (Figure 5B).

INVolVEMENT OF TRP CHANNELS IN Bone BIOLoGY

Transient receptor potential channels may affect skeletal homeostasis not only by regulating the external calcium balance but also by directly altering Ca$$^{2+}$$ signaling in bone cells. The TRP channels that are known to be involved in one of these processes will be discussed in more detail (Figure 1).

TPRV6

Active intestinal calcium transport is required when dietary calcium intake is normal/low. This pathway is stimulated by 1,25(OH)$$\text{$_2$}$D and involves calcium entry mediated in part by TPRV6, intracellular calcium binding to Calbindin-D$_{k}$, and basolateral extrusion of calcium via PMCA$_{1b}$ (see Regulation of the External Calcium Balance and its Repercussion on Bone Homeostasis; Figure 2). TPRV6 has for long been considered
to be the rate-limiting step in active intestinal calcium absorption, based on the finding that intestinal TRPV6 levels are altered in parallel with 1,25(OH)₂D activity and inversely with dietary calcium intake. Indeed, Trpv6 mRNA levels are upregulated by 1,25(OH)₂D treatment and a low dietary calcium intake, whereas Trpv6 expression is reduced in Vdr null mice or in response to a high calcium intake (Van Cromhout et al., 2001).

Genetic studies confirm that TRPV6 is involved in intestinal calcium transport, but they also show that TRPV6 is not essential for this pathway. Indeed, active intestinal calcium transport is only minimally affected in Trpv6 and Trpv6/Calbindin-D₉k double null mice when mice are fed a diet containing a normal level of calcium (Bianco et al., 2007; Benn et al., 2008). In addition, 1,25(OH)₂D treatment results in a similar increase in intestinal calcium transport in Trpv6⁻/⁻ and Trpv6/Calbindin-D₉k⁻/⁻, and wild-type mice (Benn et al., 2008; Kutuzova et al., 2008). Dietary calcium deprivation also stimulates intestinal calcium transport in all genotypes, yet the increase was less profound in the Trpv6⁻/⁻ and Trpv6/Calbindin-D₉k⁻/⁻ mice (Bianco et al., 2007; Benn et al., 2008). Altogether, these results indicate that TRPV6 is redundant for intestinal calcium absorption during normal calcium intake, but that this Ca²⁺ channel does contribute to this process when dietary calcium levels are low.

Thus, TRPV6 contributes only minimally to intestinal calcium transport during normal calcium intake. Consequently, Trpv6⁻/⁻ mice have normal serum calcium levels (Bianco et al., 2007; Benn et al., 2008) and display no abnormalities in bone homeostasis, evidenced by normal bone mass and remodeling parameters. Also the growth plate morphology is not affected which accords with normal serum calcium and phosphate levels (Lieben et al., 2010). Similarly, knock-in mice with a replacement of one amino acid of the Trpv6 gene (Trpv6D541A/D541A mice) that impairs the Ca²⁺ permeability of TRPV6, do not display changes in bone mass and morphology, yet their bone size is slightly reduced (van der Eerden et al., 2012). In contrast to the redundancy of TRPV6 during normal calcium intake, TRPV6 is required for optimal intestinal calcium absorption when dietary calcium is limited. Accordingly, Trpv6 null mice on a low calcium diet could maintain normal serum calcium levels, yet their bone matrix mineralization is more profoundly reduced compared to wild-type mice (Lieben et al., 2010). Thus, TRPV6 is essential to ensure adequate intestinal calcium absorption during dietary calcium deprivation, and hereby prevents an excessive decrease in bone mineralization. These findings also underscore the essential role of adequate dietary calcium absorption for skeletal health.

The absence of bone abnormalities during normal calcium intake indicates that TRPV6 only indirectly affects the skeleton via its involvement in intestinal calcium transport. Yet, TRPV6 is expressed in osteoblasts and osteoclasts, albeit at very low levels (1% of intestinal levels; Lieben et al., 2010; Little et al., 2011), and may thus in theory directly regulate their differentiation or functioning. Recent studies have however convincingly demonstrated that TRPV6 is not involved in osteoblastic Ca²⁺ uptake (Little et al., 2011) nor mineralization (van der Eerden et al., 2012). Altogether, these results confirm that TRPV6 lacks a direct role in bone metabolism (Figure 4).

In conclusion, TRPV6 affects skeletal metabolism by promoting intestinal calcium transport, and is especially required during dietary calcium deprivation.

**TRPV5**

TRPV5 is critically involved in renal calcium reabsorption (see Regulation of the External Calcium Balance and its Repercussion on Bone Homeostasis; Figure 2). Indeed, inactivation of Trpv5 results in a decrease in renal calcium reabsorption, leading to severe urinary calcium loss. Normal serum calcium levels are maintained via a compensatory, 1,25(OH)₂D-mediated increase in intestinal calcium absorption. Furthermore, cortical bone mass is reduced in Trpv5⁻/⁻ mice, which is associated with an increase in osteoclast number, though bone resorption parameters are reduced (Hoenderop et al., 2003). These results convincingly demonstrate that TRPV5 is important for systemic calcium homeostasis by fine-tuning renal calcium reabsorption.

The skeletal defect in Trpv5⁻/⁻ mice may be due to the abnormal external calcium balance and/or to direct changes in osteoclastic Ca²⁺ signaling. Of note, TRPV5 is not expressed in osteoblasts. In osteoclasts, on the other hand, TRPV5 is found at the ruffled border, where it likely contributes to bone resorption. van der Eerden et al. reported that the in vitro differentiation of hematopoietic precursors isolated from Trpv5 null mice leads to more and larger osteoclasts, but with severely impaired resorptive capacity. The resorption deficit is attributed to the lack of TRPV5 activity, whereas the enhanced osteoclastogenesis is explained by an increased priming of the osteoclast precursors by the high 1,25(OH)₂D levels in Trpv5⁻/⁻ null mice. These findings are in agreement with the increased osteoclast number but reduced bone resorption observed in Trpv5 null mice, yet it is still unclear how these findings explain and correlate with the reduced bone mass in vivo (van der Eerden et al., 2005). Chamoux et al. reported that TRPV5 mediates the RANKL-induced sustained Ca²⁺ influx in more differentiated human osteoclasts in vitro, and surprisingly, they observed that inhibition of TRPV5 leads to increased resorption pit formation in vitro. Unfortunately, data concerning the effect of Trpv5 inactivation on osteoclast differentiation itself is lacking in this study (Chamoux et al., 2010). Thus, TRPV5 is present at the ruffled border of resorbing osteoclasts, but its exact role in osteoclastic Ca²⁺ signaling, osteoclastogenesis, and bone resorption is still unclear (Figure 3).

In conclusion, TRPV5 mediates calcium reabsorption in the kidney, and is hereby important for the maintenance of systemic calcium and bone homeostasis. In addition, TRPV5 is expressed in osteoclasts and may directly regulate osteoclast differentiation and/or functioning.

**TRPV4**

That TRPV4 has a profound role in bone homeostasis is well demonstrated by the skeletal defects resulting from TRPV4 mutations in humans. Activating missense mutations in TRPV4 lead to a wide spectrum of autosomal-dominant skeletal dysplasias ranging from mild to lethal forms, and are characterized by cartilage and
mineralization defects (Rock et al., 2008; Krakow et al., 2009). This finding, together with the observation that TRPV4 is expressed in chondrocytes, osteoclasts, and osteoblasts, suggests that TRPV4 directly controls the functioning of skeletal cells.

We showed that TRPV4 is present on the basolateral membrane of osteoclasts and is required for osteoclast differentiation by regulating intracellular Ca\(^{2+}\) signaling (see Ca\(^{2+}\) Signaling in Osteoclasts; Figure 3). More specifically, TRPV4 mediates the sustained Ca\(^{2+}\) influx that occurs in the late stages of osteoclast differentiation, when the Ca\(^{2+}\) oscillations are no longer present (Masuyama et al., 2008; Negishi-Koga and Takayanagi, 2009). This TRPV4-mediated Ca\(^{2+}\) influx secures intracellular Ca\(^{2+}\) concentrations, ensures NFATc1-regulated gene transcription, and regulates the terminal differentiation and activity of the osteoclasts. Indeed, intracellular Ca\(^{2+}\) levels, NFATc1 activity, osteoclast differentiation and resorptive capacity are reduced in Trpv4\(^{-/-}\) osteoclasts. No difference in the percentage of oscillating cells, the frequency or the amplitude of the Ca\(^{2+}\) oscillations is observed between Trpv4\(^{-/-}\) and Trpv4\(^{+/+}\) osteoclasts, implying that TRPV4 is not involved in the generation or the maintenance of the Ca\(^{2+}\) oscillations. In agreement with the in vitro findings, bone mass is increased in adult Trpv4\(^{-/-}\) mice, and this is accompanied by a reduction in osteoclast abundance and bone resorption. Of note, TRPV4 inactivity does not affect systemic calcium homeostasis nor bone formation (Masuyama et al., 2008). Thus, Ca\(^{2+}\) oscillations and sustained Ca\(^{2+}\) influx via TRPV4 are sequentially required for osteoclast differentiation. In agreement with these findings, Trpv4\(^{-/-}\) mice are also protected against unloading-induced bone loss (Mizoguchi et al., 2008). In addition, a recent study shows that activation of TRPV4 specifically in osteoclasts increased osteoclast differentiation, which leads to bone loss. The authors also demonstrate that TRPV4 not only affects osteoclast differentiation via the Ca\(^{2+}\)-mediated activation of NFATc1, but that TRPV4 also influences osteoclast migration and fusion via Ca\(^{2+}\)-calmodulin-mediated effects on myosin II (Masuyama et al., 2012). Thus, TRPV4 is critically involved in osteoclast differentiation and migration, by mediating the sustained Ca\(^{2+}\) influx in more differentiated osteoclasts.

TRPV4 is also expressed in chondrocytes, where it modulates differentiation and osmotically induced Ca\(^{2+}\) signaling (see Ca\(^{2+}\) Signaling in Chondrocytes; Figure 5). First of all, TRPV4 activation is found to induce SOX9 expression, a master regulator of chondrogenesis (Bi et al., 1999) through a Ca\(^{2+}\)/calmodulin pathway, and by doing so, TRPV4 potentiates chondrogenic differentiation in vitro (Muramatsu et al., 2007). This finding indicates that TRPV4 is positively involved in chondrogenesis. Secondly, TRPV4 mediates the chondrocyte response to hypo-osmotic stress, which is required to maintain joint health. Indeed, suppression of TRPV4 activity abolishes the osmotic sensitivity of chondrocytes, impairs the increase in intracellular Ca\(^{2+}\) levels, and suppresses the volume decrease (Phan et al., 2009). Chemical disruption of the primary cilium also eliminates the Ca\(^{2+}\) signaling in response to an activator of TRPV channels and hypo-osmotic stimuli (Phan et al., 2009). This finding suggests that an intact cilium is required for TRPV4-mediated Ca\(^{2+}\) signaling. Remarkably, TRPV4 is distributed all over the plasma membrane, and was thus not strictly localized to the cilium (Phan et al., 2009). Consistent with these in vitro findings, Trpv4\(^{-/-}\) mice develop early and severe osteoarthritis with progressive calcification of the joint tissue (Clark et al., 2010). Altogether, TRPV4 has a chondroprotective role by mediating the response to hypo-osmotic stimuli.

Thus, TRPV4 directly regulates differentiation and functioning of osteoclasts and chondrocytes by mediating intracellular Ca\(^{2+}\) influx and signaling.

TRPV2

TRPV2 is expressed in osteoclasts and is likely involved in the Ca\(^{2+}\)-permeable pathway that mediates osteoclastic Ca\(^{2+}\) oscillations (see Ca\(^{2+}\) Signaling in Osteoclasts; Figure 3). In fact, inhibition of TRPV2 hampers the RANKL-induced Ca\(^{2+}\) oscillations, NFATc1 activation, and osteoclastogenesis in vitro (Kajiya et al., 2010), yet conclusive in vivo evidence is currently lacking.

TRPV1

TRPV1 is expressed in osteoblasts and osteoclasts and promotes the differentiation of both cell types. Indeed, pharmacological blockage of TRPV1 inhibits in vitro osteoclast and osteoblast differentiation. Moreover, in vivo suppression of TRPV1 activity protects mice against ovariectomy-induced bone loss by reducing the increase in bone resorption and formation (Idris et al., 2010). Thus, TRPV1 may directly affect osteogenic cell differentiation.

More importantly, TRPV1 is present in peripheral neurons that innervate bone, and TRPV1-mediated Ca\(^{2+}\) signaling in these neurons has an important function in the pain sensation that often accompanies bone metastases or inflammatory osteoarthrits. Indeed, growing tumor cells trigger osteoclasts to resorb bone excessively and this process induces an acid microenvironment due to the secretion of H\(^+\) by the osteoclasts. This acidity activates TRPV1 and other acid-sensing ion channels on the nociceptors that innervate bone. The associated Ca\(^{2+}\) influx triggers a series of events that are associated with pain (Nakanishi et al., 2010). Consequently, suppression of the excessive bone resorption or TRPV1 activity significantly lowers bone pain in mice (Nagae et al., 2006, 2007; Tong et al., 2010).

CONCLUSION

Bone homeostasis depends on the external calcium balance as well as on intracellular Ca\(^{2+}\) signaling that regulates bone cell functioning and differentiation. A number of TRP channels are critically involved in the regulation of these processes, including intestinal calcium absorption (TRPV6), renal calcium reabsorption (TRPV5), osteoclastogenesis (TRPV4, TRPV5, TRPV2, TRPV1), osteoblastogenesis (TRPV1), chondrocyte differentiation/functioning (TRPV4), and bone pain sensation (TRPV1). The combination of in vitro and in vivo studies have provided tremendous insight in the role of TRP channels in calcium and bone homeostasis, but some aspects still require further investigation. First of all, the finding that TRPV6 is not essential for active intestinal transport, urges the search for new Ca\(^{2+}\) channels or alternative mechanisms that mediate calcium transport in the intestine. Secondly, the mechanisms that modulate Ca\(^{2+}\) oscillations during the early of osteoclastogenesis are still largely unknown, yet of interest given that this pathway is crucial for the autoamplification of NFATc1 and thus osteoclast differentiation.
Thirdly, despite the fact that NFAT is important for osteoblast differentiation, the Ca$^{2+}$ entry mechanisms that evoke NFAT activation are largely unexplored. In a next step, in vitro studies combined with the generation of mouse models that target the different TRP channels in a cell-specific manner, will provide more insight in their role in calcium and bone homeostasis. A better understanding is required to define whether the different TRP channels may serve as therapeutic targets in conditions of bone loss. Indeed one may speculate to use TRPV5 and TRPV6 agonists to maximize the calcium availability for bone, TRPV4 modulators to suppress osteoclast differentiation or to treat genetic dysfunc-
tioning of TRPV4, etc. Thorough insight may also help to avoid possible side-effects that may occur when inhibiting/stimulating their function since TRPV channels are structurally related and thus require the development of highly specific modulators. In addition, the different TRP channels have numerous important physiological functions in different tissues, a condition that may hinder its general application. To date, only TRPV1 modulators are used in the clinic as part of analgesic therapy (Moran et al., 2011).

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