



New Advances in Osteocyte Mechanotransduction

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Accepted: 16 December 2020 / Published online: 9 January 2021

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Abstract

Purpose of Review Skeletal adaptation to mechanical loading plays a critical role in bone growth and the maintenance of bone homeostasis. Osteocytes are postulated to serve as a hub orchestrating bone remodeling. The recent findings on the molecular mechanisms by which osteocytes sense mechanical loads and the downstream bone-forming factors are reviewed.

Recent Findings Calcium channels have been implicated in mechanotransduction in bone cells for a long time. Efforts have been made to identify a specific calcium channel mediating the skeletal response to mechanical loads. Recent studies have revealed that Piezo1, a mechanosensitive ion channel, is critical for normal bone growth and is essential for the skeletal response to mechanical loading.

Summary Identification of mechanosensors and their downstream effectors in mechanosensing bone cells is essential for new strategies to modulate regenerative responses and develop therapies to treat the bone loss related to disuse or advanced age.

Keywords Mechanotransduction · Osteocyte · Bone · Mechanosensitive ion channel

Skeletal Adaptation

Bones adapt to changes in mechanical forces by changing their mass. Specifically, mechanical forces increase osteoblast number, stimulate bone formation, and increase bone mass [1, 2]. A classic example of anabolic bone adaptation is the higher bone mass that baseball players have in their throwing forearm compared with their non-throwing forearm [3]. In contrast, loss of mechanical stimuli decreases bone mass by inhibiting bone formation [4, 5] and promoting osteoclastogenesis and bone resorption [4, 6, 7]. Bone mass is rapidly lost under unloading conditions such as extreme lack of physical activity, being bedridden, or microgravity [8]. The identity of the cells responsible for directly sensing changes in mechanical loading of the skeleton is unclear.

As the most abundant resident cells in bone, osteocytes have been postulated to sense and respond to mechanical cues. They are derived from osteoblasts that become buried in the bone matrix. They are connected to one another by the lacuna-

canalicular network, which enables chemical and fluid transport between osteocytes and cells on the bone surface [9]. Changes in the mechanical forces experienced by the bone cause the rate of fluid flow inside the lacuna-canalicular system to change, and this can be detected by osteocytes [10]. In vitro evidence indicates that osteocytes are highly responsive to fluid shear stress [11, 12], and that they sense mechanical signals mainly through their long cellular processes rather than their cell bodies [13]. Being in a rigid microenvironment, osteocytes may also respond to matrix strains directly in addition to fluid shear stress [14]. The idea that osteocytes are involved in mechanosensing is partially supported by in vivo osteocyte ablation models. Tatsumi et al. showed that ablation of osteocytes in mice prevents the cancellous bone loss induced by mechanical unloading [6]. However, in the same study, when the hindlimbs of these mice are reloaded following a short period of unloading, the skeletal response is not affected by osteocyte ablation [6]. These studies indicate that osteocytes are required for the skeletal response to loss of mechanical stimulation, but not for the increase of mechanical stimulation. Other studies with loss or gain of function in osteocytes have revealed the important roles of a set of genes, such as β -catenin [15, 16], Lrp5 [17], Sost [1, 18, 19], and Piezo1 [20], in the skeletal response to mechanical load. The mouse genetic tools used in these studies to delete or over-express genes of interest in osteocytes are mainly Dmp1-Cre transgenic mice, including 8 kb Dmp1-Cre, 10 kb Dmp1-Cre,

This article is part of the Topical Collection on *Osteocytes*

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and 10 kb Dmp1-CreERT2 mice. Although osteocytes are targeted by these Cre driver strains, other cell types, including osteoblasts in particular, are also targeted [7, 21, 22]. Therefore, it is possible that the skeletal phenotype observed in these studies could be attributed to osteoblasts, osteocytes, or both. Consistent with this, *in vitro* evidence suggests that osteoblasts are also sensitive to mechanical stimulation and produce the same molecules as osteocytes in response, such as prostaglandins [23]. Thus, it is still an open question which cell type(s) in the bone senses changes in mechanical stimulation. The newly developed Sost-Cre and Sost-CreERT2 mouse models may help to answer that question since they target osteocytes but not osteoblasts [21, 24]. Studies using reporter mice showed that Dmp1-Cre transgene causes gene deletion in osteoblasts and osteocytes while the Sost-Cre transgene targets osteocytes as well as hematopoietic cells [21]. Although neither Dmp1-Cre nor Sost-Cre transgene is specific to osteocytes, they are still a common target of both transgenes. Therefore, comparison of results obtained from Sost-Cre and Dmp1-Cre models could help determine the importance of genes of interest expressed in osteocytes.

During physical activity, the skeleton encounters different types of mechanical forces, including gravitational loading and muscle contraction. While it is clear that these mechanical stimuli cause bone deformation or strain, the exact mechanisms by which strain at a whole tissue level is transduced to osteocytes and the nature of the mechanical forces that activate osteocytes *in vivo* remain unclear. Fluid shear stress has been postulated to be the major driving force for load-induced bone formation. The idea is that loading causes deformation of bone and results in interstitial pressure gradients in the bone marrow and the lacuna-canalicular system of osteocytes. These pressure gradients drive the movement of interstitial fluid in the marrow and through the lacuna-canalicular network, generating shear stress. Mechanosensitive cells in bone, such as osteocytes, perceive the change of shear stress and then orchestrate bone remodeling [25]. Based on this model, fluid shear stress has become the most widely used *in vitro* approach to study mechanotransduction in osteocytes. A more recently modified model for this transduction suggests that the fluid flow in the lacuna-canalicular system generates drag forces directly on the osteocyte tethering sites of the lacunar wall, which results in an amplified hoop strain on cell processes [26]. The magnitude of this strain is comparable with that used *in vitro* to activate osteoblastic cells. Therefore, this hoop strain has been proposed to be the driving force that activates osteocytes *in vivo*. However, an *in vitro* culture system that models this effect has not been developed.

Mechanosensors in Bone

Mechanical signals contribute to normal bone growth and skeletal homeostasis during adulthood. However, the

mechanisms by which the skeleton responds to mechanical stimulation are not fully understood. For decades, efforts have been made to identify specific mechanosensors in bone cells to pin down those mechanisms. Although a variety of cell surface proteins and structures has been proposed to facilitate perception of mechanical signals by bone cells (Fig. 1; for a revision on these mechanisms involved in osteocyte mechanotransduction, see Ref. [27–30]) [27–30], an integrated pathway explaining how osteocytes perceive and transduce mechanical signals has yet to be elucidated. Integrins are one of the surface proteins implicated in mechanosensation in osteocytes. Deletion of the $\beta 1$ integrin in osteoblasts and osteocytes using the Col1a1-cre transgene leads to a significant reduction in bone formation induced by mechanical loading compared with control mice [31]. Integrin $\alpha V/\beta 3$ is found to be expressed in osteocytes and is localized in close proximity to the purinergic channel pannexin1, the ATP-gated purinergic receptor P2X7R, and the low voltage-gated T-type calcium channel CaV3.2 in a specialized structure through which osteocyte cell processes tether to the lacunar wall [32]. These structures potentially facilitate the hoop strain amplification leading to activation of osteocytes. Recently, integrin $\alpha V/\beta 5$ has been identified as the receptor for the so-called exercise hormone Irisin [33]. Irisin levels increase dramatically with physical activity and decrease with unloading [34]. Importantly, administration of Irisin prevents the bone loss caused by hindlimb unloading [35]. *In vitro* assays show that Irisin binds to integrin $\alpha V/\beta 5$ and chemical blocking of $\beta 5$ integrin diminishes the effects of Irisin on osteocytes [33]. Therefore, integrins not only respond to physical cues directly but also respond to chemical myokines induced by mechanical stimuli.

Calcium channels are also involved in the sensing of mechanical signals by osteocytes as indicated by multiple lines of

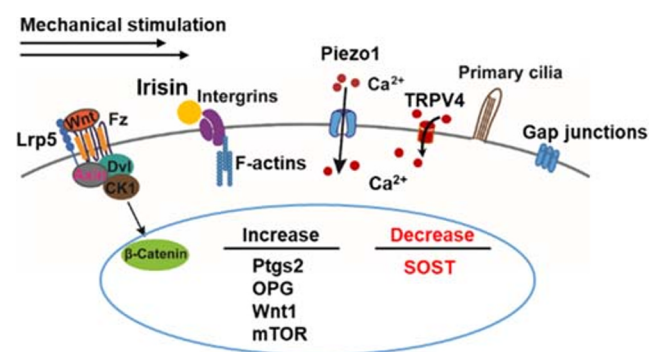


Fig. 1 Cell surface proteins and structures involved in osteocyte mechanotransduction. Wnt receptors including Lrp5, integrin-containing focal adhesions, primary cilia, voltage-gated calcium channels, and connexin-based gap junctions are the major mechanosensors being implicated in bone cells. Upon stimulation by mechanical loading, osteocytes promote osteoblast formation by increasing Wnt ligand expression and decreasing SOST expression. Mechanical stimulation also enhances osteocyte energy production by promoting mTOR signaling

evidence [36–39]. For instance, calcium influx is an early event following mechanical stimulus in osteocytes both *in vitro* and *in vivo* [37, 38]. Although the magnitude of the calcium influx within responding osteocytes does not change with increasing loading, the number of responding osteocytes does [37, 38]. This increase in intracellular calcium concentration is required for the production of *Nos2* and *Cox-2* induced by mechanical stimulation [40, 41]. In addition, chemical blocking of L-type voltage-sensitive calcium channels (VSCC) suppresses the typical loading-induced increase in bone formation in rats [42]. Similar to many other cell types, osteocytes exhibit different types of calcium channels, including transient receptor potential channels (TRPV), L-type VSCC, and T-type VSCC [39, 43, 44]. To identify a specific calcium channel involved in the response of the skeleton to mechanical loading, loss of function studies for some of these calcium channels has been implemented in the past, and some of these mutant mice have been tested for their skeletal response to loading. For the TRPV channels that expressed in osteocytes, mice lacking TRPV 1, 4, or 6 have been analyzed for their basal skeletal phenotype. TRPV 1 and TRPV4 knockout mice have high bone mass associated with decreased osteoclast number and normal bone formation rate [45–47]. This argues against a role for TRPV1 and 4 as mechanosensors in bone, and this is consistent with other observations that TRPV4 does not respond to mechanical stimulation such as membrane stretch [48]. TRPV6 is expressed in osteoblasts and osteocytes at low levels [49]. Moreover, TRPV6 knockout mice exhibit reduced intestinal calcium absorption but maintain a normal bone formation rate indicating that TRPV6 does not play a role in the osteoblast lineage [50, 51]. The role of voltage-gated calcium channels in bone has also been explored. Mice with germline deletion of the L-type VSCC *CaV* 1.3 have a reduced cross-sectional area in long bones. However, these mice respond normally to mechanical loading [52].

With recent advances in identification of mechanosensitive ion channels in neurons, Piezo1 has emerged as a critical mechanosensor in many cell types [53]. Studies in epithelial cells have shown that Piezo1 responds to various forms of mechanical forces, including membrane stretch, static pressure, and fluid shear stress [54–56]. Moreover, Piezo1 can be directly activated by mechanical perturbations of the lipid bilayer alone, demonstrating its role in mechanosensing [57]. Piezo1 is also highly expressed in osteocytes and can be up-regulated by mechanical stimulation *in vitro* as well as *in vivo* [20]. Recent studies from several different laboratories have found that deletion of the Piezo1 gene in different stages of the osteoblast lineage using the *Prx1-Cre*, *OCN-Cre*, *Col1a1-Cre*, and *Dmp1-Cre* transgene dramatically reduced both cancellous bone mass and cortical thickness [20, 58–60]. The outer and inner circumferences of cortical bone in the midshaft of the femur in Piezo1 knockout mice are also decreased and are

associated with decreased bone formation [20]. This skeletal phenotype is consistent with a reduced ability to respond to mechanical stimulation. Direct testing of this idea by performing an anabolic loading regime confirmed that the bones of the conditional knockout mice are less responsive to mechanical signals than controls [20]. This decrease was not due to an overall decrease in cell health since cell survival was not affected by Piezo1 deletion [20]. In addition, deletion of Piezo1 in osteoblast lineage cells also diminished the skeletal response to mechanical unloading induced by both hindlimb suspension [58, 59] and Botox injection [60]. Thus, these studies strongly suggest that Piezo1 plays a critical role in sensing mechanical signals in bone. The findings that the basal skeletal phenotype is similar between mice with Piezo1 deletion in different stages of osteoblast lineage as well as the evidence that the skeletal response to changes in mechanical loading is blunted in mice with Piezo1 deletion in *Dmp1-Cre* targeted cells suggest that Piezo1 expression in more mature cells including osteocytes is critical for sensing mechanical stimulation in bone.

Since the skeletal response to mechanical loading is not completely abolished in mice lacking Piezo1 in mature bone cells [20], it is most likely that there are other mechanosensors that compensate for the loss of Piezo1 in these mice. In addition, Piezo1 has been reported to be associated with integrin αV activation through $G\alpha q$ / $G\alpha 11$ signaling pathways in epithelial cells [61]. Therefore, it will also be important to understand how Piezo1 interacts with other mechanosensors in osteocytes.

Downstream Effectors Mediate Loading-Induced Bone Formation

Mechanical loading stimulates bone formation by rapidly increasing osteoblast formation and function [2]. Osteocytes have been recognized as a hub to control bone formation by communicating with osteoblasts and their progenitors. To date, several effector proteins have been implicated in the bone formation induced by mechanical signals. These include sclerostin, a canonical Wnt signaling inhibitor. Sclerostin expressed in osteocytes has been shown to be critical for osteoblast formation, and its production is governed by mechanical signals [1, 18, 19]. TRPV4 calcium channel has been shown to be required for the suppression of sclerostin by mechanical stimulation in osteocytes *in vitro* [62], but calcium oscillation is not required for this suppression [63]. Deletion of *Sost*, the sclerostin coding gene, prevented the bone loss caused by tail suspension [64] and botulinum toxin [65]. Overexpression of *Sost* in osteocytes blunted the anabolic effects of loading on bone [66]. In contrast, loss of sclerostin in mice did not prevent the increase in bone formation induced by anabolic loading [65], suggesting that sclerostin is a permissive agent for bone formation and that there are additional effector proteins mediating loading-induced bone formation.

In the Wnt/ β -catenin pathway, mechanical loading also increases the expression of several Wnt ligands including Wnt1 and Wnt7b in murine bone [67, 68]. Mechanosensitive ion channel Piezo1 has been shown to be essential for load-induced Wnt1 expression [20]. By binding to various receptors on the cell surface, Wnts, such as Wnt1, activate the Wnt signaling pathway and play critical roles in osteoblastogenesis and bone formation [69]. Importantly, earlier studies have demonstrated that Lrp5, a co-receptor for Wnt ligands, is required for loading-induced anabolic effects [17]. Consistent with this, blocking Wnt ligands' secretion using a pharmacological inhibitor diminished the anabolic effect of loading in adult mice [70]. Moreover, postnatal deletion of Wntless, a conserved transmembrane protein required for Wnts' secretion in osteoblast lineage cells in adult mice, significantly reduced the skeletal response to mechanical loading [70]. These studies suggest that Wnt ligands play an important role in mediating loading-induced bone formation. Further mouse genetic studies will be required to determine which Wnts are critical for the osteogenic response to mechanical loading.

Besides changing the expression of secreted regulatory proteins in osteocytes, mechanical loading also promotes overall health and energy production of osteocytes. Osteocyte viability is influenced by mechanical signals both in vitro and in vivo [71–73]. In addition, mechanical stimulation increases ATP production and mitochondrial function [74–77]. Although these phenomena have been observed for a long time, it is not clear whether increases in cellular metabolism contribute to anabolic effects of mechanical loading. Recent evidence from studies on the role of the mammalian target of the rapamycin (mTOR) pathway in osteoblast lineage cells has shed light on this matter. The mTOR pathway is a highly conserved central regulator for cellular metabolism [78]. Mechanical loading can stimulate mTOR activity in osteocytes in vitro [79], which in turn promotes glycolysis and energy production in bone cells [80]. Deletion of Rictor, an mTOR complex 2 subunit, in osteoblast lineage cells using Prx1-Cre blunted the anabolic response to mechanical stimuli in adult mice [81], suggesting that cell metabolism plays an important role in the response. More recent studies with deletion of Rictor in Dmp1-cre-expressing cells showed a comparable cancellous and cortical bone phenotype to that observed in mice with Rictor deletion in Prx1-Cre expressing cells [82]. Importantly, the skeletal response to mechanical loading is also prevented in mice lacking Rictor in Dmp1-cre expressing cells [82]. These results demonstrate that the positive impact of the mTOR signaling on the anabolic response to loading occurs in mature cells, possibly osteocytes.

Future Directions

Even though much progress has been made, many questions remain unanswered. For instance, are osteocytes the major mechanosensing cells in bone? If not, what are the other cell types that respond directly to mechanical stimulation in bone? With advances in generating more osteocyte specific Cre driver strains and the development of cell type-specific CRISPR interference technology, the role that particular cell types in the different stages of osteoblast lineage play in mechanosensation could be determined. Other questions, such as whether mechanosensors are specific to certain forms of mechanical stimulation and how different mechanosensors and downstream signaling pathways interact with each other upon activation, are also critical to elucidate the complex system of mechanotransduction in bone. As the exact pathways of mechanosensation and the mediating factors that promote bone formation come to light, it is feasible that bone anabolism could be achieved by pharmacological agents targeting the activation of those pathways.

Compliance with Ethical Standards

Conflict of Interest Jinhu Xiong, Xuehua Li, and Jacob Kordsmeier declare no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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