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Mechanosensation and Transduction in Osteocytes

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Abstract

There may be no single mechanoreceptor in osteocytes, but instead a combination of events that has to be triggered for mechanosensation and transduction of signal to occur. Possibilities include shear stress along dendritic processes and/or the cell body, cell deformation in response to strain, and primary cilia. These events could occur independently or simultaneously to activate mechanotransduction. Signal initiators include calcium channel activation and ATP, nitric oxide, and prostaglandin release. Means of signal transfer include gap junctions and hemichannels, and the release of signaling molecules into the bone fluid. Questions remain regarding the magnitude of strain necessary to induce an osteocyte response, how the response propagates within the osteocyte network, and the timing involved in the initiation of bone resorption and/or formation on the bone surface. Mechanical loading in the form of shear stress is clearly involved not only in mechanosensation and transduction, but also in osteocyte viability. It remains to be determined if mechanical loading can also affect mineral homeostasis and mineralization, which are newly recognized functions of osteocytes.

Osteocytes, composing over 90–95% of all bone cells in the adult animal (1), are defined as cells embedded in the mineralized bone matrix, yet clear functions have not been ascribed to these cells, in contrast to osteoblasts and osteoclasts. Osteocytes are regularly dispersed throughout the mineralized matrix within ‘caves’ called lacunae, connected to each other and cells on the bone surface through slender, cytoplasmic processes or dendrites passing through the bone in thin ‘tunnels’ (100–300 nm) called canaliculi. Not only do these cells communicate with each other and with cells on the bone surface, but their dendritic processes are also in contact with the bone marrow (2), implying that osteocytes can communicate with marrow resident cells. One means for communication with other cell types is through gap junctions, and another is through release of signaling molecules into the bone fluid that flows through the lacuno-canalicular system. The most popular theory regarding the major function of osteocytes is that they translate mechanical strain into biochemical signals between osteocytes and to cells on the bone surface to affect (re)modeling (3), yet this remains to be definitively proven. Recent data suggest additional important functions for osteocytes, such as the regulation of mineral metabolism (4) and the alteration of the properties of their surrounding matrix (5).

Osteocytes as Mechanosensors Directing Bone Formation and/or Resorption

A known key regulator of osteoblast and osteoclast activity in bone is mechanical strain. The skeleton is able to continually adapt to mechanical loading by adding new bone to withstand increased amounts of loading, and by removing bone in response to unloading or disuse (reviewed in (6;7)). Galileo, in 1638, is documented as first suggesting that the shape of bones is related to loading. Julius Wolff, in 1892, more eloquently proposed that bone accommodates or responds to strain. The cells of bone with the potential for sensing mechanical strain and

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translating these forces into biochemical signals include bone lining cells, osteoblasts, and osteocytes. Of these, the osteocytes, with their sheer numbers and distribution throughout the bone matrix and their high degree of interconnectivity, are thought to be the major cell type responsible for sensing mechanical strain and translating that strain according to the intensity of the strain signals (3).

Various studies have demonstrated load-related responses in osteocytes *in vivo*, supporting their proposed role as mechanotransducers in bone. Within a few minutes of loading, glucose 6-phosphate dehydrogenase, a marker of cell metabolism, is increased in osteocytes and lining cells (8). By 2 hours, c-fos mRNA is evident in osteocytes and by four hours, transforming growth factor- β and insulin-like growth factor-1 mRNAs are increased (9). Additional osteocyte selective markers, such as E11/gp38, dentin matrix protein 1 (DMP1), MEPE, and sclerostin, are also regulated by mechanical loading. The dentin matrix protein 1 gene, *DMP1*, is activated in a few hours in response to mechanical loading in osteocytes in the tooth movement model (10) and in the mouse ulna loading model of bone formation (11). E11/gp38, a membrane protein that is osteocyte-selective and thought to play a role in dendrite elongation, is also activated within 4 hours after mechanical load, not only in cells near the bone surface, but also in deeply embedded osteocytes (12). As detailed below, the osteocyte specific marker sclerostin, the protein product of the *SOST* gene, is decreased in response to anabolic loading (13).

Anabolic signals that are released within seconds after loading in osteocytes include nitric oxide (NO), prostaglandins, and other small molecules such as ATP. NO, a short-lived free radical that inhibits resorption and promotes bone formation, is generated within seconds in both osteoblasts and osteocytes in response to mechanical strain (14). Primary osteocytes and primary calvarial bone cells have also been shown to release prostaglandins in response to fluid flow treatment, and a number of studies have suggested that osteocytes are the primary source of these load-induced prostaglandins (15). *In vivo* studies have shown that new bone formation induced by loading can be blocked by the prostaglandin inhibitor, indomethacin (16), and agonists of the prostaglandin receptors have been shown to increase new bone formation (17).

Another anabolic pathway that appears to be activated rapidly in osteocytes within one hour in response to load is the canonical Wnt/ β -catenin pathway. Johnson and colleagues, discoverers of the high bone mass (*HBM*) gene, a mutated low-density lipoprotein receptor-related protein 5 gene (*LRP5*) encoding the LRP5 receptor, hypothesized as early as 2002 that LRP5 is a major player in the way that bone cells respond to mechanical load (18). They reasoned that the *HBM* mutation results in a skeleton that is overadapted in relation to the actual loads being applied, but yet is in homeostatic equilibrium. They found that wild-type bone experienced 40% greater strain than HBM bone with the same load (19). Based on these observations in humans and mice, they hypothesized that the set-point for load responsiveness was lower in the HBM skeleton. Loss of function mutations in LRP5 result in low bone mass, and mice with mutations in LRP5 do not respond to mechanical load (20), again supporting the notion that LRP5 is involved in mechanotransduction. At the most recent annual meeting of the ASBMR, Robling *et al.* showed that sclerostin, an inhibitor of the Wnt pathway that binds to LRP5 and that is produced exclusively by mature osteocytes, decreases 24 hours after loading (13). These investigators proposed that Wnt/ β -catenin is the initiator and *SOST*/sclerostin is the inhibitor of load-induced new bone formation. Also at this meeting, Kamel *et al.* showed that prostaglandin released by bone cells in response to fluid flow can activate the Wnt/ β -catenin pathway independent of LRP5 (21). These investigators suggested that prostaglandin can bypass the inhibitory effects of sclerostin present in the bone matrix.

Osteocytes may also send signals for bone resorption. Isolated avian osteocytes have been shown to support osteoclast formation and activation (22), as has the osteocyte-like cell line, MLO-Y4. However, unlike any previously reported stromal cell lines, MLO-Y4 cells did so in the absence of any osteotropic factors (23). These cells express RANKL along their dendritic processes and secrete large amounts of macrophage colony-stimulating factor, both essential for osteoclast formation. Expression of RANKL along osteocyte dendritic processes, and the capacity of osteocyte dendritic processes to extend into the marrow space (2), provide a potential means for osteocytes within bone to interact and stimulate osteoclast precursors at the bone surface. Another means by which osteocytes can support osteoclast activation and formation is through apoptosis. Osteocyte apoptosis occurs at sites of microdamage, where the dying osteocyte may send signals to osteoclasts for targeted removal of bone (24). Investigators found that Bax (apoptotic biomarker) was elevated in osteocytes immediately at the microcrack locus, whereas Bcl-2 (anti-apoptotic biomarker) was expressed 1–2 mm from the microcrack, suggesting that damaged osteocytes send signals of resorption, whereas those osteocytes that do not undergo apoptosis are prevented from doing so by active protective mechanisms. It is still unclear if signals of resorption sent by dying osteocytes are the same as or different from those sent by viable osteocytes.

The parameters for inducing bone formation or bone resorption *in vivo* are fairly well-known and well-characterized. Bone mass is influenced by peak applied strain (25), and bone formation rate is related to loading rate (26). At bending frequencies of 0.5 to 2.0 Hz, bone formation rates increase as much as four-fold, while no increase is observed at frequencies lower than 0.5 Hz. When rest periods are inserted, the loaded bone shows increased bone formation rates when compared to bone subjected to a single bout of mechanical loading (27). Improved bone structure and strength is greatest if loading is applied in shorter versus longer increments (28). Therefore, for optimal anabolic loading, frequency, intensity, and timing of loading are all important parameters. The major challenge has been to translate these known *in vivo* parameters of mechanical loading to *in vitro* cell culture models.

Mechanisms Whereby Osteocytes Sense Mechanical Loading

Even though osteocytes are thought to be mechanosensors, there is little conclusive data to show how mechanical loading is sensed by these cells. One of the more accepted forms of strain is the flow of bone interstitial fluid driven by extravascular pressure in combination with applied mechanical loading (29;30). Recently, the first real-time attempts to measure solute transport in bone through dye diffusion within the lacunar-canalicular system were conducted *ex vivo* (31). Fluid flow imposes a shear stress on osteocytes that appears to deform the cells within their lacunae and the dendrites within their canaliculi (30). Theoretical modeling predicts osteocyte wall shear stresses resulting from peak physiologic loads *in-vivo* in the range of 8 to 30 dynes/cm². However, it is not clear if the dendritic processes, the osteocyte cell body, and/or cilia are the mechanosensors (see Figure 1).

A model of strain amplification in osteocyte cell processes has been proposed by Weinbaum and coworkers (32). One of the requirements of the model is that osteocyte dendritic processes be tethered to the canalicular wall and anchored to hexagonal actin bundles within the cell processes. The model predicts that fluid flow through this canalicular space will deform the shape of these tethering elements, creating a drag force that then imposes a hoop strain on the central actin bundles inside the osteocyte cell process. This model, however, does not take into account that the dendritic processes of osteocytes may not always be firmly anchored to their canaliculi. The osteocyte has been viewed as a quiescent cell until recently, when Dallas and coworkers showed cell body movement and the extension and retraction of dendritic processes (33). Calvarial explants from transgenic mice with green fluorescent protein (GFP) expression targeted to osteocytes were used to dynamically image living osteocytes within their lacunae.

Surprisingly, these studies revealed that, far from being a static cell, the osteocyte may be highly dynamic. These data suggest that dendrites, rather than being permanent connections between osteocytes and with bone surface cells, may have the capacity to connect and disconnect. These studies also partially explain why a protein thought to play a role in dendrite elongation, E11/gp38, would be regulated by mechanical load in cells embedded in mineralized matrix (12).

Fluid flow shear stress may induce mechanosensation in osteocytes through perturbation of integrins (34). Integrins, comprised of heterodimers of α and β subunits, are major receptors/transducers that connect the cytoskeleton to the extracellular matrix (35) and interact with plasma membrane proteins such as metalloproteases, receptors, transporters, and channels mainly through the extracellular domain of their α subunits (36). The integrin $\alpha 5$ subunit may act as a tethering protein that, when perturbed by shear stress, opens hemichannels in osteocytes, allowing the release of prostaglandin (37).

It has also been proposed that mechanical information is relayed in part by matrix and cell deformation (38–40). Typical *in vivo* strains in humans are on the order of 1,200 to 1,900 μE and were determined using strain gauges that covered an area approximately 1.8 mm by 3.6 mm; this area would contain thousands of cells and the strains measured are therefore averages. Microstructural strains measured at or near osteocyte lacunae were up to 3 times greater than the average strains measured with an external strain gauge (39;40). This suggests that the osteocyte is subjected to larger strains than those measured on the external bone surface.

Recently, it has been shown that polycystin-1 and 2 (PKD1 and PKD2), known mechanosensory proteins in the kidney, do play a role in normal bone structure and that cilia do exist on both osteoblasts and osteocytes (41). Primary cilia clearly function as sensors of odors, light, and movement, depending on cell type (42). It remains to be determined whether the bone defect in animals with reduced or defective PKD1 function is due to defective mechanosensory function in bone cell cilia, as has been shown in kidney epithelial cells. Recently, Jacobs and coworkers provided preliminary data that loss of cilia resulted in decreased sensitivity to flow (43). It will be important to determine how a single cilium on an osteocyte cell body can mediate the mechanosensory functions ascribed to the osteocyte.

In vivo, it has been shown that physiological loading prevents osteocyte apoptosis (44) and, conversely, that reduced mechanical loading in the tail suspension model increases osteocyte apoptosis (45). *In vitro* experiments have shown that fluid flow shear stress inhibits osteocyte apoptosis induced by serum starvation (46) and that substrate stretching prevents dexamethasone-induced apoptosis (47). Fluid flow shear stress has recently been shown to prevent both dexamethasone- and tumor necrosis factor- α -induced apoptosis, and this effect was shown to be mediated by prostaglandin production (48). Mechanical loading is therefore protective against apoptosis and this effect is mediated through prostaglandin production. Prostaglandin can now be added to the list of anti-apoptotic factors for osteocytes.

Osteocytes as Regulators of Mineralization and Mineral Metabolism

The osteoid-osteocyte may control deposition of mineral that begins to surround and encase this cell while it is embedding (49;50). It is also likely that this cell is subjected and responsive to loading. Mechanosensation may play a role in the process of selection of targeted osteoblasts on the bone surface to become osteocytes. Osteocytes in cortical bone are orderly and linearly arrayed. Signals passing from embedded cells to selected cells on the bone surface may be delivered through gap junctions to select a cell that will maintain this ordered network. Mature osteocytes also have the capacity to modify their local microenvironment. Glucocorticoid treatment causes mature osteocytes to enlarge their lacunae and remove mineral from their

microenvironment (5). Osteocytes may be able to modify their microenvironment in response to other factors.

Osteocytes may also play a major role in mineral homeostasis. Genes that are highly expressed in osteocytes are known regulators of mineralization and mineral homeostasis. The most convincing evidence that osteocytes are regulators of mineralization comes from studies of *SOST*/sclerostin. The *SOST* gene encodes a protein, sclerostin, that is highly expressed in mature (not early) osteocytes and functions as an inhibitor of bone formation (51). The human conditions of sclerostosis and van Buchem disease are due to mutations in the *SOST* gene, and transgenic mice lacking sclerostin have increased bone mass. It appears that sclerostin is an indirect inhibitor of BMP, but specifically antagonizes the Wnt pathway (52) as an antagonist of LRP5, a gene shown to be important as a positive regulator of bone mass (53). Both Wnt/ β -catenin and *SOST* are regulated by mechanical strain in osteocytes, positively and negatively, respectively. Is this one means by which loading regulates the bone formation and resorption responses?

Deletion or mutation of genes that are highly expressed in embedding osteocytes and mature osteocytes, such as dentin matrix protein 1 (*DMP1*) and phosphate-regulating gene with homologies to endopeptidases on the X chromosome (*PHEX*), results in hypophosphatemic rickets (4;54). *PHEX* is a cell surface membrane metalloendoproteinase and *DMP1* is expressed along the canaliculi of osteocytes. Other players in mineral metabolism include MEPE and FGF23, also highly expressed in osteocytes (55;56). Therefore, it has been proposed that the osteocyte network be viewed as an endocrine gland that can regulate mineral metabolism.

DMP1, a promoter of mineralization and mineral homeostasis, and MEPE, an inhibitor of mineralization, both increase sequentially in response to mechanical load (10). This raises the question whether mineral metabolism could be regulated by mechanical loading. Another level of complexity, but an exciting one!

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Figure 1.

Cartoon showing potential ways that an osteocyte may sense fluid flow shear stress. (A). Fluid flow shear stress could perturb tethering elements between the canaliculus wall and the cell membrane. (B). Fluid flow shear stress may also affect the cell body, causing cell deformation. (C). Fluid flow may perturb primary cilia leading to mechanosensation. Both matrix and cell deformation are also proposed to play a role in osteocyte mechanosensation.