HHS Public Access

Author manuscript

Bone. Author manuscript; available in PMC 2022 December 01.

Published in final edited form as:

Bone. 2021 December ; 153: 116104. doi:10.1016/j.bone.2021.116104.

Tunnels in the rock: dynamics of osteocyte morphogenesis

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Abstract

Osteocytes are dynamic, bone matrix-remodeling cells that form an intricate network of interconnected projections through the bone matrix, called the lacunar-canalicular system. Osteocytes are the dominant mechanosensory cells in bone and their mechanosensory and mechanotransductive functions follow their morphological form. During osteocytogenesis and development of the osteocyte lacunar-canalicular network, osteocytes must dramatically remodel both their cytoskeleton and their extracellular matrix. In this review, we summarize our current understanding of the mechanisms that govern osteocyte differentiation, cytoskeletal morphogenesis, mechanotransduction, and matrix remodeling. We postulate that the physiologic activation of matrix remodeling in adult osteocytes, known as perilacunar/canalicular remodeling (PLR) represents a re-activation of the developmental program by which the osteocyte network is first established. While much of osteocyte biology remains unclear, new tools and approaches make the present moment a particularly fruitful and exciting time to study the development of these remarkable cells.

Hezekiah's Tunnel

The year was c.700 BC. The Assyrian empire was expanding, and King Sennacharib was about to complete his conquest of the fertile crescent. Hezekiah, king in Jerusalem, scrambled to prepare for a siege. But there was a problem. The Gihon spring that provided water to the hill-top city was outside the city walls, so Hezekiah devised a last-ditch plan: dig a tunnel to direct the water into the city. With time slipping away, workers dug from both ends, hoping to meet in the middle. Despite a few changes of direction, the engineers were successful; the pool filled with water, and the city successfully held off the siege.

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This blind tunneling through rock, from both ends, meeting in the middle, also occurs more than a trillion times in each adult human skeleton in the osteocyte lacunarcanalicular network (Figure 1) [1]. But how do osteocytes accomplish this? And how does this complex network develop? In this review, we discuss our current understanding of how the osteocyte network develops, how osteocyte dendrites dig through the bone matrix, and highlight the putative role of mechanobiology in osteocyte morphogenesis.

Osteocyte characteristics

Osteocytes are the most abundant cell type in bone. There are approximately 40 billion osteocytes per adult skeleton, and they form an incredibly complex, interconnected network that permeates the mineralized bone matrix (Figure 1) [2-5]. Osteocytes reside in lacunae of 15-20 μm in diameter and extend dendritic arms to connect with neighboring osteocytes, blood vessels, surface cells, and bone marrow [2-4]. These dendritic processes pass through small tunnels called canaliculi whose diameter ranges from 250-300 nm. This complex system is called the lacunar-canalicular network [4,5]. The lacunar-canalicular network is filled with fluid to facilitate oxygen and nutrient transport to osteocytes [3]. The dendritic processes do not float freely in the liquid, but are anchored to the canalicular wall by tethering fibers [6]. Osteocytes are surrounded by a pericellular matrix (PCM), containing collagens and non-collagenous proteins, and are separated from the extracellular matrix by a 50-80 nm thick space that enables fluid flow and nutrient transport [7]. Although osteocytes share morphological characteristics (e.g., stellate shape), many factors such as location and bone compartment (i.e., cancellous vs. cortical), age, and the magnitude of typical local mechanical stimuli define their cellular shape and connectivity. For example, trabecular bone of the mandible features less-aligned canaliculi compared to diaphyseal cortical bone in mice [8,9]. Advanced aging reduces canalicular processes length and connectivity in human subjects [10]. Osteocytes in embryonic mouse long bones exhibit short, isotropic canaliculi with low connectivity, but lengthen and re-orient their canaliculi with the principal axis of the bone by six weeks of age [11]. Further, preventing ambulatory loading by sciatic nerve resection shortly after birth blunted this canalicular network adaptation, implicating mechanical forces in osteocyte morphogenesis [11]. In adult mice, comparing osteocytes across anatomical sites further suggests a role of the mechanical environment as long bone osteocytes feature more elongated cell bodies than the relatively spherical osteocytes in calvaria [12]. Together, these observations indicate that morphogenesis and adaptation of the osteocyte lacunar-canalicular are dynamic and may be regulated by mechanical cues.

Osteocyte mechanosensation and mechanotransduction

Osteocytes are the primary mechanosensing cells in bone (Figure 2). Their abundance, architecture, and connectivity provide osteocytes unique and dynamic sensitivity to mechanical stimuli in the skeleton [13-16]. Osteocytes are exposed to a variety of mechanical stimuli, including fluid shear stress and direct matrix deformation. Under direct perilacunar bone matrix deformation, the osteocyte lacuna can act as a strain concentrator to promote osteocyte mechanotransduction [17]. Likewise, fluid flow through the canalicular system may further amplify mechanical signals as even small inhomogeneous matrix deformations can produce pressure differentials, resulting in both shear stress on the

osteocyte cell membrane and drag forces on the tethering elements that attach the dendrites to the canalicular wall [18]. Primary cilia may also function as "cellular antennae" that enable the osteocyte to sense mechanical signals [19]. Osteocyte mechanotransduction translates these mechanical cues into biochemical signals that communicate to osteoblasts and osteoclasts and direct bone remodeling [20-22]. Demonstrating the functional roles of osteocytes in bone physiology, Tatsumi and colleagues conditionally ablated osteocytes using diphtheria toxin (D.T.), and observed bone fragility, microfractures, osteoblastic dysfunction, and resistance to unloading-induced bone loss [23].

Osteocyte mechanotransduction translates mechanical cues to biochemical signals. The mechanisms that mediate mechanotransduction in osteocytes are reviewed thoroughly in other articles [14,24-30]. Briefly, osteocytes transduce mechanical cues by activation of a concert of signaling pathways. Osteocytes transduce mechanical signals at the membrane through multiple signals, including matrix adhesions and calcium channels such as Piezo and TRPV4 [31-33]. Intracellularly, these signals are transmitted by pathways that activate transcriptional regulators, including β-catenin and YAP/TAZ [4,31,34,35]. For example, the osteocyte-secreted signaling protein, sclerostin, antagonizes Wnt/β-catenin signaling by binding to the Wnt co-receptors, LRP5/6, to prevent bone anabolism. In response to mechanical stimulation, osteocytes both decrease Sost mRNA expression and rapidly degrade the sclerostin protein to enable bone formation [33,36-38]. Interestingly, bones from lactating mice exhibit greater ex vivo load-induced β-catenin expression and sclerostin suppression than bones from virgin controls, suggesting a greater response to loading during lactation [39,40]. Other intracellular mediators of mechanotransduction include intracellular calcium (Ca^{2+}), adenosine triphosphate (ATP), prostaglandin E2 (PGE_2), and nitric oxide (NO) [13,41-43], the latter of which serve also as second messengers to promote differentiation and induce bone formation [44-46].

In addition to experimental methods, multiscale computational approaches have contributed to our understanding of the osteocyte mechanical environment and mechanotransduction. For instance, osteocytic strain-amplification was identified using theoretical and computational approaches to provide a physically-consistent solution to the paradox that physiologic tissue-level strains are too small to initiate the cellular responses [6,47-49]. Such multiscale modeling approaches can also be applied to the osteocyte cytoskeleton and mechanotransduction [50]. Multiscale finite element analyses recapitulate the location-dependent osteocytic mechano-response depending on anatomic location [51].

Osteocyte mechanosensory function follows morphological form

Osteocyte mechanosensation and mechanotransduction arise from the osteocyte’s unique stellate shape and interconnected architecture. The osteocyte is made up of an ellipsoid cell body and many long dendritic processes, both of which may contribute to mechanosensation. Direct *in vitro* stimulation data suggest that the dendritic processes are more mechanosensitive than the cell body, though the cell body also has important roles in mechanotransduction. Adachi et al. measured intracellular calcium transients in response to local mechanical deformation of either the cell body or of the dendrites [52]. Intracellular calcium wave propagation was faster and was induced at smaller deformations

when stimulation was applied to the dendrites than to the cell body [52]. Similarly, Thi et al. showed that osteocyte-like cells respond to fluid flow through $\alpha_V\beta_3$ integrin attachment sites in the dendritic processes but not in the cell body [53]. However, Burra et al. observed a significant cell body response to mechanical stimulation through connexin 43 (Cx43) hemichannel opening while dendritic hemichannel opening was minimal under the same stimulus [54]. Together, these observations show that osteocyte morphology is critical to their mechanosensory function.

Conversely, osteocyte mechano-activation may also alter osteocyte morphology. This has been observed both in cell culture and animal models. *In vitro*, for example, Ponik et al. observed a dramatic increase in the number of dendrites in MLO-Y4 osteocyte-like cells after exposing the cells to 24 hours of oscillatory fluid flow. [32]. *In vivo*, osteocytes align with the direction of principal mechanical stresses across anatomical sites, with increased alignment in bones that experience more loading [12]. During development and growth, reducing ambulatory loading can impair osteocyte morphogenesis. Sugawara et al. performed sciatic nerve resection in 5-day-old mice and observed development of rounded-shape osteocytes at 6 weeks of age, in contrast to spindleshaped osteocytes in mice allowed to develop normally [11]. Similarly, Britz et al. performed sciatic nerve resection on 3-week-old rats and observed decreased lacunar density and volume at week 30 [55]. Sasaki et al. found that osteocytes in bone formed around mechanically-loaded orthopaedic implants exhibited greater ellipticity and dendrite density than in non-loaded implants [56]. Together, these observations suggest that osteocyte morphogenesis is influenced by the mechanical environment. However, whether osteocytes are capable of remodeling their matrix to re-orient the lacunae or canaliculi in response to adult bone loading remains an open question.

Osteocyte morphogenesis and the cytoskeleton

Osteocyte morphology is supported by the osteocyte cytoskeleton. The cytoskeleton forms a mechanical link between the extracellular environment and the intracellular compartment and is a central effector of mechanotransduction. Cytoskeletal filaments consist of three main types: actin filaments, microtubules, and intermediate filaments. Filament length, crosslinking geometry, binding proteins, and the cytoskeletal components' mechanical properties determine the cytoskeletal network's mechanical characteristics and the osteocytes [57]. Kamioka et al. showed that actin filaments abundant in isolated osteocyte processes and extend toward the end of the processes compared to intermediate filaments that were most abundant in the proximal end of the dendrites [45]. Actin filaments facilitate mechanotransduction by allowing the direct transmission of the mechanical signals from the dendrites to the cell body [58,59]. The microtubule cytoskeleton is also important in osteocyte mechanotransduction. For example, Lyons et al. identified a microtubule-dependent mechanotransduction pathway by which fluid shear stress (FSS) de-tyrosinates and stabilizes microtubules to induce reactive oxygen and calcium signaling to reduce sclerostin abundance in cultured osteocyte-like cells [60]. This rapid reduction in sclerostin abundance is effected by lysosomal degradation of sclerostin protein [38]. Together, these observations point to the cytoskeleton as important contributors to osteocyte shape.

Osteocyte morphogenesis requires biologic regulation of the cytoskeleton. Osteocyte morphology differs dramatically from that of the cuboidal osteoblasts from which they differentiate, including a dramatic rearrangement of the cytoskeleton (Figure 3). In osteoblasts, the microtubules radiate from the perinuclear microtubule organizing center and extend throughout the cell body. As osteoblasts differentiate into osteocytes, the microtubule filaments extend toward the immature dendrites; however, in mature osteocytes *in vivo*, the microtubule filaments are primarily localized in the cell body rather than the processes [61]. For example, actin bundling proteins such as fimbrin, α-actinin and villin are abundant in osteocyte dendrites but absent from osteoblast stress fibers [58][59]. Similarly, motor proteins that regulate cytoskeletal tension are expressed highly in osteocytes *in vivo* [62], but classic methods to directly measure cytoskeletal tension are difficult to perform on osteocytes *in vivo* due to their architecture and location in the mineralized bone matrix. However, cytoskeletal properties and dynamics can be studied using osteocyte-like cells *in vitro*. Cell culture models suggest that the significant cytoskeletal dissimilarities between osteoblasts and osteocytes contribute to their differential mechanosensitivity. For example, Ponik et al. compared the morphological and biochemical response to unidirectional and oscillatory fluid flow in MC3T3-E1 osteoblasts and MLO-Y4 osteocyte-like cells. They noticed that the MLO-Y4cells required a prolonged time to form and organize their stress fibers under unidirectional fluid flow. Whereas, in osteoblasts, the time needed for the formation and organization of the stress fibers under similar fluid flow was shorter [63]. In vivo, however, the density and organization of cytoskeletal elements in the dendrites combined with the dynamic mechanical environment of the canalicular system support unique mechanoresponsiveness [6]. Despite this understanding, the regulatory mechanisms that mediate cytoskeletal remodeling during osteocyte morphogenesis and the extent to which the cytoskeleton directs or is directed by osteocyte differentiation are still unclear.

Osteocyte development and differentiation

Osteocytes differentiate from osteoblasts (Figure 3). During the final stages of bone formation, osteoblasts undergo three potential fates: 1) to embed in the bone matrix to become osteocytes, 2) to become bone lining cells or inactive osteoblasts, 3) to die (apoptosis). The type of bone, age, and animal species determine the proportion of the osteoblasts undergoing any of these fates [64]. However, the causes that determine which osteoblasts embed and become osteocytes are still unclear. This transition coincides with a dramatic change in morphology from cuboidal osteoblasts to highly dendritic cells and a reduction in the cell body size of approximately 70% [65]. Three cell types can be distinguished during this transition: osteoblastic osteocytes (Type 1), osteoid-osteocytes (Type 2), and osteocytes surrounded by mineralized matrix (Type 3) [66]. The transition process was once thought to be a passive process by which a new generation of osteoblasts covered existing osteoblasts on the bone surface, which would then decrease their matrix deposition rate and become trapped by the secretion of their neighboring cells [67-69]. However, it is now clear that osteocytes attain their unique morphology through an active invasive process. Zhao et al. observed collagen cleavage at the peri-osteocytic extracellular matrix, demonstrating that this remodeling during embedding was mediated by Matrix Metalloproteinases (MMP) [70]. Holmbeck et al., showed that collagen fibril cleavage along

the processes is mediated by an MMP type collagenase (MT1-MMP/MMP14) [71]. The establishment of live imaging techniques in recent years has further confirmed that the transitional process is active. For example, using time-lapse intravital imaging of DMP1-GFP-labeled cells, the Dallas group observed osteocytically-differentiating osteoblasts migrating along the formed bone surface accompanied by extension of dendritic processes [3].

Dynamics of osteocyte development

Osteoblasts differentiate into osteocytes by dynamic regulation of transcriptional programs. Some osteoblastic genes are downregulated with osteocyte differentiation; others are upregulated. Downregulated genes include Type I Collagen and alkaline phosphatase (ALP), while upregulated genes include dentin matrix protein (DMP1), fibroblast growth factor 23 (FGF23), extracellular matrix phosphoglycoprotein (MEPE), and phosphate regulating endopeptidase homolog X-linked (PHEX) (Figure 4). Many of these genes are particularly regulated by mechanotransduction in osteocytes, including DMP1, MEPE, and PHEX [26]. For example, mechanical loading of mouse alveolar bone causes localization of DMP1 protein to the osteocytes canalicular wall [72,73]. Interestingly, a similar mechanical loading approach, reveals distinct regulation of MEPE compared to DMP1 [74]. These observations suggest unique interacts between cellular state, context, mechanical environment, and inducible gene expression in osteocytes. Besides genes associated with matrix deposition and mineralization, osteocytes are also a significant source of the pro-osteoclastic cytokine, RANKL, which is negatively regulated by fluid shear stress (FSS) and is upregulated with osteocytic differentiation [75]. Similarly, Sost, which encodes sclerostin, a negative regulator of osteoblast activity, is induced in mature osteocytes and is robustly suppressed by mechanical loading [4][76]. In addition to genes that more stably mark cell of the osteoblast vs. osteocyte differentiation state, some genes are also transiently expressed, only during the transition. The most well-described of these genes is E11/gp33, also known as podoplanin. E11 is localizes to nascent osteocytic dendritic processes, which have begun to embed in the bone matrix. Upon osteocyte maturation, E11 expression effectively disappears [77,78]. Like many other osteocyte genes, E11 is regulated by loading; for example, fluid shear stress (FSS) induction of E11 expression has been suggested as one of the fundamental mechanisms leading to dendritic elongation [79]. Identification of the transient signals expressed uniquely during the osteoblast-osteocyte transition may be a particularly fruitful approach to understanding osteocytogenesis. Still, much remains to be discovered about the mechanisms by that control osteocyte morphogenesis and the development of the lacunar-canalicular system. Yet, similarities between canalicular network development and adult osteocytic remodeling may provide some clues.

Perilacunar/canalicular remodeling: re-activation of a developmental program?

We postulate that perilacunar/canalicular remodeling (PLR) in adult osteocytes is a reactivation of the developmental program by which the canalicular network is first established. Mature osteocytes dynamically remodel their extracellular matrix to mobilize

calcium for lactation and to regulate bone matrix quality [2,5,80]. We pose the hypothesis, yet to be tested, that the mechanisms by which new osteocytes form their interconnected dendritic network in the bone matrix and the mechanisms by which mature osteocytes remodel that same network are functionally equivalent. Indeed, the first descriptions of "osteocyte osteolysis" in the 1960s were made by observing the morphology and peri-osteocytic matrix acidification of chick embryo osteocytes [81,82]. Functional and mechanistic convergence is also supported by recent studies of osteocytic (re)modeling. During both canalicular network development and adult PLR, osteocytes actively alter their own connectivity and their extracellular matrix collagen organization and mineralization [71,83]. Recent data from Marc Wein and colleagues support a molecular and functional link between osteocyte morphogenesis and canalicular remodeling [84]. They show that the transcription factor osterix-1, which is encoded by the Sp7 gene, regulates osteocyte expression of osteocrine (Ostn) to promote dendrite formation during development [84]. Sp7 deletion impaired canalicular network morphogenesis, but, remarkably, systemic injection of adenoviral particles to exogenously express osteocrin in 3-week-old Sp7 knockout mice rescued dendrite formation by 6 weeks of age [84]. These data identify a developmental program can be re-activated post-weaning to induce dramatic canalicular remodeling.

Adult perilacunar/canalicular remodeling occurs in both male and female bones, but is sexually dimorphic [85]. In female rats, loss of estrogen by experimental ovariectomy (OVX), increases lacunar size compared to non-OVX controls [86]. Dole et al. showed that TGFβ regulation of PLR is sexually dimorphic, such that osteocyte-conditional TGFβ receptor II deletion in male mice impaired PLR and caused bone fragility, but female mice were protected from TGFβ-dependent defects in PLR and bone quality [85]. In adults, PLR is most prominently observed during lactation [39,40,87,88], but is also activated by exercise [89] and fracture repair [90]. During lactation, osteocytes actively degrade the mineralized matrix to mobilize calcium and meet the demands of milk production [2]. Osteocyte lacunar size increases in lactating mice but is reversed by osteocytic mineral deposition after weaning when the resorption pressure is lifted [88]. Although the mechanisms remain unclear, parathyroid hormone (PTH) is a key mediator of lactation-induced PLR [39]. Lactation elevates levels of PTH-related Peptide (PTHrP) in the maternal circulation to induce bone resorption [91]. Mechanistically, continuous PTH signaling upregulates ATPase H+ Transporting V0 Subunit D2 (ATP6V0D2), a proton pump on the cell membrane acidifying the extracellular environment [88]. During lactation, PTHrP promotes ATP6V0D2 expression to acidify and demineralize the perilacunar matrix [2]. In contrast, intermittent PTH(1-34) administration promotes bone formation and alters perilacunar matrix remodeling to promote fatigue resistance [92]. Physiologically, exercise produces intermittent endogenous PTH release to promote bone formation and perilacunar matrix remodeling [89]. Notably, PTH signaling is critical for embryonic and post-natal bone development [93,94], but mapping the multifactorial roles of PTH signaling between osteocyte morphogenesis and adult PLR requires further study. Enzymes and membrane pumps such as Acp5/TRAP, the Na+/H+ exchanger and the domain containing 2 (NHEDC2) contribute to matrix degradation and demineralization [88] and may explain the early observations of periosteocyte acidification in embryonic bone explants [81,82,95]. The collagen-degrading enzymes, matrix-type 1 MMP. (MT1-MMP/MMP14), MMP13, and

Cathepsin K (Ctsk) are critical to form the canalicular network in development [70,71,83] and mediate PLR by degradation and remodeling of the organic matrix [35,83]. Lactation enhances Ctsk expression [96], and Ctsk deletion from osteocytes increases PTHrP levels and prevents the decrease in PTH that is induced by lactation [97]. Taken together, these studies of bone development and adaptation suggest convergent mechanisms for osteocyte-mediated matrix remodeling.

Molecular regulation of osteocytic remodeling

Recent studies are beginning to shed light on the osteocyte-intrinsic mechanisms that enable osteocyte morphogenesis and perilacunar/canalicular remodeling. For example, Alliston and colleagues found that osteocyte TGF-β signaling mediates bone adaptation to mechanical loads [99] and mediates PLR [98]. Specifically, consistent with their prior observation that global MMP13 deletion disorganized collagen matrix [83], osteocyte-conditional TGF-β receptor deletion impaired bone matrix organization and canalicular network density and connectivity (Figure 5A and C) [98. In our pursuit of the molecular mediators of mechanotransduction in osteocytes, we found that osteocyte-conditional deletion of the mechanosensitive transcriptional regulators, Yes-associated protein (YAP) and Transcriptional co-activator with PDZ-binding motif (TAZ) produced a nearly-identical phenotype, with disorganized collagen matrix organization and disrupted canalicular network development [35] (Figure 5B and D). Together, these observations suggest a role for osteocyte TGF-β signaling in mechanoregulation of osteocyte development, though causative relationships remain to be established. Notably all of these findings were made using constitutive knockout mouse models, resulting in a disruption of lacunar-canalicular network morphogenesis. Decoupling the mechanisms of osteocyte development from adult PLR and osteocyte-intrinsic mechanotransduction will require further research using inducible genetic models and rescue experiments.

Conclusion

Like King Hezekiah's engineers, who dug through the bedrock to let the springwater flow, osteocytes are dynamic cells that tunnel through mineralized bone matrix to form a network of fluid-filled tunnels. These canalicular tunnels communicate a wide variety of signals, from mechanical to endocrine. Osteocyte mechanosensory function follows morphological form, which are both effected and supported by the cytoskeleton. Osteocytic differentiation from osteoblasts and the development of the lacunar-canalicular network requires dynamic signaling and both cytoskeletal and matrix remodeling. This osteocyte-mediated remodeling can be re-activated by physiologic events in adult osteocytes in perilacunar/canalicular remodeling. We postulate that PLR represents the re-activation of this developmental program, though further research on these remarkable cells is required. Given the emerging roles of osteocytes as direct regulators of both bone quality and systemic organ crosstalk, osteocytes represent an exciting frontier for therapeutic intervention.

Acknowledgements:

This work was supported by NIH/NIAMS grants R21 AR071559 (to J.D.B.) and P30 AR069619 (to J.D.B.) and), and the Center for Engineering Mechanobiology (CEMB), an NSF Science and Technology Center, under grant agreement CMMI: 15-48571.

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Highlights

- Osteocytes are the dominant mechanosensory cells in bone and their mechanosensory and mechanotransductive functions follow their morphological form.
- In this review, we summarize our current understanding of the mechanisms that govern osteocyte differentiation, cytoskeletal morphogenesis, mechanotransduction, and matrix remodeling.
- We postulate that the physiologic activation of matrix remodeling in adult osteocytes, known as perilacunar/canalicular remodeling (PLR) represents a re-activation of the developmental program by which the osteocyte network is first established.

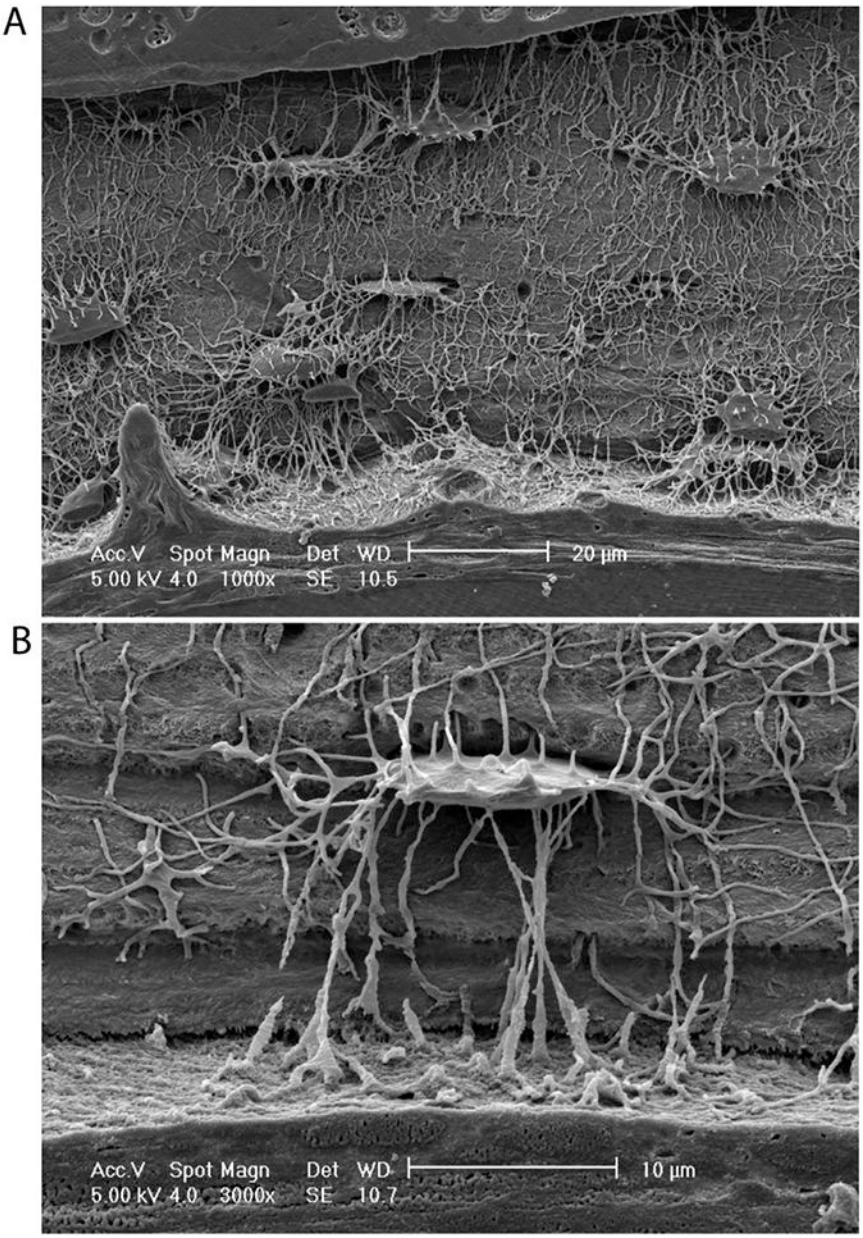

Figure 1: Acid-etched human cortical bone, showing the complexity of the osteocyte canalicular network.

A) 1000x and B) 3000x magnified images courtesy of Lilian Plotkin and Lynda Bonewald (Indiana University School of Medicine), and Teresita Bellido (University of Arkansas for Medical Sciences).

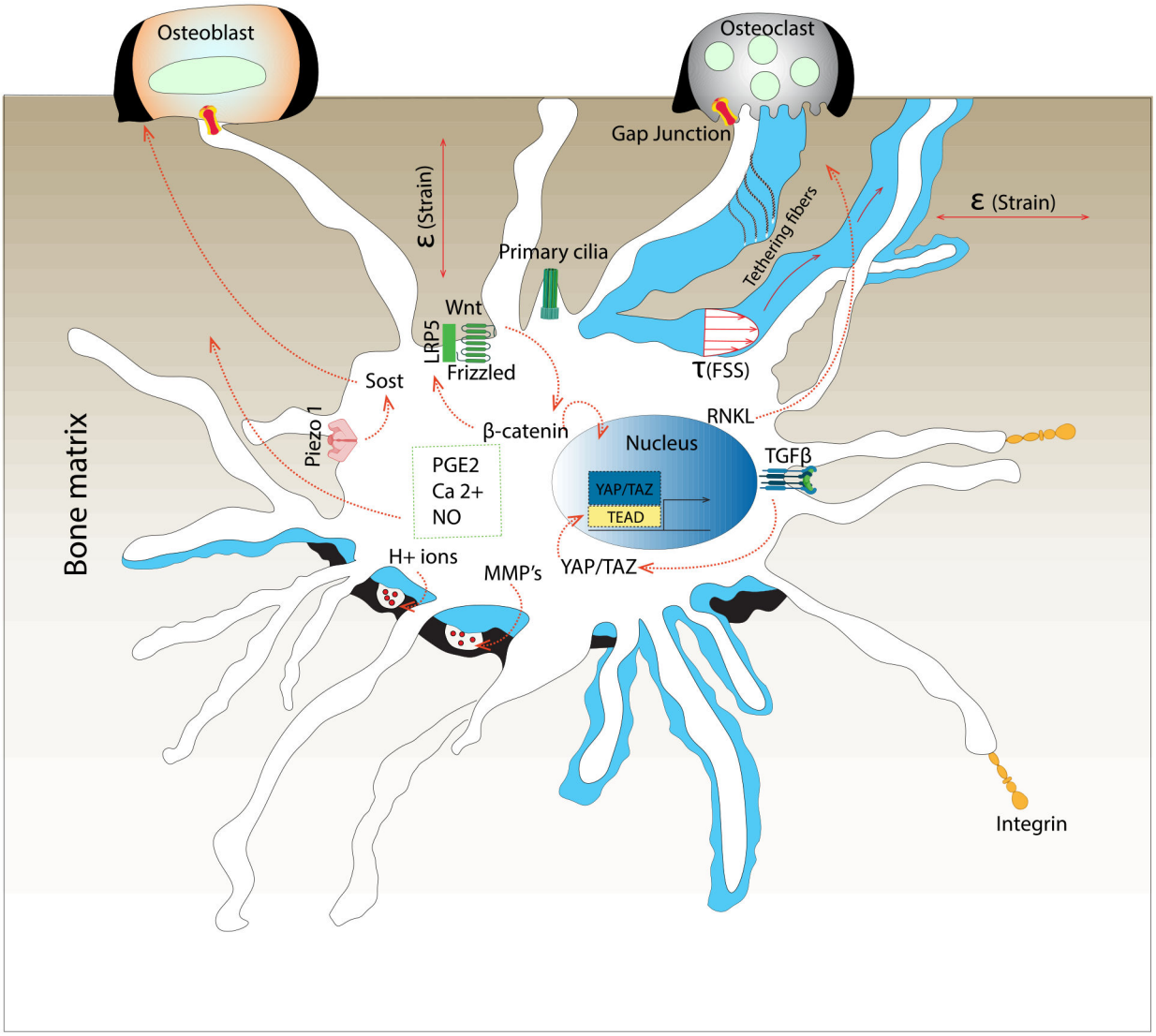

Figure 2:

Mechanisms of osteocyte mechanosensation and mechanotransduction and communication with osteoblast and osteoclasts.

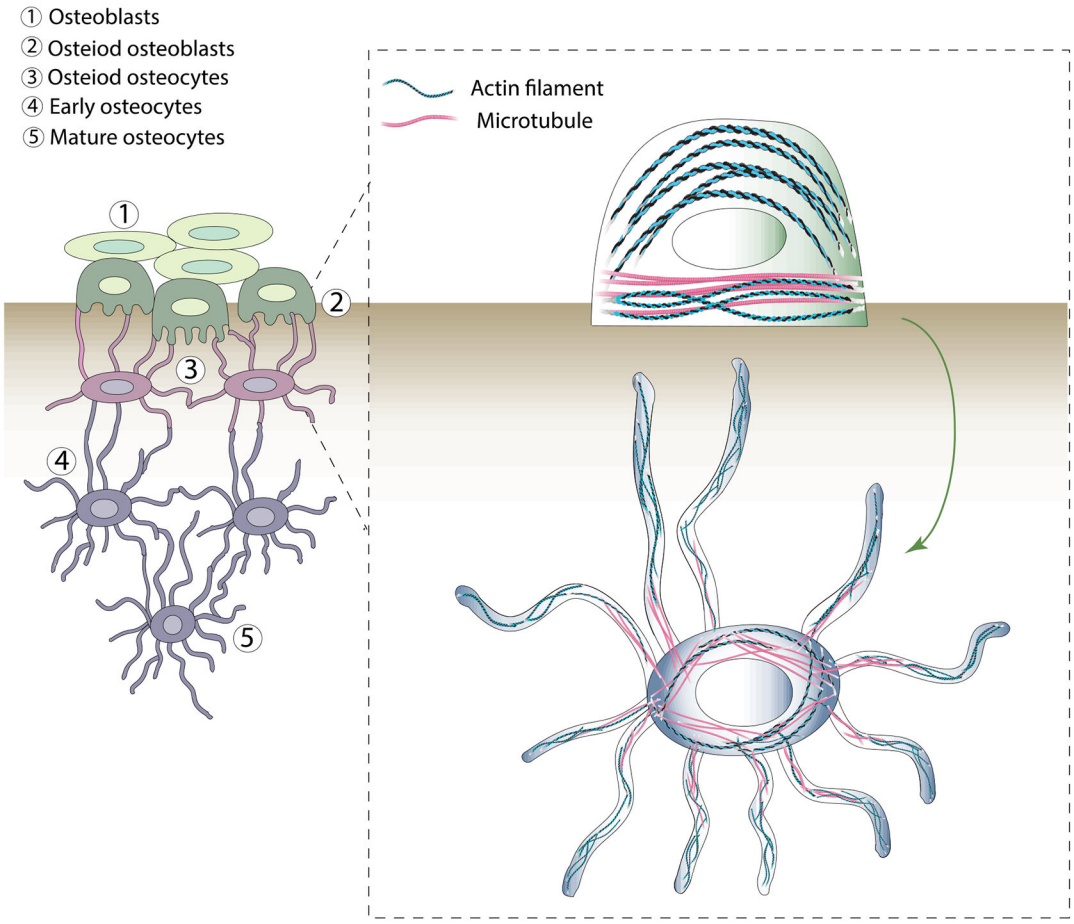

Figure 3: Osteocyte morphogenesis requires dramatic reorganization of cytoskeletal architecture during osteoblast-osteocyte differentiation.

The extent to which these cytoskeletal changes are required for or consequent to osteocytogenesis remains an open question.

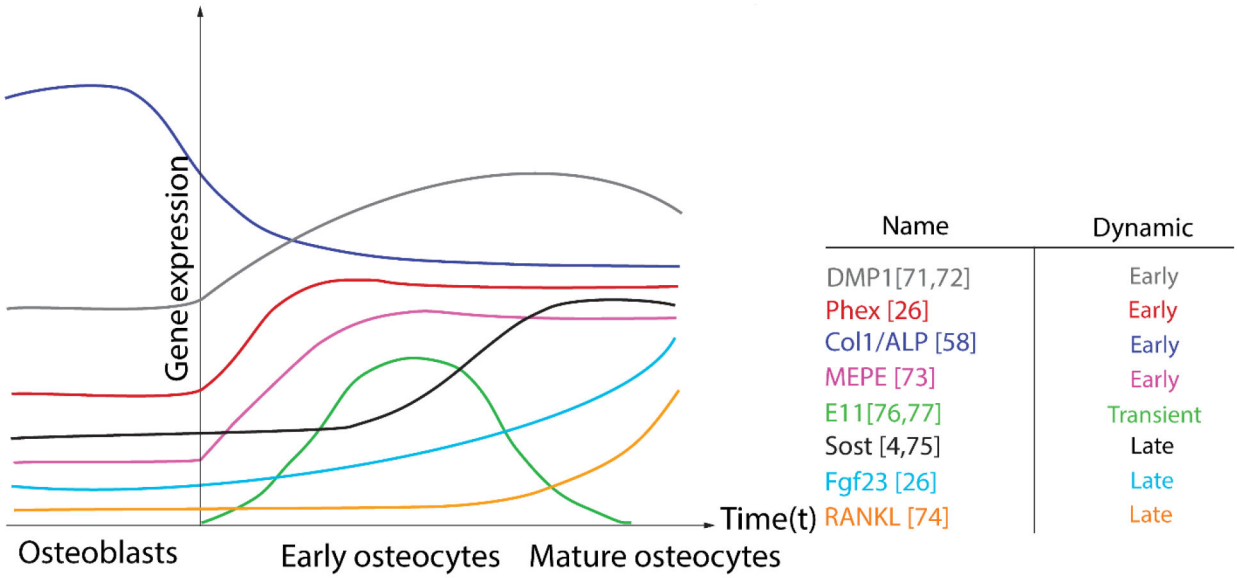

Figure 4: Osteocyte differentiation requires dynamic gene expression.

Genes associated with osteocytogenesis include both upregulated (DMP1, Phex, MEPE) and downregulated (Col1/ALP) early osteocyte genes, transient genes (E11/gp38), and late genes (Sost, Fgf23, RANKL).

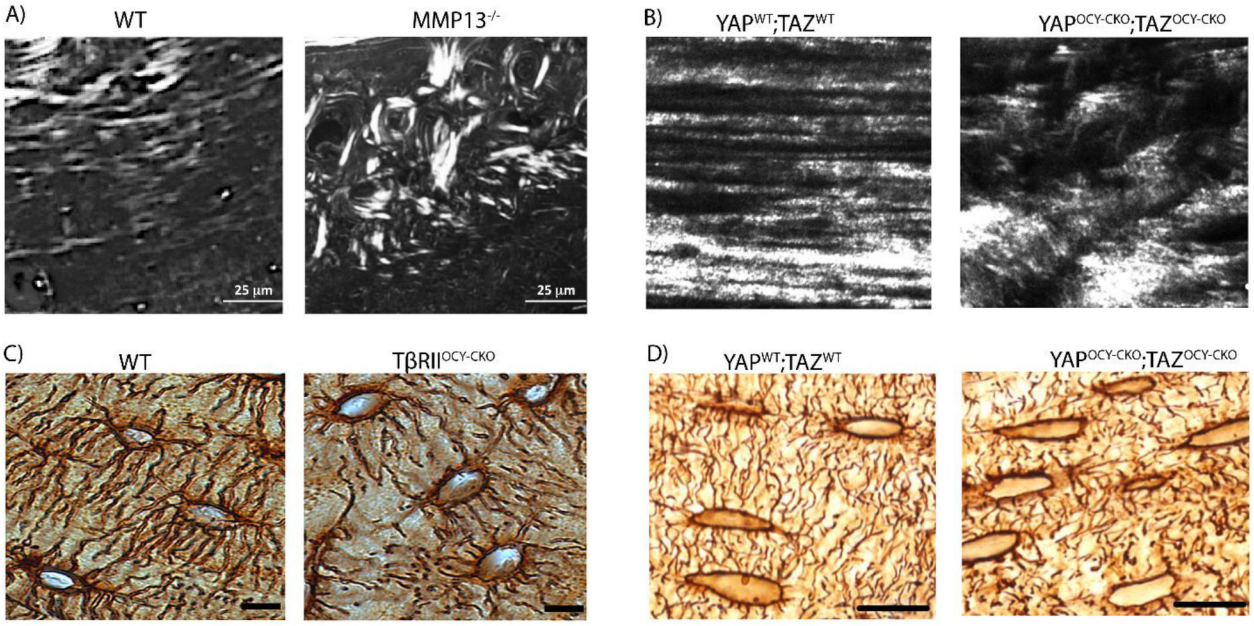

Figure 5: Convergent consequences of osteocyte-conditional deletion of TGF-β receptor II and YAP/TAZ.

A) Second harmonic generated (SHG) images of tibial cortical bone to show the collagen matrix in WT and MMP-13-deficient bone adapted with permission from Ref. [83]. B) Second harmonic generated (SHG) images of femoral cortical bone to show the collagen matrix in WT and Ocy-conditional YAP/TAZ KO mice adapted with permission from Ref. [35] C) Silver staining of mouse femoral cortical bone canalicular networks in W.T. and Ocy-conditional TβRII KO mice adapted with permission from Ref. [98].. D) Silver staining of mouse femoral cortical bone canalicular networks in WT and Ocy-conditional YAP/TAZ KO mice, adapted with permission from Ref. [35].