



Review

The Mechanotransduction Signaling Pathways in the Regulation of Osteogenesis

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Abstract: Bones are constantly exposed to mechanical forces from both muscles and Earth's gravity to maintain bone homeostasis by stimulating bone formation. Mechanotransduction transforms external mechanical signals such as force, fluid flow shear, and gravity into intracellular responses to achieve force adaptation. However, the underlying molecular mechanisms on the conversion from mechanical signals into bone formation has not been completely defined yet. In the present review, we provide a comprehensive and systematic description of the mechanotransduction signaling pathways induced by mechanical stimuli during osteogenesis and address the different layers of interconnections between different signaling pathways. Further exploration of mechanotransduction would benefit patients with osteoporosis, including the aging population and postmenopausal women.

Keywords: mechanotransduction; osteogenesis; microgravity; mechanical stimuli; bone formation



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1. Introduction

Currently, a large number of elderly people, especially elderly women, suffer from osteoporosis. Of adults aged 50 years and above, 12.6% (19.6% in women and 4.4% in men) had osteoporosis at the femur neck, lumbar spine, or both in the USA in 2017–2018 [1]. Typical symptoms of osteoporosis include a decrease in bone mass and deterioration in the bone microstructure. Aging and menopause are the main risk factors for osteoporosis in women [2,3]. Estradiol deficiency increases the risk of osteoporosis in women during and after menopause [4,5]. In addition to estradiol deficiency, low mechanical load is another factor that increases the risk of osteoporosis. The occurrence of disuse osteoporosis can be attributed to the lack of mechanical loading on the skeletal system [6].

Bone mass loss occurs not only in individuals diagnosed with osteoporosis, but also in astronauts. Bone loss caused by microgravity was first observed in crews of the Skylab space station, which showed 1–2% decrease in bone mass every month [7–9]. A continuous decrease in bone density caused by space flight requires more time to recover to the pre-flight level [10,11]. Consistently, after seven days of space flight, mice showed 47–55% loss in tibial trabecular bone mass, 20–24% decrease in the thickness of trabecular bone, and 40–43% reduction in bone density [12]. Typical indicants of both osteoporosis and space bone loss include bone mass loss, decline in bone density, and structural impairment in bone tissue. Mechanical unloading is the primary factor contributing to disuse osteoporosis and space bone loss [6,13]. Therefore, studies on space bone loss due to microgravity will be inspiring to explore targets and therapeutic measures for osteoporosis. Nevertheless, due to the high expense and difficulties in space flight, there are limited numbers of space experiments on microgravity-induced bone loss.

Mechanotransduction is a process that includes a sense of the existence and changes in mechanical signals and signal transduction into the nucleus to regulate gene expression [14]. Here, we describe how microgravity affects osteogenesis by drawing mechanotransduction signaling pathways in detail and depicting the crosstalk between the pathways. A systemic overview on the mechanotransduction in osteoblast-lineage cells will provide clues to explore novel therapeutic targets against osteoporosis and bone loss during spaceflight.

2. Osteogenesis and the Differentiation of Osteoblast Lineage Cells

Osteogenesis, the process of bone development and formation, is crucial to maintain the skeletal system [15]. Osteogenesis is involved in the process of bone remodeling and the subsequent repair of bone fractures [16,17]. Osteogenesis is tightly regulated by mechanical and biochemical signaling pathways [18]. During osteogenesis, the sequential differentiation occurs in osteoblast lineage cells, including mesenchymal stem cells (MSCs), osteoprogenitors, pre-osteoblasts, osteoblasts, and osteocytes [16,17,19–22]. Osteoblasts secrete the organic matrix osteoid, which is subsequently calcified to form the bone. The calcification of osteoid is induced by the deposition of mineral salt. Subsequently, osteoblasts differentiate into osteocytes resulting in the formation of trabecular bone and periosteum [23].

The differentiation of osteoblast lineage cells undergo four sequential stages: commitment, proliferation, maturation, and mineralization (Figure 1) [24]. MSCs have multiple differentiation fates, including osteoblasts, fibroblasts, adipocytes, and chondrocytes [25]. During the commitment stage, osteoprogenitor cells originate from MSCs and differentiate into osteoblasts [25,26]. Subsequently, the committed osteoprogenitors proliferate and differentiate into pre-osteoblasts [27]. Pre-osteoblasts express collagen type I alpha 1 (COL1A1) and alkaline phosphatase (ALP), which are necessary for the bone matrix formation and mineralization [28]. For mature osteoblasts, the expression of osteopontin (OPN), bone sialoprotein (BSP), and osteocalcin (OCN) are all elevated along with ALP and COL1A1 [24]. The glycoprotein ALP on cell surface hydrolyzes the mineralization inhibitor pyrophosphate into phosphate, which promotes the mineral deposition on the collagen fiber scaffold [29–31]. OCN has high affinity to the hydroxyapatite matrix, and BSP enhances hydroxyapatite crystal formation [24]. OCN adjusts the alignment of apatite crystals parallel to collagen fibrils, to maintain bone mineral density and strength [32]. OPN inhibits hydroxyapatite formation, and decreases the differentiation of MSCs into osteoblasts [33,34]. After the matrix mineralization, mature osteoblasts undergo apoptosis, or form lining cells, or differentiate into osteocytes (Figure 1) [24,35]. Osteoblasts embedded in the mineralized bone matrix differentiate into osteocytes [36]. Non-apoptotic osteoblasts on bone surface become bone lining cells, which regulate bone remodeling by communicating with osteocytes [37].

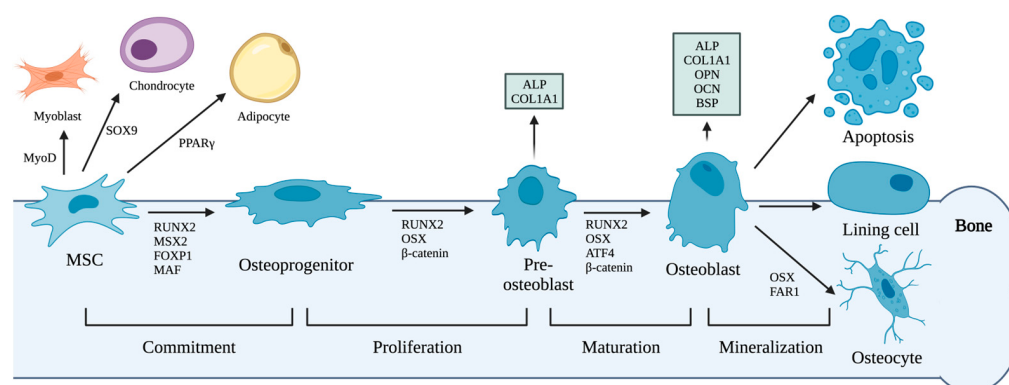


Figure 1. Schematic representation of the differentiation of osteoblast lineage cells. The differentiation process is divided into four stages. The representative markers for pre-osteoblasts and osteoblasts are presented in the corresponding boxes above the arrows, while TFs involved in the differentiation are presented beneath the arrows.

The differentiation of osteoblast lineage cells is tightly regulated by transcription factors (TFs) (Figure 1) [38]. For MSCs, PPAR γ , SOX9, and MyoD induce the differentiation into adipocytes, chondrocytes, and myoblasts, respectively [39–41]. Runt-related transcription factor 2 (RUNX2) is considered as the master switch for the initiation of osteogenesis, as RUNX2 is expressed in MSCs and further upregulated in pre-osteoblasts, while in osteoblasts, the expression of RUNX2 is decreased [42,43]. Osterix (OSX/SP7) induces the osteoblast differentiation, and inhibits the adipocyte differentiation [44]. Both RUNX2 and OSX induce the expression of ALP, OCN, OPN, BSP, and COL1A1 [24]. The co-activator β -catenin promotes the differentiation from pre-osteoblasts to osteoblasts [45]. Activating transcription factor 4 (ATF4), as one of the main transcription factors involved in osteoblast differentiation, functions via the upregulation of β -catenin [46–48]. TF Msh homeobox 2 (MSX2) promotes the osteogenic differentiation of MSCs and the calcification of osteoblasts [49]. Both AP1 protein FOS-related antigen 1 (FRA1) and JUNB, which are classic members of the Activator Protein 1 (AP1) transcription factor family, stimulate the osteoblast differentiation [50,51]. MAF bZIP transcription factor (MAF) positively regulates the osteogenic differentiation of MSCs, as well [52]. TF Forkhead box P1 (FOXP1) stimulates both the osteogenic differentiation of MSCs and the osteoblast mineralization [53,54].

Among multiple TFs regulating osteoblast differentiation, RUNX2 and OSX (encoded by gene *SP7*) are master TFs in the differentiation from MSCs into osteoblasts [55]. Therefore, expressions of *RUNX2* and *SP7* are commonly used as markers of osteogenic differentiation of MSCs. In addition to TFs, there are multiple makers characterized for different stages of osteoblast differentiation. As the contact between osteoblasts and collagen type I is essential for the differentiation of osteoblasts, collagen type I, especially COL1A1, is another maker for the differentiation from MSCs into osteoblasts [56,57]. As ALP enhances mineralization of ECM, the quantification of ALP at both mRNA and protein levels has been used to describe the differentiation of osteoblasts [28]. BSP, OPN, and OCN, which promote mineralization of ECM, are used as makers of differentiation of mature osteoblasts [58,59].

3. Mechanotransduction and Osteogenesis

3.1. Mechanical Stimuli and Osteogenesis

Both osteogenesis and the differentiation of osteoblast lineage cells are regulated by alterations of mechanical stimuli, such as microgravity. Microgravity simulation inhibits the osteogenic differentiation of MSCs and the process of mineralization, but promotes the adipogenic differentiation [60]. After 24 h of microgravity simulation, the mRNA levels of *Alp*, *Ocn* (osteocalcin), and *Runx2* in osteoblasts decreased by approximately 80%, 50%, and 60%, respectively [61]. After seven days of microgravity simulation, the activity of *Alp* was significantly reduced, and the expression of osteoblastic differentiation genes, including *Ocn*, *Col1a1*, and *Runx2*, was downregulated [62]. To dissect the underlying mechanisms between bone loss and microgravity, it will be helpful to explore new strategies or therapeutic targets to alleviate osteoporosis and space bone loss.

Mechanical stimuli directly affect the differentiation of MSCs and osteoblasts in vitro, whereas osteocytes are the major mechanosensitive sensors in bone tissue. Via mechanotransduction, mechanical signals regulate the osteogenic gene expressions and the release of signaling molecules. Subsequently, osteocytes influenced by mechanical signals regulated MSCs and osteoblasts. The lacunar canalicular system (LCS) is the fundamental structure for mechanosensing in osteocytes. The lacunae outside the cell body of osteocytes together with the tubules outside the dendrites of osteocytes form the LCS [63]. Both the osteocyte network and LCS are essential for the functions of osteocytes. Between the mineralized matrix and osteocytes, there is a 50–100 nm lacunae [64]. The tissue fluid fills the gaps within the collagen fiber layer [64]. Osteocytes, which are the primary cells responding to mechanical stimuli, are regulated by fluid shear stress under normal physiological conditions [65]. Mechanical unloading by microgravity during space flight induces bone matrix resorption and rebuilding around osteocytes [66,67]. Osteocytes transmit mechanical signals from the external environment to osteoblasts and osteoclasts

by releasing molecules including ATP, prostaglandin E2 (PGE₂), nitric oxide (NO), and growth factors [68–71]. Once osteocytes sense mechanical signals, mechanotransduction is initiated to induce the production and release of signaling molecules, such as ATP and PGE₂. Signaling molecules activate intracellular pathways in osteoblasts by binding to the receptors on cell membrane or by translocating into cells via channels. Ultimately, the activities of osteoblasts, such as differentiation, are altered in response to the mechanical signals, via both intracellular mechanotransduction and cell-cell communications (Figure 2). Generally speaking, the process of mechanotransduction includes three sequential steps: mechanosensing, mechanotransduction pathways, and transcriptional regulation.

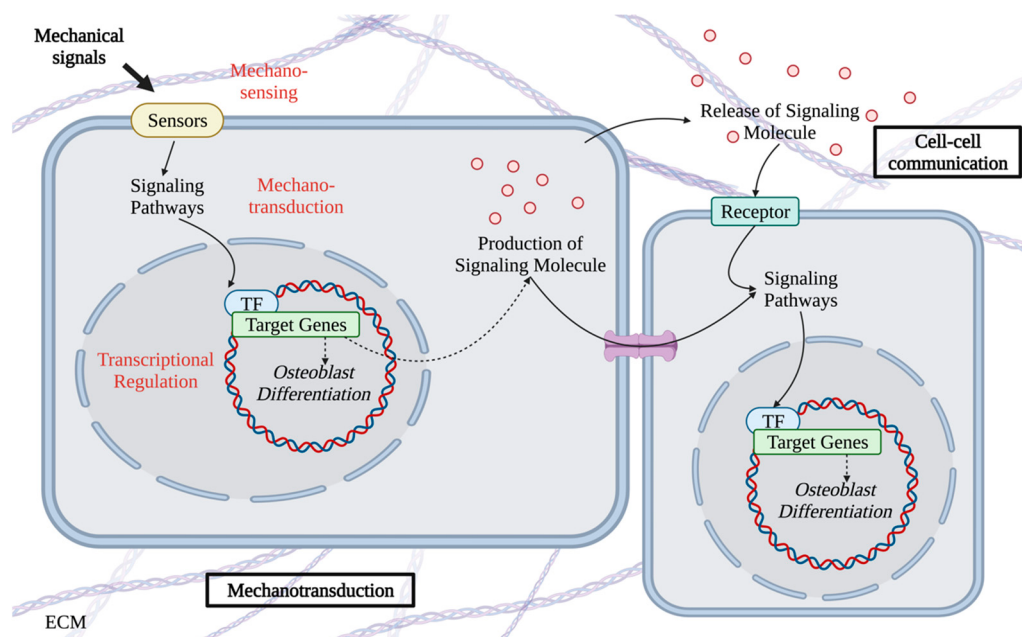


Figure 2. The mechanotransduction within or between bone cells. The signaling molecules transmit the mechanical signals into cells or nearby cells. The red fonts highlight the three sequential steps of mechanotransduction.

3.2. Mechanosensing on the Cell Membrane

Cells respond to mechanical signals, including physical forces, ultrasonic waves, and electromagnetic waves, to adapt to environmental changes. Since mechanical load stimulates bone growth, there have been numerous studies on the mechanisms of mechanosensing, the first step of mechanotransduction [72]. Mechanosensing is the process including the altered structure of sensors and transmission of extracellular signal into intracellular pathways [73]. Integrins, Piezo channels, primary cilia, and gap junction (GJ)-mediated mechanosensing have been extensively studied. In response to mechanical stimuli, integrin proteins change their structure to induce the formation of focal adhesions (FAs). Mechanosensitive calcium channels, represented by the Piezo calcium channel protein family, switch from closed to open in response to mechanical stimuli, resulting in extracellular calcium ion influx and a series of biochemical reactions. Activated by fluid flow shear stress, the cAMP level recedes in primary cilia. Mechanical stimuli switch GJs to the open state to allow signaling molecules, including calcium ions, ATP, and PGE₂, to translocate into neighbor cells. Signaling molecules translocate to ECM via GJs, as well. The mechanical sensors, including integrins, Ca²⁺ channels, and GJs, will be introduced one-by-one in detail.

3.2.1. Integrins Sense Mechanical Signals through Integrin-ECM Interaction

Integrins, a family of transmembrane proteins, include non-covalently linked α and β subunits. Integrins function as receptors in heterodimer to initiate the assembly of signaling

complexes, which subsequently link ECM to cytoskeleton [74]. The integrin heterodimers $\alpha1\beta1$, $\alpha2\beta1$, $\alpha5\beta1$, $\alpha6\beta1$, $\alpha V\beta3$, and $\alpha V\beta5$ have been characterized in human MSCs (hMSCs) [75]. The $\alpha1$, $\alpha3$, and $\beta1$ integrin subunits are predominantly expressed, whereas $\alpha2$ is weakly expressed in osteoblasts [76]. Integrin $\alpha1$ -5, αV , $\beta1$, and $\beta3$ are expressed in osteocytes [77,78]. Since the abnormality in integrin is associated with bone diseases, integrins are potential therapeutic targets for bone loss. For example, the impairment of $\alpha V\beta3$ integrins in osteocytes results in the attenuation of mechanosensing [78].

ECM, as a three-dimensional non-cellular macromolecular network, is composed of collagens, proteoglycans, and glycosaminoglycans, such as elastin, fibronectin, and laminins [79]. Type I, III, and V collagen proteins, the most abundant constituents of ECM in bones, mainly act as scaffolds for mechanical support in bone cells [80]. Small leucine-rich proteoglycans interact with the collagen framework, cytokines, and receptors to regulate the proliferation, differentiation, and especially mineralization [81]. As an essential ECM component, BSP, a heavily glycosylated and phosphorylated protein, promotes osteoblast differentiation and initiates matrix mineralization [82].

The mechanotransduction is initiated by the interactions between integrins and ECM [83]. Integrin heterodimers are inactivated by the cytoplasmic salt bridges. When integrins are inactivated, the extracellular domains are in a close and bent conformation with a low ligand-binding affinity (Figure 3A) [84]. Mechanical forces stretch the integrin binding sites on cell surfaces to switch them into an open and extended conformation, with high affinity to ECM ligands, such as laminin (ligand for $\alpha6\beta1$), fibronectin (ligand for $\alpha5\beta1$ and $\alpha V\beta3$), or vitronectin (ligand for $\alpha V\beta3$) (Figure 3A) [84–86]. The activated integrins then aggregate and reinforce the connections between cells and ECM [87,88].

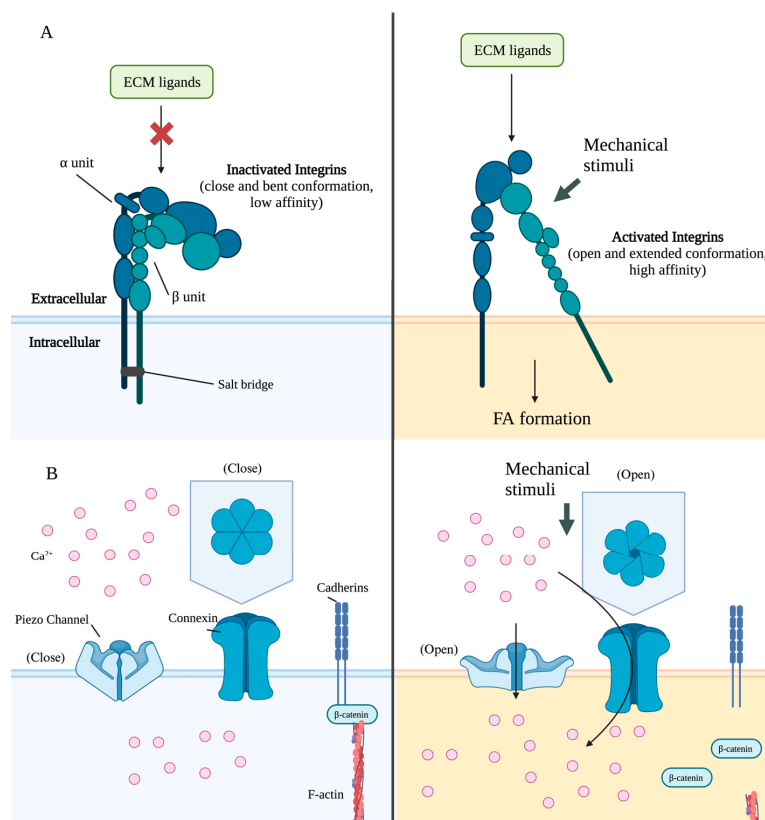


Figure 3. The activation of sensors by mechanical stimuli. (A) The conformation of integrins switches from the close and bent conformation (left) to the open and extended conformation (right) by mechanical stimuli. The activated integrins initiate the FA formation for mechanotransduction. (B) Mechanical stimuli transform channel proteins, including Piezo and connexin 43, from closed status (left) to open status (right). β -catenin combined with cadherins in a static state (left), but are released into cytoplasm with mechanical stimuli (right).

A series of integrins, including $\alpha 5 \beta 1$ and $\alpha 3 \beta 1$, mediate the direct interactions between osteoblast-lineage cells and ECM. For example, specific $\alpha 5 \beta 1$ integrins interact with fibronectin, which is a type of glycoprotein distributed ubiquitously in ECM during osteogenic differentiation [89,90]. Under Rotating Cell Culture System (RCCS) microgravity simulation for seven days, the differentiation of hMSCs into osteoblasts was severely impaired, the expression of the ECM proteins and type I collagen (Col I) was decreased, and the expression of Col I-specific $\alpha 2$ and $\beta 1$ integrin proteins was enhanced [91]. However, FA formation and the activation of FA signal proteins were subsequently decreased. The activation of downstream pathways is influenced by the alterations in ligand accessibility and the quantity of integrins [91]. Above all, integrins are involved in mechanotransduction in response to mechanical changes.

3.2.2. Ca^{2+} Channels as Mechanical Sensors

Calcium ion channels transform extracellular mechanical signals into intracellular biochemical signals [92,93]. Calcium intake benefits bone health via the regulation on cell-cell communications and cell-ECM interactions [94,95]. Intracellular calcium signaling is widely involved in the regulation of cell proliferation, differentiation, and metabolism [96].

Piezo proteins, including Piezo1 and Piezo2, were identified as mechanosensitive Ca^{2+} channels in 2010 [97]. Using cryoelectron microscopy, the structures of mouse Piezo1 and Piezo2 have been depicted in detail [98,99]. Piezo1 is a three-bladed propeller-like homotrimer with two modules: a central ion-conduction pore module and a peripheral mechanotransduction module [100,101]. And the structures and functions of Piezo1 and Piezo2 proteins are highly similar.

Piezo proteins play important roles in osteogenic differentiation. In MSCs, Piezo1 senses hydrostatic pressure to promote osteogenic differentiation and inhibits differentiation into adipocytes [102]. *Piezo1* knockout results in bone formation failure and stunted bone in adulthood, and consistently, the decrease of Piezo1 channels is one of the causes of osteoporosis [103]. Fluid shear stress upregulates the expression of *Piezo1* and induces the expression of *Runx2* in pre-osteoblasts MC3T3-E1 cells [104]. As a type of mechanical stimuli, low-intensity pulsed ultrasound (LIPUS) transduced by Piezo1 increases the concentration of intracellular calcium [105]. Mechanical signals induce cell membrane deformation, and the tension from the deformed cell membrane stretches the Piezo1 channel to open the central pore module (Figure 3B) [106,107]. Extracellular cations, including calcium ions, enter cells through Piezo channels to activate downstream pathways [108,109]. The microgravity simulation significantly reduced the expression of *Piezo1* and *Alp*, whereas fluid shear stress upregulated their expression [103].

The primary cilium, a non-motile microtubule-based organelle protruding from cell surface, senses extracellular chemical and mechanical signals and transduces mechanical signals into cells [110,111]. Primary cilia are in hMSCs, osteoblasts, and osteocytes [111–113]. When cells are exposed to fluid flow shear, the primary cilia deflect and recoil accordingly with fluid flow [114]. Using siRNAs to inhibit Polaris, a protein necessary to the primary cilia formation, resulted in the transcriptional reduction of *RUNX2* and *PPARG* [115]. The expression level of *OPN* was increased in osteoblasts 1 h after exposure to oscillatory fluid flow [111]. The removal of primary cilia by chloral hydrate treatment via the disruption of the connection between primary cilia and the basal body, results in the failure of response to fluid flow for osteoblasts. The mRNA expression of *OPN* is increased by fluid flow [116]. However, the transcriptional increase of *OPN* is damaged by the removal of primary cilia [111]. Therefore, without primary cilia, cells fail to respond to fluid flow.

The mechanosensitive calcium channel TRPV4 in primary cilia is a member of the transient receptor potential (TRP) channel family [117]. TRPV4, 871 amino acids with six transmembrane α -helices, is expressed in MSCs, osteoblasts, and osteocytes [118]. TRPV4 regulates bone homeostasis by controlling the Ca^{2+} influx [119–123]. Dominant mutations in *TRPV4* cause several types of skeletal dysplasia, including metatropic dysplasia, spondylometaphyseal dysplasia Kozlowski type (SMDK), and autosomal dominant

brachyolmia [124]. Mechanical forces promote osteoblastic differentiation by enhancing the expression of *TRPV4* [125,126]. The primary cilia-dependent calcium ion channel *TRPV4* mediates fluid shear signal transduction by inducing calcium ion into primary cilia. Even when *TRPV4* is activated, the osteogenic differentiations of MSCs with defective primary cilia are inhibited [127]. Therefore, primary cilia and *TRPV4* channels collaborate to sense mechanical signals.

3.2.3. Gap Junctions and Bone Cell Communication

Both GJs and hemichannels, which are composed of connexins, are involved in the transmission of mechanical signals. The hexameric connexin protein is composed of six homogenous or heterogeneous connexin subunits. Hemichannels provide communication between the intracellular cytoplasm and extracellular ECM [128]. GJs between adjacent cells enable the communication of neighboring cells [129]. For example, GJs between osteoblasts and MSCs promote the transcription of *ALP* in MSCs [130]. Connexin 43, expressed in osteocytes, osteoblasts, and MSCs, is responsible for the gap junction formation [131].

Mechanical stimuli are transmitted via altering the expression of connexin 43 and status of GJs. In bone tissue, signaling molecules, including PGE_2 , ATP, cAMP, and Ca^{2+} , diffuse from cells via GJs or hemichannels to stimulate signaling pathways in neighbor cells [132–135]. The expression of connexin 43 was increased in MC3T3-E1 cells after 5 h treatment with microstrain [136]. The mechanical signal transforms GJs and hemichannels from closed to open status to exchange signaling molecules (Figure 3B) [77]. Response to fluid shear stress, PGE_2 , and ATP are released via hemichannels from osteocytes into ECM [132,133].

In addition to GJs, cadherins implement the communication between nearby cells. In osteoblast lineage cells, N-cadherin (cadherin-2, *CDH2*) and cadherin-11 (*CDH11*) are predominantly expressed [137]. The expression of cadherins, including *Cdh2* and *Cdh11*, is low in MSCs, while the expression of *Cdh2* and *Cdh11* is increased with the differentiation commitment to the pre-osteoblasts [138]. During the progress of the osteoblast differentiation, *Cdh2* is downregulated and subsequently rarely expressed in mature osteoblasts, but *Cdh11* is present throughout the osteoblast differentiation [138]. The adhesion mediated by cadherin is crucial for the early stage of osteoblast differentiation [139]. The function loss of cadherin inhibits osteoblast differentiation, but enhances adipogenic differentiation [140]. Cadherins are essential to osteogenesis, as there are developmental defects and low bone mass in *Cdh2* mutant mice [138].

As a type of sensor, cadherins mediate the transmission of mechanical stimuli. In a static state, β -catenin mediates the connections between cadherins on cell membrane and cytoplasmic cytoskeleton. In response to mechanical stimuli, such as fluid flow shear, the release of β -catenin from cadherins and the β -catenin accumulates in the cytoplasm subsequently (Figure 3B). The cytoplasmic β -catenin undergoes translocation into the nucleus and triggers transcription in response to mechanical stimuli [141,142].

3.3. The Cytoplasmic Mechanotransduction Pathways

For the pathways involved in mechanotransduction during osteogenesis, both the integrin-focal adhesion (FA)-cytoskeleton pathway and the RhoA pathway depend on the activation of integrins. Both the NFAT-Ppp3Ca pathway downstream of Piezo and mitogen-activated kinase (MAPK) pathways depend on the influx of calcium ions. The decrease in cAMP level in primary cilia enhances the promotion of osteogenesis by cyclooxygenase-2 (COX-2) and inhibits the activity of β -catenin by regulating its activation or the translocation into the nucleus.

3.3.1. Cytoskeleton Reorganization by FA and RhoA Pathway

The cytoskeleton, as a prestressed tensegrity structure, receives and sustains force, stabilizes cells, and facilitates cells to adapt to environmental alterations [143]. Filamentous actin (F-actin), as the main form of cytoskeleton, is a polar polymer of globular actin

(G-actin). At the barbed and pointed end of F-actin, G-actin is polymerized and depolymerized, respectively. The binding of cofilin induces the severing of F-actin [144]. The reorganization of the actin cytoskeleton is a response to mechanical forces. Actin filaments are extended and stabilized in a direction parallel to the force, and then myosin II replaces cofilin to associate with actin. Microtubules (MTs) and intermediate filaments (IFs), other types of cytoskeletons, are involved in mechanotransduction, as well. MTs are acetylated by integrin-mediated substrate-rigidity sensing [145]. IFs are crucial in the regulation of cell shape and maintenance of mechanical integrity [146].

The activated integrins mediate the formation of focal adhesions (FAs), which connect integrins to the F-actin cytoskeleton. FAs are composed of signal proteins and structural proteins. Signal proteins, including Src and focal adhesion kinase (FAK), are crucial for mechanical transmission [147]. Once the external mechanical stimuli are sensed, FAK is recruited to FA first, and the structural proteins, including talin, paxillin, vinculin, and zyxin, are recruited to the complex, subsequently [147–151]. The binding between vinculin and talin further stabilizes the interaction between talin and F-actin, and thus transfers the mechanical signal inward [152]. The structural protein p¹³⁰Cas is phosphorylated by the FAK-Src complex to respond to mechanical stress, such as the attachment and spreading [153–155]. The connection between integrin and actin mediated by FA reorganizes cytoskeleton to adapt to environmental changes, such as mechanical stimuli (Figure 4).

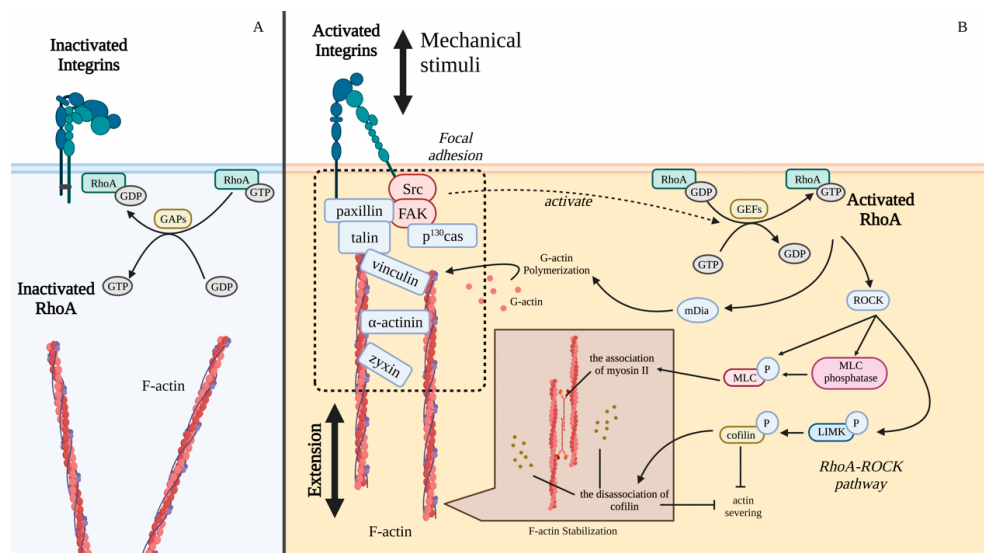


Figure 4. The activated integrin recruits signal proteins and structural proteins to form FA to respond to mechanical stimuli. (A) Without mechanical stimuli, RhoA is inactive as RhoA-GDP. (B) Mechanical stimuli activate integrins to initiate FA formation. Through FAK-Src complex, RhoA pathway is activated by integrin-mediated mechanical stimuli. mDia activated by RhoA promotes the polymerization of G-actin. ROCK activated by RhoA promotes the dephosphorylation of MLC and cofilin to stabilize the cytoskeleton. Integrin-FA complex reorganizes the actin cytoskeleton and transmits the extracellular mechanical signals into cells.

RhoA signaling is involved in mechanotransduction via the regulation of cytoskeletal stabilization [156]. FAs mediate mechanical signals, including fluid shear stress, from activated integrins to RhoA [157–159]. After the sensing of external mechanical stimuli, FAK and Src are recruited and activated, which subsequently activate RhoA. RhoA, a GTPase, regulates various cellular activities, including actomyosin dynamics, adhesion, proliferation, and survival [160]. The interaction between GDP and RhoA is essential to maintain RhoA in an inactive state in the cytoplasm [161]. GTPase-activating proteins (GAPs) transform RhoA into an inactive state by converting GTP to GDP (Figure 4A) [162]. In contrast, RhoA is activated by guanine nucleotide exchange factors (GEFs) by catalyzing GDP to GTP (Figure 4B) [163].

The stabilization of the actin cytoskeleton is enhanced via the actin polymerization and the F-actin-severing inhibition by the RhoA pathway. Both mDia and ROCK are downstream effectors of the activated RhoA (Figure 4B) [164,165]. The effector mDia promotes the extension of F-actin through enhancing the polymerization of G-actin [166]. Another effector ROCK alters the association between F-actin of myosin II and cofilin. ROCK increases the phosphorylation of myosin light chain (MLC) to promote the assembly of myosin II into bipolar filaments, and inhibits the dephosphorylation of phosphorylated MLC [167,168]. The ATPase activity of myosin II is enhanced by ROCK. The association between myosin II and the phosphorylated MLC further increases the stability of actin. LIM kinase (LIMK) is phosphorylated by ROCK. The activated LIMK inactivates cofilin, which is an F-actin-severing protein [169]. The dissociation of cofilin from actin results in the inhibition of F-actin severing (Figure 4B).

Cell density alters cell shapes and drives hMSC commitment via the activation of RhoA, which subsequently regulates ROCK and cytoskeletal integrity [170]. Under enhanced or minimized mechanical stimuli, such as forces or microgravity, cell shape will be changed [171,172]. The intracellular cytoskeleton is resistant to the deformation induced by the extracellular mechanical stimuli, as the cytoskeleton is highly dynamic and adaptive [173]. Similarly, in response to a reduced gravitational load, from 1 G to microgravity, cells change cytoskeletal structures accordingly [174,175]. The reorganization of actin filaments in simulated microgravity provides clues of the altered cytoskeleton function in mechanotransduction [176].

3.3.2. Downstream Pathways of Ca^{2+}

The open Piezo channel activates the downstream pathways using calcium ions as second messengers. Calcium ions were first characterized as second messengers in the excitation-contraction coupling in skeletal muscles [177]. Calcium influx through the Piezo channel in osteoblasts promotes the phosphorylation of ERK1/2 and the polymerization of perinuclear F-actin filaments [105]. In addition, calcium influx through the open Piezo channels induced by mechanical signals activates calmodulin-dependent heterodimer serine/threonine phosphatase calcineurin (Ppp3ca). Nuclear factor of activated T cells (NFAT), Yes1 associated transcriptional regulator (YAP1), and β -catenin are activated via Ca^{2+} /Ppp3ca activated by Piezo1 (Figure 5) [178]. Nuclear NFAT and SP7 (Osterix) form transcriptional complexes to trigger the expression of osteogenic genes, including *COL1A1*, *ALP*, *SPP1(OPN)*, and *BGLAP (OCN)* [179,180]. The activated calmodulin-calcineurin pathway dephosphorylates NFAT in osteoblasts [181].

Calcium influx induced by mechanical signaling activates Runx2 via the Ras/ERK-MAPK pathway, a subfamily of MAPK pathway families (Figure 5) [182–184]. The activation of the MAPK pathway by tensile and shear stress, hence, facilitates the transmission of mechanical signals [185]. Via the ERK-MAPK pathway, mechanical stress promotes osteogenic differentiation and osteogenesis [182,183]. The phosphorylated ERK activated by the MAPK pathway is translocated into the nucleus to phosphorylate RUNX2, which decompresses the chromosome and promotes the transcription of osteogenic genes [186].

The deflection of primary cilia caused by fluid shear activates Ca^{2+} channels, and intracellular Ca^{2+} influx inhibits the activity of adenylyl cyclase 6 (AC6), resulting in the decreased levels of cAMP [115]. The drop in cAMP in the primary ciliary activates osteogenesis by promoting the expression of COX-2, which produces PGE_2 and further regulates RUNX2 and SP7 to mediate osteogenesis and bone repair (Figure 5) [187,188]. Protein kinase A (PKA), another downstream effector in primary cilia, activates the ERK1/2-CREB signaling pathway and inhibits glycogen synthase kinase 3 β (GSK3 β)-mediated degradation of β -catenin [116,141,189]. Through the MAPK and Ras/Raf-dependent ERK1/2 pathways, mechanical stress increases the activated Runx2 and promotes osteoblast differentiation.

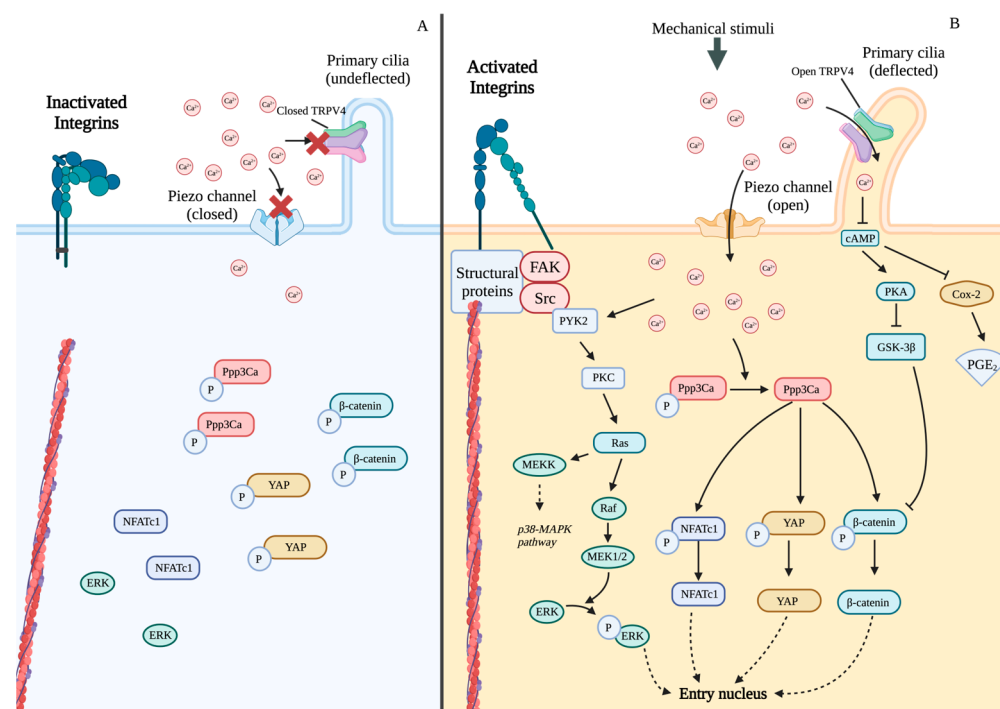


Figure 5. Mechanosensitive Ca²⁺ channels are open with mechanical signals, and subsequent Ca²⁺ influx initiates downstream pathways. **(A)** The mechanosensitive Ca²⁺ channels are close in static environment. **(B)** The mechanical stimuli switch the mechanical-sensitive Ca²⁺ channels to the open state. The influx of Ca²⁺ activates multiple pathways. In deflected primary cilia, influx of Ca²⁺ results in the reduction of cAMP.

3.4. Nuclear Alterations and the Transcriptional Regulation

For the transmission of mechanical signals into the nuclei, the linker of the nucleoskeleton and cytoskeleton (LINC) is necessary to connect the nucleoskeleton, nuclear envelope, and cytoskeleton [190]. Inner SUN and outer KASH domain proteins form the core of the LINC complex [191]. The nuclear envelope consists of inner and outer nuclear membranes (INM and ONM), where SUN and KASH anchor (Figure 6) [192]. By the interaction with LINC and F-actin, the nucleus acts as a mechanical-sensitive subcellular compartment [193]. The alteration in nucleus shape, induced by cell deformation and mediated by cytoskeleton-LINC, influences the intranuclear transcription. Under a 1 G gravity environment, the nucleus has a large, round shape, but microgravity makes the nucleus 30% smaller [194]. Nuclear pore complexes (NPCs) on the nuclear membrane mediate the transport between the cytoplasm and the nucleus [195]. Stimulated by mechanical force, the permeability of nuclear pores is increased by the LINC-regulated nuclear stretch, and more TF proteins enter the nucleus subsequently (Figure 6) [196].

In the nucleus, RUNX2, YAP, TAZ, and β-catenin are responsive to mechanical stimuli. In the ERK-MAPK pathway, Runx2 is activated and transmits the mechanical signals to gene expression regulation (Figure 6). In the nucleus, the phosphorylated ERK of the MAPK pathway phosphorylates RUNX2 protein to enhance the binding between the histone acetyltransferase p300 to achieve the acetylation of histone via H3K9ac and H4K5ac [186]. The epigenetic changes decondense chromatin and increase the transcription of osteogenic genes via the recruitment of RNA polymerase II [186].

As both YAP and TAZ lack DNA-binding domains, YAP and TAZ must bind to coactivators to activate the transcription of target genes [197]. Both in vitro and in vivo experiments showed that the inhibition on the interaction between YAP/TAZ and the transcriptional enhanced associate domain (TEAD) reduced the expression of osteogenic genes (Figure 6) [198]. Compared to the control group, in YAP or TAZ knockout mice, matrix collagen contents were reduced and bone microstructures were damaged [198]. YAP

promotes osteogenesis and suppresses adipogenesis by interacting with and stabilizing β -catenin protein to maintain the nuclear level of β -catenin [199]. TAZ stimulates osteoblast differentiation via activating Runx2 [200]. YAP/TAZ is involved in diverse steps of osteogenesis; for example, YAP/TAZ inhibits MSC differentiation into osteoblasts, promotes bone formation, and inhibits bone resorption in mature osteoblasts and osteocytes [201].

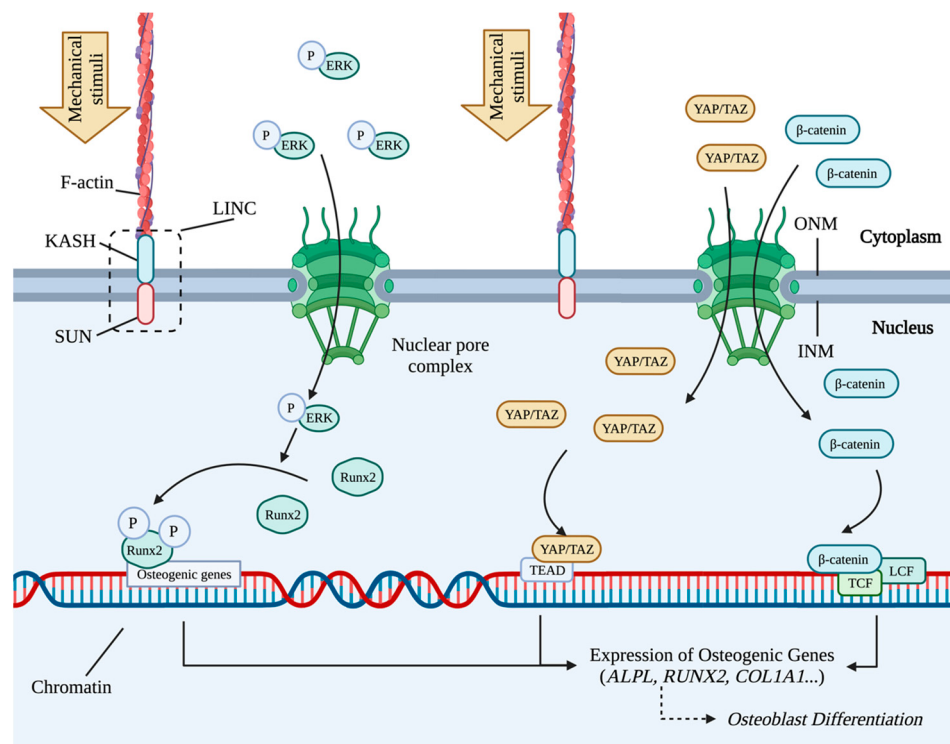


Figure 6. The responsive translocation of TFs and the transcriptional regulation by mechanotransduction during osteogenesis. Mechanical stimuli are transformed to nuclear stretch via the interactions between LINC and F-actin. Subsequently, NPCs on nuclear membrane are allowed increasing TFs and coactivators to enter into the nucleus. More YAP/TAZ and β -catenin proteins enter nucleus and promote the expression of osteogenic genes. The binding by the phosphorylated Runx2 transforms the nearby chromosomal region to open status and stimulates the expression of osteogenic genes.

β -catenin, encoded by *CTNNB1*, is a multifunctional protein [202,203]. β -catenin shuttles between the cytoplasm and nucleus [204]. Cytosolic stable β -catenin enters the nucleus to bind to T-cell factor/lymphoid enhancer-binding factor (TCF/LEF) proteins and activate transcription subsequently, including osteogenic gene expressions (Figure 6) [202,205]. In bone tissue, β -catenin promotes bone formation and inhibits bone resorption in both mice and human [206,207]. β -catenin stimulates the differentiation of pre-osteoblasts into osteoblasts [46].

3.5. Crosstalks during Mechanotransduction

Mechanotransduction is summarized as three sequential steps as above: mechanical sensors on cell membrane, cytoplasmic mechanotransduction pathways, and transcriptional regulation. There are crosstalks at each layer, which make the mechanotransduction an interconnected network.

The cooperation between different sensors enhances the mechanotransduction initiation (Figure 7A). For osteocytes, fluid shear stress stimulated sensors connexin 43 hemichannels via activating α V and α 5 integrins [208]. Sensor Piezo1 binds to integrins and promotes the formation of FA [209]. Fluid shear stress leads to Piezo1-mediated integrin activation resulting in FAK activation [210].

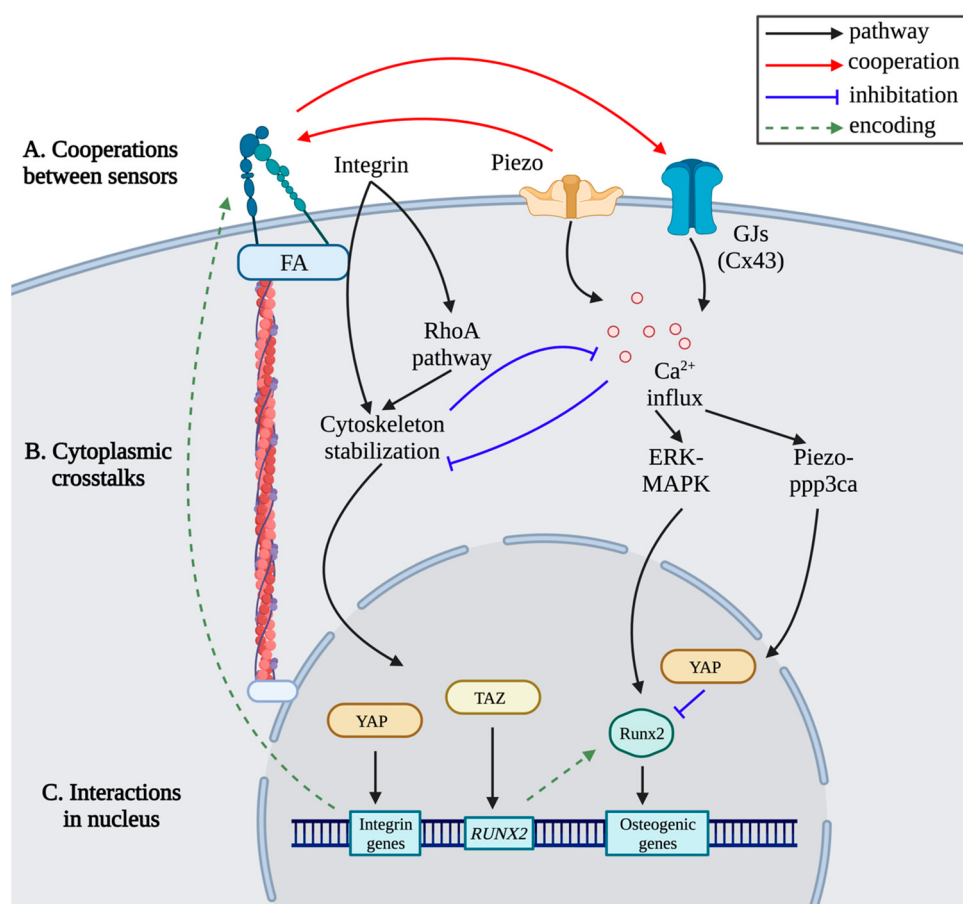


Figure 7. Crosstalks during mechanotransduction. In mechanotransduction, sensors cooperate with each other. Within the cytoplasm, cytoskeleton and Ca^{2+} -involved pathways have crosstalks. The expression of osteogenic genes is coordinated by different TFs and coactivators, including *RUNX2* and integrin protein coding genes.

Cytoskeleton and cytoplasmic Ca^{2+} -mediated pathways are responsible for the transformation of mechanical stimuli from sensors to nuclear. In mechanotransduction pathway networks, cytoskeleton and cytoplasmic Ca^{2+} are central components. Cytoskeleton and Ca^{2+} interact with each other (Figure 7B). The increase in calcium inhibits the elongation of filaments [211]. In osteoblasts, the polymerization of actin increases the influx of calcium ions, while depolymerization decreases the influx [212]. After the sensing of mechanical signals, cytoskeletal mechanical sensors activate mechanical transducers including Ca^{2+} influx [213].

The expressions of osteogenic genes are regulated by intranuclear interactions (Figure 7C). TAZ coactivates the transcription of *RUNX2*-dependent genes, and promotes the osteogenic differentiation of MSCs [214]. Interactions between YAP and *RUNX2* suppress the transcriptional activity of *RUNX2* [215]. As the *Runx2* promoter region has TCF response elements, β -catenin positively regulates *Runx2* expression [216]. In addition, TFs and coactivators affect sensors and components of pathways in mechanotransduction via expression regulation. For example, integrin genes *ITGA1*, *ITGA4*, and *ITGAV* are target genes of YAP [217].

4. Perspectives and Conclusions

Osteogenesis plays a crucial role in the maintenance of bone mass and strength. Mechanical signals influence osteogenesis via mechanotransduction, which is the process that transmits mechanical signals to the nucleus to regulate gene expression. Further investigation on mechanotransduction will potentially provide a comprehensive understanding

of the molecular mechanisms of bone loss and facilitate the development of a therapeutic strategy against osteoporosis, as well.

The principles of osteoporosis therapy are to improve bone formation and/or to decrease bone resorption. Bisphosphonates inhibit bone resorption via the inhibition on the activity of osteoclasts [218,219]. Bisphosphonates are widely prescribed in clinical practice, including alendronate, risedronate, ibandronate, and zoledronic acid [220–223]. Among multiple bisphosphonates, alendronate has been proven to alleviate bone loss of the astronauts on long-term space missions [224]. It is convenient to take bisphosphonates orally, but bisphosphonates are commonly employed for a maximum duration of 10 years in the long-term management of osteoporosis [225,226]. In addition, bisphosphonates are associated with adverse effects, such as osteonecrosis of the jaw, delayed dental eruption, atypical femoral fracture, and ocular side effects [227,228]. Another commonly prescribed anti-bone resorption drug, Denosumab, is more effective against bone loss than bisphosphonate [229]. But denosumab increases the incidence of adverse events, including hypocalcemia, osteonecrosis of the jaw, and atypical fractures [230]. From the perspective of bone formation, bone-building drugs have been developed to alleviate osteoporosis, and teriparatide, abaloparatide, and romosozumab are commonly recommended [231–233]. In comparison to anti-bone resorption drugs, bone-building medications are more efficient to enhance bone mass density and have fewer side effects [234,235]. Bone-building medications are appropriate for patients experiencing complications from bisphosphonate treatment, but the maximum usage lasts for only 2 years [236,237]. And the subcutaneous injection makes bone-building medications less convenient to take [232,238]. As bone mass was increased by bone-building medications in mice models under microgravity simulation, it is likely that the bone-building medications will bring benefits to astronauts against space bone loss [239]. However, the hypothesis remains to be validated by experiments in orbit. Since both bisphosphonates and bone-building medications have limitations, it is demanding to explore pharmaceuticals against bone loss with less adverse effects and more convenience.

In addition to pharmacological interventions, mechanical stimuli represent an alternative therapeutic approach for mitigating bone loss by promoting bone growth. LIPUS, as a type of mechanical stimulus, has significant advantages for osteogenesis by promoting the differentiation of osteoblasts, thereby effectively facilitating bone regeneration [240]. In 1994, LIPUS was approved as an adjuvant therapy in the healing of primary fractures [241]. In Canada and the UK, LIPUS is available for patients as a prescribed treatment [242]. An extremely low-frequency pulsed electromagnetic field, which is a type of mechanical stimuli, enhances the proliferation and differentiation of osteoblasts [243]. The utilization of mechanical stimulation is associated with minimal side effects, mostly due to the non-invasiveness. Studies on mechanotransduction may provide new potential therapeutic targets by mechanical stimulation to cure osteoporosis.

Further investigation on the molecular basis of mechanotransduction in bone physiology is essential to explore mechanically oriented therapeutic strategies. The components of mechanotransduction are potential targets that facilitate the responses to mechanical loading. Previous research has demonstrated that the activation of integrin α V, a sensor involved in mechanotransduction, represents a potential for the treatment of osteoporosis [244]. Delivery strategies have been explored to target bone cells, specifically MSCs and osteoblasts. Lipid nanoparticles and liposomal transport have been employed for the targeted delivery of medicines into osteoblasts and MSCs, as documented in previous studies [245,246]. A bone-targeting technology has been developed for the delivery of siRNA, and the efficacy has been evaluated in a preclinical investigation [247,248]. In mice, exosomes deliver S8178, a Wnt agonist, to bone specifically, thereby facilitating the osteogenic differentiation of MSCs [249]. Bisphosphonates, which have an affinity to hydroxyapatite, are applied to implement bone tissue-specific targeting [246]. By integrating iron oxide nanoparticles and bisphosphonates, the treatment of osteoporosis was efficient by increasing the bone mechanical strength [250].

Above all, this review provides a comprehensive overview of mechanotransduction during osteogenesis. The mechanotransduction pathways and crosstalks described in this review provide potential targets against bone loss. Among the components of mechanotransduction, molecules, which promote the corresponding responses to mechanical load, are potential targets. Via the inhibition or augmentation of targets, therapies mimicking the enhanced mechanotransduction may increase osteogenesis to improve lost bone mass in osteoporosis patients or astronauts.

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