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Review

Molecular mechanisms in bone mechanotransduction

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Summary. Bone is one of the most adaptable tissues in the body as it is continuously subjected to load bearing. In fact, mechanical loading is an important regulator of bone mass. The skeleton adjusts to load by changing its mass, shape and microarchitecture, depending on the magnitude of the strain. Mechanical stimulation is necessary for the development of the skeleton, whereas in adults physiological levels of strain help maintain bone mass by reducing bone resorption. On the other hand, an excessive level of strain or bone disuse induces bone loss. Osteocytes are long-lived cells comprising more than 90% of bone cellularity, which are embedded in the bone matrix forming a functional syncytium extending to the bone surface. These cells are considered to be the main bone cells responsible for translating mechanical strain into regulatory signals for osteoblasts and osteoclasts, leading to adapting bone responses to environmental changes. In this review, we discuss the complexity and well-orchestrated events that occur in bone mechanotransduction, focusing on osteocyte viability as an important biological response in this respect. Elucidation of the molecular mechanisms of bone mechanotransduction and the key role of osteocytes is opening new avenues for the treatment of bone loss-related diseases.

Key words: Osteocytes, Mechanical stimulation, Bone mechanotransduction, Mechanoreceptors, Osteocyte survival

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Introduction

Bone is one of the most adaptable tissues in the human body. In fact, the primary function of the skeleton is to give support to the whole body and to withstand the biomechanical loads imposed by daily life, thus preventing damage (Lanyon, 1992). Both cortical and cancellous bone work together to provide mechanical support, and can respond and adapt to mechanical stimuli (Ito et al., 2002; De Souza et al., 2005), but in a specific manner, related to their different structure and mechanical properties (Currey, 1984; Martin et al., 2015). Whereas cortical bone carries a considerable share of the total load bearing (Martin et al., 1989), cancellous bone -with the interconnected architecture of trabeculae- provides structural support by funneling mechanical stresses towards the stiff cortical layer on the

Abbreviations. Akt, protein kinase B; COX, cyclooxygenase; Cx, connexin; ER, estrogen receptors; ERKs, extracellular signal-regulated kinases; FAK, focal adhesion kinase; FRET, fluorescence resonance energy transfer; FZD, frizzled receptors; G-proteins, guanine nucleotide-binding proteins; GPCRs, G protein-coupled receptors; GSK-3β, Glycogen synthase kinase 3 beta; iNOS, inducible form of NOS; LRP5/6, low-density lipoprotein receptor-related protein 5 or 6 co-receptors; MSCs, mechanosensitive channels; NO, nitric oxide; NOS, NO synthase; NS-398, N-[2-(Cyclohexyloxy)-4-nitrophenyl] methanesulfonamide; PC, polycystin; PGE2, prostaglandin E2; PTH1R, parathyroid hormone/parathyroid hormone related protein type 1 receptor; PI3K, phosphatidylinositol-4,5-bisphosphate 3-kinase; PYK2, proline-rich tyrosine kinase 2; RANKL, receptor activator of nuclear factor kappa-B ligand; TRV4, transient receptor potential vanilloid 4; VEGF, vascular endothelial growth factor; VEGFR2, vascular endothelial growth factor receptor 2; VSCs, voltage-sensitive channels.

bone surface (Huiskes et al., 2000).

Mechanical loading is an important regulator of bone mass. Hence, the skeleton can adjust to load by changing its mass, shape and microarchitecture, and this response differs with the magnitude of the imposed strain (Turner et al., 1991; Robling et al., 2006; Schulte et al., 2013). The concept that the skeleton adapts its structure to mechanical loads that create bone stresses was initially proposed by Julius Wolff and enshrined in Wolff's Law (Wolff, 1892). In the late 1980s, this idea was extended by developing a conceptual model -the *mechanostat* hypothesis- to explain bone adaptation to mechanical inputs (Frost, 1987). This is based on the piezoelectric properties of bone and proposes several mechanisms through which bone strength can be increased upon demand. This hypothesis has been a useful tool for understanding how the mechanical environment may regulate bone modeling and remodeling, and the manner whereby osteoporotic bone can respond to mechanical stimulation (Frost, 1987).

Mechanical stimulation appears to be necessary for the development of the skeleton (Roddy et al., 2011; Sharir et al., 2011). It has been described that limbs may decrease up to 50-70% of their normal bone mass in the absence of mechanical usage during human bone growth (Ralis et al., 1976; Rodriguez et al., 1988). In this context, studies in both humans and animals have shown that bone reshaping in response to mechanical loading occurs during skeletal growth (Kannus et al., 1995; Turner et al., 1995; Bass et al., 1998); in the adult skeleton, a physiological level of strain reduces bone resorption, thus contributing to bone mass maintenance (Flieger et al., 1998; Rubin et al., 2001). On the other hand, an excessive level of strain or bone disuse induces bone resorption and bone loss (Bikle et al., 2003; Aguirre et al., 2006).

Nevertheless, the molecular basis of bone mechanotransduction is not completely understood, and many questions still remain unanswered. In this review, we address the complexity and well-orchestrated events that occur in bone mechanotransduction, resulting in adaptive changes to maintain bone mass. We will start by dealing with osteocytes, the most abundant cell type in bone, and those principally responsible for sensing mechanical strain. We will then focus on the main osteocyte mechanoreceptors as well as different mechanisms of signaling resulting in increased osteocyte viability, a key factor for bone mass maintenance. As bone mechanical stimulation leads to activation of bone anabolic pathways, their characterization might provide clues to identify potential targets to treat bone loss-related diseases.

Osteocytes

Osteocytes are terminally differentiated osteoblasts that become embedded into the mineralized bone matrix during the process of bone deposition. Osteocytes constitute the most abundant cell type in bone, comprising more than 90% of all bone cells in mature bone (Parfitt, 1977). The physical environment in which osteocytes are located -buried in the mineralized matrix- is quite different from the bone surface, where osteoblasts, osteoclasts and lining cells localize, or from the bone marrow containing osteoblast and osteoclast progenitors (Bonewald, 2011). The fact that osteocytes are completely surrounded by mineralized bone would in theory hamper cell communication. However, each osteocyte is fitted in a cavity ("lacuna") but contains 40-60 dendritic processes emerging from the cell body in all directions and traveling inside narrow (260 nm of diameter) canals (canaliculi) (Knothe et al., 2004). Neighboring osteocytes contact with each other by these cell processes that transmit molecular signals through gap junction channels, permitting a rapid cell-cell communication. This lacunae-canalicular system thus provides a complex and extensive network of communication that links not only osteocytes with each other but also with other cells on the bone surface and the bone marrow (Kamioka et al., 2004). Thus, osteocytes are unique in being long-lived and abundant bone cells, which form a functional syncytium from the mineralized bone matrix to the bone surface and the bone marrow, and are capable of sensing variations in mechanical strain. All these features confer osteocytes the capacity to orchestrate osteoblast and osteoclast function, leading to adaptive responses of bone to environmental changes.

Role of osteocytes in bone mechanotransduction

Currently, both theoretical considerations and experimental evidence support the osteocyte role as the primary mechanosensitive cell in bone (Lanyon, 1993; Klein-Nulend et al., 1995; Mullender and Huiskes, 1997; You et al., 2008). Of note in this respect, transgenic mice with inducible and specific ablation of osteocytes but completely functional osteoblasts and osteoclasts show resistance to disuse-related bone loss in contrast to their wild controls, indicating that an intact and functional osteocyte network is necessary to sense and respond to these mechanical loading changes (Tatsumi et al., 2007). The question thus is how osteocytes are able to sense mechanical changes in their physical environment. The lacuno-canalicular system in which osteocytes are inserted is filled with bone interstitial fluid (Knothe et al., 2004; Bonewald, 2011). It has been proposed that upon bone loading, such fluid is pushed back and forth through the extracellular space. This causes deformation of both osteocytes within the lacunae and also osteocyte processes within the canaliculi, modifying the normally tight space between the cell membrane and the canalicular wall (Cowin et al., 1991). As a consequence, fluid velocity can increase from that supposedly corresponding to physiologic loads, in the range of 8-30 dynes/cm^2; the magnitude of this fluid force is proportional to the body load (Wang et al., 2005).

In the early 1990s, a model of strain amplification in osteocytes was proposed, including their sensitivity to

relatively small fluid shear stress as a consequence of fluid drag by the proteoglycan matrix on the osteocyte membrane (Weinbaum et al., 1994). This model has recently been further extended by emphasizing the importance of tethering the osteocyte processes to the canalicular wall via integrins and the glycocalix (Han et al., 2004). The fluid flow movement through the canalicular space would produce deformation of the latter tethering complex by imposing a hoop strain on the central actin bundles inside the osteocyte cell process. This has been estimated to represent an increase in osteocyte membrane strain of 10-100-fold over physiological values (Han et al., 2004).

The primary cilium as a mechanosensor organelle

In the last few years, the putative role of osteocyte primary cilium in bone mechanotransduction has emerged. This microtubule structure is a single and non-motile organelle protruding from the cell surface of many mammalian cell types, including kidney and liver cells. Of interest, it has been shown that fluid flow-induced bending of the renal epithelial cell primary cilium leads to a rapid increase of intracellular Ca^{2+} (Praetorius and Spring, 2001, 2003). In addition, this mechanical response appears to be mediated by the polycystin (PC) 1/2 receptor-ion channel complex consisting of PC1, a large membrane protein, and PC2, a cationic channel (Nauli et al., 2003). Recently, Xiao et al. found that PC1 and PC2 localize to the primary cilium in osteoblasts and osteocytes (Xiao et al., 2006). Heterozygous mutant *Pkd1* mice with a missense mutation in the PC1gene have a decrease in bone mineral density, trabecular bone volume and cortical thickness. Moreover, both MC3T3-E1 osteoblasts and MLO-Y4 osteocytes have been shown to respond to fluid flow with a cilium-dependent stimulation of both cyclooxygenase (COX) 2 and prostaglandinE2 (PGE2), a well characterized system in bone mechanotransduction (Malone et al., 2007). Of interest, prevention of cilium formation by knocking down the polaris gene - which is necessary for cilium development- in both cell types did not interfere with Ca^{2+} influx in response to fluid flow, suggesting independence of primary cilium (Malone et al., 2007). More recently, the same team of investigators using fluorescence resonance energy transfer (FRET) technology has been able to measure Ca^{2+} influx in the primary cilium of MLO-Y4 osteocytes. They showed that at least some mechanical responses in these cells appear to be dependent on Ca^{2+} entry through the transient receptor potential vanilloid 4 (TRV4) in a distinct microdomain on the surface of the primary cilium (Lee et al., 2015). These aggregated findings suggest that fluid flow stimulation in osteocytes can lead to a primary cilium-independent Ca^{2+} influx, but also to localized Ca^{2+} peaks in the cilium itself.

Taken together, these studies support the potential role of the osteocyte primary cilium in bone mechanotransduction. However, further studies are necessary to unravel the underlying molecular mechanisms whereby this osteocyte structure may act as a true mechanosensor in these cells.

Bone mechanoreceptors

Once the mechanical stimulus or its lack thereof is sensed by the osteocyte, a cascade of signaling events takes place in order to generate biological responses eventually leading to bone gain or bone loss, respectively. The molecular entities that initiate the process of transducing a physical input into a biochemical response are known as mechanoreceptors. They can rapidly (in seconds) trigger intracellular signals with two main functions: activation of transcriptional mechanisms affecting bone modeling/remodeling, and propagation of mechanical stimuli to other osteocytes through paracrine effectors such as NO and PGE2 secretion. Currently, mechanoreceptors of three categories have been described: mechanosensitive ion channels; cell adhesion/cytoskeletal signaling molecules; and certain G protein-coupled receptors (GPCRs).

Ion channels

Ion channels are multimeric pore-forming proteins located in the plasma membrane. They have the ability to open and close in response to different chemical or mechanical signals, such as changes in cell membrane voltage [voltage-sensitive channels (VSCs)], biochemical ligands and physical stimuli [mechanosensitive channels (MSCs)]. Fluid forces are thought to activate MSCs in osteocytes (Wang et al., 2007), and this activation might secondarily induce calcium-dependent VSCs (Duncan and Turner, 1995). Moreover, pre-treatment of bone cells with gadolinium chloride, a blocker of MSCs, before mechanical stimulation, inhibits loading-related release of PGE2 and NO (Rawlinson et al., 1996). Furthermore, blocking VSCs by using the L-type VSC antagonists verapamil and nifedipine has been shown to suppress bone mechanotransduction (Li et al., 2002).

Cell adhesion, focal adhesion and cytoskeleton molecules

Integrins are transmembrane proteins with a dual role: a structural function, in which they are linked to the cytoskeleton network, and as signal transducers, whereby they are associated with adaptor proteins such as talin, paxillin, and focal adhesion kinase (FAK) (focal adhesion proteins). Both functions of integrins seem to be implicated in the mechanosensory apparatus of osteocytes. Regarding their structural role, some evidence supports the notion that mechanical stimulation can be sensed by integrins, which transduce the mechanical input to the nucleus via the actin cytoskeleton, giving rise to nuclear realignment and

chromatin remodeling (Maniotis et al., 1997). There is also growing evidence for a signal transduction role of an integrin-focal adhesion complex in bone mechanobiology (Pommerenke et al., 2002). Therefore, bone regeneration around a mechanically loaded implant is suppressed in FAK-deficient mice (Leucht et al., 2007). In addition, another focal adhesion signaling protein with an important role in bone mechanotransduction is proline-rich tyrosine kinase 2 (PYK2). Like unloading itself, PYK2 activation induces osteocyte apoptosis (Aguirre et al., 2006; Plotkin et al., 2007), while mechanical loading suppresses this activation (Plotkin et al., 2005). Of note, PYK2 knockout mice have high bone mass resulting from increased bone formation (Buckbinder et al., 2007) and decreased bone resorption (Gil-Henn et al., 2007).

GPCRs

GPCRs are seven-transmembrane domain receptors coupled to trimeric guanine nucleotide-binding proteins (G-proteins), and represent the largest family of cell surface receptors. They can be activated by a variety of ligands triggering several signal transduction pathways. Recent studies using biophysical, biological and biochemical methods suggest that certain GPCRs, including bradykinin receptor in endothelial cells (Chachisvilis et al., 2006), angiotensin II type 1 receptor in cardiomyocytes and osteoblasts (Zou et al., 2004; Bandow et al., 2007), formyl peptide receptor in neutrophils (Makino et al., 2006) and the parathyroid hormone/parathyroid hormone related protein type 1 receptor (PTH1R) in osteoblasts (Zhang et al., 2009) are all able to sense mechanical perturbation of the plasma membrane leading to ligand-independent conformational transitions. In this regard, our group has recently demonstrated that PTH1R is an important component of the mechanical signal transduction machinery in MLO-Y4 osteocytic cells (Maycas et al., 2015a). In these cells, a ligand-independent PTH1R activation, related to an increased Ca_i^{2+} influx, was found to occur immediately after mechanical stimulation by fluid flow (Maycas et al., 2015a). Moreover, this mechanical stimulation induced an increase in PTH1R protein in the plasma membrane of MLO-Y4 cells (Maycas et al., 2015b). Interestingly, it was found that the endogenous PTH1R ligand PTHrP was reduced in rodent bone by microgravity, and that PTHrP can protect mouse trabecular osteoblasts from microgravity-induced apoptosis (Torday, 2003; Camirand et al., 2016). Also recently, exogenous PTHrP was shown to exert an additive anabolic effect with mechanical loading on diabetic mouse bone (Maycas et al., 2016).

Other molecules

Other molecules have also been postulated to act as mechanoreceptor candidates in osteocytes. Thus, the essential role of membrane-associated estrogen receptors for the transduction of mechanical forces into cell survival signals in a ligand-independent fashion has been reported in osteoblasts and osteocytes (Aguirre et al., 2007). In addition, the vascular endothelial growth factor (VEGF) receptor 2 (VEGFR2) can be mechanically activated in a VEGF-independent manner in endothelial cells (Jin et al., 2003). We recently demonstrated that a similar VEGFR2 activation -dependent on the transmembrane protein caveolin-1- occurs in mechanically stimulated MLO-Y4 osteocytes (Castro et al., 2015).

Other well known molecules that have been proposed as putative mechanotransductors are connexins (Cx), a family of transmembrane proteins which cluster together in groups of 6 forming hemichannels; a combination of 2 hemichannels from adjacent cells forms a gap junction channel, which is essential for intercellular communication. Due to the role of osteocytes as primary mechanosensor cells in bone and their extensive communication network, there has long been proposed an important role of Cxs in the response of these cells to mechanical stimulation (Plotkin and Bello, 2013; Lloyd et al., 2014). Several Cxs, including Cx43, Cx45, Cx46 and Cx37, have been characterized in osteoblasts and osteocytes (Stains et al., 2005; Pacheco-Costa et al., 2014); although Cx43 has been the best studied so far in osteocytes (Schirrmacher et al., 1992; Civitelli et al., 1993; Yellowley et al., 2000). Fluid flow-induced stress has been shown to trigger the opening of Cx43 hemichannels associated with the anti-apoptotic response in MLO-Y4 osteocytes (Cheng et al., 2001a). Furthermore, Cx43 interacts with integrins (Batra et al., 2012), and also seems to be involved in the ATP and PGE2 release occurring after mechanical stimulation (as discussed below) (Cheng et al., 2001b; Genetos et al., 2007). Despite this *in vitro* evidence pointing to a role of Cx43 in bone mechanotransduction, recent *in vivo* studies have shown unexpected results in this respect. Thus, mice lacking Cx43 in osteoblastic or osteocytic cells surprisingly exhibited an increased bone anabolic response to loading (Zhang et al., 2011; Bivi et al., 2013). These mice also showed a blunted catabolic response to unloading by immobilization. Therefore, current data suggest that the role of Cx43 in bone mechanotransduction might be complex and deserves further studies (Lloyd et al., 2012).

Recently, the Notch pathway has also received some attention in this regard. Notch receptors 1-4 are a family of single-pass transmembrane proteins, which play a critical role in skeletal development and homeostasis. Interestingly, Notch activation in osteoblasts leads to osteopenia while its activation in osteocytes shows a completely different phenotype with an increase in bone mass (Canalis et al., 2013a). However, the underlying mechanisms leading to these bone alterations are still unknown. Fluid flow shear stress has been shown to activate notch signaling in MLO-Y4 osteocytes, supporting the notion that this pathway in these cells may play a role in skeletal adaptation to mechanical

inputs (Canalis et al., 2013b). Further work is needed, though, to validate this hypothesis.

Mechanisms of signal propagation in osteocytes

Following activation of mechanoreceptors in osteocytes, a series of secondary biochemical signaling events takes place inducing adaptive changes in gene expression, protein metabolism and secretion, and cell structure reorganization, eventually affecting cell proliferation and viability that contribute to maintain bone mass. As all of these actions are often mediated through multiple, overlapping and cross-talking signaling pathways, a challenge arises at elucidating those pathways that are critical in the anabolic response to mechanical loads. The most studied pathways in this regard include an increase of intracellular Ca_i^{2+} and ATP, PGE2 release, NO production and Wnt pathway activation.

Changes in Ca_i^{2+} and ATP release

The increase of both Ca_i^{2+} and ATP is among the earliest responses, taking place within seconds, to mechanical stimulation of osteocytes (and osteoblasts) (Hung et al., 1996; Genetos et al., 2005, 2007). Inhibition of voltage-sensitive calcium channels has been shown to avoid ATP release in response to fluid flow shear stress in MC3T3-E1 osteoblasts, indicating that Ca_i^{2+} influx is needed for ATP release in this scenario (Genetos et al., 2005). Furthermore, *in vivo* studies have confirmed the importance of both Ca_i^{2+} and ATP in bone mechanotransduction. Rats treated with L-type VSC antagonists before mechanical stimulation decreased load-induced bone formation by 50-60% (Li et al., 2002), whereas transgenic mice carrying a loss-of-function mutation of an ionotropic ATP receptor similarly show a reduced bone anabolic response to mechanical loading (Li et al., 2005).

COX2-PGE2 pathway

Another pathway likely involved in bone mechanotransduction is the COX2-PGE2 pathway (Ajubi et al., 1999). Both *in vivo* and *in vitro* studies point to the important role of COX-2 (the inducible isoform of COX)-PGE2 system in the anabolic response to loading. *In vitro*, in MLO-Y4 cells, fluid flow shear stress or stretching rapidly induces PGE2 release through a mechanism involving Cx43 hemichannels (Cherian et al., 2005) and/or the purogenic P2X7 protein complex (Li et al., 2005). The released PGE2 thereafter binds to PGE2 receptors (EP1-4) that signal through cAMP/protein kinase (PK)A, phosphatidyli-nositol-4,5-bisphosphate 3-kinase (PI3K)/Akt and glycogen synthase kinase 3β (GSK-3β)/β-catenin pathways, which likely crosstalk to block osteocyte apoptosis (Kitase et al., 2010). Furthermore, pre-treatment of MLO-Y4 cells with indomethacin, a potent inhibitor of PGE2 synthesis, prevents the pro-survival effect of fluid flow shear stress (Kitase et al., 2010). *In vivo* studies also support the crucial role of the COX-PGE2 pathway in mechanotransduction. Hence, rats with pharmacologic inhibition of either COX-1 and -2 by indomethacin administration, or of COX-1 alone via NS-398 (N-[2-(cyclohexyloxy)-4-nitrophenyl]metha-nesulfonamide) administration showed a decreased osteogenic response to mechanical loading (Chow and Chambers, 1994; Forwood, 1996). Moreover, an immediate release of PGE2 in the proximal tibia occurs in humans after jumping (Thorsen et al., 1996).

NO pathway

NO synthase (NOS) catalyzes NO generation from L-arginine. NO is a short-lived free radical that inhibits bone resorption but promotes bone formation *in vivo*, and is generated within seconds of mechanical strain in both osteoblasts and osteocytes *in vitro* (Tan et al., 2008). Mechanically induced NO overproduction lowers the receptor activator of nuclear factor kappa-B ligand (RANKL), a major promoter of osteoclast formation and survival (Rahnert et al., 2008). In addition, mice lacking the inducible form of NOS (iNOS) are unable to generate an osteogenic response to mechanical loading (Watanuki et al., 2002).

Wnt/β-catenin pathway

The canonical Wnt/β-catenin plays an important role in osteoblast differentiation, proliferation and apoptosis (Glass and Karsenty, 2007). The bulk of *in vivo* and *in vitro* studies have consistently shown that mechanical stimulation activates this pathway (Robinson et al., 2006; Santos et al., 2010) . This pathway comprises Wnt glycoproteins as ligands and a receptor complex of frizzled receptors (FZD) and low-density lipoprotein receptor-related protein 5 or 6 (LRP5 or LRP6) co-receptors. Upon mechanical activation, this complex leads to intracellular stabilization and cell relocalization of β-catenin in both the cell membrane and nucleus (Castro et al., 2015; Maycas et al., 2015a), which then stimulates transcription of osteogenic genes in the latter compartment. Deletion of LRP5 in mice prevents mechanical-induced bone formation (Sawakami et al., 2006). On the other hand, endogenous inhibitors of the canonical Wnt pathway such as sclerostin (the protein product of the SOST gene, specific for osteocytes) have been shown to be down-regulated by mechanical loading (Robling et al., 2008). Furthermore, there seems to be a crosstalk between the Wnt/β-catenin and PGE2 pathways upon their activation in response to mechanical loading to preserve osteocyte viability (as discussed below) (Kitase et al., 2010).

Other pathways

In vitro studies show that extracellular signal-

regulated kinase (ERK) phosphorylation and subsequent nuclear translocation occur shortly (<10 min) after mechanical stimulation of osteocytes (Plotkin et al., 2005; Gortazar et al., 2013; Castro et al., 2015; Maycas et al., 2015a,b). Of interest, recent studies indicate that activation and nuclear translocation of ERK in this scenario occur through a signalsome that includes integrin signaling, Src kinase activity and intact caveolae (Plotkin et al., 2005). In fact, caveolin-1, the structural component of caveolae, interacts with ERK and also with integrin β1 in MLO-Y4 osteocytes. Both ERK activation and osteocyte survival induced by mechanical stimulation are abolished by β-cyclodextrin, a cholesterol chelator that disrupts membrane caveolae micro-domains. Thus, current evidence points to the importance of caveolin-1 in the pro-survival response to mechanical loads in osteocytes (Plotkin et al., 2005; Gortazar et al., 2013).

Osteocyte survival as a major response to mechanical stimulation

Preserving osteocyte viability is a paramount feature for maintaining skeletal integrity. It has been demonstrated that different drugs with anti-fracture efficacy, namely bisphosphonates, PTH (by intermittent administration) and estrogens, all prevent osteocyte apoptosis (Tomkinson et al., 1998; Plotkin et al., 1999; Weinstein et al., 2010). On the other hand, an excess of glucocorticoids and sex steroids deficiency -conditions increasing bone fragility- are both associated with decreased osteocyte survival (Weinstein et al., 2010; Jilka et al., 2013). Reduced mechanical loading or bone disuse *in vivo* increases the number of osteocytes undergoing apoptosis (Aguirre et al., 2006). However, in this situation, inhibition of osteocyte apoptosis does not appear to be sufficient to prevent bone loss (Plotkin et al., 2015). In contrast, mechanical stimulation of osteocytes *in vitro* inhibits apoptosis induced by low-serum conditions or pro-apoptotic agents (Plotkin et al., 2007; Kitase et al., 2010; Gortazar et al., 2013; Maycas et al., 2015a,b). The biochemical signals whereby mechanical loading helps maintain osteocyte viability are only starting to be characterized. The role of β-catenin signaling in the prevention of apoptosis is well established in several cell systems, and also in osteocytes upon mechanical stimulation (Gortazar et al., 2013; Castro et al., 2015; Maycas et al., 2015a,b). In this regard, our recent study has proposed the existence of a crosstalk between caveolin-1/ERK and Wnt/β-catenin pathways related to cell survival in mechanically stimulated MLO-Y4 cells (Gortazar et al., 2013). Moreover, the recognition of ERK and β-catenin signaling pathways as early mediators of osteocyte survival has helped identify other upstream components of the mechanotransduction machinery implicated in the preservation of osteocyte viability. In this respect, mechanical activation of several membrane receptors, namely estrogen receptors, the PTH1R and VEGFR2, as well as PGE2, all appear to contribute to osteocyte protection by promoting accumulation of ERK and β-catenin (Aguirre et al., 2007; Gortazar et al., 2013;

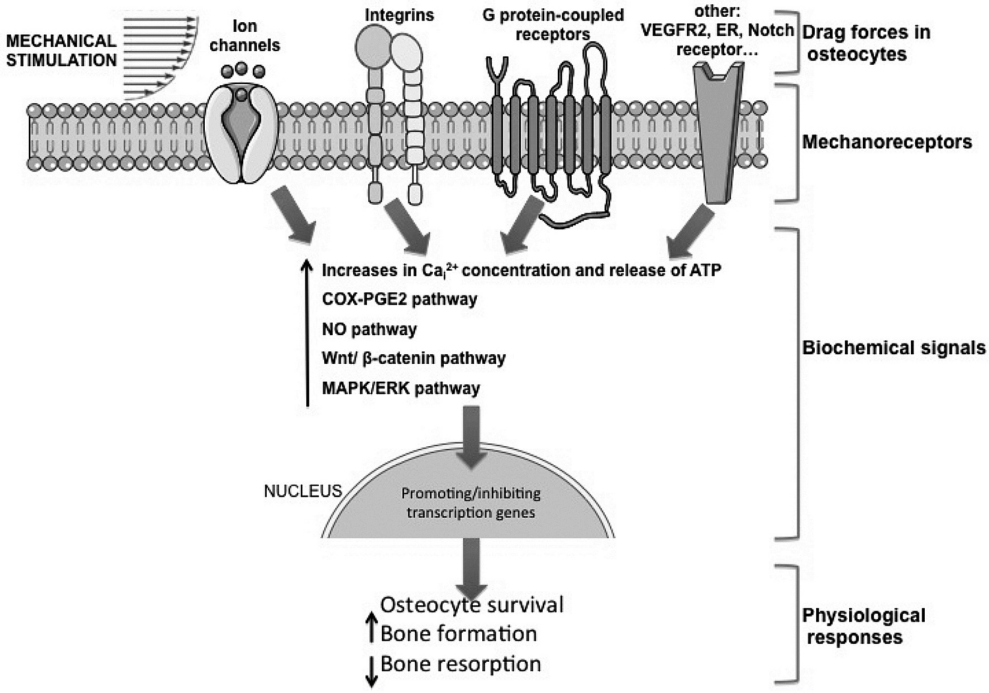

Fig. 1. Mechanisms of bone mechanotransduction. This cell process converts mechanical stimuli into biochemical activities leading to physiological responses. In this scheme, we show currently characterized biological components of mechanotransduction in osteocytes. These cells can be stimulated by relatively small fluid shear stresses caused by fluid drag affecting proteoglycan matrix on the osteocyte surface and on its cell processes. This deforms the cell membrane, which then activates several mechanoreceptors: ion channels, integrins and GPCRs. Other receptors such as VEGFR2, estrogen receptors, and Notch receptors, as well as Cxs might also play a role in this respect. Activation of mechanoreceptors propagates the response through various secondary signaling events including increase in Cai2+and ATP, activation of COX-PGE2, as well as NO, Wnt/β-catenin and MAPK/ERK pathways, which eventually affect transcription of target genes leading to bone anabolic responses.

Castro et al., 2015; Maycas et al., 2015a,b).

Concluding comments and perspectives

In this review, we have dealt with current perspectives on the cellular and molecular elements involved in bone mechanotransduction (Fig. 1). This is a continuously evolving area with the aim of better understanding the molecular mechanisms involved in this process. The complexity of bone mechanotransduction is related to the special characteristics of the osteocyte, as a cell buried in the mineralized bone matrix which has developed a unique sensitivity to translate mechanical stimuli into autocrine and paracrine cell responses. Mechanical loading leads to increased bone mass, decreased bone loss and improved bone strength. Further studies ongoing in several laboratories are expected to provide a better characterization of the underlying mechanisms of bone mechanotransduction in the near future. This characterization may prove useful for designing new paradigms for therapeutic intervention in bone diseases.

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