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Control of the Osteoblast Lineage by Mitogen-Activated Protein Kinase Signaling

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Abstract
Purpose of the Review This review will provide a timely assessment of MAP kinase actions in bone development and homeostasis with particular emphasis on transcriptional control of the osteoblast lineage.
Recent Findings Extracellular signal-regulated kinase (ERK) and p38 MAP kinase function as transducers of signals initiated by the extracellular matrix, mechanical loading, TGF-β, BMPs, and FGF2. MAPK signals may also affect and/or interact with other important pathways such as WNT and HIPPO. ERK and p38 MAP kinase pathways phosphorylate specific osteogenic transcription factors including RUNX2, Osterix, ATF4, and DLX5. For RUNX2, phosphorylation at specific serine residues initiates epigenetic changes in chromatin necessary for decondensation and increased transcription. MAPK also suppresses marrow adipogenesis by phosphorylating and inhibiting PPARγ, which may explain the well-known relationship between reduced skeletal loading and marrow fat accumulation.
Summary MAPKs transduce signals from the extracellular environment to the nucleus allowing bone cells to respond to changes in hormonal/growth factor signaling and mechanical loading, thereby optimizing bone structure to meet physiological and mechanical needs of the body.

Keywords MAP kinase · Transcription · Osteoblast · Chromatin · RUNX2

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Introduction

The mitogen-activated protein kinase (MAPK) pathways function as important regulators of cell growth, differentiation, and morphogenesis in most tissues including bone. Extracellular signal-regulated kinases (ERKs), p38 kinases, and c-June N-terminal kinases (JNKs) constitute the three main classes of MAP kinases to be discussed in this review. Canonical components of the ERK pathway are Ras, RAF, the MAP kinases, MEK1 and MEK2, and the terminal MAP kinases, ERK1 and ERK2. Intermediates of the p38 pathway are MAP kinases 3 and 6 (MKK3, MKK6) and p38 α, β, γ, and δ. JNK pathway components are MAP kinases 4 and 7 and JNK1 and 2. Because MAPKs are activated by a wide variety of factors including growth factors, morphogens, extracellular matrix (ECM) components, and biomechanical signals, they have the potential to mediate the skeletal response to both internal and external environmental cues. This article will specifically focus on possible mechanisms used by MAPKs to mediate bone responses to ECM and other signals as well as delve into potential control mechanisms and the major intracellular target, tissue-specific transcription factors. This work is not intended to be comprehensive and instead reflects the unique perspective of the authors. For a more comprehensive treatment of this subject in bone and other tissues, the reader is referred to other recent reviews [1•, 2].

MAPK Signaling in Skeletal Development

The MAPKs are among the most fundamental signal transduction pathways in biology, being present in all eukaryotes including yeasts, plants, insects, and vertebrates. They are required at the earliest stages of vertebrate development including initial animal cap differentiation to mesenchyme in *Xenopus laevis* embryos and formation of primary mesenchyme in mice [3, 4]. In addition, JNK1 and JNK2 are required for development of the neural tube, while ERK, p38, and JNK all participate in formation of various components of the immune system [5, 6]. In skeletal development, clear in vivo roles have been established for both ERK and P38 MAPK pathways, both of which will be discussed in detail.

Transgenic overexpression of a constitutively active form of the ERK/MAPK intermediate, *Mek1*, in osteoblasts using the osteoblast-specific *Bglap2* promoter accelerates formation of the both cranial and appendicular skeletons, while a dominant-negative *Mek1* slows development [7]. Epistasis was demonstrated between *Mek1* transgene activity and the osteoblast-related transcription factor, RUNX2, thereby providing strong evidence that major aspects of the MAPK response are mediated by this factor (see below). Specifically, crossing mice expressing constitutively active MEK1 with $Runx2^{+/-}$ mice partially rescued the hypoplastic clavicles and hypomineralized calvaria characteristic of *Runx2* haploinsufficiency in mice and humans [8], while crossing dominant-negative *Mek1* mice with $Runx2^{+/-}$ animals exacerbated clavicular hypertrophy and calvarial hypomineralization resulting in embryonic lethality [7]. Consistent with these results, calvarial osteoblasts isolated from transgenic mice showed the expected changes in differentiation with cells from dominant-negative Mek1 mice differentiating less than cells from wild-type littermates and cells from constitutively active Mek1 mice showing enhanced differentiation. Similarly, in loss of function studies, *Prx1-cre*-mediated inactivation of *Erk2* in mesenchymal cells of the appendicular skeleton in *Erk1*-null mice blocked osteoblast differentiation leading to ectopic cartilage formation in regions of the perichondrium that normally form bone. Furthermore, increased ERK/MAPK signaling in the same cell population increased osteoblast differentiation and inhibited chrondrogenesis [9]. In these mice, osteoclast numbers are also reduced, consistent with ERK also being important for RANKL induction in both hypertrophic chondrocytes and osteoblasts, possibly via a RUNX2-dependent mechanism. This may explain the inability of these mice to remove hypertrophic chondrocytes to make way for new bone formation.

An important role for ERK/MAPK signaling was also identified in FGF-mediated cranial suture fusion. FGF ligands, particularly FGF8, are necessary for normal growth and development of craniofacial structures [10]. Gain-of-function mutations in FGFR2 cause certain forms of craniosynostosis (premature suture fusion) including Apert syndrome and Crouzon syndrome [11]. Activated FGFRs signal through the ERK/MAPK pathway leading to elevated levels of P-ERK1/2 [12]. In mice harboring the $Fgfr2^{S252W}$ mutation, which is causal for Apert syndrome, inhibition of ERK phosphorylation with the specific inhibitor, U0126, or with an shRNA specific to mutant *Fgfr2*, can block pathological suture fusion [13]. These studies strongly support a model wherein FGFR2 stimulates suture fusion via activation of the ERK/MAPK pathway.

The p38 MAPK pathway is also required for osteoblast differentiation where it functions as a downstream signal activated by the TGF-β and BMP responsive kinase, TAK1 [14, 15]. Conditional deletion of *Tak1* in preosteoblasts using an *Osx-cre* leads to reduced cortical and trabecular bone, clavicular hypoplasia, and delayed fontanelle fusion. Effects of TAK1 deficiency were specifically attributed to reduced p38 signaling in that mice deficient in the p38 intermediates, *Mkk3*, *Mkk6*, *p38α*, or *p38β* all have decreased bone mass [14]. Interestingly, some selectivity was seen in the requirement for *p38α* or *p38β* in bone development with *p38β*-deficient mice showing defects in long bone formation without major effects on calvarial development. The phenotype of *Tak1*-deficient mice is similar to that observed with Runx2 haploinsufficiency, which suggest that p38 may regulate RUNX2 (see below).

Involvement of the JNK pathway in bone development has not been extensively examined, although several studies suggest a role for this pathway in osteoblast differentiation. For example, accumulation of MEKK2 as a consequence of Smurf1 inactivation leads to JNK activation and increased bone mass, while overexpression of a constitutively active JNK1 increases in vitro osteoblast differentiation [16]. Also, the craniosynostosis-associated factor, Nell-1, activates JNK1 and ERK1/2 in calvarial osteoblasts and is associated with enhanced differentiation [17]. However, the interpretation of these studies is complicated by the known role of JNK signaling in cell survival and apoptosis [5].

MAPK Targets

All MAPKs are serine/threonine kinases with a broad range of substrates. Of specific relevance to bone is the observation that a number of osteoblast-related transcription factors can be phosphorylated by ERK and/or p38 MAPKs.

RUNX2 The first MAPK target to be identified was RUNX2, an essential transcription factor for osteoblast and hypertrophic chondrocyte development [18–20]. This discovery arose from initial observations showing that the electrophoretic mobility of RUNX2 was slightly altered in differentiating osteoblast cultures. Changes in mobility were correlated with

increased binding of RUNX2 to DNA and increase ability of RUNX2 to stimulate transcription in the absence of changes in RUNX2 protein levels [21, 22]. Because RUNX2 activation was dependent on de novo collagen synthesis and binding of type I collagen to $\alpha_2\beta_1$ integrins, the author's laboratory postulated that RUNX2 is phosphorylated and activated by the ERK MAPK pathway, a known mediator of integrin signaling [21–25]. Subsequent mass spectroscopy and related analysis established RUNX2 as a direct ERK1/2 target that is phosphorylated at Ser^{43}, Ser^{301}, Ser^{319}, and Ser^{510} (murine type II RUNX2 isoform). Of these, Ser^{301} and Ser^{319} were most important for MAPK-dependent transcriptional activation of osteoblast-related genes such as *Bglap2*, *Ibsp*, and *Alpl* [26]. Significantly, Ser to Ala mutations of individual phosphorylation sites had little effect on transcriptional activity. Only when combined S301A/S319A mutations were examined was a major drop in MAPK-stimulated transcription observed. In addition, ERK was shown to directly bind RUNX2 using a consensus ERK docking "D" site between amino acid residues 201–215 of the runt DNA-binding domain [27]. This site is similar to those for other MAPK-responsive transcription factors in that it is immediately N-terminal to a transcription activation domain, called the proline/serine/threonine-rich (PST) domain in RUNX2, which also contains the critical Ser^{301} and Ser^{319} phosphorylation sites [28]. Although initial studies focused on ECM/integrin-mediated activation of ERK/MAPK and RUNX2 phosphorylation, other factors known to signal through MAPK such as FGF2 and BMPs were subsequently shown to also increase RUNX2 phosphorylation and activity [29–31].

Relationship Between RUNX2 Phosphorylation and Chromatin Remodeling Interestingly, binding of P-ERK to RUNX2 occurs on the chromatin of target genes rather than in the cytoplasm or other nuclear/perinuclear sites [32]. In undifferentiated preosteoblasts, RUNX2 is already present in the nucleus bound to specific enhancer regions of *Ibsp* and *Bglap2*. Once cells are exposed to differentiating conditions, ERK translocates from perinuclear to nuclear sites where it is detected in complex with chromatin-associated RUNX2. The binding of P-ERK to chromatin requires RUNX2 and intact RUNX2-binding enhancer sequences in both genes. In subsequent work, nuclear translocation of P-ERK was shown to be tightly correlated with appearance of chromatin-associated Ser^{319} P-RUNX2, p300/CBP (CREB binding protein), and RNA polymerase II as well as specific changes in histone acetylation and methylation [33••]. The following differentiation-dependent histone changes were measured: increased H3K9 and H4K5 acetylation and H3K4 di-methylation, histone marks associated with transcriptional activation, and decreased H3K9 mono-, di-, and tri-methylation, histone marks associated with transcriptional repression [34]. Significantly, most of these chromatin changes as well as RUNX2-dependent transcription were blocked by MAPK inhibition or by mutation of Ser^{301} and Ser^{319} residues in RUNX2 to alanine. These results support a model wherein ERK-dependent RUNX2 phosphorylation plays a pivotal role in recruiting chromatin-modifying factors such as p300/CBP to gene enhancers, thereby allowing the epigenetic changes necessary for transcription and osteoblast differentiation. Consistent with this model, genetic interactions have been demonstrated between RUNX2, p300/CBP, and its binding partner, CREB in the maintenance of bone mass [35].

It should be noted that the full repertoire of factors recruited to chromatin after RUNX2 phosphorylation is still not known. The observed increase in chromatin-associated p300/CBP, which contains histone acetyltransferase (HAT) activity, may explain some of the observed increases in histone acetylation [36]. Other changes in acetylation may be due to inhibition of histone deacetylases (HDACs) such as HDACs 3, 7, and 8, which are known to affect bone formation and osteoblast activity [37, 38]. Similarly, the basis for changes in histone methylation remains to be explored. KDM4B, which demethylates trimethyl H3K9, and KDM6B, which demethylates H3K27, were shown to be required for osteoblast differentiation of mesenchymal cells [39], but their relationship to RUNX2 phosphorylation has not been examined.

Thus far, effects of MAPK-dependent RUNX2 phosphorylation on chromatin have only been examined in the proximal promoter regions of two osteoblast-related genes (*Bglap2* and *Ibsp*). However, recent ChIP-Seq analysis suggests that the changes observed may be generally associated with RUNX2-dependent transcriptional activation [40•, 41••, 42••]. In all these studies, chromatin-associated RUNX2 was widely distributed throughout the genome; 30% of bound RUNX2 were in the promoter regions of putative target genes and 70% in non-promoter regions including intron, exon, and intergenic regions. Significantly, in differentiated osteoblasts where RUNX2 is likely in the phosphorylated state, bound RUNX2 was generally near chromatin regions enriched in Me_2H3K4 and AcH3K9 and AcH4K5, the same histone marks shown to be dependent on MAPK activity and RUNX2 phosphorylation [33••]. Based on this work, it may be inferred that MAPK-dependent RUNX2 phosphorylation at Ser^{301} and Ser^{319} has broad effects on the genome leading to the accumulation of activating histone marks in genes necessary for osteoblast differentiation and function. Through this pathway, extracellular signals can be broadly delivered throughout the genome to alter global patterns of gene expression.

The association of ERK with RUNX2 on target gene chromatin is a specific example of a more general concept where tissue-specific transcription factors can serve as docking sites for nuclear kinases in progenitor cells of various lineages. Subsequent transcription factor phosphorylation can then direct a tissue-specific pattern of gene expression necessary for

differentiation or response to specific stimuli. Examples of this include the association of p38α/β with MyoD and MEF2 on the chromatin of muscle-related genes, which are necessary for myoblast differentiation; the binding of P-ERK to Beta2, MafA, and PDX-1 transcription factors in pancreatic β cells in response to elevated glucose; and binding of yeast homologs of p38, ERK, and PKA with the chromatin of multiple genes to globally alter patterns of gene expression in response to external stimuli such as osmotic stress, pheromones, or glucose [43–46].

ERK1/2 and p38 Interactions in the Control of RUNX2 Activity Consistent with the role of p38 in bone formation described above, RUNX2 is also a substrate for p38α and β, which phosphorylate at Ser^{31}, Ser^{254}, and Ser^{319} [14, 31]. As was the case with ERK1/2 phosphorylation, separate mutation of each site had a minimal effect on transcriptional activity while combined mutation blocked most p38-dependent transcription. Also like ERK phosphorylation, p38 increased binding of RUNX2 to p300/CBP, which suggests that both MAPKs activate RUNX2 through a similar mechanism involving phosphorylation on shared as well as separate serine residues.

In spite of these similarities, more recent evidence suggests that ERK and p38 may be responsible for different osteoblast responses [31]. Specifically, although ERK1, p38α, and p38β all stimulate RUNX2 Ser^{319} phosphorylation and transcriptional activity, direct comparisons indicated that ERK1 was the most active of the three kinases. Also, although ERK and p38 bind RUNX2 through a common MAPK D site, the apparent affinity of this site for ERK is greater than for p38. Lastly, RUNX2 Ser^{319} phosphorylation and osteoblast differentiation whether measured in calvarial organ cultures, primary osteoblasts, or osteoblast cell lines is preferentially sensitive to ERK, but not p38 inhibition. In contrast, p38 inhibition does partially inhibit BMP2/7-dependent differentiation/gene expression, although this response is not accompanied by a reduction in RUNX2 Ser^{319} phosphorylation. These results suggest involvement either of other RUNX2 phosphorylation sites or p38 regulation of other nuclear factors and are consistent with p38 preferentially mediating TGF-β/BMP responses likely through the MAPK, TAK1 [14].

Effects of MAPK Phosphorylation on RUNX2 Stability Although the increased osteoblast gene expression associated with ERK or p38-dependent RUNX2 phosphorylation has been attributed to P-RUNX2 effects on nuclear factor recruitment and transcription, phosphorylation may also stabilize RUNX2 by facilitating acetylation and subsequent resistance to proteosomal degradation [47–49]. Specifically, BMP2 or FGF2 can both increase p300-dependent RUNX2 acetylation which renders RUNX2 resistant to Smurf-1 mediated ubiquitination and proteosomal degradation. Both BMP2 and FGF2 increase ERK signaling, which is required for subsequent RUNX2 stabilization. In the case of FGF2, ERK activation requires the RUNX2 Ser^{319} phosphorylation site [49].

Other MAPK Targets Both p38 and ERK have additional substrates that affect osteoblast differentiation using either direct or indirect mechanisms of action.

In the first category are the transcription factors, Osterix (OSX or SP7) and DLX5, which are substrates for p38. Osterix is a bone-specific transcription factor functioning downstream of RUNX2. It is necessary for overt osteoblast differentiation, but unlike RUNX2, it is not required for cartilage hypertrophy [50]. In addition to being indirectly induced by BMP2 at the mRNA level, it is activated and phosphorylated by p38 [51–53]. Furthermore, in MKK6-transfected cells, OSX was phosphorylated at Ser^{73} and Ser^{77}. Phosphorylation at these sites was necessary for recruitment of RNA polymerase II, p300, and Brg-1 to promoter regions of *Ibsp* and *Fmod* genes as well transcriptional activation [51]. The second transcription factor, DLX5, is preferentially associated with craniofacial development; *Dlx5*-deficient mice have defects in cranial ossification with only minor changes in the axial and appendicular skeletons [54]. DLX5 regulates expression of osteoblast-related genes like *Ibsp* and *Sp7* by directly binding homeobox sequences in the promoter region [53, 55]. DLX5 is induced by BMP2 with a time course that precedes induction of osteoblast markers including SP7 and IBSP. DLX5-dependent transcriptional activity is dependent on p38-dependent phosphorylation at Ser^{34} and Ser^{217} [53]. As suggested by Greenblatt and coworkers, it is possible that the preferential effects of p38α deficiency on craniofacial versus long bone mineralization may be explained by selective phosphorylation of DLX5 by this MAPK, although the specific p38 (α or β) phosphorylating DLX5 has not been determined [1•].

MAPK substrates that regulate osteoblast differentiation through indirect mechanisms include RSK2, ERF, and GSK-3β. RSK2, a protein kinase A/protein kinase G/protein kinase C (AGC) family member, is activated by ERK phosphorylation [56]. RSK2 then phosphorylates and activates ATF4, an osteoblast-enriched transcription factor and mediator of the unfolded protein response that is critical for proper collagen production in mature osteoblasts [57, 58]. Activation of MAPK signaling by disruption of the Ras GTPase-activating protein (RasGAP), neurofibromin (NF1), in mature osteoblasts increases ATF4 phosphorylation and transcriptional activity as well as bone mass in mice [59]. Paradoxically, neurofibromatosis patients with germline loss-of-function mutations in *NF1* have a low bone mass phenotype [60]. As was subsequently shown, this discrepancy may be explained by the stage at which *Nf1* is disrupted since crossing mice harboring a conditional *Nf1* allele with a *Col2α1-Cre* expressed in cartilage and osteochondroprogenitor cells of bone marrow

resulted in mice with reduced bone mass, osteoblast number, and increased resorption [61]. Thus, NF1 can function in a stage-specific manner to either suppress or stimulate bone mass and this response is mediated by ATF4. The ETS family transcription factor, ERF, is an additional ERK1/2 nuclear target that may function to suppress RUNX2 activity [62•]. Loss-of-function and hypomorphic mutations in *Erf* cause craniosynostosis in humans and mice due to accelerated cranial suture fusion. ChIP-seq analysis revealed frequent localization of RUNX2 binding motifs within ERF target genes, suggesting possible interactions between these two factors. It was further shown that ERF could inhibit RUNX2 transcriptional activity by interacting with a hybrid RUNX2-ETS binding site. Since nuclear export of ERF is stimulated by ERK phosphorylation [63], the MAPK pathway was proposed to stimulate osteogenesis by reversing ERF inhibition of RUNX2. This pathway may work in consort with direct ERK phosphorylation and activation of RUNX2 to stimulate osteogenesis. A final MAPK target that indirectly regulates osteoblast differentiation is GSK-3β. In the absence of WNT signaling, GSK-3β is a component of an inhibitory complex that phosphorylates β-catenin to target it for proteasome-mediated degradation. The ERK/MAPK pathway indirectly activates the WNT pathway by phosphorylating and inactivating GSK-3β (serine^{9} phosphorylation), thereby allowing nuclear translocation of β-catenin and activation of osteoblast gene expression [64]. This ERK-dependent activation of the WNT pathway is negatively regulated by the scaffolding protein, Schnurri-3 (SHN3), which selectively inhibits the phosphorylation and inactivation of GSK-3β by ERK, to inhibit WNT signaling [65]. Consistent with this model, *Shn3*-deficient mice have a high bone mass sclerotic phenotype that is partially rescued by crossing with *Lrp5*$^{-/-}$ mice.

MAPKs and the Response of Skeletal Progenitor Cells to Mechanical Stimuli

Bone has the remarkable ability to alter its structure in response to changes in mechanical loading. Well-known examples of this are the increase in bone mass associated with weight-bearing exercise and, conversely, loss of bone after prolonged bed rest or exposure to microgravity during space flight [66–69]. Loading stimulates a large number of systemic responses in bone including activation of WNT, BMP, and nitric oxide signaling (for review, see Knapik et al. [70]). However, this review will selectively focus on cellular responses to load with emphasis on the role of MAPK signaling.

Both dynamic and static loads can alter the differentiation of skeletal progenitor cells in bone marrow. For example, when mice are exposed to low-magnitude mechanical signals, marrow stromal cells (MSCs), which include skeletal progenitors, preferentially differentiate to osteoblasts while adipocyte differentiation is suppressed [71]. In contrast, skeletal unloading of mice stimulates marrow adipogenesis and decreases bone formation [72]. This is also seen in paraplegic patients whose disuse osteopenia is associated with increased marrow fat [73]. Some of these responses to loading are also seen when MSCs are exposed to biaxial mechanical strain or fluid shear in vitro, leading to inhibition of adipocyte differentiation and stimulation of osteoblastogenesis [74–76]. Variation of static loads (tension) on cells also profoundly affects MSC differentiation. This is accomplished by altering adhesive surface and cell spreading or through the use of synthetic scaffolds of varying stiffness [77–80]. In all cases, the same general trends prevail; high static loads favor differentiation to osteoblasts while progressively decreasing loads favor cartilage, then muscle and, finally, adipocyte differentiation.

One of the primary mechanisms used by cells to detect mechanical forces is through the integrin-mediated activation of focal adhesion kinase (FAK), a component of focal adhesions. These multiprotein structures link the extracellular matrix, primarily composed of type I collagen in bone, with the actin cytoskeleton, and act as force transducers to match intracellular contractility generated by the cytoskeleton with extracellular tension provided by the ECM. The main integrins responsible for the binding of bone cells to type I collagen are $\alpha_1\beta_1$, $\alpha_2\beta_1$, and $\alpha_{11}\beta_1$, although other integrins such as $\alpha_5\beta_1$ may also be involved in mechanotransduction [81, 82]. Exposure of integrins to mechanical force activates FAK and Src kinases and stimulates RhoA and Rho-associated coiled-coil containing protein kinase (ROCK). Rho/ROCK signaling then stimulates strengthening of the actin-myosin cytoskeleton resulting in cell stiffening (reviewed in [83]). FAK also activates ERK, p38, and JNK MAP kinases as well as phosphatidylinositol 3-kinase (PI3K)/protein kinase B (AKT) pathways followed by induction of mechanoresponsive genes and cell differentiation [84–86]. Consistent with its role in mechanoresponsiveness, conditional deletion of FAK in osteoblasts using a *Col1a1-Cre* renders mice resistant to the local effects of mechanical loading [87].

FAK-mediated activation of ERK/MAPK signaling and subsequent transcription factor phosphorylation are likely responsible for at least some of the observed loading-dependent shift in MSCs differentiation from adipocytes to osteoblasts. The transcription factors, RUNX2 and PPARγ, are two critical regulators of this process. As recently shown, MAPK-dependent phosphorylation of RUNX2 and PPARγ in MSCs can simultaneously increase osteoblast differentiation and suppress adipogenesis [88••]. This is accomplished using the two activating phosphorylation sites in RUNX2 described above (i.e., Ser^{301} and Ser^{319}) and a single inhibitory MAPK phosphorylation site in PPARγ (Ser^{112}) [89, 90]. Mechanical stimulation of osteoblasts by exposure to fluid flow shear

stress rapidly (i.e., within minutes) increases P-ERK-dependent phosphorylation of RUNX2 at Ser[301] and Ser[319] on target gene chromatin, and this phosphorylation is necessary for subsequent induction of histone acetylation and transcription [91]. It is not currently known if P-ERK also phosphorylates PPARγ on the chromatin of adipocyte genes versus at other nuclear or cytoplasmic sites.

Interestingly, the phosphatase, PP5, was recently shown to reverse effects of P-ERK phosphorylation on RUNX2 and PPARγ [92•]. By preferentially dephosphorylating both transcription factors at their respective ERK phosphorylation sites, PP5 suppresses RUNX2 and stimulates PPARγ transcriptional activity. Consistent with these actions, mice deficient in *Pp5* have increased osteoblast numbers, high rates of bone formation, increased bone mass, and decrease marrow fat. In the presence of the PPARγ agonist, rosiglitazone, PP5 translocates to the nucleus where it binds RUNX2 and PPARγ and dephosphorylates both factors to promote marrow adipogenesis and inhibit bone formation. Interestingly, *Pp5* deficiency blocks the negative effects of rosiglitazone on the skeleton.

Yes-associated protein (YAP) and transcriptional co-activator with PDZ-binding motif (TAZ) are two additional mediators of cellular responses to static and dynamic loads that function downstream of Rho/ROCK activation (for reviews, see [93, 94•]). Both factors exist in a phosphorylated, inactive form in the cytoplasm following phosphorylation by the Hippo pathway kinases, LATS1/LATS1, and in an active dephosphorylated nuclear form. Through a mechanism that is not completely understood, the Rho/ROCK-mediated cytoskeletal stiffening prevents YAP/TAZ phosphorylation and promotes nuclear translocation. Once in the nucleus, YAP and TAZ interact with several transcription factors including TEA domain (TEAD) factors, T-box 5 (TBX5) and, interestingly, RUNX2 and PPARγ [95]. On complexing with RUNX2, TAZ stimulates transcriptional activity; in contrast, TAZ inhibits PPARγ. While being highly dependent on Rho/ROCK activity and cytoskeletal reorganization, nuclear translocation of YAP/TAZ is surprisingly independent of FAK [96]. Thus, FAK-mediated MAPK activation and subsequent regulation of RUNX2 and PPARγ activities and YAP/TAZ nuclear translocation can be viewed as separate arms of a coordinated cellular response to mechanical loads. Nevertheless, both pathways must be active for stimulation of osteoblast differentiation and suppression of adipogenesis since inhibition of either Rho/ROCK or ERK blocks osteoblast differentiation of MSCs grown of stiff hydrogels [79, 80, 97•]. Furthermore, there is apparent cross talk between MAPK and Rho/ROCK signaling since ERK or JNK inhibitors can block TAZ nuclear localization [97•].

Discoidin Receptors and Bone

Although integrins/focal adhesions are generally considered to be responsible for sensing mechanical signals in bone, this tissue contains other adhesion receptors including cadherins, syndecans, hyaluronan receptors, and discoidin receptors (for review, see [98]). With regard to MAPK signaling, the discoidin receptors are of particular interest. Unlike integrins, these molecules have intrinsic tyrosine kinase activity that is activated by binding to fibrillar collagens. Once activated, DDRs can stimulate several signal transduction pathways that, depending on the tissue, include ERK, JNK, and p38 MAPKs, the PI-3 kinase/AKT and NFKβ pathways (for review, see [99•]). The two DDRs in mammals, DDR1 and DDR2, have different tissue distributions and are preferentially activated by different collagens. DDR1 is mainly expressed by epithelium and has broad ligand specificity for most collagens, while DDR2 is expressed by mesenchymal cells including osteoblasts and preferentially binds collagens I, II, III, and X. Consistent with its distribution and collagen selectivity, DDR2 has important functions in bone. Loss-of-function mutations in DDR2 cause spondylo-meta-epiphyseal dysplasia (SMED) in humans, resulting in dwarfism, bone weakness, abnormal calcifications, and craniofacial abnormalities [100]. Furthermore, polymorphisms in the DDR2 locus are associated with increased fracture risk and low bone density [101]. DDR2-deficient mice have SMED-like characteristics including dwarfism and reduced bone mineral density [102–104]. Detailed analysis of the bone phenotype of mice globally deficient in DDR2 revealed dramatic reductions in mineral density in bones of the appendicular, axial, and cranial skeletons in males and females that were explained by reduced osteoblast activity and bone formation in the absence of changes in resorption [104••]. Bone changes were accompanied by a large increase in marrow fat. Furthermore, MSCs from deficient mice showed reduced ability to differentiate into osteoblasts and increased adipogenesis. The defective osteoblast differentiation in DDR2-deficient cells was directly attributed to a reduction in ERK/MAPK signaling and RUNX2 Ser[301] Ser[319] phosphorylation and could be rescued with a phosphomimetic RUNX2 mutant (S301E/S319E mutant) that did not require phosphorylation for optimal activity. Consistent with these results, overexpression of a constitutively active DDR2 stimulated ERK/MAPK signaling and phosphorylation of RUNX2 and PPARγ, leading to increased RUNX2-dependent transcriptional activity and inhibition of PPARγ. Similar effects of DDR2 on the MAPK/RUNX2 axis were previously reported in studies with osteoblast and chondrocyte cell lines [105•].

Summary and Conclusions

Many factors important in bone metabolism including growth factors, TGF-β/BMPs, extracellular matrix, and mechanical loads stimulate MAP kinase signaling. In vivo gain- and loss-of-function studies established clear roles for ERK and p38 MAP kinases in osteoblast differentiation. Both kinases largely act by directly phosphorylating and activating osteoblast-related transcription factors including RUNX2, OSX, and DLX5. In addition, they can function indirectly by activating secondary kinases such as RSK2 to stimulate osteoblast gene expression or, alternatively, to inhibit the activity of factors like ERF and GSK-3β that themselves directly or indirectly suppress osteoblast activity. During MSC differentiation, ERK can simultaneously phosphorylate RUNX2 and PPARγ to stimulate osteoblast differentiation and suppress adipogenesis. Static and dynamic mechanical loads are detected in skeletal progenitor cells through focal adhesions and activation of focal adhesion kinase and subsequent activation of Src, Rho, and ROCK activities. The Rho/ROCK-dependent stiffening of the actin-myosin cytoskeleton promotes dephosphorylation and nuclear translocation of YAP and TAZ, while FAK activates ERK, p38, JNK, and PI3 kinase. These two pathways may cooperate to stimulate osteoblast gene expression and suppress adipogenesis. DDR2 is a non-integrin receptor tyrosine kinase activated by fibrillar collagens including the type I collagen of bone that provides a second route for the ECM to activate MAPK signaling, leading to increased osteoblast differentiation and suppression of adipogenesis. The nuclear functions of MAP kinases in bone are likely explained by their direct binding to and phosphorylation of tissue-specific transcription factors such as RUNX2 on the chromatin of target genes. This and related phosphorylation events stimulate recruitment of chromatin modifying factors leading to the epigenetic modification of histones necessary for osteoblast gene expression while also suppressing other lineages such as adipocytes. This provides a mechanism whereby an extracellular signal can be conveyed to the nucleus via translocation of MAPKs, which can then be targeted to all relevant genes necessary for specific control of mesenchymal stem cell lineage to osteoblasts (Fig. 1 uses the specific example of RUNX2-dependent transcription to illustrate these control mechanisms).

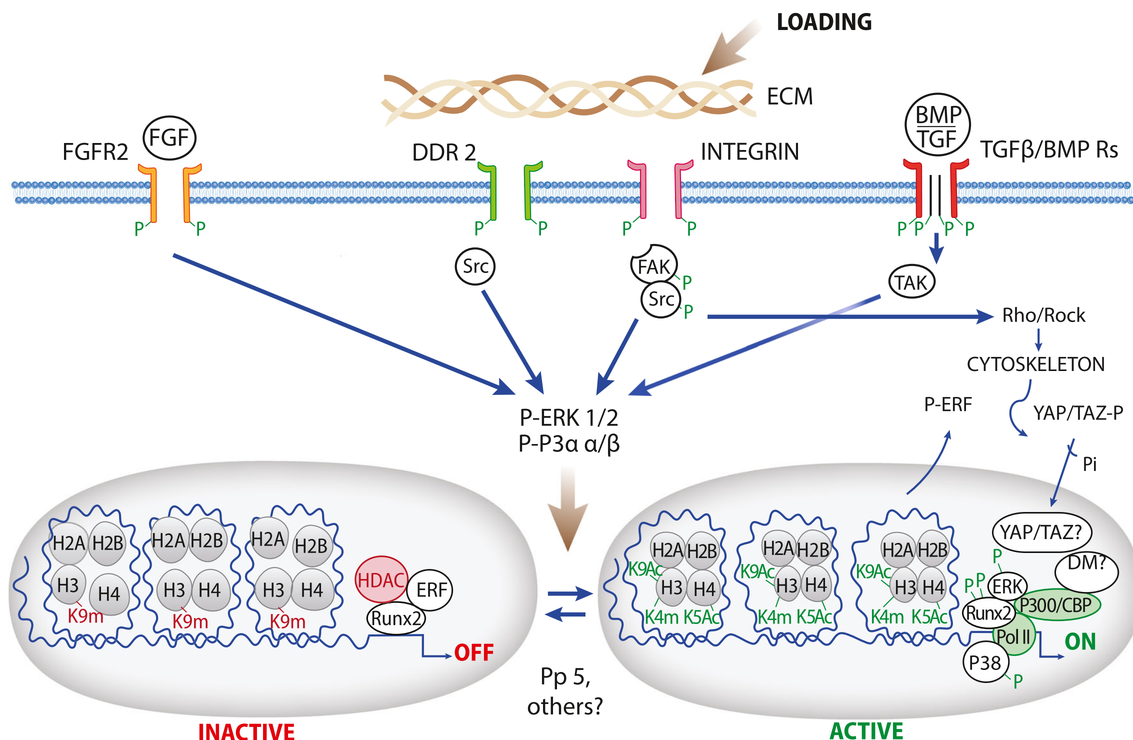

Fig. 1 Model for MAP kinase regulation of osteoblast gene expression with emphasis on RUNX2-dependent transcription. Major pathways in bone that signal at least in part through MAPKs are shown in *top part* of figure. *Bottom of figure* shows specific chromatin/histone modifications associated with ERK (and possibly p38)-dependent RUNX2 phosphorylation. Both stimulatory changes (H3K9Ac, H4K5Ac and H3K4m, YAP/TAZ dephosphorylation, and nuclear translocation) as well as removal of inhibitory chromatin modifications/factors (decreased H3K9Me and increased DM activity, decreased HDAC activity, ERF-P/translocation to cytoplasm) are shown. The *question mark* next to nuclear YAP/TAZ and histone demethylase (DM) reflect uncertainty as to whether their association with chromatin is related to MAPK signaling

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflicts of interest.

Human and Animal Rights and Informed Consent All reported studies/experiments with human or animal subjects performed by the authors have been previously published and complied with all applicable ethical standards (including the Helsinki declaration and its amendments, institutional/national research committee standards, and international/national/institutional guidelines).

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