

MAP KINASE SIGNALING CASCADES AND GENE EXPRESSION IN OSTEOBLASTS

Robert A. Hipskind¹ and Graeme Bilbe²

¹*Institut de Genetique Moleculaire de Montpellier, CNRS, 1919 Route de Mende, 34293 Montpellier cedex 5, France* ²*Novartis Pharma A.G., Postfach, K681.4.43, CH-4002 Basel, Switzerland*

Received 1/16/98 Accepted 2/17/ 98

TABLE OF CONTENTS

1. Abstract
2. Introduction
3. Early events in osteoblast proliferation and differentiation
4. The human *c-fos* promoter
5. MAP kinase signaling cascades
 - 5.1. ERKs
 - 5.2. SAPK/JNKs
 - 5.3. p38s
 - 5.4. MAPK cascades in osteoblasts
 - 5.5. MAPK cascades in vivo
6. AP-1
 - 6.1. AP1 as a key regulatory point of MAPK cascades
 - 6.2. AP-1 and MAP kinase pathways in osteoblasts
7. Perspective
8. Acknowledgements
9. References

1. ABSTRACT

Environmental cues direct osteoblasts to proliferate and differentiate. The mitogen-activated protein (MAP) kinase pathways provide a key link between the membrane bound receptors that receive these cues and changes in the pattern of gene expression. The three MAPK cascades in mammalian cells are: the extracellular signal-regulated kinase (ERK) cascade, the stress activated protein kinase/c-jun N-terminal kinase (SAPK/JNK) cascade and the p38MAPK/RK/HOG cascade. Each has varied roles, depending upon the cell type and context, that include transmitting stress, growth, differentiative and apoptotic signals to the nucleus. These pathways target an overlapping set of transcription factors that lead to the differential activation of rapid response genes, particularly members of the *fos* and *jun* family of proto-oncogenes. These proteins are the principal components of the transcription factor AP-1, which plays a central role in regulating genes activated early in osteoblast differentiation. We discuss in detail a) the nature and activation of these pathways b) how they induce *c-fos* expression and c) how these MAPK cascades can differentially regulate the activity of AP-1 and thereby osteoblast-specific gene expression.

2. INTRODUCTION

The control of bone mass is attributable to the function of two cell types: the osteoblast and the osteoclast. There is a vast descriptive literature about the effects of

cytokines, growth factors and other agents on these cells. One underlying assumption inherent in all these analyses is that these various agents lead to changes in phenotype via altering the pattern of gene expression (1, 2). The genes that are both induced and repressed play key roles in driving cells toward the desired phenotype, either osteoblastic or osteoclastic. Nevertheless, little is known or understood of how extracellular signals are transduced to the nucleus to effect these changes in gene expression. This review concentrates on one important signaling network linking ligand-activated membrane receptors to the nucleus, the MAP kinase signaling cascades.

The MAP kinase signaling cascades, which will be described in detail below, are likely to play a crucial role in the immediate osteoblast response to a variety of bone active agents. MAP kinases, namely ERKs, SAPKs and p38MAPK, are activated downstream of many different types of receptors, among them are the receptor tyrosine kinases (RTKs), cytokine receptors and serpentine G-protein-coupled receptors. MAP kinases can also be induced by elevated intracellular Ca^{2+} concentrations, PKC activation, almost all types of stress, including mechanical stress, and in certain contexts by elevated [cAMP]/PKA activity (3-7). Given this global response to all these factors, it seems likely that MAP kinase cascades play an important but not unique role in the response of osteoblasts to their environment. An important component of this response occurs at the transcriptional level, and the

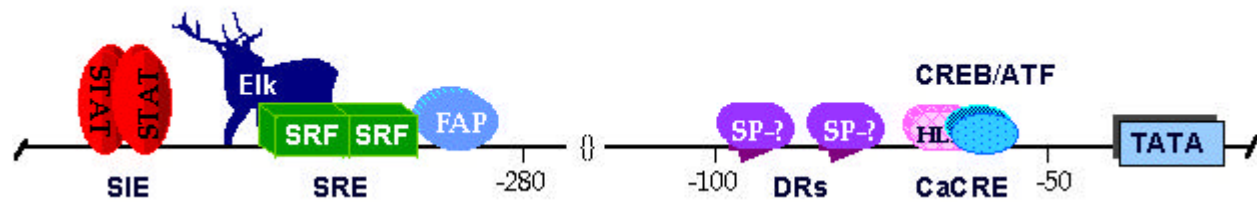


Figure 1. The human *c-fos* promoter. The different regulatory elements of the *c-fos* promoter are indicated below the line and the proteins thought to interact with these elements are indicated above the line. For abbreviations see the text; TATA, TATA box; DRs, GC-rich direct repeats; SP-?, transcription factor related to SP-1; FAP, Fos AP-1-like complex.

rapid activation of these cascades strongly implicates them as an essential intermediate in this process.

3. EARLY EVENTS IN OSTEOBLAST PROLIFERATION AND DIFFERENTIATION

Since commitment to the bone lineage is dependent upon extracellular agents, genes that are rapidly activated by these stimuli are likely to play a key role early in this process. This rapid induction is mediated by the signaling-driven modification of proteins that interact specifically with the different promoter elements of these genes. These transcription factors do not necessarily have to be expressed in a tissue-specific fashion; rather it is the nature and combination of signaling pathways targeting them that forms the basis of the differential response to similar extracellular stimuli in various cell types, e.g., osteoclasts and osteoblasts. Among the genes rapidly activated by external agents, perhaps the best characterized is the *c-fos* proto-oncogene. Since this gene is responsive to the various MAP kinase signaling pathways, we have used it to evaluate the activation of MAP kinases by a variety of bone active agents (8-10). This approach is further supported by the key role of *c-fos* in the normal control of osteoblast growth and in the generation of osteosarcomas upon its overexpression (11).

4. THE HUMAN C-FOS PROMOTER

The promoter of the *c-fos* gene is comprised of three major elements that mediate the response to distinct intracellular signaling pathways (figure 1). Since genomic footprinting shows no change in the occupation of two of these elements throughout the process of induction, these elements and the proteins bound to them *in vivo* are likely to be directly targeted by intracellular signals (12). The calcium/cAMP responsive element (CaCRE; 5), located just upstream of the TATA box, can drive transcriptional activation in response to elevation of intracellular Ca^{2+} or cAMP concentrations. Proteins of the family, cAMP responsive element binding protein/activating transcription factor (CREB/ATF), bind to this element *in vitro* and can activate CaCRE-directed reporter genes in transient transfection experiments (13). This activation is dependent upon the phosphorylation of CREB on Ser133, which leads to the recruitment of the coactivator CREB binding protein (CBP)/p300 and thereby potentiation of transcription complex assembly on the promoter (14). The serum

response element (SRE; 15,16) is sufficient to drive the transcriptional response of a reporter gene to a wide variety of signals, including growth factors, tumor promoters, and elevated Ca^{2+} levels in some instances (17). The protein complex assembled on the SRE contains a dimer of serum response factor (SRF) together with one molecule of ternary complex factor (TCF). The major component of TCF in human cells is Elk-1 (18,19), and Elk-1 is one of the major nuclear substrates of activated MAP kinases (20). The resulting phosphorylation of Elk-1 transforms this protein into a potent transactivator of transcription, thus the MAPK/Elk-1 cassette plays a major role in signaling-driven gene activation *in vivo*. In the absence of MAP kinase activation SRF can also mediate a moderate transcriptional activation to signals downstream of serum and Ca^{2+} elevation (21,22). The third major element, the *v-sis*-inducible element (SIE), lies just upstream of the SRE (23). Proteins of the Signal Transducers and Activators of Transcription (STAT) family of transcription factors bind this element, namely homodimers of STAT1 and STAT3 and heterodimers between the two (24). Their phosphorylation on tyrosine in the cytoplasm, mediated by JAK/TYK kinases (Janus kinase/Tyrosine kinase) associated with cytokine receptors and by certain RTKs, drives their dimerization and nuclear translocation. The dimerized factor can then bind the SIE and participate in promoter activation (23,24). Accordingly, genomic footprints show that the SIE becomes occupied shortly after induction by agents that activate this pathway (12). As will be discussed below, all of these elements are potential targets of MAP kinase cascades via their respective transcription factors.

5. MAP KINASE SIGNALING CASCADES

Extracellular signals are transmitted to the nucleus in a variety of ways (figure 2). In many instances this occurs via the activation of a kinase localized in the cytoplasm in an inactive form. Upon its activation the kinase translocates to the nucleus where it phosphorylates target transcription factors, thereby modifying their capacity to regulate gene expression (20). In the case of the MAP kinase cascades, the signal is propagated through the sequential activation of a multiple kinase cassette that allows small changes in an input signal to be amplified into

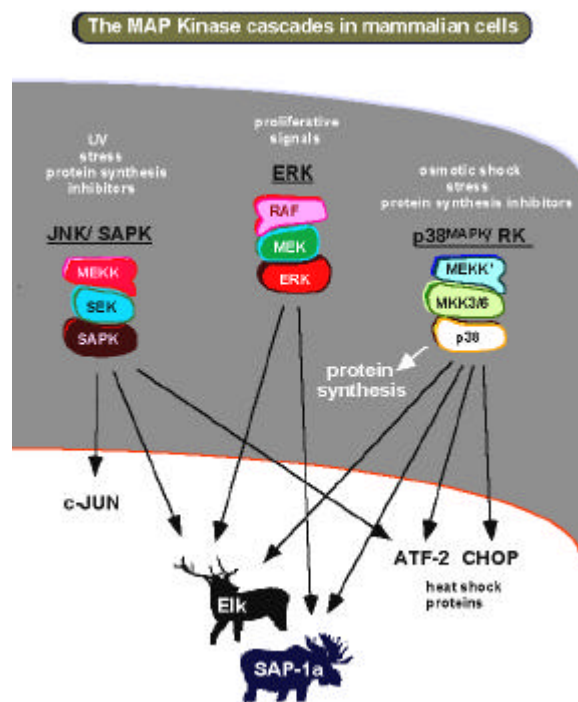


Figure 2. MAP kinase signaling cascades in mammalian cells. Shown in schematic form are the three major MAPK signaling cascades in the cytoplasmic compartment with the factors generally thought to activate them shown above each cascade. Arrows indicate the specificity of each cascade for different nuclear transcription factors (redline indicates the nuclear envelope). For abbreviations see text.

large changes in output signal, i.e. transcriptional regulation via phosphorylation (25, 26). Furthermore this allows both positive and negative modulation of the signal at each level of the cascade, thereby greatly expanding the possibilities of regulating the signal. The MAP kinases share two characteristics: their activation by dual phosphorylation on a Thr-Xaa-Tyr motif, after which they function as proline-directed Ser/Thr kinases with a minimal target sequence of Ser/Thr-Pro (25). The composition and regulation of each MAP kinase cascade is briefly discussed below. For a more exhaustive and detailed description the reader is advised to consult any of the large number of recent reviews concentrating on these signaling systems.

5.1 ERKs

Growth factors and other extracellular agents that drive cells to proliferate rapidly activate the ERK (Extracellular signal-regulated kinase, previously called the MAP kinase) signaling cascade. The sequential series of kinases at the core of this cascade are Raf >> MEK >> ERK. The first, Raf or MAPKKK, is activated by a still-unclear mechanism that is dependent upon Ras in the vast majority of cases (25,27). This interaction with Ras relocalizes Raf to the membrane, which seems to be an important component for its activation (25). The Raf family currently contains three members (27), c-Raf (or Raf-1), B-

Raf and A-Raf, and all three proteins can function as MAPKKK depending upon the cell type. c-Raf has been generally described as the major activator; however, a considerable body of data suggests other functions for c-Raf, since its activity can be uncoupled from the ERK cascade (28,29). Furthermore B-Raf shows the strongest activation of the ERK cascade *in vivo* (30,31). Other kinases can also function in this capacity, notably MEKKs 1 and 3 (25). However, these two kinases can also target the SAPK/JNK pathway (see below), so the possibility remains open for other specific activators of the ERK cascade.

Raf activates a second kinase, the MAPKK MEK, that has the singular ability to phosphorylate both the Thr and Tyr residues in the Thr-Glu-Tyr activation motif in ERK (25). Both MEK1 and MEK2 can activate ERK, and it is still unclear whether the two isoforms have different physiological roles in signal transduction. Upon its activation ERK relocalizes to the nucleus, where it can target both transcription factors and the basal transcription complex (32,33). One of its major targets is TCF, namely Elk-1 and its homologue SAP-1a, which then play a key role in the activation of immediate early genes, like *c-fos*, controlled by SREs in their promoters (20). Phosphorylation by ERK is also implicated in potentiating the activity of other transcription factors, e.g. STATS, Myc, various ETS proteins and C/EBPbeta (34,35); however, the degree of activation in most of these cases is much less impressive than that observed with TCF and possibly reflects non-physiological levels of overexpression by transient transfection.

ERK activity rapidly decreases to a low level that persists well into the G1 phase of the cell cycle (32, 36). ERK is inactivated by dephosphorylation, and two classes of phosphatases likely play separate roles in this process (37). One, the MKP class of phosphatases (MAP kinase phosphatase), demonstrate a dual specificity for phospho-Tyr and -Thr (25, 37). The majority of these phosphatases are encoded by immediate early genes, which demonstrates the existence of a feedback regulation to insure that ERKs are only transiently induced. Nevertheless the induction of MKPs is often too slow to account for ERK inactivation, which occurs via PP-2A (protein phosphatase 2A) mediated Thr dephosphorylation in these cases (38, 39).

There are five members of the ERK family to date. p44ERK1 and p42ERK2 appear to be the major transducers of proliferative signals to the nucleus (25). ERK3 is predominantly nuclear in quiescent cells, and neither the signals leading to its activation nor its targets have been characterized (40). ERK4 remains equally enigmatic, while ERK5/BMK1 (for Big MAP Kinase) is a redox-sensitive kinase that might be targeted to the cytoskeleton (41).

ERK has a number of downstream effectors in addition to transcription factors. One, the p90RSK family of kinases, is also involved in gene regulation. RSK (for ribosomal protein S6 kinase) appears to be complexed with ERK in the cell (42) and, like ERK, translocates to the

nucleus shortly after its activation (33). Nuclear substrates for RSK phosphorylation include SRF (43) and CREB (44), thus providing a mechanism through which the ERK cascade can simultaneously target multiple elements in the *c-fos* promoter. In addition the c-Fos protein is synergistically phosphorylated by the combination of ERK and RSK on sites that have been implicated in transcriptional repression (45,46), as well as by the MAPK-related kinase Frk-1 in its transactivation domain (47). Thus the ERK cascade can regulate the immediate early transcriptional response at several different levels.

5.2. SAPKs/JNKs

This complex family of MAP kinases was identified in two ways: by their activation by different environmental stresses (therefore, Stress Activated Protein Kinase; 48) and by their ability to bind and phosphorylate the NH₂-terminal transactivation domain of *c-jun* on Ser63 and Ser73 (hence *c-jun* N-terminal Kinase; 49). They are induced by a wide range of cellular stresses (among them UV irradiation, chemical, oxidative, hypoxia and anoxia, heat shock, protein synthesis inhibitors, inflammatory cytokines), but also by a wide range of stimuli, including cytokines and G-protein-coupled receptors among others (25, 50). Depending upon the cell context, SAPK/JNK activation can lead to proliferation, differentiation or apoptosis (25, 50).

Three major isoforms of SAPKs/JNKs, namely p46, p54alpha and p55beta have been identified biochemically and by cloning, although alternative splicing could give rise to as many as 10 distinct proteins encoded by three genes (51). No consistent correlation can be made between different isoforms and the varied signals activating these kinases.

As with the ERK cascade, their activation occurs via the sequential activation of a kinase signaling cassette that, unlike the ERK cascade, cannot be as clearly defined. Several MAPKKs for SAPKs/JNKs have been described that phosphorylate the Thr-Pro-Tyr activation motif in SAPK/JNK. MKK4 (also called SEK1 and JNKK) was the first identified, but it can also activate P38MAPK (see below) when overexpressed by transient transfection in culture cells (25, 50). More recently a much more selective and active SAPKK/JNKK was cloned, namely MKK7, and this kinase is the current favorite as the activator *in vivo* (52). Biochemical fractionation hints at other direct activators which remain to be cloned (50).

At the MAPKKK level, functionally equivalent to Rafs in the ERK cascade, are MEKKs 1-4, along with the mixed lineage kinases (53, 54). While MEKK1 was originally described to activate the ERK cascade (55), it clearly initiates the signaling cascade leading to SAPK/JNK induction (56). Interestingly MEKK1 is also activated by caspase cleavage early in certain apoptotic signaling pathways (57). Some MAPKKKs are activated by the Rho family of GTPases that are related to Ras (25,50). Intermediates in this pathway are the p21-activated Ser/Thr kinases (PAKS) that bind to and are activated by Cdc42 and Rac1, as well as several effectors downstream of RhoA.

These GTPases play a critical role in signaling-driven changes in cell morphology and are also implicated in gene regulation. SAPKs/JNKs clearly play a key role in the latter process through phosphorylation of transcription factors. Primary among them are c-Jun, Elk-1 and ATF-2 (25, 50). Thus the ERK and the SAPK/JNK cascades target an overlapping but not identical set of genes, thereby allowing for a complex pattern of regulation through the phosphorylation of a limited number of factors.

Given the strong similarity between the three MAP kinase cascades, it seems likely that there will be a kinase downstream of the SAPKs/JNKs that would be a functional and possibly structural homologue of RSK and MAPKAPK (for MAP kinase-activated protein kinase-see below). This would help explain the activation of *c-fos* and other IEGs (immediate early genes) by the SAPK/JNK pathway and further expand the regulatory possibilities. Also consistent with the strong conservation between MAPK cascades, several MKPs show a preference for SAPK/JNK as substrates, providing a mechanism for inducible inactivation of this cascade (58).

What none of these pathways explains is the mechanism by which the small GTPase RhoA can activate the SRE/SRF complex independently of TCF and SAPK/JNK (59). Nevertheless this observation agrees with others suggesting that the TCF:SRF:SRE complex can also integrate signals arising outside of the MAP kinase signaling network (60).

5.3. p38s

This stress-activated protein kinase cascade was identified and cloned in three independent approaches. p38 was the kinase activated by high osmolarity and bacterial lipopolysaccharide analogous to the yeast HOG1 pathway (61,62). p40 was reactivating kinase, capable of directly activating MAPKAP kinase-2 (see below; 63,64). Finally it also proved to be the cytokine suppressive anti-inflammatory drug binding proteins 1 and 2 (CSBP 1 & 2), revealed by a screen for drug-sensitive targets involved in the regulation of IL-1 and TNFalpha synthesis in stimulated monocytes (65). This explains the confusing terminology used in the first publications describing this pathway. As alluded to above, it is activated in response to various stresses and by inflammatory cytokines, thus its activation coincides with that of SAPKs/JNKs in many instances (25, 50). Sodium arsenite has been reported to selectively activate the p38 pathway (63), while UV-C is specific for the SAPK/JNK cascade (25, 50). This demarcation is not true in all cell lines, which suggests that the differences between the two cascades will be subtle, probably determined by the kinetics and degree of activation of the two pathways.

Four isoforms of p38 have been identified by cloning, termed alpha (61-65), beta (66), gamma (67) and delta (68,69). Given their activation by stress, alpha and beta have also been referred to as SAPK2, gamma has also been cloned as ERK6 and SAPK3, and delta is identical to SAPK4. The isoforms can be differentiated by their

sensitivity to the pyridinyl-imidazole drugs used in the screening described above, namely that isoforms alpha and beta are inhibited, whereas gamma and delta are not (68,69). All 4 isoforms are activated by dual phosphorylation on a Thr-Gly-Tyr motif by MKKs 3, 4 and 6 (70,71). Their activation by MKK4 seems to be an anomaly of transient transfection, since it does not activate in all cases and MEKK1 does not induce p38 activity (50). Interestingly MKKs 3 and 6 can selectively activate different isoforms depending upon the cell type analyzed (66,68,72,73). Upstream of MKK the situation remains to be clarified. At present the MAPKKK candidates are TAK1 (Transforming Growth Factor beta Activated kinase, 74), ASK1 (Apoptosis Signal-Regulating Kinase, 75) and MTK1 (MAP Three Kinase 1 for Mitogen Activated Protein Kinase Kinase, 76), where the last two also activate the SAPK/JNK pathway. Thus more p38-specific MAPKKKs are likely to be identified soon. Similarly nothing is published concerning the molecular events linking stress and cytokines to this pathway.

In analogy to the ERK cascade, a family of kinases, MAPKAPKs 2 and 3, serves as an important downstream effector in the p38 cascade. *In vitro* and in transfected cells, certain p38 isoforms can be linked to either of the MAPKAPKs (77,78). More germane to this review are their potential substrates in the nucleus. p38 can phosphorylate and thereby activate several transcription factors, including the TCFs Elk-1 and SAP-1a, ATF-2 and CHOP (79-82). MAPKAPKs can target CREB, HSP27 and possibly SRF (83). Thus the same strategy noted above applies in the p38 cascade. Several components in the cascade can target different factors involved in controlling the expression of rapid response genes, therefore generating a complex network with several possible levels of regulation.

5.4. MAPK cascades in osteoblasts

In osteoblastic cells a variety of bone-active agents have been described to induce ERKs. These include the growth factors PDGF-BB, EGF and basicFGF, phorbol ester, estrogen, and fluoride at mitogenic doses (84-91). These compounds have been variously tested in osteoblast-like cell lines of human (84,87,88), mouse (85) and rat origin (83,89,90), as well as in normal human (84) and rat osteoblastic cells (86). Several studies have evaluated the functional consequences of ERK activation at the level of gene induction; they report the same strong linkage between ERK and *c-fos* activation observed in other types of cells (87,89). Interestingly parathyroid hormone (PTH) antagonizes ERK activation via a PKA-dependent pathway in UMR106 osteosarcoma cells (86), an observation that would correlate with the anti-mitogenic effects of PTH in these cells (92). As in fibroblasts, this PKA-dependent suppression of ERK is variable between cell lines, since PTH does not antagonize growth factor induction of ERKs and *c-fos* in several human osteosarcoma cell lines (93). While the ERK cascade appears to be an important

component of intracellular signals initiated by many agents having proliferative effects on osteoblasts, its *in vivo* role remains unanswered. This will require selective inactivation of this cascade by pharmacological inhibition, e.g. the MEK inhibitor PD98059 (94), or by gene disruption.

The activation of the SAPK/JNK and p38 cascades has yet to be documented in osteoblasts. In several studies, using various osteoblast-like osteosarcoma cell lines, we observe the induction of both 46kD and 54/55kD SAPKs/JNKs by TNFalpha, IL-1 and different stresses (10). Certain signals also strongly induce a yet-uncharacterized isoform of p38, which is likely to represent either p38alpha or delta given their expression patterns (72). Thus all three pathways are present in these cells and can be activated by extracellular factors.

5.5. MAP kinase cascades *in vivo*

The functions of the various cascades have been principally defined by experiments using cultured cell lines. A number of recent studies nevertheless suggest that these cascades might have other functions *in vivo*. In T cells and megakaryocytes ERKs are not essential for proliferation but rather for differentiation (95-97). The SAPK/JNK pathway appears to play an important role in hematopoiesis (98), T cell proliferation and survival (99,100), and in liver regeneration (101), as well as in metabolically stressed liver (102). In this model, these stresses led to the downregulation of constitutively active p38. In contrast, the p38 cascade is involved in IL-2-driven T cell proliferation (103), stimulated by insulin in a neuronal growth/differentiation system (104) and activated in skeletal muscle by exercise (105). These examples, by no means comprehensive, strongly suggest that these kinase cascades are likely to play important roles during development and differentiation. Considering the important role played by the components of AP-1 (detailed below) in early stages in osteoblast differentiation, their selective targeting by separate or the combined activation of different MAPK cascades might play a key role in osteoid stem cell proliferation and differentiation.

6. AP-1

6.1. AP1 as a key regulatory point of MAPK cascades

AP-1 was originally described as a transcription factor that mediated gene expression in response to the phorbol ester 12-O-tetradecanoylphorbol-13-acetate (TPA) and bound to TPA-response elements (TRE) as found in the *c-jun* promoter (106). AP-1 activity is now known to be induced by a wide array of stimuli including growth factors, cytokines, UV radiation and cellular stress, in other

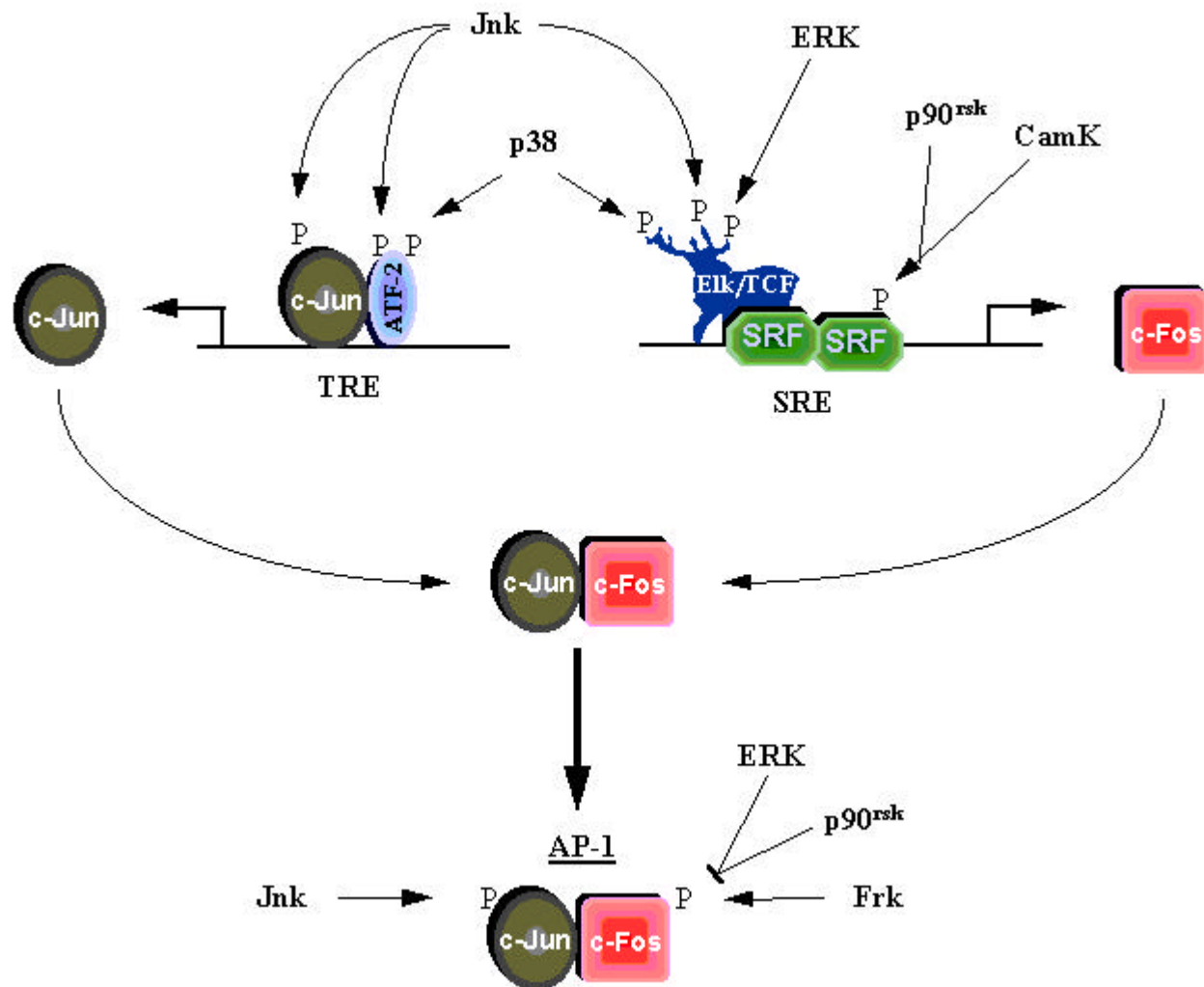


Figure 3. AP-1 as the regulation point for MAP kinase signaling pathways. The possible modifications of the transcription factors are indicated by the arrows leading from the kinases to the transcription factors. Jnk potentiates the activation of *c-jun* and Frk likewise for *c-fos*. On the other hand the cooperative phosphorylation of *c-fos* by ERK and p90^{rsk} mediates transcriptional repression by *c-fos*. For abbreviations see text.

words activators of the MAPK cascades described above (25). It is often found as a heterodimer of c-Jun and c-Fos but can actually consist of a heterodimer comprised of any of the Jun proteins (JunB and JunD) and any of the Fos proteins (FosB, Fra1 and Fra2)(106). Although less stable than heterodimers, c-Jun homodimers can also form and bind to AP-1 sites in promoters, notably the promoters of early response genes, increasing the possible combination of Fos/Jun family members contributing to AP-1 action (25,107). AP-1 activity is determined by not only its composition but also the phosphorylation state of each Fos/Jun heteropartner. In addition the combination of AP-1 with other transcription factor families, including CREB/ATF, NFAT, ETS, NFkappaB and nuclear hormone receptors, considerably expands the repertoire of genes controlled by AP-1 (106,107).

As described above, several MAPK cascades can target and phosphorylate the partners of the c-Jun/ATF-2 dimer bound to the TRE elements of the *c-jun* promoter, thereby determining the extent to which *c-jun* is expressed (figure 3). Both preexisting and newly synthesised AP-1 can also be regulated by posttranslational phosphorylation of c-Fos and c-Jun (107). Early reports indicated that COOH-terminal phosphorylation of c-Jun and other family members may negatively affect their function by interfering with binding of homodimers but not heterodimers with c-Fos (107,108). The current significance of these observations is unclear. What is much clearer is the link between Jun phosphorylation and transcriptional activation. c-Jun is bound by SAPK/JNK in the domain located near the amino-terminus, which recruits the kinase to phosphorylate Ser63 and Ser73 in the c-Jun

transactivation domain (109). Interestingly this kinase/Jun interaction may also allow SAPK/JNK to target certain dimerization partners, e.g. JunD but not JunB (110). Although the sequences around the c-Jun phosphorylation sites are similar to those located in the transcriptional activation region of c-Fos, a different protein kinase, Frk, mediates this in a Ras-dependent manner (47). Similarly the activity of other members of the Fos family is also dependent upon phosphorylation mediated by both MAPK-dependent and -independent pathways (111-113).

Differential phosphorylation of Jun and Fos has obvious functional implications for AP-1 activity. Recruitment of the ubiquitous coactivators in the p300/CBP family by c-Jun is dependent upon this amino-terminal phosphorylation (114). This provides a mechanism for connecting AP-1, via SAPK/JNK-driven phosphorylation of c-Jun, to the basal transcription machinery. In contrast, the binding of CBP to c-Fos is not phosphorylation-dependent *in vitro*, although it is unclear whether (the Ras-dependent) Frk phosphorylation of c-Fos is required for CBP-mediated transcriptional activity (115). On the other hand, the capacity of c-Fos to repress transcription, as well as the rapid turnover of c-Fos, is strongly potentiated by c-Fos phosphorylation on COOH-terminal sites, phosphorylation that can be mediated by the synergistic interplay between ERK and RSK *in vitro* (45,46). Thus differential Fos phosphorylation can account for its function as both an activator and repressor of transcription.

6.2. AP-1 and MAP kinase pathways in osteoblasts

A number of factors including cytokines, growth factors and steroid hormones affect osteoblast activity, and many of them activate MAPK cascades. PDGF, FGF and IGF strongly activate the ERK pathway (116), LIF does so weakly (117) and IL-1 and TNF α activate both the SAPK/JNK and the p38 pathways (10,116). While all of these factors can stimulate the expression of *c-fos* mRNA to a greater or lesser extent, the activation of kinase-mediated signaling pathways would assume a different complexity dependent on the degree of activation. For example, PDGF, a strong activator of the ERK cascade, would affect osteoblast gene expression by leading to a strong, transient increase in the level of c-Fos available to form AP-1 heterodimers. PDGF would likely have a second effect on AP-1 activity via Ras-dependent activation of Frk and the activation of both ERK and RSK, with the corresponding positive and negative effects on c-Fos discussed above. There would also be the corresponding induction of JunB and other Fos/Jun family members, thereby modulating the spectrum of AP-1 partner interactions, as well as those with other transcription factors, on osteoblast-specific promoters (1, 2). In contrast, however, IL-1 and TNF α have a more complex effect on the signal transduction machinery. IL-1 and TNF α act predominantly by activating the stress MAPK pathways (25,50). Nevertheless both factors only weakly activate *c-fos* transcription in osteoblast-like osteosarcoma cells (10,116). In contrast they should strongly potentiate c-jun transcription via phosphorylation of both Jun and ATF-2 bound to the TREs in the *c-jun* promoter, along with c-Jun transactivation. This would alter

the species of AP-1 available for gene control as compared to PDGF activation. Cytokines like LIF primarily activate the Jak/STAT pathway but also activate the ERK cascade (7). In osteosarcoma cells both pathways are required for a robust induction of *c-fos* mRNA (117). In this instance there would be a strong induction of c-Fos protein but only a feeble Jun activation since LIF does not activate the SAPK/JNK cascade. Once again the composition of AP-1 would be significantly different. Thus the spectrum of osteoblast gene expression in response to various factors is dependent on the MAP kinase pathway involved.

These three examples illustrate the complexity of the intracellular signaling network controlling gene expression when viewed from the perspective of the MAPK cascades and AP-1 alone. Nevertheless they also illustrate the enormous potential for differential regulation of gene expression via the selective or combined activation of each cascade, as well as the effects of weak versus strong activation. Although our understanding of these phenomena is rudimentary, it is clear that their dissection will be crucial to fully comprehend the process leading to osteoblast proliferation and differentiation. AP-1 sites are implicated in the control of various osteoblast-specific genes, e.g. collagen type I, osteopontin, osteonectin and osteocalcin (1,2). We will use the osteocalcin gene to briefly illustrate this point. The osteocalcin promoter is composed of three regulatory regions that contain a series of AP-1 sites (2). These contribute to different expression of the osteocalcin gene depending on the status of the osteoblast. Basal and bone-specific transcription is regulated by the OC (osteocalcin) gene box which binds homeodomain-containing transcription factors and AP-1. High levels of AP-1 activity promote binding of AP-1 to this site and inhibit osteocalcin gene expression in proliferating cells, whereas the low levels of AP-1 activity found in growth-inhibited cells permit osteocalcin gene expression through the same promoter element. For example, TGF β -driven phosphorylation of Fra-1 correlates strongly with repression of OC mediated via this site (118). In contrast, developmental and physiological cues for osteocalcin expression are at least partially regulated by glucocorticoid and 1,25(OH) $_2$ D $_3$ response elements. Both types of receptors are known to interact with AP-1 and CBP, thus varying levels of AP-1 activity are likely to have an important modulatory role in this response (119-126).

7. PERSPECTIVE

It is apparent that MAP kinase signaling pathways are essential for regulating the response of osteoblasts to a variety of extracellular stimuli. They play an important role in osteoblastic responses to developmental signals and bone-active factors via the activation of distinct cascades targeting specific transcription factors. Although the notion that one extracellular factor activates one signaling pathway holds in many cases, the degree of cross-talk between MAP kinase pathways will play an important role in controlling the complexity of osteoblastic gene expression. A further level of complexity is added by the integration of signaling inputs by both components of the transcription factor AP-1. Thus

this factor acting alone or in concert with other transcription factors allows both developmental and physiological conditions to target genes at the promoter level. Our limited understanding of the mechanism behind this process shows that much remains to be learned of the integration of kinase-mediated signaling pathways and the activation of gene expression in the control of osteoblast function and its contribution to the control of bone modelling.

8. ACKNOWLEDGEMENTS

For practical purposes, we have limited the references to more detailed review articles and an appropriate selection of original research articles. We apologize to our colleagues for any egregious omissions. We thank Jocelyne Caboche for constructive comments on the review. This work was supported by grants to RAH from Novartis Pharma A.G. and the "Fondation pour la Recherche Medicale. RAH is a researcher of the CNRS.

9. REFERENCES

1. Rodan, G.A. & M. Noda: Gene expression in osteoblastic cells. *Crit Rev Eukaryot Gene Expr* 1, 85-98 (1991)
2. Stein, G.S., J.B. Lian, J.L. Stein, A.J. van Wijnen & M. Montecino: Transcriptional control of osteoblast growth and differentiation. *Physiol Rev* 76, 593-629 (1996)
3. Karin, M. & T. Hunter: Transcriptional control by protein phosphorylation: signal transmission from the cell surface to the nucleus. *Curr Biol* 5, 747-57 (1995)
4. Sugden, P. & A. Clerk: Regulation of the ERK subgroup of MAP kinase cascades through G protein-coupled receptors. *Cell Signal* 9, 337-51 (1997)
5. Ghosh, A. & M.E. Greenberg: Calcium signaling in neurons: molecular mechanisms and cellular consequences. *Science* 268, 239-47 (1995)
6. Burgering, B.M.T. & J.L. Bos: Regulation of Ras-mediated signalling: more than one way to skin a cat. *Trends Biochem Sci* 20, 18-22 (1995)
7. Yin, T. & Y.C. Yang: Mitogen-activated Protein Kinases and Ribosomal S6 Protein Kinases Are Involved in Signaling Pathways Shared by Interleukin-11, Interleukin-6, Leukemia Inhibitory Factor, and Oncostatin M in Mouse 3T3-L1 Cells. *J Biol Chem* 269, 3731-8 (1994)
8. Hipskind, R.A., M. Baccarini & A. Nordheim: Transient activation of RAF-1, MEK and ERK2 coincides kinetically with TCF phosphorylation and IEG promoter activity *in vivo*. *Mol Cell Biol* 14, 6219-31 (1994)
9. Zinck, R., M.A. Cahill, M. Kracht, C. Sachsenmaier, R.A. Hipskind & A. Nordheim: Protein synthesis inhibitors reveal differential regulation of MAPK and SAPK pathways that converge on Elk-1. *Mol Cell Biol* 15, 4930-38 (1995)
10. Bebie, M. & R.A. Hipskind, manuscript in preparation
11. Grigoriadis, A.E., Z.Q. Wang & E.F. Wagner: Fos and bone cell development: lessons from a nuclear oncogene. *Trends Genet* 11, 436-41 (1995)
12. Herrera, R.E., P.E. Shaw & A. Nordheim: Occupation of the *c-fos* serum response element *in vivo* by a multi-protein complex is unaltered by growth factor induction. *Nature* 340, 68-70 (1989)
13. Ginty, D.D.: Calcium regulation of gene expression: Isn't that spatial? *Neuron* 18, 183-6 (1997)
14. Janknecht, R. & T. Hunter: Transcriptional control: Versatile molecular glue. *Curr.Biol* 6, 951-4 (1996)
15. Hill, C.S. & R. Treisman: Transcriptional regulation by extracellular signals: mechanisms and specificity. *Cell* 80, 199-211(1995)
16. Cahill, M.A., R. Janknecht & A. Nordheim: Signalling pathways: Jack of all cascades. *Curr Biol* 6, 16-9 (1996)
17. Xia, Z., H. Dudek, C.K. Miranti & M.E. Greenberg: Calcium influx via the NMDA receptor induces immediate early gene transcription by a MAP kinase/ERK-dependent mechanism. *J Neurosci* 16, 5425-36 (1996)
18. Pingoud, V., R. Zinck, R.A. Hipskind, R. Janknecht & A. Nordheim: Heterogeneity of ternary complex factors in HeLa cell extracts. *J Biol Chem* 269, 23310-7 (1994)
19. Hipskind, R.A., Rao VN, Mueller CFG, Reddy ESP and Nordheim A: Ets-related protein Elk-1 is homologous to the *c-fos* regulatory factor p62TCF. *Nature* 354, 531-534 (1991)
20. Treisman, R.: Regulation of transcription by MAP kinase cascades. *Curr Opin Cell Biol* 8, 205-15 (1996)
21. Miranti, C.K., D.D. Ginty, G. Huang, T. Chatila & M.E. Greenberg: Calcium activates serum response factor-dependent transcription by a Ras- and Elk-1-independent mechanism that involves a Ca²⁺/calmodulin-dependent kinase. *Mol Cell Biol* 15, 3672-84 (1995)
22. Treisman, R.: Journey to the surface of the cell: Fos regulation and the SRE. *EMBO J* 14, 4905-13 (1995)
23. Wagner, B.J., T.E. Hayes, C.J. Hoban & B.H. Cochran: The SIF binding element confers sis/PDGF inducibility onto the *c-fos* promoter. *EMBO J* 9, 4477-4484 (1990)
24. Sadowski, H.B., K. Shuai, J.E. Darnell & M.Z. Gilman: A Common Nuclear Signal Transduction Pathway Activated by Growth Factor & Cytokine Receptors. *Science* 261, 1739-44 (1993)

25. Whitmarsh, A.J. & R.J. Davis: Transcription factor AP-1 regulation by mitogen-activated protein kinase signal transduction pathways. *J Mol Med* 74, 589-607 (1996)
26. Ferrel, J.E.: Tripping the switch fantastic: how a protein kinase cascade can convert graded inputs into switch-like outputs. *Trends Biochem Sci* 21, 460-6 (1996)
27. Daum, G., I. Eisenmann-Tappe, H.W. Fries, J. Troppmair & U. R. Rapp: The ins and outs of Raf kinases. *Trends Biochem Sci* 19, 474-80 (1994)
28. Chao T.S., D.A. Foster, U.R. Rapp & M.R. Rosner: Differential Raf requirement for activation of mitogen-activated protein kinase by growth factors, phorbol esters, and calcium. *J Biol Chem* 269, 7337-41 (1994)
29. Kuo W.L., M. Abe, J. Rhee, E.M. Eves, S.A. McCarthy, M. Yan, D.J. Templeton, M. McMahon & M.R. Rosner: Raf, but not MEK or ERK, is sufficient for differentiation of hippocampal neuronal cells. *Mol Cell Biol* 16, 1458-70 (1996)
30. Reuter C.W., A.D. Catling, T. Jelinek & M.J. Weber: Biochemical analysis of MEK activation in NIH3T3 fibroblasts. Identification of B-Raf and other activators. *J Biol Chem* 270, 7644-7655 (1995)
31. Vossler M.R., H. Yao, R.D. York, M.G. Pan, C.S. Rim & P.J. Stork: cAMP activates MAP kinase and Elk-1 through a B-Raf- and Rap1-dependent pathway. *Cell* 89, 73-82 (1997)
32. Lenormand, P., C. Sardet, G. Pages, G. L'Allemain, A. Brunet & J. Pouyssegur: Growth factors induce nuclear translocation of MAP kinases (p42mapk and p44mapk) but not of their activator MAP kinase kinase (p45mapkk) in fibroblasts. *J Cell Biol* 122, 1079-88 (1993)
33. Chen, R.H., C. Sarnecki & J. Blenis: Nuclear localization and regulation of erk- and rsk-encoded protein kinases. *Mol Cell Biol* 12, 915-27 (1992)
34. Wen, Z., Z. Zhong & J.E. Darnell: Maximal Activation of Transcription by Stat1 and Stat3 Requires Both Tyrosine and Serine Phosphorylation. *Cell* 82, 241-50 (1995)
35. Davis, R.J.: Transcriptional regulation by MAP kinases. *Mol Reprod Dev* 42, 459-467 (1995)
36. Piumiglia, K.M. & S.J. Decker: Cell cycle arrest mediated by the MEK/mitogen-activated protein kinase pathway. *Proc Natl Acad Sci USA* 94, 448-52 (1997)
37. Hunter, T.: Protein kinases and phosphatases: the yin and yang of protein phosphorylation and signaling. *Cell* 80, 225-236 (1995)
38. Wu, J., L.F. Lau & T.W. Sturgill: Rapid deactivation of MAP kinase in PC12 cells occurs independently of induction of phosphatase MKP-1. *FEBS Lett* 353, 9-12 (1994)
39. Alessi, D.R., N. Gomez, G. Moorhead, T. Lewis, S.M. Keyse & P. Cohen: Inactivation of p42 MAP kinase by protein phosphatase 2A and a protein tyrosine phosphatase, but not CL100, in various cell lines. *Curr Biol* 5, 283-95 (1995)
40. Cheng M., T.G. Boulton & M.H. Cobb: ERK3 is a constitutively nuclear protein kinase. *J Biol Chem* 271, 8951-8 (1996)
41. Zhou, G., Z.Q. Bao & J.E. Dixon: Components of a new human protein kinase signal transduction pathway. *J Biol Chem* 270, 12665-12669 (1995)
42. Hsiao, K.M., S.Y. Chou, S.J. Shih & J.E. Ferrell: Evidence that inactive p42 mitogen-activated protein kinase and inactive Rsk exist as a heterodimer *in vivo*. *Proc Natl Acad Sci USA* 91, 5480-4 (1994)
43. Rivera, V.M., C.K. Miranti, R.P. Misra, D.D. Ginty, R.H. Chen, J. Blenis & M.E. Greenberg: A growth factor-induced kinase phosphorylates the serum response factor at a site that regulates its DNA binding activity. *Mol Cell Biol* 13, 6260-73 (1993)
44. Xing, J., D.D. Ginty & M.E. Greenberg: Coupling of the ras-MAPK pathway to gene activation by RSK2, a growth-factor-regulated CREB kinase. *Science* 273, 959-963 (1996)
45. Chen, R.H., C. Abate & J. Blenis: Phosphorylation of the c-Fos transrepression domain by mitogen-activated protein kinase and 90-kDa ribosomal S6 kinase. *Proc Natl Acad Sci USA* 90, 10952-6 (1993)
46. Ofir, R., V.J. Dwarki, D. Rashid & I.M. Verma: Phosphorylation of the C terminus of Fos protein is required for transcriptional transrepression of the *c-fos* promoter. *Nature* 348, 80-2 (1990)
47. Deng, T. & M. Karin: c-Fos transcriptional activity stimulated by H-Ras-activated protein kinase distinct from JNK and ERK. *Nature* 371, 171-5 (1994)
48. Kyriakis, J.M., P. Banerjee, E. Nikolakaki, T. Dai, E.A. Rubie, M.F. Ahmad, J. Avruch & J.R. Woodgett: The stress-activated protein kinase subfamily of c-Jun kinases. *Nature* 369, 156-60 (1994)
49. Hibi, M., A. Lin, T. Smeal, A. Minden & M. Karin: Identification of an oncoprotein- and UV-responsive protein kinase that binds and potentiates the c-Jun activation domain. *Genes Dev* 7, 2135-48 (1993)
50. Paul, A., S. Wilson, C.M. Belham, C.J.M. Robinson, P.H. Scott, G.W. Gould & R. Plevin: Stress-activated protein kinases: activation, regulation and function. *Cell Signal* 9, 403-10 (1997)

51. Gupta, S., T. Barrett, A.J. Whitmarsh, J. Cavanagh, H.K. Sluss, B. Derijard & R.J. Davis: Selective interaction of JNK protein kinase isoforms with transcription factors. *EMBO J* 15, 2760-70 (1996)
52. Tournier, C., A.J. Whitmarsh, J. Cavanagh, T. Barrett & R.J. Davis: Mitogen-activated protein kinase 7 is an activator of the c-Jun NH2-terminal kinase. *Proc Natl Acad Sci U S A* 94, 7337-42 (1997)
53. Fanger, G.R., P. Gerwins, C. Widmann, M.B. Jarpe & G.L. Johnson: MEKs, GCKs, MLKs, PAKs, TAKs, and Tpls: upstream regulators of the c-Jun amino-terminal kinases? *Curr Opin Genet Devel* 7, 67-74 (1997)
54. Hirai S., M. Katoh, M. Terada, J.M. Kyriakis, L.I. Zon, A. Rana, J. Avruch & S. Ohno: MST/MLK2, a member of the mixed lineage kinase family, directly phosphorylates and activates SEK1, an activator of c-Jun N-terminal kinase/stress-activated protein kinase. *J Biol Chem* 272,15167-73 (1997)
55. Lange-Carter, C.A., C.M. Pleiman, A.M. Gardner, K.J. Blumer, & G. L. Johnson: A divergence in the MAP kinase regulatory network defined by MEK kinase and Raf. *Science* 260, 315-319 (1993)
56. Yan, M., T. Dai, J.C. Deak, J.M. Kyriakis, L.I. Zon, J.R. Woodgett & D.J. Templeton: Activation of stress-activated protein kinase by MEKK1 phosphorylation of its activator SEK1. *Nature* 372 798-800 (1994)
57. Cardone, M.H., G.S. Salvesen, C. Widmann, G. Johnson & S.M. Frisch: The regulation of anoikis: MEKK-1 activation requires cleavage by caspases. *Cell* 90, 315-323 (1997)
58. Muda M., A. Theodosiou, N. Rodrigues, U. Boschert, M. Camps, C. Gillieron, K. Davies, A. Ashworth & S. Arkinstall: The dual specificity phosphatases M3/6 and MKP-3 are highly selective for inactivation of distinct mitogen-activated protein kinases. *J Biol Chem* 271, 27205-8 (1996)
59. Hill, C.S., J. Wynne & R. Treisman R: The Rho family GTPases RhoA, Rac1, and CDC42Hs regulate transcriptional activation by SRF. *Cell* 81, 1159-1170 (1995)
60. Hippskind, R.A., D. Buescher, A. Nordheim & M. Baccarini: Ras/MAP kinase-dependent and -independent signaling pathways target distinct ternary complex factors. *Gene Dev* 8, 1803-16 (1994)
61. Galcheva-Gargova, Z., B. Derijard, I.H. Wu & R.J. Davis: A osmosensing signal transduction pathway in mammalian cells. *Science* 265, 806-8 (1994)
62. Han, J., J.D. Lee, L. Bibbs & R.J. Ulevitch: A MAP kinase targeted by endotoxin and hyperosmolarity in mammalian cells. *Science* 265, 808-11 (1994)
63. Rouse, J., P. Cohen, S. Trigon, M. Morange, A. Alonso-Llamazares, D. Zamanillo, T. Hunt & A. Nebreda: A novel kinase cascade triggered by stress and heat shock that stimulates MAPKAP kinase-2 and phosphorylation of the small heat shock proteins. *Cell* 78, 1027-37 (1994)
64. Freshney, N.W., L. Rawlinson, F. Guesdon, E. Jones, S. Cowley, J. Hsuan & J. Saklatvaia: Interleukin-1 activates a novel protein kinase cascade that results in the phosphorylation of Hsp27. *Cell* 78, 1039-49 (1994)
65. Lee, J.C., J.T. Laydon, P.C. McDonnell, T.F. Gallagher, S. Kumar, D. Green, D. McNulty, M.J. Blumenthal, J.R. Heys, S.W. Landvatter, J.E. Strickler, M.M. McLaughlin, I.R. Siemens, S. M. Fischer, G.P. Livi, J.R. White, J.L. Adams & P.R. Young: A protein kinase involved in the regulation of inflammatory cytokine biosynthesis. *Nature* 372, 739-446 (1994)
66. Jiang Y., C. Chen, Z. Li, W. Guo, J.A. Gegner, S. Lin & J. Han: Characterization of the structure and function of a new mitogen-activated protein kinase (p38beta). *J Biol Chem* 271, 17920-6 (1996)
67. Li Z., Y. Jiang, R.J. Ulevitch & J. Han: The primary structure of p38 gamma: a new member of p38 group of MAP kinases. *Biochem Biophys Res Commun* 228, 334-40 (1996)
68. Wang, X. S., K. Diener, C.L.Manthey, S. Wang, B. Rosenzweig, J. Bray, J. Delaney, C.N. Cole, P.Z. Chan-Hui, N. Mantlo, H.S. Lichenstein, M. Zukowski & Z. Yao: Molecular cloning and characterization of a novel p38 mitogen-activated protein kinase. *J Biol Chem* 272, 23668-74 (1997)
69. Kumar, S., P.C. McDonnell, R.J. Gum, A.T. Hand, J.C. Lee & P.R. Young: Novel homologues of CSBP/p38 MAP kinase: activation, substrate specificity and sensitivity to inhibition by pyridyl imidazoles. *Biochem Biophys Res Commun* 235, 533-8 (1997)
70. Derijard, B., J. Raingeaud, T. Barrett, I.H. Wu, J. Han, R.J. Ulevitch & R.J. Davis: Independent human MAP kinase signal transduction pathways defined by MEK and MKK isoforms. *Science* 267, 682-5 (1995)
71. Raingeaud J., A.J. Whitmarsh, T. Barrett, B. Derijard & R.J. Davis: MKK3- and MKK6-regulated gene expression is mediated by the p38 mitogen-activated protein kinase signal transduction pathway. *Mol Cell Biol* 16, 1247-55 (1996)
72. Moriguchi, T., N. Kuroyanagi, K. Yamaguchi, Y. Gotoh, K. Irie, T. Kano, K. Shirakabe, Y. Muro, H. Shibuya, K. Matsumoto, E. Nishida & M. Hagiwara: A novel kinase cascade mediated by mitogen-activated protein

- kinase kinase 6 and MKK3. *J Biol Chem* 271, 13675-9 (1996)
73. Meier, R., J. Rouse, A. Cuenda, A.R. Nebreda & P. Cohen: Cellular stresses and cytokines activate multiple mitogen-activated-protein kinase kinase homologues in PC12 and KB cells. *Eur J Biochem* 236, 796-805 (1996)
74. Yamaguchi, K., K. Shirakabe, H. Shibuya, K. Irie, I. Oishi, N. Ueno, T. Taniguchi, E. Nishida & K. Matsumoto: Identification of a member of the MAPKKK family as a potential mediator of TGF-beta signal transduction. *Science* 270, 2008-11 (1995)
75. Ichijo H., E. Nishida, K. Irie, P. ten Dijke, M. Saitoh, T. Moriguchi, M. Takagi, K. Matsumoto, K. Miyazono & Y. Gotoh: Induction of apoptosis by ASK1, a mammalian MAPKKK that activates SAPK/JNK and p38 signaling pathways. *Science* 275, 90-4 (1997)
76. Takekawa M., F. Posas & H. Saito: A human homolog of the yeast Ssk2/Ssk22 MAP kinase kinase kinases, MTK1, mediates stress-induced activation of the p38 and JNK pathways. *EMBO J* 16, 4973-82 (1997)
77. Cuenda A., P. Cohen, V. Buee-Scherrer & M. Goedert: Activation of stress-activated protein kinase-3 (SAPK3) by cytokines and cellular stresses is mediated via SAPKK3 (MKK6); comparison of the specificities of SAPK3 and SAPK2 (RK/p38). *EMBO J* 16, 295-305 (1997)
78. McLaughlin M.M., S. Kumar, P.C. McDonnell, S. Van Horn, J.C. Lee, G.P. Livi & P.R. Young: Identification of mitogen-activated protein (MAP) kinase-activated protein kinase-3, a novel substrate of CSBP p38 MAP kinase. *J Biol Chem* 271, 8488-92 (1996)
79. Price, M.A., F.H. Cruzalegui & R. Treisman: The p38 and ERK MAP kinase pathways cooperate to activate Ternary Complex Factors and *c-fos* transcription in response to UV light. *EMBO J* 15, 6552-63 (1996)
80. Janknecht, R. & T. Hunter: Convergence of MAP kinase pathways on the ternary complex factor Sap-1a. *EMBO J* 16, 1620-7 (1997)
81. Whitmarsh, A.J., S.H. Yang, M.S. Su, A.D. Sharrocks & R.J. Davis: Role of p38 and JNK mitogen-activated protein kinases in the activation of ternary complex factors. *Mol Cell Biol* 17, 2360-71 (1997)
82. Wang X.Z. & D. Ron: Stress-induced phosphorylation and activation of the transcription factor CHOP (GADD153) by p38 MAP Kinase. *Science* 272, 1347-9 (1996)
83. Tan Y., J. Rouse, A. Zhang, S. Cariaty, P. Cohen & M.J. Comb: FGF and stress regulate CREB and ATF-1 via a pathway involving p38 MAP kinase and MAPKAP kinase-2. *EMBO J* 15, 4629-42 (1996)
84. Chaudhary, L.R. & L.V. Avioli: Activation of extracellular signal-regulated kinases 1 and 2 (ERK1 and ERK2) by FGF-2 and PDGF-BB in normal human osteoblastic and bone marrow stromal cells: differences in mobility and in-gel renaturation of ERK1 in human, rat, and mouse osteoblastic cells. *Biochem Biophys Res Commun* 238, 134-9 (1997)
85. Zhen, X., J.P. Bonjour & J. Caverzasio: Platelet-derived growth factor stimulates sodium-dependent Pi transport in osteoblastic cells via phospholipase Cgamma and phosphatidylinositol 3'-kinase. *J Bone Miner Res* 12, 36-44 (1997)
86. Verheijen, M.H. & L.H. Defize: Parathyroid hormone inhibits mitogen-activated protein kinase activation in osteosarcoma cells via a protein kinase A-dependent pathway. *Endocrinology* 136, 3331-7 (1995)
87. Evans, D.B., R.A. Hipskind & G. Bilbe: Analysis of signaling pathways used by parathyroid hormone to activate the *c-fos* gene in human SaOS2 osteoblast-like cells. *J Bone Miner Res* 11, 1066-74 (1996)
88. Zhang, W., R.M. Dziak & J.M. Aletta: EGF-mediated phosphorylation of extracellular signal-regulated kinases in osteoblastic cells. *J Cell Physiol* 162, 348-58 (1995)
89. Hurley, M.M., K. Marcello, C. Abreu & M. Kessler: Signal transduction by basic fibroblast growth factor in rat osteoblastic Pyla cells. *J Bone Miner Res* 11, 1256-63 (1996)
90. Endho, H., H. Sasaki, K. Maruyama, K. Takeyama, I. Waga, T. Shimizu, S. Kato & H. Kawashima: Rapid activation of MAP kinase by estrogen in the bone cell line. *Biochem Biophys Res Commun* 235, 99-102 (1997)
91. Wu, L.W., H.K. Yoon, D.J. Baylink, L.M. Graves & K.H. Lau: Fluoride at mitogenic doses induces a sustained activation of p42mapk, but not p42mapk, in human TE85 osteosarcoma cells. *J Clin Endocrinol Metab* 82, 1126-35 (1997)
92. Fang, M.A., D.A. Kujubu & T.J. Hahn: The effects of prostaglandin E2, parathyroid hormone, and epidermal growth factor on mitogenesis, signaling, and primary response genes in UMR106-01 osteoblast-like cells. *Endocrinology* 131, 2113-9 (1992)
93. Hipskind, R.A., D.B. Evans & G. Bilbe: manuscript in preparation
94. Alessi, D.R., A. Cuenda, P. Cohen, D.T. Dudley & A.R. Saltiel: PD098059 is a specific inhibitor of the activation of mitogen-activated protein kinase kinase *in vitro* and *in vivo*. *J Biol Chem* 270, 27489-27494 (1995)
95. Alberola-Ila J., K.A. Forbush, R. Seger, E.G. Krebs & R.M. Perlmutter: Selective requirement for MAP kinase

activation in thymocyte differentiation. *Nature* 373, 620-623 (1995)

96. Crompton T., K.C. Gilmour & M.J. Owen: The MAP kinase pathway controls differentiation from double-negative to double-positive thymocyte. *Cell* 86, 243-51 (1996)

97. Whalen A.M., S.C. Galasinski, P.S. Shapiro, T.S. Nahreini & N.G. Ahn: Megakaryocytic differentiation induced by constitutive activation of mitogen-activated protein kinase kinase. *Mol Cell Biol* 17, 1947-58 (1997)

98. Hu M.C., W.R. Qiu, X. Wang, C.F. Meyer & T.H. Tan: Human HPK1, a novel human hematopoietic progenitor kinase that activates the JNK/SAPK kinase cascade. *Genes Dev* 10, 2251-64 (1996)

99. Nishina, H., M. Bachmann, A.J. Oliveira-dos-Santos, I. Kozieradzki, K.D. Fischer, B. Odermatt, A. Wakeham, A. Shahinian, H. Takimoto, A. Bernstein, T.W. Mak, J.R. Woodgett, P.S. Ohashi & J.M. Penninger: Impaired CD28-mediated interleukin 2 production and proliferation in stress kinase SAPK/ERK1 kinase (SEK1)/mitogen-activated protein kinase kinase 4 (MKK4)-deficient T lymphocytes. *J Exp Med* 186, 941-53 (1997)

100. Nishina, H., K.D. Fischer, L. Radvanyi, A. Shahinian, R. Hakem, E.A. Rubie, A. Bernstein, T.W. Mak, J.R. Woodgett & J.M. Penninger: Stress-signalling kinase Sek1 protects thymocytes from apoptosis mediated by CD95 and CD3. *Nature* 385, 350-3 (1997)

101. Westwick, J.K., C. Weitzel, H.L. Leffert & D.A. Brenner: Activation of Jun kinase is an early event in hepatic regeneration. *J Clin Invest* 95, 803-10 (1995)

102. Mendelson, K.G., L.R. Contois, S.G. Tevosian, R.J. Davis & K.E. Paulson: Independent regulation of JNK/p38 mitogen-activated protein kinases by metabolic oxidative stress in the liver. *Proc Natl Acad Sci U S A* 93, 12908-13 (1996)

103. Crawley, J.B., L. Rawlinson, F.V. Lali, T.H. Page, J. Saklatvala & B.M. Foxwell: T cell proliferation in response to interleukins 2 and 7 requires p38MAP kinase activation. *J Biol Chem* 272, 15023-7 (1997)

104. Heidenreich, K.A. & J.L. Kummer: T cell proliferation in response to interleukins 2 and 7 requires p38MAP kinase activation. *J Biol Chem* 271, 9891-4 (1996)

105. Goodyear, L.J., P.Y. Chang, D.J. Sherwood, S.D. Dufresne & D.E. Moller: Effects of exercise and insulin on mitogen-activated protein kinase signaling pathways in rat skeletal muscle. *Am J Physiol* 271, E403-8 (1996)

106. Karin, M., Z.G. Liu & E. Zandi: AP-1 function and regulation. *Curr Opin Cell Biol* 9, 240-6 (1997)

107. Karin, M.: Signal transduction from the cell surface to the nucleus through the phosphorylation of transcription factors. *Curr Opin Cell Biol* 6, 415-24 (1994)

108. Nikolakaki E., P.J. Coffey, R. Hemelsoet, J.R. Woodgett & L.H. Defize: Glycogen synthase kinase 3 phosphorylates Jun family members *in vitro* and negatively regulates their transactivating potential in intact cells. *Oncogene* 8, 833-40 (1993)

109. Derijard, B., M. Hibi, I.H. Wu, T. Barrett, B. Su, T. Deng & M. Karin: JNK1: a protein kinase stimulated by UV light and Ha-Ras binds and phosphorylates the c-Jun activation domain. *Cell* 76, 1025-37 (1994)

110. Kallunki T., T. Deng, M. Hibi & M. Karin: c-Jun can recruit JNK to phosphorylate dimerization partners via specific docking interactions. *Cell* 87, 929-39 (1996)

111. Skinner M., S. Qu, C. Moore & R. Wisdom: Transcriptional activation and transformation by FosB protein require phosphorylation of the carboxyl-terminal activation domain. *Mol Cell Biol* 17, 2372-80 (1997)

112. Gruda M.C., K. Kovary, R. Metz & R. Bravo: Regulation of Fra-1 and Fra-2 phosphorylation differs during the cell cycle of fibroblasts and phosphorylation *in vitro* by MAP kinase affects DNA binding activity. *Oncogene* 9, 2537-47 (1994)

113. Lallemand D., G. Spyrou, M. Yaniv & C.M. Pfarr: Variations in Jun and Fos protein expression and AP-1 activity in cycling, resting and stimulated fibroblasts. *Oncogene* 14, 819-30 (1997)

114. Arias, J., A.S. Alberts, P. Brindle, F.X. Claret, T. Smeal, M. Karin, J. Feramisco & M. Montminy: Activation of cAMP and mitogen responsive genes relies on a common nuclear factor. *Nature* 370, 226-9 (1994)

115. Bannister, A.J. & T. Kouzarides: CBP-induced stimulation of c-Fos activity is abrogated by E1A. *EMBO J* 14, 4758-62 (1995)

116. Hippskind, R.A., D.B. Evans, M. Baccarini & G. Bilbe: manuscript in preparation

117. Hippskind, R.A., C. Halleux, D.B. Evans, M. Bebie, S. Decker & G. Bilbe: manuscript in preparation

118. Banerjee, C., J.L. Stein, A.J. Van Wijnen, B. Frenkel, J.B. Lian & G.S. Stein: Transforming growth factor-beta 1 responsiveness of the rat osteocalcin gene is mediated by an activator protein-1 binding site. *Endocrinology* 137, 1991-2000 (1996)

119. Ponta, H., A.C. Cato & P. Herrlich: Interference of pathway specific transcription factors. *Biochim Biophys Acta* 1129, 255-261 (1992)

120. Pfahl, M.: Signal transduction by retinoid receptors. *Skin Pharmacol* 6 Suppl 1, 8-16 (1993)

121. Schule, R., K. Umesonu, D.J. Mangelsdorf, J. Bolado, J.W. Pike & R.M. Evans: Jun-Fos and receptors for vitamins A and D recognize a common response element in the human osteocalcin gene. *Cell* 61, 497-504 (1990)

122. Jonat, C., H.J. Rahmsdorf, K.K. Park, A.C. Cato, S. Gebel, H. Ponta & P. Herrlich: Antitumor promotion and antiinflammation: down-modulation of AP-1 (Fos/Jun) activity by glucocorticoid hormone. *Cell* 62, 1189-1204 (1990)

123. Yang-Yen, H.F., J.C. Chambard, Y.L. Sun, T. Smeal, T.J. Schmidt, J. Drouin & M. Karin: Transcriptional interference between c-Jun and the glucocorticoid receptor: mutual inhibition of DNA binding due to direct protein-protein interaction. *Cell* 62, 1205-15 (1990)

124. Schule, R., P. Rangarajan, S. Kliewer, L.J. Ransone, J. Bolado, N. Yang, I.M. Verma & R.M. Evans: Functional antagonism between oncoprotein c-Jun and the glucocorticoid receptor. *Cell* 62, 1217-26 (1990)

125. Kamei, Y., L. Xu, T. Heinzel, J. Torchia, R. Kurokawa, B. Gloss, S.C. Lin, R.A. Heyman, D.W. Rose, C.K. Glass & M.G. Rosenfeld: A CBP integrator complex mediates transcriptional activation and AP-1 inhibition by nuclear receptors. *Cell* 85, 403-14 (1996)

126. Stein, G.S., J.B. Lian, A.J. van Wijnen & J.L. Stein: The osteocalcin gene: a model for multiple parameters of skeletal-specific transcriptional control. *Mol Biol Rep* 24, 185-96 (1997)

Key words: Bone, ERK, SAPK, JNK, p38, Elk-1, fos, jun, AP-1

Send correspondence to: Robert A. Hipskind, Institut de Genetique Moleculaire de Montpellier, CNRS, 1919 Route de Mende, 34293 Montpellier cedex 5, France Tel: (33).467.613667, Fax (33).467.040231, E-mail: hipskind@igm.cnrs-mop.fr, Dr Graeme Bilbe, Novartis Pharma A.G., Postfach, K681.4.43, CH-4002 Basel, Switzerland, Tel: (41).61.6967745, Fax (41).61.6964145, E-mail graeme.bilbe@pharma.novartis.com