CHAPTER 13

Genes and Proteins Involved in the Regulation of Osteogenesis

G. R. Kirkham and S. H. Cartmell*

Summary

Skeletal tissue is exposed to mechanical forces throughout a vertebrate life span, and bone mass is adjusted in response, either absorbing existing skeletal material or synthesising new bone in a site-specific manner. The precise cellular signalling mechanisms by which bone synthesis is controlled within developing osteoblasts and progenitor cells remains undefined. The process is likely to include early electrophysiological responses, possibly mediated by integrins and stretch activated ion channels, followed by activation of intracellular signalling pathways. These mechanisms can then act to regulate the actions and transcription levels of various transcription factors involved in the regulation of the osteoblast phenotype, which in turn regulate the genes that code for bone matrix proteins. This review will briefly discuss role of the key genetic regulators of the osteoblast phenotype, starting with various transcription factors, moving on to some key matrix proteins.

Keywords: Osteogenesis, Differentiation, Regulation, Proteins, Transcription Factors

*Correspondence to: Dr Sarah Cartmell. Tel: +44 (0)1782 554 253. Fax: +44 (0)1782 717 079.
E-mail: s.h.cartmell@bemp.keele.ac.uk

Topics in Tissue Engineering, Vol. 3, 2007. Eds. N Ashammakhi, R Reis & E Chiellini © 2007.

Transcription factors

Runx2

Runt-related transcription factor 2 (Runx2, (Cbfa1/PEBP2αA/AML-3/Osf2)) is considered to be the central control gene within the osteoblast phenotype. Numerous studies have indicated its importance in the skeletal development of numerous mammalian organisms. Mutations in the gene have been shown to have dramatic, even life threatening, physiological consequences. Inactivating mutations can cause the condition known as Cleidocranial dysplasia (CCD), an autosomal dominant condition, characterised by abnormal skeletal genesis and the arrest of osteoblast development (Mundlos, *et al*., 1997). Cbfa1 -/- mice have also been shown to have a higher rate of proliferation (Pratap, *et al*., 2003), indicating its possible role as a growth regulator. Furthermore, the ability of Cbfa1 to redirect a committed pre-muscle cell into an osteoblast lineage (Lee, *et al*., 2000, Lee, *et al*., 1999), and its ability to inhibit the adipogenic phenotype (Gori, *et al*., 1999), reinforce the key role of Cbfa1 in osteoblast development. Mutations resulting in overexpression of the Cbfa1 protein have been linked to skeletal cancer development (Perinpanayagam, *et al*., 2004), and ectopic breast cancer (Barnes, *et al*., 2003, Brubaker, *et al*., 2003). These dramatic effects illustrate the importance of this gene in skeletal development and regulation. It should be noted however that although the importance of Cbfa1 within the osteoblast phenotype has been clearly established, the gene is not osteoblast specific. Its expression has been observed in the early development of numerous cell types, a key example is its role in chondrocyte differentiation (Lian and Stein, 2003).

In earlier studies Runx2 regulation of osteocalcin was more widely recognised, but in recent years numerous investigations have indicated its role in the regulation of a broad spectrum of osteoblast specific genes. The Runx2 transcript has the ability to facilitate the convergence of numerous osteogenic signalling pathways. Factors produced as a consequence of these extracellular signals are recruited by Runx2-specific subnuclear domains, producing a multi-component transcriptional complex. Runx2 has also be shown to interact with other native proteins: C/EBPδ, the tumour suppressor protein pRB, homeodomain proteins, Ets1, Smad factors and LEF-1 (Kahler and Westendorf, 2003, Westendorf and Hiebert, 1999), along with its binding partner CBFβ (Yoshida, *et al*., 2002, Kundu, *et al*., 2002, Miller, *et al*., 2002). It is this multi-component complex that interacts with the promoter regions of most major

osteoblast specific genes. This enables Cbfa1 to directly stimulate genes such as: osteocalcin, osteopontin, collagen I, collagenase 3 (matrix metaloproteinase 1), bone sialoprotein, alkaline phosphatase (ALP) TGFβ receptor 1, C/EBPδ, and RANKL (receptor activator of nuclear factor kappa B ligand (see Figures 1 and 2)) (Ducy, *et al*., 1997, Selvamurugan, *et al*., 1998, Kern, *et al*., 2001, Harada, *et al*., 1999, Shimizu-Sasaki, *et al*., 2001, Newberry, *et al*., 1997, Otto, *et al*., 2003).

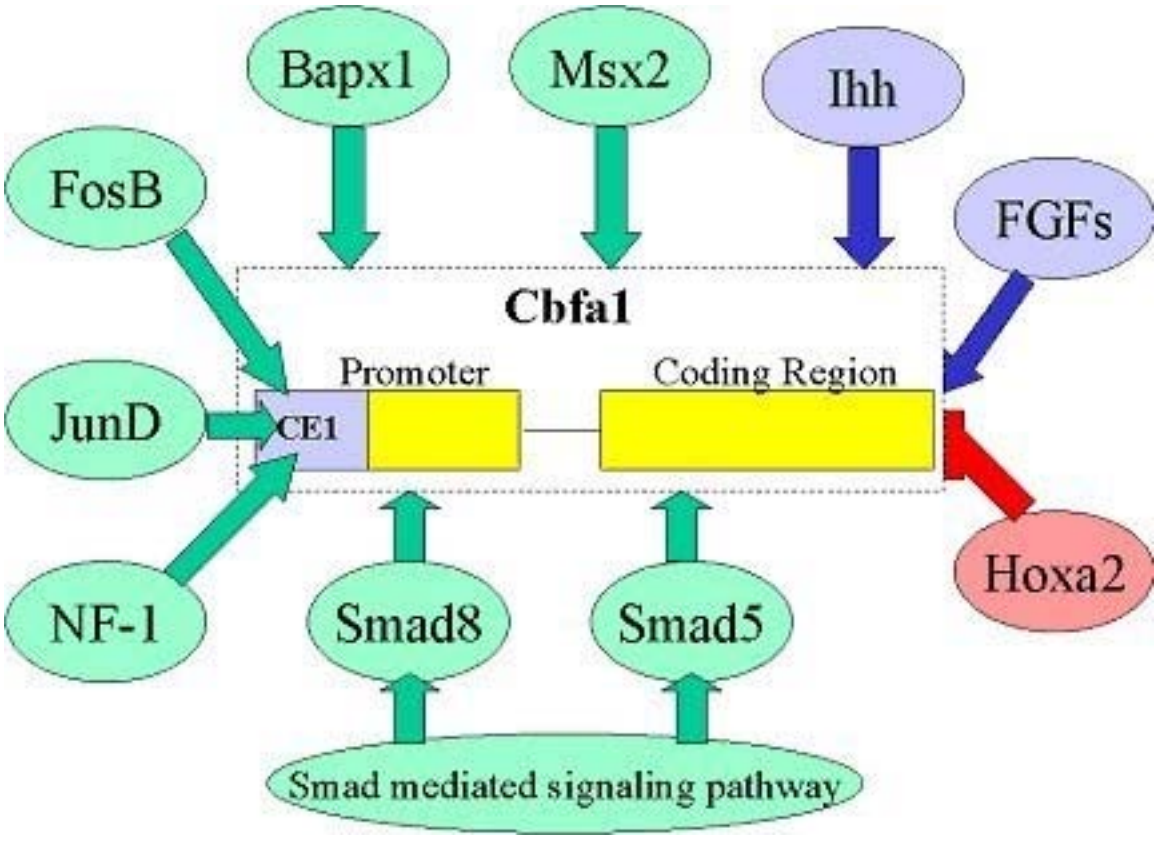

Figure 1. Examples of transcriptional regulation of the Runx2 gene. Transcription factors that have an up-regulatory action are shown green. Transcription factors that have an inhibitory action are shown in red with truncated arrows. Finally growth factors are shown in blue.

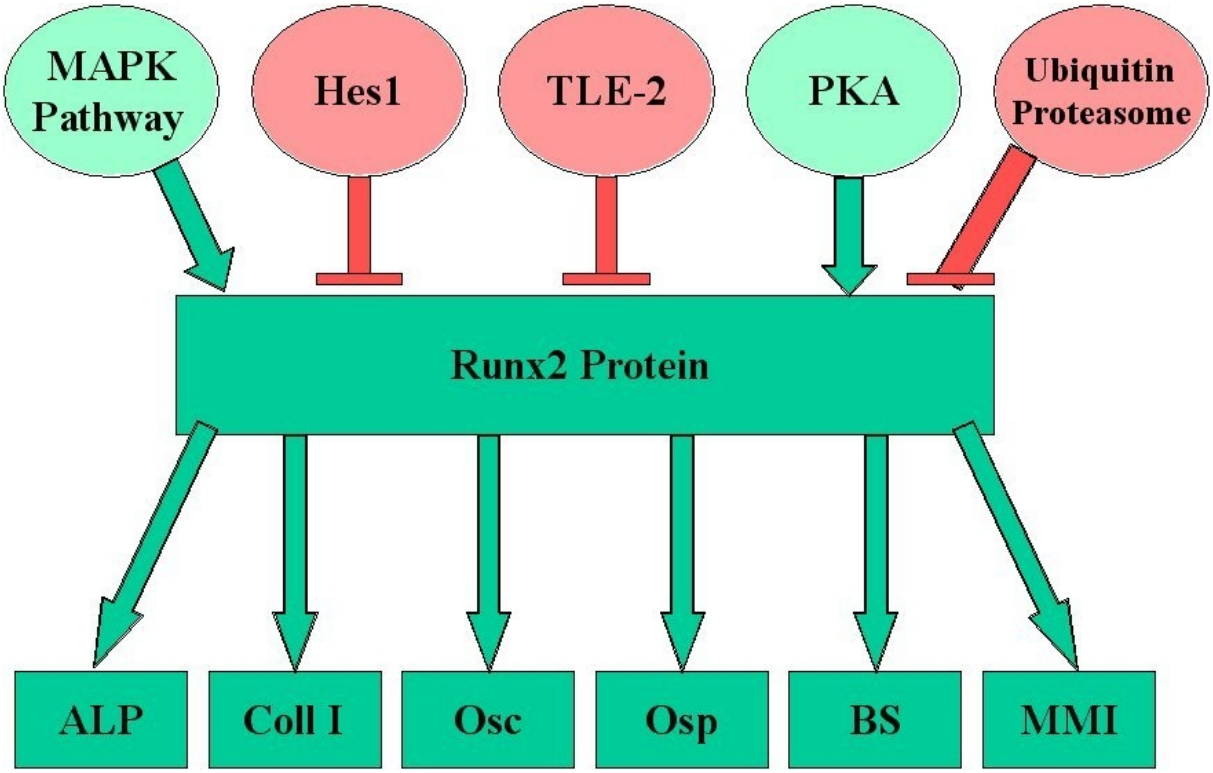

Figure 2. The actions and regulation of the Runx2 transcript. Transcription factors that have an up-regulatory action are shown in green. Transcription factors that have an inhibitory action are shown in red with truncated arrows. Gene abbreviation stand for the following: Alkaline Phosphatase (ALP), Collagen I (Coll I), Osteocalcin (OCN), Osteopontin (OPN), Bone Sialoprotein (BSP) and matrix metalloproteinase I (MMPI).

The mechanisms responsible for the regulation of the Runx2 gene itself have only recently begun to be understood. It was previously assumed that Runx2 regulation of osteogenic genes was mediated solely by levels of Runx2 protein, and therefore mRNA levels. This assumption was based on data gathered from non-human (usually mouse) models. Recent studies have shown that levels of Runx2 during *in vitro* differentiation of primary human osteoblasts showed no major changes, whereas levels of downstream genes such as bone sialoprotein and alkaline phosphatase were dramatically increased (Xiao, *et al*., 1998). The data indicated that Runx2 mRNA levels were constitutively expressed, with a distinct lack of correlation between Runx2 mRNA/protein levels and the acquisition of the osteoblast phenotype. Furthermore, in a recent study by Shui *et al* (Shui, *et al*., 2003) real time PCR and western blot analyses indicated that there was no significant increase in the levels of Cbfa1 protein or mRNA during human osteoblast differentiation. This contradicts data obtained from rodent samples, indicating that human Runx2 may be regulated at multiple levels, including changes

in mRNA and protein levels (Banerjee, *et al*., 2001, Prince, *et al*., 2001, Sudhakar, *et al*., 2001). Immonoprecipitation experiments indicated a high degree of Runx2 protein phosphorylation as osteoblastic differentiation continued. The binding of the Runx2 protein to Runx regulatory elements was monitored using electrophoretic mobility shift assays (EMSA), binding efficiency increased with the onset of differentiation, suggesting the activity of the Runx2 transcript be involved in the regulation of osteogenic genes (see figure 3).

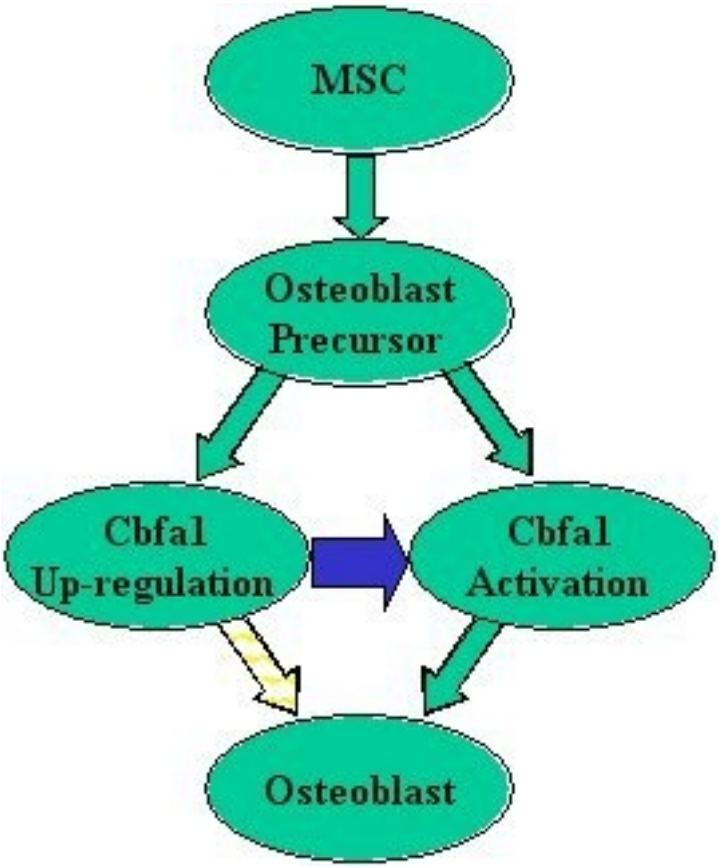

Figure 3. A proposed model of Cbfa1 based regulation of osteoblast differentiation. The actions of the gene may be mediated by either up-regulation of protein levels or activation of the Runx2 transcript, or a combination of the two mechanisms.

Osteoblast cells respond to and differentiate as a consequence of two main factors: chemical signals and physical stress application, activating specific signalling pathways. One such pathway is the MEK extra-cellular regulated kinase (MEK/ERK) branch of the mitogen-activated protein kinase (MAPK) pathway, this provides a link between mechanosensitive cell

surface integrin interactions with the ECM and activation of Runx2 (Xiao, *et al*., 2002). This was confirmed by U0126 (inhibitor) inactivation of the integrin mediated MAPK pathway, selectively blocking the extracellular matrix (ECM) mediated up-regulation of osteocalcin. Furthermore, over activity of the MAPK pathway, by the transfection of constitutively active MAPK genes, significantly increased the levels of Runx2 phosphorylation and ostreocalcin mRNA (Xiao, *et al*., 1998, Xiao, *et al*., 2002). This data is further reinforced by the actions of MAPK in adipogenic fibroblasts, *in vivo* endogenous Runx2 was phosphorylated and ultimately potentiated by the actions of the pathway. Protein kinase A (PKA) is another signalling system implicated in the regulation of Runx2 activity.

Osterix

Osterix (Osx) is one of the few characterized osteoblast specific genes. The gene was first identified within murine models, and later the human homologue Sp7 in a variety of bone cell types (fetal and carniofacial cells, and in cell lines (MG63 and HOS) (although not in adult femoral cells (Milona, *et al*., 2003)). Osx -/- mice die at birth, due to the inability of their rib cage to sustain breathing. These same mice also demonstrated the presence of an intact and perfectly patterned skeleton, although this was composed entirely of cartilage (Nakashima, *et al*., 2002, Otto, *et al*., 1997, Komori, *et al*., 1997). This clearly indicates that the presence of Osx is vital to the development of the mature skeleton within murine fetal development.

Osx is thought to act in the regulation of numerous osteoblast genes that including: osteocalcin, osteonectin, osteopontin, bone sialoprotein and collagen type I (see figure 4) (Ducy, *et al*., 1996, Karsenty, 2000). The gene may also act in conjunction with other known regulators, such as; pNmp4 (Torrungruang, *et al*., 2002), Aj18 (Jheon, *et al*., 2002, Jheon, *et al*., 2001) Runx2, and cox-2 (Zhang, *et al*., 2002), although any such interactions remain undefined. Removal of the Osx gene from mouse models results is a complete lack of ossification; the mice also lack the presence of osteoblasts although they do have partially differentiated mesenchymal stem cells (MSCs) (Nakashima, *et al*., 2002, Huang, *et al*., 2004). Interestingly the levels of Runx2 expression within the Osx -/- mice remain unaffected by the genes absence, indicating that Osx may act downstream or independently of Runx2 (Harada, *et al*., 1999, Nakashima, *et al*., 2002, Komori, *et al*., 1997). Furthermore, the removal of Runx2 (but with BMP-2 treatment) has no affect on the levels of Osx expression (Lee, *et al*.,

2003), this provides further indications that Osx acts independently of Cbfa1. It should be noted that although Osx/Sp7 is widely thought to be osteoblast specific in nature, its expression has been detected within early chondrocyte cells. However, this seems to be of little consequence as the expression is weak and transient, and the removal of the Osx gene incur no obvious cartilagonous defect (Ducy, *et al*., 1997, Nakashima, *et al*., 2002, Takeda, *et al*., 2001).

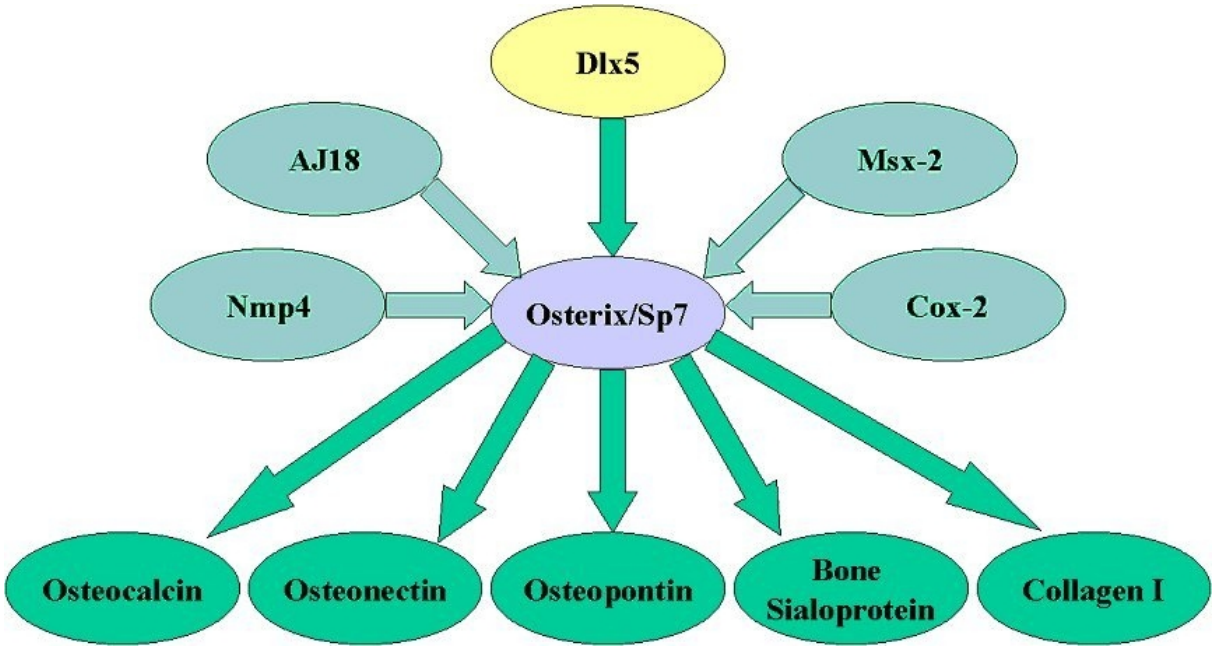

Figure 4. The regulation and actions of osterix. Genes responsible for osterix/Sp7 regulation are shown in light green. Genes thought to be up-regulated by the osterix protein are shown in lightly dark green. Osterix has been indicated in the regulation of the listed genes, although it has not yet been determined if these are due to direct regulation as apposed to indirect mechanisms.

Osx is a comparatively new gene to be identified within the osteoblast phenotype and as a consequence very little is known about the control mechanisms that govern its expression. Early experiments seem to indicate the gene Dlx5 in the control of Osx, as its inactivation (by antisense blocking) completely abrogated the expression of Osx (Lee, *et al*., 2003). Dlx5 must therefore play a key role in the regulation of Osx expression within differentiating

osteoblastic cells. The chemical and mechanical regulatory mechanisms that control Osx are not currently fully understood.

Dlx5

It should be mentioned at the outset that distal-less homeobox protein 5 (Dlx5) is not osteoblast specific, although it is thought to play a key role in the differentiation and maturation of the osteoblast phenotype, having been observed in tissues that undergo both intramenbranous and endochondral ossification (Simeone, *et al*., 1994, Zhao, *et al*., 1994, Chen, *et al*., 1996, Davideau, *et al*., 1999, Ferrari, *et al*., 1995, Davideau, *et al*., 1999). The function of the gene and its affects in terms of osteoblastic gene promotion are highly ambiguous in nature, with numerous studies providing contradictory data. In the first instance Dlx5 may not act alone, it may work in conjunction with other transcription factors such as Dlx6 and Msx-2 (Shirakabe, *et al*., 2001, Ducy, 2000). In the case of Msx-2 the Dlx5 transcript may act as an indirect negative regulator, down-regulating the Msx-2 transcript as the osteoblasts cease to proliferate (Ryoo, *et al*., 1997).

The levels of Dlx5 expression seem to increase with the progression of the osteoblast phenotype. Low levels of the transcript are observed in the early differentiation (skull clavarial cells), higher levels develop after the osteoblasts reach confluency (may provide further evidence for Dlx5 regulation of Msx-2), the levels of transcription then increase steadily over time (Ryoo, *et al*., 1997). Forced expression of Dlx5 leads to an increased rate of mineralised matrix production in culture (Lee, *et al*., 2003), but the effect of the protein on other osteoblast genes (such as osteocalcin) is highly contradictory. Studies have indicated; an activation of osteocalcin gene expression (Lee, *et al*., 2003, Miyama, *et al*., 1999), a repression of expression (Ryoo, *et al*., 1997), or little effect on the levels of osteocalcin (Newberry, *et al*., 1998). The role of mechanical forces in the regulation of Dlx5 remains unknown.

Alkaline Phosphatase

Alkaline phosphatase (ALP) is a ubiquitous cellular protein and consequently cannot be considered bone specific. The function of ALP is not well defined, acting within a prolifery of different cells types. Numerous studies have indicated that ALP may act as an early indicator of cellular activity and differentiation. The protein levels have also been shown to be up-

regulated in response to mechanical force application. Levels of ALP mRNA have been shown to increase as little as 2 days post stimulation with steady increases with the progression of osteoblastic differentiation (up to 14 days) (Shui, *et al*., 2003, Qi, *et al*., 2003). Various types of force and the *in vitro* method of application have also been shown to illicit this response (Shui, *et al*., 2003, Qi, *et al*., 2003, Roelofsen, *et al*., 1995, D'Addario, *et al*., 2003, Jagodzinski, *et al*., 2004, Yoshikawa, *et al*., 1997, Klein-Nulend, *et al*., 1997, Pavlin, *et al*., 2000, Pavlin and Gluhak-Heinrich, 2001, Pavlin, *et al*., 2001, Yuge, *et al*., 2003)

Msx-2

Msx-2 (Hox-8) is another transcription factor that cannot be considered bone specific, although recent studies have indicated the role of Msx-2 in the differentiation and regulation of the osteoblast phenotype. In murine models removal of the Msx-2 gene enduces a significant down-regulation of both Cbfa1 and osteocalcin, bone growth is delayed and the overall bone mass of the mouse decreases (Ducy, *et al*., 2000). A separate study indicated that Msx-2 does indeed have a positive affect on osteocalcin transcription (Hoffmann, *et al*., 1996), binding to the Oc-box homeodomain motif of the osteocalcin gene (Ryoo, *et al*., 1997). The gene may also play a role in the regulation of bone sialoprotein (Barnes, *et al*., 2003). In a recent study by Chen *et al* (Chen, *et al*., 2003) myofibroblasts (which are capable of undergoing osteoblastic differentiation under certain conditions) were transfected with a virus encoding high levels of Msx-2. The levels of osterix expression increased 10-fold, ALP transcription levels concomitantly increased 50-fold, and mineralised nodule formation was also increased by a factor of 30. The decreased levels of adipocyte markers does not seem to involve Msx-2 binding, rather it seem in part to occur due to protein-protein interaction with C/EBPδ (Davideau, *et al*., 1999). The mode of action by which Msx-2 elicits this response is not fully understood. The gene may act through osterix (see osterix section) or may act independently. Due to the comparatively recent indication of the role of Msx-2 in osteoblast differentiation, the exact regulatory systems (including mechanical stimulation) that determine Msx-2 function are not currently known.

NF-κB

Nuclear factor of kappa light polypeptide gene enhancer in B-cells (NF-κB) is a member of the Rel family which control the expression of numerous genes involved in; immune and

inflammatory responses, cell adhesion, and cell growth (Kurokouchi, *et al*., 2001). The gene is a down-stream promoter of osteoclastogenesis and may be involved in the activation of later osteoclast genes such as tartate resistant acid phosphatase, and carbonic anhydrase II. The NF-κB gene has been shown to be activated by many extra-cellular simuli including; viral infection, bacterial products, oxidative stress and physical stress (Kurokouchi, *et al*., 2001). Physical stress has been shown to inhibit the actions of the NF-κB gene, most likely by regulation of protein binding efficiency. The gene is down-regulated during osteoblastic differentiation as a result of the Smad regulatory pathway (see figure 3) (Qi, *et al*., 2003), therefore providing a further indicator of osteoblastic differentiation.

Osteoprotegerin

Osteoprotegerin (OPG) is a member of the TNF super-family and is an excretory protein produced by the osteoblast phenotype. The protein acts as a soluble decoy receptor by binding with RANKL and thus preventing the subsequent activation of NF-κB and JNK (Lian and Stein, 2003), blocking osteoclastic bone resorption (Rubin, *et al*., 2002). The protein seems to be produced by developing osteoblasts, arresting bone resorption and therefore enhancing the synthesis bone matrix.

c-fos

C-fos is expressed during vertebrate growth, differentiation and development and is one of the earliest mechano-sensitive genes within the osteoblast phenotype (Moalli, *et al*., 2000). Studies within developing foetuses have shown that c-fos expression *in vivo* is associated with areas of foetal bone that have the highest growth potential during embryonic bone formation and fracture healing. Murine models have further reinforced these findings, with the development of bone tumors within c-fos transgenic mice (Closs, *et al*., 1990) and a block in osteoblastic and osteoclast differentiation within mutant animals (Grigoriadis, *et al*., 1994). Clearly c-fos plays a key role in the development and maintenance of human bone tissue. The gene encoding the c-fos protein has been shown to be induced by numerous pathways including; tyrosine kinase, p21 ras, MAPK, PKA, cAMPs, and possibly PKC (Fitzgerald and Hughes-Fulford, 1999). For example PKA regulation of c-fos and c-jun (a similar transcription factor) has been shown to act via the up-regulation of cAMP response element binding protein (CREBP) (Franceschi and Xiao, 2003).

It should be noted however, that the gene is not osteoblast specific with induction in response to load observed in numerous cell types. These include: vascular smooth muscle (Li and Xu, 2000), cardiac myocytes (Komuro, *et al*., 1990, Komuro, *et al*., 1990, Sadoshima, *et al*., 1992), endothelial cells (Ranjan and Diamond, 1993, Hsieh, *et al*., 1993). The pattern of response between the various cell types is conserved and has been observed in response to: shear stress (Pavalko, *et al*., 1998), tension (Kawata and Mikuni-Takagaki, 1998), gravitational loading (Fitzgerald and Hughes-Fulford, 1996), vibration (Tjandrawinata, *et al*., 1997), and hypertonic swelling (Sadoshima, *et al*., 1996). The response of the gene is rapid and large, with transcriptional responses typically observed within 30-60 minutes post-stimulation (Moalli, *et al*., 2000, Kawata and Mikuni-Takagaki, 1998, Lean, *et al*., 1996), both *in vitro* (Glanstchnig, *et al*., 1996) and *in vivo* (Lean, *et al*., 1996). The response is also short, with levels of expression returning to basal levels within 12 hours post stimulation (Moalli, *et al*., 2000, Kawata and Mikuni-Takagaki, 1998). The long term response of c-fos not full understood, one study by Moalli *et al* (Moalli, *et al*., 2000) has indicated the response of the c-fos gene may be biphasic, with a further up-regulation at 24 hours post stimulation.

The mechanisms of action of the c-fos protein on various major osteoblast genes are not fully understood, however it is thought to act in conjunction with numerous other promotion factors (e.g. c-jun) ultimately acting in conjunction with the AP-1 complex. This complex is a multifaceted transcription factor that plays a regulatory role within numerous gene regulatory systems. Its role within the osteoblast phenotype and its interactions with c-fos in the regulation of osteoblastic genes remains undefined. AP-1 binding sites have been detected in a number of osteoblast gene promoters including: osteocalcin (Goldberg, *et al*., 1996, Aslam, *et al*., 1999, Owen, *et al*., 1990), osteopontin (Bidder, *et al*., 2002) alkaline phosphatase (Owen, *et al*., 1990, Matsuura, *et al*., 1990), matrix metalloproteinase I and bone sialoprotein (Sodek, *et al*., 1996).

Mechanical activation of the c-fos promoter requires the activation of multiple intra-cellular signalling pathways. Experiments involving cell types other than bone and also using chemical stimulation of osteoblasts (Bowler, *et al*., 1999, Fitzgerald, *et al*., 2000) indicate that multiple c-fos response element are involved in the transcriptional regulation of c-fos.

Cox-2

Cytochrome c oxidase subunit (Cox) 1 and 2 encode cyclooxygenases and are known to play a rate limited step in the control of prostaglandin production. The exact role of cox-2 in the regulation of bone formation is not fully understood but seems to affect both the osteoblasts and osteoclast lineages. Cox-2 negative mice have demonstrated a decrease in bone density (Okada, *et al*., 2000), as well as significantly reduced levels of Cbfa1 and osterix (Zhang, *et al*., 2002). Addition of BMP-2 increased levels of Cbfa1 and osterix in both cox-2 -/- and wild type cultures, PGE2 has a similar affect (Zhang, *et al*., 2002). Furthermore studies have shown that increased laminar bone formation in response to strain may be mediated by cox-2 (Forwood, 1996, Duncan and Turner, 1995). Other mice models have also shown decreased levels of bone resorption in response to parathyroid hormone or 1.25-hydroxyl vitamin D (Okada, *et al*., 2000), and in an *in vitro* mouse clavaria models the gene has been indicated as a candidate for the control of wear induced osteoclastogenesis and osteolysis (Zhang, *et al*., 2001).

Clearly cox-2 plays a key role in the regulation of bone formation and resorption. The evidence also suggests that this may not be limited to the osteoblast phenotype and that the gene does not control solely bone deposition. The gene seems to play a role in osteogenisis be considered as a candidate in any assessment of very short-term osteogenic differentiation. Studies in various cell types have demonstrated an increase in the mRNA levels of cox-2 in response to mechanical force application. Fluid flow has been shown to induce increased levels of expression with a peak in expression at varying time point post stimulation. For example, peak levels of Cox-2 expression where observed in: primary murine osteoblasts 60 minutes after force application by pulsed fluid flow (PFF) (Bakker, *et al*., 2003), MC3T3-E1 cells 4-5 hours after 10 and 12dynes/cm^2 of fluid flow (Wadhwa, *et al*., 2002), and MC3T3-E1 cells 30 minutes after 10dynes/cm^2.

FGF2

It should be noted at the outset that basic fibroblast growth factor 2 (FGF2) is a not a bone specific transcription factor, having been detected in numerous cell types (Carreras, *et al*., 2001). However, an increase in the levels of expression of FGF2 has been shown to cause premature mineralisation and shortening of the long bones, while a decrease in transcription levels encured a significant decrease in bone mass and formation (Coffin, *et al*., 1995,

Montero, *et al*., 2000). The exact mechanisms of action associated with FGF2 are not fully understood although several key osteoblastic genes have been indicated. For example, activating mutations of the FGF2 gene can cause an up-regulation of Cbfa1 (Zhou, *et al*., 2000). Increased levels of osteocalcin can also be stimulated by the actions of FGF2 in preosteoblast cells, as well as treatment with the FGF2 protein (as mentioned in the Cbfa1 section of this review) (Boudreaux and Towler, 1996). Other osteoblastic genes known to be stimulated by the action of FGF2 include bone sialoprotein and matrix MMPI (Shimizu-Sasaki, *et al*., 2001, Newberry, *et al*., 1997), although it is not known if this response is mediated by the Cbfa1 gene.

Bapx1

Homo sapiens bagpipe homeobox homolog 1 (*Drosophila*) (Bapx1) is the human homologue of the *Drosophila, bagpipe* homeobox gene. This transcription factor is a member of the *NK* homeobox superfamily, detected in higher vertabrate organisms. Lower organisms such as phyla *cnidaria* and *platyhelmintes* show no evidence of Bapx1 (Tribioli, *et al*., 1997). Loss of the gene in murine models has been shown to cause a down-regulation of both Cbfa1 and osteocalcin. The loss of the Bapx1 gene also affects the distribution of sclerotomal cells, intervertebral disks and centra of the vertebral bodies fail to form, as well as abnormalities in the basal skull (Tribioli, *et al*., 1997).

Matrix Proteins

Collagen I

Collagen I is an important component of bone extra-cellular matrix, forming connections with cell surface integrins and other ECM proteins. The protein cannot be considered bone specific having been identified in numerous unrelated cell types. The protein has been shown to play a role in cell adhesion, proliferation and differentiation of the osteoblast phenotype. Up-regulation of the collagen I gene has been observed in response to a number of different methods of *in vitro* force application (Jagodzinski, *et al*., 2004, Klein-Nulend, *et al*., 1997), with mRNA levels increasing as little as 2 days post stimulation (Pavlin and Gluhak-Heinrich, 2001, Pavlin, *et al*., 2001). The protein would therefore seem to play a key role within the osteoblast phenotype and can be considered as an early indicator of osteoblastic differentiation. A related protein known as collagen III is also produced by developing

osteoblasts. The protein has shown increases in its levels of expression as little as 4 to 7 days post mechanical stimulation.

Osteocalcin

Osteocalcin is one of the few osteoblast specific genes (Lian, *et al*., 1989) and is one of the most abundant proteins present in bone, second only to collagen type I (Lian and Stein, 2003). It is thought to play an important role in the differentiation of osteoblast progenitor cells, with significant up-regulation observed in both matrix synthesis and mineralisation (Ryoo, *et al*., 1997). The gene seems to be regulated by numerous intracellular factors, having numerous receptor binding sites specific for many different hormones. Two main OSE promoter regions have been identified, OSE 1 and 2. The most understood of these two promoter regions is OSE2, the promoter to which the Cbfa1 protein binds and incurs the gene's up-regulation (figure 5). OSE1 activity may also be required for OSE2 promoter activity (Schinke and Karsenty, 1999). Blocking of α2-integrin-ECM interactions has been shown to block ascorbic acid dependent OSE2 activation, indicating that integrin interactions may play a key role in the regulation of the osteocalcin gene.

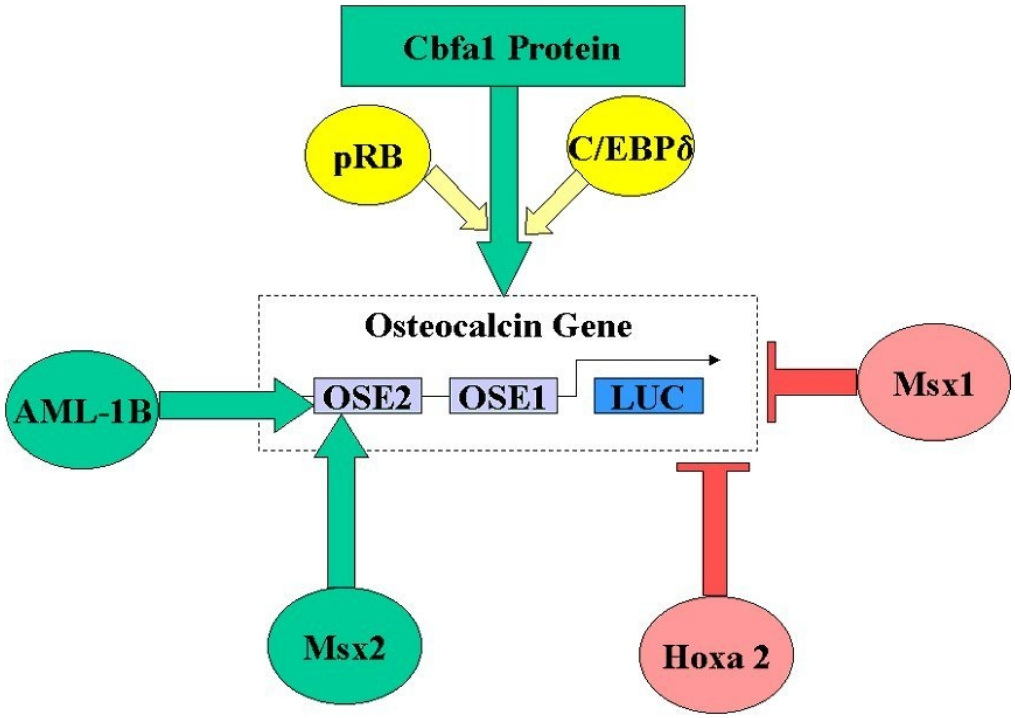

Figure 5. The regulation of the osteocalcin gene. Transcription factors that have an up-regulatory action are shown in green. Transcription factors that have an inhibitory action are shown in red with truncated arrows. Binding partner proteins are shown in yellow.

Numerous extra-cellular factors have been identified that regulate the expression of the osteocalcin protein. Studies have also indicated the role of mechanical force in the regulation of osteocalcin mRNA levels. Up-regulation of the genes has been observed as little as 1-2 days post force application (Pavlin and Gluhak-Heinrich, 2001), with steady increases as osteoblastic differentiation progresses (Shui, *et al*., 2003, Jagodzinski, *et al*., 2004). Based on established data, the regulation of osteocalcin by mechanical force is most likely mediated by the action of the Cbfa1 protein.

Osteopontin

Osteopontin is a secreted adhesive glycophosphoprotein that has been detected within; bone, teeth, kidneys, epithelial lining tissues, blood plasma, and breast milk. As a consequence osteopontin cannot be considered bone specific, although it does perform important bone related functions. Within bone tissue it plays key functions in cell; adhesion, migration and survival. Malfunctions within the gene encoding osteopontin can lead to tumorigenisis (Standal, *et al*., 2004, Standal, *et al*., 2004).

It has also been established that the gene encoding the osteopontin protein responds to mechanical forces. Numerous studies have demonstrated the up-regulation of the gene after the application of *in vitro* artificial mechanical forces. This significant increase in mRNA levels has been observed as little as 9-24 hours post stimulation (You, *et al*., 2001, Toma, *et al*., 1997). However, the mechanotransductive mechanisms involved in the force based up-regulation of osteopontin are not fully understood. The signaling pathways that have been indicated thus far include tyrosine kinase (s) and protein kinase A. It has also been postulated that these pathways may initially be activated by cell surface integrins, this may in tern activate the focal adhesion kinase (FAK) (Schaller and Parsons, 1994, Wang, *et al*., 1993). The subsequent phophorylation cascade includes paxillin and tensin (Schaller and Parsons, 1994).

Bone Sialoprotein

Bone sialoprotein (BSP) is a highly sulfated, phosphorylated, and glycosylated protein present within bone matrix. The protein is characterised by its ability to bind to hydroxyapetite through polyglutamic acid sequences and to mediate cell attachment through an RGD sequence (Oldberg, *et al*., 1988, Ogata, *et al*., 1995, Ganss, *et al*., 1999). The presence of BSP

within the extracellular matrix and its ability to nucleate hydroxyapatite crystal formation, indicates a potential role of the protein in the early mineralization of osteoblasts (Hunter and Goldberg, 1993). In addition, it has been reported that BSP is mitogenic for pre-osteoblast cells and can promote their differentiation into mature osteoblasts, ultimately stimulating bone mineralization (Zhou, *et al*., 1995).

As with other osteoblast genes BSP is regulated by both chemical and physical cues. Levels of BSP mRNA and protein have been shown to be significantly upregulated in response to mechanical load *in vitro* in Saos-2 (Mitsui, *et al*., 2005), and *in vitro* mouse models (Sasaguri, *et al*., 1998). Investigations have indicated that BSP expression is upregulated within 1-3 days post force application.

Other Genes

The genes mentioned thus far form only part of the complex regulatory systems involved in osteoblast differentiation. Although this process remains largely undefined there are numerous other genes involved in the control process. LIM mineralised protein 1 (LMP-1) is an essential positive regulator of osteoblast differentiation and when over-expressed is highly osteoinductive (Liu, *et al*., 2002). The LMP-1 protein mediates BMP-6 induction of bone nodule formation (Boden, *et al*., 1998). Another important osteoblast regulatory gene is erythroblastosis virus E26 oncogene homologue 1 (avian) (Ets1). This transcription factor activates the expression of alkaline phosphatase and osteopontin, it can also up-regulate PthR1 which plays an important role in the control of osteoblast intracellular calcium levels (Qi, *et al*., 2003) (Figure 6). This action may in part be achieved by the ability of Ets1 to bind with Cbfa1, affecting the resulting down-stream pathways for the previously mentioned genes. If this is the case the gene may also have an indirect regulatory role on numerous other osteoblastic genes.

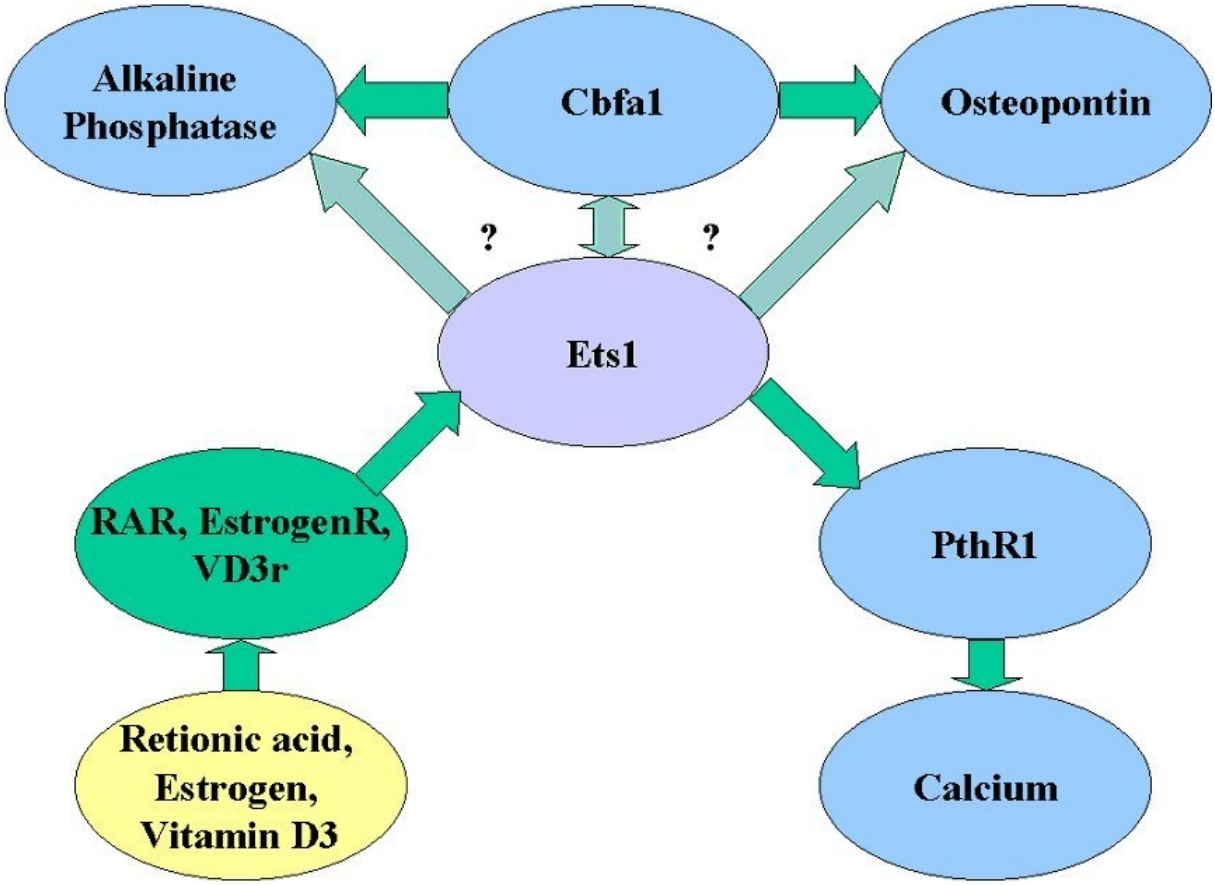

Figure 6. Ets regulation of osteoblastic genes. Transcription factors that have an up-regulatory action are shown in green. Genes that are/may be regulated by Ets1 are shown in circles, light green arrows represent possible regulatory events.

The actions of the Cbfa1 gene have been previously discussed, but the function of Cbfa2 and 3, which are closely related to the Cbfa1 protein, are an important consideration in any review of early osteoblastic genes. Cbfa2 (Runx1/AML1/PEBP2αB) is essential for definitive hematopoietic differentiation, but does correlate with the early stages of chondrocyte/osteoblast differentiation . Cbfa3 (Runx3/AML2/PEBP2αC) is necessary for nerve development and is a tumor suppressor gene in gastric cancer. The gene is also expressed in pre-hypertrophic and hypertrophic chondrocytes (Lian and Stein, 2003) and has been detected osteogenic bone regions (Yamashiro, *et al*., 2002). It should also be noted that the expression of Cbfa1 and 2 seems to overlap in initial skeletal element formation, later Cbfa1 increases in osteogenic cells.

Fra1 may also play an important role in osteoblast differentiation, its over-expression significantly increases bone mass but has no affect on Cbfa1 expression indicating it may be a

down-stream factor or act independently (Zambotti, *et al*., 2002). The mechanical and interferon induced expression of Best 5 has also been observed approximately 28 days after initial stimulation (Grewal, *et al*., 2000). Matrix metalloproteinase I (MMPI) (collagenase 3) may also play a role in differentiating osteoblasts. The gene has been shown to have Cbfa1 and AP1 binding sites (Selvamurugan, *et al*., 1998, D'Alonzo, *et al*., 2002, Hess, *et al*., 2001) and mutations abrogated c-fos/c-jun respectively (Franceschi and Xiao, 2003).

References

1 Mundlos, S., Otto, F., Mundlos, C., Mulliken, J. B., Aylsworth, A. S., Albright, S., Lindhout, D., Cole, W. G., Henn, W., Knoll, J. H., Owen, M. J., Mertelsmann, R., Zabel, B. U. and Olsen, B. R.Cell 1997; 89:773-9

2 Pratap, J., Galindo, M., Zaidi, S. K., Vradii, D., Bhat, B. M., Robinson, J. A., Choi, J. Y., Komori, T., Stein, J. L., Lian, J. B., Stein, G. S. and van Wijnen, A. J.Cancer Res 2003; 63:5357-62

3 Lee, K. S., Kim, H. J., Li, Q. L., Chi, X. Z., Ueta, C., Komori, T., Wozney, J. M., Kim, E. G., Choi, J. Y., Ryoo, H. M. and Bae, S. C.Mol Cell Biol 2000; 20:8783-92

4 Lee, M. H., Javed, A., Kim, H. J., Shin, H. I., Gutierrez, S., Choi, J. Y., Rosen, V., Stein, J. L., van Wijnen, A. J., Stein, G. S., Lian, J. B. and Ryoo, H. M.J Cell Biochem 1999; 73:114-25

5 Gori, F., Thomas, T., Hicok, K. C., Spelsberg, T. C. and Riggs, B. L.J Bone Miner Res 1999; 14:1522-35

6 Perinpanayagam, H., Schneider, G., Holtman, K., Zaharias, R. and Stanford, C.J Orthop Res 2004; 22:404-10

7 Barnes, G. L., Javed, A., Waller, S. M., Kamal, M. H., Hebert, K. E., Hassan, M. Q., Bellahcene, A., Van Wijnen, A. J., Young, M. F., Lian, J. B., Stein, G. S. and Gerstenfeld, L. C.Cancer Res 2003; 63:2631-7

8 Brubaker, K. D., Vessella, R. L., Brown, L. G. and Corey, E.Prostate 2003; 56:13-22

9 Lian, J. B. and Stein, G. S.Curr Pharm Des 2003; 9:2677-85

10 Kahler, R. A. and Westendorf, J. J.J Biol Chem 2003; 278:11937-44

11 Westendorf, J. J. and Hiebert, S. W.J Cell Biochem 1999; Suppl 32-33:51-8

12 Yoshida, C. A., Furuichi, T., Fujita, T., Fukuyama, R., Kanatani, N., Kobayashi, S., Satake, M., Takada, K. and Komori, T.Nat Genet 2002; 32:633-8

13 Kundu, M., Javed, A., Jeon, J. P., Horner, A., Shum, L., Eckhaus, M., Muenke, M., Lian, J. B., Yang, Y., Nuckolls, G. H., Stein, G. S. and Liu, P. P.Nat Genet 2002; 32:639-44

14 Miller, J., Horner, A., Stacy, T., Lowrey, C., Lian, J. B., Stein, G., Nuckolls, G. H. and Speck, N. A.Nat Genet 2002; 32:645-9

15 Ducy, P., Zhang, R., Geoffroy, V., Ridall, A. L. and Karsenty, G.Cell 1997; 89:747-54

16 Selvamurugan, N., Chou, W. Y., Pearman, A. T., Pulumati, M. R. and Partridge, N. C.J Biol Chem 1998; 273:10647-57

17 Kern, B., Shen, J., Starbuck, M. and Karsenty, G.J Biol Chem 2001; 276:7101-7

18 Harada, H., Tagashira, S., Fujiwara, M., Ogawa, S., Katsumata, T., Yamaguchi, A., Komori, T. and Nakatsuka, M.J Biol Chem 1999; 274:6972-8

19 Shimizu-Sasaki, E., Yamazaki, M., Furuyama, S., Sugiya, H., Sodek, J. and Ogata, Y.J Biol Chem 2001; 276:5459-66

20 Newberry, E. P., Willis, D., Latifi, T., Boudreaux, J. M. and Towler, D. A.Mol Endocrinol 1997; 11:1129-44
21 Otto, F., Lubbert, M. and Stock, M.J Cell Biochem 2003; 89:9-18
22 Xiao, G., Wang, D., Benson, M. D., Karsenty, G. and Franceschi, R. T.J Biol Chem 1998; 273:32988-94
23 Shui, C., Spelsberg, T. C., Riggs, B. L. and Khosla, S.J Bone Miner Res 2003; 18:213-21
24 Banerjee, C., Javed, A., Choi, J. Y., Green, J., Rosen, V., van Wijnen, A. J., Stein, J. L., Lian, J. B. and Stein, G. S.Endocrinology 2001; 142:4026-39
25 Prince, M., Banerjee, C., Javed, A., Green, J., Lian, J. B., Stein, G. S., Bodine, P. V. and Komm, B. S.J Cell Biochem 2001; 80:424-40
26 Sudhakar, S., Li, Y., Katz, M. S. and Elango, N.Biochem Biophys Res Commun 2001; 289:616-22
27 Xiao, G., Gopalakrishnan, R., Jiang, D., Reith, E., Benson, M. D. and Franceschi, R. T.J Bone Miner Res 2002; 17:101-10
28 Milona, M. A., Gough, J. E. and Edgar, A. J.BMC Genomics 2003; 4:43
29 Nakashima, K., Zhou, X., Kunkel, G., Zhang, Z., Deng, J. M., Behringer, R. R. and de Crombrugghe, B.Cell 2002; 108:17-29
30 Otto, F., Thornell, A. P., Crompton, T., Denzel, A., Gilmour, K. C., Rosewell, I. R., Stamp, G. W., Beddington, R. S., Mundlos, S., Olsen, B. R., Selby, P. B. and Owen, M. J.Cell 1997; 89:765-71
31 Komori, T., Yagi, H., Nomura, S., Yamaguchi, A., Sasaki, K., Deguchi, K., Shimizu, Y., Bronson, R. T., Gao, Y. H., Inada, M., Sato, M., Okamoto, R., Kitamura, Y., Yoshiki, S. and Kishimoto, T.Cell 1997; 89:755-64
32 Ducy, P., Desbois, C., Boyce, B., Pinero, G., Story, B., Dunstan, C., Smith, E., Bonadio, J., Goldstein, S., Gundberg, C., Bradley, A. and Karsenty, G.Nature 1996; 382:448-52
33 Karsenty, G.Matrix Biol 2000; 19:85-9
34 Torrungruang, K., Shah, R., Alvarez, M., Bowen, D. K., Gerard, R., Pavalko, F. M., Elmendorf, J. S., Charoonpatrapong, K., Hock, J., Rhodes, S. J. and Bidwell, J. P.Bone 2002; 30:931-6
35 Jheon, A., Chen, J., Teo, W., Ganss, B., Sodek, J. and Cheifetz, S.J Histochem Cytochem 2002; 50:973-82
36 Jheon, A. H., Ganss, B., Cheifetz, S. and Sodek, J.J Biol Chem 2001; 276:18282-9
37 Zhang, X., Schwarz, E. M., Young, D. A., Puzas, J. E., Rosier, R. N. and O'Keefe, R. J.J Clin Invest 2002; 109:1405-15
38 Huang, L., Teng, X. Y., Cheng, Y. Y., Lee, K. M. and Kumta, S. M.Bone 2004; 34:393-401
39 Lee, M. H., Kwon, T. G., Park, H. S., Wozney, J. M. and Ryoo, H. M.Biochem Biophys Res Commun 2003; 309:689-94
40 Takeda, S., Bonnamy, J. P., Owen, M. J., Ducy, P. and Karsenty, G.Genes Dev 2001; 15:467-81
41 Simeone, A., Acampora, D., Pannese, M., D'Esposito, M., Stornaiuolo, A., Gulisano, M., Mallamaci, A., Kastury, K., Druck, T., Huebner, K. and et al.Proc Natl Acad Sci U S A 1994; 91:2250-4
42 Zhao, G. Q., Zhao, S., Zhou, X., Eberspaecher, H., Solursh, M. and de Crombrugghe, B.Dev Biol 1994; 164:37-51
43 Chen, X., Li, X., Wang, W. and Lufkin, T.Ann N Y Acad Sci 1996; 785:38-47
44 Davideau, J. L., Demri, P., Gu, T. T., Simmons, D., Nessman, C., Forest, N., MacDougall, M. and Berdal, A.Mech Dev 1999; 81:183-6

45 Ferrari, D., Sumoy, L., Gannon, J., Sun, H., Brown, A. M., Upholt, W. B. and Kosher, R. A.Mech Dev 1995; 52:257-64
46 Davideau, J. L., Demri, P., Hotton, D., Gu, T. T., MacDougall, M., Sharpe, P., Forest, N. and Berdal, A.Pediatr Res 1999; 46:650-6
47 Shirakabe, K., Terasawa, K., Miyama, K., Shibuya, H. and Nishida, E.Genes Cells 2001; 6:851-6
48 Ducy, P.Dev Dyn 2000; 219:461-71
49 Ryoo, H. M., Hoffmann, H. M., Beumer, T., Frenkel, B., Towler, D. A., Stein, G. S., Stein, J. L., van Wijnen, A. J. and Lian, J. B.Mol Endocrinol 1997; 11:1681-94
50 Miyama, K., Yamada, G., Yamamoto, T. S., Takagi, C., Miyado, K., Sakai, M., Ueno, N. and Shibuya, H.Dev Biol 1999; 208:123-33
51 Newberry, E. P., Latifi, T. and Towler, D. A.Biochemistry 1998; 37:16360-8
52 Qi, H., Aguiar, D. J., Williams, S. M., La Pean, A., Pan, W. and Verfaillie, C. M.Proc Natl Acad Sci U S A 2003; 100:3305-10
53 Roelofsen, J., Klein-Nulend, J. and Burger, E. H.J Biomech 1995; 28:1493-503
54 D'Addario, M., Arora, P. D., Ellen, R. P. and McCulloch, C. A.J Biol Chem 2003;
55 Jagodzinski, M., Drescher, M., Zeichen, J., Hankemeier, S., Krettek, C., Bosch, U. and van Griensven, M.Eur Cell Mater 2004; 7:35-41; discussion 41
56 Yoshikawa, T., Peel, S. A., Gladstone, J. R. and Davies, J. E.Biomed Mater Eng 1997; 7:369-77
57 Klein-Nulend, J., Roelofsen, J., Semeins, C. M., Bronckers, A. L. and Burger, E. H.J Cell Physiol 1997; 170:174-81
58 Pavlin, D., Dove, S. B., Zadro, R. and Gluhak-Heinrich, J.Calcif Tissue Int 2000; 67:163-72
59 Pavlin, D. and Gluhak-Heinrich, J.Crit Rev Oral Biol Med 2001; 12:414-24
60 Pavlin, D., Zadro, R. and Gluhak-Heinrich, J.Connect Tissue Res 2001; 42:135-48
61 Yuge, L., Okubo, A., Miyashita, T., Kumagai, T., Nikawa, T., Takeda, S., Kanno, M., Urabe, Y., Sugiyama, M. and Kataoka, K.Biochem Biophys Res Commun 2003; 311:32-8
62 Ducy, P., Schinke, T. and Karsenty, G.Science 2000; 289:1501-4
63 Hoffmann, H. M., Beumer, T. L., Rahman, S., McCabe, L. R., Banerjee, C., Aslam, F., Tiro, J. A., van Wijnen, A. J., Stein, J. L., Stein, G. S. and Lian, J. B.J Cell Biochem 1996; 61:310-24
64 Chen, Y. J., Wang, C. J., Yang, K. D., Chang, P. R., Huang, H. C., Huang, Y. T., Sun, Y. C. and Wang, F. S.FEBS Lett 2003; 554:154-8
65 Kurokouchi, K., Jacobs, C. R. and Donahue, H. J.J Biol Chem 2001; 276:13499-504
66 Rubin, J., Ackert-Bicknell, C. L., Zhu, L., Fan, X., Murphy, T. C., Nanes, M. S., Marcus, R., Holloway, L., Beamer, W. G. and Rosen, C. J.J Clin Endocrinol Metab 2002; 87:4273-9
67 Moalli, M. R., Caldwell, N. J., Patil, P. V. and Goldstein, S. A.J Bone Miner Res 2000; 15:1346-53
68 Closs, E. I., Murray, A. B., Schmidt, J., Schon, A., Erfle, V. and Strauss, P. G.J Cell Biol 1990; 111:1313-23
69 Grigoriadis, A. E., Wang, Z. Q., Cecchini, M. G., Hofstetter, W., Felix, R., Fleisch, H. A. and Wagner, E. F.Science 1994; 266:443-8
70 Fitzgerald, J. and Hughes-Fulford, M.Faseb J 1999; 13:553-7
71 Franceschi, R. T. and Xiao, G.J Cell Biochem 2003; 88:446-54
72 Li, C. and Xu, Q.Cell Signal 2000; 12:435-45
73 Komuro, I., Kurabayashi, M., Shibazaki, Y., Katoh, Y., Hoh, E., Kaida, T., Ieki, K., Takaku, F. and Yazaki, Y.Jpn Circ J 1990; 54:526-34

74 Komuro, I., Kaida, T., Shibazaki, Y., Kurabayashi, M., Katoh, Y., Hoh, E., Takaku, F. and Yazaki, Y.J Biol Chem 1990; 265:3595-8
75 Sadoshima, J., Jahn, L., Takahashi, T., Kulik, T. J. and Izumo, S.J Biol Chem 1992; 267:10551-60
76 Ranjan, V. and Diamond, S. L.Biochem Biophys Res Commun 1993; 196:79-84
77 Hsieh, H. J., Li, N. Q. and Frangos, J. A.J Cell Physiol 1993; 154:143-51
78 Pavalko, F. M., Chen, N. X., Turner, C. H., Burr, D. B., Atkinson, S., Hsieh, Y. F., Qiu, J. and Duncan, R. L.Am J Physiol 1998; 275:C1591-601
79 Kawata, A. and Mikuni-Takagaki, Y.Biochem Biophys Res Commun 1998; 246:404-8
80 Fitzgerald, J. and Hughes-Fulford, M.Exp Cell Res 1996; 228:168-71
81 Tjandrawinata, R. R., Vincent, V. L. and Hughes-Fulford, M.Faseb J 1997; 11:493-7
82 Sadoshima, J., Qiu, Z., Morgan, J. P. and Izumo, S.Embo J 1996; 15:5535-46
83 Lean, J. M., Mackay, A. G., Chow, J. W. and Chambers, T. J.Am J Physiol 1996; 270:E937-45
84 Glanstchnig, H., Varga, F., Rumpler, M. and Klaushofer, K.Eur J Clin Invest 1996; 26:544-8
85 Goldberg, D., Polly, P., Eisman, J. A. and Morrison, N. A.J Cell Biochem 1996; 60:447-57
86 Aslam, F., McCabe, L., Frenkel, B., van Wijnen, A. J., Stein, G. S., Lian, J. B. and Stein, J. L.Endocrinology 1999; 140:63-70
87 Owen, T. A., Bortell, R., Yocum, S. A., Smock, S. L., Zhang, M., Abate, C., Shalhoub, V., Aronin, N., Wright, K. L., van Wijnen, A. J. and et al.Proc Natl Acad Sci U S A 1990; 87:9990-4
88 Bidder, M., Shao, J. S., Charlton-Kachigian, N., Loewy, A. P., Semenkovich, C. F. and Towler, D. A.J Biol Chem 2002; 277:44485-96
89 Matsuura, S., Kishi, F. and Kajii, T.Biochem Biophys Res Commun 1990; 168:993-1000
90 Sodek, J., Li, J. J., Kim, R. H., Ogata, Y. and Yamauchi, M.Connect Tissue Res 1996; 35:23-31
91 Bowler, W. B., Dixon, C. J., Halleux, C., Maier, R., Bilbe, G., Fraser, W. D., Gallagher, J. A. and Hipskind, R. A.J Biol Chem 1999; 274:14315-24
92 Fitzgerald, A. M., Gaston, P., Wilson, Y., Quaba, A. and McQueen, M. M.Br J Plast Surg 2000; 53:690-3
93 Okada, Y., Voznesensky, O., Herschman, H., Harrison, J. and Pilbeam, C.J Cell Biochem 2000; 78:197-209
94 Forwood, M. R.J Bone Miner Res 1996; 11:1688-93
95 Duncan, R. L. and Turner, C. H.Calcif Tissue Int 1995; 57:344-58
96 Okada, Y., Lorenzo, J. A., Freeman, A. M., Tomita, M., Morham, S. G., Raisz, L. G. and Pilbeam, C. C.J Clin Invest 2000; 105:823-32
97 Zhang, X., Morham, S. G., Langenbach, R., Young, D. A., Xing, L., Boyce, B. F., Puzas, E. J., Rosier, R. N., O'Keefe, R. J. and Schwarz, E. M.J Bone Miner Res 2001; 16:660-70
98 Bakker, A. D., Klein-Nulend, J. and Burger, E. H.Biochem Biophys Res Commun 2003; 305:677-83
99 Wadhwa, S., Choudhary, S., Voznesensky, M., Epstein, M., Raisz, L. and Pilbeam, C.Biochem Biophys Res Commun 2002; 297:46-51
100 Carreras, I., Rich, C. B., Jaworski, J. A., Dicamillo, S. J., Panchenko, M. P., Goldstein, R. and Foster, J. A.Am J Physiol Lung Cell Mol Physiol 2001; 281:L766-75

101 Coffin, J. D., Florkiewicz, R. Z., Neumann, J., Mort-Hopkins, T., Dorn, G. W., 2nd, Lightfoot, P., German, R., Howles, P. N., Kier, A., O'Toole, B. A. and et al.Mol Biol Cell 1995; 6:1861-73

102 Montero, A., Okada, Y., Tomita, M., Ito, M., Tsurukami, H., Nakamura, T., Doetschman, T., Coffin, J. D. and Hurley, M. M.J Clin Invest 2000; 105:1085-93

103 Zhou, Y. X., Xu, X., Chen, L., Li, C., Brodie, S. G. and Deng, C. X.Hum Mol Genet 2000; 9:2001-8

104 Boudreaux, J. M. and Towler, D. A.J Biol Chem 1996; 271:7508-15

105 Tribioli, C., Frasch, M. and Lufkin, T.Mech Dev 1997; 65:145-62

106 Lian, J., Stewart, C., Puchacz, E., Mackowiak, S., Shalhoub, V., Collart, D., Zambetti, G. and Stein, G.Proc Natl Acad Sci U S A 1989; 86:1143-7

107 Schinke, T. and Karsenty, G.J Biol Chem 1999; 274:30182-9

108 Standal, T., Borset, M. and Sundan, A.Exp Oncol 2004; 26:179-84

109 Standal, T., Hjorth-Hansen, H., Rasmussen, T., Dahl, I. M., Lenhoff, S., Brenne, A. T., Seidel, C., Baykov, V., Waage, A., Borset, M., Sundan, A. and Hjertner, O.Haematologica 2004; 89:174-82

110 You, J., Reilly, G. C., Zhen, X., Yellowley, C. E., Chen, Q., Donahue, H. J. and Jacobs, C. R.J Biol Chem 2001; 276:13365-71

111 Toma, C. D., Ashkar, S., Gray, M. L., Schaffer, J. L. and Gerstenfeld, L. C.J Bone Miner Res 1997; 12:1626-36

112 Schaller, M. D. and Parsons, J. T.Curr Opin Cell Biol 1994; 6:705-10

113 Wang, N., Butler, J. P. and Ingber, D. E.Science 1993; 260:1124-7

114 Oldberg, A., Franzen, A. and Heinegard, D.The Journal of Biological Chemistry 1988; 263:19430-19432

115 Ogata, Y., TYamauchi, M., Kim, R. H., Li, J. J., Freedman, L. P. and Sodek, J.European Journal of Biochemistry 1995; 230:185-192

116 Ganss, B., Kim, R. H. and Sodek, J.Critical Reviews in Oral Biology and Medicine 1999; 10:79-98

117 Hunter, G. K. and Goldberg, H. A.Proceedings of the National Academy of Sciences of the United States 1993; 90:8562-8565

118 Zhou, H. Y., Takita, H., Fujisawa, R., Mizuno, M. and Kuboki, Y.Calcified Tissue International 1995; 56:403-407

119 Mitsui, N., Suziki, N., Maeno, M., Mayahara, K., Yanagisawa, M., Otsuka, K. and Shimuzi, N.Life Sciences 2005; 77:3168-3182

120 Sasaguri, K., Jiang, H. and Chen, J.Arch Oral Biol 1998; 43:83-92

121 Liu, Y., Hair, G. A., Boden, S. D., Viggeswarapu, M. and Titus, L.J Bone Miner Res 2002; 17:406-14

122 Boden, S. D., Liu, Y., Hair, G. A., Helms, J. A., Hu, D., Racine, M., Nanes, M. S. and Titus, L.Endocrinology 1998; 139:5125-34

123 Yamashiro, T., Aberg, T., Levanon, D., Groner, Y. and Thesleff, I.Mech Dev 2002; 119 Suppl 1:S107-10

124 Zambotti, A., Makhluf, H., Shen, J. and Ducy, P.J Biol Chem 2002; 277:41497-506

125 Grewal, T. S., Genever, P. G., Brabbs, A. C., Birch, M. and Skerry, T. M.Faseb J 2000; 14:523-31

126 D'Alonzo, R. C., Selvamurugan, N., Karsenty, G. and Partridge, N. C.J Biol Chem 2002; 277:816-22

127 Hess, J., Porte, D., Munz, C. and Angel, P.J Biol Chem 2001; 276:20029-38