Journal of Cellular Biochemistry Supplements 30/31:62–72 (1998)

Osteocalcin Gene Promoter: Unlocking the Secrets for Regulation of Osteoblast Growth and Differentiation

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Abstract The bone tissue-specific osteocalcin gene remains one of a few genes that exhibits osteoblast-restricted expression. Over the last decade, characterization of the promoter regulatory elements and complexes of factors that control suppression of the osteocalcin gene in osteoprogenitor cells and transactivation in mature osteoblasts has revealed transcriptional regulatory mechanisms that mediate development of the osteoblast phenotype. In this review, we have focused on emerging concepts related to molecular mechanisms supporting osteoblast growth and differentiation based on the discoveries that the osteocalcin gene is regulated by homeodomain factors, AP-1 related proteins, and the bone restricted Cbfa1/AML3 transcription factor. J. Cell. Biochem. Suppls. 30/31:62–72, 1998.

Key words: osteocalcin gene; osteoblast growth; osteoblast differentiation

OSTEOCALCIN: A BONE SPECIFIC PROTEIN LINKED TO SKELETAL DEVELOPMENT AND BONE REMODELING

Osteocalcin was first discovered as a calcium binding protein in bone characterized by three residues of the vitamin K dependently synthesized γ-carboxyglutamic acid (Gla) which interacts with divalent cations. The mRNA of the osteocalcin gene encodes a 10 kD pre-propeptide and its processing may be dependent on the Gla residues analogous to the vitamin K clotting proteins. The protein was found not only to be unique when compared to the hepatic synthesized vitamin K dependent clotting proteins, but was determined to be bone-specific. Osteocalcin is expressed by osteoblasts, odontoblasts, and hypertrophic chondrocytes at the onset of tissue mineralization and accumulates in the bone extracellular matrix [reviewed in Price, 1988; Hauschka et al., 1989].

The 6 kD secreted protein has a high affinity for hydroxyapatite in bone that is dependent on the Gla residues. This property presumes a critical role for osteocalcin in regulating formation of the mineral phase of bone. However, in both warfarin-treated rats which exhibit greater than 90% depletion of osteocalcin from bone [Price, 1988] and in osteocalcin null mutant mice [Desbois et al., 1995], postnatal bone formation, and mineral deposition appeared normal. Although no defects in cell and tissue organization were observed in the knockout mice at birth, increased bone density is observed several months postnatally. Analysis of the mineral phase by FT-IRM suggests a crystal maturational defect in the absence of osteocalcin [Boskey et al., 1998]. The increase in bone mass implicates osteocalcin as a potential inhibitor of bone formation, although a role for osteocalcin in bone resorption is strongly supported by a series of in vivo and in vitro studies relating osteocalcin in bone to osteoclast recruitment and activity [Lian et al., 1984; Glowacki et al., 1991]. The hypothesis that osteocalcin may inhibit osteogenesis is consistent with the developmentally regulated expression profile of osteocalcin during osteoblast differentiation, where strong suppression signals predominate in early osteoprogenitors, while enhancer factors for osteocalcin transcription are found in mature postproliferative osteoblasts [Owen et al., 1990a; Banerjee et al., 1996a; Towler et al., 1994; Hoffmann et al., 1994; Newberry et al., 1998; Ryoo et al., 1997].

REGULATED EXPRESSION OF OSTEOCALCIN DURING OSTEOBLAST DIFFERENTIATION

Bone-specific gene expression must be understood within the context of transcriptional con-

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Received 19 October 1998; Accepted 21 October 1998

trol during progressive development of the osteoblast phenotype. A characteristic feature of primary osteoblasts, in vitro, (but not osteocytes) is the ability of the cells to reinitiate proliferation and differentiation as they multilayer and synthesize an extracellular matrix (ECM) with a bone tissue-like organization [reviewed in Aubin and Liu, 1996; Lian and Stein, 1996]. A temporal pattern of gene expression reflects three major periods of cell and tissue development in bone formation-proliferation, matrix maturation, and mineralization as the osteoblast matures to an osteocyte within its mineralized matrix (Fig. 1). The early stage cells synthesize significant levels of growth factors and type I collagen to support both proliferation and matrix formation. The cessation of growth and accumulated extracellular matrix signals maturation of the phenotype reflected by upregulation of alkaline phosphatase, an early stage marker essential for matrix mineralization. Phenotypic proteins that have affinity for the mineral phase in bone (osteocalcin, osteopontin, bone sialoprotein) are expressed at maximal levels in the subsequent stage of differentiation at the onset of mineralization. Osteocalcin is only expressed postproliferatively in osteoblasts, preosteocytes, and mature osteocytes.

Characterization of osteocalcin gene regulation has greatly contributed to understanding factors regulating development of the osteoblast phenotype [Hoffmann et al., 1994, 1996; Heinrichs et al., 1993, 1995; Ducy and Karsenty, 1995; Bidwell et al., 1993; Merriman et al., 1995; Towler et al., 1994]. By addressing the molecular mechanisms which contribute to activation of bone-specific genes in the early stages of osteoblast differentiation, transcription factors regulating the progression of osteoblast differentiation have been identified. Equally important has been the understanding of mechanisms which contribute to suppression of genes in proliferating osteoprogenitors, like osteocalcin which functions at later stages of bone formation in the regulation of mineral deposition and turnover of bone. This regulation is facilitated by the organization of overlapping and contiguous regulatory elements in the osteocalcin promoter, as illustrated by the TATA/GRE, E Box/AP-1/OC Box; AP-1/Cbfa, and AP-1/YY1/VDRE which provides a basis for combined activities that support both positive and negative control, as well as responsiveness to physiologic mediators. The majority of the regulatory elements have been identified in the region which spans the promoter from the VDRE domain to the first exon using assays involving promoter-reporter constructs transiently expressed in cells (Fig. 1). However, additional upstream sequences that contribute to both basal and enhancer-mediated control of transcription for fidelity of tissue-specific expression when the osteocalcin gene is chromosomally integrated in vivo, must be further defined.

TRANSCRIPTIONAL CONTROL OF OSTEOBLAST DIFFERENTIATION

Osteoblasts originate from pluripotent mesenchymal stem cells which give rise to a number of committed cell lineages. The exact pathways in the earliest phases of commitment to the osteoblast lineage and cellular differentiation is an area of intense investigation. The growth period of osteoblast phenotype development encompasses critical mechanisms for control of the cell cycle and for assurance that the cell is competent to exit the cell cycle and assume phenotypes that are distinct from other cells of mesenchymal origin such as chondrocytes or adipocytes. AP-1 factors, bone morphogenetic protein 2, TGF-β1, and homeodomain proteins DLX-5 and Msx-2, are some examples of regulatory factors that mediate commitment of a mesenchymal cell to the osteoblast lineage expressed at maximal levels in the proliferating stage and contribute to regulation of osteocalcin promoter activity (Fig. 1). It is here in the proliferative period that cells are maximally responsive to steroid hormones and transcriptional control mechanisms that promote acquisition of phenotypes.

Roles for Cell Cycle Regulatory Factors in Differentiation

Because committment of tissue-specific phenotypes is normally associated with growth arrest, most of the attention to cell cycle regulatory factors within the context of differentiation has been focused on mechanisms that ensure exit from the cell cycle as a prerequisite for tissue-specific gene expression. These mechanisms include abundance of hypophosphorylated Rb in a variety of postmitotic cell types [reviewed in Weinberg, 1995], decreased representation or activity of cyclin-CDK complexes, and the upregulation of CDIs during differentiation. While these concepts in relation to carcino-

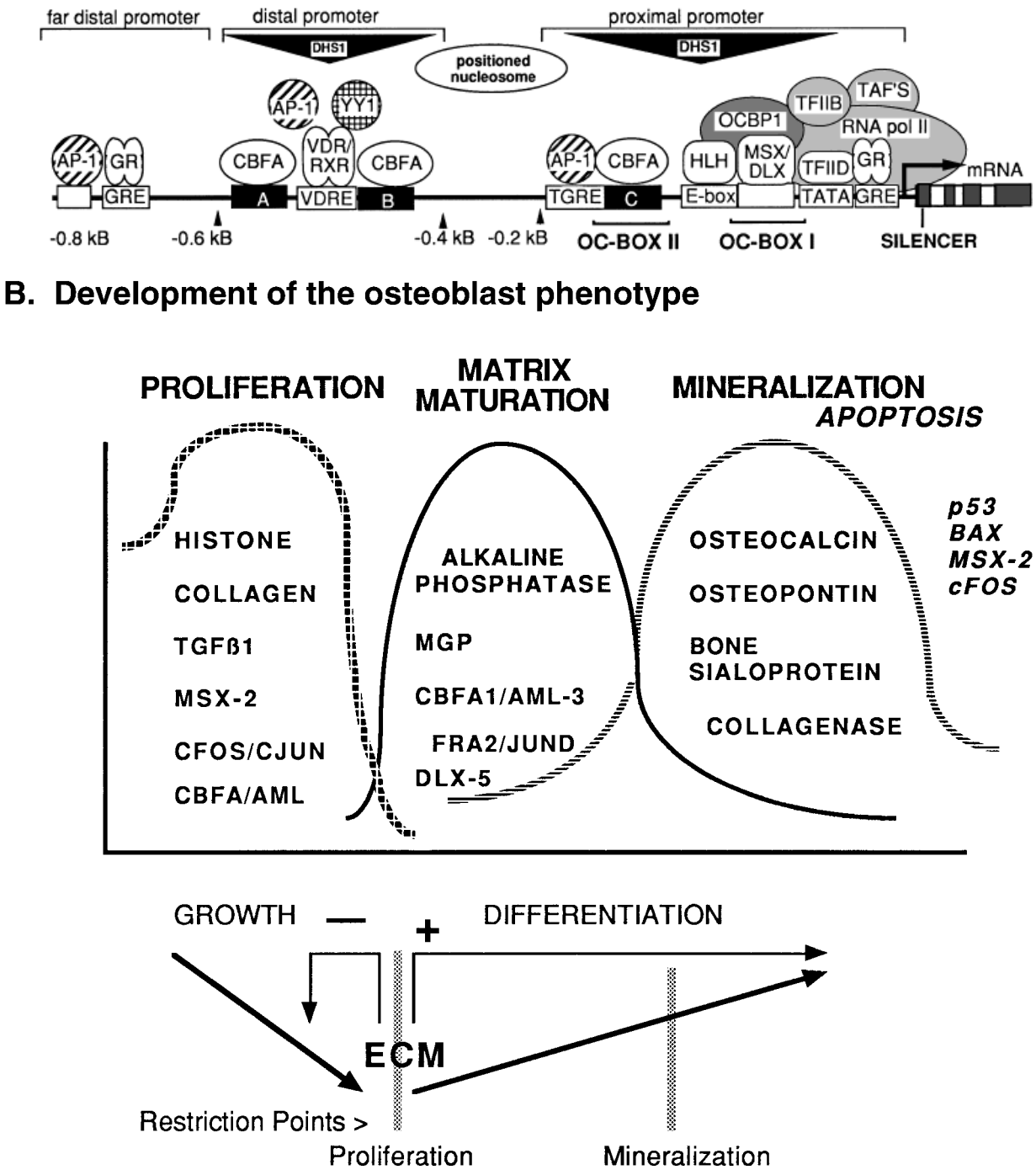

Fig. 1. Regulated expression of the osteocalcin promoter during osteoblast differentiation. **A:** Promoter elements of the rat osteocalcin gene directing basal tissue expression and responsiveness to hormones and growth factors. **B:** Osteocalcin is expressed at the onset of mineralization during development of the osteoblast phenotype and reaches peak levels during mineralization. Three stages of maturation are indicated reflected by maximal expression of growth and differentiation markers. Expression of transcriptional regulators of osteocalcin and osteoblast differentiation are also indicated. Apoptosis of osteoblasts occurs in mineralized nodules related to tissue organization. The lower schematic illustrates the two restriction points influenced by extracellular matrix formation, the downregulation of cell growth and ECM mineralization, that provide signals for progression of osteoblast maturation (see text for details).

genesis have been addressed in several reviews [Weinberg, 1995; Marks et al., 1996], recent evidence has demonstrated that factors controlling cell cycle progression acquire additional roles postproliferatively and are in fact active in the differentiation process of various cell types [Chen et al., 1995].

Postproliferative roles for pRb are provided by pRb gene ablation studies. Cell proliferation in early mouse embryos lacking functional pRb initially appears normal, possibly attributable to functional redundancy with the pRb-related proteins p107 and/or p130. However, at midgestation, neural development and erythropoiesis is abrogated, resulting in prenatal lethality [reviewed by Weinberg, 1995; Slack and Miller, 1996]. Biochemical and cellular analyses of the pRb-deficient cells further show maturation defects in myoblasts [Novitch et al., 1996] and adipocyte differentiation with competency for differentiation restored upon re-expression of pRb [Chen et al., 1996]. The proposed mechanisms for pRB control of differentiation involves interactions with phenotypic transcription factors, such as MyoD [Gu et al., 1993], adipogenic inducers of the C/EBP family [Chen et al., 1996], NF-IL6, the glucocorticoid receptor [Singh et al., 1995], and ATF-2 [reviewed by Sellers and Kaelin, 1996]. Finally, inhibition of apoptosis by pRb family members is an important mechanism for maintaining a differentiated phenotype.

The p107 and p130 null mice also do not exhibit a generalized cell cycle defect. Specific defects in chondrocytic growth and limb development, with neonatal lethality are observed [Cobrinik et al., 1996]. While pRb plays a role in neural and hematopoietic development early during embryogenesis, p107 and p130 interact with cyclin-CDK complexes and are critical later during skeletal development. p107 has been recently found in a transcription factor complex bound to the bone-specific osteocalcin gene promoter [van Gurp et al., 1998]. Thus, all three members of the Rb gene family seem to play tissue-specific differentiation-related roles in addition to their more traditional regulatory roles in cell cycle progression.

There is increasing evidence of cell type-specific postproliferative retention and upregulation of cyclins in differentiating cells [reviewed in Stein et al., 1998b] including osteoblasts [Smith et al., 1995]. However, in most cases no kinase activity is associated with these postproliferative cyclins, and therefore their function may be related to association with other, nonkinase, proteins, such as pRb [Chen et al., 1995] or possibly nuclear hormone receptors [Zwijsen et al., 1997]. In one case, the postproliferative upregulation of cyclin E has been shown to support an osteoblast differentiation-related kinase activity, suppressible by inhibitory activity residing in proliferating osteoblasts [Smith et al., 1997]. Additional evidence for involvement of cyclins in cell differentiation come from experiments manipulating their levels [Stein et al., 1998a]. For example, the myeloid cell line 32D fails to differentiate when cyclins D2 or D3 are overexpressed, probably due to interaction of these cyclins with pRb and/or p107 [Kato and Sherr, 1993]. Overexpression of cyclin D1 in mouse mammary epithelial cells resulted in a more differentiated phenotype [Han et al., 1996]. These findings may be attributed to CDK-independent activation of the estrogen receptor by cyclin D1 [Zwijsen et al., 1997]. In some other cases, however, cyclins may support the induction or maintenance of a differentiated phenotype by activation of CDKs [Bartkova et al., 1996; Dobashi et al., 1996; Smith et al., 1997] or association with pRB/E2F differentiation-related complexes [Kiyokawa et al., 1994].

In summary, it is becoming clear that as cells acquire specific phenotypic properties, some cell cycle regulatory factors persist or even become more abundant postproliferatively. Not only Rb family members but also cyclins and CDKs seem to play roles in cell type-specific differentiation processes. We are currently witnessing the initial steps in a growing field, investigating how these cell cycle regulatory molecules acquire new functions during differentiation, including interactions among themselves, with E2F and with other transcription factor families. The regulatory mechanisms appear to involve interactions with promoter elements of growth- and differentiation-related genes.

Oncogenic Factors

In vivo, c-fos has been established as a key regulator of bone formation [Grigoriadis et al., 1993; Wang et al., 1992]. In vitro, c-fos expression and its AP-1 activity is maximal in proliferating cells. Expression of the entire fos and jun family of oncogene-encoded early response genes (e.g., c-Fos, Fra-1, Fra-2, c-Jun, Jun-B, Jun-D) has been examined as a function of osteoblast

differentiation with respect to protein [McCabe et al., 1996] and mRNA [McCabe et al., 1995; Machwate et al., 1995] levels, as well as influences on osteocalcin gene expression. In the growth period of osteoblasts, c-fos and c-jun heterodimers form the complexes predominantly represented at AP-1 sites, while fra-2 and jun-B are the abundant AP-1-related factors in differentiated rat osteoblast cultures and MC3T3 cells [McCabe et al., 1996]. This profile of expression and activity in osteoblasts (i.e., binding to AP-1 sites in gene promoters) is consistent with recent findings from studies in other systems [Rezzonico et al., 1995; Gandarillas and Watt, 1995; Szabo et al., 1991], which has led to an appreciation for involvement of AP-1-related transcription factors in regulating cellular differentiation. In osteoblasts the functional significance of relatively high levels of fra-2 compared to other AP-1 proteins for maturation of the differentiation program is supported by antisense studies in cultured rat calvarial derived cells. Inhibited translation of fra-2, but not fra-1, blocked the ability of osteoblasts to produce a mineralizing matrix [McCabe et al., 1996]. Initiation of antisense inhibition of c-fos during the growth period blocked maturation at the onset of differentiation, the matrix maturation period, when alkaline phosphatase levels are maximal. This inhibition further suggests c-fos is an important determinant for bone formation as reflected by in vivo studies [Grigoriadis et al., 1993].

The consequences of the osteoblast stage specific representation of different fos and jun family proteins is appreciated from regulation of osteocalcin by AP-1 family members. There are three AP-1 motifs within the osteocalcin promoter. In proliferating osteoblasts, c-fos and c-jun heterodimers contribute to suppression of osteocalcin promoter activity. In contrast, when fra-2 and jun-D co-expressed in osteoblasts, a several fold induction of promoter activity is observed, consistent with the sustained representation of these factors in postproliferative osteoblasts. Fra-2 binding to the TGFβ response element supports basal expression. However, in the presence of high levels of the TGF-β1, osteocalcin promoter activity is decreased and the mechanism involves inactivation of fra-2 by modifications in phosphorylation [Banerjee et al., 1996b]. The AP-1 site in the vitamin D response element is also necessary to support vitamin D responsiveness as demonstrated by mutational analysis [Aslam et al., 1998]. However, in proliferating osteoblasts in the absence of basal expression, vitamin D enhancer activity does not occur; again, supporting the hypothesis that c-fos/c-jun heterodimers block binding activity of the VDR/RXR complex [Owen et al., 1990b], whereas fra-2 binding to the VDRE [McCabe et al., 1996] may facilitate VDR/RXR binding [Aslam et al., 1998].

Homeodomain Protein Developmental Factors

The OC Box I regulatory element (nt -99 to -76) is highly conserved among mammalian osteocalcin genes. The 24 nucleotide sequences which has a homeodomain motif as an essential core, has been established as required for rendering the gene transcribable in a tissue-specific manner [Hoffmann et al., 1996]. OC Box I also binds a non-homeodomain protein, as yet unidentified [Hoffmann et al., 1998], but is related to transcriptional enhancer activity, while homeodomain protein binding suppresses osteocalcin promoter activity [Hoffmann et al., 1994; Towler et al., 1994, 1998; Ryoo et al., 1997].

Several homeobox containing genes have been shown to be critical to skeletal patterning and limb development in embryos [Johnson et al., 1994; Balling et al., 1989; Davis et al., 1995; Martin et al., 1995; Jabs et al., 1993; Lufkin et al., 1992; Satokata and Maas, 1994]. Particularly relevant to bone formation are the mammalian Msx-1 and Msx-2 members of the *Msh* homeodomain gene family and the Dlx gene family. These factors are expressed in tissues which require epithelial-mesenchymal interaction in the developing embryo and have been implicated as regulators of inductive events in the vertebrae, limbs, and cranium. The importance of Msx-2 and related proteins in orchestrating normal bone development is illustrated by expression of Msx-2 in early developing bone tissue [Liu et al., 1995; Iimura et al., 1994] and by skeletal abnormalities resulting from mutations of the gene in human [Jabs et al., 1993] or in mice [Satokata and Maas, 1994]. Msx-2 also provides important signals for apoptosis during limb development [Coelho et al., 1991; Graham et al., 1993]. During osteoblast differentiation in vitro, Msx-2 mRNA levels decline from the growth to differentiation periods and then become upregulated in the late stage of mineralization when apoptosis is ongoing [Lynch et al., 1998]. In contrast, Msx-1 mRNA levels are con-

stitutive, consistent with this factor being ubiquitous, compared to the skeletal tissue restricted expression of Msx-2. Furthermore, antisense inhibition of Msx-2, but not Msx-1 transcripts, block inhibited osteoblast differentiation [Hoffmann et al., 1996].

These findings support the concept that expression of the Msx-2 homeodomain proteins in early development of bone may be necessary for dictating this outcome of osteoprogenitor differentiation to the final stages of extracellular matrix mineralization. Notably, several osteoblast cell lines that do not express osteocalcin also lack Msx-2 transcripts [Hoffmann et al., 1994]. However, downregulation of Msx-2 is required for enhanced expression of osteoblast products that includes collagen [Dodig et al., 1996] and osteocalcin [Hoffmann et al., 1994; Towler et al., 1994]. The suppressor activity of homeodomain proteins in mature osteoblasts may be functionally related to maintaining necessary critical levels of osteoblast expressed genes involved in bone turnover, like osteocalcin and osteopontin. Excessive levels of these proteins may inhibit osteogenesis as suggested by osteocalcin null mice [Ducy et al., 1996]. In this regard, other homeodomain proteins regulate development of the osteoblast phenotype.

Dlx-5, a member of the distal-less family of homeobox-containing genes, is also essential for limb development [Lufkin et al., 1992; Ferrari et al., 1995]. Dlx-5 is co-expressed with Msx-2 in several cell types at key developmental stages required for normal limb development and craniofacial morphogenesis [Zhang et al., 1997; Liu et al., 1997]. During osteoblast differentiation, Dlx-5 exhibits a reciprocal pattern of expression when compared to Msx-2 [Ryoo et al., 1997]. Dlx-5 is detectable only in the postproliferative period and becomes upregulated during the mineralization period reaching maximal levels in concert with the osteocalcin marker of maturation. Notably, Dlx-5 is the only developmentally regulated transcription factor characterized to date which is specifically upregulated during osteoblast differentiation [Ryoo et al., 1997]. Dlx-5 is co-expressed with CBFA1/AML-3 which has been shown to be a key regulator of osteoblast differentiation and skeletal development in vivo [Otto et al., 1997; Komori et al., 1997] and activator of osteocalcin transcription [Banerjee et al., 1996a]. Analogous to CBFA1/AML3, Dlx-5 targets a principal tissue-specific transcriptional element (OC-box I) of the bone-specific osteocalcin gene [Hoffmann et al., 1996]. In mature osteoblasts, Dlx-5 may function as a suppressor of osteocalcin gene transcription to stringently regulate cellular levels in an environment where strong enhancer factors, such as Cbfa1/AML-3, are enriched. Alternatively, since Msx-2 and Dlx-5 can heterodimerize [Zhang et al., 1997], the postproliferative expression of Dlx-5 may relieve Msx-2 suppression of osteoblast genes having homeodomain motifs (e.g., collagen, bone sialoprotein, osteopontin). Taken together, these findings suggest that Dlx-5 may be an important regulator of gene expression in mature osteoblasts as well as playing an important role in early limb formation. Dlx-5 null mice have not been generated to date to address this critical aspect of in vitro expression.

The Runt Homology Domain (Rhd)-Cbfa1 Factors

In the characterization of bone-specific transcription factor complexes associated with osteocalcin gene sequences, three consensus sequences for CBFα/AML-related proteins were identified in the rat osteocalcin promoter (Fig. 1) [Bidwell et al., 1993; Merriman et al., 1995; Banerjee et al., 1996a]. Site C is equivalent to OSE2, the osteoblast specific element of the mouse promoter [Ducy et al., 1997]. The *runt* homology domain related core binding factors (CBFα), also named polyoma enhancer binding protein (PEBPα), were initially identified as a pair rule genes controlling *Drosophila* development and subsequently as key regulators of mammalian hematopoietic gene expression [Kagoshima et al., 1993; Levanon et al., 1994]. In human these factors were also designated AML/protein because they are encoded by gene loci that are rearranged in acute myelogenous leukemia (AML) [Speck and Stacy, 1995]. There are three related genes that each encode numerous splice variants having different functional activities [Speck and Stacy, 1995; Stewart et al., 1997; Ogawa et al., 1993; Lenny et al., 1997]. Cbfa2 (AML-1B/PEBP2αB) is critical for T and B cell differentiation and Cbfa3 (AML-2/PEBP2αC) exhibits more restricted expression.

The specific requirement of Cbfa1 (AML-3/PEBP2αA) for bone formation is demonstrated by the inhibition of bone tissue formation in the Cbfa1 null mutation mouse model [Komori et al., 1997; Otto et al., 1997]. Null mutation of the *Cbfa2/PEBP2αB/AML-1* gene in mice resulted in embryonic lethality due to disruption of he-

matopoieses and hemorrhage in the central nervous system prior to development of the skeleton [Okuda et al., 1996]. However, mice survived to, but not beyond birth, with *Cbfa1/AML-3* gene ablation. While the cartilage anlage of long bone developed and intramembranous skeletal tissue developed alkaline phosphatase positive cells, skeletal formation was disrupted at the mineralization stage. At the same time that this knockout phenotype was reported by two independent laboratories, the mutational defect in the human disorder, cleidocranial dysplasia, was identified in different families as deletions of the *CBFA1* gene [Mundlos et al., 1997; Lee et al., 1997]. The significance of the Cbfa1/AML-3 null mutation with respect to our understanding of transcription factors required for development of the skeleton and maturation of the osteoblast phenotype is quite remarkable because neither of the other two Cbfa/AML genes, having conserved DNA binding domains, can compensate for the skeletal defect.

During mouse development, transient expression of Cbfa1 is observed at high levels from gestational age 9.5 to 12. Expression levels then rise in the bony tissues in osteoblasts at later stages of development and post-natally [Komori et al., 1997; Otto et al., 1997; Ducy et al., 1997]. These findings coupled with increased representation of Cbfa during osteoblast differentiation [Banerjee et al., 1997] suggests that the factor may be important in early specification of the phenotype, but may play a sustained role in the final stages of osteoblast maturation. Several studies suggest that the Cbfa factors may be necessary, but not sufficient for induction of osteogenesis. Recent findings [Ryoo et al., 1998] show that both TGFβ and BMP-2 induce Cbfa1 expression in the myogenic C2C12 line, but only BMP-2 transdifferentiates cells into osteoblasts. We do not yet understand if Cbfa1 can provide the cascade of signals induced by the bone morphogenic proteins that leads to both commitment, and osteogenic differentiation, which is ultimately defined by production of bone tissue.

All *Cbfa* family members are detected in isolated osteoblasts in vitro at the protein level using a panel of antibodies generated by the Hiebert laboratory [Banerjee et al., 1997]. Cbfa1 and Cbfa3 appear to be expressed predominantly in the postproliferative mature osteoblast. However, it is Cbfa1 that is a component of the osteoblast-specific complex which interacts with the three Cbfa elements in the osteocalcin promoter [Banerjee et al., 1997]. Northern blot analyses show multiple mRNAs in osteoblasts [Merriman et al., 1995]; however, a particular splice variant of Cbfa1 predominates. This isoform [first identified by Stewart et al., 1997, and confirmed by Xiao et al., 1998; Thirunavukkarasu et al., 1998, and unpublished observations of this laboratory] has a different N-terminal extension than the Cbfa1 isoform associated with hematopoiesis [Levanon et al., 1994; Ogawa et al., 1993; Bae et al., 1995; Speck and Stacy, 1995]. The isoforms arise from alternative usage of two promoters and multiple ATGs, resulting in proteins with different N-terminal sequences [Stewart et al., 1997; Ducy et al., 1997; Ogawa et al., 1993]. The til-1 isoform initiated from Met2 (MASNS) [Stewart et al., 1997], and not Met1 (encoding the putative bone-specific protein, Osf2) [Ducy et al., 1997], is the major translated product of Cbfa1 mRNAs in osteoblasts [Thirunavukkarasu et al., 1998]. Since all functionally active Cbfa/AML factors can equally transactivate the osteocalcin gene in transient transfection assays and in nonosseous cells [Banerjee et al., 1996a, 1997], the biological significance and activities of the osteoblast-represented Cbfa1 splice variants must be further clarified [Geoffroy et al., 1998; Banerjee et al., 1998].

CONCLUSIONS

Important concepts are emerging regarding the transcriptional control of osteoblast phenotype development. First, factors that have established roles in specifying spatial differentiation and pattern formation during early embryonic stages are re-expressed at selective stages of osteoblast maturation in the postnatal animal and target genes phenotypic of the mature osteoblast, such as osteocalcin. Because bone remodels, there is a continuing requirement to re-establish and sustain a complex tissue organization. Secondly, it is apparent that many transcription factors must be expressed in the undifferentiated mesenchymal cell or early osteoprogenitor and either downregulated for further maturation of the osteoblast (e.g., Msx-2) or require additional modifications for competency to transactivate genes expressed later in development (e.g., Cbfa1). This observation suggests that transient expression of such factors

in the early stages of embryogenesis or development of the osteoblast phenotype may dictate the final commitment of a progenitor to the osteoblast lineage. Lastly, related family members of specific classes of regulatory proteins may be expressed reciprocally during osteoblast differentiation to ensure maintenance of the osteoblast phenotype and/or activities of regulatory proteins may be controlled through interactions with partner proteins. A typical molecular mechanism may involve a change in protein-protein interactions to form a transactivating complex rather than one that suppresses transcription of the gene at specific stages of osteoblast maturation. Recent results suggest that partner proteins acetylate or deacetylate histones to modify chromatin organization. A basis is thereby provided for controlling accessibility of promoter elements to cognate transcription factors and supporting integration of activities at independent promoter domains. The osteocalcin gene is a paradigm for these types of mechanisms. Notably, the c-fos and c-jun heterodimers downregulate osteocalcin expression in proliferating osteoblasts through their interaction at AP-1 sites and in differentiated osteoblasts, the higher levels of fra-2 and jun-D mediate enhancement of osteocalcin gene expression. The Cbfa1-Cbfβ heterodimer complex [Speck and Stacy, 1995] transactivates the osteocalcin gene [Bannerjee et al., 1997]; in contrast, Cbfa1 interaction with Groucho proteins [Fisher and Caudy, 1998] leads to suppression of osteocalcin promoter activity [Guo et al., 1998]. The repertoire of transcription factors that control either bone development or the expression of bone phenotypic genes is expanding. Screens such as differential display, expression cloning and two hybrid assays are providing insight into parameters of transcriptional mechanisms that control osteoblast differentiation to support bone development and remodelling.

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