# DEVELOPMENT OF THE OSTEOBLAST PHENOTYPE: MOLECULAR MECHANISMS MEDIATING OSTEOBLAST GROWTH AND DIFFERENTIATION

Jane B. Lian, Ph.D.
Gary S. Stein, Ph.D.

## ABSTRACT

The formation of bone tissue involves multiple activities of the osteoblast. The combined application of molecular, biochemical, histochemical and ultrastructural approaches has defined stages in the development of the osteoblast phenotype with each subpopulation of cells exhibiting unique morphologic and functional properties in relation to the ordered deposition of the mineralized bone extracellular matrix (ECM). Peak levels of expressed genes reflect a maturational sequence of osteoblast growth and differentiation characterized by three principal periods: proliferation, ECM maturation and mineralization. A plethora of new information in the past several years provides the basis for insight into molecular mechanisms regulating the development and activities of differentiating osteoblasts. These new concepts will be discussed within the context of understanding cellular responses of bone tissue. To be considered are the following: 1) maturational stages of the osteoblast reflected by the selective expression of transcription factors (e.g., oncogenes, cyclins, homeodomain proteins) and phenotypic genes that provide signals for differentiation through the osteoblast lineage; 2) role of the extracellular matrix in mediating osteoblast growth and differentiation; 3) osteoblast stage specific responses to physiologic mediators (e.g., growth factors and hormones); 4) the developmentally regulated expression and selective responses of osteoblast phenotypic genes are supported by cooperative, synergistic and/or antagonistic activities at multiple basal and enhancer or suppressor sequences in gene promoters; and 5) deregulation of these control mechanisms in transformed osteoblasts and osteosarcoma cells.

## I. INTRODUCTION

Understanding the factors regulating bone growth, the continued remodelling of bone throughout life and the regeneration of injured tissue are basic biological questions and concerns of today's clinician for the treatment of bone related disorders. Practical applications in orthopedics include knowledge of bone formation and resorption events related to abnormalities in skeletal development, fracture repair, metabolic bone diseases and implant stability. To allow for a rational intervention of these processes clinically, one must gain knowledge of the mechanisms that regulate tissue formation, maturation and stability. One fundamental approach has been to isolate the bone cells which in culture exhibit tissue-specific metabolic responses and biosynthesis of structural components of bone. From these studies, several classes of factors have been identified that allow us to define regulatory mechanisms operative in control of growth and differentiation of the cell osteoblasts and/or osteoprogenitors. These include but are not restricted to bone morphogenic proteins[27], cell growth factors[23], hormones[98], cytokine modulators[57] and mechanical physical forces[22,136]. It is also recognized that a broad spectrum of physiologic mediators of bone formation have differential and highly selective effects on the numerous cells involved in osseous tissue formation and turnover. A clear understanding of effects on osteoblasts is somewhat compromised by species differences or the experimental system, although such variations are now being appreciated as related to the stage of cellular maturation or differentiation which impacts on the responsiveness of the osteoblast to physiologic modulators of bone cell functions.

The focus of this review is a description of the sequelae of events associated with osteoblast growth and the progression of differentiation as related to formation and organization of bone tissue by normal diploid cells derived from fetal bone *in vitro*. For each maturational stage, characteristic activities and responses to physiologic mediators of osteoblast growth and differentiation will be described. The signalling pathways and molecular mechanisms mediating development of the osteoblast phenotype will be described as these approaches provide a basis for development of specific modes of therapeutic intervention in the treatment of skeletal disorders.

## II. DEVELOPMENT OF THE OSTEOBLAST PHENOTYPE

For many years, bone was defined anatomically and examined largely in a descriptive manner by ultrastructural analysis and by biochemical and histochemical methods[21,123,139]. These studies provided the basis for our understanding of bone tissue organization and orchestration of the progressive recruitment, proliferation and

Correspondence to: Jane B. Lian, Ph.D., Department of Cell Biology, University of Massachusetts Medical Center, 55 Lake Avenue North, Worcester, MA 01655., Telephone: (508)856-5625

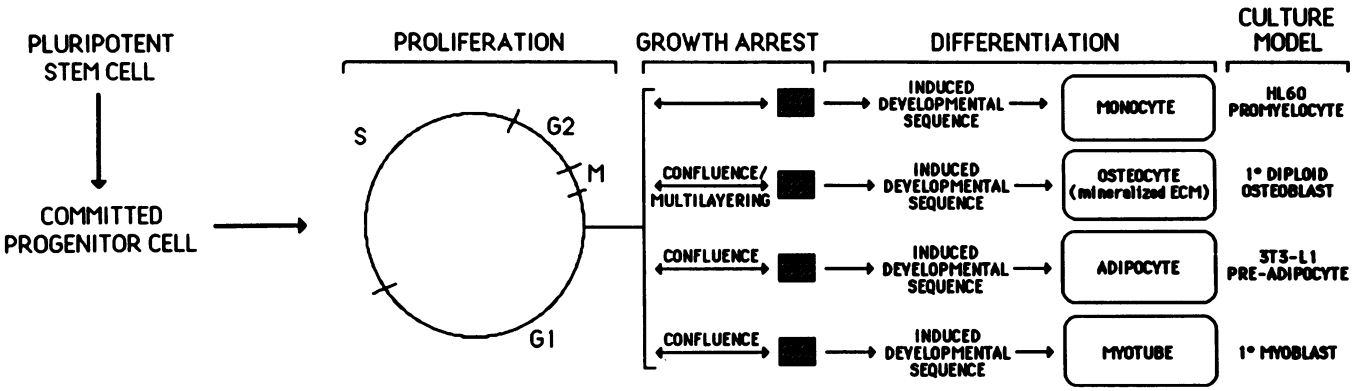


**Fig. 1. Schematic representation of the events associated with progressive expression of monocyte, granulocyte, osteoblast, adipocyte, and myotube phenotypes. Initially the cells (HL-60 promyelocytic leukemia cells, primary cultures of embryonic calvarial osteoblasts, 3T3-L1 preadipocytes, or myoblasts) actively proliferate, expressing cell cycle and cell growth-regulated genes as well as genes encoding extracellular matrix proteins. After growth arrest, a developmental sequence involving the sequential and selective expression of genes that results in the differentiated cell and tissue phenotype occurs. Completion of the proliferation period marks an important transition point where expression of tissue-specific genes, often functionally coupled to the down-regulation of proliferation, is initiated.**

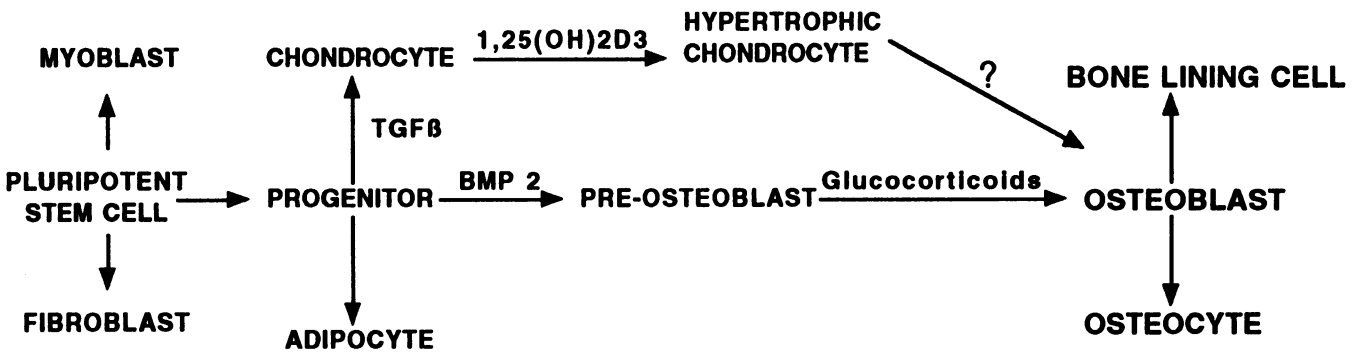


**Fig. 2. The osteoblast differentiation pathway. Progression through the osteoblast lineage from a pluripotent mesenchymal stem cell which has multiple options is illustrated. Several physiologic mediators including, but not limited to, transforming growth factor b (TGFb), the bone morphogenic protein 2 (BMP-2) and glucocorticoids, which have been documented to influence progression through the chondrocyte or osteoblast phenotype, are indicated. A question mark placed between the hypertrophic chondrocyte and osteoblast reflects recent reports which have indicated the hypertrophic chondrocyte can further differentiate to an osteoblast-like cell expressing genes observed in mature osteoblasts. The cuboidal osteoblast that synthesizes bone matrix will further differentiate to either an osteocyte when enveloped in mineralized matrix or to a flattened lining cell on a quiescent surface of mineralized bone. Each of these subpopulations of osteoblasts exhibit different properties and express osteoblast phenotypic genes to varying extents (see text for details).**

differentiation of the various cellular components of bone tissue. Now, complemented by an increased knowledge of molecular mechanisms that are associated with and regulate expression of genes encoding phenotypic components of bone and those which may control the progressive development and maturation of the bone cell phenotype, our understanding of bone cell and tissue differentiation is rapidly expanding.

It has been recognized that defining mechanisms operative in the development and maintenance of the osteoblast phenotype necessitates comprehensive knowledge of cell growth and tissue-specific genes expressed with regard to: 1) the time course and extent of expression; 2) temporal vs. functionally coupled relationships; 3) control of gene expression at both transcriptional and post-transcriptional levels; and 4) signalling pathways and macromolecules that integrate the activities of physiological regulatory mediators involved in lineage development of the osteoblast.

A functional relationship between cell growth and the initiation and progression of events associated with differentiation has been viewed as a fundamental question by developmental biologists for more than a century. In the case of bone, as observed with other systems (Figure 1), the relationship of growth and differentiation must be maintained and stringently regulated, both during development and throughout the life of the organism to support tissue remodeling. Understanding biological regulatory mechanisms that support bone cell development within the context of the proliferation/differentiation relationship provides a viable basis for addressing skeletal disorders and particularly bone cell cancer, where aberrations in the proliferation/differentiation relationship may facilitate both diagnosis and evaluation of targeted therapy.

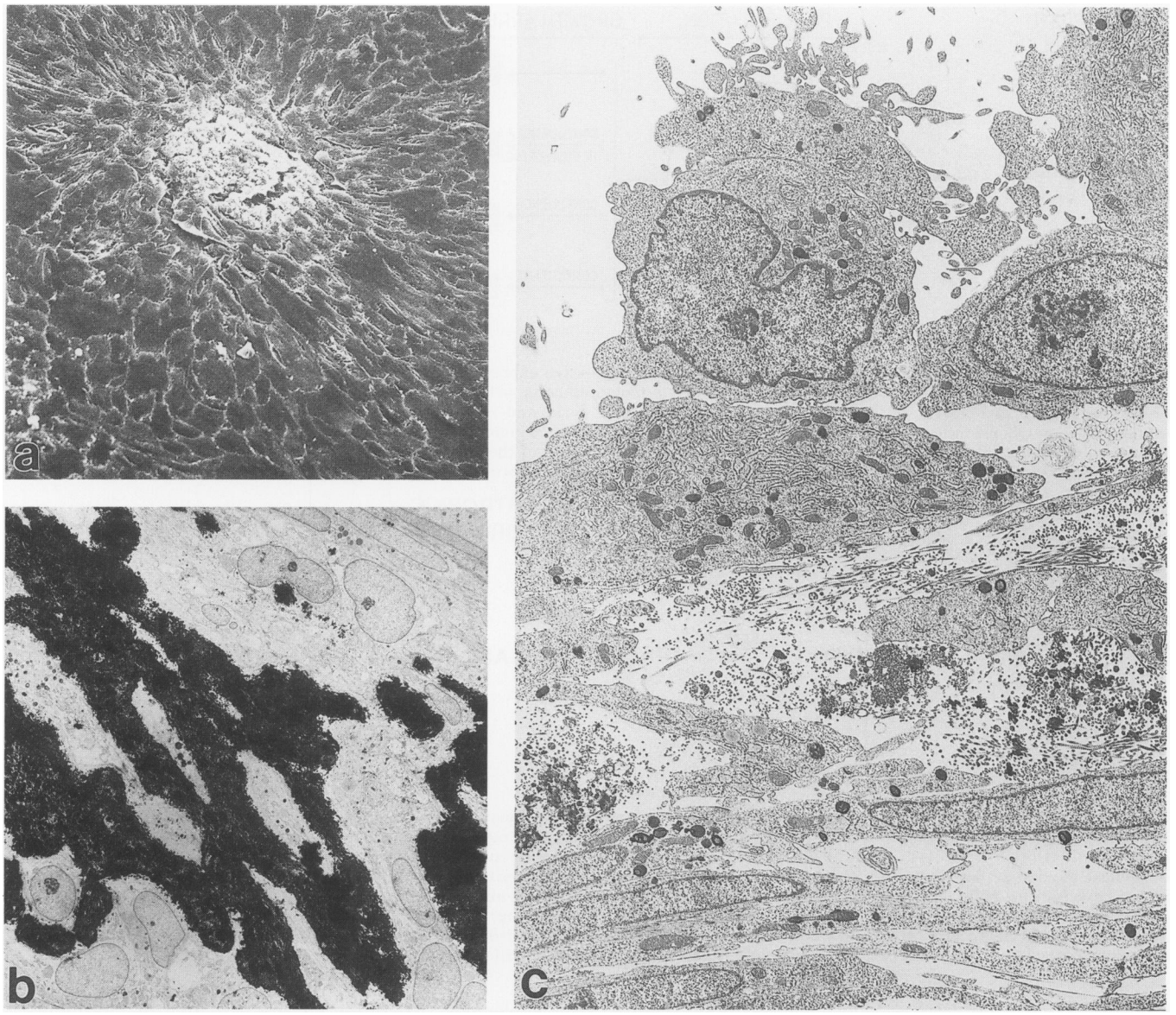


**Fig. 3. Morphology and ultrastructure of fetal rat calvaria-derived osteoblast cultures. (a) Scanning electron micrograph showing cuboidal osteoblasts both at monolayer confluency and in multi-layers forming a typical bone nodule having a mineralized extracellular matrix, 100X. (b) Low power transmission electron micrograph of an undemineralized sectioned bone nodule to illustrate the mineralized extracellular matrix surrounding osteoblasts. Mineral deposits are hydroxyapatite crystals associated with collagen fibers, 500X. (c) Transmission electron micrograph of a demineralized nodule in cross-section to reveal the orthogonal layers of collagen interspersed between osteoblasts. Note the large cuboidal surface osteoblast having a large central nucleus rich in ribosomes and with extensive Golgi, 8000X.**

It is now generally accepted that the progenitor stem cell of the osteoblast is a pluripotent mesenchymal fibroblast which prior to commitment can develop as an osteoblast, chondrocyte or other phenotypes[46]. An important aspect of bone cell biology is to experimentally address the regulatory events related to the recruitment and commitment of pluripotent mesenchymal cells into the developmental pathway which initially restricts their options for specialization to either the osteoblast and chondrocyte pathways and subsequently to solely the osteoblast phenotype (Figure 2). Potential cell culture systems to study growth and differentiation of progenitors include recent use of bone marrow cultures which offer the possibility of examining events associated with stem cell differentiation to osteoblasts[28,45,86,135,191]. Particularly successful has been culture of human derived marrow cells that can be induced to differentiate to osteoblasts producing a bone like matrix by the synthetic glucocorticoid, dexamethasone, or by the bone morphogenic protein, BMP-2[51,76].

## A. The ordered expression of genes during development of the osteoblast phenotype

The development of methods for culture of normal diploid calvarial-derived osteoblasts under conditions which support development of a tissue-like organization[16,141] (Figure 3) similar to embryonic bone provided the basis for experimentally addressing the proliferation/ differentiation relationship within the context of physiological regulation[131]. By trypsin-collagenase digestion of fetal rat calvaria, a population of cells from the third sequential digest will express significant levels of osteoblast phenotypic markers including alkaline phosphatase, osteopontin and osteocalcin mRNA[4,131]. When placed into culture these bone cell genes are downregulated and nearly every plated cell initiates proliferation as indicated from $^{3}$H thymidine autoradiography and *in situ* hybridization studies using the H4 histone gene as a probe which is coupled to DNA synthesis (Figure 4A). Only a few osteocytes released, having a distinct morphology, will not reinitiate proliferation[178]. The osteoblasts proliferate forming multilayers of cells as sheets[48,72,78] or focal nodules[4,6]. When proliferation ceases, alkaline phosphatase levels increase sufficiently for histochemical detection. An ordered deposition of mineral is initiated within the extracellular matrix of these nodules resulting in the development of a bone tissue-like organization, with an orthogonal arrangement of collagen layers between osteoblasts and a mineralized extracellular matrix (Figure 3).

The sequential expression of cell growth and tissue-specific genes has been mapped by immunocytochemical methods[4,108], Northern blot analysis[9,131] and *in situ* hybridization[100,141] during progressive development of the bone cell phenotype from a proliferating cell to a mature osteocytic cell in a mineralized Type I collagen extracellular matrix. This temporal sequence of gene expression has defined three distinct periods: growth (proliferation) and extracellular matrix biosynthesis; extracellular matrix development, maturation and organization; and extracellular matrix mineralization (Figure 4B).

During the first 10 to 12 days of culture following isolation of fetal rat calvarial-derived osteoblasts, a period of active proliferation is reflected by mitotic activity with expression of cell cycle (e.g. histone) and cell growth (e.g. c-myc, c-fos, and c-jun) regulated genes. These genes encode proteins that support proliferation by functioning as transactivation factors in the case of c-myc and c-fos and as proteins that play a primary role in packaging newly replicated DNA into chromatin in the case of histones. Modifications in expression of proliferation related genes, e.g. c-fos or c-src expression *in vivo* (in transgenic animals), results in altered bone formation[58,75,147,158] indicating the importance of their regulated expression during the proliferative period for control of cell growth as well as for control of genes later during osteoblast development that directly involve bone cell differentiation. This concept is further supported by evidence for the selective expression of fos and jun related family members through the course of osteoblast differentiation[114].

During this proliferation period, and fundamental to development of the bone cell phenotype, several genes associated with formation of the extracellular matrix (Type I collagen, fibronectin, and TGFβ) are actively expressed and then gradually decline with collagen mRNA being maintained at a low basal level during subsequent stages of osteoblast differentiation. The accumulation of collagen contributes, in part, to the cessation of cell growth. Immediately following the down-regulation of proliferation, proteins associated with bone cell phenotype are detected. For example, alkaline phosphatase enzyme activity and mRNA are increased greater than 10-fold. At this time, a differentiation-specific histone gene is expressed[30,160]. During the immediate post-proliferative period (from 12 to 18 days), the extracellular matrix undergoes a series of modifications in composition and organization that renders it competent for mineralization. As the cultures progress into the mineralization stage, all cells become alkaline phosphatase positive histochemically (Figure 3). Histochemical detection of APase is considered one of the earliest phenotypic markers of the osteoblast lineage. In heavily mineralized cultures, cellular levels of alkaline phosphatase mRNA decline (Figure 4B). At this time maximal levels of collagenase gene expression are observed and may relate to a remodelling of the matrix to support tissue organization and mineral deposition[154]. Apoptotic cells are also detected suggesting programmed cell death may provide a mechanism for regulating the number of surface osteoblasts that further differentiate to osteocytes[102].

With the onset of mineralization, several other bone-expressed genes are induced to maximal levels, paralleling the accumulation of mineral. These are bone-synthesized proteins known to associate with the mineralized matrix *in vivo*[40,43,61,189] including bone sialoprotein[119], osteopontin and osteocalcin[48,131]. Osteopontin is expressed both during the period of active proliferation (at 25% of maximal levels), decreases post-proliferatively and exhibits induction at the onset of mineralization, achieving peak levels of expression during mineralization (days 16 to 20). This pattern of osteopontin expression has been observed in other osteoblastic developmental systems[49,165] and may reflect multiple functional properties. Increased osteopontin expression can occur following serum stimulation of quiescent fibroblasts, oncogene transformation or phorbol ester treatment of fibroblasts[31]. Here one can speculate that proliferation or tumorigenic related functions of osteopontin may be related to control of relationships be-

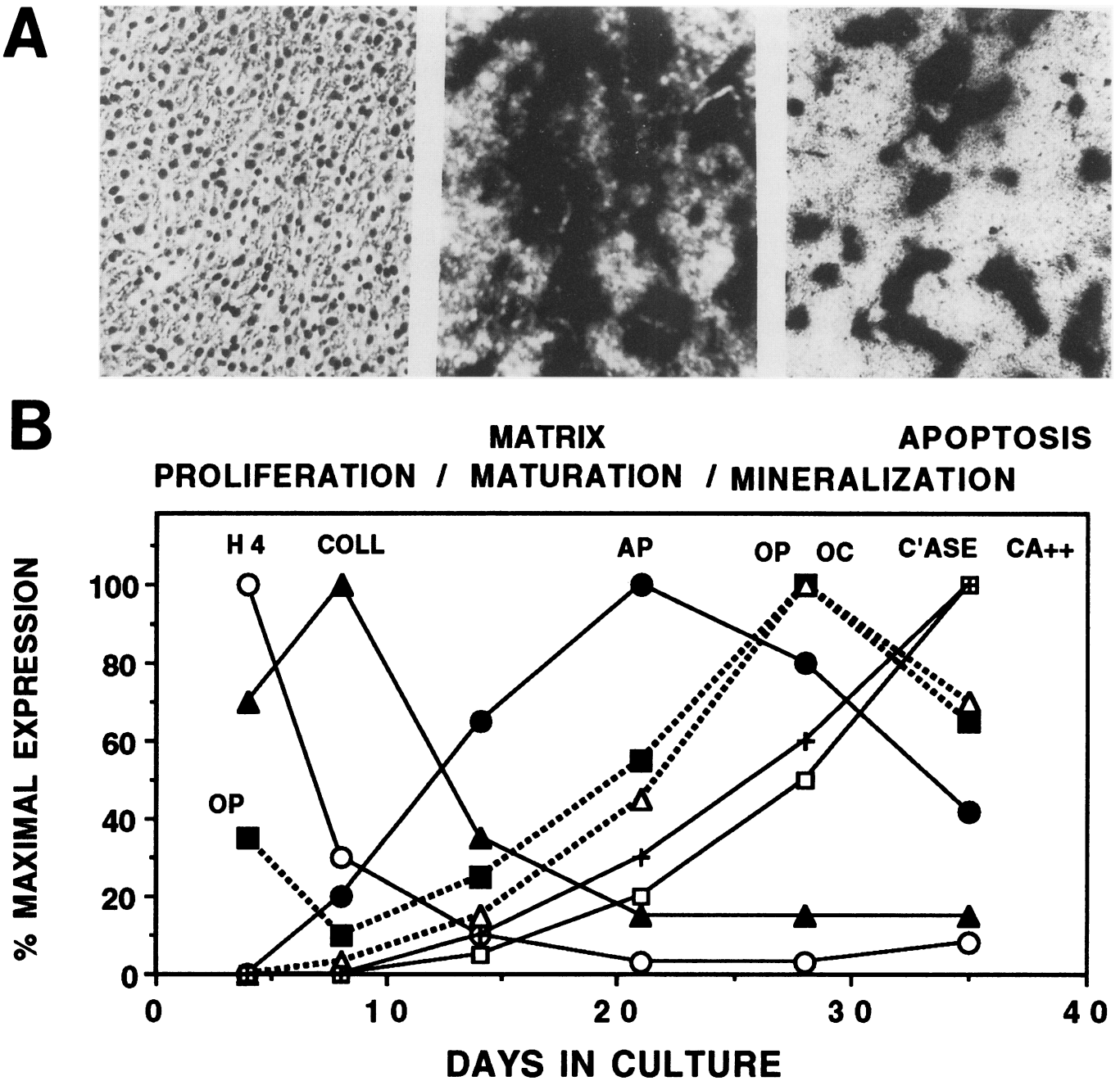


**Fig. 4** The osteoblast development sequence. (A) Histochemical staining of fetal rat calvaria derived osteoblast cultures during the development of in vitro formed bone-like tissue. Left panel, tritiated thymidine autoradiography illustrating the proliferation period; middle panel represents alkaline phosphatase staining demonstrating nearly every cell is positive; and the right panel represents a typical von Kossa silver stain plate revealing mineralized bone nodules throughout the culture dish, 10X. (B) Temporal expression of cell growth and osteoblast phenotype related genes during 35 days of culture. Cells were harvested on the indicated days and total cellular RNA prepared for assay of various transcripts by Northern blot analysis. The resulting blots were quantitated by scanning densitometry and the results plotted relative to the maximal expression of each transcript. Conditions and origin of the probes are detailed in Owen et al., 1990 and Shalhoub et al., 1992. In the growth period, histone H4 collagen (COLL), and osteopontin (OP) are shown. The peak levels of alkaline phosphatase mRNA (AP) represent the matrix maturation period. In the mineralization period, osteopontin (OP) and osteocalcin (OC) reach their peak levels. The increase in mRNA transcripts of OC and OP during the culture period reflect calcium (Ca++) deposition. The increase and peak levels of collagenase (C'ASE) are related to remodeling or editing of the extracellular matrix. Apoptotic cells are observed during the mineralization period (see text for details).

tween cells and extracellular matrices, particularly in light of the arg-gly-asp containing sequence that mediates cell attachment[130]. Consistent with high levels of osteopontin expression during the mineralization period are the calcium binding properties of this acidic glycoprotein containing O-phosphoserine[53]. Several studies indicate osteopontin interact with osteoclasts implicating this phosphoprotein in the resorption process[115,116,144].

The vitamin K-dependent protein, osteocalcin (bone Gla protein), in contrast to osteopontin, is expressed only post-proliferatively with the onset of nodule formation. This 5.7kD calcium-binding protein, characterized by 3-gammacarboxy-glutamic acid residues (Gla), binds tightly to hydroxyapatite and is maximally expressed with mineralization of the extracellular matrix *in vivo*[61] and *in vitro*[131]. Osteocalcin mRNA and synthesis correlates (0.92) to calcium deposition *in vitro* in rat osteoblast cultures[4], similar to expression and protein accumulation in the bone extracellular matrix *in vivo*[61,187]. Several modifications of culture conditions of fetal rat calvarial derived osteoblasts[131] demonstrate that induction of high osteocalcin and osteopontin mRNA levels are dependent upon formation of a mineralized extracellular matrix. However, post-natal derived cells or osteoblasts from more mature bone exhibit osteocalcin expression in non-mineralizing cultures. Several studies suggest osteocalcin may contribute to either regulation of mineral deposition or turnover of the mineral phase in bone. *In vitro* osteocalcin is an inhibitor of mineral nucleation[19,146]. *In vivo* increased synthesis by pharmacologic doses of $1,25(OH)_2D_3$ results in undermineralization[64], while absence of osteocalcin accumulation in bone (induced by warfarin anticoagulants) leads to punctate calcifications[39,68]. Other studies demonstrate osteocalcin serves as a bone matrix signal that promotes osteoblast differentiation and acivation demonstrated in several biological assays *in vivo* [33,55,56,89,93,185] and *in vitro*[10,29,90,99]. Thus, expression late in the osteoblast development sequence[91,94] reflects osteocalcin as a marker of the mature osteoblast, consistent with a possible role for the synthesis and binding of osteocalcin to mineral for participation in bone resorption related events. Clinically, serum osteocalcin levels reflect bone turnover[92], and the gene is regulated by hormones (e.g. $1,25(OH)_2D_3$) and growth factors (e.g. TGFβ) involved in bone turnover (see next section).

Analysis of these osteoblasts at the single cell resolution by *in situ* hybridization for expressed genes indicates that the temporal pattern of gene expression does not occur synchronously in all cells[140,141] but is related to tissue organization. For example, osteocalcin expressing cells are found only within mineralizing nodules; in contrast, alkaline phosphatase positive cells occur between nodules. The biological relevance of the osteoblast culture system as a model for bone cell differentiation is supported by a sequence of gene expression (cell growth and osteoblast related genes) that is similar to the pattern of gene expression and tissue distribution determined during fetal calvarial development *in vivo*[192] and in neonatal bones[104,126,148,187]. The significance of the osteoblast developmental sequence is that it provides a framework for evaluating aberrations in osteoblast growth or differentiation responses in bone disorders and allows for development of targeted therapeutic approaches.

Several striking examples illustrate therapeutic approaches that can be extrapolated from an understanding of the osteoblast development sequence. The effects of substances including caffeine[8], warfarin[39,68] and dioxanes[1], which have caused abnormalities in skeletal development were examined in cultured osteoblasts[11,52,170]. Each inhibited the osteoblast developmental sequence *in vitro*, but by impinging on different regulatory mechanisms. In other studies, it has been shown that potential defects in bone formation *in vivo* can be reiterated through the osteoblast *in vitro*. For example, osteoblast cultures established from calvaria of two rat osteopetrotic mutants, the tl/tl and op/op, show alterations in expression of genes and nodule formation. For the tl mutant derived osteoblasts, the developmental sequence is abbreviated compared to cells from normal littermates[74]. Mineralization occurs prematurely in the osteoblasts derived from osteopetrotic calvaria, and some of the abnormalities in the developmental pattern of osteoblast genes expressed *in vivo*[152] that contribute to the sclerotic features of the disease are retained *in vitro*[74].

### B. Two transition-restriction points during development of the osteoblast phenotype characterize the proliferation/differentiation relationship.

The sequential and stringently regulated expression of genes that define three principal periods of osteoblast phenotype development (proliferation, extracellular matrix development and maturation, and mineralization) is schematically illustrated in Figure 5 as a reciprocal and functionally coupled relationship between proliferation and differentiation. Two transition points are key elements of this temporal expression of genes that support cell growth and differentiation: the first transition point, at the completion of the proliferation period when genes for cell cycle and cell growth control are down-regulated and expression of genes encoding proteins for extracellular matrix maturation is initiated; and the second, at the onset of extracellular matrix mineralization. These transitions have been experimentally established[131] and functionally defined as restriction points during osteoblast differentiation to which developmental expression of genes can proceed but cannot pass without additional cellular signalling[reviewed in 163,164]. Thus, establish-

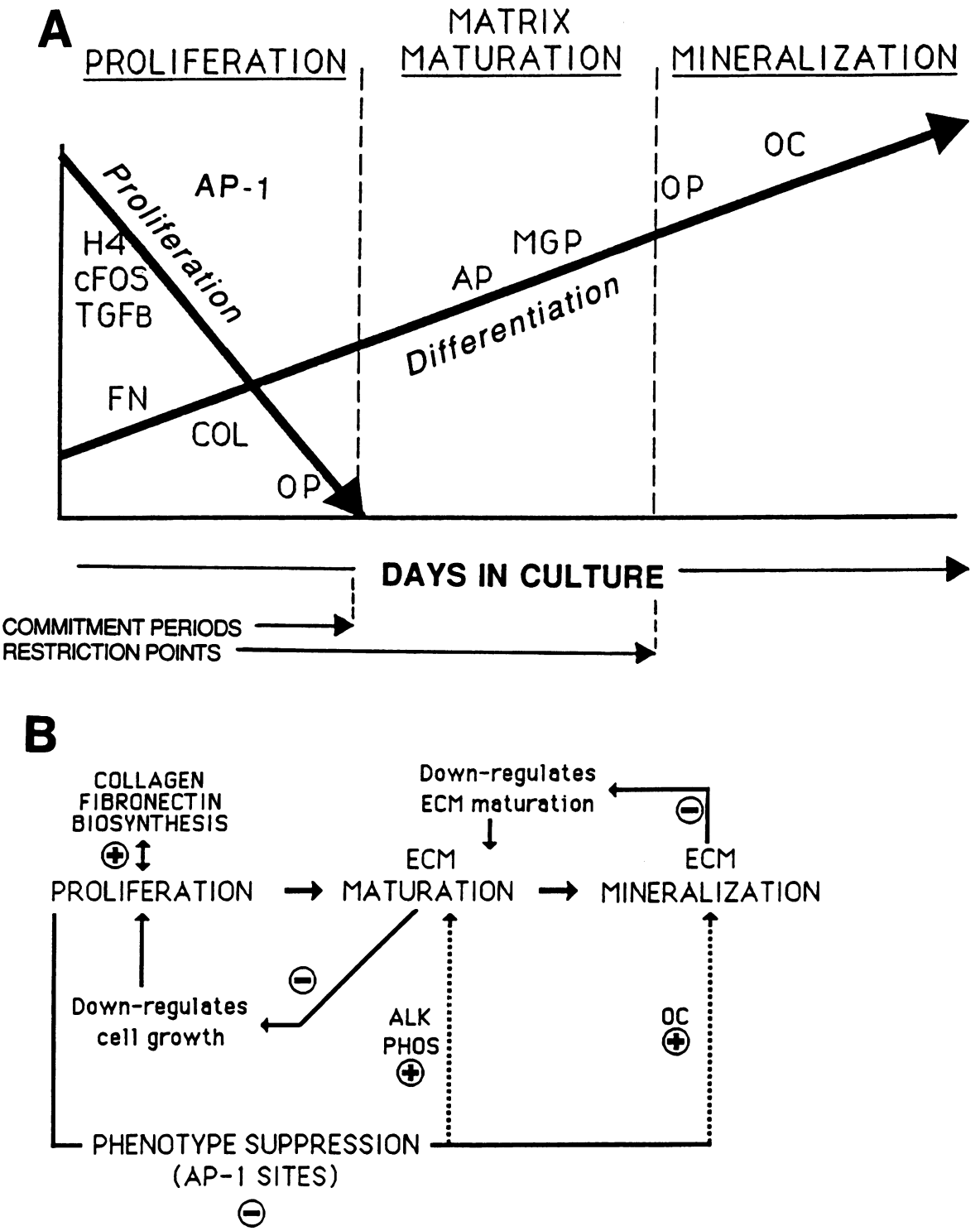


**Fig. 5. Reciprocal and functionally coupled relationship between cell growth and differentiation-related gene expression. (A) These relationships are schematically illustrated as arrows representing changes in expression of cell cycle- and cell growth-regulated genes (proliferation arrow) and genes associated with the maturation (differentiation arrow) of the osteoblast phenotype as the extracellular matrix develops and mineralizes in normal diploid cell cultures. Here, the three principal periods of the osteoblast developmental sequence are designated within broken vertical lines (proliferation, matrix development and maturation, and mineralization). These broken lines indicate the two experimentally established principal transition points in the developmental sequence exhibited by normal diploid osteoblasts during the progressive acquisition of the bone cell phenotype. The first is at the completion of proliferation when genes associated with matrix development and maturation are up-regulated, and the second occurs at the onset of extracellular matrix mineralization. (B) A series of signalling mechanisms are illustrated whereby the proliferation period supports the synthesis of a Type I collagen-fibronectin ECM, which continues to mature and mineralize. The formation of this matrix down-regulates proliferation, and matrix mineralization down-regulates the expression of genes associated with the ECM maturation period. The occupancy of AP-1 sites in the osteocalcin and the alkaline phosphatase gene promoters by Fos-Jun and/or related proteins are proposed to suppress both basal and vitamin D-induced expression before upregulation post-proliferatively (phenotype suppression).**

ing the basis of cellular competency for progression toward development of the mature osteoblast phenotype necessitates identification of the signalling pathways operative at the developmental transition points by which genes are selectively activated and/or suppressed. The absence during the proliferation period of expression of genes observed in post-proliferative mature osteoblasts is called "phenotype suppression." The model is supported by binding of regulatory factors abundant in proliferating osteoblasts (e.g. oncogenes[94,95,114] encoded or helix-loop-helix proteins[118,129]) to regulatory elements of genes expressed later in development (see Section IV).

By abbreviating or extending either the proliferation or differentiation period, one can determine which genes associated with extracellular matrix biosynthesis, maturation or mineralization are prematurely upregulated or delayed in initiation of expression. The biochemical modifications that include inhibition of proliferation, decreased extracellular matrix biosynthesis, delayed extracellular matrix mineralization, and accelerated differentiation by glucocorticoid on culture in collagen matrices provided support for several signalling pathways that promote differentiation. Inhibition of proliferation upregulates expression of genes that are expressed only during progression of the osteoblast developmental sequence up to the stage where mineralization is initiated (e.g. alkaline phosphatase and osteopontin but not osteocalcin). Additionally, there is evidence for the concept that expression of at least a second set of genes is not directly coupled to the down-regulation of proliferation, but rather to development of the more differentiated osteoblast in a mineralized matrix. Inhibition of collagenous extracellular matrix formation prevents induction of alkaline phosphatase levels and mineralization. In the absence of mineralization, osteopontin and osteocalcin are not induced to high levels and alkaline phosphatase does not decline. From these studies, one can postulate that proliferation is functionally related to the synthesis of a bone specific extracellular matrix and that the maturation and organization of the extracellular matrix contributes to the shutdown of proliferation. This then promotes expression of genes that render the matrix competent for mineralization, a final process that is essential for complete expression of the mature osteoblast phenotype. The onset of extracellular matrix mineralization and/or events earlier during the mineralization period may be responsible for the down-regulation of genes expressed during extracellular matrix maturation and organization. Clearly, in this model the development of an extracellular matrix is integrally related to the differentiation stages. This relationship is evident from a series of studies in which cells were cultured at various concentrations of ascorbic acid[131]. With resulting higher levels of collagen synthesis and accumulation of the extracellular matrix, proliferation ceases at a lower cell density, and alkaline phosphatase mRNA levels and enzyme activity per cell are greater. Thus, there is a contribution of signals from the extracellular matrix that promotes progressive differentiation of the osteoblast, and this is indicated from findings in other cell culture systems[4,42,142]. This working model for a functionally coupled relationship provides a basis for understanding aberrations in skeletal development and the consequences of intervention by factors that effect bone formation and turnover.

A complementary experimental approach substantiating the support and/or inductive effect of the collagen extracellular matrix in osteoblast differentiation is culturing primary diploid osteoblasts on Type I collagen film or in a collagen gel[2,112]. Here, with rat osteoblasts, we observe an accelerated progression of osteoblast phenotype development[103] indicated by early and enhanced expression of alkaline phosphatase and osteocalcin. This is in agreement with the studies of Reddi and coworkers[184] demonstrating induction of osteocytic cells from osteoprogenitors when grown on matrigel. Thus, our working model provides a basis for addressing whether particular stages of osteoblast differentiation exhibit selective responsiveness to actions of hormones and other physiological factors that influence osteoblast activity as well as other questions related to molecular mechanisms associated with bone formation.

## III. GROWTH FACTOR AND HORMONE MODIFICATIONS ON DEVELOPMENT OF THE OSTEOBLAST PHENOTYPE

### A. Windows of Responsiveness for Altering the Course of Osteoblast Differentiation

Hormonal and growth factor modulation of osteoblast growth and differentiation is also illustrated by several striking examples, including the steroid hormones, glucocorticoids, and 1,25(OH)2D3, the active vitamin D metabolite. From an historical perspective, it is well known that vitamin D anabolically and catabolically modulates bone cell metabolic activities[64,110,190], and more recently it has become apparent that this occurs through selective expression of a series of vitamin D responsive genes[reviewed in 96,98]. Vitamin D treatment of osteoblasts alters the level of gene expression to one that reflects a more mature differentiated cell. For example, with short term exposure (up to 48 hours), proliferation is down regulated with collagen and alkaline phosphatase mRNA levels while osteocalcin, matrix Gla protein and osteopontin are upregulated. However, chronic treatment of rat osteoblasts with $10^{-8}$M $1,25(OH)_2D_3$, if initiated during the proliferation period, can block differentiation of osteoblasts (Figure

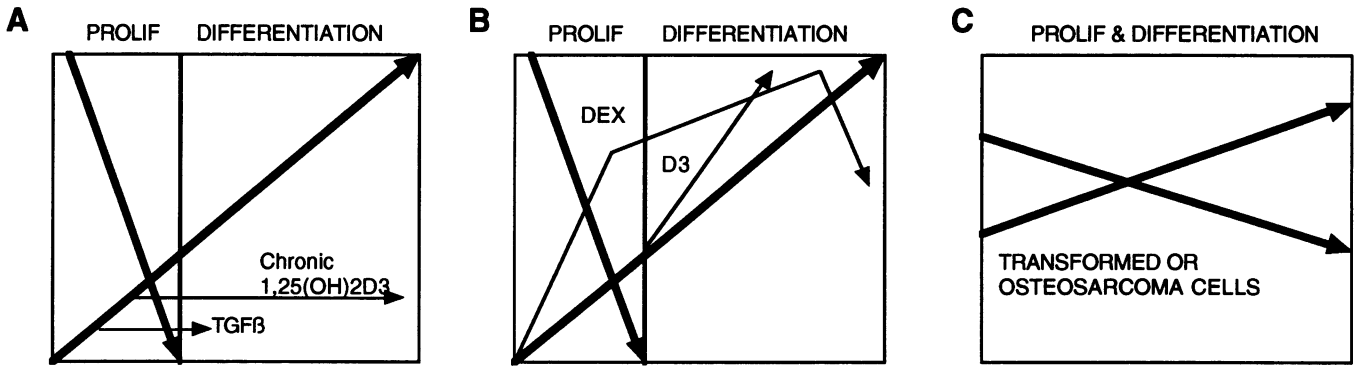


**Fig. 6. Modifications of the growth differentiation relationship by growth factors, hormones and in osteosarcoma cells. (A) Inhibition of differentiation is indicated by treatment of fetal rat calvaria osteoblasts during the proliferation period. The mitogenic effects extend the proliferation period and irreversibly alter the phenotype development. Exposure of proliferating osteoblasts to the active metabolite of vitamin D expression inhibits proliferation and downregulates collagen and alkaline phosphatase, blocking differentiation. (B) Induction of differentiation by exposure of proliferating osteoblasts to glucocorticoids, represented by dexamethasone (DEX) or by acute exposure of the cultures in the post-proliferative period to 1,25(OH)2D3 (D3). (C) The deregulation of the relationship between growth and differentiation in transformed osteoblasts or osteosarcoma cells is illustrated. The proliferation vector reflects the continuous expression of cell growth and expression of cell cycle- and proliferation-related genes. In contrast to normal diploid cells, the constitutive expression of osteoblast differentiation phenotype markers in transformed cells in the absence of the two developmentally important transition points (cessation of proliferation and mineralization) observed in normal diploid cells. In osteosarcoma cells, cell growth and tissue-specific gene expression occur concomitantly.**

6). Here the down-regulation of collagen and alkaline phosphatase gene expression by vitamin D prevents formation and mineralization of the bone extracellular matrix. Thus, there is a consequential absence of expression of the mature osteoblast phenotype genes such as osteocalcin and osteopontin which are coupled to mineral deposition.

The proliferating osteoblast *in vitro* (analogous to the osteoprogenitor cell *in vivo*) is highly responsive to a phenotype modification, as also illustrated by the effects of glucocorticoids[24]. Glucocorticoids promote differentiation of progenitor cells to the osteoblast phenotype[86] and accelerate the differentiation time course of rat osteoblasts *in vitro*. Dexamethasone, a synthetic glucocorticoid, increases the number of mineralized bone nodules in primary fetal rat calvarial osteoblast cultures[15]. In subcultivated cells, the effect is even more pronounced. Glucocorticoids stimulate the growth of cells competent to progress through the osteoblast developmental sequence. In the absence of dexamethasone, passaged osteoblasts lose the ability to differentiate. This responsiveness diminishes when cells reach confluency indicating the importance in targeting the proliferating cell. However, there is a limited capacity of the cells for this reinitiation of osteoblast growth and differentiation. It is likely that glucocorticoids push the cells to terminal differentiation, depleting the pool of proliferating uncommitted cells.

The molecular mechanisms associated with glucocorticoid mediated osteoblast differentiation are not clear[153,167]. Early response genes (e.g., cfos) are induced. Osteopontin responds rapidly and expression is increased by dexamethasone during the proliferation period. Alkaline phosphatase and osteocalcin are upregulated parallel to an increase in the number of bone nodules and earlier induction of mineralization compared to non-dexamethasone treated cultures. Whether some of the relationships between genes that are coupled or functionally related have been altered due to direct modulation by glucocorticoids or whether the accelerated differentiation is secondary to other modifications in osteoblast developmental parameters remains to be established.

TGFβ1 is a principal mediator of cell growth and differentiation[reviewed in 27] which regulates bone matrix formation and turnover *in vivo*[14,109,124]. However, TGFβ1 has been shown to inhibit, in a dose dependent manner, development of the osteoblast phenotype in cultures of chick periosteal cells[73] and fetal rat calvarial derived cells[3,20,60]. This observation appears inconsistent with (a) several *in vivo* studies demonstrating local application of TGFβ1 stimulates bone formation and (b) knowledge that osteoblasts produce TGFβ1 [147,131]. The discrepancy is easily reconciled. *In vivo*, numerous cell types are present in the local bone environment that are targets for TGFβ1. Synergistic effects on local factors produced by non-osseous cells that stimulate osteoblast activity are not present in the more homogeneous osteoblast preparations isolated for *in vitro* studies. Secondly, *in vitro*, TGFβ1 mRNA levels are highest during the proliferating period and downregulated post-proliferatively during nodule formation[131]. Another level of regulation is production of protein in a latent form[32]. Thus, the addition of active TGFβ1 peptide to bone cells *in vitro* reveals effects on the osteoblast phenotype not appreciated from *in vivo* studies. Harris et al.[60] (1994) reported that TGFβ1 also inhibited

BMP2 expression in these fetal rat calvarial cell cultures implicating this bone morphogenic protein, as well as the bone matrix related proteins, as components of the TGFβ1 inhibition of osteoblast differentiation. Post-proliferative osteoblasts and second passage cells that are accelerated to differentiate by dexamethasone are refractory to the inhibitory effects of TGFβ1[20] emphasizing the window of responsiveness to physiologic mediators for phenotype modifications in proliferating osteoblasts.

### B. Selective Responses are Related to the Stage of Osteoblast Maturation

Not only do hormones and growth factors influence the osteoblast growth and differentiation relationship, but responsiveness of a particular gene to a physiologic regulator is a function of the developmental stage of the osteoblast. This is illustrated by several examples. Several studies reported differential stimulation of alkaline phosphatase by vitamin D, dependent upon the basal level of expression in the ROS 17/2.8 cell[44,107,159]; $1,25(OH)_2D_3$ exerts either stimulatory or inhibiting effects on collagen synthesis in various cell lines and culture systems[26,41,85]. In the normal diploid rat osteoblast cultures at different stages of phenotype development, from the proliferation period to the mature osteocyte-like cell in the mineralized matrix, we observed pleiotropic effects on several vitamin D regulated genes between immature and differentiated osteoblasts[50,133]. For example, histone H4, collagen, and alkaline phosphatase are down-regulated in proliferating osteoblasts, but in mature osteoblasts in a mineralized matrix, an upregulation is observed. The selective effects of the hormone are illustrated by a change in morphology of osteoblasts on the surface of a mineralized nodule, with no effect on internodular cells[140]. This observation may be relevant to the selective functional activities of osteoblast populations *in vivo*. *In vivo* $1,25(OH)_2D_3$ can promote differentiation of osteoclasts and mediates bone resorption through activities on the osteoblast. Lining cell osteoblasts must retract from the bone surface and secrete factors for osteoclast activation. Other osteoblasts need to be involved in bone formation activities. The release of local active growth factors or cytokines to stimulate osteoblast proliferation is one such mechanism to increase or recruit a local osteoblast population to the resorption site.

Confirmation of this concept of developmental responsiveness of the osteoblast is also observed in relation to glucocorticoid effects[153,175]. Osteopontin and alkaline phosphatase are increased 20-30 fold greater in alkaline phosphatase negative cells compared to mature osteoblasts. Growth factors also exhibit differential effects in modulating cellular activities depending on the cell phenotype, stage of maturation and level of gene expression[25,54,145]. Fibroblast growth factor (αFGF) can stimulate bone formation *in vivo*[113,120], and *in vitro* can promote osteoblast differentiation of marrow progenitor cells in conjunction with dexamethasone[125] or bone organ culture[25]. However, exposure of osteoblast cultures to αFGF can block differentiation via its mitogenic effects and induction of collagenase[122,145,169] and inhibit collagen expression in mature MC3T3-E1 cells[70].

In summary, these findings support the concepts that (1) a proliferating osteoblast-like cell is selectively sensitive to phenotype alterations by steroid hormones and growth factors; and (2) genes exhibit developmental stage-specific responses reflecting subtle differences in the cohort of transcription factor complexes regulating the cognate promoter regulatory elements. Glucocorticoids, $1,25(OH)_2D_3$ and TGFβ have irreversible and sustained effects that modify the progression of differentiation of proliferating cells of the osteoblast lineage. Several questions are raised regarding the relevance of these *in vitro* observations to physiologic and therapeutic effects *in vivo*. Considerations need to be given to osteoblast subpopulations as well as to dose and timing or duration of hormone exposure. More fundamental questions are related to defining the specific mechanisms operative in proliferating cells that allow for such dramatic phenotypic alterations. Possibilities include the involvement of early response genes and modifications in transcription factors by hormones and growth factors, e.g. the oncogenes[94,95,132], homeobox genes[66,101,173] and helix-loop-helix proteins[118,129,168]. Each of these classes of transcription factors is regulated by steroid hormones and modulates bone-specific gene expression reflecting a cascade of mechanisms contributing to phenotypic modifications.

## IV. ADDRESSING MOLECULAR MECHANISMS MEDIATING OSTEOBLAST GROWTH AND DIFFERENTIATION

Proliferation is a fundamental requirement for developmental establishment and renewal of tissues. A broad spectrum of signalling mechanisms, which integrate and amplify growth-related regulatory cues, regulate the expression of genes requisite for proliferation and cell cycle control. Equally important are the regulatory cues in proliferating cells that allow for exit from the cell cycle and commitment to a phenotypic lineage. Consequently, the modularly organized promoter elements of genes which support the onset and progression of proliferation provide a blueprint for growth control within the context of responsiveness to mediators of proliferative status. These include, but are not restricted to, cytokines, growth factors and steroid hormones. To define these molecular mechanisms, we have focused on characterization of the transcription factors regulating the promoters of two genes; the histone gene which is tightly coupled to DNA synthesis and the osteocalcin gene which is developmen-

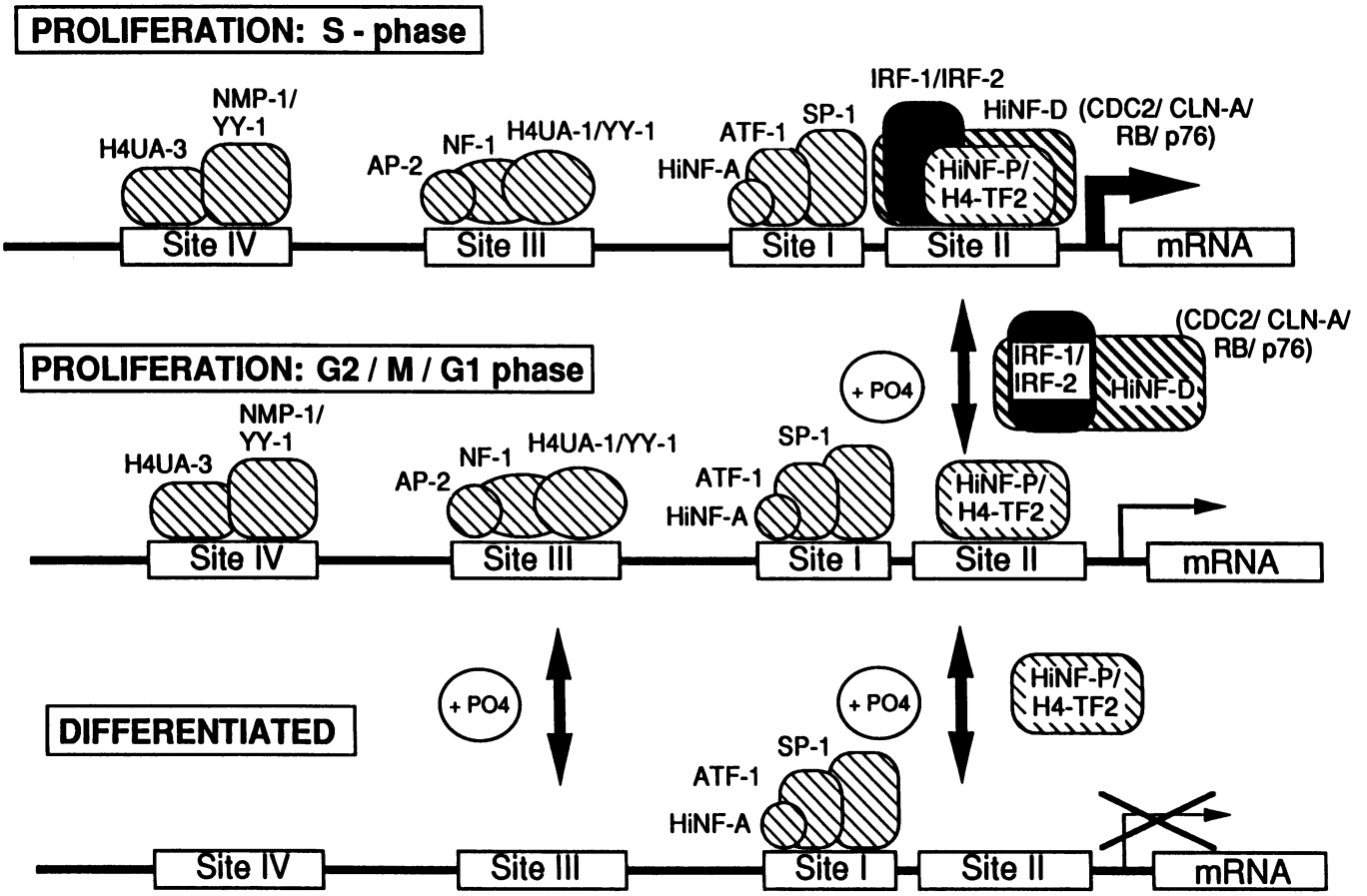


**Fig. 7. Regulation of histone gene expression during osteoblast growth and differentiation. Organization of the human histone H4 gene promoter regulatory elements (Sites I-IV) is illustrated. The transcription factors which exhibit sequence specific interactions with these domains are indicated during the S phase of the cell cycle when the gene is actively transcribed (top panel). Site II contributes to cell cycle regulation of transcription, Site IV binds a nuclear matrix protein complex (NMP-1), while the protein-DNA interactions at Sites III and I support general transcriptional enhancement. The Site II transcription factor complex is modified by phosphorylation during the G1/G2/ mitotic periods of the cell cycle resulting in lower levels of transcription. The lower panel (differentiated cells) illustrates complete loss of transcription factor complexes at Sites II, III, and IV following exit from the cell cycle with the onset of differentiation. Assembly of the Site II complex during S phase involves cyclin A, the cyclin dependent kinase cdc2, an RB related protein and IRF2 growth regulatory factors, reflecting integration of phosphorylation mediated control of histone gene expression.**

tally regulated and a tissue specific marker of differentiation.

The regulatory sequences are established in gene promoters by one or more criteria that include: (1) demonstration of an influence on transcriptional activity by deletion, substitution or site specific mutagenesis *in vitro* and *in vivo*; (2) identification and characterization of sequence specific regulatory element occupancy by cognate transcription factors *in vitro* and *in vivo*; (3) modifications in protein-DNA interactions as a function of biological activity; and (4) consequential modifications in functional activity following overexpression or suppression of factors which exhibit sequence-specific recognition for regulator domains.

### A. The Histone Gene Promoter is a Paradigm for the Integration of Regulator Signals Mediating Cell Cycle Control and Proliferation/ Differentiation Interrelationships.

From a biological perspective, an understanding of growth control necessitates defining the stringent regulation of molecular parameters associated with competency for initiation of proliferation and progression through the cell cycle[12]. Regulatory parameters have been defined that mediate both initiation of proliferation and cell cycle progression[155]. The emerging concept is that the cyclins and cyclin-dependent kinases are responsive to regulation by the phosphorylation-dependent signalling pathways associated with activities of the early response genes which are upregulated following mitogen-stimulation of proliferation[reviewed in 71,69,80,106]. Cyclin-dependent phosphorylation is functionally linked to activation and suppression of both p53 and Rb-related tumor suppressor genes which

mediate transcriptional events involved with passage into S phase. The activities of the cyclin-dependent kinases are downregulated by a series of inhibitors designated CDI's and mediators of ubiquitination which signal destabilization and/or destruction of these regulatory complexes in a cell cycle-dependent manner. Particularly significant is the accumulating evidence for functional interrelationships between activities of cyclin-cdk complexes and growth arrest at G1 and G2 checkpoints where competency for cell cycle traverse is monitored[37,38,128,156], editing functions are operative, and decisions for continued proliferation, growth arrest or apoptotic cell death are executed[188].

The histone gene promoter is a paradigm for understanding molecular mechanisms related to cell cycle-mediated transcriptional control[reviewed in 160,162]. Transcription of the gene is constitutive throughout the cell cycle, upregulated at the onset of S phase and completely suppressed in quiescent cells or following the onset of differentiation[13,36] (Figure 7). Consequently, activity of the promoter is responsive to regulatory signals which contribute to transcriptional competency for cell cycle progression at the G1/S phase transition point and to transcriptional downregulation postproliferatively. The modularly organized promoter regulatory elements of the histone H4 gene promoter and the cognate transcription factors have been characterized within the context of cell cycle dependent regulatory parameters[138,143,176,177,179,180] (Figure 7). There is a direct indication that the cell cycle regulatory element exhibits phosphorylation-dependent modifications in transcription factor interactions which parallel and are functionally related to cell cycle as well as growth control of histone gene expression. The S phase transcription factor complexes assembling at the H4 promoter include cdc2, cyclin A, and RB related protein and IRF-2[181,183], reflecting an integration of phosphorylation-mediated control of histone gene expression, enzymes involved in DNA replication, as well as growth stimulation and growth suppression at the G1/S phase transition point.

### B. The Osteocalcin Gene is a Paradigm for Integration of Regulator Signals Mediating Developmental and Hormonal Regulation of Bone Formation.

Influences of promoter regulatory elements that are responsive to basal and tissue-restricted transactivation factors, steroid hormones, growth factors and other physiologic mediators has provided the basis for understanding regulatory mechanisms contributing to developmental expression of osteocalcin, tissue specificity and biological activity. These regulatory elements and cognate transcription factors support postproliferative transcriptional activation and steroid hormone (e.g. vitamin D) enhancement at the onset of extracellular matrix mineralization during osteoblast differentiation. The bone-specific osteocalcin gene is organized in a manner which supports responsiveness to homeostatic physiologic mediators and developmental expression in relation to bone cell differentiation (Figure 8).

A series of elements contributing to basal expression[91] include a TATA sequence (located at -42 to -39) and the osteocalcin box (OC box), a 24 nucleotide element with a CCAAT motif as a central core. Both are required for rendering the gene transcribable[62,77,172,173]. The OC box is a highly conserved regulatory sequence required for basal expression of the rat, mouse and human OC genes. The OC box additionally serves a regulatory function in defining the threshold for initiation of transcription and contributes to bone tissue-specific expression of the osteocalcin gene[62]. However caution must be exercised in attributing tissue-specific transcriptional control to a single element. Contributions of multiple sequences appear to be operative in tissue-specific regulation thereby providing opportunities for expression of the osteocalcin gene in bone under diverse biological circumstances.

Two osteocalcin gene promoter regulatory domains which exhibit recognition for transcription factors that mediate developmental pattern formation are an MSX homeodomain protein binding site within the OC box[62,66,173] and an AML runt homology sequence protein binding site[161,182]. The AML sites also bind an osteoblast restricted nuclear matrix protein complex (NMP-2),[17] and our preliminary studies indicate an AML related protein is present in osteoblasts[161,182]. These sequences may represent components of regulatory mechanisms that contribute to pattern formation associated with bone tissue organization during initial developmental stages and subsequently during tissue remodelling. Involvement of other homeodomain-related genes that are expressed during skeletal development in control of osteoblast proliferation and differentiation is worthy of consideration. These include, but are not restricted to, the families of Dlx and PAX[59,83,101,157] genes.

Multiple glucocorticoid responsive elements (GRE) with sequences that exhibit both strong and weak affinities for glucocorticoid receptor binding have been identified in the proximal promoter[5,63,166]. Interactions of other transcription factors with the proximal glucocorticoid responsive elements which include NF-IL6 have been reported[174], further expanding the potential of the OC gene to be transcriptionally regulated by glucocorticoids. A distal GRE (nt -697 to -683) is in close proximity to an AP-1 site. It is reasonable to consider that the OC gene GRE'S may be selectively utilized in a developmentally and/or physiologically responsive manner.

The vitamin D responsive element (VDRE) functions as an enhancer[20,34,79,111,117,171]. The VDRE transcription

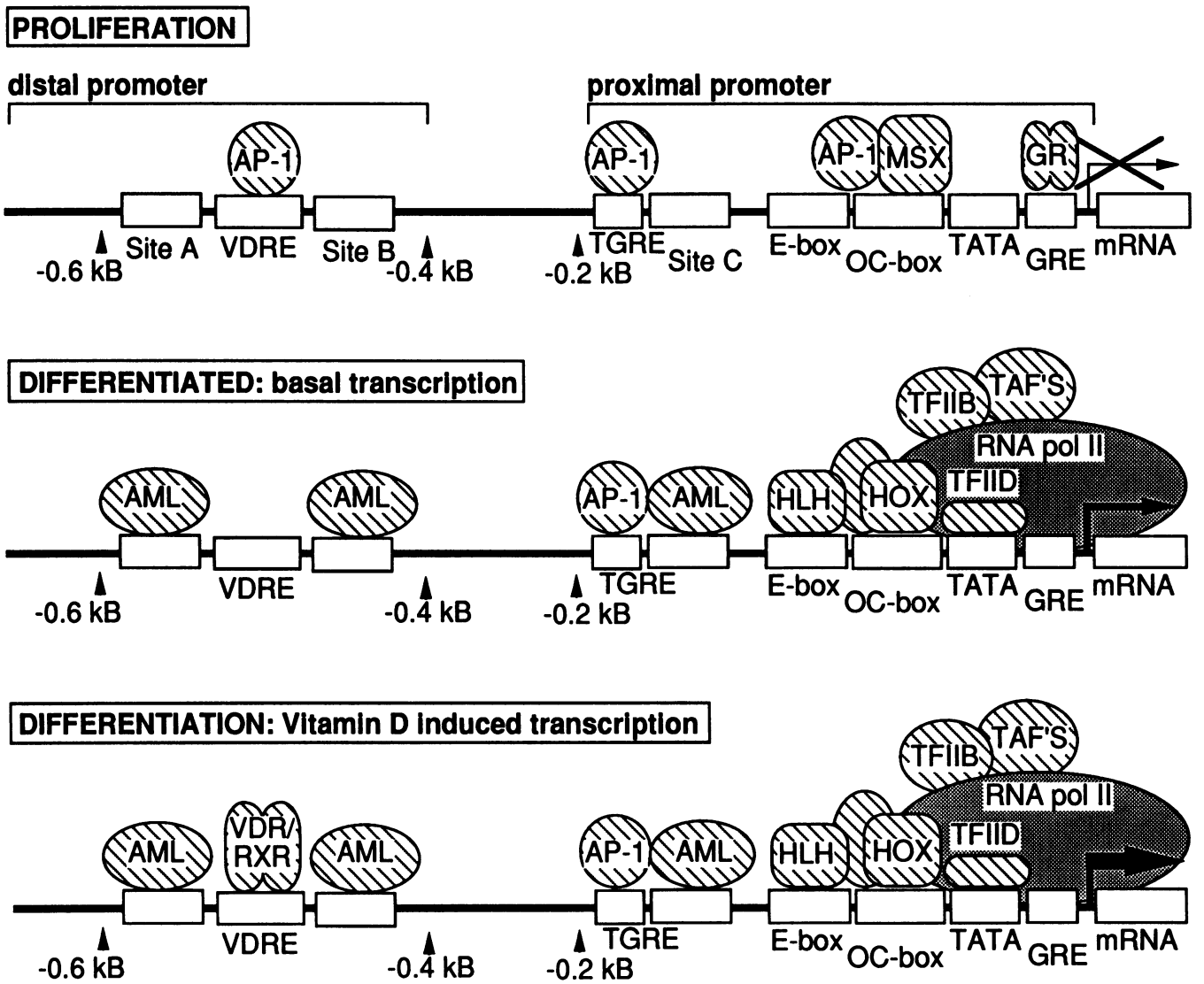


**Fig. 8. Regulation of osteocalcin gene expression during osteoblast growth and differentiation. The regulatory domains within the initial 700 nucleotides 5' to the transcription start are indicated. In the proximal promoter, several classes of transcription factors are represented which bind to key regulatory elements. The OC Box is the primary tissue specific transcriptional element that binds both homeodomain proteins (MSX), oncogene encoded proteins forming heterodimers at AP-1 sites or a cryptic tissue-specific complex (not shown). Helix-loop-helix protein (HLH) bind to the contiguous E Box motif. Three nuclear matrix protein binding Sites (A, B and C) interact with an AML related transcription factor. Several glucocorticoid response elements (GRE) are indicated as well as the vitamin D response element (VDRE), which is a primary enhancer element. Additional AP-1 sites are indicated that overlap either the TGFb response element (TGRE) or the VDRE. The combined and integrated activities of overlapping regulatory elements and associated transcription factors provide a mechanism for developmental control of expression during osteoblast growth and differentiation. Occupancy of the elements by cognate transcription factors parallels and is functionally related to either (a) suppression of transcription in proliferating osteoblasts (top panel); (b) activation of expression in differentiated cells (middle); and (c) enhancement of transcription by vitamin D (lower panel).**

factor complex appears to be a target for modifications in vitamin D mediated transcription by other physiologic factors including TNF-$\alpha$[84] and retinoic acid[18,82,105,149,150]. Additional regulatory sequences include an interferon response domain[121], an NFkB site reported to be involved in regulation mediated by TNF$\alpha$,[88] a series of AP-1 sites[35,94,137] (one of which mediates TGF$\beta$ responsiveness[7]), an E box[168] that binds HLH containing transcription factor complexes, and a sequence in the proximal promoter that binds a multi subunit complex containing CP1/NR-Y/CBF-like CAAT factor complex[174].

The overlapping and contiguous organization of regulatory elements, as illustrated by the TATA/GRE, E Box/AP-1/CCAAT/homeodomain, and TNFa/VDRE AP-1/VDRE, provide a basis for combined activities that support post-proliferative expression and responsiveness to physiologic mediators[reviewed in 96-98]. Additionally, hormones modulate binding of transcription factors other than the cognate receptor to non-steroid regulatory sequences. For example, vitamin D induced interactions occur at the basal TATA domain,[134] and $1,25(OH)_2D_3$ upregulates MSX-2 binding to the OC box homeodomain motif and supports increased MSX-2 expression[65,66,173]. It is this complexity of OC gene promoter element upregulation that allows for hormone responsiveness in relation to either basal levels of expression or other activities.

## V. DEREGULATION OF GROWTH-DIFFERENTIATION RELATIONSHIP IN TRANSFORMED OR OSTEOSARCOMA CELLS

In several transformed osteoblasts and osteosarcoma cell lines such as the ROS 17/2.8 rat osteosarcoma cell line, there is a deregulation of the sequential pattern of gene expression observed in diploid osteoblasts. A relaxation of control mechanisms permits sequentially expressed genes in diploid osteoblasts to be expressed simultaneously during proliferation, as reflected by concomitant expression of the histone H4 gene and tissue specific genes including alkaline phosphatase, osteopontin, and osteocalcin[164]. It should be emphasized that, while in normal diploid osteoblasts some genes (e.g. osteocalcin, osteopontin) are expressed or induced to high levels only at the onset of extracellular matrix mineralization, the genes in osteosarcoma cells are expressed in the absence of a bone-like extracellular matrix. Perturbations are implicated in the signalling mechanisms that interface the down-regulation of cell growth and induction of genes that support extracellular matrix maturation and specialization with those that control gene expression associated with extracellular matrix mineralization. Our understanding of how gene responsiveness to regulators of osteoblast growth and differentiation is compromised in osteosarcoma cells can thereby provide insight into molecular mechanisms that are deregulated.

Transcriptional control of the H4 histone gene is strikingly modified in osteosarcoma cells. An abrogation of the cell cycle and proliferation-regulated changes in transcription factor binding to the histone promoter elements, most notably at the cell cycle regulatory element by nuclear extracts from tumor cells, is observed[67]. Enzymatic dephosphorylation of nuclear extracts results in loss of HiNF-D-site II interactions and increased electrophoretic mobility of the site III protein-DNA complex in normal cells. Therefore, involvement of phosphorylation at these two histone gene promoter elements is implicated[81]. Indeed, specific phosphorylation events (kinase activities) play a key role in cell cycle progression e.g. cdc2 regulation of the cyclin gene[127]. Thus, for a tumor cell, continual traverse of the cell cycle may be associated with modifications in the level of transcription factor phosphorylation or the utilization of specific phosphorylation sites. The observations are not unique to the ROS 17/2.8 cell, but a comparison of several tumor cell lines revealed similar differences when compared to their normal diploid counterparts. The identical relationship has been shown in WI38 (normal fibroblasts) compared to the transformed HeLa cell line[177].

The post-proliferative and developmental expression of osteocalcin in normal diploid osteoblasts and its regulation by steroid hormones only when osteocalcin expression is ongoing postproliferatively has been well documented. We have proposed that AP-1 sites which overlap the basal regulatory CAAT-containing element, the OC box, and the vitamin D responsive element (VDRE), bind the nuclear proto-oncogene encoded fos-jun protein complex as a mechanism by which osteocalcin expression and vitamin D modulation is suppressed in normal actively dividing cells[94,133]. In ROS 17/2.8 cells, osteocalcin expression occurs in the presence of AP-1 activity in contrast to the diploid cells. To understand osteocalcin expression in proliferating tumor cells, we have examined the transcription factor complexes at basal regulatory sequences and at the vitamin D responsive element of the osteocalcin gene promoter. In normal diploid osteoblasts, nuclear extracts from proliferating cells and in post-proliferative osteocalcin expressing cells exhibit differences in protein-DNA interactions. In contrast, the tumor cell nuclear extracts from proliferating or confluent cultures are similar and support protein-DNA interactions that are strikingly different than those of the normal osteoblasts[18]. These differences are found at each of the basal elements and the VDRE[20,151].

We can speculate that the deregulation of growth control in osteosarcoma cells may reflect modifications in the activity of tumor suppressor genes[reviewed by 87,186]. Alternatively, or together with the loss of stringent growth control, there may be modifications in the regulatory sequences and/or in the factors that control the

progressive expression of tissue-specific genes and their response to cell growth or morphogenic regulatory factors. While the specific molecular mechanisms remain to be established, by further understanding the deregulation of growth in bone tumors, we can anticipate gaining additional insight into control of the tightly coupled relationship between proliferation and development of the osteoblast phenotype.

## VI. ACKNOWLEDGEMENTS

Studies reported from the authors' laboratories were supported by grants from the National Institutes of Health (AR33920, AR35166, AR39588, GM32010, AR42262) and the March of Dimes Birth Defects Foundation.

## REFERENCES

[1] Abbott, B.D.; Birnhaum, L.L.S.: TCDD Alters Medial Epithelial Cell Differentiation During Palatogenesis. Toxicol. Appl. Pharmacol., 99:276-288, 1989.

[2] Andrianarivo, A.G.; Robinson, J.A.; Mann, K.G. and Tracy, R.P.: Growth on Type I Collagen Promotes Expression of the Osteoblast Phenotype in Human Osteosarcoma MG-63 Cells. J. Cell. Physiol., 153:256-265, 1992.

[3] Antosz, M.E.; Bellows, C.G. and Aubin, J.E.: Effects of Transforming Growth Factor b and Epidermal Growth Factor on Cell Proliferation and the Formation of Bone Nodules in Isolated Fetal Rat Calvaria Cells. J. Cell. Physiol., 140:386-395, 1989.

[4] Aronow, M.A.; Gerstenfeld, L.C.; Owen, T.A.; Tassinari, M.S.; Stein, G.S. and Lian, J.B.: Factors that Promote Progressive Development of the Osteoblast Phenotype in Cultured Fetal Rat Calvaria Cells. J. Cell. Physiol., 143:213-221, 1990.

[5] Aslam, F.; Shalhoub, V.; van Wijnen, A.J.; Banerjee, C.; Bortell, R.; Shakoori, A.R.; Litwack, G.; Stein, J.L.; Stein, G.S. and Lian, J.B.: Contributions of Distal and Proximal Promoter Elements to Glucocorticoid Regulation of Osteocalcin Gene Transcription. Mol. Endocrin., in press.

[6] Aubin, J.E.; Heersche, J.N.M.; Merriless, M.J. and Sodek, J.: Isolation of Bone Cell Clones with Differences in Growth, Hormone Responses and Extracellular Matrix Production. J. Cell Biol., 92:452-461, 1982.

[7] Banerjee, C.; Stein, J.L.; van Wijnen, A.J.; Kovary, K.; Bravo, R.; Lian, J.B. and Stein, G.S.: Identification of a TGF-b1 Response Element in the Rat Osteocalcin Gene. J. Bone Min. Res., 9:S161 (Abstract), 1994.

[8] Barone, L.M.; Tassinari, M.S.; Bortell, R.; Owen, T.A.; Zeroogian, J.; Gagne, K.; Stein, G.S. and Lian, J.B.: Inhibition of Induced Endochondral Bone Development in Caffeine-Treated Rats. J. Cell. Biochem., 52:171-182, 1993.

[9] Barone, L.M.; Owen, T.A.; Tassinari, M.S.; Bortell, R.; Stein, G.S. and Lian, J.B.: Developmental Expression and Hormonal Regulation of the Rat Matrix Gla Protein (MGP) Gene in Chondrogenesis and Osteogenesis. J. Cell. Biochem., 46:351-365, 1991a.

[10] Barone, L.M.; Boyd, B.; Bettencourt, B.; Minkin, C.; Stein, G.S. and Lian, J.B.: Osteoclcin (OC), a Bone Specific Protein, Mediates Differentiation of Osteoclasts in Two Model Systems. J. Cell Biol., 115:448A, 1991b.

[11] Barone, L.M.; Aronow, M.A.; Canalis, E.; Stein, G.S. and Lian, J.B.: Differential Effects of Warfarin on mRNA Levels of Developmentally Regulated Vitamin K Dependent Proteins, Osteocalcin and Matrix Gla Protein, In Vitro. J. Cell. Physiol., 160:255-264, 1994.

[12] Baserga, R. and Rubin, R.: Cell Cycle and Growth Control. Crit. Rev. Eukary., Gene Exp., 3:47-61, 1993.

[13] Baumbach, L.; Marashi, F.; Plumb, M.; Stein, G.S. and Stein, J.L.: Inhibition of DNA Replication Coordinately Reduces Cellular Levels of Core and H1 Histone mRNAs: Requirement for Protein Synthesis. Biochem., 23,1618-1625, 1984.

[14] Beck, L.S.; Amento, E.P.; Xu, Y.; Deguzman, L.; Lee, W.P.; Nguyen, T. and Gillett, N.A.: TGF-b1 Induces Bone Closure of Skull Defects: Temporal Dynamics of Bone Formation in Defects Exposed to rhTGF-b1. J. Bone Min. Res., 8:753-761, 1993.

[15] Bellows, C.G.; Aubin, J.E. and Heersche, J.N.M.: Physiological Concentrations of Glucocorticoids Stimulate Formation of Bone Nodules from Isolated Rat Calvaria Cells In Vitro. Endocrinology, 121:1985-1992, 1987.

[16] Bhargava, U.; Bar-Lev, M.; Bellows, C.G. and Aubin, J.E.: Ultrastructural Analysis of Bone Nodules Formed In Vitro by Isolated Fetal Rat Calvaria Cells. Bone, 9:155-163, 1988.

[17] Bidwell, J.P.; van Wijnen, A.J.; Fey, E.G.; Dworetzky, S.; Penman, S.; Stein, J.L.; Lian, J.B. and Stein, G.S.: Osteocalcin Gene Promoter Binding Factors are Tissue-Specific Nuclear Matrix Components. Proc. Natl. Acad. Sci. USA, 90:3162-3166, 1993.

[18] Bortell, R.; Owen, T.A.; Shalhoub, V.; Heinrichs, A.; Aronow, M.A.; Rochette-Egly, C.; Lutz, Y.; Stein, J.L.; Lian, J.B. and Stein, G.S.: Constitutive Transcription of the Osteocalcin Gene in Osteosarcoma Cells is Reflected by Altered Protein/DNA Interactions at Promoter Regulatory Elements. Proc. Natl. Acad. Sci. USA, 90:2300-2304, 1993.

[19] Boskey, A.L.; Wians Jr., F.H, and Hauschka, P.V.: The Effect of Osteocalcin on In Vitro Lipid-Induced Hydroxyapatite Formation and Seeded Hydroxyapatite Growth. Calc. Tiss. Int., 37:57-62, 1985.

[20]Breen, E.C.; van Wijnen, A.J.; Lian, J.B.; Stein, G.S. and Stein, J.L.: In Vivo Occupancy of the Vitamin D Responsive Element in the Osteocalcin Gene Supports Vitamin D Dependent Transcriptional Upregulation in Intact Cells. Proc. Natl. Acad. Sci. USA, 91:12902-12906, 1994.

[21]Bruder, S.P. and Caplan, A.I.: Discrete Stages within the Osteogenic Lineage are Revealed by Alterations in the Cell Surface Architecture of Embryonic Bone Cells. Conn. Tissue Res., 20:073-79, 1989.

[22]Buckley, M.J.; Banes, A.J. and Jordan, R.D.: The Effects of Mechanical Strain on Osteoblasts In Vitro. J. Oral & Maxillofacial Surg., 48:276-282, 1990.

[23]Canalis, E.; Pash, J. and Varghese, S.: Skeletal Growth Factors. Crit. Rev. Eukary. Gene Exp., 3:155-156, 1993.

[24]Canalis, E.: Effect of Glucocorticoids on Type I Collagen Synthesis, Alkaline Phosphatase Activity and Deoxyribonucleic Acid Content in Cultured Rat Calvariae. Endocrinology, 112:931-939, 1983.

[25]Canalis, E.; Centrella, M. and McCarthy, T.: Effects of Basic Fibroblast Growth Factor on Bone Formation In Vitro. J. Clin. Invest., 81:1572-7, 1988.

[26]Canalis, E. and Lian, J.B.: 1,25(OH)2D3 Effects on Collagen and DNA Synthesis in Periosteum and Periosteum-Free Calvariae. Bone, 6:457-460, 1985.

[27]Centrella, M.; Horowitz, M.C.; Wozney, J.M. and McCarthy, T.L.: Transforming Growth Factor-b Gene Family Members and Bone. Endocr. Rev., 15:27-39, 1994.

[28]Cheng, S.L.; Yang, J.W.; Rifas, L.; Zhang, S.F. and Avioli, L.: Differentiation of Human Bone Marrow Osteogenic Stromal Cells In Vitro: Induction of the Osteoblast Phenotype by Dexamethasone. Endocrinol., 134:277-286, 1994.

[29]Chenu, C.; Colucci, S.; Grano, M.; Zigrino, P.; Barattolo, R.; Zambonin, G.; Baldini, N.; Vergnaud, P.; Delmas, P.D. and Zallone, A.Z.: Osteocalcin Induces Chemotaxis, Secretion of Matrix Proteins, and Calcium-Mediated Intracellular Signaling in Human Osteoclast-like Cells. J. Cell Biol., 127:1149-1158, 1994.

[30]Collart, D.; Ramsey-Ewing, A.; Bortell, R.; Lian, J.; Stein, J. and Stein, G.: Isolation and Characterization of a cDNA from a Human Histone H2B Gene which is Reciprocally Expressed in Relation to Replication-Dependent H2B Histone Genes during HL60 Cell Differentiation. Biochem., 30:1610-1617, 1991.

[31]Craig, A.M.; Smith, J.H. and Denhardt, D.T.: Osteopontin, a Transformation-Associated Cell Adhesion Phosphoprotein, is Induced by 12-0-Tetradecanoylphorbol 13-Acetate in Mouse Epidermis. J. Biol. Chem., 264:9682-9689, 1989.

[32]Dallas, S.L.; Park-Snyder, S.; Miyazono, K.; Twardzik, D.; Mundy, G.R. and Bonewald, B.L.: Characterization and Autoregulation of Latent Transforming Growth Factor b (TGFb) Complexes in Osteoblast-like Cell Lines: Production of a Latent Complex Lacking the Latent TGFb-Binding Protein (LTBP). J. Biol. Chem., 269:6815-21, 1994.

[33]DeFranco, D.J.; Glowacki, J.; Cox, K.A. and Lian, J.B.: Normal Bone Particles Are Preferentially Resorbed in the Presence of Osteocalcin-Deficient Bone Particles In Vivo. Calcif. Tissue Int., 49:43-50, 1991.

[34]Demay, M.B.; Gerardi, J.M.; DeLuca, H.F. and Kronenberg, H.M.: DNA Sequences in the Rat Osteocalcin Gene that Bind the 1,25-Dihydroxyvitamin D3 Receptor and Confer Responsive to 1,25-Dihydroxyvitamin D3. Proc. Natl. Acad. Sci. USA, 87:369-373, 1990.

[35]Demay, M.B.; Kiernan, M.S.; DeLuca, H.F. and Kronenberg, H.M.: Characterization of 1,25-Dihydroxyvitamin D3 Receptor Interactions with Target Sequences in the Rat Osteocalcin Gene. Molec. Endocrinol., 6:557-562, 1992.

[36]Detke, S.; Lichtler, A.; Phillips, I.; Stein, J. and Stein, G.: Reassessment of Histone Gene Expression during the Cell Cycle in Human Cells by using Homologous H4 Histone cDNA. Proc. Natl. Acad. Sci. USA, 76:4995-4999, 1979.

[37]Dou, Q.; Markell, P.J. and Pardee, A.B.: Retinoblastoma-like Protein and cdc2 Kinase are in Complexes that Regulate a G1/S Event. Proc. Natl. Acad. Sci USA, 89:3256-3260, 1992.

[38]Dowdy, S.F.; Hinds, P.W.; Louie, K.; Reed, S.I.; Arnold, A. and Weinberg, R.A.: Physical Interaction of the Retinoblastoma Protein with Human D Cyclins. Cell, 73:499-511, 1993.

[39]Feteih, R.; Tassinari, M.S. and Lian, J.B.: Effects of sodium Warfarin on Development of the Rat Fetus and Vitamin K-Dependent Proteins. J. Bone Min. Res., 5:885-894, 1990.

[40]Fisher, L.W.; Hawins, G.R.; Turcoss, N. and Termine, J.D.: Purification and Partial Characterization of Small Proteoglycans I and II, Bone Sialoproteins I and II, and Osteonectin from the Mineral Compartment of Developing Human Bone. J. Biol. Chem., 262:9702-9708, 1987.

[41]Franceschi, R.T.; Romano, P.R. and Park, K.Y.: Regulation of Type I Collagen Synthesis by 1,25-Dihydroxyvitamin D3 in Human Osteosarcoma Cells. J. Biol. Chem., 263:18938-18945, 1988.

[42]Franceschi, R.T. and Young, J.: Regulation of Alkaline Phosphatase by 1,25-Dihydroxyvitamin D3 and Ascorbic Acid in Bone-Derived Cells. J. Bone Min. Res., 5:1157-1167, 1990.

[43]Franzen, A. and Heinegard, D.: Isolation and Characterization of Two Sialoproteins Present only in Bone Calcified Matrix. Biochem. J., 232:715-724, 1985.

[44]Fraser, J.D. and Price, P.A.: Lung, Heart, and Kidney Express High Levels of mRNA for the Vitamin K-Dependent Matrix Gla Protein: Implications for the Possible Functions of Matrix Gla Protein and for the Tissue Distribution of the g-Carboxylase. J. Biol. Chem., 263:11033-11036, 1988.

[45]Freid, A.; Benayahu, D. and Weintroub, S.: Marrow Stroma-Derived Osteogenic Clonal Cell Lines: Putative Stages in Osteoblastic Differentiation. J. Cell. Physiol, 155:472-482, 1993.

[46]Friedenstein, A.J. and Chailakhyan, R.K.: Bone Marrow Osteogenic Stem Cells: In Vitro Cultivation and Transplantation in Diffusion Chambers. Cell Tissue Kinet., 20:263-272, 1987.

[47]Gehron-Robey, P.; Young, M.F.; Flanders, K.C.; Roche, N.S.; Kondaiah, P; Reddi, A.H.; Termine, J.D.; Sporn, M.B. and Roberts, A.B.: Osteoblasts Synthesize and Respond to Transforming Growth Factor-Type b (TGF-b) In Vitro. J. Cell Biol., 105:457-463, 1987.

[48]Gerstenfeld, L.C.; Chipman, S.D.; Glowacki, J. and Lian, J.B.: Expression of Differentiated Function by Mineralizing Cultures of Chicken Osteoblasts. Dev. Biol., 122:49-60, 1987.

[49]Gerstenfeld, L.C.; Gotoh, Y.; McKee, M.D.; Nanci, A.; Landis, W.J. and Glimcher, M.J.: Ultrastructural Immunolocalization of a Major 66 kDa Phosphoprotein Synthesized by Chicken Osteoblasts during Mineralization In Vitro. Anatomical Record, 228:3-103, 1990.

[50]Gerstenfeld, L.C.; Broess, M.; Bruder, S.; Caplan, A. and Landis, W.J.: Regulation of Osteoblast Extracellular Matrix Formation: Post-Translational Regulation of Extracellular Matrix Deposition and Relationship Between Embryonic Development and Hormonal Response. In Chemistry & Biology of Mineralized Tissues, pp 287-315, Elsevier Science Publishers B.V., Slavkin H, Price P, 1992.

[51]Ghosh-Choudhury, N.; Harris, M.A.; Feng, J.Q.; Mundy, G.R. and Harris, S.E.: Expression of the BMP 2 Gene during Bone Cell Differentiation. Crit. Rev. Eukary. Gene Exp., 4:345-355, 1994.

[52]Gierthy, J.F.; Silkworth, J.B.; Tassinari, M.; Stein, G.S. and Lian, J.B.: 2,3,7,8-Tetrachlorodibenzo-P-Dioxin Inhibits Differentiation of Normal Diploid Rat Osteoblasts In Vitro. J. Cell. Biochem., 54:231-238, 1994.

[53]Glimcher, M.J.: Mechanism of Calcification: Role of Collagen Fibrils and Collagen-Phosphoprotein Complexes In Vitro and In Vivo. Anatomical Rec., 224:139-153, 1989.

[54]Globus, R.K.; Patterson-Buckendahl, P. and Gospodarowicz, D.: Regulation of Bovine Bone Cell Proliferation by Fibroblast Growth Factor and Transforming Growth Factor b. Endocrinol., 123:98-105, 1988.

[55]Glowacki, J.; Rey, C.; Glimcher, M.J.; Cox, K.A. and Lian, J.B.: A Role for Osteocalcin in Osteoclast Differentiation. J. Cell. Biochem., 45:292-302, 1991.

[56]Glowacki, J. and Lian J.: Impaired Recruitment of Osteoclast Progenitors in Deficient Osteocalcin-Depleted Bone Implants. Cell Differ., 21:247-254, 1987.

[57]Goldring, M.B. and Goldring, S.R.: Basic Science and Pathology: Skeletal Tissue Response to Cytokines. Clin. Ortho. Rel. Res., 258:245-278, 1990.

[58]Grigoriadis, A.E.; Wang, Z.-Q. and Wagner, E.F.: c-fos Oncogene Expression in Cartilage and Bone Tissues of Transgenic and Chimeric Mice. In Cellular and Molecular Biology of the Bone. pp 497-537. Academic Press, New York., 1993

[59]Gruss, P., and Walther, C.: Pax in Development. Cell, 69,719-722, 1992.

[60]Harris, S.E.; Bonewald, L.F.; Harris, M.A.; Sabatini, M.; Dallas, S.; Feng, J.; Ghosh-Choudhury, N.; Wozney, J. and Mundy, G.R.: Effects of Transforming Growth Factor b on Bone Nodule Formation and Expression of Bone Morphogenetic Protein 2, Osteocalcin, Osteopontin, Alkaline Phosphatase and Type 1 Collagen mRNA in Long-Term Cultures of Fetal Rat Calvarial Osteoblasts. J. Bone Min. Res., 9:855-863, 1994.

[61]Hauschka, P.V.; Lian, J.B.; Cole, D.E.C. and Gundberg, C.M.: Osteocalcin and Matrix Gla Protein: Vitamin K-Dependent Proteins in Bone. Physiol. Rev., 990-1047, 1989.

[62]Heinrichs, A.A.J.; Bortell, R.; Bourke, M.; Lian, J.B.; Stein, G.S. and Stein, J.L.: Proximal Promoter Binding Protein Contributes to Developmental, Tissue-Restricted Expression of the Rat Osteocalcin Gene. J. Cell. Biochem., 57:90-100, 1995.

[63]Heinrichs, A.A.J.; Bortell, R.; Rahman, S.; Stein, J.L.; Alnemri, E.S.; Litwack, G.; Lian, J.B. and Stein, G.S.: Identification of Multiple Glucocorticoid Receptor Binding Sites in the Rat Osteocalcin Gene Promoter. Biochem., 32:11436-11444, 1993.

[64]Hock, J.M.; Gunness-Hey, M.; Poser, J.; Olson, H.; Bell, N.H. and Raisz, L.G.: Stimulation of Undermineralized Matrix Formation by 1,25 Dihydroxyvitamin D3 in Long Bones of Rats. Calc. Tiss. Int., 28:79-86, 1986.

[65]Hodgkinson, J.E.; Davidson, C.L.; Beresford, J. and Sharpe, P.T.: Expression of a Human Homeobox-Containing Gene is Regulated by 1,25(OH)2D3 in Bone Cells. Biochim. et Biophys. Acta, 1174:11-16, 1993.

[66]Hoffmann, H.M.; Catron, K.M.; van Wijnen, A.J.; McCabe, L.R.; Lian, J.B.; Stein, G.S. and Stein, J.L.: Transcriptional Control of the Tissue-Specific Developmentally Regulated Osteocalcin Gene Requires a Binding Motif for the MSX-Family of Homeodomain Proteins. Proc. Natl. Acad. Sci. USA, 91:12887-12891, 1994.

[67]Holthuis, J.; Owen, T.A.; van Wijnen, A.J.; Wright, K.L.; Ramsey-Ewing, A.; Kennedy, M.B.; Carter, R.; Cosenza, S.C.; Soprano, K.J.; Lian, J.B.; Stein, J.L. and Stein, G.S.: Tumor Cells Exhibit Deregulation of the Cell Cycle Histone Gene Promoter Factor HiNF-D. Science, 247:1454-1457, 1990.

[68]Howe, A.M. and Webster, W.S.: The Warfarin Embryopathy: A Rat Model Showing Maxillonasal Hypoplasia and Other Skeletal Disturbances. Teratology, 46:379-390, 1992.

[69]Hunter, T. and Pines, J.: Cyclins and Cancer II: Cyclin D and CDK Inhibitors Come of Age. Cell, 79:573-582, 1994.

[70]Hurley, M.M.; Abreu, C.; Harrison, J.R.; Lichtler, A.C.; Raisz, L.G. and Kream, B.E.: Basic Fibroblast Growth Factor Inhibits Type 1 Collagen Gene Expression in Osteoblastic MC3T3-E1 Cells. J. Biol. Chem., 268:5588-5593, 1993.

[71]Hyder, S.M.; Stancel, G.M. and Loose-Mitchell, D.S.: Steroid Hormone-Induced Expression of Oncogene Encoded Nuclear Proteins. Crit. Rev. Eukary. Gene Exp. 4:55-116, 1994.

[72]Ibaraki, K.; Termine, J.D.; Whitson, W.S. and Young, M.F.: Bone Matrix mRNA Expression in Differentiating Fetal Bovine Osteoblasts. J. Bone Min. Res., 7:743-754, 1992.

[73]Iwasaki, M.; Nakata, K.; Nakahara, H.; Nakase, T.; Kimura, T.; Kimata, K.; Caplan, A.L. and Ono, K.: Transforming Growth Factor-beta 1 Stimulates Chondrogenesis and Inhibits Osteogenesis in High Density Culture of Periosteum-Derived Cells. Endocrinol., 132:1603-1608, 1993.

[74]Jackson, M.E.; Shalhoub, V.; Lian, J.B.; Stein, G.S. and Marks, S.C. Jr.: Aberrant Gene Expression in Cultured Mammalian Bone Cells Demonstrates an Osteoblast Defect in Osteopetrosis. J. Cell. Biochem., 55:366-372, 1994.

[75]Johnson, R.S.; Spiegelman, B.M. and Papaioannou, V.: Pleiotropic Effects of a Null Mutation in the c-fos Proto-Oncogene. Cell, 71:577-586, 1992.

[76]Katagiri, T.; Yamaguchi, A.; Komaki, M.; Abe, E.; Takahashi, N.; Ikeda, T.; Rosen, V.; Wozney, J.M.; Fujisawa-Sehara, A. and Suda, T.: Bone Morphogenetic Protein-2 Converts the Differentiation Pathway of C2C12 Myoblasts into the Osteoblast Lineage. J. Cell Biol., 127:1755-1766, 1994.

[77]Kawaguchi, N.; DeLuca, H.F. and Noda, M.: Id Gene Expression and Its Suppression by 1,25-Dihydroxyvitamin D3 in Rat Osteoblastic Osteosarcoma Cells. Proc. Natl. Acad. Sci. USA, 89:4569-4572, 1992.

[78]Keeting, P.H.; Scott, R.E.; Colvard, D.S.; Anderson, M.A.; Oursler, M.J.; Spelsberg, T.C. and Riggs, B.L.: Development and Characterization of Rapidly Proliferating, Well-Differentiated Cell Line Derived from Normal Adult Human Osteoblast-like Cells Transfected with SV40 Large T Antigen. J. Bone Min. Res., 7:127-136, 1992.

[79]Kerner, S.A.; Scott, R.A., and Pike, J.W.: Sequence Elements in the Human Osteocalcin Gene Confer Basal Activation and Inducible Response to Hormonal Vitamin D3. Proc. Natl. Acad. Sci. USA, 86:4455-4459, 1989.

[80]King, R.W.; Jackson, P.K. and Kirschner, M.W.: Mitosis in Transition. Cell, 79:563-571, 1994.

[81]Kleinsmith, L.J.; Stein, J. and Stein, G.: Dephosphorylation of Nonhistone Proteins Specifically Alters the Pattern of Gene Transcription in Reconstituted Chromatin. Proc. Natl. Acad. Sci. USA, 73:1174-1178, 1976.

[82]Kliewer, S.A.; Umesono, K.; Mangelsdorf, D.J. and Evans, R.M.: Retinoid X Receptor Interacts with Nuclear Receptors in Retinoic Acid, Thyroid Hormone and Vitamin D3 Signalling. Nature, 355:446-450, 1992.

[83]Krumlauf, R.: Hox Genes and Pattern Formation in the Branchial Region of the Vertebrate Head. Trends Gen., 9:106-112, 1993.

[84]Kuno, H.; Kurian, S.M.; Hendy, G.N.; White, J.; DeLuca, H.F.; Evans, C-O. and Nanes, M.S.: Inhibition of 1,25-Dihydroxyvitamin D3 Stimulated Osteocalcin Gene Transcription by Tumor Necrosis Factor-a: Structural Determinants within the Vitamin D Response Element. Endocrinol., 134:2524-2531, 1994.

[85]Kyeyune-Nyombi, E.; Lau, W.K.-H.; Baylink, D.J. and Strong, D.D.: Stimulation of Cellular Alkaline Phosphatase Activity and Its Messenger RNA Level in a Human Osteosarcoma Cell Line by 1,25-Dihydroxyvitamin D3. Arch. Biochem. Biophys., 275:363-370, 1989.

[86]Leboy, P.S.; Beresford, J.N.; Devlin, C. and Owen, M.E.: Dexamethasone Induction of Osteoblast mRNAs in Rat Marrow Stromal Cell Cultures. J. Cell. Physiol., 146:370-378, 1991.

[87]Levine, A.J.: Tumor Suppressor Genes. Bioessays, 12:60-66, 1990.

[88]Li, Y. and Stashenko, P.: Characterization of a Tumor Necrosis Factor-Responsive Element which Downregulates the Human Osteocalcin Gene. Molec. Cell. Biol., 13:3714-3721, 1993.

[89]Lian, J.B.; Tassinari, M. and Glowacki, J.: Resorption of Implanted Bone Prepared from Normal and Warfarin-Treated Rats. J. Clin. Invest., 73:1223-1226, 1984.

[90]Lian, J.B.; Dunn, K. and Key, L.L.: In Vitro Degradation of Bone Particles by Human Monocytes is Decreased with the Depletion of the Vitamin K-Dependent Bone Protein from the Matrix. Endocrinol., 118:1636-1642, 1986.

[91]Lian, J.; Stewart, C.; Puchacz, E.; Mackowiak, S.; Shalhoub, V.; Collart, D.; Zambetti, G. and Stein, G.:

Structure of the Rat Osteocalcin Gene and Regulation of Vitamin D-Dependent Expression. Proc. Natl. Acad. Sci. USA, 86:1143-1147, 1989.

[92] Lian, J.B. and Gundberg, C.M.: Osteocalcin: Biochemical Considerations and Clinical Applications. Clin. Orthop. Rel. Res., 266:267-291, 1988.

[93] Lian, J.B. and Marks, S.C.: Osteopetrosis in the Rat: Coexistence of Reduction in the Osteocalcin and Bone Resorption. Endocrinol., 126:955-962, 1990.

[94] Lian, J.B.; Stein, G.S.; Bortell, R. and Owen, T.A.: Phenotype Suppression: A Postulated Molecular Mechanism for Mediating the Relationship of Proliferation and Differentiation by fos/jun Interactions at AP-1 Sites in Steroid Responsive Promoter Elements of Tissue-Specific Genes. J. Cell. Biochem., 45:9-14, 1991.

[95] Lian, J.B. and Stein, G.S.: Proto-Oncogene Mediated Control of Gene Expression during Osteoblast Differentiation. Ital. J. Min. Elect. Metab., 7:175-83, 1993a.

[96] Lian, J.B. and Stein G.S.: Vitamin D Regulation of Osteoblast Growth and Differentiation. In Nutrition and Gene Expression, pp. 391-429, CRC Press, Boca Raton, FL, C.D. Berdanier and J.L. Hargrove (eds.), 1993b.

[97] Lian, J.B.; Stein, G.S.; Stein, J.L.; van Wijnen, A.J.; Montecino, M.; Desai, R.; Shakoori, A.R.; Breen, E.; Hoffmann, H. and Pockwinse, S.M.: Vitamin D Regulation of Osteocalcin Gene Transcription: A Model for Defining Molecular Mechanisms of 1,25(OH)2D3 Control of Osteoblast Growth and Differentiation. In Vitamin D: a pluripotent steroid hormone: structural studies, molecular endocrinology, and clinical applications: Proceedings of the Ninth Workshop on Vitamin D, Orlando, Florida, pp. 264-272, Walter de Gruyter, Berlin, NY, A.W. Norman, R. Bouillon, M. Thomasset (eds), 1994.

[98] Lian, J.B. and Stein, G.S.: Osteocalcin Gene Expression: A Molecular Blueprint for Developmental and Steroid Hormone Mediated Regulation of Osteoblast Growth and Differentiation. Endocr. Rev., in press.

[99] Liggett, W.H.; Lian, J.B.; Greenberger, J.S. and Glowacki, J.: Osteocalcin Promotes Differentiation of Putative Osteoclast Progenitors from Murine Long-Term Bone Marrow Cultures. J. Cell. Biochem., 55:190-199, 1994.

[100] Liu, F.; Malaval, L.; Gupta, A.K. and Aubin, J.E.: Simultaneous Detection of Multiple Bone-Related mRNAs and Protein Expression during Osteoblast Differentiation: Polymerase Chain Reaction and Immunocytochemical Studies at the Single Cell Level. Dev. Biol., 166:220-234, 1994.

[101] Lufkin, T.; Mark, M.; Hart, C.P.; LeMeur, M. and Chambon, P.: Homeotic Transformation of the Occipital Bones of the Skull by Ectopic Expression of a Homeobox Gene. Nature, 359:835-841, 1992.

[102] Lynch, M.P.; Stein, J.S.; Stein, G.S. and Lian, J.B.: Apoptosis during In Vitro Bone Formation. J. Bone Min. Res., 9:S352 (Abstract), 1994.

[103] Lynch, M.P.; Stein, J.L.; Stein, G.S. and Lian, J.B.: The Influence of Type I Collagen on the Development and Maintenance of the Osteoblast Phenotype in Primary & Passaged Rat Calvarial Osteoblasts: Modification of Expression of Genes Supporting Cell Growth, Adhesion, and Extracellular Matrix Mineralization. Exp. Cell Res., 216:35-45, 1995.

[104] Lyons, K.M.; Pelton, R.W. and Hogan, B.L.M.: Patterns of Expression of Murine Vgr-1 and BMP-2a RNA Suggest that Transforming Growth Factor-b-like Genes Coordinately Regulate Aspects of Embryonic Development. Genes Dev., 1:1657-1668, 1989.

[105] MacDonald, P.N.; Dowd, D.R.; Nakajima, S.; Galligan, M.A.; Reeder, M.C.; Haussler, C.A.; Ozato, K. and Haussler, M.R.: Retinoid X Receptors Stimulate and 9-cis Retinoic Acid Inhibits 1,25-Dihydroxyvitamin D3-Activated Expression of the Rat Osteocalcin Gene. Mol. Cell. Biol., 3:5907-5917, 1993.

[106] MacLachlan, T.K.; Sang, N. and Giordano, A.: Cyclins, Cyclin-Dependent Kinases and Cdk Inhibitors: Implications in Cell Cycle Control and Cancer. Crit. Rev. Eukary. Gene Exp., in press, 1995.

[107] Majeska, R.J.; and Rodan, G.A.: The Effect of 1,25(OH)2D3 on Alkaline Phosphatase in Osteoblastic Osteosarcoma Cells. J. Biol. Chem., 257:3362, 1982.

[108] Malaval, L.; Modrowski, D.; Gupta, A.K. and Aubin, J.E.: Cellular Expression of Bone-Related Proteins during In Vitro Osteogenesis in Rat Bone Marrow Stromal Cell Cultures. J. Cell. Physiol., 158:555-572, 1994.

[109] Marcelli, C.; Yates, A.J. and Mundy, G.R.: In Vivo Effects of Human Recombinant Transforming Growth Factor b on Bone Turnover in Normal Mice. J. Bone Min. Res., 5:1087-1096, 1990.

[110] Marie, P.J.; Hott, M. and Garba, M.-T.: Contrasting Effects of 1,25-Dihydroxyvitamin D3 on Bone Matrix and Mineral Appositional Rates in the Mouse. Metabolism, 34:777-783, 1985.

[111] Markose, E.R.; Stein, J.L.; Stein, G.S. and Lian, J.B.: Vitamin D-Mediated Modifications in Protein-DNA Interactions at Two Promoter Elements of the Osteocalcin Gene. Proc. Natl. Acad. Sci. USA, 87:1701-1705, 1990.

[112] Masi, L.; Franchi, A.; Santucci, M.; Danielli, D.; Atganini, L.; Giannone, V.; Formigli, L.; Benvenuti, S.; Tanini, A.; Beghe, F.; Mian, M. and Brandi, M.L.: Adhesion, Growth and Matrix Production by Osteoblasts on Collagen Substrata. Calcif. Tiss. Int., 51:202-212, 1992.

[113] Mayahara, H.; Ito, T.; Nagai, H.; Miyajima, H.; Tsukuda, R.; Taketomi, S.; Mizoguchi, J. and Kato, K.: In

Vivo Stimulation of Endosteal Bone Formation by Basic Fibroblast Growth Factor in Rats. Growth Factors, 9:73-80, 1993.

[114] McCabe, L.R.; Kockx, M.; Lian, J.B.; Stein, J.L.; Stein, G.S.: Selective Expression of fos- and jun-related Genes during Osteoblast Proliferation and Differentiation. Exp. Cell Res., in press.

[115] Merry, K.; Dodds, R.; Littlewood, A. and Gowen, M.: Expression of Osteopontin mRNA by Osteoclasts and Osteoblasts in Modeling Adult Human Bone. J. Cell. Sci. 104:1013-1020, 1993.

[116] Miyauchi, A.; Alvarez, J.; Greenfield, E.M.; Teti, A.; Grano, M.; Colucci, S.; Zambonin-Zallone, A.; Ross, F.P.; Teitelbaum, S.L.; Cheresh, D., and Hruska, K.A.: Recognition of Osteopontin and Related Peptides by an $a_v\beta3$ Integrin Stimulates Immediate Cell Signals in Osteoclasts. J. Biol. Chem., 266:20369-20374, 1991.

[117] Morrison, N.A.; Shine, J.; Fragonas J.-C.; Verkest, V.; McMenemy, L. and Eisman, J.A.: 1,25-Dihydroxyvitamin D-Responsive Element and Glucocorticoid Repression in the Osteocalcin Gene. Science, 246:1158-1161, 1989.

[118] Murray, S.; Glackin, C.; Winters, K.A.; Gazit, D.; Kahn, A.J. and Murray, E.J.: Expression of Helix-Loop-Helix Regulatory Genes during Differentiation of Mouse Osteoblastic Cells. Bone Min. Res., 7:1131-1138, 1992.

[119] Nagata, T.; Bellows, C.G.; Kasugai, S.; Butler, W.T. and Sodek, J.: Biosynthesis of Bone Proteins [SPP-1 (Secreted Phosphoprotein-1, Osteopontin), BSP (Bone Sialoprotein) and SPARC (Osteonectin)] in Association with Mineralized-Tissue Formation by Fetal-Rat Calvarial Cells in Culture. J. Biochem., 274:513-520, 1991.

[120] Nakamura, T.; Hanada, K.; Tamura, M.; Shibanushi, T.; Nigi, H.; Tagawa, M.; Fukumoto, S. and Matsumoto, T.: Stimulation of Endosteal Bone Formation by Systemic Injections of Recombinant Basic Fibroblast Growth Factor in Rats. Endocrinol., 136:1276-1284, 1995.

[121] Nanes, M.S.; Rubin, J.; Titus, L.; Hendy, G.N. and Catherwood, B.D.: Interferon-g Inhibits 1,25-Dihydroxyvitamin D3-Stimulated Synthesis of Bone GLA Protein in Rat Osteosarcoma Cells by a Pretranslational Mechanism. Endocrinol., 127:588-594, 1990.

[122] Nicolas, V.; Nefussi, J.R.; Collin, P. and Forest, N.: Effects of Acidic Fibroblast Growth Factor and Epidermal Growth Factor on Subconfluent Fetal Rat Calvaria Cell Cultures: DNA Synthesis and Alkaline Phosphatase Activity. Bone Min., 8:145-56, 1990.

[123] Nijweide, P.J. and Mulder, R.J.P.: Identification of Osteocytes in Osteoblast-like Cell Cultures Using a Monoclonal Antibody Specifically Directed Against Osteocytes. Histochemistry, 84:342-347, 1986.

[124] Noda, M. and Camilliere, J.J.: In Vivo Stimulation of Bone Formation by Transforming Growth Factor-beta. Endocrinol., 124:2991-2994, 1989.

[125] Noff, P.; Pitaru, S. and Savion, N.: Basic Fibroblast Growth Factor Enchances the Capacity of Bone Marrow Cells to Form Bone-like Nodules In Vitro. FEBS, 250:619-621, 1989.

[126] Nomura, S.; Wills, A.J.; Edwards, D.R.; Heath, J.K. and Hogan, B.L.M.: Developmental Expression of 2ar (Osteopontin) and SPARC (Osteonectin) RNA as Revealed by In Situ Hybridization. J. Cell Biol., 106:441-450, 1988.

[127] Nurse, P.: Universal Control Mechanism Regulating Onset of M-Phase. Nature, 344:503-508, 1990.

[128] Nurse, P.: Ordering S Phase and M Phase in the Cell Cycle. Cell, 79:547-550, 1994.

[129] Ogata, T. and Noda, M.: Expression of ID, a Negative Regulator of Helix-Loop-Helix DNA Binding Proteins, is Down-regulated at Confluence and Enhanced by Dexamethasone in a Mouse Osteoblastic Cell Line, MC3T3E1. Biochem. Biophys. Res. Com., 180:1194-1199, 1991.

[130] Oldberg, dA.; Franzén, A. and Heinegard, D.: Cloning and Sequence Analysis of Rat Bone Sialoprotein (Osteopontin) cDNA Reveals an Arg-Gly-Asp Cell-Binding Sequence. Proc. Natl. Acad. Sci. USA, 83:8819-8823, 1986.

[131] Owen, T.A.; Aronow, M.; Shalhoub, V.; Barone, L.M.; Wilming, L.; Tassinari, M.S.; Kennedy, M.B.; Pockwinse, S.; Lian, J.B. and Stein, G.S.: Progressive Development of the Rat Osteoblast Phenotype In Vitro: Reciprocal Relationships in Expression of Genes Associated with Osteoblast Proliferation and Differentiation during Formation of the Bone Extracellular Matrix. J. Cell. Physiol., 143:420-430, 1990a.

[132] 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.; Stein, J.L.; Curran, T.; Lian, J.B. and Stein, G.S.: Coordinate Occupancy of AP-1 Sites in the Vitamin D Responsive and CCAAT Box Elements by fos-jun in the Osteocalcin Gene: A Model for Phenotype Suppression of Transcription. Proc. Natl. Acad. Sci. USA, 87:9990-9994, 1990b.

[133] Owen, T.A.; Aronow, M.A.; Barone, L.M.; Bettencourt, B.; Stein, G. and Lian, J.B.: Pleiotropic Effects of Vitamin D on Osteoblast Gene Expression are Related to the Proliferative and Differentiated State of the Bone Cell Phenotype: Dependency Upon Basal Levels of Gene Expression, Duration of Exposure and Bone Matrix Competency in Normal Rat Osteoblast Cultures. Endocrinol., 128:1496-1504, 1991.

[134] Owen, T.A.; Bortell, R.; Shalhoub, V.; Heinrichs, A.; Stein, J.L.; Stein, G.S. and Lian, J.B.: Postproliferative Transcription of the Rat Osteocalcin Gene is Reflected by Vitamin D-Responsive Developmental Modifications in

Protein-DNA Interactions at Basal and Enhancer Promoter Elements. Proc. Natl. Acad. Sci. USA, 90:1503-1507, 1993.

[135] Owen, M. and Friedenstein, A.J.: Stromal Stem Cells: Marrow-Derived Osteogenic Precursors. In Cell and Molecular Biology of Vertebrate Hard Tissues, pp. 42-60, D. Evered and S.Harnett (eds.), John Wiley and Sons, Chichester, 1988.

[136] Ozawa, H.; Imamura, K.; Abe, E.; Takahashi, N.; Hiraide, T.; Shibasaki, Y.; Fukuhara, T. and Suda, T.: Effect of a Continuously Applied Compressive Pressure on Mouse Osteoblast-like Cells (MC3T3-E1) In Vitro. J. Cell. Phys., 142:177-185, 1990.

[137] Ozono, K.; Sone, T. and Pike, J.W.: The Genomic Mechanism of Action of 1,25-Dihydroxyvitamin D3. J. Bone Min. Res., 6:1021-1027, 1991.

[138] Pauli, U.; Chrysogelos, S.; Stein, J.; Stein, G.S. and Nick, H.: Protein-DNA Interactions In Vivo Upstream of a Cell Cycle Regulated Human H4 Histone Gene. Science, 236:1308-1311, 1987.

[139] Pechak, D.G.; Kujawa, J.J. and Caplan, A.I.: Morphological and Histochemical Events during First Bone Formation in Embryonic Chick Limbs. Bone, 7:441-458, 1986.

[140] Pockwinse, S.M.; Stein, J.L.; Lian, J.B. and Stein, G.S.: Developmental Stage-Specific Cellular Responses to Vitamin D and Glucocorticoids During Differentiation of the Osteoblast Phenotype: Interrelationships of Morphology and Gene Expression by In Situ Hybridization. Exp. Cell Res., 216:244-260, 1995.

[141] Pockwinse, S.; Wilming, L.; Conlon, D.; Stein, G.S. and Lian, J.B.: Expression of Cell Growth and Bone Specific Genes at Single Cell Resolution during Development of Bone Tissue-like Organization in Primary Osteoblast Cultures. J. Cell. Biochem. 49:310-323, 1992.

[142] Quarles, L.D.; Yohay, D.A.; Lever, L.W.; Caton, R. and Wenstrup, R.J.: Distinct Proliferative and Differentiated Stages of Murine MC3T3-E1 Cells in Culture: An In Vitro Model of Osteoblast Development. J. Bone Min. Res., 7:683-692, 1992.

[143] Ramsey-Ewing, A.; van Wijnen, A.; Stein, G.S. and Stein, J.L.: Delineation of a Human Histone H4 Cell Cycle Element In Vivo: The Master Switch for H4 Gene Transcription. Proc. Natl. Acad. Sci. USA, 91:4475-4479, 1994.

[144] Reinholt, F.P.; Hultenby, K.; Oldberg, A. and Heinegard, D.: Osteopontin—A Possible Anchor of Osteoclasts to Bone. Proc. Natl. Acad. Sci. USA, 87:4473-4475, 1990.

[145] Rodan, S.B.; Wesolowski, G.; Thomas, K. and Rodan, G.A.: Growth Stimulation of Rat Calvaria Osteoblastic Cells by Acidic Fibroblast Growth Factor. Endocrinol., 121:1917-23, 1987.

[146] Romberg, R.W.; Werness, P.G.; Riggs, B.L. and Mann, K.G.: Inhibition of Hydroxyapatite Crystal Growth by Bone-Specific and Other Calcium-Binding Proteins. Biochem., 25:1176-1180, 1986.

[147] Rüther, U.; Garber, C.; Komitowski, D.; Müller, R. and Wagner, E.F.: Deregulated c-fos Expression Interferes with Normal Bone Development in Transgenic Mice. Nature, 325:412-416, 1987.

[148] Sandberg, M.; Autio-Harmainen, H. and Vuorio, E.: Localization of the Expression of Types I, III, and IV Collagen, TGF-b1 and c-fos Genes in Developing Human Calvarial Bones. Dev. Biol., 130:324-334, 1988.

[149] Schrader, M.; Bendik, I.; Becker-Andre, M. and Carlberg, C.: Interaction Between Retinoic Acid and Vitamin D Signaling Pathways. J. Bio. Chem., 268:17830-17836, 1993.

[150] Sch[00fc]le, R.; Umesono, K.; Mangelsdorf, D.J.; Bolado, J.; Pike, J.W. and Evans, R.M.: Jun-Fos and Receptors for Vitamins A and D Recognize a Common Response Element in the Human Osteocalcin Gene. Cell, 61:497-504, 1990.

[151] Shakoori, A.R.; Owen, T.A.; Shalhoub, V.; Stein, J.L.; Bustin, M.; Stein, G.S. and Lian, J.B.: Differential Expression of the Chromosomal High Mobility Group Proteins 14 and 17 During the Onset of Differentiation in Mammalian Osteoblasts and Promyelocytic Leukemia Cells. J. Cell. Biochem., 51:479-487, 1993.

[152] Shalhoub, V.; Jackson, M.E.; Lian, J.B.; Stein, G.S. and Marks Jr., S.C.: Gene Expression during Skeletal Development in Three Osteopetrotic Rat Mutations: Evidence for Osteoblast Abnormalities. J. Biol. Chem., 266:9847-9856, 1991.

[153] Shalhoub, V.; Conlon, C.; Tassinari, M.; Quinn, C.; Partridge, N.; Stein, G.S. and Lian, J.B.: Glucocorticoids Promote Development of the Osteoblast Phenotype by Selectively Modulating Expression of Cell Growth and Differentiation Associated Genes. J. Cell. Biochem., 50:425-440, 1992.

[154] Shalhoub, V.; Bortell, R.; Jackson, M.E.; Marks, S.C. Jr.; Stein, J.L.; Lian, J.B. and Stein, G.S.: Transcriptionally Active Nuclei Isolated from Intact Bone Reflect Modified Levels of Gene Expression in Skeletal Development and Pathology. J. Cell. Biochem., 55:182-189, 1994.

[155] Sherr, C.J.: G1 Phase Progression: Cycling on Cue. Cell, 79:551-555, 1994.

[156] Sherr, C.J.: Mammalian G1 Cyclins. Cell, 73:1059-1065, 1993.

[157] Simeone, A.; Acampora, D.; Pannese, M.; D'Esposito, M.; Stornaiuolo, A.; Gulisano, M.; Mallamaci, A.; Kastury, K.; Druck, T.; Huebner, K. and Boncinelli, E.: Cloning and Characterization of Two New Members of the Vertebrate Dlx Family. Proc. Natl. Acad. Sci. USA, 91:2250-2254, 1994.

[158.]Soriano, P.; Montgomery, C.; Geske, R. and Bradley, A.: Targeted Disruption of the c-src Proto-Oncogene Leads to Osteopetrosis in Mice. Cell, 64:693-702, 1991.

[159.]Speiss, Y.H.; Price, P.A.; Deftos, J.L. and Manolagas, S.C.: Phenotype-Associated Changes in the Effects of 1,25-Dihydroxyvitamin D3 on Alkaline Phosphatase and Bone Gla-Protein of Rat Osteoblastic Cells. Endocrinol., 118:1340-1346, 1986.

[160.]Stein, G.S.; Stein, J.L.; van Wijnen, A.J. and Lian, J.B.: Regulation of Histone Gene Expression. Curr. Opin. Cell Biol., 4:166-173, 1992.

[161.]Stein, G.S.; Lian, J.B.; Dworetzky, S.I.; Owen, T.A.; Bortell, R.; Bidwell, J.P. and van Wijnen, A.J.: Regulation of Transcription-Factor Activity during Growth and Differentiation: Involvement of the Nuclear Matrix in Concentration and Localization of Promoter Binding Proteins. J. Cell. Biochem., 47:300-305, 1991.

[162.]Stein, G.S.; Stein, J.L.; van Wijnen, A.J. and Lian, J.B.: Histone Gene Transcription: A Model for Responsiveness to an Integrated Series of Regulatory Signals Mediating Cell Cycle Control and Proliferation/Differentiation Interrelationships. J. Cell. Biochem., 54:393-404, 1994.

[163.]Stein, G.S. and Lian, J.B.: Molecular Mechanisms Mediating Proliferation/Differentiation Interrelationships during Progressive Development of the Osteoblast Phenotype. Endocr. Rev., 14:424-442, 1993.

[164.]Stein, G.S.; Lian, J.B. and Owen, T.A.: Relationship of Cell Growth to the Regulation of Tissue-Specific Gene Expression during Osteoblast Differentiation. FASEB J., 4:3111-3123, 1990.

[165.]Strauss, P.G.; Closs, E.I.; Schmidt, J. and Erfle, V.: Gene Expression during Osteogenic Differentiation in Mandibular Condyles In Vitro. J. Cell Biol., 110:1369, 1990.

[166.]Stromstedt, P.E.; Poellinger, L.; Gustafsson, J.A. and Cralstedt-Duke J.: The Glucocorticoid Receptor Binds to a Sequence Overlapping the TATA Box of the Human Osteocalcin Promoter: A Potential Mechanism for Negative Regulation. Mol. Cell. Biol., 11:3379-3383, 1991.

[167.]Subramaniam, M.; Colvard, D.; Keeting, P.E.; Rasmussen, K.; Riggs, B.L. and Spelsberg, T.C.: Glucocorticoid Regulation of Alkaline Phosphatase, Osteocalcin, and Proto-Oncogenes in Normal Human Osteoblast-like Cells. J. Cell. Biochem., 50:411-424, 1992.

[168.]Tamura, M. and Noda, M.: Identification of a DNA Sequence Involved in Osteoblast-Specific Gene Expression via Interaction with Helix-Loop-Helix (HLH)-Type Transcription Factors. J. Cell Biol., 126:773-782, 1994.

[169.]Tang, K.-T.; DeVito, W.J.; Capparelli, C.; Stein, J.L.; Stein, G.S.; Lian, J.B. and Braverman, L.E.: Acidic Fibroblast Growth Factor Inhibits Osteoblast Differentiation In Vitro: Altered Expression of Collagenase, Cell Growth-Related and Mineralization-Associated Genes. Manuscript in Preparation.

[170.]Tassinari, M.S.; Gerstenfeld, L.C.; Stein, G.S. and Lian, J.B.: Effect of Caffeine on Parameters of Osteoblast Growth and the Differentiation of a Mineralized Extracellular Matrix In Vitro. J. Bone Min. Res., 6:1029-1036, 1991.

[171.]Terpening, C.M.; Haussler, C.A.; Jurutka, P.W.; Galligan, M.A.; Komm, B.S. and Haussler, M.R.: The Vitamin D-Responsive Element in the Rat Bone Gla Protein Gene is an Imperfect Direct Repeat that Cooperates with Other Cis-Elements in 1,25-Dihydroxyvitamin D3-Mediated Transcriptional Activation. Mol. Endocrin., 5:373-385, 1991.

[172.]Towler, D.A.; Bennett, C.D. and Rodan, G.A.: Activity of the Rat Osteocalcin Basal Promoter in Osteoblastic Cells is Dependent Upon Homeodomain and CP1 Binding Motifs. Mol. Endocrinol., 8:614-624, 1994a.

[173.]Towler, D.A.; Rutledge, S.J. and Rodan, G.A.: Msx-2/Hox 8.1: A Transcriptional Regulator of the Rat Osteocalcin Promoter. Mol. Endocrinol., 8:1484-1493, 1994b.

[174.]Towler, D.A. and Rodan, G.A.: Cross-Talk Between Glucocorticoid and PTH Signaling in the Regulation of the Rat Osteocalcin Promoter. J. Bone Min. Res., 9:S282 (Abstract), 1994c.

[175.]Turksen, K. and Aubin, J.E.: Positive and Negative Immunoselection for Enrichment of Two Classes of Osteoprogenitor Cells. J. Cell Biol., 114:373-384, 1991.

[176.]van den Ent, F.M.I.; van Wijnen, A.J.; Lian, J.B.; Stein, J.L. and Stein, G.S.: Cell Cycle Controlled Histone H1, H3 and H4 Genes Share Unusual Arrangements of Recognition Motifs for HiNF-D Supporting a Coordinate Promoter Binding Mechanism. J. Cell. Physiol., 159:515-530, 1994.

[177.]van der Houven van Oordt, C.W.; van Wijnen, A.J.; Carter, R.; Soprano, K.; Lian, J.B.; Stein, G.S. and Stein, J.L.: Protein-DNA Interactions at the H4-Site III Upstream Transcriptional Element of a Cell Cycle Regulated Histone H4 Gene: Differences in Normal Versus Tumor Cells. J. Cell. Biochem., 49:93-110, 1992.

[178.]Van Der Plas, A.; Aarden, E.M.; Feijen, J.H.M.; De Boer, A.H.; Wiltink, A.; Alblas, M.J.; De Leij, L. and Nijweide, P.J.: Characteristics and Properties of Osteocytes in Culture. J. Bone Min. Res., 9:1697-1704, 1994.

[179.]van Wijnen, A.J.; Wright, K.L.; Lian, J.B.; Stein, J.L. and Stein, G.S.: Human H4 Histone Gene Transcription Requires the Proliferation-Specific Nuclear Factor HiNF-D. J. Biol. Chem., 264:15034-15042, 1989.

[180.]van Wijnen, A.J.; van den Ent, F.M.I.; Lian, J.B.; Stein, J.L. and Stein, G.S.: Overlapping and CpG Methylation-Sensitive Protein/DNA Interactions at the Histone H4 Transcriptional Cell Cycle Domain: Distinc-

tions Between Two Human H4 Gene Promoters. Mol. Cell. Biol., 12:3273-3287, 1992.

[181.] van Wijnen, A.J.; Aziz, F.; Grana, X.; De Luca, A.; Desai, R.K.; Jaarsveld, K.; Last, T.J.; Soprano, K.; Giordano, A.; Lian, J.B.; Stein, J.L. and Stein, G.S.: Transcription of Histone H4, H3 and H1 Cell Cycle Genes: Promoter Factor HiNF-D Contains CDC2, Cyclin A and an RB-Related Protein. Proc. Natl. Acad. Sci. USA, 91:12882-12886, 1994a.

[182.] van Wijnen, A.J.; Merriman, H.; Guo, B.; Bidwell, J.P.; Stein, J.L.; Lian, J. and Stein, G.: Nuclear Matrix Interactions With the Osteocalcin Gene Promoter: Multiple Binding Sites for the Runt-Homology Related Protein NMP-2 and for NMP-1, a Heteromeric CREB-2 Containing Transcription Factor. J. Bone Min. Res., 9:S148 (Abstract), 1994b.

[183.] Vaughan, P.S.; van Wijnen, A.; Stein, G.S. and Stein, J.L.: The Histone H4 Cell Cycle Element Recognition Factor HiNF-M is Identical to Interferon Regulatory Factor 2 (IRF2). Manuscript submitted.

[184.] Vukicevic, S.; Luyten, F.P.; Kleinman, H.K. and Reddi, A.H.: Differentiation of Canalicular Cell Processes in Bone Cells by Basement Membrane Matrix Components: Regulation by Discrete Domains of Laminin. Cell, 63:437-445, 1990.

[185.] Webber, D.; Osdoby, P.; Hauschka, P. and Krukowski, M.: Correlation of an Osteoclast Antigen and Ruffled Border on Giant Cells Formed in Response to Resorbable Substrates. J. Bone Min. Res., 5:401-410, 1990.

[186.] Weinberg, R.A.: Oncogenes, Antioncogenes, and the Molecular Bases of Multistep Carcinogenesis. Cancer Res., 49:3713-3721, 1989.

[187.] Weinreb, M.; Shinar, D. and Rodan, G.A.: Different Pattern of Alkaline Phosphatase, Osteopontin, and Osteocalcin Expression in Developing Rat Bone Visualized by In Situ Hybridization. J. Bone Min. Res., 5:831-842, 1990.

[188.] White, E.: p53, Guardian of Rb. Nature, 371:21-22, 1994.

[189.] Whitson, S.W.; Harrison, W.; Dunlap, M.K.; Bowers, D.E. Jr.; Fisher, L.W.; Robey, P.G. and Termine, J.D.: Fetal Bovine Cells Synthesize Bone Specific Matrix Proteins. J. Cell Biol., 88:607-614, 1984.

[190.] Wronski, T.J.; Halloran, B.P.; Bikle, D.D.; Globus, R.K. and Morey-Holton, E.R.: Chronic Administration of 1,25-Dihydroxyvitamin D3: Increased Bone but Impaired Mineralization. Endocrinol., 119:2580-2585, 1986.

[191.] Yao, K.L.; Todescan, R. and Sodek, J.: Temporal Changes in Matrix Protein Synthesis and mRNA Expression during Mineralized Tissue Formation by Adult Rat Bone Marrow Cells in Culture. J. Bone Min. Res., 9:231-240, 1994.

[192.] Yoon, K., Buenaga, R. and Rodan, G.A.: Tissue Specificity and Developmental Expression of Osteopontin. Biochem. Biophys. Res. Comm., 148:1129-1136, 1987.