Journal of Cellular Biochemistry 55:4–15 (1994)

PROSPECTS SYMPOSIUM

Nuclear Architecture Supports Integration of Physiological Regulatory Signals for Transcription of Cell Growth and Tissue-Specific Genes During Osteoblast Differentiation

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Abstract During the past several years it has become increasingly evident that the three-dimensional organization of the nucleus plays a critical role in transcriptional control. The principal theme of this prospect will be the contribution of nuclear structure to the regulation of gene expression as functionally related to development and maintenance of the osteoblast phenotype during establishment of bone tissue-like organization. The contributions of nuclear structure as it regulates and is regulated by the progressive developmental expression of cell growth and bone cell related genes will be examined. We will consider signalling mechanisms that integrate the complex and interdependent responsiveness to physiological mediators of osteoblast proliferation and differentiation. The focus will be on the involvement of the nuclear matrix, chromatin structure, and nucleosome organization in transcriptional control of cell growth and bone cell related genes. Findings are presented which are consistent with involvement of nuclear structure in gene regulatory mechanisms which support osteoblast differentiation by addressing four principal questions: 1) Does the representation of nuclear matrix proteins reflect the developmental stage-specific requirements for modifications in transcription during osteoblast differentiation? 2) Are developmental stage-specific transcription factors components of nuclear matrix proteins? 3) Can the nuclear matrix facilitate interrelationships between physiological regulatory signals that control transcription and the integration of activities of multiple promoter regulatory elements? 4) Are alterations in gene expression and cell phenotypic properties in transformed osteoblasts and osteosarcoma cells reflected by modifications in nuclear matrix proteins? There is a striking representation of nuclear matrix proteins unique to cells, tissues as well as developmental stages of differentiation, and tissue organization. Together with selective association of regulatory molecules with the nuclear matrix in a growth and differentiation-specific manner, there is a potential for application of nuclear matrix proteins in tumor diagnosis, assessment of tumor progression, and prognosis of therapies where properties of the transformed state of cells is modified. It is realistic to consider the utilization of nuclear matrix proteins for targeting regions of cell nuclei and specific genomic domains on the basis of developmental phenotypic properties or tissue pathology.

Key words: nucleus, gene expression, cell growth, osteoblast, nucleosome

INTRODUCTION

An understanding of transcriptional control in vivo necessitates resolution of a fundamental biological paradox. How can a threshold for initiation of transcription be achieved within the nuclei of intact cells with a limited concentration of tissue-specific and gene-specific regulatory elements and a similar restricted availability of cognate promoter binding factors. From a cellular perspective the transcription factor binding domains of promoter regulatory elements are less than 20 nucleotides, reflecting a cellular representation lower than 68 angstroms per 2 meters of DNA. Transcription factors which exhibit restricted recognition for specific genes are present in nuclei at extremely low levels. It is therefore necessary to define transcriptional regulatory mechanisms that are operative under either of the following conditions: those where transcription initiation complexes are as-

Received and accepted December 30, 1993.

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sembled by diffusion within the nucleus or those where functional interactions between promoter regulatory sequences and cognate DNA binding proteins is facilitated by co-localization and compartmentalized nuclear domains.

It is tempting to discount the requirement for invoking the complexities of nuclear architecture (nuclear compartmentalization) when seeking an explanation for nuclear regulatory components of gene expression, since reconstituted cell free systems exhibit principal components of gene-specific transcription. However, even skeptics accept that such in vitro systems include concentrations of both the genes and transcription factors which are considerably higher than those observed in cells and transcription occurs at extremely low rates. In addition, explanations for transcriptional control in an in vivo biological context must address the integration of a broad spectrum of regulatory mechanisms that support interrelationships between intracellular and extracellular signalling pathways which are responsive to both the establishment and maintenance of cell and tissue phenotypic properties. These transcriptional regulatory parameters modulate expression of genes during progressive stages of differentiation as well as those requisite for sustaining the commitment of differentiated cells to defined structural and functional properties. Here long-term dedication to expression of phenotypic genes are involved together with transient modifications in gene expression to accommodate homeostatic responsiveness to physiological mediators associated with metabolic control.

During the past several years it has become increasingly evident that the three-dimensional organization of the cell nucleus plays a central role in transcriptional control. Multiple findings support involvement of the nuclear matrix as a functional mediator of RNA synthesis. Initially, the nuclear matrix was identified by the Coffey and Penman laboratories [Berezney and Coffey, 1975; Fey et al., 1986] as the non-chromatin nuclear substructure which is organized as a proteinaceous network of polymorphic anastomosing fibers. It was the observation that the nuclear matrix is composed of a broad spectrum of cell-type and tissue-specific proteins, as well as hnRNAs [Fey and Penman, 1988; Nickerson et al., 1990; Getzenberg and Coffey, 1990a; Fey et al., 1984a; Nickerson and Penman, 1992; Berezney, 1991; Berezney and Coffey, 1975; Fey et al., 1984b; Pienta et al., 1991; Getzenberg et al., 1990; Fey et al., 1988; Penman, 1991; Getzenberg et al., 1991a,b; Wan et al., 1994], which provided compelling evidence for nuclear matrix participation in the regulation of gene expression. Further indications for contributions of the nuclear matrix to regulation of gene expression includes sites for DNA replication [Pardoll and Vogelstein, 1980; Berezney, 1991; Berezney and Coffey, 1975; Hozak et al., 1993; Pienta et al., 1991], preferential association with actively transcribed genes [Nelkin et al., 1980; Robinson et al., 1982; Schaack et al., 1990; Stief et al., 1989; Zenk et al., 1990; Cockerill and Garrard, 1986; Dworetzky et al., 1992; Gasser and Laemmli, 1986; Ward and Coffey, 1990], RNA synthesis at fixed transcriptional complexes and pre-mRNA splicing [He et al., 1990; Lawrence et al., 1989; Zeitlin et al., 1987; Carter et al., 1993; Xing and Lawrence, 1991; Spector et al., 1991; Spector, 1990; Xing et al., 1993; Fey et al., 1986; Penman, 1991], as well as specific association of some but not all steroid hormone receptors [Barrack and Coffey, 1983; Kumara-Siri et al., 1986; Landers and Spelsberg, 1992; Bidwell et al., 1994a; van Steensel et al., 1991; Ciejek et al., 1983; Thorburn and Knowland, 1993; Barrack and Coffey, 1980; Metzger et al., 1990; Metzger and Kovach, 1991; Alexander et al., 1987; Barrack, 1987; Landers and Spelsberg, 1992] and ubiquitous transcription factors [van Wijnen et al., 1993; Mancini et al., 1994] with the nuclear matrix. Recent results suggest partitioning of oncogene encoded transcription factors between the nuclear matrix and non-matrix nuclear fraction in a proliferation-dependent manner [van Wijnen et al., 1993]. Modifications in nuclear matrix proteins in osteosarcoma cells following parathyroid hormone treatment [Bidwell et al., in press, b] reflect involvement of the nuclear matrix in polypeptide hormone-mediated signal transduction pathways.

Matrix attachment regions (MARs), containing extensive runs of AT residues, have been shown to confer position and orientation independence to transcriptional properties of several genes in vitro and in vivo [Stief et al., 1989; Phi-Van and Stratling, 1990; van Driel et al., 1991; Gasser et al., 1989; von Kries et al., 1991; Luderus et al., 1992; Klehr et al., 1991; Bonifer et al., 1990]. These stable gene attachment sites mediate long-range influences covering several hundred kb [Jarman and Higgs, 1988]. It remains to be established how transient expression of genes and long-term commitment to

transcription of tissue-specific genes is related to interactions with nuclear architecture. Here a second class of nuclear matrix attachment sites for genes may be involved where the transcribed genes are tethered from the nuclear matrix through interactions with consensus sequences in proximal promoter regions. Both types of transcriptional control are required for the onset, progression, and maintenance of tissue-specific phenotypic properties which are responsive to physiologic regulatory signals.

The central theme of this prospect will be the contribution of nuclear structure to the regulation of gene expression as functionally related to development and maintenance of the osteoblast phenotype during establishment of bone tissue-like organization. The contributions of nuclear structure as it regulates and is regulated by the progressive developmental expression of cell growth and bone cell-related genes will be examined. We will consider signalling mechanisms that integrate the complex and interdependent responsiveness to physiological mediators of osteoblast proliferation and differentiation. The focus will be on the involvement of the nuclear matrix, chromatin structure, and nucleosome organization in transcriptional control of cell growth and bone cell-related genes.

The working hypothesis we have developed is that nuclear structure contributes to the onset, progression, and maintenance of osteoblast phenotypic properties by participating in the regulation of gene expression both at the transcriptional and at a series of post-transcriptional levels. Additionally we are postulating that nuclear structure is required for the expression of genes related to phenotypic properties of the bone cell. We will present an overview of findings which are consistent with involvement of nuclear architecture in gene regulatory mechanisms which support osteoblast differentiation by addressing four principal questions: 1) Does the representation of nuclear matrix proteins reflect the developmental stage-specific requirements for modifications in transcription during osteoblast differentiation? 2) Are developmental stage-specific transcription factors components of nuclear matrix proteins? 3) Can the nuclear matrix facilitate interrelationships between physiological regulatory signals that control transcription and the integration of activities at multiple promoter regulatory elements? 4) Are alterations in gene expression and cell phenotypic properties in transformed osteoblasts and osteosarcoma cells reflected by modifications in nuclear matrix proteins?

THE REPRESENTATION OF NUCLEAR MATRIX PROTEINS REFLECT DEVELOPMENTAL STAGE-SPECIFIC REQUIREMENTS FOR MODIFICATIONS IN TRANSCRIPTION DURING DIFFERENTIATION

It has been well established that differentiation of proliferating, normal diploid osteoblasts to post-proliferative cells in a mineralized type I collagen extracellular matrix is associated with a three-stage developmental sequence of gene expression which supports progressive establishment of bone tissue organization [Owen et al., 1990]. This developmental expression of cell growth and tissue-specific genes has been observed in primary cultures of normal diploid mammalian osteoblasts during formation of nodules of multi-layered osteocytic cells in a mineralized extracellular matrix with a bone tissue-like organization as well as during osteoblast differentiation in vivo reviewed in Stein et al., 1992; Lian and Stein, 1992; Stein and Lian, 1993; Rodan et al., 1991] (Fig. 1). At first, proliferating osteoblasts express genes which support cell cycle and cell growth control (e.g., histone, c-myc, c-fos, and c-jun) together with genes for the initial biosynthesis of the bone extracellular matrix (e.g., TGFβ, type I collagen, and fibronectin). Equally important, regulatory mechanisms are operative at this time which suppress expression of genes associated with mature osteoblast phenotypic properties. During a second developmental period immediately following the down-regulation of proliferation, genes involved with the continued biosynthesis, maturation, and organization of the bone extracellular matrix are expressed. Then during a third developmental period, gene expression associated with competency for the ordered deposition of mineral occurs.

Initial results providing a linkage of the nuclear matrix to transcriptional control of osteoblast genes is provided by two-dimensional electrophoretic profiles of nuclear matrix proteins analyzed throughout the osteoblast developmental sequence. Changes in the protein composition of the nuclear matrix parallel sequential expression of genes during the progressive development of the osteoblast phenotype. The composition of the nuclear matrix is consistent within each of the three principal periods of osteoblast differentiation but is modified dramatically at the two key transition points: at the completion of the proliferation period, and at the onset of extracellular matrix mineralization. This relationship between nuclear matrix protein composition and expression of specific genes is further

supported by retention of the characteristic stage-specific representation of nuclear matrix proteins when the osteoblast developmental sequence is delayed [Dworetzky et al., 1990].

DEVELOPMENTAL STAGE-SPECIFIC TRANSCRIPTION FACTORS ARE ASSOCIATED WITH THE NUCLEAR MATRIX

More direct evidence linking the nuclear matrix with the regulation of gene expression is provided by the recent demonstrations that 1) the histone gene, when actively transcribed, is associated with the nuclear matrix in proliferating cells and 2) the H4 histone gene distal promoter binding factor NMP-1 is a component of the nuclear matrix. Results from gel mobility shift analysis demonstrate that these sequence-specific nuclear matrix protein-DNA interactions are localized to a distal element (−650 to −654) in the histone H4 gene promoter (Fig. 2). This element was further established at single nucleotide resolution by copper phenanthroline footprint analysis, methylation interference and competition analysis [Dworetsky et al., 1992b]. The molecular weight of NMP-1 was determined by ultraviolet crosslinking studies and DNA affinity chromatography using the NMP-1 recognition site oligonucleotide [Dworetsky et

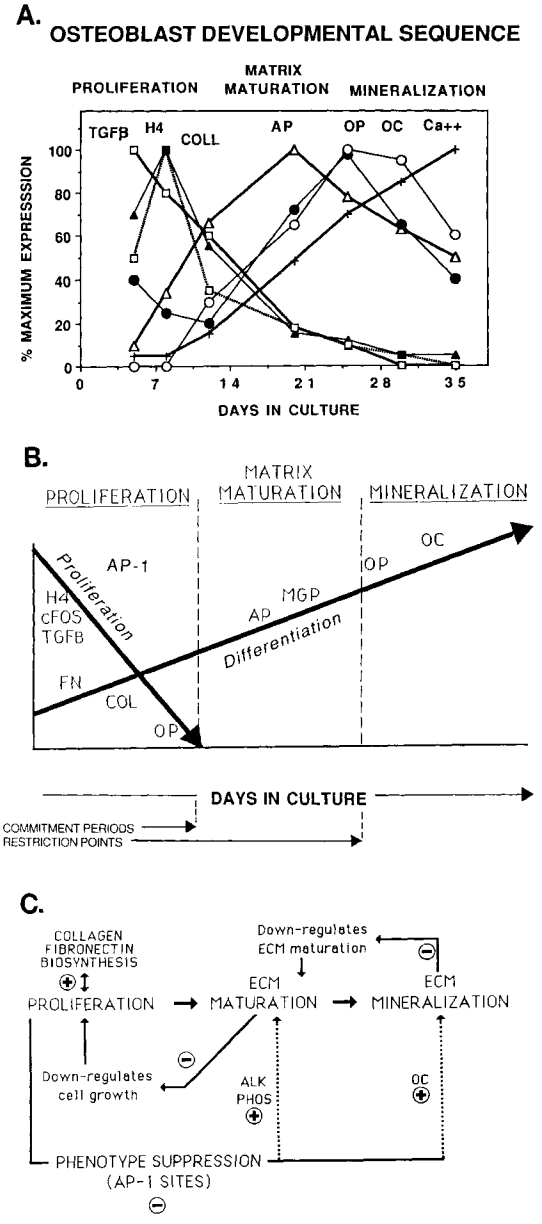

Fig. 1. **A:** Temporal expression of cell growth and osteoblast phenotype-related genes during development of the osteoblast phenotype. Isolated primary cells were initially cultured in minimum essential medium (MEM) with 10% fetal calf serum (FCS), then after confluence, in BGJb medium supplemented with 10% FCS, 50 μg/ml ascorbic acid and 10 mM β-glycerol phosphate. Cellular RNA was isolated at the times indicated (3, 5, 7, 10, 12, 16, 21, 28, and 35 days) during the differentiation time course and assayed for the steady state levels of various gene transcripts by Northern blot analysis. The resulting blots were quantitated by scanning densitometry and the results plotted relative to the maximal expression of each gene. The three periods of gene expression are illustrated with expression of representative genes: *Proliferation,* H4 histone reflects DNA synthesis, c-fos and c-jun (AP-1), and type I collagen (COL-I); *Matrix maturation,* Alkaline phosphatase (AP) is expressed post-proliferatively; *Mineralization,* Genes represented are induced to high levels with onset of extracellular matrix mineralization are osteopontin (OP) and osteocalcin (OC). Calcium (Ca^{+2}) accumulation is indicated. **B:** Reciprocal and functionally coupled relationship between cell growth and differentiation-related gene expression. 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 is at the onset of extracellular matrix mineralization. **C:** A series of signaling 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 extracellular matrix 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).

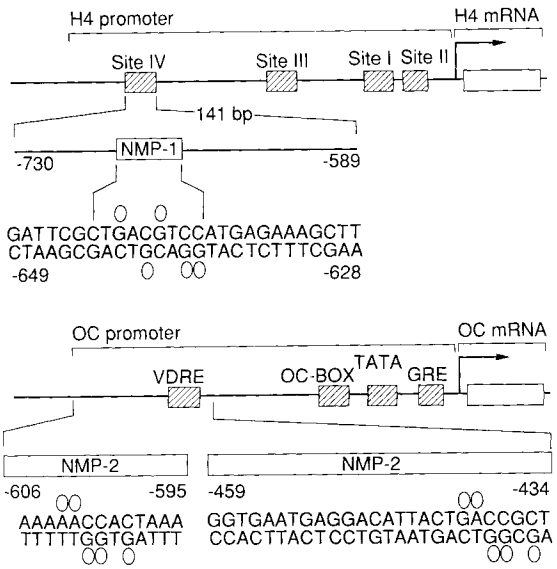

Fig. 2. Schematic illustration of the 5' promoter regions of the proliferation-related histone H4 (top) and differentiation-related osteocalcin genes (lower). Within the H4 gene is indicated a series of proximal cis acting elements (Sites III, I, and II) involved in cell cycle regulation of histone gene transcription. Site IV is a nuclear matrix attachment region spanning nt −730 to −589 which binds nuclear matrix protein designated NMP-1, an ATF-like promoter binding factor. The open circles indicate G residue contacts established by methylation interference [Dworetzky et al., 1992]. Within the OC promoter is indicated a series of basal (CAAT containing OC-Box and TATA motifs) and steroid hormone responsive elements (vitamin D responsive element, VDRE; glucocorticoid responsive element, GRE). NMP-2 is a bone related 38 kD nuclear matrix-associated protein which binds to two sites flanking the VDRE. Open circles indicate G residue contacts established by methylation interference [Bidwell et al., 1993].

al., 1992b]. These results indicate that NMP-1 represents a family of ATF-like proteins of 43 kD and 54 kD.

Further support for involvement of the nuclear matrix in transcriptional control during development of the bone cell phenotype is provided by our recent identification of a nuclear matrix-derived osteocalcin gene promoter binding factor designated NMP-2 (Fig. 2). NMP-2 is a tissue and sequence-specific DNA binding protein associated with the nuclear matrix when the osteocalcin gene is expressed. Bidirectional deletion analysis using gel shift assays revealed two sites that each interact with NMP-2. The first partially overlaps the vitamin D responsive element, whereas the second is 0.1 kB upstream of the vitamin D responsive element [Bidwell et al., 1993].

Developmentally regulated partitioning of transcription factors between the nuclear matrix and the non-matrix nuclear fraction supports a role for the nuclear matrix in sequestering promoter binding factors in a manner that may render sequence-specific DNA-binding proteins available or inaccessible for interaction with promoter regulatory elements [van Wijnen et al., 1993] (Fig. 3). A summary of the representation of transcription factors in the nuclear matrix and/or in the non-matrix nuclear fraction indicate a potential relationship between functional properties and subnuclear distribution (Fig. 3). It appears that general transcription factors which are abundant and ubiquitously associated with a broad spectrum of promoters reside in both the nuclear matrix and non-matrix nuclear fractions. Modifications in the extent to which at least a subset of the general transcription factors are nuclear matrix associated is developmentally dependent. In contrast, low abundant transcription factors which are tissue specific and present in nuclei solely during specific developmental periods are only observed as nuclear matrix-associated proteins. NMP-2 is a striking example of a low abundant transcription factor present only in mature os-

Selective partioning of transcription factors in nuclear matrix and non-matrix nuclear compartments

	confluent ROS 17/2.8		proliferating HeLa	
	NE	NM	NE	NM
NMP-1 (ATF)	+++	+++	++	++
NMP-2 (C/EBP)	±	+++	±	±
AP-1 (FOS/JUN)	±	+++	+++	++
CCAAT	±	±	±	++
OCT-1	±	±	+++	++
SP-1	+++	++	+++	++

Fig. 3. Selective partitioning of transcription factors in nuclear matrix and non-matrix nuclear compartments. The relative presence of transcription factor DNA binding activities in two distinct nuclear compartments (NE, nuclear extract or non-matrix nuclear compartment; NM, solubilized nuclear matrix fraction) as monitored by gel shift assays is schematically depicted by (+) symbols [van Wijnen et al., 1993]. Based on results with confluent ROS 17/2.8 osteosarcoma cells and proliferating HeLa cervical carcinoma cells, several transcription factors appear to be nuclear matrix-specific (e.g., NMP-2) [Bidwell et al., 1993], whereas others distribute similarly between nuclear matrix and non-matrix nuclear fractions (e.g., NMP-1) [Dworetzky et al., 1992]. In addition, the partioning of AP-1 binding activity in these two nuclear compartments appears to be cell growth and/or cell type related.

teoblasts undergoing extracellular matrix mineralization and associated with the nuclear matrix.

These results are consistent with a role for the nuclear matrix in localization of actively transcribed genes as well as in the concentration and targeting of transcription factors. This may explain how, with low representation of factors and sequence-specific regulatory elements in the nucleus, a threshold to initiate transcription can be obtained. In a broader biological context, these results provide a basis for understanding transcriptional control mediated by nuclear architecture. The two nuclear matrix protein binding sites associated with the osteocalcin gene promoter, both located within the region flanking the vitamin D responsive element, [Bidwell et al., in press, a], support involvement of the nuclear matrix, together with changes in chromatin structure, in mediating modifications in gene expression in response to hormones or

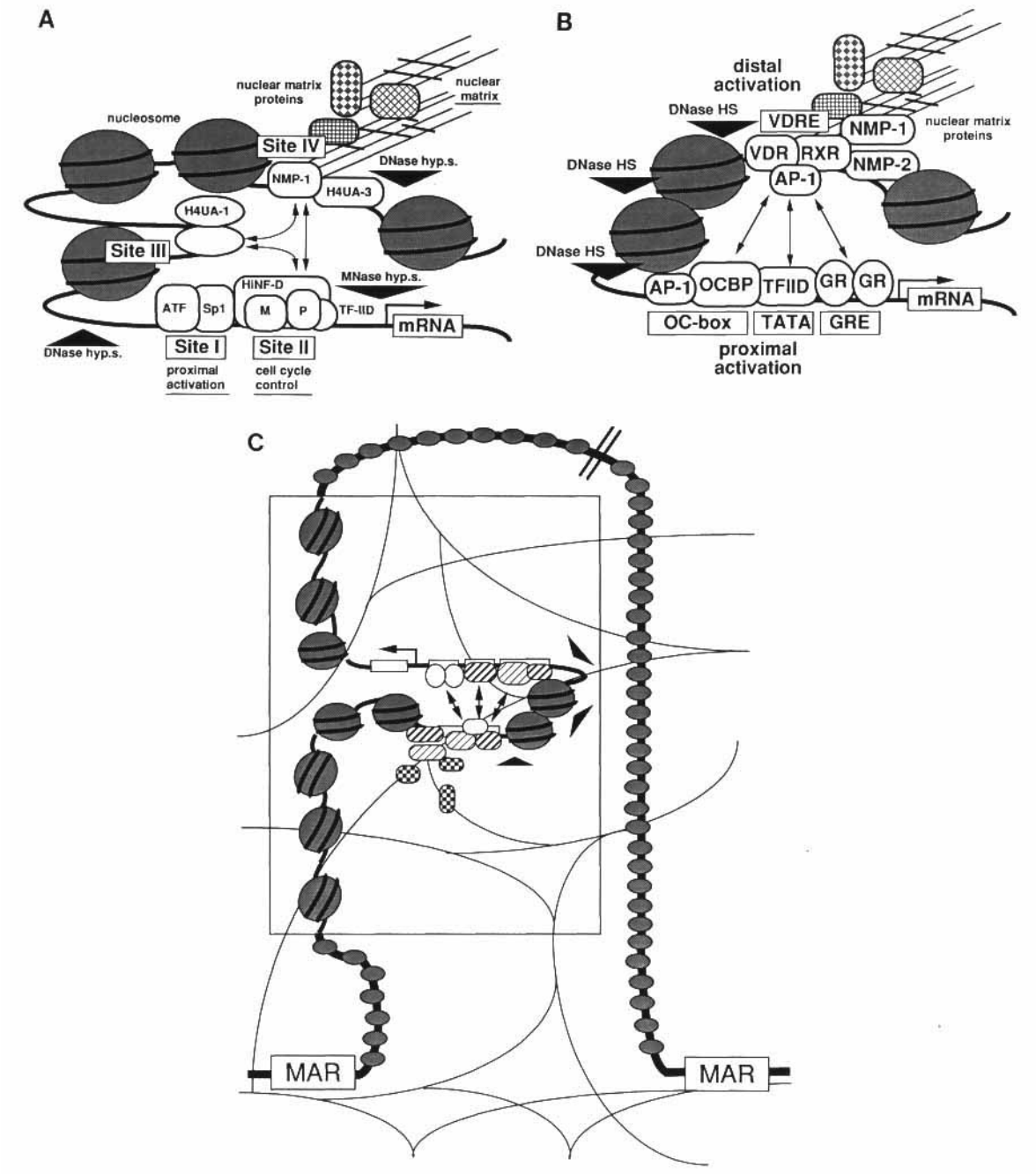

Figure 4. (Legend appears on page 10.)

other physiological regulatory factors. However, it must be appreciated that despite the multiple lines of evidence which temporarily and functionally relate nuclear matrix proteins with developmental and tissue-responsive modifications in transcription, cause and effect relationships have not been formally established. The extent to which the associations of actively transcribed genes with the nuclear matrix are determinants of or consequential to association of transcription factor complexes with nuclear architecture must be defined experimentally.

THE NUCLEAR MATRIX FACILITATES INTERRELATIONSHIPS BETWEEN PHYSIOLOGICAL REGULATORY SIGNALS THAT CONTROL TRANSCRIPTION AND THE INTEGRATION OF ACTIVITIES AT MULTIPLE PROMOTER REGULATORY ELEMENTS

A basic question with respect to transcriptional regulation of histone and osteocalcin genes during development of the osteoblast phenotype is how can transcription of specific genes be selectively initiated within the nucleus of an intact cell? Several features of chromatin structure may contribute to developmental modifications in competency of regulatory sequences for transactivation factor binding, both independently and by functional cooperativity between the multiple basal and enhancer elements of the histone and osteocalcin gene promoters.

The presence of nucleosomes in the human H4 histone gene promoter [Chrysogelos et al., 1985, 1989; Moreno et al., 1986; Pauli et al., 1989] provides the possibility for increasing the proximity of independent regulatory elements that support synergistic and/or antagonistic cooperative interactions between histone gene DNA binding activities. Involvement of chromatin structure with transcriptional regulation as related to growth control is consistent with variations in nucleosome organization as a function of cell cycle progression [Moreno et al., 1986]. Such growth regulated changes in chromatin structure and nucleosome organization may enhance and/or restrict accessibility of transcription factors and may modulate the extent to which DNA bound factors are phosphorylated. Cell cycle and growth related modifications in chromatin organization of the human histone

Fig. 4. **A:** Model for three-dimensional configurations of the histone and osteocalcin gene promoters showing potential interactions between independent regulatory elements. Spatial integration of intra- and extra-cellular signals modulating H4-F0108 gene transcription by reversible alterations in chromatin structure and nucleosomal organization of the promoter is shown. Relationship of distal and proximal protein/DNA interaction sites where H4-F0108 promoter DNA (solid black line) is packaged into nucleosomes (open ovals) is depicted. Indicated are possible cooperative and/or mutually exclusive higher-order nucleoprotein interactions (thin arrows) between various DNA bound trans-acting factors. The presence of a putative attachment site (Site IV) with the nuclear matrix (network of lines), containing matrix associated sequence-specific DNA binding proteins (rounded boxes filled with alternative symbols), provides a basis for restricted mobility of the promoter to a confined position within the nucleus as well as for the concentration and localization of transcription factors. **B:** Three-dimensional organization of the rat OC gene promoter is shown with interactions between the vitamin D responsive element and the basal regulatory OC box and TATA/GRE elements. Schematically illustrated are nuclear matrix protein/DNA interactions near the VDRE which could serve to structurally anchor this region of the promoter to impose conformational constraints on chromatin organization and/or to concentrate transcription factors that facilitate vitamin D receptor binding to the VDRE. Association of the OC gene promoter with the nuclear matrix together with the presence of nucleosomes between the VDRE, OC box, and TATA/GRE reduces the distance between these regulatory elements. A basis for modulating cooperative interactions that enhance osteocalcin gene expression is thereby provided. The question mark indicates the potential involvement of auxiliary proteins in mediating the interactions between these regulatory complexes. VDR, vitamin D receptor; AF, accessory factor; OCBBP, OC box binding proteins; and TBP, TATA/GRE binding proteins; NMP, nuclear matrix proteins. **C:** Possible relationships between stable and transient nuclear matrix-attachment sites. The diagram depicts stable nuclear matrix attachment sites (MARs) that establish gene-regulatory boundaries of chromatin (thick black line; grey ovals represent nucleosomes) by tethering DNA to nuclear matrix filaments (thin interconnecting curved lines). The resultant loop-domains (10 to 100 kB) may function as transcriptional units acting independently of adjacent loop-domains. The relationship between matrix attachment regions and "locus control regions" remains to be explored experimentally. The boxed area represents an enlargement that depicts short (1 to 2 kB) chromatin curves within the loop-domain near a transcribed gene (box with hooked arrow) with short nuclease hypersensitive chromatin regions (black triangles). Formation of these local chromatin curves may be facilitated by transient attachment with the nuclear matrix which may impose structural constraints and facilitate gene-activation by bringing remotely positioned transcriptional elements in close proximity of the transcriptional start site (hooked arrow) through protein/protein interactions between transcription factors (bi-directional arrows between rounded boxes). Transient nuclear matrix attachments may be mediated by protein/DNA and protein/protein interactions involving constitutive nuclear matrix proteins (checkered rounded boxes) and transcription factors (diagonally striped or open rounded boxes) whose presence in the nuclear matrix is cell stage-specific to different degrees (thick or thin stripes, or absence of stripes).

gene promoter include modifications in nucleosome spacing as well as protein-protein and protein-DNA interactions both within nucleosomes and in the internucleosomal sequences [Moreno et al., 1986]. This is reflected by accessibility to micrococcal nuclease, DNase I, SI nuclease, and a series of restriction enzymes [Chrysogelos et al., 1985; Moreno et al., 1986; Pauli et al., 1989].

The determinants of cell cycle-regulated changes in nucleosome organization remains to be established. Histone-histone and histone-DNA interactions, together with contributions of non-histone proteins that associate with nucleosomes or internucleosomal chromatin domains, are viable possibilities. The well-documented post-translational modifications of histone proteins, particularly acetylation, which are associated with transcriptionally active chromatin, suggest a potential regulatory mechanism. Here the findings of Davie and co-workers indicate that histone acetylases and deacetylases are associated with the nuclear matrix, providing an additional example of a manner in which nuclear architecture, may determine transcriptional competency of chromatin [Hendzel et al., 1991; Hendzel and Davie, 1992; Brandes et al., 1992].

The presence of nucleosomes within the rat osteocalcin gene promoter sequences spanning the vitamin D responsive element and the proximal basal regulatory region reduces the potential distance between multiple promoter regulatory domains [Bortell et al., 1992]. This raises the possibility of functional cooperativity between a series of basal and enhancer elements that are responsive to physiological mediators of osteocalcin gene expression. Additionally, reduced distances between the vitamin D responsive element and basal elements of the osteocalcin gene 5′ regulatory region provide a basis for interactions of the vitamin D receptor and proximal promoter transactivation factors bound at elements which enhance transcriptional activity. Such possibilities are supported by our recent demonstration that sequence-specific modifications in chromatin structure of the osteocalcin gene promoter reflect the level of transcription. We have observed differential DNase I hypersensitivity of the vitamin D responsive element and proximal basal regulatory elements (osteocalcin box and glucocorticoid responsive element/TATA box), which is tissue-specific (bone cell restricted) and related to the level of basal transcription and vitamin D responsiveness in ROS 17/2.8 osteosarcoma cells [Montecino et al., 1994] and developmentally in normal diploid osteoblasts (Montecino et al., in preparation).

A model for the three dimensional organization of the histone and osteocalcin gene promoters can be constructed on the basis of observed parameters of chromatin structure and gene-nuclear matrix associations (Fig. 4). Such conformational properties of the genes within the nucleus of intact cells may modulate regulation of transcriptional activity by a) imposing conformational constraints on chromatin structure, b) increasing the proximity of independent regulatory elements that support synergistic and/or antagonistic interactions between independent gene transcriptional DNA binding activities, c) enhancing and/or restricting accessibility of transcription factors, and d) providing a mechanism for modulating the extent to which DNA-bound factors are phosphorylated.

ABERRATIONS IN GENE EXPRESSION AND CELL PHENOTYPIC PROPERTIES IN TRANSFORMED OSTEOBLASTS AND OSTEOSARCOMA CELLS ARE REFLECTED BY MODIFICATIONS IN NUCLEAR MATRIX PROTEINS

Transformed and tumor cells exhibit aberrations in expression of cell growth and tissue-specific genes during both the onset and progression of neoplasia. A fundamental question is tumor related modifications in regulatory parameters that selectively restrict or render developmentally expressed genes competent for transcription.

Osteosarcoma cells such as the ROS 17/2.8 line exhibit loss of the developmental sequence of gene expression characteristic of normal diploid osteoblasts [Bortell et al., 1993; Rodan and Noda, 1991]. Abrogation of stringent growth control in these rodent osteosarcoma cells is accompanied by and potentially functionally related to concomitant expression of genes involved with both proliferation and post-proliferative phenotypic properties of mature bone cells.

To experimentally address inter-relationships between nuclear architecture and gene expression in bone tumor cells we compared nuclear matrix proteins of normal diploid osteoblasts during differentiation with those in ROS 17/2.8 osteosarcoma cells. Consistent with co-expression of genes in osteosarcoma cells which are sequentially and independently expressed dur-

ing osteoblast differentiation, we observed a composite representation of nuclear matrix proteins in ROS 17/2.8 cells which are characteristic of both the proliferation and post-proliferative periods of the osteoblast developmental sequence [Bidwell et al., 1994c]. We identified proliferation-dependent and growth-independent nuclear matrix proteins that are present in rat as well as human osteosarcoma cells but absent in normal diploid osteoblasts. Expression of other nuclear matrix proteins is restricted to normal diploid bone cells. The nuclear matrix proteins observed in normal diploid osteoblasts and in osteosarcoma cells is schematically presented in Figure 5.

Distinct nuclear matrix proteins characterize tumors of diverse origin and phenotypic properties. Examples include prostate, breast, colon, and bone [Fey and Pegman, 1988; Getzenberg et al., 1991; Pienta et al., 1991a,b; Stuurman et al., 1989; Partin et al., 1989; Bidwell et al., 1994]. It must be acknowledged that the transcription regulatory mechanisms by which the nuclear matrix contributes to modifications in the extent to which proliferation and phenotypic genes are expressed in transformed and tumor cells remains to be determined. However, there is a striking representation of nuclear matrix proteins unique to cells, tissues as well as developmental stages of differentiation and tissue organization. Together with selective association of regulatory molecules with the nuclear matrix in a growth and differentiation-specific manner there is a potential for application of nuclear matrix proteins to tumor diagnosis, assessment of tumor progression, and prognosis of therapies where properties of the transformed state of cells is modified. It is realistic to consider the utilization of nuclear matrix proteins for targeting regions of cell nuclei and specific genomic domains on the basis of developmental phenotypic properties or tissue pathology.

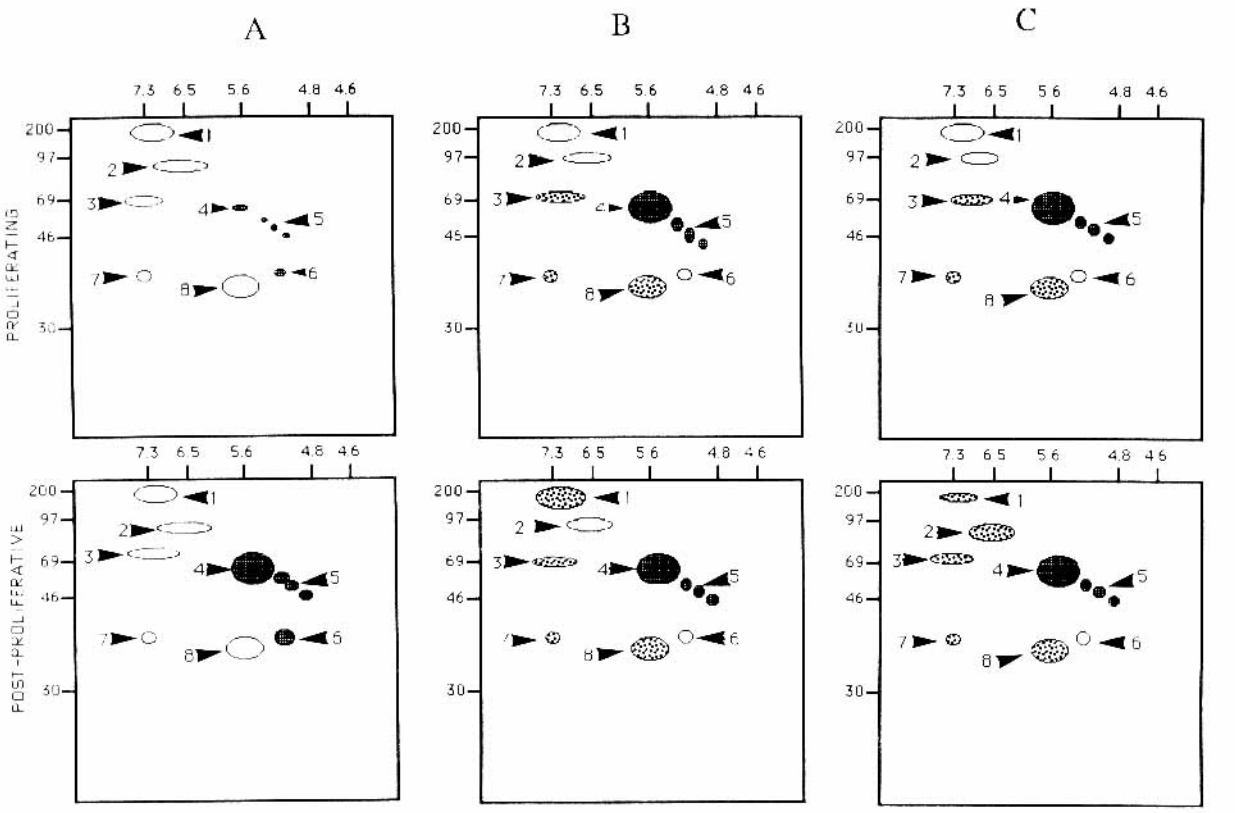

Fig. 5. Schematic representation of nuclear matrix proteins in normal diploid osteoblasts **(A)**, ROS 17/2.8 rat osteosarcoma cells **(B)**, and MG-63 human osteosarcoma cells **(C)**. The nuclear matrix proteins resolved in two-dimensional gels are designated as follows: solid symbols (designated 4 and 5) indicate the presence of nuclear matrix proteins characteristic of post-proliferative normal diploid osteoblasts that are co-expressed in both proliferating and post-proliferative osteosarcoma cells or protein 6, which is proliferation independent but only observed in normal diploid cells. The tumor-specific nuclear matrix proteins are designated by stippled symbols. Unfilled symbols indicate the absence of designated nuclear matrix proteins.

ACKNOWLEDGMENTS

Results reported are from studies supported by grants AR33920, AR35166, AR39588, AR42262 and GM32010 from the National Institutes of Health. The authors thank Ms. Elizabeth Bronstein for editorial assistance with preparation of the manuscript.

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