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Functional Interrelationships Between Nuclear Structure and Transcriptional Control: Contributions to Regulation of Cell Cycle- and Tissue-Specific Gene Expression

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Abstract Multiple levels of nuclear structure contribute to functional interrelationships with transcriptional control in vivo. The linear organization of gene regulatory sequences is necessary but insufficient to accommodate the requirements for physiological responsiveness to homeostatic, developmental, and tissue-related signals. Chromatin structure, nucleosome organization, and gene–nuclear matrix interactions provide a basis for rendering sequences accessible to transcription factors supporting integration of activities at independent promoter elements of cell cycle- and tissue-specific genes. A model is presented for remodeling of nuclear organization to accommodate developmental transcriptional control. © 1996 Wiley-Liss, Inc.

Key words: nuclear structure, gene regulatory sequences, tissue-related signals, transcriptional control, cell cycle

INTRODUCTION

Development and tissue renewal necessitates the stringently regulated expression of genes that support proliferation as well as structural and functional parameters of specific phenotypes. A common denominator to both cell growth- and tissue-specific transcriptional regulatory mechanisms, it is the requirement for insight into a fundamental paradox. How, with a limited representation of regulatory sequences and transcription factors, can a threshold concentration for initiation of transcription be achieved? Let us phrase the question in a biological perspective. The concentration of a gene-specific regulatory element within the nucleus is approximately 15 nucleotides per 2.5 yards of DNA and the representation of cognate transcription factors is extremely restricted. In this *Prospect* article, we explore involvement of nuclear architecture in facilitation of in vivo transcriptional control and support for the requisite plasticity to accommodate cell cycle-dependent and steroid hormone responsive modulation of gene expression. Evidence is presented that is consistent with the emerging concept that multiple components of nuclear organization contribute to competency for transcription and the extent to which genes are transcribed. Redundancy of regulatory mechanisms that interface with structural components of the nucleus is considered within the context of options for integration of physiological regulatory signals under diverse biological circumstances functionally linked to transcription during proliferation and differentiation. The dynamics of chromatin remodeling are examined in relation to developmental and phenotypic requirements for gene expression.

BIOLOGICAL MODEL FOR CELL CYCLE AND STEROID HORMONE RESPONSIVE DEVELOPMENTAL TRANSCRIPTIONAL CONTROL

The cell cycle-dependent histone gene promoter provides a paradigm for pursuit of transcriptional regulatory mechanisms operative at the G_1/S phase transition point in the cell cycle and competency for transcriptional control me-

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diating cell cycle progression at the onset of DNA replication [reviewed in Stein et al., 1992, 1994, 1995, in press]. Regulation of histone gene expression is a key component of growth control in proliferating osteoblasts and downregulation occurs at the onset of differentiation [van Wijnen et al., 1992, 1994; Ramsey-Ewing et al., 1994; Pauli et al., 1987; Vaughan et al., 1995; Holthuis et al., 1990]. The bone tissue-specific osteocalcin gene promoter is a paradigm for steroid hormone and growth factor-responsive transcriptional control that is functionally linked to expression of phenotypic properties characteristic of postproliferative mature osteoblasts [reviewed in Stein et al., 1990; Stein and Lian, 1995; Lian and Stein, 1992]. Consequently, examining histone and osteocalcin gene transcription during osteoblast differentiation permits investigation of mechanisms supporting both the activation and suppression of genes in relation to cell cycle and postproliferative endocrine-mediated control. The availability of in vivo and culture models for bone cell differentiation allows exploration of transcriptional regulatory parameters associated with development, as well as maintenance of tissue organization and function [reviewed in Stein et al., 1990; Stein and Lian, 1995; Lian and Stein, 1992].

MULTIPLE LEVELS OF NUCLEAR ARCHITECTURE SUPPORT REGULATION OF TRANSCRIPTION

It is becoming increasingly apparent that nuclear architecture provides a basis for support of stringently regulated modulation of cell growth- and tissue-specific transcription, which is necessary for the onset and progression of differentiation. Here, multiple lines of evidence point to contributions by three levels of nuclear organization to in vivo transcriptional control where structural parameters are functionally coupled to regulatory events. The primary level of gene organization establishes a linear ordering of promoter regulatory elements. Overlapping transcription factor-binding sites are present within many promoter regulatory domains. This representation of regulatory sequences reflects competency for responsiveness to cascades of physiological regulatory signals defining specificity for protein–protein as well as protein–DNA interactions. However, interspersion of sequences between promoter elements that exhibit coordinate and synergistic activities indicates the requirement of a structural basis for integration of activities at independent regulatory domains. Parameters of chromatin structure and nucleosome organization are a second level of genome architecture that reduce the distance between promoter elements thereby supporting interactions between the modular components of transcriptional control [reviewed in Felsenfeld, 1992; Owen-Hughes and Workman, 1994; van Holde, 1988; Richard-Foy and Hager, 1987]. Each nucleosome (approximately 140 nucleotide base pairs wound around a core complex of two each of H3, H4, H2A, and H2B histone proteins) contracts linear spacing by sevenfold. Higher-order chromatin structure further reduces nucleotide distances between regulatory sequences. Folding of nucleosome arrays into solinoid-type structures provides potential for interactions that support synergism between promoter elements and responsiveness to multiple signaling pathways. A third level of nuclear architecture that contributes to transcriptional control is the nuclear matrix. The anastomosing network of fibers and filaments that constitute the nuclear matrix supports the structural properties of the nucleus as a cellular organelle and accommodates the structural modifications associated with proliferation, differentiation, and changes necessary to sustain phenotypic requirements of specialized cells [Berezney and Coffey, 1975; Fey et al., 1984a, 1984b, 1986; Fey and Penman, 1988; Nickerson et al., 1990; Getzenberg and Coffey, 1990; Nickerson and Penman, 1992; Berezney, 1991; Wan et al., 1994; Pienta and Coffey, 1991; Getzenberg et al., 1990, 1991a,b; Dworetzky et al., 1990; Penman, 1991; Pienta et al., 1991; Bidwell et al., 1994a,b,c]. Regulatory functions of the nuclear matrix include, but are by no means restricted to, gene localization, imposition of physical constraints on chromatin structure that support formation of loop domains, concentration and targeting of transcription factors, RNA processing and transport of gene transcripts, concentration and targeting of transcription factors, as well as imprinting and modifications of chromatin structure [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; Guo et al., 1995; Gasser and Laemmli, 1986, 1989; Ward and Coffey, 1990; He et al., 1990; Lawrence et al., 1989; Zeitlin et al., 1987; Carter et al., 1993; Guo et al., (Gasser); Spector, 1990;

Spector et al., 1991; Xing et al., 1993; Merriman et al., 1995; Bidwell et al., 1993; Barrack and Coffey, 1980, 1983; Kumara-Siri et al., 1986; Landers and Spelsberg, 1992; van Steensel et al., 1991; Ciejek et al., 1983; Thorburn and Knowland, 1993; Metzger and Korach, 1990; Metzger et al., 1991; Alexander et al., 1987; Barrack, 1987; Jarman and Higgs, 1988; Mancini et al., 1994; Blencowe et al., 1994; van Wijnen et al., 1993; Bonifer et al., 1990; Phi-Van and Stratling, 1988, 1990; van Driel et al., 1991; von Kries et al., 1991; Luderus et al., 1992; Klehr et al., 1991; Berezney and Jeon, 1995; Stein et al., in press].

Taken together, these components of nuclear architecture facilitate biological requirements for physiologically responsive modifications in gene expression within the contexts of (1) homeostatic control involving rapid, short-term, and transient responsiveness; (2) developmental control that is progressive and stage-specific; and (3) differentiation-related control associated with long-term phenotypic commitments to gene expression for support of structural and functional properties of cells and tissues.

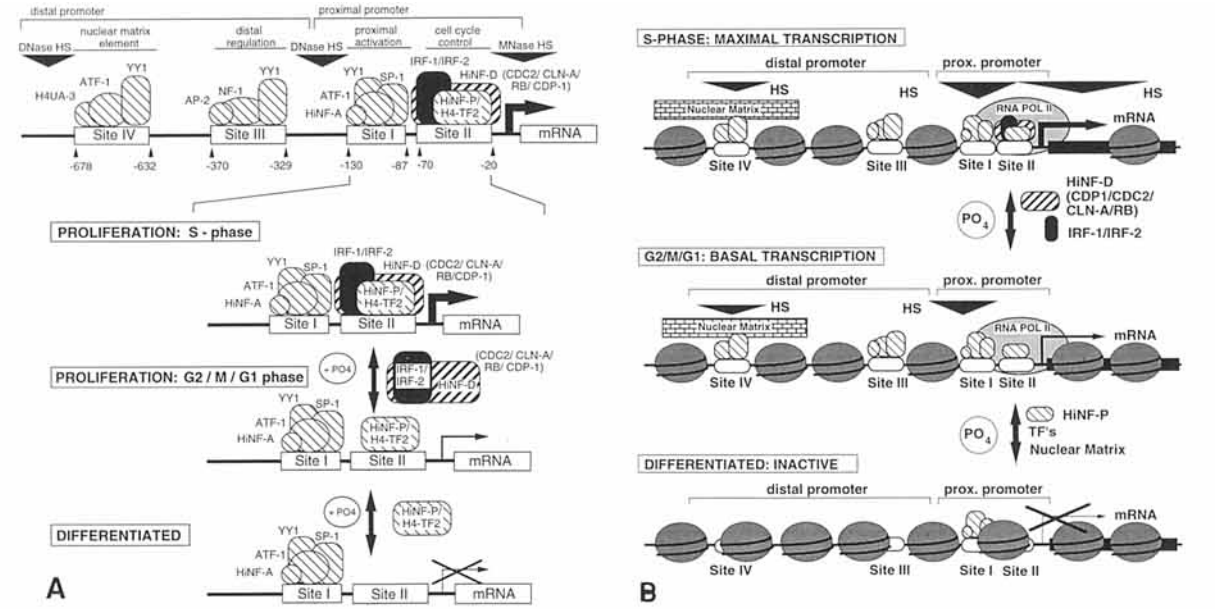
We are just beginning to appreciate the significance of nuclear domains in the control of gene expression. However, it is already apparent that local nuclear environments generated by the multiple aspects of nuclear structure are intimately tied to developmental expression of cell growth- and tissue-specific genes (see *Prospects* by Clemson and Lawrence and by Huang and Spector in this issue). From a broader perspective, it is becoming increasingly evident that, reflecting the diversity of regulatory requirements as well as the phenotype-specific and physiologically responsive representation of nuclear structural proteins, there is a reciprocally functional relationship between nuclear structure and gene expression. Nuclear structure is a primary determinant of transcriptional control, and expressed genes modulate the regulatory components of nuclear architecture. The power of addressing gene expression within the three-dimensional context of nuclear structure would be difficult to overestimate. Membrane-mediated initiation of signaling pathways that ultimately influence transcription have been recognized for some time. Extending the structure–regulation paradigm to nuclear architecture expands the cellular context in which cell–structure gene expression interrelationships are operative.

CHROMATIN STRUCTURE AND NUCLEOSOME ORGANIZATION FACILITATE DEVELOPMENTAL CELL CYCLE-DEPENDENT AND STEROID HORMONE-RESPONSIVE TRANSCRIPTIONAL CONTROL

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 (Fig. 1) and osteocalcin gene promoters (Fig. 2).

The presence of nucleosomes in the human H4 histone gene promoter [Chrysogelos et al.,

Fig. 1. **A:** Regulation of histone gene expression during the cell cycle. *First panel:* Organization of the human histone H4 gene promoter regulatory elements (sites I–IV). The transcription factors that exhibit sequence-specific interactions with these domains are indicated during the S phase of the cell cycle, when the gene is maximally transcribed. Site II contributes to cell cycle regulation of transcription. Site IV binds a nuclear matrix protein complex (NMP-1/YY-1), while the protein–DNA interactions at sites III and I support general transcriptional enhancement. The site II complex includes cyclin A, cyclin-dependent kinase cdc2, and RB-related protein, CDP-1 and IRF growth regulatory factors, reflecting integration of phosphorylation-mediated control of histone gene expression. *Second panel:* Occupancy of the four principal regulatory elements of the histone H4 gene promoter during the S phase of the cell cycle when transcription is maximal is schematically illustrated. *Third panel:* Site II transcription factor complex is modified by phosphorylation during the G_1/G_2/mitotic periods of the cell cycle resulting in altered levels of transcription. Phosphorylation-dependent dissociation of the IRF and HiNF-D (cdc2, cyclin A, CDP-1, and RB-related protein) factors occurs in non-S-phase cells. *Fourth panel:* Complete loss of transcription factor complexes at site II following exit from the cell cycle with the onset of differentiation. At this time, transcription is completely downregulated. **B:** Schematic illustration of the remodeling of chromatin structure and nucleosome organization, which accommodates cell cycle stage-specific and developmental parameters of histone gene promoter architecture to support modifications in level of expression. Placement of nucleosomes and representation as well as magnitude of nuclease-hypersensitive sites (▲) are designated. The principal regulatory elements and transcription factors are shown. **C:** Three-dimensional organization of the histone gene promoter. Model schematically presented for the spatial organization of the rat osteocalcin gene promoter based on evidence for nucleosome placement and the interaction of DNA-binding sequences with the nuclear matrix. These components of chromatin structure and nuclear architecture restrict mobility of the promoter and impose physical constraints that reduce distances between proximal and distal promoter elements. Such a postulated organization of the osteocalcin gene promoter can facilitate cooperative interactions for crosstalk between elements that mediate transcription factor binding and consequently determine the extent to which the gene is transcribed.


distal promoter
proximal promoter
nuclear matrix element
distal regulation
proximal activation
cell cycle control
DNase HS
DNase HS
MNase HS
H4UA-3
ATF-1
YY1
NF-1
AP-2
YY1
ATF-1
HiNF-A
YY1
SP-1
IRF-1/IRF-2
HiNF-D (CDC2/ CLN-A/ RB/ CDP-1)
HiNF-P/ H4-TF2
Site IV
Site III
Site I
Site II
mRNA
-678
-632
-370
-329
-130
-87
-70
-20
PROLIFERATION: S - phase
PROLIFERATION: G2 / M / G1 phase
DIFFERENTIATED
+ PO4
A
S-PHASE: MAXIMAL TRANSCRIPTION
distal promoter
prox. promoter
HS
Nuclear Matrix
RNA POL II
mRNA
Site IV
Site III
Site I Site II
HiNF-D (CDP1/CDC2/ CLN-A/RB)
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G2/M/G1: BASAL TRANSCRIPTION
HiNF-P
TF's
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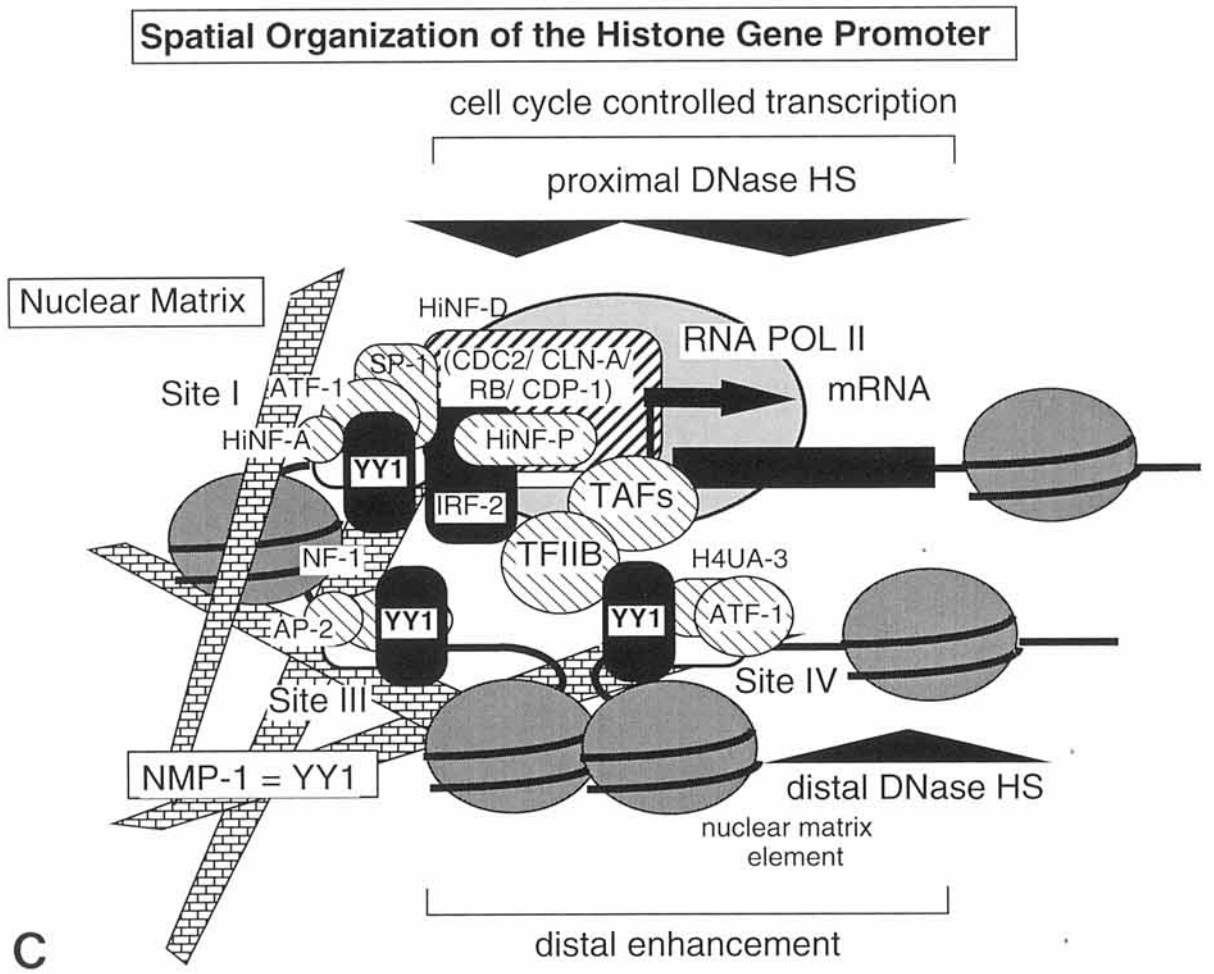
Spatial Organization of the Histone Gene Promoter
cell cycle controlled transcription
proximal DNase HS
Nuclear Matrix
HiNF-D
RNA POL II
Site I
ATF-1
SP-1
(CDC2/ CLN-A/ RB/ CDP-1)
mRNA
HiNF-A
HiNF-P
YY1
IRF-2
TAFs
NF-1
TFIIB
H4UA-3
AP-2
YY1
YY1
ATF-1
Site IV
Site III
NMP-1 = YY1
distal DNase HS
nuclear matrix element
distal enhancement
C

Figure 1.

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

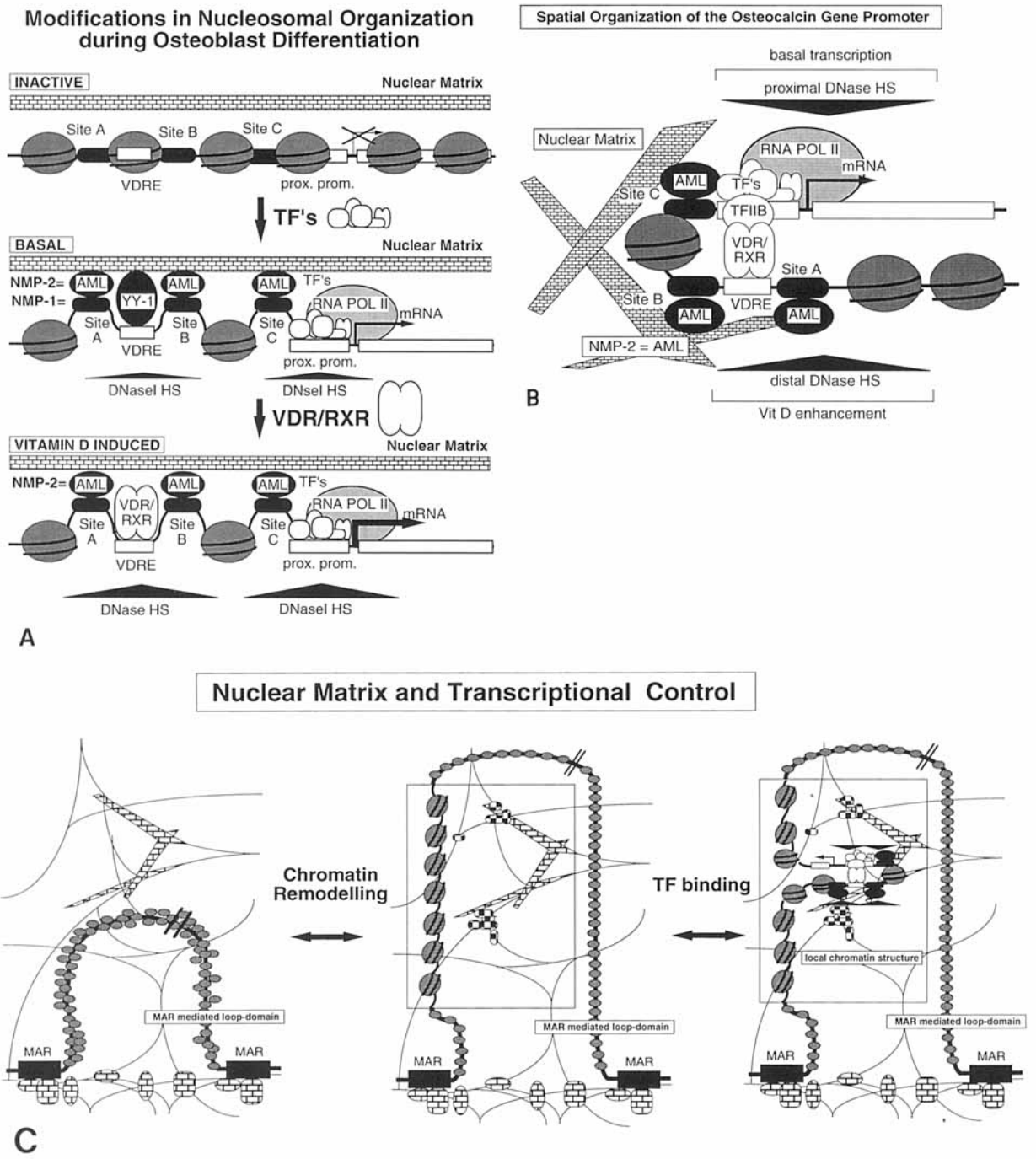

Figure 2.

chromatin organization of the human histone 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, S1 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. Factors that control chromatin remodeling and that sustain conformations that support long-term phenotypic contributions to gene expression must be defined. Histone–histone and histone–DNA interactions, together with contributions of nonhistone proteins associated 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]. Recent characterization of regulatory mechanisms that accompany acetylation-mediated remodeling of yeast chromatin may be extrapolated to higher eukaryotic systems [Wright et al., 1992; Renauld et al., 1993; Hecht et al., 1995; Marsolier et al., 1995].

Modifications in parameters of chromatin structure and nucleosome organization parallel both competency for transcription and the extent to which the osteocalcin gene is transcribed [Bortell et al., 1992; Montecino et al., 1994; in press]. The biological significance of chromatin organization in fidelity of osteocalcin gene transcriptional control in intact cells is provided by promoter sequence requirements for expression of transfected genes. Significant differences are observed when comparing transcriptional activity in transiently transfected cells to cell lines and transgenic animals in which constructs are stably integrated and packaged as chromatin [Montecino et al., in press; Frenkel et al., 1996]. Specific changes in chromatin organization occur in response to physiological mediators of basal expression and steroid hormone responsiveness. Thus, a conceptual and experimental basis is provided for the involvement of nuclear architecture in developmental, homeostatic, and physiologic control of osteocalcin gene expression during the establishment and maintenance of bone tissue structure and activity.

In both normal diploid osteoblasts and osteosarcoma cells, basal expression and enhancement of osteocalcin gene transcription are accompanied by two alterations in structural properties of chromatin. Nuclease hypersensitivity of sequences flanking the tissue-specific osteocalcin box and the vitamin D-responsive element enhancer domain are observed [Montecino et al., 1994, in press; Breen et al., 1994]. Together with modifications in nucleosome placement [Montecino et al., in press], a basis for accessibility of transactivation factors to basal and steroid hormone-dependent regulatory sequences can

Fig. 2. **A:** Schematic representation of the osteocalcin gene promoter organization and occupancy of regulatory elements by cognate transcription factors paralleling and supporting functional relationships. *Top:* Suppression of transcription in proliferating osteoblasts. *Middle:* Activation of expression in differentiated cells. *Bottom:* Alternatively, enhancement of transcription by vitamin D. The placement of nucleosomes is indicated. Remodeling of chromatin structure in nucleosome organization to support suppression, basal, and vitamin D-induced transcription of the osteocalcin gene is indicated. (▲) representation and magnitude of DNase I hypersensitive sites. Gene–nuclear matrix interactions are shown. **B:** Three-dimensional organization of the rat osteocalcin gene promoter. Model schematically presented for the spatial organization of the rat osteocalcin gene promoter based on evidence for nucleosome placement and the interaction of DNA binding sequences with the nuclear matrix. These components of chromatin structure and nuclear architecture restrict mobility of the promoter and impose physical constraints that reduce distances between proximal and distal promoter elements. Such postulated organization of the osteocalcin gene promoter can facilitate cooperative interactions for crosstalk between elements that mediate transcription factor binding and consequently determine the extent to which the gene is transcribed. **C:** Postulated remodeling of chromatin structure, nucleosome organization, and the nuclear matrix to support transcriptional activation and repression of the osteocalcin gene. Contributions of multiple components of nuclear architecture to gene–nuclear matrix interrelationships and the association of transcription factors with both gene regulatory elements and the nuclear matrix are schematically illustrated. Modifications of structure–function relationships are shown that mediate transitions from the repressed to the transcriptionally active regulatory states. *Right:* Model for osteocalcin gene–nuclear matrix interactions with occupancy of basal, tissue-specific, and enhancer sequences by cognate transcription factors. Also shown is a model for crosstalk between proximal and distal regulatory domains of the osteocalcin gene promoter via direct interactions between factors that are parameters of nuclear architecture.

be explained. In early-stage proliferating normal diploid osteoblasts, when the osteocalcin gene is repressed, a nucleosome is placed in the osteocalcin box, which is required for control of basal tissue-specific expression. At this time, a nucleosome is also placed in the VDRE promoter sequence, which supports steroid hormone-dependent transcriptional enhancement. Nuclease-hypersensitive sites are not present in the vicinity of these regulatory elements. By contrast, when osteocalcin gene expression is transcriptionally upregulated postproliferatively and vitamin D-mediated enhancement of transcription occurs, the osteocalcin box and VDRE become nucleosome free; these regulatory domains are flanked by DNase I-hypersensitive sites. The complete absence of hypersensitivity and the presence of nucleosomes in the VDRE and osteocalcin box domains of the osteocalcin gene promoter in ROS 24/1 cells that lack the vitamin D receptor additionally corroborate these findings. Strikingly, the initial 1.1 kb of the osteocalcin promoter is sufficient to restore major components of chromatin structure that are absent following random integration in the genome of transfected osteoblastic cells [Frenkel et al., 1996]. Taken together, these studies provide evidence for functional relationships between structural modifications in chromatin and physiologically regulated levels of osteocalcin gene transcription.

Despite the compelling experimental basis for these structure–function interrelationships, the cause-and-effect parameters remain to be established. Sequences and regulatory factors that are rate-limiting for modulation of chromatin structure and nucleosome organization from the perspectives of determinants or immediate consequences of osteocalcin transcriptional control require definition. It should be noted that a placed nucleosome in a promoter domain does not preclude contributions of the element to transcriptional regulatory activity. Nucleosomes are organized as octomeric nucleoprotein complexes, each containing a core of H3–H4 and H2A–H2B heterodimers, with DNA on the outside and potentially accessible for functional interactions with transcription factors.

NUCLEAR MATRIX CONTRIBUTES TO CELL CYCLE-REGULATED DEVELOPMENTAL AND STEROID HORMONE-DEPENDENT, TISSUE-SPECIFIC TRANSCRIPTION

When actively transcribed in early-stage proliferating osteoblasts, the cell cycle-regulated histone gene is associated with the nuclear matrix. Consistent with involvement of the nuclear matrix in developmental control of gene expression, the histone H4 gene distal promoter binding factor NMP1/YY1 has been shown to be a nuclear matrix component. The specificity and functionality of the nuclear matrix-associated NMP1/YY1 transcription factor with the histone gene promoter is indicated by gel mobility shift analysis, footprint analysis, ultraviolet (UV) cross-linking studies, and in vivo expression experiments [Dworetzky et al., 1992; Guo et al., 1995] (Fig. 1).

Involvement of the nuclear matrix in control of osteocalcin gene transcription is provided by several lines of evidence (Fig. 2). A parallel representation of nuclear matrix proteins with developmental expression of the osteocalcin gene during osteoblast differentiation was the initial suggestion of functional linkage between the nuclear matrix and osteocalcin gene expression [Dworetzky et al., 1990]. There are developmental modifications in the selective partitioning of the ubiquitous fos/jun-related transcription factors that bind to a series of osteocalcin gene promoter elements between the nuclear matrix and nonmatrix nuclear fractions during osteoblast differentiation [van Wijnen et al., 1993].

One of the most compelling lines of support for a role of nuclear matrix proteins in steroid hormone-dependent osteocalcin gene transcriptional control is the demonstration that two nuclear matrix proteins [Banerjee et al., 1996, Merriman et al., 1995; Bidwell et al., 1993; Guo et al., 1995, submitted] designated NMP1 and NMP2 bind with specificity to sequences associated with the osteocalcin gene vitamin D-responsive element (VDRE). NMP2, which we have established is an AML-related, bone phenotype-specific transcription factor [Banerjee et al., 1996, Merriman et al., 1995; Bidwell et al., 1993], interacts with sequences flanking, but not overlapping, the steroid half-element motifs of the osteocalcin gene VDRE. NMP1, which we have shown is the YY1 transcription factor, interacts with the proximal steroid half-element within the osteocalcin gene VDRE. Taken together, these relationships between organization of nuclear matrix protein binding domains within and contiguous to the osteocalcin gene VDRE suggests options for both positive and negative control of vitamin D-mediated transcriptional enhancement. Based on overlapping binding domains within the VDRE for the VDR and the NMP1/YY1 nuclear matrix protein transcrip-

tion factor [Guo et al., 1996], one can speculate that reciprocal interactions of NMP1 and VDR complexes may contribute to competency of the VDRE to support transcriptional enhancement. Binding of NMP2 at the VDRE flanking sequence may establish permissiveness for VDR interactions by gene–nuclear matrix associations that facilitate conformational modifications in the transcription factor recognition sequences. Direct evidence for modulation of steroid hormone-responsive transcriptional regulation of osteocalcin gene expression by nuclear matrix proteins includes (1) upregulation of osteocalcin gene transcription following in vivo overexpression of AML genes transfected into osteoblastic cells [Banerjee et al., 1996, Merriman et al., 1995; Bidwell et al., 1993], and (2) a dose-dependent abrogation of ligand- and receptor-mediated vitamin D enhancement of osteocalcin gene transcription following overexpression of YY1 in intact cells [Guo et al., submitted]. Taken together, these findings implicate nuclear matrix proteins in facilitation of osteocalcin gene expression by NMP2/AML as well as mutual exclusive binding of VDR/RXR or NMP1/YY1 transcription factors. Support for involvement of nuclear organization in facilitation of steroid hormone-dependent transcriptional control is further provided by the demonstration of interactions between the vitamin D receptor and TFIIB, implicating crosstalk between the VDRE and TATA domains. These interactions have been shown to support enhancement of osteocalcin gene transcription [Blanco et al., 1995; MacDonald et al., 1995].

NUCLEAR ARCHITECTURE FACILITIES INTEGRATION OF ACTIVITIES AT INDEPENDENT PROMOTER REGULATORY ELEMENTS

It is apparent from available findings that the linear organization of gene regulatory sequences is necessary but insufficient to accommodate the requirements for physiological responsiveness to homeostatic, developmental, and tissue-related signals. It would be presumptive to propose a formal model for the three-dimensional organization of the histone and osteocalcin gene promoters. However, the working model presented in Figures 1 and 2 represents postulated interactions between histone and osteocalcin gene promoter elements that reflect the potential for integration of activities by nuclear architecture to support transcriptional control within the three-dimensional context of cell structure and regulatory requirements at the cell and tissue levels.

It is becoming increasingly evident that developmental transcriptional control and modifications in transcription to accommodate homeostatic regulation of cell and tissue function is modulated by the integration of a complex series of physiological regulatory signals. Fidelity of responsiveness necessitates the convergence of activities mediated by multiple regulatory elements of gene promoters. Our current knowledge of promoter organization and the repertoire of transcription factors that mediate activities provides a single-dimensional map of options for biological control. We are beginning to appreciate the additional structural and functional dimensions provided by chromatin structure, nucleosome organization, and subnuclear localization and targeting of both genes and transcription factors. Particularly exciting is increasing evidence for dynamic modifications in nuclear structure that parallel developmental expression of genes. The extent to which nuclear structure regulates and/or is regulated by modifications in gene expression remains to be experimentally established.

Despite the emerging evidence for nuclear structure-gene expression interrelationships, a number of fundamental questions remain to be experimentally addressed. The complexities to levels of nuclear organization is becoming increasingly apparent. Our awareness of nuclear domains that are dedicated to specific components of gene expression has evolved from considering the nucleolar localization of ribosomal RNA transcription an exception, to defining a broad spectrum of nuclear domains within the context of support for expression of specific genes. There is a quest for understanding of interrelationships between multiple components of nuclear structure with subtleties in transcription and transcript processing. Among the challenges that we now face is the necessity to define functionally the cause and/or effect components of interrelationships between structural parameters of the nucleus associated with specific transcriptional regulatory mechanisms.

During the past several years, there has been a rapid accrual in definitions of structures that are functionally linked to steps in transcriptional activation and RNA processing. However, many represent advances that have been descriptive at both the structural and molecular levels. We are now in a position to pursue biochemical determinants and functional consequences of

activities associated with gene expression as it regulates, and is regulated by, components of nuclear architecture. Elucidation of rate-limiting regulatory components of nuclear structure–function interrelationships will provide insight into control of plasticity required for remodeling of nuclear structure in relation to transcriptional control. Here, physiologically responsive accommodations to modulations in gene expression include, but are not restricted to, changes in nucleosome placement, intranucleosomal properties, as well as higher-order nuclear organization.

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REFERENCES

Alexander RB, Greene GL, Barrack ER (1987): Estrogen receptors in the nuclear matrix: Direct demonstration using monoclonal antireceptor antibody. Endocrinology 120:1851–1857.

Banerjee C, Hiebert SW, Stein JL, Lian JB, Stein GS (1996): An AML-1 consensus sequence binds an osteoblast-specific complex and transcriptionally activates the osteocalcin gene. Proc Natl Acad Sci USA 93:4968–4973.

Barrack ER (1987): Steroid hormone receptor localization in the nuclear matrix: Interaction with acceptor sites. J Steroid Biochem 27:115–121.

Barrack ER, Coffey DS (1980): The specific binding of estrogens and androgens to the nuclear matrix of sex hormone responsive tissues. J Biol Chem 255:7265–7275.

Barrack ER, Coffey DS (1983): In Roy, Clark (eds): "Gene Regulation by Steroid Hormones." Vol. II. New York: Springer, pp 239–266.

Berezney R (1991): The nuclear matrix: A heuristic model for investigating genomic organization and function in the cell nucleus. J Cell Biochem 47:109–123.

Berezney R, Coffey DS (1975): Nuclear protein matrix: association with newly synthesized DNA. Science 189:291–292.

Berezney R, Jeon KW (eds) (1995): "Structural and Functional Organization of the Nuclear Matrix." Vols. 162A,B: "International Review of Cytology: A Survey of Cell Biology." San Diego: Academic Press.

Bidwell JP, Fey EG, Merriman H, van Wijnen AJ, Penman S, Stein JL, Stein GS, Lian JB (1994a): Subnuclear distribution of the vitamin D receptor. J Cell Biochem 54:494–500.

Bidwell JP, Fey EG, van Wijnen AJ, Penman S, Stein JL, Lian JB, Stein GS (1994b): Nuclear matrix proteins distinguish normal diploid osteoblasts from osteosarcoma cells. Cancer Res 54:28–32.

Bidwell JP, van Wijnen AJ, Banerjee C, Fey EG, Merriman H, Penman S, Stein JL, Lian JB, Stein GS (1994c): PTH-responsive modifications in the nuclear matrix of ROS 17/2.8 rat osteosarcoma cells. Endocrinology 134:1738–1744.

Bidwell JP, van Wijnen AJ, Fey EG, Penman S, Stein JL, Lian JB, Stein GS (1993): Osteocalcin gene promoter binding factors and nuclear matrix components. Proc Natl Acad Sci USA 90:3162–3166.

Blanco JM, Wang M, Tsai S, Tsai M, O'Malley B, Jurutka P, Haussler M, Ozato K (1995): Transcription factor TFIIB and the vitamin D receptor cooperatively activate ligand-dependent transcription. Proc Natl Acad Sci USA 92:1535–1539.

Blencowe BJ, Nickerson JA, Issner R, Penman S, Sharp PA (1994): Association of nuclear matrix antigens with exon-containing splicing complexes. J Cell Biol 127:593–607.

Bonifer C, Vidal M, Grosveld F, Sippel AE (1990): Tissue specific and position independent expression of the complete gene domain for chicken lysozyme in transgenic mice. EMBO J 9:2843–2848.

Bortell R, Owen TA, Bidwell JP, Gavazzo P, Breen E, van Wijnen AJ, DeLuca HF, Stein JL, Lian JB, Stein GS (1992): Vitamin D-responsive protein–DNA interactions at multiple promoter regulatory elements that contribute to the level of rat osteocalcin gene expression. Proc Natl Acad Sci USA 89:6119–6123.

Brandes LJ, Bogdanovic RP, Tong J, Davie JR, LaBella FS (1992): Intracellular histamine and liver regeneration: high affinity binding of histamine to chromatin, low affinity binding to matrix, and depletion of a nuclear storage pool following partial hepatectomy. Biochem Biophys Res Commun 184:840–847.

Breen EC, van Wijnen AJ, Lian JB, Stein GS, Stein JL (1994): 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.

Carter KC, Bowman D, Carrington W, Fogarty K, McNeil JA, Fay FS, Lawrence JB (1993): A three-dimensional view of precursor messenger RNA metabolism within the mammalian nucleus. Science 259:1330.

Chrysogelos S, Riley DE, Stein GS, Stein JL (1985): A human histone H4 gene exhibits cell cycle dependent changes in chromatin structure that correlate with its expression. Proc Natl Acad Sci USA 82:7535–7539.

Chrysogelos S, Pauli U, Stein G, Stein J (1989): Fine mapping of the chromatin structure of a cell cycle-regulated human H4 histone gene. J Biol Chem 264:1232–1237.

Ciejek E, Tsai MH, O'Malley BW (1983): Actively transcribed genes are associated with nuclear matrix. Nature 306:607–609.

Cockerill PN, Garrard WT (1986): Chromosomal loop anchorage of the Kappa immunoglobulin gene occurs next to the enhancer in a region containing topoisomerase 11 sites. Cell 44:273–282.

Dworetzky SI, Fey EG, Penman S, Lian JB, Stein JL, Stein GS (1990): Progressive changes in the protein composition of the nuclear matrix during rat osteoblast differentiation. Proc Natl Acad Sci USA 87:4605–4607.

Dworetzky S, Wright KL, Fey EG, Penman S, Lian JB, Stein JL, Stein GS (1992): Sequence-specific DNA binding proteins are components of a nuclear matrix attachment site. Proc Natl Acad Sci USA 89:4178–4182.

Felsenfeld G (1992): Chromatin as an essential part of the transcriptional mechanism. Nature 355:219–224.

Fey EG, Penman S (1988): Nuclear matrix proteins reflect cell type of origin in cultured human cells. Proc Natl Acad Sci USA 85:121–125.

Fey EG, Wan KM, Penman S (1984a): Epithelial cytoskeletal framework and nuclear matrix-intermediate filament scaffold: Three-dimensional organization and protein composition. J Cell Biol 98:1973–1984.

Fey EG, Capco DG, Krochmalnic G, Penman S (1984b): Epithelial structure revealed by chemical dissection and unembedded electron microscopy. J Cell Biol 99:203s–208s.

Fey EG, Krochmalnic G, Penman S (1986): The nonchromatin substructures of the nucleus: The ribonucleoprotein (RNP)-containing and RNP-depleted matrices analyzed by sequential fractionation and resinless section electron microscopy. J Cell Biol 102:1654–1665.

Frenkel B, Montecino M, Green J, Aslam F, Desai R, Banerjee C, Stein JL, Lian JB, Stein GS (1996): Basal and vitamin D-responsive activity of the rat osteocalcin promoter in stably transfected osteosarcoma cells: Requirement of upstream sequences for control by the proximal regulatory domain. Endocrinology 137:1080–1088.

Gasser SM, Laemmli UK (1986): The organization of chromatin loops: Characterization of a scaffold attachment site. EMBO J 5:511–518.

Gasser SM, Amati BB, Cardenas ME, Hofmann JF (1989): Studies on scaffold attachment sites and their relation to genome function. Int Rev Cytol 119:57–96.

Getzenberg RH, Coffey DS (1990): Tissue specificity of the hormonal response in sex accessory tissues is associated with nuclear matrix protein patterns. Mol Endocrinol 4:1336–1342.

Getzenberg RH, Pienta KJ, Coffey DS (1990): The tissue matrix: Cell dynamics and hormone action. Endocr Rev 11:399–417.

Getzenberg RH, Pienta KJ, Huang EYW, Coffey DS (1991a): Identification of nuclear matrix proteins in the cancerous and normal rat prostate. Cancer Res 51:6514–6520.

Getzenberg RH, Pienta KJ, Ward WS, Coffey DS (1991b): Nuclear structure and the three-dimensional organization of DNA. J Cell Biochem 47:289–299.

Guo B, Odgren PR, van Wijnen AJ, Last TJ, Fey EG, Penman S, Stein JL, Lian JB, Stein GS (1995): The nuclear matrix protein NMP-1 is the transcription factor YY-1. Proc Natl Acad Sci USA 92:10526–10530.

Guo B, Aslam F, van Wijnen AJ, Frenkel B, DeLuca H, Lian JB, Stein GS, Stein JL (submitted): YY1 transcriptionally regulates RXR/VDR mediated transactivation of the vitamin D responsive osteocalcin gene.

He D, Nickerson JA, Penman S (1990): The core filaments of the nuclear matrix. J Cell Biol 110:569–580.

Hecht A, Laroche T, Strahl-Bolsinger S, Gasser SM, Grunstein M (1995): Histone H3 and H4 N-termini interact with SIR3 and SIR4 proteins: A molecular model for the formation of heterochromatin in yeast. Cell 80:583–592.

Hendzel MJ, Davie JR (1992): Nuclear distribution of histone deacetylase: A marker enzyme for the internal nuclear matrix. Biochim Biophys Acta 1130:307–313.

Hendzel MJ, Delcuve GP, Davie JR (1991): Histone deacetylase is a component of the internal nuclear matrix. J Biol Chem 266:21936–21942.

Holthuis J, Owen TA, van Wijnen AJ, Wright KL, Ramsey-Ewing A, Kennedy MB, Carter R, Cosenza SC, Soprano KJ, Lian JB, Stein JL, Stein GS (1990): Tumor cells exhibit deregulation of the cell cycle histone gene promoter factor HiNF-D. Science 247:1454–1457.

Jarman AP, Higgs DR (1988): Nuclear scaffold attachment sites in the human globin, gene complexes. EMBO J 7:3337–3344.

Klehr D, Maass K, Bode J (1991): Scaffold-attached regions from the human interferon-β domain can be used to enhance the stable expression of genes under the control of various promoters. Biochemistry 30:1264–1270.

Kumara-Siri MH, Shapiro LE, Surks MI (1986): Association of the 3,5,3′-triiodo-L-thyronine nuclear receptor with the nuclear matrix cultured growth hormone-producing rate pituitary tumor cells (GC cells). J Biol Chem 261:L2844–2852.

Landers JP, Spelsberg TC (1992): New concepts in steroid hormone action: Transcription factors, proto-oncogenes, and the cascade model for steroid regulation of gene expression. Crit Rev Eukaryotic Gene Expr 2:19–63.

Lawrence JB, Marselle LM, Singer RH (1989): Highly localized distribution of specific transcripts within interphase nuclei visualized by in situ hybridization. Cell 57:493–502.

Lian JB, Stein GS (1992): Concepts of osteoblast growth and differentiation: basis for modulation of bone cell development and tissue formation. Crit Rev Oral Biol Med 3:269–305.

Luderus ME, de Graaf A, Mattia E, den Blaauwen JL, Grande MA, de Jong L, van Driel R (1992): Binding of matrix attachment regions to lamin B1. Cell 70:949–959.

MacDonald P, Sherman D, Dowd D, Jefcoat S, DeLisle R (1995): The vitamin D receptor interacts with general transcription factor IIB. J Biol Chem 270:4748–4752.

Mancini MA, Shan B, Nickerson JA, Penman S, Lee WH (1994): The retinoblastoma gene product is a cell cycle-dependent, nuclear matrix-associated protein. Proc Natl Acad Sci USA 91:418–422.

Marsolier MC, Tanaka S, Livingstone-Zatchej M, Grunstein M, Thoma F, Sentenac A (1995): Reciprocal interferences between nucleosomal organization and transcriptional activity of the yeast SNR6 gene. Genes Dev 9:410–422.

Merriman HL, van Wijnen AJ, Hiebert S, Bidwell JP, Fey E, Lian JB, Stein JL, Stein GS (1995) The tissue-specific nuclear matrix protein, NMP-2, is a member of the AML/PEBP2/*runt domain* transcription factor family: Interactions with the osteocalcin gene promoter. Biochemistry 34:13125–13132.

Metzger DA, Korach KS (1990): Cell-free interaction of the estrogen receptor with mouse uterine nuclear matrix: evidence of saturability, specificity, and resistance to KC1 extraction. Endocrinology 126:2190–2195.

Metzger DA, Curtis S, Korach KS (1991): Diethylstilbestrol metabolites and analogs: Differential ligand effects on estrogen receptor interactions with nuclear matrix sites. Endocrinology 128:1785–1791.

Montecino M, Pockwinse S, Lian J, Stein G, Stein J (1994): DNase I hypersensitive sites in promoter elements associated with basal and vitamin D dependent transcription of the bone-specific osteocalcin gene. Biochemistry 33:348–353.

Montecino M, Lian JB, Stein GS, Stein JL (1996): Changes in chromatin structure support constitutive and developmentally regulated transcription of the bone specific osteocalcin gene in osteoblastic cells. Biochemistry 35:5093–5102.

Montecino M, Frenkel B, Lian JB, Stein JL, Stein GS (in press): Requirement of distal and proximal promoter sequences for chromatin organization of the osteocalcin gene in bone-derived cells. J Cell Biochem.

Moreno ML, Chrysogelos SA, Stein GS, Stein JL (1986): Reversible changes in the nucleosomal organization of a human H4 histone gene during the cell cycle. Biochemistry 25:5364–5370.

Nelkin BD, Pardoll DN, Vogelstein B (1980): Localization of SV40 genes with supercoiled loop domains. Nucleic Acids Res 8:5623–5633.

Nickerson JA, Krockmalnic G, He D, Penman S (1990): Immunolocalization in three dimensions: Immunogold staining of cytoskeletal and nuclear matrix proteins in resinless electron microscopy sections. Proc Natl Acad Sci USA 87:2259–2263.

Nickerson JA, Penman S (1992): The nuclear matrix: structure and involvement in gene expression. In Stein G, Lian J (eds): "Molecular and Cellular Approaches to the Control of Proliferation and Differentiation." San Diego: Academic Press, pp 343–380.

Owen-Hughes T, Workman J (1994): Experimental analysis of chromatin function in transcription control. Crit Rev Eukaryot Gene Expr 44:403–441.

Pauli U, Chrysogelos S, Stein J, Stein G, Nick H (1987): Protein-DNA interactions in vivo upstream of a cell cycle regulated human H4 histone gene. Science 236:1308–1311.

Pauli U, Chrysogelos S, Nick H, Stein G, Stein J (1989): In vivo protein binding sites and nuclease hypersensitivity in the promoter region of a cell cycle regulated human H3 histone gene. Nucleic Acids Res 17:2333–2350.

Penman S (1991): If genes just make proteins and our proteins are the same, then why are we so different? J Cell Biochem 47:95–98.

Phi-Van L, Stratling WH (1988): The matrix attachment regions of the chicken lysozyme gene co-map with the boundaries of the chromatin domain. EMBO J 7:655–664.

Phi-Van L, Stratling WH (1990): Association of DNA with nuclear matrix. Prog Mol Subcell Biol 11:1–11.

Pienta KJ, Coffey DS (1991): Correlation of nuclear morphometry with progression of breast cancer. Cancer 68: 2012–2016.

Pienta KJ, Getzenberg RH, Coffey DS (1991): Cell structure and DNA organization. Crit Rev Eukaryot Gene Expr 1:355–385.

Ramsey-Ewing A, van Wijnen A, Stein GS, Stein JL (1994): 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.

Renauld H, Aparicio OM, Zierath PD, Billington BL, Chablani SK, Gottschling DE (1993): Silent domains are assembled continuously from the telomere and are defined by promoter distance and strength, and by SIR3 dosage. Genes Dev 7:1133–1145.

Richard-Foy H, Hager G (1987): Sequence-specific positioning of nucleosomes over the steroid-inducible MMTV promoter. EMBO J 6:2321–2328.

Robinson SI, Nelkin BD, Vogelstein B (1982): The ovalbumin gene is associated with the nuclear matrix of chicken oviduct cells. Cell 28:99–106.

Schaack J, HO WY-W, Freimuth P, Shenk T (1990): Adenovirus terminal protein mediates both nuclear–matrix association and efficient transcription of adenovirus DNA. Genes Dev 4:1197–1208.

Spector DL (1990): Higher order nuclear organization: Three-dimensional distribution of small nuclear ribonuclearprotein particles. Proc Natl Acad Sci USA 87:147–151.

Spector DL, Fu X-D, Maniatis T (1991): Associations between distinct pre-mRNA splicing components and the cell nucleus. EMBO J 10:3467–3481.

Stein GS, Lian JB (1995): Molecular mechanisms mediating proliferation-differentiation interrelationships during progressive development of the osteoblast phenotype: Update 1995. Endocr Rev 4:290–297.

Stein GS, Lian JB, Owen TA (1990): Relationship of cell growth to the regulation of tissue-specific gene expression during osteoblast differentiation. FASEB J 4:111–3123.

Stein GS, Stein JL, van Wijnen AJ, Lian JB (1992): Regulation of histone gene expression. Curr Opin Cell Biol 4:166–173.

Stein GS, Stein JL, van Wijnen AJ, Lian JB (1994): 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.

Stein GS, Stein JL, van Wijnen AJ, Lian JB, Quesenberry PJ (1995): Molecular mechanisms mediating cell cycle and cell growth control. Exp Hematol 23:1053–1061.

Stein GS, van Wijnen AJ, Stein JL, Lian JB, Montecino M (in press): Nuclear architecture supports developmental transcriptional control of cell growth and tissue-specific genes. In Bird RC, Stein GS, Lian JB, Stein JL (eds): "Nuclear Structure and Gene Expression." San Diego: Academic Press.

Stief A, Winter DM, Stratling WH, Sippel AE (1989): A nuclear DNA attachment element mediates elevated and position-independent gene activity. Nature 341:343–345.

Thorburn A, Knowland J (1993): Attachment of vitellogenin genes to the nucleoskeleton accompanies their activation. Biochem Biophys Res Commun 191:308–313.

van Driel R, Humbel B, de Jong L (1991): The nucleus: a black box being opened. J Cell Biochem 47:311–316.

van Holde K (1988): "Chromatin." New York: Springer-Verlag.

van Steensel B, van Haarst AD, de Kloet ER, van Driel R (1991): Binding of corticosteroid receptors to rat hippocampus nuclear matrix. FEBS Lett 292:229–231.

van Wijnen AJ, van de Ent FMI, Lian JB, Stein JL, Stein GS (1992): Overlapping and CpG methylation-sensitive protein/DNA interactions at the histone H4 transcriptional cell cycle domain: distinctions between two human H4 gene promoters. Mol Cell Biol 12:3273–3287.

van Wijnen AJ, Bidwell JP, Fey EG, Penman S, Lian JB, Stein JL, Stein GS (1993): Nuclear matrix association of multiple sequence specific DNA binding activities related to SP-1, ATF, CCAAT, C/EBP, OCT-1 and AP-1. Biochemistry 32:8397–8402.

van Wijnen AJ, Aziz F, Grana X, De Luca A, Desai RK, Jaarsveld K, Last TJ, Soprano K, Giordano A, Lian JB, Stein JL, Stein GS (1994): 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.

Vaughan PS, Aziz F, van Wijnen AJ, Wu S, Soprano K, Stein GS, Stein JL (1995): Activation of a cell cycle regulated histone gene by the oncogenic transcription factor IRF2. Nature 377:362–365.

von Kries JP, Buhrmester H, Stratling WH (1991): A matrix/scaffold attachment region binding protein: Identification, purification, and mode of binding. Cell 64:123–135.

Wan KM, Nickerson JA, Krockmalnic G, Penman (1994): The B1CS protein is in the dense assemblies of the nuclearmatrix and relocates to the spindle and pericentriolarfilaments at mitosis. Proc Natl Acad Sci USA 91:594–598.

Ward WS, Coffey DS (1990): Specific organization of genes in relation to the sperm nuclear matrix. Biochem Biophys Res Commun 173:20–25.

Wright JH, Gottschling DE, Zakian VA (1992): Saccharomyces telomeres assume a non-nucleosomal chromatin structure. Genes Dev 6:197–210.

Xing Y, Johnson CV, Dobner PR, Lawrence JB (1993): Higher level organization of individual gene transcription and RNA splicing. Science 259:1326.

Zeitlin S, Parent A, Silverstein S, Efstratiadis A (1987): Pre-mRNA splicing and the nuclear matrix. Mol Cell Biol 7:111–120.

Zenk DW, Ginder GD, Brotherton TW (1990): A nuclearmatrix protein binds very tightly to DNA in the avian β-globin gene enhancer. Biochemistry 29:5221–5226.