Journal of Cellular Biochemistry 47:300–305 (1991)

Regulation of Transcription-Factor Activity During Growth and Differentiation: Involvement of the Nuclear Matrix in Concentration and Localization of Promoter Binding Proteins

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Abstract Several lines of evidence are presented which support involvement of the nuclear matrix in regulating the transcription of two genes, histone and osteocalcin, that are reciprocally expressed during development of the osteoblast phenotype. In the 5′ regulatory region of an H4 histone gene, which is expressed in proliferating osteoblasts early during the developmental/differentiation sequence, a dual role is proposed for the nuclear matrix binding domain designated NMP-1 (−589 to −730 upstream from the transcription start site). In addition to functioning as a nuclear matrix attachment site, the sequences contribute to the upregulation of histone gene transcription, potentially facilitated by concentration and localization of an 84kD ATF DNA binding protein. A homologous nuclear matrix binding domain was identified in the promoter of the osteocalcin gene, which is expressed in mature osteoblasts in an extracellular matrix undergoing mineralization. The NMP binding domain in the osteocalcin gene promoter resides contiguous to the vitamin D responsive element. Together with gene and transcription factor localization, a model is proposed whereby nuclear matrix-associated structural constraints on conformation of the osteocalcin gene promoter facilitates vitamin D responsiveness mediated by cooperativity at multiple regulatory elements.

Key words: CCAAT box, osteocalcin promoter, histone promoter, regulatory elements, vitamin D gene regulation, hormone control, transcription factors

Fundamental to understanding the expression of genes during cell and tissue specialization are mechanisms operative in modulating the sequence-specific interactions of promoter regulatory elements with their cognate DNA binding proteins. The complexity of the biological mechanism is reflected by requirements which include recognition and selective binding of transcription factors at defined stages of differentiation with the modularly organized promoter elements. Each element is multipartite and responsive to an integrated series of physiological regulatory signals that exert both positive and negative control. Here the complexity provides a basis for diversity and flexibility in accommodating requirements for the extent to which specific genes are expressed as differentiation progresses.

While over the past several years, considerable progress has been made in the identification and characterization of such gene regulatory elements and transcription complexes, a basic question has emerged with respect to transcriptional regulation within the nucleus of an intact cell. How can transcription of specific genes be selectively initiated with a limited representation of the regulatory factors and the regulatory elements? In this article, we will explore transcriptional regulation within the three-dimensional context of nuclear architecture and chromatin structure. We will consider the potential involvement of the nuclear matrix in the concentration and localization of promoter regulatory elements, as well as sequence-specific transcription factors.

INVOLVEMENT OF THE NUCLEAR MATRIX IN MEDIATING GENE EXPRESSION AND DNA REPLICATION

The nuclear matrix is operationally defined as the proteinaceous nuclear substructure that re-

Received May 22, 1991; accepted June 6, 1991.

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sists both nuclease digestion and salt extractions. While considerable controversy exists regarding the extent to which various nuclear matrix preparations retain in vivo fidelity of composition and organization [1,2], the existence of the nuclear matrix as a network of polymorphic, anastomosing filaments within the nucleus is undeniable. Historically, the nuclear matrix was independently identified as a "scaffold" associated with metaphase chromosomes [3,4], and as the non-nucleic acid structural component of the interphase nucleus potentially involved with DNA replication and transcription [5,6]. In both cases, characterization was initially by microscopy, subsequently complemented by biochemical analysis. As with all structural components of the cell, composition is at least in part operationally defined by the isolation procedure. However, it is becoming apparent that the nuclear matrix is extremely dynamic structurally and functionally, undergoing modifications in both composition and organization which are responsive to and supporting cellular requirements for chromatin organization and gene expression. Distinct differences are observed in proliferating (mitotic and interphase), quiescent, and differentiated cells. Indications of a role for the nuclear matrix in the regulation of gene expression includes: sites for DNA attachment [7–13], DNA replication [14], preferential association with actively transcribed genes [15–22], association with HN-RNA [23–29], RNA synthesis at fixed transcriptional complexes, pre-mRNA splicing [30], and specific association of steroid receptors [31,32]. Involvement of the nuclear matrix in the regulation of cell and tissue specific gene expression is further suggested by recent demonstrations of variations in the nuclear matrix protein composition of different cells, tissues, and tumors [6,33–35].

Additional support for participation of the nuclear matrix in transcriptional control is provided by recent results (Fig. 1) from two-dimensional electrophoretic analysis of nuclear matrix proteins during a developmental sequence associated with osteoblast growth and differentiation. Changes in the protein composition of the nuclear matrix parallel sequential expression of genes during progressive expression of the osteoblast phenotype in primary cultures of calvarial-derived mammalian osteoblasts, which during a 35-day period develop a tissue-like organization of osteocytic cells in a mineralized extracellular matrix similar to embryonic bone [36–40]. The composition of the nuclear matrix is constant within each of the three principal periods of osteoblast differentiation (proliferation and extracellular matrix biosynthesis, extracellular matrix maturation and organization, and extracellular matrix mineralization), but is modified dramatically at the two key transition points—at 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 [41].

CONTRIBUTION OF THE NUCLEAR MATRIX TO TRANSCRIPTIONAL CONTROL OF GENE EXPRESSION SUPPORTING THE PROLIFERATION/DIFFERENTIATION RELATIONSHIP DURING TISSUE SPECIALIZATION

More direct evidence linking the nuclear matrix with transcriptional control is provided by a relationship of the nuclear matrix to activity of two genes which are reciprocally expressed during development of the osteoblast phenotype in primary cultures of calvarial-derived osteoblasts—the cell cycle-regulated histone genes which are expressed only in proliferating osteoblasts early in the developmental/differentiation sequence [36,40,42] and the osteocalcin gene, which is expressed in mature osteoblasts undergoing an ordered deposition of hydroxyapatite in the mineralizing extracellular matrix [36,40,42]. Transcriptional control is operative in the stringently regulated expression of both genes; and the modularly organized promoters, each with well-defined transcription factor binding elements, contribute synergistically and antagonistically to determine the levels of transcription [39,43–48].

The H4 histone gene is associated with the nuclear matrix only during the proliferation period of the osteoblast developmental sequence when actively transcribed, at which time the distal promoter factor NMP-1 that binds to a strong positive regulatory element is a nuclear matrix component [49]. Sequence-specific binding to the nuclear matrix by a 141 bp element containing the NMP-1 site, which resides between −589 and −730 upstream from the transcription start site was initially established by assaying a sequential series of radiolabelled pro-

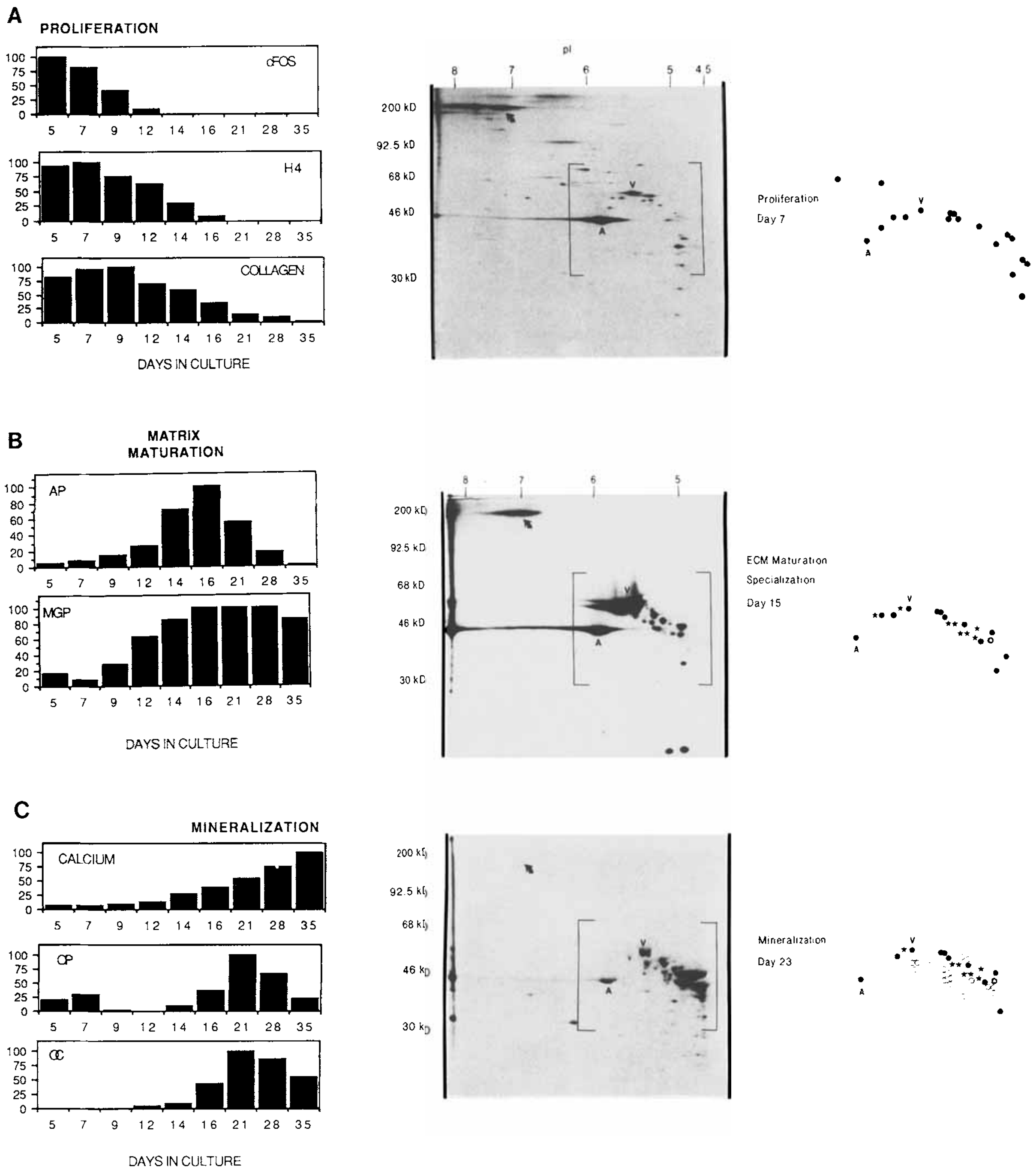

Fig. 1. Progressive modifications in the protein composition of the nuclear matrix during development of the osteoblast phenotype. The panels on the left indicate the temporal expression of cell growth, extracellular matrix, and osteoblast phenotype related genes during development of the osteoblast phenotype in vitro. Isolated primary cells were cultured after confluence in BGJb medium supplemented with 10% fetal calf serum, 50 μg/ml ascorbic acid, and 10 mM β-glycerol phosphate. Cellular RNA was isolated at times indicated during the proliferation/differentiation time course and assayed for steady state levels of various RNA 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. **(A)** Examples of genes expressed during proliferation include the cell growth regulated histone (reflects DNA synthesis), c-fos, and Type I collagen involved in synthesis of the bone extracellular matrix. **(B)** Genes associated with extracellular matrix maturation shown are alkaline phosphatase and matrix gla protein. **(C)** Genes induced with extracellular matrix mineralization represented are osteopontin and osteocalcin. Calcium accumulation is additionally indicated. The fluorographs on the right represent 2-dimensional gel electrophoresis of ^{35}S-methionine labeled nuclear matrix proteins isolated from primary rat osteoblast cultures on day 7 (active proliferation), day 15 (extracellular matrix maturation and specialization) and day 23 (extracellular matrix mineralization) of the osteoblast developmental sequence. Schematically illustrated are proteins in the bracketed regions which are representative of stage-specific changes in the nuclear matrix composition. The arrow points to a 190 kD protein that completely disappears as the cell differentiates. Symbols represent proteins that appear on that particular day. ●-day 7; ✪-day 15; ○-day 23. The symbols are removed when the given protein is no longer present.

moter segments. These sequence-specific protein-DNA interactions have been confirmed and further localized by gel mobility shift analysis and defined at single nucleotide resolution by OP-CU footprint analysis, DMS protection, and competition analysis. Characterization of the NMP-1 nuclear matrix protein with respect to molecular weight by ultraviolet crosslinking studies and DNA binding site recognition by NMP-1 oligonucleotide affinity chromatography indicates that NMP-1 is a unique member of the ATF transcription factor family, suggesting a dual role for the nuclear matrix binding domain in the H4 histone gene promoter. In addition to functioning as a nuclear matrix attachment site, the sequences contribute to the upregulation of histone gene transcription, potentially facilitated by the nuclear matrix serving to concentrate and localize an 84 kD ATF DNA binding protein [49].

Further support for involvement of the nuclear matrix in transcriptional control during development of the bone cell phenotype is provided by our recent observation that a homologous nuclear matrix attachment site resides in the osteocalcin gene promoter and that the sequence-specific DNA binding proteins are associated with the nuclear matrix only when the osteocalcin gene is expressed [50]. Identification of an NMP-1 site in the osteocalcin gene promoter adjacent to the vitamin D responsive element serves as the basis for postulating a model for the three dimensional organization of the osteocalcin 5′ regulatory sequences that is consistent with synergistic interactions of two regulatory elements which are vitamin D responsive [50] and contribute to upregulation of osteocalcin gene transcription (Fig. 2). Here, in addition to gene and transcription factor localization, a potential mechanism for nuclear matrix-mediated structural constraints on the conformation of the osteocalcin gene promoter that facilitates vitamin D responsiveness may be operative.

Sequence-specific vitamin D receptor binding at the vitamin D responsive element (−462 to −440) which functions as the primary site for 1,25 dihydroxyvitamin D_3 interaction [44,45] has been demonstrated by vitamin D inducible and antibody sensitive protein-DNA interactions verified by footprint competition analysis. Sequence-specific protein-DNA interactions in response to vitamin D have also been shown in the proximal regulatory region of the osteocalcin gene promoter where sequences responsible for basal

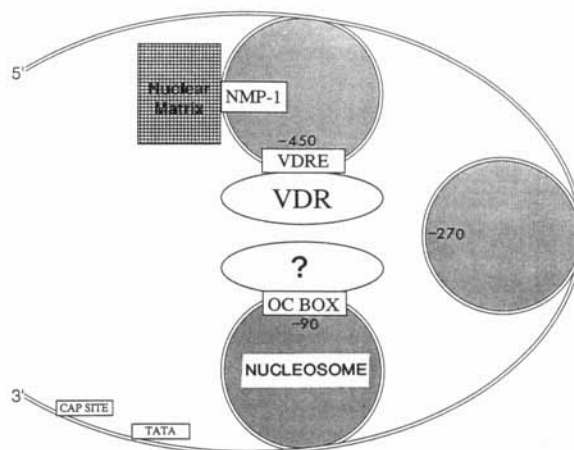

Fig. 2. Model of three dimensional organization of the osteocalcin gene promoter. The proposed model accounts for cooperative interactions between two regulatory regions in the osteocalcin gene promoter, the proximal basal regulatory elements (e.g. the osteocalcin box (−99 to −76) (OC Box and/or the TATA motif), and the vitamin D responsive element (−462 to −440) (VDRE), for controlling levels of basal transcription and vitamin D modulation during development of the osteoblast phenotype. Experimental evidence for close proximity of the VDRE and the proximal promotor sequences includes: (1) vitamin D-dependent and receptor antibody sensitive protein-DNA interactions at both the VDRE; (2) vitamin D-inducible sequence-specific protein-DNA interactions in the proximal promoter region; (3) the presence of 3 nucleosomes each 180 nucleotides, as detected by micrococcal nuclease digestion in the 5′ promoter of the osteocalcin gene spanning the regions of the OC Box and the VDRE, thereby reducing distance between these elements; and (4) a nuclear matrix attachment consensus sequence (NMP-1) associated with the VDRE. A question mark signifies several possibilities for receptor mediated interactions between the vitamin D receptor binding complex and proximal regulatory elements that may be function associated with basal transcription factors.

transcription reside. Results indicating the presence of nucleosomes [50], each encompassing approximately 180 bp's within the promoter sequence spanning the vitamin D responsive element and the osteocalcin box region, reduces the potential distance between the two promotor regulator domains. This raises the possibility of functional cooperativity between vitamin D receptor and proximal promoter transactivation factors bound in the two regions, which thereby enhances transcriptional activity. Alternatively, the steroid receptor binding motif in the osteocalcin box may be utilized as a low affinity site for interaction with the vitamin D receptor complex. Then, from the proximity of vitamin D receptors bound at both the osteocalcin box and the vitamin D responsive element, reflecting the nucleosome-associated chromatin organization of the osteocalcin gene proximal

promoter sequences, an elevated concentration of vitamin D receptor may be established. Hence, increased potential for interactions at the vitamin D responsive element can occur and contribute to the vitamin D enhancement of osteocalcin gene transcription.

Equally important is consideration of the possibility that gene nuclear matrix-association, together with structural properties of chromatin, may be functionally related to other protein-DNA interactions at the vitamin D responsive element and osteocalcin box that influence transcriptional activity of the osteocalcin gene, possibly further contributing to cooperativity between the two regulatory elements. The coordinate occupancy of AP-1 sites within the vitamin D responsive element and osteocalcin box by the nuclear proto-oncogene-encoded fos and jun proteins which overlap hormone receptor binding domains is such an example and one where suppression may be the resulting activity [45,51].

Formal proof for such models remains to be provided, and, unquestionably, the proposed mechanisms are simplifications of regulatory parameters for extremely complex biological relationships. However, it is difficult to dismiss the involvement of chromatin structure, or a role for the nuclear matrix, in the concentration and localization of steroid receptors and other promoter regulatory factors in modulating the transcriptional activity of genes associated with cell and tissue differentiation in response to physiological mediators. The viability of our model is that it may explain regulation in relation to cell structure and is testable experimentally.

ACKNOWLEDGMENTS

Studies reported from the authors' laboratories were supported by the National Institutes of Health (AR33920, AR35166, AR39588, and GM32010), the March of Dimes Defects Foundation, and the Northeast Osteogenesis Imperfecta Society. The authors are indebted to Ms. Patricia Jamieson for her expert editorial assistance in the preparation of this manuscript.

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