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Intranuclear Trafficking of Transcription Factors: Requirements for Vitamin D-Mediated Biological Control of Gene Expression

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Abstract The architecturally associated subnuclear organization of nucleic acids and cognate regulatory factors suggest functional interrelationships between nuclear structure and gene expression. Mechanisms that contribute to the spatial distribution of transcription factors within the three-dimensional context of nuclear architecture control the sorting of regulatory information as well as the assembly and activities of sites within the nucleus that support gene expression. Vitamin D control of gene expression serves as a paradigm for experimentally addressing mechanisms that govern the intranuclear targeting of regulatory factors to nuclear domains where transcription of developmental and tissue-specific genes occur. We will present an overview of molecular, cellular, genetic, and biochemical approaches that provide insight into the trafficking of regulatory factors that mediate vitamin D control of gene expression to transcriptionally active subnuclear sites. Examples will be presented that suggest modifications in the intranuclear targeting of transcription factors abrogate competency for vitamin D control of skeletal gene expression during development and fidelity of gene expression in tumor cells. J. Cell. Biochem. 88: 340–355, 2003.

Key words: RUNX/cbfa/AML; nuclear matrix; osteocalcin; vitamin D

THE REQUIREMENTS FOR PHYSIOLOGICAL CONTROL OF SKELETAL GENE EXPRESSION IN VIVO

Bone formation during development and skeletal remodeling throughout life requires the complex and interdependent expression of cell growth and phenotypic genes [reviewed in Bilezikian et al., 2002; Stein et al., 2002]. There is a requirement for responsiveness to a broad spectrum of regulatory cues that transduce physiological signals from the extracellular matrix to sites within the nucleus where genes that mediate skeletogenesis reside [Stein et al., 1997; Lian and Stein, 1999; Xiao et al., 2002; Zaidi et al., 2002b]. As our understanding of gene regulatory mechanisms expand, it becomes increasingly evident that there are unique parameters of transcriptional control that support the transient activation and suppression of genes for skeletal development and bone homeostasis. Other mechanisms are invoked for long-term obligations to gene expression that sustain the specialized properties of bone cells. Vitamin D serves as a principal modulator of skeletal gene transcription necessitating an understanding of interfaces between activity of this steroid hormone with regulatory cascades that are functionally linked to the activity of skeletal genes [Lian et al., 1999].

There is growing appreciation for the repertoire of factors that influence gene expression for commitment to the osteoblast lineage. It is well documented that sequentially expressed

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genes support progression of osteoblast differentiation through developmental transition points where responsiveness to phosphorylation-mediated regulatory cascades determine competency for establishing and maintaining the structural and functional properties of bone cells [Aubin and Liu, 1996; Lian and Stein, 1999; Schinke and Karsenty, 2002; Stein et al., 2002]. However, the catalogue of promoter elements and cognate regulatory proteins that govern skeletal gene expression offer essential but insufficient insight into mechanisms that are operative in vivo. Gene promoters serve as regulatory infrastructure by functioning as blueprints for responsiveness to the flow of cellular regulatory signals. But to access the specific genetic information necessitates understanding transcriptional control of skeletal genes within the contexts of the subnuclear organization of nucleic acids and regulatory proteins. Explanations are required for: (1) convergence of multiple regulatory signals at promoter sequences; (2) the integration of regulatory information at independent promoter domains; (3) selective utilization of redundant regulatory pathways; (4) thresholds for initiation or downregulation of transcription with limited intranuclear representation of promoter elements and regulatory factors; (5) mechanisms that render the promoters of cell growth and phenotypic genes competent for protein–DNA and protein–protein interactions in a physiologically responsive manner; (6) the composition, organization, and assembly of sites within the nucleus that support transcription; and (7) the intranuclear trafficking of regulatory proteins to transcriptionally active foci.

GENE EXPRESSION WITHIN THE THREE-DIMENSIONAL CONTEXT OF NUCLEAR ARCHITECTURE: REQUIREMENTS FOR BOUNDARIES AND DIRECTIONS

Evidence is accruing that the architectural organization of nucleic acids and regulatory proteins within the nucleus support functional interrelationships between nuclear structure and gene expression (Fig. 1). There is increasing acceptance that components of nuclear architecture are functionally linked to the organization and sorting of regulatory information in a manner that permits selective utilization [Berezney and Jeon, 1995; Berezney et al., 1996; Zeng et al., 1997, 1998; Lamond and Earnshaw, 1998; Ma et al., 1998, 1999; McNeil et al., 1998, 1999; Stein et al., 2000a; Choi et al., 2001; DeFranco, 2002; Gasser, 2002]. The primary level of nuclear organization, the representation and ordering of genes and promoter elements, provide alternatives for physiological control. The molecular organization of regulatory elements, the overlap of regulatory sequences within promoter domains, and the multipartite composition of regulatory complexes increase options for responsiveness. Chromatin structure and nucleosome organization reduce distances between regulatory sequences, facilitate crosstalk between promoter elements and render elements competent for interactions with positive and negative regulatory factors [Bresnick et al., 1990, 1991; Archer et al., 1991, 1992; Breen et al., 1994; Cairns et al., 1994, 1996; Côté et al., 1994; Kwon et al., 1994; Vettese-Dadey et al., 1994, 1996; Tsukiyama and Wu, 1995; Bartsch et al., 1996; Brownell et al., 1996; Wang et al., 1996a,b; Ito et al., 1997; Ura et al., 1997; Varga-Weisz et al., 1997; Cote et al., 1998; Imbalzano, 1998; Lorch et al., 1998; Peterson et al., 1998; Schnitzler et al., 1998; Chen et al., 1999; Montecino et al., 1999; Gasser, 2002; Strahl et al., 2002]. The components of higher order nuclear architecture that includes nuclear pores [Blobel, 1995; Ullman et al., 1997; Bangs et al., 1998; Mattaj and Englmeier, 1998; Moroianu, 1999; Iborra et al., 2000], the nuclear matrix and subnuclear domains contribute to the subnuclear distribution and activities of genes and regulatory factors [reviewed in Berezney and Jeon, 1995; Penman, 1995; Misteli, 2000]. Compartmentalization of regulatory complexes is illustrated by focal organization of PML bodies [Dyck et al., 1994; Weis et al., 1994; Grande et al., 1996; Melnick and Licht, 1999], Runx bodies [Aubin and Liu, 1996; Zeng et al., 1997; McNeil et al., 1999; Harrington et al., 2002; Javed et al., 2000], the nucleolus, chromosomes [Ma et al., 1999] as well as by the punctate intranuclear distribution of sites for replication [Leonhardt et al., 1998; Wei et al., 1998; Cook, 1999], DNA repair, transcription [Ciejek et al., 1983; Guo et al., 1995; Merriman et al., 1995; van Steensel et al., 1995; Htun et al., 1996; McNeil et al., 1998; Stenoien et al., 1998; Tang et al., 1998b; Cook, 1999; Kimura et al., 1999; Verschure et al., 1999; Wei et al., 1999] and the processing of gene transcripts [Misteli and Spector, 1999; Smith et al., 1999; Misteli, 2000]. There is

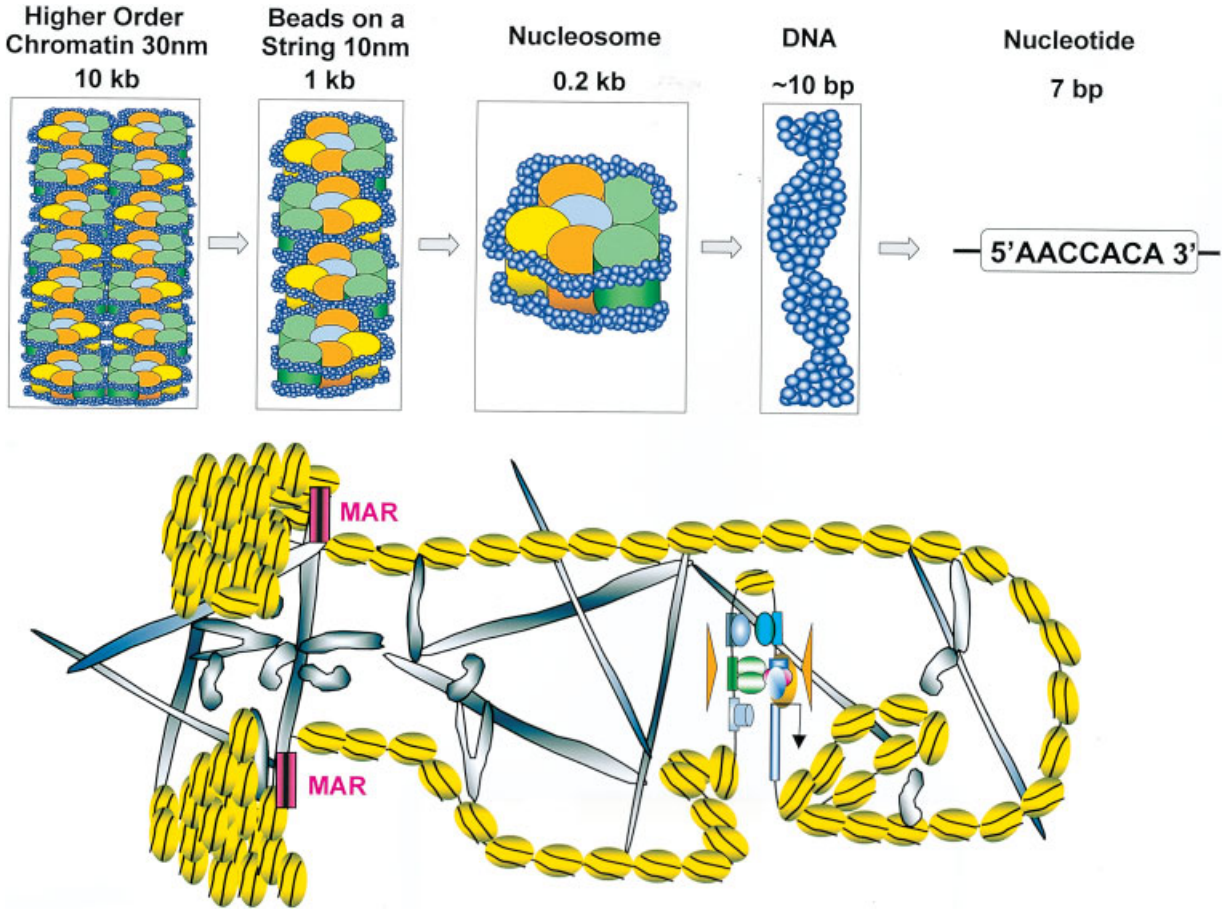
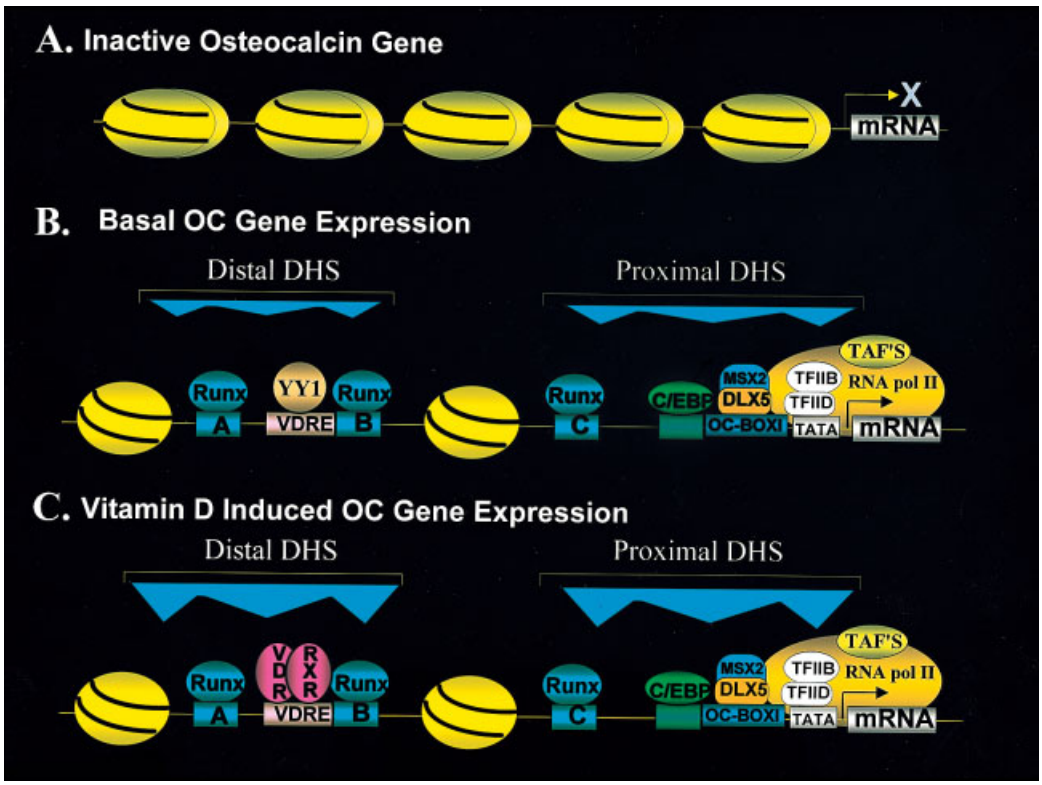


Fig. 1. Levels of chromatin architecture within nucleus. The upper panel schematically illustrates sequential packaging of DNA from a linear double helix to higher order chromatin organization. The seven nucleotide Runx binding element is shown. The lower panels schematically illustrate a loop domain. Elements at the base of the loop structure designated matrix attachment regions (MARs) [MARs, or alternatively locus control regions (LCR), or scaffold attachment region (SCA)] mediate association of these genomic domains with the nuclear matrix or scaffold. Genes within the loop domain undergo local chromatin remodeling to establish competency for protein–DNA and protein–protein interactions that support transcriptional activation or suppression. The machinery for chromatin remodeling includes ATP dependent and independent enzymes (e.g., SWI/SNIF related proteins) and factors that mediate post-translational modification of the histones (e.g., Histone acetyl transferases, histone deacytalases, kinases, phosphatases, and methyalases). The nuclear matrix provides anchorage for both nucleic acids and regulatory as well as coregulatory factors that control transcription.

emerging recognition that nuclear structure and function are causally interrelated. With mounting evidence for organization of nucleic acids and regulatory proteins into subnuclear domains that are associated with components of nuclear architecture, the perception of a dichotomy between nuclear architecture and control of gene expression is difficult to justify. Rather, the challenges are to design experiments to define mechanisms that direct genes and regulatory factors to sites within the nucleus where localization integrates regulatory parameters of gene expression and establish microenvironments with boundaries between regulatory complexes that are required for fidelity of activity.

The bone-specific osteocalcin gene and skeletal-restricted RUNX2 (AML3/CBFA1/PEBP2α) transcription factor serve as paradigms for obligatory relationships between nuclear structure with physiological control of skeletal gene expression [Merriman et al., 1995; Banerjee et al., 1996, 1997; Ducy et al., 1997; Javed et al., 1999, 2001] (Fig. 2). The modularly organized promoter of the bone specific osteocalcin gene contains proximal and distal regulatory elements that support basal, tissue-specific as well as growth factor, homeodomain, signaling protein, and steroid hormone responsive transcriptional control [reviewed in Demay et al., 1990; Markose et al., 1990; Bortell et al., 1992; Hoffmann et al., 1994; Tamura and Noda,



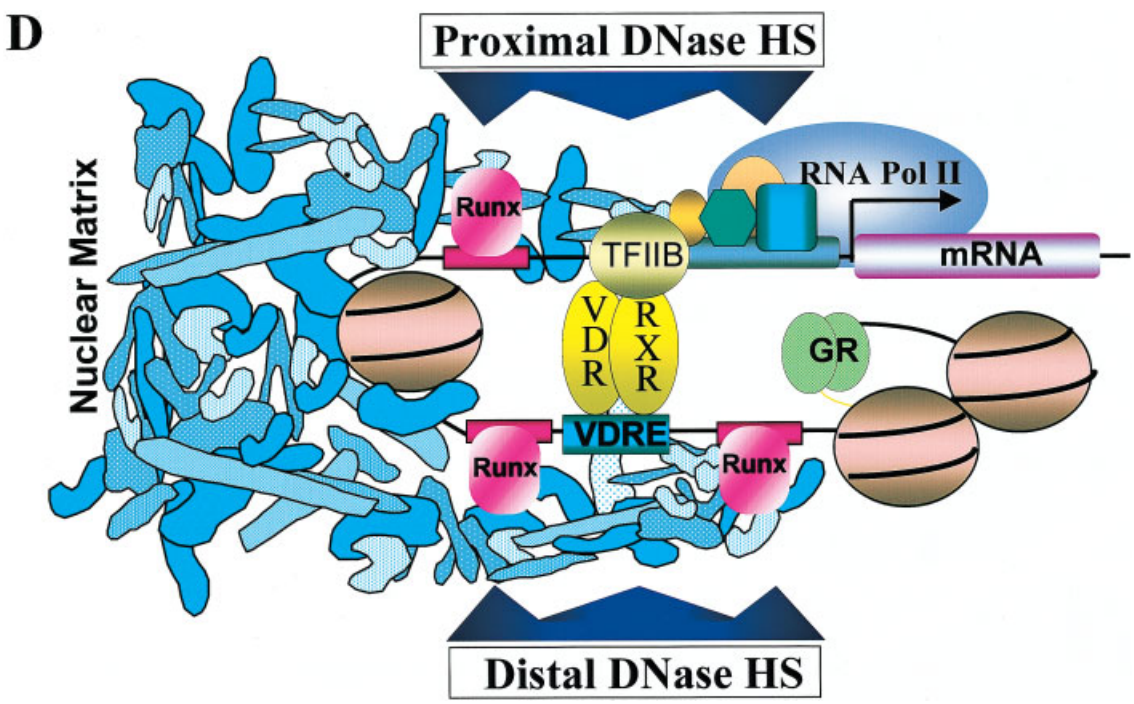


Fig. 2. Remodeling of the osteocalcin gene promoter during developmental stages of osteoblast differentiation. Schematic illustration of the inactive rat osteocalcin gene with nucleosomes placed in the proximal tissue-specific and upstream enhancer region of the promoter (**panel A**). Factors that support basal tissue-specific transcription are recruited to the OC gene promoter and are organized in the proximal and upstream promoter domains. Modifications in chromatin structure that mediate assembly of the regulatory machinery for gene expression are reflected by the nuclease hypersensitive sites (DHS). Positioned nucleosome resides between the proximal basal and distal enhancer regions of the promoter (**panel B**). In response to Vitamin D, chromatin remodeling renders the upstream VDRE (vitamin D responsive element) competent for binding the VDR/RXR heterodimer at its cognate element (**panel C**). Higher order chromatin organization permits cross talk between basal transcription machinery and the Vitamin D receptor complex that involves direct interactions of the Vitamin D receptor with the TFIIB regulatory factor (**panel D**).

1994; Ducy and Karsenty, 1995; Guo et al., 1995; Merriman et al., 1995; Banerjee et al., 1996; Bilezikian et al., 2002]. Modulation of osteocalcin gene expression during bone formation and remodeling requires physiologically responsive accessibility of these proximal and upstream promoter sequences to regulatory and coregulatory proteins as well as protein–protein interactions that integrate independent promoter domains. The RUNX transcription factors contribute to the control of skeletal gene expression by sequence-specific binding to promoter elements of target genes and serving as scaffolds for the assembly and organization of coregulatory proteins that mediate biochemical and architectural control of promoter activity.

Chromatin Remodeling Facilitates Vitamin D-Mediated Promoter Accessibility and Integration of Regulatory Activities

It is well recognized that genomic DNA is packaged as chromatin. These "bead on a string" structures designated nucleosomes are structurally remodeled to accommodate requirements for transcription, emphasizing the extent to which architectural organization of genes is causally related to functional activity. The identification and characterization of proteins that catalyze histone acetylation, deacetylation, and phosphorylation [Bresnick et al., 1990, 1991; Georgieva et al., 1991; Brosch et al., 1992; Lopez-Rodas et al., 1992; Toh et al., 1994, 1997, 1999; Vettese-Dadey et al., 1994, 1996; Chen and Evans, 1995; Horlein et al., 1995; Bartsch et al., 1996; Brownell et al., 1996; Fondell et al., 1996; Lechner et al., 1996; Yang et al., 1996; Ura et al., 1997; Cui et al., 1998; Davie, 1998; Feng et al., 1998; Grant et al., 1998; Janknecht et al., 1998; Laherty et al., 1998; Lavinsky et al., 1998; Siddique et al., 1998; Workman and Kingston, 1998; Zhang et al., 1998; Zhou et al., 1998; Ayer, 1999; Kornberg and Lorch, 1999; Montecino et al., 1999; Safadi et al., 1999; Agalioti et al., 2000; Hassan et al., 2001] as well as the SWI/SNF-related proteins [Cairns et al., 1994, 1996; Cote et al., 1994, 1998; Côté et al., 1994; Kwon et al., 1994; Tsukiyama et al., 1994, 1999; Tsukiyama and Wu, 1995; Wang et al., 1996a,b; Ito et al., 1997; Varga-Weisz et al., 1997; Imbalzano, 1998; Lorch et al., 1998; Peterson et al., 1998; Schnitzler et al., 1998; Workman and Kingston, 1998; de la Serna et al., 2001] that facilitate chromatin remodeling and potentially the accessibility of promoter sequences to regulatory and coregulatory factors, represent an important dimension in control of the structural and functional activities of genes and promoter regulatory elements. Relationships of regulatory signaling pathways to enhance activities that modulate gene, chromatin, and chromosome organization can now be directly investigated. Additional levels of specificity are provided by structural modifications of gene promoters that influence competency for factor interactions. Simply stated, changes in the architectural properties of promoter elements determine effectiveness of gene regulatory sequences as substrates for interactions with regulatory factors. The regulatory and regulated parameters of chromatin remodeling and the rate limiting steps in the relevant signaling cascades are being actively pursued and will unquestionably provide insight into skeletal gene regulatory mechanisms from structural and functional perspectives.

The chromatin organization of the osteocalcin gene illustrates dynamic remodeling of a promoter to accommodate requirements for phenotype-related developmental and steroid hormone responsive activity. Nuclease digestion and ligation-mediated PCR analysis as well as in vitro nucleosome reconstitution studies establish the placement of nucleosomes in the proximal basal/tissue specific domain and at the upstream vitamin D responsive element, blocking accessibility of these promoter sequences to regulatory proteins in immature bone cells when this skeletal restricted gene is suppressed [Breen et al., 1994; Montecino et al., 1994a,b, 1996, 1999]. In response to developmental and skeletal regulatory signals the striking removal of a nucleosome and modifications in chromatin structure renders the proximal promoter of the OC gene accessible to regulatory and coregulatory proteins that support basal level activity [Breen et al., 1994; Montecino et al., 1994a,b, 1996, 1999; Javed et al., 1999]. Vitamin D enhancement of osteocalcin gene transcription is associated with removal of the nucleosome at the upstream vitamin D responsive element that permits binding of the vitamin D receptor-RXR heterodimer [Breen et al., 1994; Montecino et al., 1994a,b, 1996, 1999; Javed et al., 1999]. The retention of a nucleosome between the proximal and upstream enhancer domain reduces distance between the basal and vitamin D

responsive element and supports a promoter configuration that is conducive to protein–protein interactions between the vitamin D receptor and the basal TF2B transcription factor [Blanco et al., 1995; MacDonald et al., 1995; Guo et al., 1997]. Interaction of the vitamin D receptor at the distal promoter region of the bone specific osteocalcin gene requires nucleosomal remodeling [Paredes et al., 2002].

Thus, insight into control of skeletal gene expression can be obtained from the understanding of mechanisms that alter osteocalcin gene chromatin organization under biological conditions. Site directed mutagenesis of osteocalcin genes that are genetically integrated in stable cell lines have established that RUNX elements flanking the proximal and upstream promoter sequences are responsible for developmental and vitamin D-induced chromatin remodeling [Javed et al., 1999]. Reduced CpG methylation is associated with transcriptional activation of the bone-specific osteocalcin gene in osteoblasts [Villagra et al., 2002]. In vitro and in vivo genetic approaches have demonstrated that RUNX2 controls developmental and steroid hormone-responsive chromatin reconfiguration of the osteocalcin gene promoter [Javed et al., 1999; Gutierrez et al., 2000a]. Chromatin immunoprecipitation analyses have shown that developmental and vitamin D-linked remodeling of osteocalcin gene promoter organization is accompanied by acetylation of histones in the proximal basal and upstream vitamin D responsive element domains [Shen et al., 2002]. This post-translational modification of histone proteins reduces the tenacity of histone DNA interactions in a manner that is conducive to an open chromatin organization with increased access to regulatory factors. The most compelling evidence for a functional involvement of chromatin organization in skeletal gene expression is the obligatory relationship of dynamic changes in the biochemical and structural properties of osteocalcin gene promoter organization with competency for bone tissue-restricted and enhanced transcription in response to vitamin D [Javed et al., 1999]. Yet, despite the cogent support for a central role of chromatin remodeling in transcriptional control of the osteocalcin gene, there are open-ended questions. It is not justifiable to extrapolate from these findings to conclude that all genes that are activated and suppressed during skeletogenesis employ identical mechanisms. From a broader biological perspective there are multiple levels of control that must be mechanistically characterized to explain physiologically responsive regulation of chromatin structure within restricted and global genomic contexts.

Nuclear Microenvironments: Accommodating the Rules That Govern In Vivo Transcriptional Control

Key components of the basal transcription machinery and several tissue-specific transcription factor complexes are functionally compartmentalized as specialized subnuclear domains [Robinson et al., 1982; Stief et al., 1989; Schaack et al., 1990; Dworetzky et al., 1992; van Wijnen et al., 1993; Htun et al., 1996; Banerjee et al., 1997; Zeng et al., 1997, 1998, Bangs et al., 1998; Lamond and Earnshaw, 1998; McNeil et al., 1998; Stenoien et al., 1998; Tang et al., 1998b; Kimura et al., 1999; McNeil et al., 1999; Misteli and Spector, 1999; Wei et al., 1999; Stein et al., 2000b; DeFranco, 2002]. Such compartmentalization may, at least in part, accommodate biological constraints on the control of transcription in nuclei of intact bone cells. The low representation of promoter regulatory elements and cognate transcription factors necessitate a subnuclear organization of nucleic acids and regulatory proteins that supports threshold concentrations for the activation and repression of gene expression. From an historical perspective, compartmentalization of the regulatory machinery for ribosomal genes in nucleoli and the organization of chromosomes during mitosis provide paradigms for intranuclear localization of genes and regulatory complexes.

During the past several years there has been growing recognition that the organization of nucleic acids and regulatory proteins is functionally linked to the assembly, organization, and activity of gene regulatory machinery. Cellular, molecular, biochemical, and genetic evidence indicate an obligatory relationship between sites within the nucleus where regulatory complexes reside and fidelity of transcriptional control. The biological relevance for the intranuclear distribution of regulatory complexes is directly reflected by aberrant nuclear structure-gene expression interrelationships that are associated with perturbations in skeletal development [Choi et al., 2001] and leukemia [McNeil et al., 1999].

Intranuclear Trafficking of Skeletal Regulatory Factors to Subnuclear Sites That Support Transcription: "to be in the Right Place at the Right Time"

There is a need to gain insight into mechanisms that vectorially direct skeletal factors to subnuclear sites where regulatory events occur. Association of osteoblast, myeloid, and lymphoid RUNX transcription factors that mediate tissue-specific transcription [Bae et al., 1993; Meyers et al., 1993, 1995, 1996; Wang et al., 1993; Nuchprayoon et al., 1994; Frank et al., 1995; Merriman et al., 1995; Satake et al., 1995; Banerjee et al., 1996, 1997; Ducy et al., 1997; Zeng et al., 1997] with the nuclear matrix has permitted direct examination of mechanisms for targeting regulatory proteins to transcriptionally active subnuclear domains. Both biochemical and immunofluorescence analyses have shown that RUNX transcription factors exhibit a punctate nuclear distribution that is associated with the nuclear matrix in situ [Tang et al., 1998a; Zeng et al., 1997, 1998; Zaidi et al., 2002]. Taken together, these observations are consistent with the concept that the nuclear matrix is functionally involved in gene localization and in the concentration and subnuclear localization of regulatory factors [Dworetzky et al., 1992; Bidwell et al., 1993; van Wijnen et al., 1993; Blencowe et al., 1994; Mancini et al., 1994; Stein et al., 1994, 1996, 1997; Nickerson et al., 1995; Zeng et al., 1997].

The initial indication that nuclear matrix association of RUNX factors is required for maximal activity was provided by the observation that transcriptionally active RUNX proteins associate with the nuclear matrix but inactive C-terminally truncated RUNX proteins do not [Zeng et al., 1997, 1998; Choi et al., 1999, 2001; Javed et al., 2000; Zaidi et al., 2001] (Fig. 3). This localization of RUNX was established by biochemical fractionation and in situ immunofluorescence as well as by green fluorescent protein tagged RUNX proteins [Harrington et al., 2002] in living cells. Colocalization of RUNX1, 2, and 3 at nuclear matrix-associated sites indicate a common intranuclear targeting mechanism may be operative for the family of RUNX transcription factors [Tang et al., 1998a; Javed et al., 2000; Harrington et al., 2002]. Variations in the partitioning of transcriptionally active and inactive RUNX between subnuclear fractions permitted development of a strategy to identify a region of the RUNX transcription factors that directs the regulatory proteins to nuclear matrix-associated foci. A series of deletions and internal mutations was constructed and assayed for competency to associate with the nuclear matrix by Western blot analysis of biochemically prepared nuclear fractions and by in situ immuno staining following transfection into intact cells. Association of osteogenic and hematopoietic RUNX proteins with the nuclear matrix is independent of DNA binding and requires a nuclear matrix targeting signal, a 31 amino acid segment near the C-terminus that is distinct from nuclear localization signals [Zeng et al., 1997]. The nuclear matrix targeting signal functions autonomously and is necessary as well as sufficient to direct the transcriptionally active RUNX transcription factors to nuclear matrix-associated sites where gene expression occurs [Zeng et al., 1997].

These findings indicate mechanisms involved in the selective trafficking of proteins to specialized domains within the nucleus where they become components of functional regulatory complexes. At least two trafficking signals appear to be required for subnuclear targeting of RUNX transcription factors; the first supports nuclear import (nuclear localization signal) and a second mediates association with the nuclear matrix (nuclear matrix targeting signal). The multiplicity of determinants for nuclear localization and alternative splicing of RUNX messenger RNA may provide the requisite complexity to support targeting to specific sites within the nucleus in response to diverse biological conditions. Furthermore, because gene expression by RUNX involves contributions by factors and coregulatory proteins that include CBFβ [Ogawa et al., 1993; Banerjee et al., 1996], ETS-1 [Giese et al., 1995; Mao et al., 1999; Xie et al., 1999] and C/EBP [Zhang et al., 1996; Gutierrez et al., 2000b], Groucho/TLE [Levanon et al., 1998; Javed et al., 2000], HES and SMAD [Zaidi et al., 2002b; Zhang et al., 2000], RUNX may facilitate recruitment of these factors to the nuclear matrix.

Properties of transcriptionally active subnuclear compartments. Association of genes and cognate factors with the nuclear matrix may support the formation and/or activities of nuclear domains that facilitate transcriptional control [Guo et al., 1995; Merriman et al., 1995; Nickerson et al., 1995; Berezney

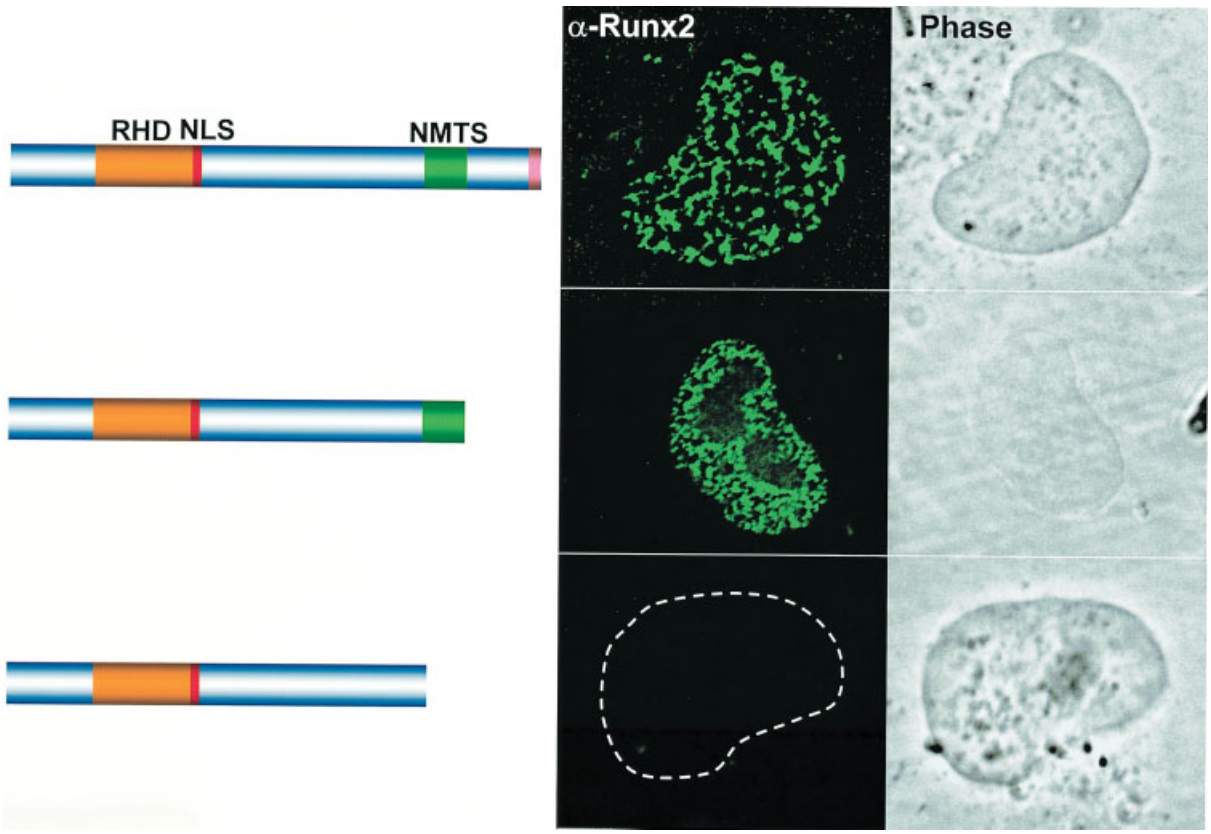


Fig. 3. The intranuclear trafficking signal of the Runx/AML/Cbfa transcription factor supports targeting to punctate subnuclear sites. The panel shows immunofluorescence and phase images of the nuclear matrix intermediate filament preparations of cells transfected with constructs encoding epitope tagged Runx proteins that possesses or lack the intranuclear trafficking signal (NMTS). The location of the runt homology DNA binding domain (RHD) and nuclear import signal (NLS) in the N-terminal region as well as the intranuclear trafficking signal (NMTS) that includes a context-dependent transactivation domain in the C-terminal region are indicated. The presence of the NLS and NMTS supports nuclear import and trafficking of the Runx/AML transcription factor to punctate nuclear matrix associated sites. Deletion of the NMTS does not compromise nuclear import but the truncated protein is not architecturally localized at nuclear matrix-associated subnuclear sites.

et al., 1996; Chen et al., 1996; Nardozza et al., 1996; Spelsberg et al., 1996; Stein et al., 1996; Alvarez et al., 1997; Davie, 1997; Grande et al., 1997; Jackson, 1997; Lindenmuth et al., 1997]. Results from our laboratory indicate that the association of RUNX transcription factors with the nuclear matrix is obligatory for activity [Zeng et al., 1998; Choi et al., 2001]. The promoter recognition function of the runt homology domain of RUNX, and thus the consequential interactions with RUNX-responsive genes, is essential for formation of transcriptionally active foci containing RUNX and RNA polymerase II that are nuclear matrix associated [Zeng et al., 1998]. Additionally, the nuclear matrix-targeting signal supports transactivation when associated with an appropriate promoter, and transcriptional activity of the nuclear matrix targeting signal depends on association with the nuclear matrix [Zeng et al., 1998]. Taken together, targeting of RUNX transcription factors to the nuclear matrix is important for their function and transcription. However, components of the nuclear matrix that function as acceptor sites remain to be established. Characterization of such nuclear matrix components will provide an additional dimension to characterizing molecular mechanisms associated with gene expression—the targeting of regulatory proteins to specific spatial domains within the nucleus. An initial indication of transcription factor interactions with the nuclear matrix is provided by crystal structure of the RUNX nuclear matrix targeting signal that was determined by X-ray diffraction analysis at 2.7 Å [Tang et al., 1998a].

Subnuclear targeting and integration of signaling pathways. Gene expression during skeletal development and bone remodeling is controlled by a broad spectrum of regulatory

signals that converge at promoter elements to activate or repress transcription in a physiologically responsive manner. The subnuclear compartmentalization of transcription machinery necessitates a mechanistic explanation for directing signaling factor to sites within the nucleus where gene expression occurs under conditions that support integration of regulatory cues. The interactions of YAP and SMAD coregulatory proteins with C-terminal segments of the RUNX2 transcription factor permits assessment of requirements for recruitment of cSRC and BMP/TGFβ-mediated signals to skeletal target genes. Our findings indicate that nuclear import of YAP and SMAD coregulatory factors is agonist dependent. However, there is a stringent requirement for fidelity of RUNX subnuclear targeting for recruitment of these signaling proteins to transcriptionally active subnuclear foci. Our results demonstrate that the interactions and spatial-temporal organization of RUNX and SMAD as well as YAP coregulatory proteins are essential for assembly of transcription machinery that supports expression of skeletal genes [Zaidi et al., 2002a,b]. Competency for intranuclear trafficking of RUNX proteins has similarly been functionally linked with the subnuclear localization and activity of TLE/Groucho coregulatory proteins [Javed et al., 2000]. These findings are consistent with proteins serving as a scaffold for interactions with coregulatory proteins that contribute to biological control.

In vivo consequences of aberrant intranuclear trafficking of RUNX transcription factors. Using RUNX2 and its essential role in osteogenesis as a model, we investigated the fundamental importance of fidelity of subnuclear localization for tissue differentiating activity by deleting the intranuclear targeting signal via homologous recombination. Mice homozygous for the deletion (RUNX2ΔC) do not form bone due to perturbed maturation or arrest of osteoblasts. Heterozygotes do not develop clavicles, but are otherwise normal. These phenotypes are indistinguishable from those of the RUNX2 homozygous and heterozygous null mutants, indicating that the intranuclear targeting signal is a critical determinant for function. The expressed truncated RUNX2ΔC protein enters the nucleus and retains normal DNA binding activity, but shows complete loss of intranuclear targeting. These results establish that the multifunctional N-terminal region of the RUNX2 protein is not sufficient for biological activity. Our results demonstrate that subnuclear localization of RUNX factors in specific foci together with associated regulatory functions is essential for control of RUNX-dependent genes involved in tissue differentiation during embryonic development [Choi et al., 2001]. The importance of subnuclear localization of RUNX transcription factors for biological control is further indicated by compromised subnuclear organization and activity of RUNX1 hematopoietic regulatory proteins in acute myelogenous leukemia [McNeil et al., 1999].

THE REGULATED AND REGULATORY PARAMETERS OF SUBNUCLEAR ORGANIZATION

Multiple lines of evidence suggest that components of nuclear architecture contribute both structurally and enzymatically to control gene expression during osteoblast differentiation. Sequences have been identified that direct RUNX transcription factors to nuclear matrix-associated sites that support transcription in a cell cycle dependent manner [Young et al., 2002]. Insight is thereby provided into mechanisms linked to the assembly and activities of subnuclear domains where transcription occurs. In a restricted sense, the foundation has been provided for experimentally addressing intranuclear trafficking of gene regulatory factors and control of association with the nuclear matrix to establish and sustain domains that are competent for transcription. The unique sequences [Zeng et al., 1997, 1998] and crystal structure for the 31 amino acid nuclear matrix targeting signal of RUNX transcription factors [Tang et al., 1998a] supports specificity for localization at intranuclear sites where the regulatory machinery for gene expression is assembled, rendered operative, and/or suppressed. In a broader context, there is a growing appreciation for involvement of nuclear architecture in a dynamic and bidirectional exchange of gene transcripts and regulatory factors between the nucleus and cytoplasm, as well as between regions and structures within the nucleus [Lamond and Earnshaw, 1998; Wei et al., 1998; Misteli, 2000; Stein et al., 2000b; Gasser, 2002].

It would be presumptuous to propose a single model to account for the specific pathways that

direct transcription factors to sites within the nucleus that support transcription. However, findings suggest that parameters of nuclear architecture functionally interface with components of transcriptional control. The involvement of nuclear matrix-associated transcription factors with recruitment of regulatory components to modulate transcription remains to be defined. Working models that serve as frameworks for experimentally addressing components of transcriptional control within the context of nuclear architecture can be compatible with mechanisms that involve architecturally or activity driven assembly of transcriptionally active intranuclear foci. The diversity of targeting signals must be established to evaluate the extent to which regulatory discrimination is mediated by encoded intranuclear trafficking signals. It will additionally be important to biochemically and mechanistically define the checkpoints, which are operative during subnuclear distribution of regulatory factors, and the editing steps, which are invoked to ensure that structural and functional fidelity of nuclear domains, where replication and expression of genes occur. There is emerging recognition that placement of regulatory components of gene expression must be temporally and spatially coordinated to optimally mediate biological control. It is realistic to anticipate that further understanding of mechanisms that position genes and regulatory factors for establishment and maintenance of the bone cell phenotype will clarify nuclear structure-function interrelationships that are operative during osteoblast differentiation and vitamin D modulation of regulatory activity.

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