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Linkages of Nuclear Architecture to Biological and Pathological Control of Gene Expression

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Abstract Functional interrelationships between components of nuclear architecture and control of gene expression are becoming increasingly evident. There is growing appreciation that multiple levels of nuclear organization integrate the regulatory cues that support activation and suppression of genes as well as the processing of gene transcripts. The linear organization of genes and promoter elements provide the potential for responsiveness to physiological regulatory signals. Parameters of chromatin structure and nucleosome organization support synergism between activities at independent regulatory sequences and render promoter elements accessible or refractory to transcription factors. Association of genes, transcription factors, and the machinery for transcript processing with the nuclear matrix facilitates fidelity of gene expression within the three-dimensional context of nuclear architecture. Mechanisms must be defined that couple nuclear morphology with enzymatic parameters of gene expression. The recent characterization of factors that mediate chromatin remodeling and intranuclear targeting signals that direct transcription factors to subnuclear domains where gene expression occurs, reflect linkage of genetic and structural components of transcriptional control. Nuclear reorganization and aberrant intranuclear trafficking of transcription factors for developmental and tissue-specific control that occurs in tumor cells and in neurological disorders provides a basis for high resolution diagnostics and targeted therapy. J. Cell. Biochem. Suppls. 30/31:220–231, 1998.

Key words: nuclear architecture; gene expression; tumor cells

It is readily acknowledged that transcriptional and post-transcriptional control is governed by complex and interdependent regulatory events. The biochemical components of transcription, processing of gene transcripts, and the bidirectional exchange of regulatory macromolecules between the nucleus and cytoplasm must be stringently modulated to ensure the fidelity of cell growth and phenotype-restricted gene expression. However, there is growing appreciation that the representation of factors involved with each component of gene expression are necessary but insufficient to facilitate the integration of regulatory signals required for transient and long-term commitments to physiologically responsive transcriptional control. How, with a limited representation of gene-specific or phenotype-restricted promoter regulatory elements and cognate factors, can a threshold concentration for initiation of expression be attained in intact cells? How are genes and regulatory proteins directed to sites within the nucleus that support replication and expression? How are genes and transcripts compositely assembled into complexes and biochemically modified to support activation and suppression of genes? These fundamental questions provide a basis for experimentally addressing the functional implications of nucleic acid compartmentalization within the nucleus as well as the requirements for fidelity of interrelationships between nuclear architecture and parameters of gene expression that are required to sustain biological control.

CONCEPTUAL AND EXPERIMENTAL BASIS FOR NUCLEAR STRUCTURE—GENE EXPRESSION INTERRELATIONSHIPS

General Considerations

Gene regulatory mechanisms that are operative in vivo must be understood within the three-dimensional context of nuclear architec-

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ture. Historically there was a perceived dichotomy between regulatory mechanisms supporting gene expression and components of nuclear structure. However, this parochial view is rapidly changing. The emerging concept is that both transcription and DNA synthesis occur in association with structural parameters of the nucleus. Consequently, it has become evident that the cellular and molecular mechanisms must be defined which contribute to both the regulated and regulatory relationships of nuclear morphology to the expression and replication of genes [reviewed in Berezney and Jeon, 1995; Lamond and Earnshaw, 1998; Bird et al., 1997].

Our concept of a promoter has evolved from the initial expectation of a single regulatory sequence that determines transcriptional competency and level of expression. We now appreciate that transcriptional control is mediated by an interdependent series of regulatory sequences that reside 5', 3' and within transcribed regions of genes. Rather than focusing on the minimal sequences required for transcriptional control to support biological activity, efforts are being directed towards defining functional limits. Contributions of distal flanking sequences to regulation of transcription and long-range chromosomal contexts are being experimentally addressed. Cross-talk between a series of regulatory domains must be understood under diverse biological circumstances where expression of genes supports cell and tissue functions. The overlapping binding sites for transcription factors within promoter regulatory elements and protein-protein interactions that influence transcription factor activity provide further components of the requisite diversity to accommodate regulatory options for physiologically responsive gene expression.

Levels of Nuclear Organization Mediating Gene Expression

There is growing appreciation that nuclear architecture provides a basis for support of stringently regulated modulation of cell growth and tissue-specific transcription. Here, evidence points to contributions by multiple 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. The representation of regulatory sequences reflects competency for responsiveness to physiological regulatory signals. However, interspersion of sequences between promoter elements that exhibit coordinate and synergistic activities indicates that a structural basis is required for integration of activities at independent regulatory domains. Parameters of chromatin structure and nucleosome organization are a second level of genome architecture that reduces the distance between promoter elements thereby supporting interactions between the modular components of transcriptional control (reviewed in Kingston et al., 1996; Zlatanova and van Holde, 1992). Each nucleosome contracts linear spacing by seven-fold. Higher order chromatin structure further reduces nucleotide distances between regulatory sequences. Folding of nucleosome arrays into solenoid-type structures provides a potential for interactions which support synergism between promoter elements and responsiveness to multiple signaling pathways. Chromatin organization renders promoter elements accessible or refractory to interactions with transcription factors under a broad spectrum of biological circumstances and mediators of chromatin remodeling are being defined [Kingston et al., 1996; Grunstein, 1997]. A third level of nuclear architecture which contributes to transcriptional control is provided by the nuclear matrix. The anastomosing network of fibers and filaments which constitute the nuclear matrix supports the structural properties of the nucleus as a cellular organelle and accommodates structural modifications associated with proliferation, differentiation and changes necessary to sustain phenotypic requirements of specialized cells [Bidwell et al., 1994; Dworetzky et al., 1990; Getzenberg and Coffey, 1990; Nickerson et al., 1990]. Regulatory functions of the nuclear matrix include but are by no means restricted to: DNA replication [Berezney and Coffey, 1975], gene localization [Zeng et al., 1997], imposition of physical constraints on chromatin structure which support formation of loop domains, concentration, and targeting of transcription factors [Dworetzky et al., 1992; van Wijnen et al., 1993; Nelkin et al., 1980; Robinson et al., 1982; Schaack et al., 1990; Stief et al., 1989], RNA processing and transport of gene transcripts [Lawrence et al., 1989; Zeitlin et al., 1987; Carter et al., 1993; Spector, 1990; Blencowe et al., 1994], post-translational modifications of chromosomal proteins, as well as imprinting

and modifications of chromatin structure [Davie, 1997].

We are just beginning to comprehend the significance of nuclear domains in the control of gene expression. These local nuclear environments that are generated by the multiple aspects of nuclear structure are tied to developmental expression of cell growth and tissue-specific genes. Initially, control of gene expression and characterization of structural features of the nucleus were conceptually and experimentally pursued as minimally integrated questions. However, independent pursuit of nuclear structure and function has occurred in parallel with the appreciation that several components of nuclear architecture are associated with parameters of gene expression or control of specific classes of genes. There is long-standing acceptance that the nucleolus is the site of ribosomal gene expression. The nuclear pore is recognized as a site for facilitating the import and retention of gene regulatory factors, as well as the export of gene transcripts [Silver et al., 1984; reviewed in Ullman et al., 1997]. SC35 domains have been extensively studied from the standpoints of RNA splicing and the dynamic recruitment of transcript processing factors [Carter et al., 1993; Clemson et al., 1996; Dyck et al., 1994; Nickerson et al., 1995; Pombo and Cook, 1996]. PML bodies and coiled bodies have been associated with control of gene expression and undergo modifications in structure and potentially function in cancer cells [Dyck et al., 1994; Grande et al., 1996]. Because these components of nuclear architecture have been defined by immunofluorescence microscopy and/or ultrastructural imaging as well as by biochemical criteria, a viable basis has been established for linkage with gene regulatory mechanisms. Taken together, these components of nuclear architecture facilitate the 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 which is progressive and stage-specific; and 3) differentiation-related control which is associated with long-term phenotypic commitments to gene expression for support of structural and functional properties of cells and tissues.

From a broader perspective, reflecting diverse regulatory requirements as well as 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 critical determinant of transcriptional control and the expressed genes modulate the regulatory components of nuclear architecture.

INTRANUCLEAR TARGETING OF TRANSCRIPTION FACTORS TO SUBNUCLEAR DOMAINS THAT SUPPORT EXPRESSION

An understanding of interrelationships between nuclear structure and gene expression necessitates knowledge of the composition, organization, and regulation of sites within the nucleus that are dedicated to replication, transcription, and processing of gene transcripts. During the past several years there have been developments in reagents and instrumentation to enhance the resolution of nucleic acid and protein detection by in situ hybridization and immunofluorescence analyses. The combined application of isotopic and non-isotopic methods, together with a new generation of high resolution techniques for quantitation and three-dimensional construction of "captured images" is providing new insights into the intranuclear distribution of genes and regulatory factors. We are beginning to make the transition from descriptive in situ mapping of genes, transcripts, and regulatory factors to visualization of gene expression from the three-dimensional perspective of nuclear architecture. Initially, in situ approaches were primarily utilized for intracellular localization of nucleic acids and proteins that were subsequently shown by biochemical analyses to contribute to control of gene expression. We are now applying high resolution in situ analyses for the primary characterization of gene regulatory mechanisms under in vivo conditions.

Transcription Factor Organization Reflects Linkages of Nuclear Structure With Gene Expression

The organization and activities of transcription factors provide a paradigm for addressing interrelationships of nuclear architecture with transcriptional control. Association of CBFA/AML transcription factors with the nuclear matrix has permitted direct examination of mechanisms for targeting regulatory factors to subnuclear domains that support transcription. CBFA/AML- related factors (core binding factor α/acute myelogenous leukemia factors) are expressed in tissues of the lymphoid, my-

eloid and osteoblast lineages where they are key components of mechanisms mediating tissue-specific transcription [Bae et al., 1993; Banerjee et al., 1996, 1997; Meyers et al., 1996; Satake et al., 1995; Merriman et al., 1995; Ducy et al., 1997; Nuchprayoon et al., 1994; Frank et al., 1995]. There are three genes designated CBFA1/AML-3, CBFA2/AML-1, and CBFA3/AML-2, which share a runt homology DNA binding domain first observed in the drosophila *runt* pair rule gene [Wang et al., 1993; Meyers et al., 1993, 1995, 1996; Bae et al., 1993]. Control of hematopoietic and osteogenic transcription is mediated by interactions with CBFA/AML recognition sequences (5' TGYGGT; Y= C or T) that reside in promoters of genes which exhibit developmental and tissue-restricted expression.

Insight into the regulated and regulatory activities of AML transcription factors are provided by functional interactions with nuclear architecture. Both biochemical and immunofluorescence analyses have shown that CBFA/AML transcription factors associate with the nuclear matrix in situ [Banerjee et al., 1997; Merriman et al., 1995; Zeng et al., 1997]. Antibody staining patterns indicate a punctate nuclear distribution of CBFA/AML proteins. 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 [Zeng et al., 1997; Stein et al., 1994, 1996, 1997; Dworetzky et al., 1992; van Wijnen et al., 1993; Nickerson et al., 1995; Blencowe et al., 1994; Mancini et al., 1994; Bidwell et al., 1993].

The initial indication that nuclear matrix association of AML factors is required for maximal activity was provided by the observation that transcriptionally active AML-1B (amino acid 1–480) associates with the nuclear matrix but inactive AML-1 (amino acids 1–250) does not [Zeng et al., 1997]. This localization of AML was established by biochemical fractionation and in situ immunofluorescence. A similar association of AML-1B, AML-2, and AML-3 with the nuclear matrix occurs indicating that a common intranuclear targeting mechanism may be operative for the family of AML transcription factors [Zeng et al., 1997]. Variations in the partitioning of the transcriptionally active AML-1B and the inactive AML-1 between subnuclear fractions permitted development of a strategy to identify a region of the AML transcription factors which are directing the regulatory proteins to the nuclear matrix. A series of deletion and internal mutations were constructed and assayed for competency to associate with the nuclear matrix by Western analysis of biochemically prepared nuclear fractions and by in situ immunostaining following transfection into intact cells. As schematically illustrated in Figure 1 and shown by immunofluorescence images [Zeng et al., 1997], association of AML-1B 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]. A similar nuclear matrix targeting signal is present in AML-2 and the bone-related AML-3 transcription factors. Fusion of the AML-1B nuclear matrix targeting signal to the heterologous GAL4-(1–147) protein directs GAL4 to the nuclear matrix [Zeng et al., 1997]. Thus, the nuclear matrix targeting signal functions autonomously and is necessary as well as sufficient to target the transcriptionally active AML-1B to the nuclear matrix.

These results provide insight into mechanisms by which gene regulatory factors are targeted to the nuclear matrix. The existence of a nuclear matrix targeting module that functions independently of the AML-1B DNA binding domain provides evidence for the specificity of these factors/nuclear matrix interactions. Specific targeting argues against indiscriminate attachment of such proteins to the nuclear matrix during subcellular fractionation. These findings are an indication of mechanisms involved in the selective trafficking of proteins to specialized domains within the nucleus to become components of functional complexes. At least two trafficking signals appear to be required for subnuclear targeting of AML transcription factors; the first supports nuclear import (Nuclear Localization Signal) and a second mediates association with the nuclear matrix (Nuclear Matrix Targeting Signal; Fig. 2). The multiplicity of determinants for nuclear localization and alternative splicing of CBFA/AML mRNA may provide the requisite complexity to support targeting to specific sites within the nucleus in response to diverse biological conditions. Furthermore, because gene regulation by AML-1B involves contributions by other factors such as CBFβ [Ogawa et al., 1993; Banerjee et al., 1996], ETS-1 [Giese et al., 1995], and C/EBP

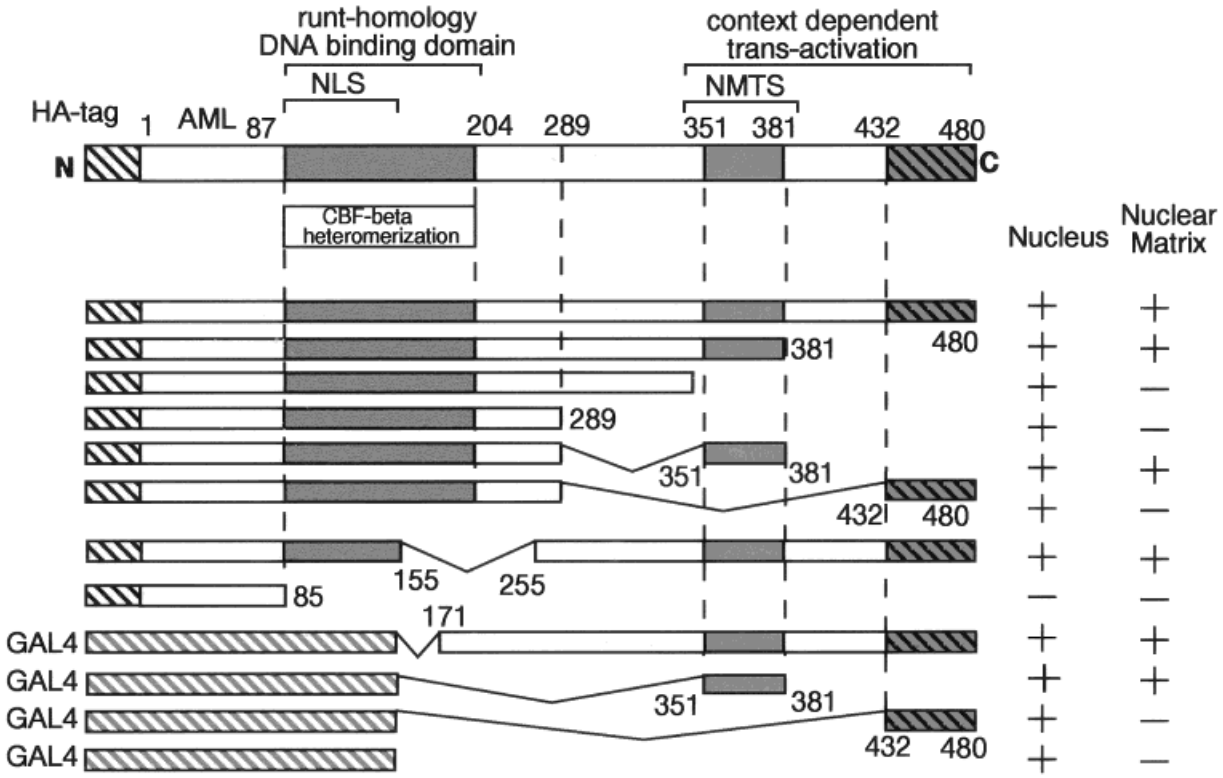

Fig. 1. Delineation of the nuclear matrix targeting signal of CBFA2/AML-1B. The CBFA2/AML-1B transcription factor is schematically illustrated with designations for the nuclear localization signal (NLS), the highly conserved runt homology domain that functions as a sequence-specific DNA binding protein and interacts with the heterodimerization partner CBFβ, the nuclear matrix targeting signal (NMTS), and two C-terminal context-dependent transactivation domains in the NMTS and the amino acid 432–480 segment. **Color plate on page 322.**

[Zhang et al., 1996], AML-1B may facilitate recruitment of these factors to the nuclear matrix.

Characterization 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 [Nickerson et al., 1995; Stein et al., 1996; Berezney et al., 1996; Jackson, 1997; Davie, 1997; Guo et al., 1995; Merriman et al., 1995; Lindenmuth et al., 1997; Grande et al., 1997; Alvarez et al., 1997; Chen et al., 1996; Nardozza et al., 1996; Spelsberg et al., 1996]. Recent results from our laboratory indicate that the association of AML transcription factors with the nuclear matrix is obligatory for activity [Zeng et al., 1998]. Active transcription is required for colocalization of AML-1B and RNA polymerase II at the nuclear matrix [Zeng et al., 1998] (Fig. 3). The promoter recognition function of the *runt* homology domain of AML-1B, and thus the consequential interactions with AML responsive genes is essential for formation of transcriptionally active foci containing AML and RNA polymerase II in the nuclear matrix [Zeng et al., 1998]. In addition, 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 AML 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 add an additional dimension to characterizing molecular mechanisms associated with gene expression—the targeting of regulatory proteins to specific spatial domains within the nucleus.

Functional Implications for Modified Nuclear Structure in Tumor Cells

Transformed and tumor cells exhibit striking alterations in nuclear morphology as well as in

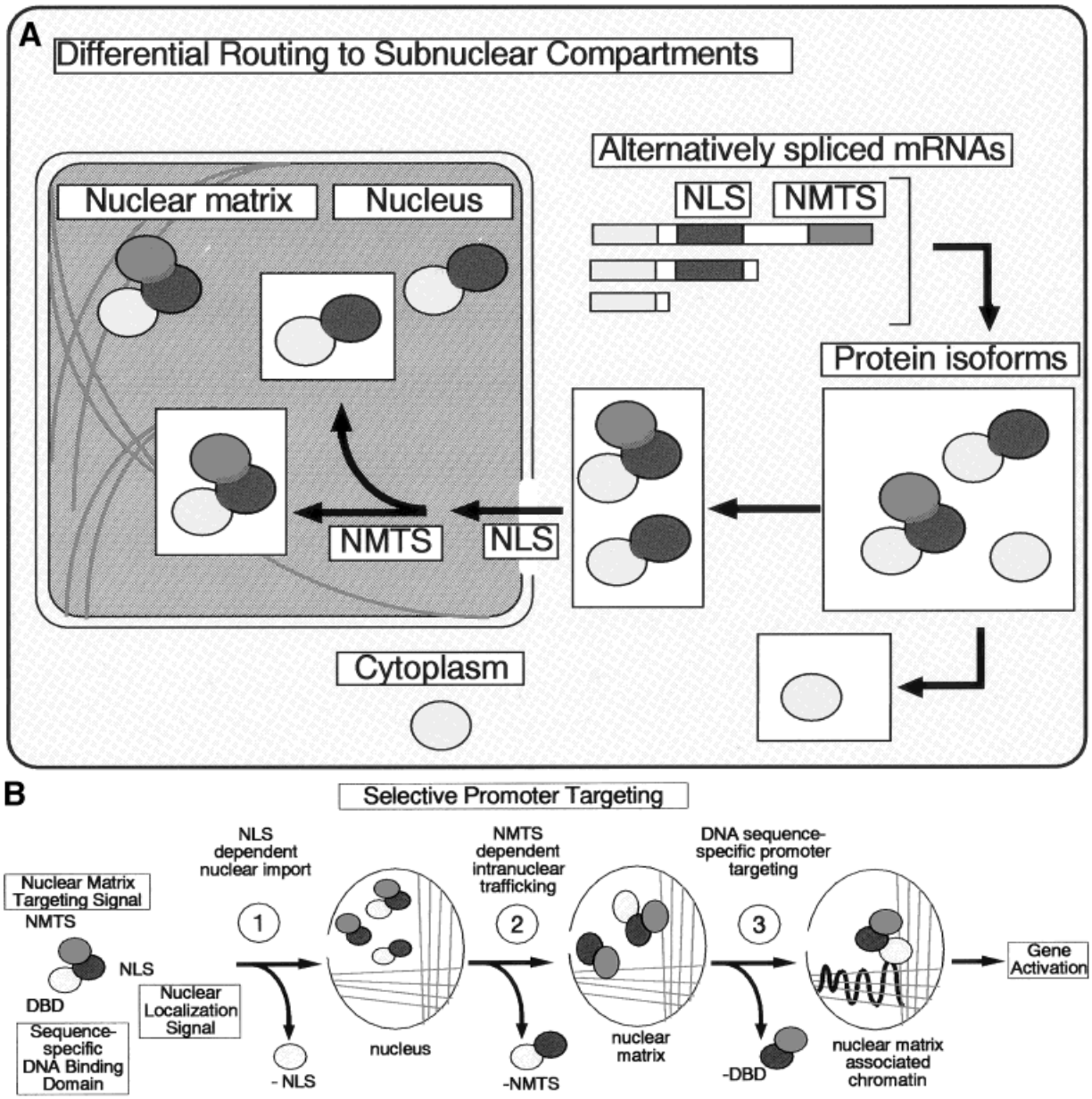

Fig. 2. Intracellular trafficking of the CBFA/AML class of transcription factors supports gene activation. **A.** The differential intra-cellular routing of distinct CBFA/AML factors depending on presence of specific subcellular targeting signals (green, red) in protein isoforms encoded by mRNA splice variants. **B.** Provides a model of the molecular sorting mechanisms which occur to support selective targeting of CBFA/AML factors to transcriptionally active domains. This involves nuclear localization signal (NLS; green) dependent nuclear import (Step 1), specific association with the nuclear matrix (vertical and horizontal lines) in response to the presence of a nuclear matrix targeting signal (NMTS; red; Step 2), and a requirement for a promoter recognition function of a sequence-specific DNA binding domain (DBD; yellow; Step 3) to associate with active chromatin (thick wavy line). These three steps together result in RNA pol II_0-mediated activation of AML responsive genes. **Color plate on page 323.**

the representation and intranuclear distribution of nucleic acids and regulatory factors. In both leukemias and solid tumor cells there are modifications in components of nuclear architecture that are involved in control of gene expression. Examples include mutations of the AML, ALL, and PML loci in leukemias that accompany changes in gene expression and the subnuclear organization of encoded transcription factors. In colon tumor cells, modifications in the subnuclear distribution of the APC factor is observed [Joslyn et al., 1993]. These factors are associated with nuclear architecture and the alterations in relationships with nuclear archi-

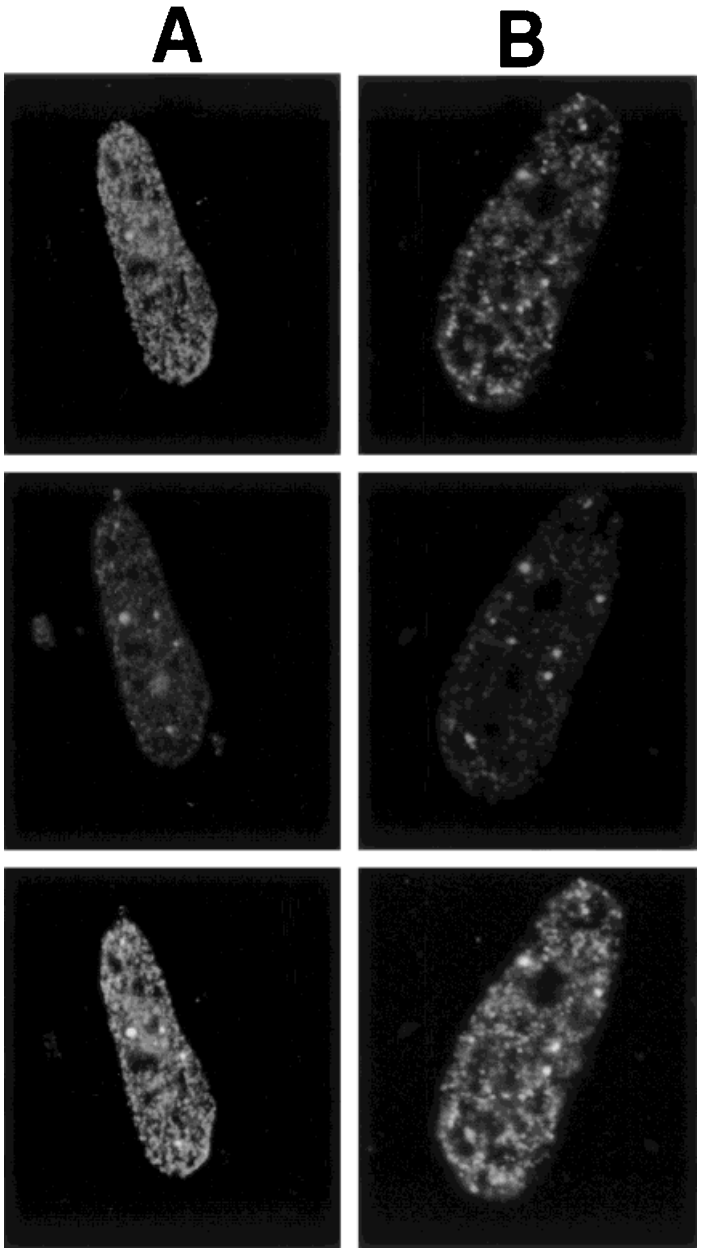

Fig. 3. CBFA2/AML-1B is directed to transcriptionally active nuclear foci which contain the hyperphosphorylated form of RNA polymerase II (pol II_0). **A,B.** Colocalization of a subset of AML-1B with RNA pol II_0 in the nuclear matrix of human SAOS-2 osteosarcoma cells. The images were obtained by immunofluorescence microscopy using antibodies against AML-1B (green) and RNA pol II_0 (red), while colocalization is reflected by yellow signals. Immunofluorescence signals were recorded using standard 35 mm slide photography (A) or a CCD camera interfaced with a digital microscope system (B). **Color plate on page 324.**

tecture appear to be related to changes in gene control. Identification of nuclear import signals in transcription factors and the recent characterization of intranuclear targeting signals that direct regulatory proteins to subnuclear domains, support transcription reinforce linkages between nuclear structure and aberrant transcriptional control. These observations provide an opportunity to develop high resolution in situ immunofluorescence analysis to diagnose and stage leukemias as well as to monitor remission, relapse, and effectiveness of treatment.

Reflecting alterations in nuclear organization that are the hallmarks of cancer cells, the gene locus encoding the CBFα2/AML-1 transcription factor is frequently the target of chromosomal translocations in human leukemia. Mapping of the nuclear matrix targeting signal to exon 8 reveals that this domain is not present in the t(8;21) fusion protein (AML-1/ETO), but is replaced by sequences from the MTG8 gene [Hiebert et al., 1996]. Thus, intranuclear targeting of the AML-1B transcription factor may be abrogated because of gene rearrangements in leukemic cells. Fidelity of transcriptional control may involve localization of gene regulatory proteins to the correct subnuclear region. PML bodies are another example of

nuclear structures that are associated with the nuclear matrix and modified in leukemia cells [Dyck et al., 1994]. In normal cells the PML protein resides in discrete PML bodies. However, in promyelocytic leukemic cells the PML protein is genetically rearranged and dispersed throughout the nucleus [Dyck et al., 1994; Weis et al., 1994]. A further example of chromosomal translocations involving a locus encoding a nuclear matrix-associated transcription factor occurs in acute lymphocytic leukemia (ALL/MLL). Recently, a translocation has been described in which the ALL/MLL protein is fused with a histone acetyltransferase. The chimeric protein may promote leukemia by modifying histone acetylation of specific genomic regions. Consequential modifications in the intranuclear distribution of factors encoded by the rearranged ALL locus occur [Sobulo et al., 1997; Yano et al., 1997; Rogaia et al., 1997] while the chimeric transcription factors remain nuclear matrix associated. Hence, these results suggest that perturbations in subnuclear location and/or nuclear matrix association of proteins may be related to modifications in gene expression that are linked to leukemias.

Perturbations in nuclear organization that may impact on gene expression are not confined to cancer cells. Skinner et al. [1997] recently reported perturbations in the subnuclear distribution of ataxin-1 in spinocerebellar ataxia type 1. These investigators demonstrated that this neurological disorder which is characterized by progressive motor deterioration and loss of cerebellar purkinje cells involves a dramatic modification in the nuclear localization of ataxin-1. Because ataxin-1 is nuclear matrix associated, it is reasonable to anticipate that the pathogenesis of spinocerebellar ataxia involves the disruption of a nuclear matrix domain.

CONCLUSIONS

Multiple lines of evidence suggest that components of nuclear architecture contribute both structurally and enzymatically to control of gene expression. Sequences have been identified that direct transcription factors to nuclear matrix-associated sites which support transcription. 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 factor association with the nuclear matrix to establish and sustain domains which 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 CBF/AML transcription factors [Tang et al., 1998] supports specificity for localization at intranuclear sites where the machinery for gene expression is assembled, rendered operative and/or suppressed. In a broader context, there is 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 [Wei et al., 1998; Lamond and Earnshaw, 1998].

It is difficult to arbitrarily separate nuclear structure and function or distinguish the regulated and regulatory parameters of control. The challenges we now face are to further define the targeting of transcription factors and control which reside at the level of nuclear matrix-associated acceptor sites. The result will unquestionably be further insight into fundamental processes which are involved with directing components of gene expression to specific regions within the nucleus. It would be presumptuous to propose a single model to account for the specific pathways which 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 (Fig. 4). The involvement of nuclear matrix-associated transcription factors with recruitment of regulatory components to modulate transcription remains to be defined. However, working models are presented in Figure 4 which serve as a framework for experimentally addressing components of transcriptional control within the context of nuclear architecture. 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 both 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 medi-

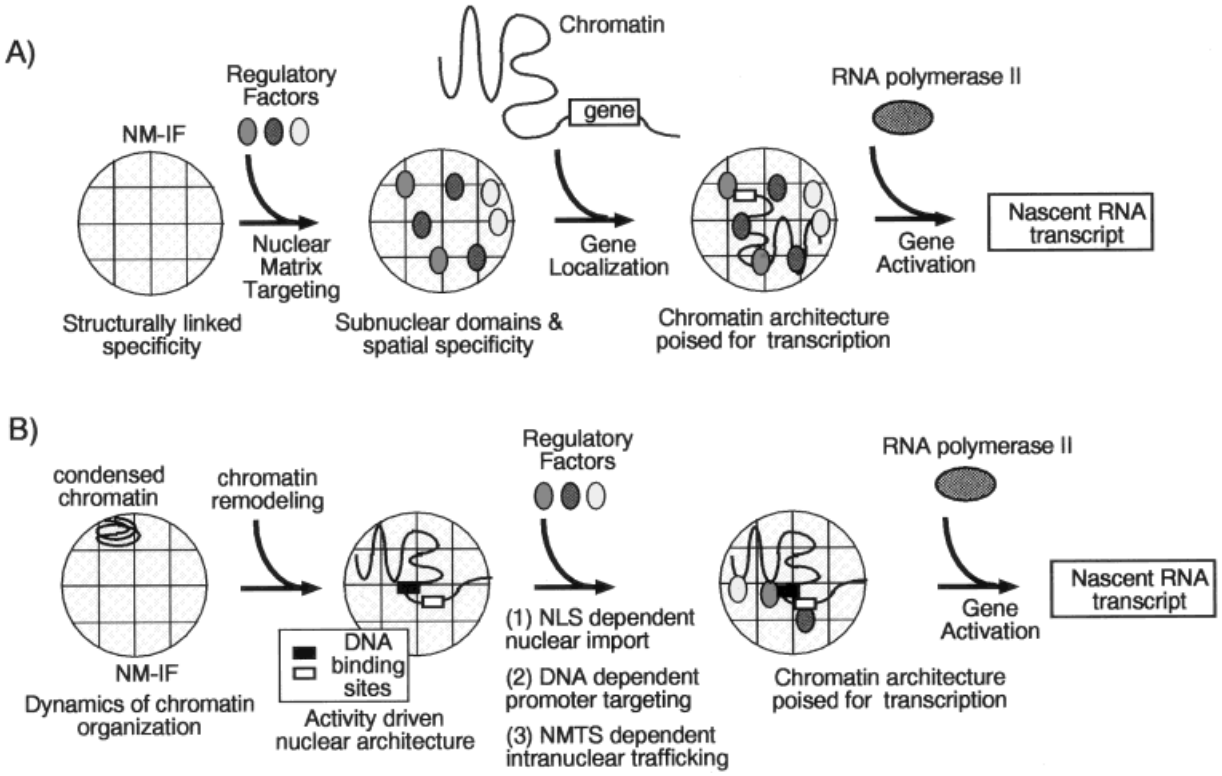

Fig. 4. Molecular mechanisms generating functional specificity of subnuclear domains which support modulations of transcriptional activity. **A.** Regional differences in the nucleus may be a direct reflection of architectural proteins of the nuclear matrix-intermediate filament scaffold. The assembly of these filamentous structures in situ may provide specific niches for protein/protein and protein/DNA interactions, e.g., at the intersections of assemblies involving different architectural proteins. These niches attract regulatory factors with specific nuclear matrix targeting signals. This mechanism results in the recruitment of additional factors and modification of chromatin structure to support entry of RNA polymerase II and gene activation. **B.** Specific subnuclear domains may arise at defined positions within chromosomal regions by the (de-) condensation of chromatin, which is mediated by nucleosome accessory factors and histone-modifying enzymes. The reorganization of chromatin results in increased accessibility of gene regulatory elements that function as DNA binding sites for transcription factors. Regulatory factors are targeted to chromatin by nuclear localization signal (NLS) dependent nuclear import and scan chromatin for accessible high affinity DNA binding sites. Upon stable binding to specific gene promoters, association with the nuclear matrix occurs in a nuclear matrix targeting signal (NMTS) dependent manner. This event may stabilize or further modulate local chromatin structure which ultimately supports entry of RNA polymerase II and formation of nascent RNA transcripts. The models presented in A and B are not mutually exclusive. Both postulated mechanisms may operate concurrently within the same nucleus and/or in the regulation of the same or different genes. Both models reflect a dynamic organization of gene regulatory factors that directly influence and/or are influenced by the spatial functions of subnuclear domains and the architecture of chromatin. **Color plate on page 325.**

ate biological control optimally. The consequences of breaches in nuclear structure-function interrelationships are observed in an expanding series of diseases, providing options for high resolution diagnosis and targeted therapy.

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