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Interrelationships of Nuclear Structure and Transcriptional Control: Functional Consequences of Being in the Right Place at the Right Time

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Abstract Functional interrelationships between components of nuclear architecture and control of gene expression are becoming increasingly evident. In this article we focus on the concept that association of genes and cognate transcription factors with the nuclear matrix may support the formation and/or activities of nuclear domains that facilitate transcriptional regulation. Several lines of evidence are consistent with the concept that association of transcription factors with the nuclear matrix may be obligatory for fidelity of gene expression and maximal transcriptional activity. The identification of specific regions of transcription factors that are responsible for intranuclear trafficking to nuclear matrix-associated sites that support transcription, reinforces the linkage of nuclear structure to regulation of genes. CBFA2/AML-1 and CBFA1/AML-3 provide paradigms for directing gene regulatory factors to RNA polymerase II containing foci within the nucleus. The implications of modifications in the intranuclear trafficking of transcription factors for developmental and tissue-specific control, as well as for aberrations in gene expression that are associated with cancer and neurological disorders, are addressed. J. Cell. Biochem. 70:200–212, 1998.

Key words: gene expression; AML/CBF transcription factors; nuclear matrix; cancer; nuclear domains

Selective expression of genes to support proliferation and differentiation as well as maintenance of phenotypic properties necessitates control of transcription and posttranscriptional processing of gene transcripts. There are requirements for responsiveness to a broad range of physiological regulatory signals and the integration of activities at multiple, independent promoter elements of cell growth and tissue-specific genes. This interfacing of regulatory cues must be sufficiently flexible to accommodate transient expression of genes during development, homeostatic control, and sustained expression of genes in specialized cells and tissues. The extensive database of gene promoter elements and regulatory factors that has been developed over the past several years provides insight into the parameters of transcriptional control and transcript processing. However, it is becoming increasingly evident that the linear representation of gene regulatory information is necessary but insufficient to support the plasticity of gene regulatory mechanisms that must be operative in vivo.

Functional interrelationships between nuclear structure and gene expression are emerging. Evidence is accruing that the regulatory information encoded in promoter sequences is rendered accessible to transcription factors by remodeling of chromatin structure and nucleosome organization [Kingston et al., 1996]. Nucleosomal architecture regulates competency for cross-talk between resident promoter domains. Modifications in chromatin architecture have been documented during development, in response to steroid hormones and within the context of cell cycle and growth control as well as differentiation (reviewed in Zlatanova and van Holde [1992] and Stein et al. [1997]). Nuclear reorganization is the striking and clinically relevant hallmark of many can-

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cer cells. In addition to providing diagnostic markers for transformation and tumor progression, these alterations in components of nuclear structure reflect abrogation of regulatory mechanisms that mediate cell growth and phenotypic control. Recently there have been significant reports of nuclear reorganization in several neurological disorders, extending the paradigm of a requirement for structural integrity of the nucleus to support fidelity of gene expression.

Historically, control of gene expression was conceptually and experimentally pursued as independent and minimally integrated questions. This 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; Xing et al., 1993]. Promyelocytic Leukemia (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; Everett and Maul, 1994; Grande et al., 1996; Weis et al., 1994]. Because these components of nuclear architecture have been defined by immunoreactive proteins and/or ultrastructural imaging as well as by biochemical criteria, a viable basis has been established for linkage with gene regulatory mechanisms.

The rules that govern interrelationships between nuclear structure and gene expression remain to be established. However, several structural and functional components of nuclear architecture are conducive to experimentally addressing the interfacing of morphology with gene regulatory mechanisms. Understanding of gene organization in a three-dimensional context has been significantly facilitated by a transition from the descriptive to the mechanistic pursuit of chromatin structure and nucleosome organization. For many years, studies of chromatin were dominated by high-resolution ultrastructural and biophysical analyses with the objective of precisely defining structural features of the histone–DNA complexes under in vivo and in vitro conditions. But recently, pursuit of regulatory mechanisms that interrelate nuclear structure and function have been successful (reviewed in Kingston et al. [1996]). Genetic and biochemical approaches have defined factors and sequences that mediate "heterochromatinization," accessibility of nucleosomal DNA to transcription factors, and integration of activities at multiple promoter elements [reviewed in Grunstein, 1997a,b]. There have been important advances in characterizing activities involving nuclear pores. Biochemical and morphological determinants for nuclear import, export, and retention have provided valuable insight into the regulated and regulatory features of this principal interface for informational exchange between the nucleus and cytoplasm [Silver et al., 1984; Ohno et al., 1998; Nigg, 1997; Moroianu, 1997]. Similarly, there have been significant increments in our understanding of contributions by the nuclear matrix to control of gene expression at the transcriptional and posttranscriptional levels.

Initial studies indicated that the representation of nuclear matrix proteins reflect cell and tissue phenotypic properties as well as modifications in gene expression which occur during differentiation and in tumors [Bidwell et al., 1994; Dworetzky et al., 1990; Getzenberg and Coffey, 1990; Getzenberg et al., 1991; Nickerson et al., 1990; Pienta and Coffey, 1991; van Holde et al., 1988]. The nuclear matrix has been shown to be involved with DNA replication [Berezney, 1991; Berezney and Coffey, 1975], transcription [Dworetzky et al., 1992; Nelkin et al., 1980; Robinson et al., 1982; Schaack et al., 1990; Stief et al., 1989; van Wijnen et al., 1993], and RNA processing [Blencowe et al., 1994; Carter et al., 1993; Lawrence et al., 1989; Spector, 1990; Spector et al., 1991; Xing et al., 1993; Zeitlin et al., 1987; Zeng et al., 1998]. The recent identification of specific regions of transcription factors that are responsible for intranuclear trafficking of regulatory proteins to the nuclear matrix-associated sites (within the nucleus) which support transcription reinforces the linkage of nuclear structure to regulation of genes [Zeng et al., 1997].

In this article we will focus on the concept that association of genes and cognate factors with the nuclear matrix may support the formation and/or activities of nuclear domains that facilitate transcriptional control. We will review several lines of evidence that are consistent with the hypothesis that association of transcription factors with the nuclear matrix may be obligatory for fidelity of gene expression and maximal transcriptional activity. We will address implications of modifications in the intranuclear trafficking of transcription factors for developmental and tissue-specific control as well as for aberrations in gene expression that are associated with cancer.

Organization and Activities of Acute Myelocytic Leukemia (AML) Transcription Factors Provide a Paradigm for Interrelationships of Nuclear Architecture With Transcriptional Control

CBFα/AML-related factors (core binding factor α/acute myelogenous leukemia factors) are expressed in tissues of the lymphoid, myeloid, and osteoblast lineages, where they are key components of mechanisms mediating tissue-specific transcription [Bae et al., 1993; Banerjee et al., 1996; Banerjee et al., 1997; Ducy et al., 1997; Frank et al., 1995; Gottschalk and Leiden, 1990; Hernandez-Munain and Krangel, 1994; Ho et al., 1989; Levanon et al., 1994; Merriman et al., 1995; Meyers et al., 1996; Miyoshi et al., 1991; Nimer et al., 1996; Nuchprayoon et al., 1994; Rodan and Harada, 1997; Satake et al., 1995; Takahashi et al., 1995]. There are three genes designated CBFα1/AML-3, CBFα2/AML-1, and CBFα3/AML-2, which share a *runt* homology DNA binding domain first observed in the *Drosophila runt* pair rule gene [Bae et al., 1993; Levanon et al., 1994; Meyers et al., 1993, 1995, 1996; Ogawa et al., 1993a, 1993b; Wang et al., 1993]. Control of hematopoietic and osteogenic transcription is mediated by interactions with CBFα/AML recognition sequences (5' TGYGGT (Y = C or T)), which reside in promoters of genes that exhibit developmental and tissue-restricted expression. Consequently, there is a necessity to understand the mechanisms that mediate selective utilization of AML regulatory elements.

From biochemical and molecular perspectives, control of AML-responsive transcription can in part but not completely be accounted for by selective expression of AML genes, alternative splicing of gene transcripts, and interactions with non-DNA binding partner proteins. The modular organization of the AML proteins (Fig. 1) indicates the shared functional domains and superimposed organizational complexity which can contribute to selective activities under diverse biological conditions. Variations in expression of AML-1, 2, and 3 occur during the developmental periods of osteoblast differentiation, showing the option for control of gene expression by variations in cellular levels of the AML-related transcription factors [Banerjee et al., 1997]. Additional components of the regulated and regulatory activities of AML transcription factors are provided by interrelationships with nuclear architecture. Both biochemical and immunofluorescence analyses have shown that 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 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 [Bidwell et al., 1993; Blencowe et al., 1994; Dworetzky et al., 1992; Mancini et al., 1994; Nickerson et al., 1995; Stein et al., 1994, 1996, 1997; van Wijnen et al., 1993; Zeng et al., 1997].

Intranuclear Targeting of AML Transcription Factors to Subnuclear Domains That Support Transcription

Identification of a nuclear matrix targeting signal. Association of AML transcription factors with the nuclear matrix has provided the basis for directly addressing mechanisms which target regulatory factors to subnuclear domains that support transcription. 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

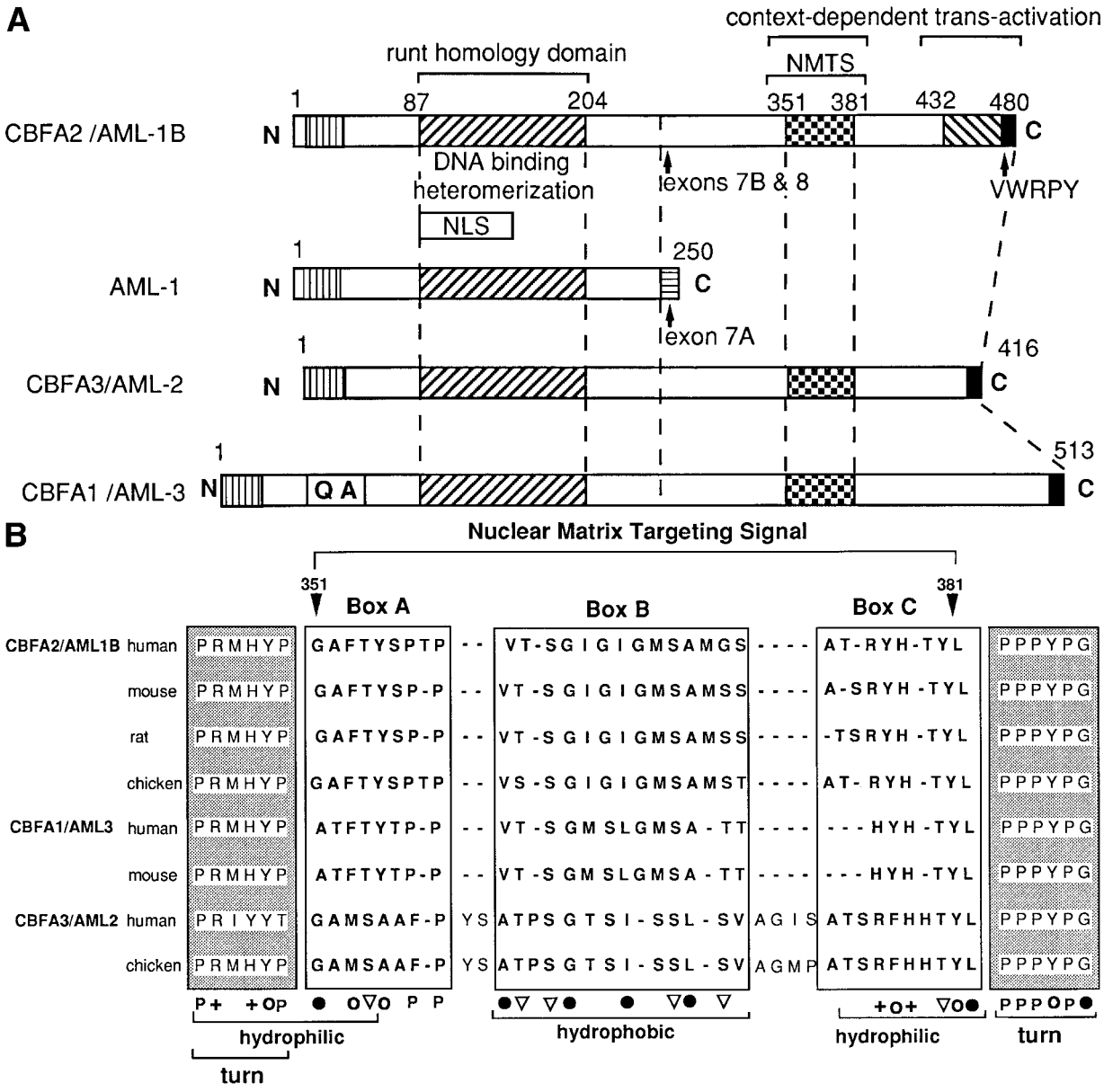

Fig. 1. **A:** Schematic representation of the CBFα/AML class of transcription factors. This class of factors is encoded by three distinct genes, designated CBFA1 (AML-3), CBFA2 (AML-1), and CBFA3 (AML-2), that each produce multiple protein isoforms. Most isoforms share an intact and highly conserved *runt* homology domain (rhd), which functions as a sequence-specific DNA binding domain and interacts with the heterodimerization partner CBFβ. The rhd contains a nuclear localization signal (NLS). Two key splice-variants of the CBFA2 gene are shown on the first two lines: (i) the full length, nuclear matrix-associated AML-1B protein contains a C-terminal domain, which spans at least two promoter context-dependent transactivation domains and the nuclear matrix targeting signal (NMTS); and (ii) the truncated and transcriptionally inactive AML-1 protein, which lacks these regulatory domains. Nuclear matrix-associated active transcription factors that are homologous to AML-1B are encoded by the CBFA3/AML-2 and CBFA1/AML-3 genes. All three proteins share an NMTS (blocked filling), the *runt* homology domain (right diagonal stripes), an N-terminal region (vertical stripes), and a highly conserved C-terminal VWRPY motif (black filling). The bone-related CBFA1/AML-3 protein contains a unique internal region rich in glutamine (Q) and alanines (A). **B:** The nuclear matrix targeting signal is a 31 amino acid domain that is highly conserved among vertebrate species as well as different subtypes of the CBFA/AML transcription factor family. The diagram indicates three groups of conserved amino acids (boxes A, B, and C), which are flanked by putative turn-motifs (gray boxes).

for the family of AML transcription factors [Banerjee 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 that 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 2A and shown by immunofluorescence images in Figure 2B, 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 (Fig. 1B). 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 the second mediates association with the nuclear matrix (nuclear matrix targeting signal) (Fig. 3).

The multiplicity of determinants for nuclear localization and alternative splicing of 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 [Banerjee et al., 1996; Ogawa et al., 1993a, 1993b], ETS-1 [Giese et al., 1995], and C/EBP [Zhang et al., 1996], AML-1B may facilitate recruitment of these factors to the nuclear matrix.

Functional consequences of transcription factor association with the nuclear matrix. Association of genes and cognate factors with the nuclear matrix may support the formation and/or activities of nuclear domains that facilitate transcriptional control [Alvarez et al., 1997; Berezney et al., 1996; Chen et al., 1996; Davie, 1997; Grande et al., 1997; Guo et al., 1995; Jackson, 1997; Lindenmuth et al., 1997; Merriman et al., 1995; Nardozza et al., 1996; Nickerson et al., 1995; Spelsberg et al., 1996; Stein 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]. Support for this conclusion is provided by results which establish that, one, active transcription is required for colocalization of AML-1B and RNA polymerase II at the nuclear matrix [Zeng et al., 1998] (Fig. 4); two, 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]; and three, 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, namely, the targeting of regulatory proteins to specific spatial domains within the nucleus.

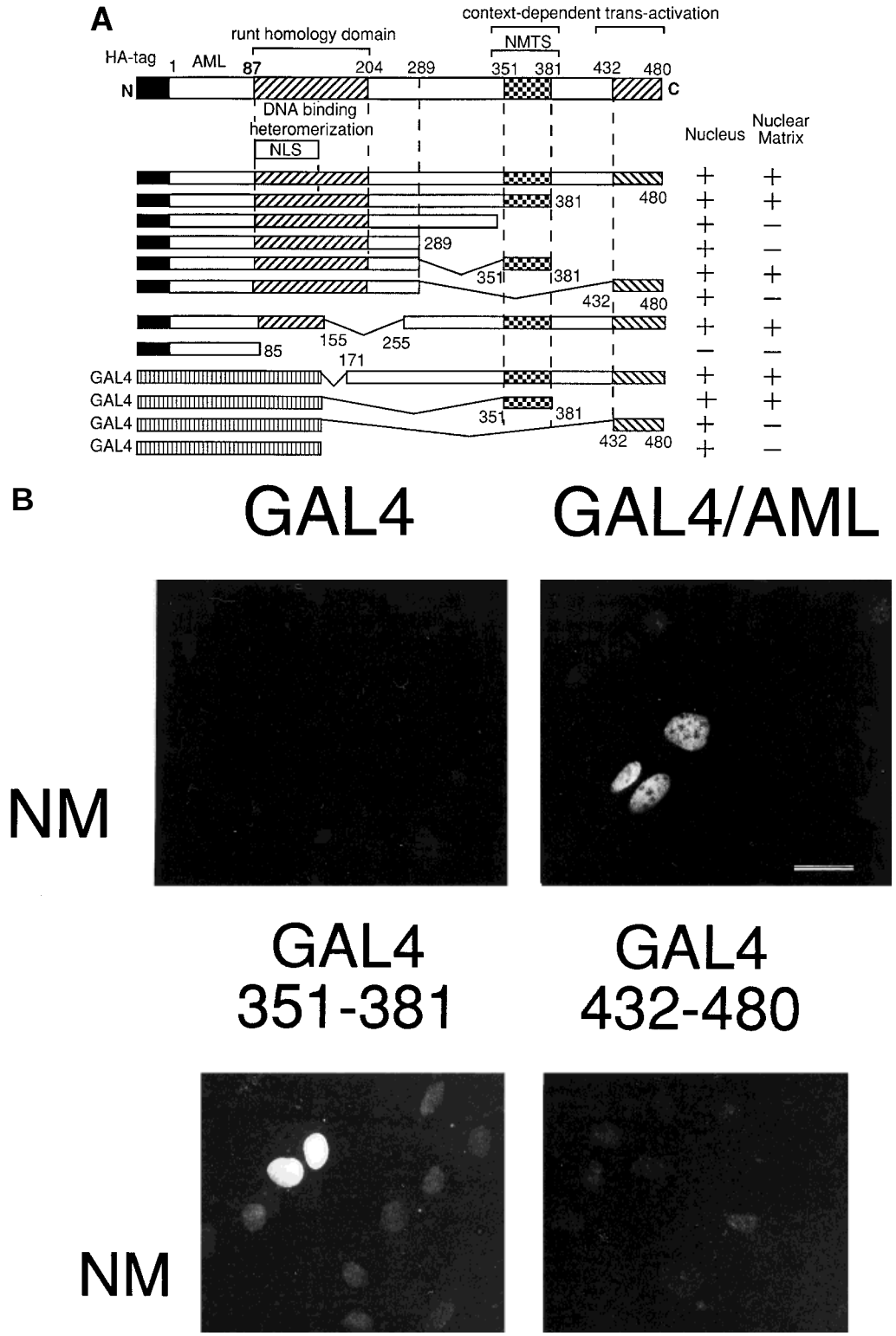

Fig. 2. **A:** Delineation of the nuclear matrix targeting signal of CBFA2/AML-1B. A panel of HA-epitope tagged deletion mutants of AML-1B was assayed by immunofluorescence analysis for nuclear import (column "Nucleus") and nuclear matrix association (column "Nuclear Matrix"). C-terminal segments of AML-1B were also fused to the heterologous GAL4 DNA binding domain (aa 1–147) and analyzed similarly. The key finding is that the NMTS (aa 351–381) autonomously mediates nuclear matrix association of the GAL4 reporter protein (third line from below). **B:** Immunofluorescence analysis of nuclear matrix association using fusion proteins between the GAL4 DNA binding domain (aa 1–147) and the C-terminus of AML-1B (GAL4/AML)(top panel, right), and segments of the C-terminus between aa 351–381 (GAL4/351–381) (bottom panel, left) and aa 432–480 (GAL4/432–480) (bottom panel, right). The GAL4 DNA binding domain alone (top panel, left) and GAL4/432–480 are not nuclear matrix-associated (grey background signals), whereas GAL4/AML and GAL4/351–381 are targeted to the nuclear matrix (white signal).

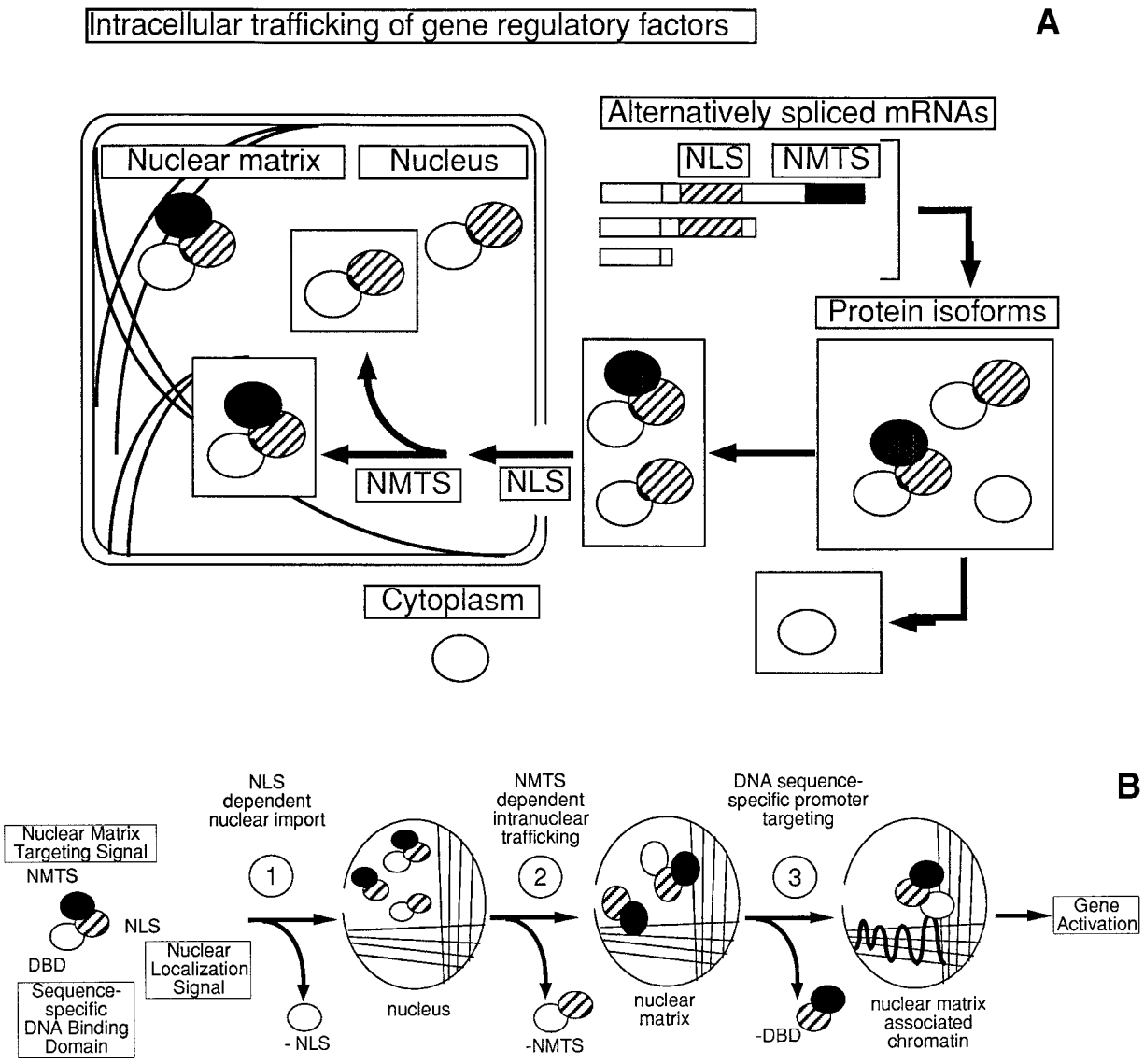

Fig. 3. Intracellular trafficking of the CBFA/AML class of transcription factors supports gene activation. **A**: Differential intracellular routing of distinct CBFA/AML factors depending on presence of specific subcellular targeting signals (diagonal stripes, black filling) in protein isoforms encoded by mRNA splice variants. **B:** Model of the molecular sorting mechanisms that occur to support selective targeting of CBFA/AML factors to transcriptionally active domains. This involves nuclear localization signal (NLS) (diagonal stripes) 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) (black filling) (step 2), and a requirement for a promoter recognition function of a sequence-specific DNA binding domain (DBD) (white filling) (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.

Implications of Aberrant Intranuclear Transcription Factor Targeting for Linkage of Modified Nuclear Architecture to Biological and Pathological Control of Transcription

Alterations in nuclear organization 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; Matera and Ward, 1993]. 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. For example, PML

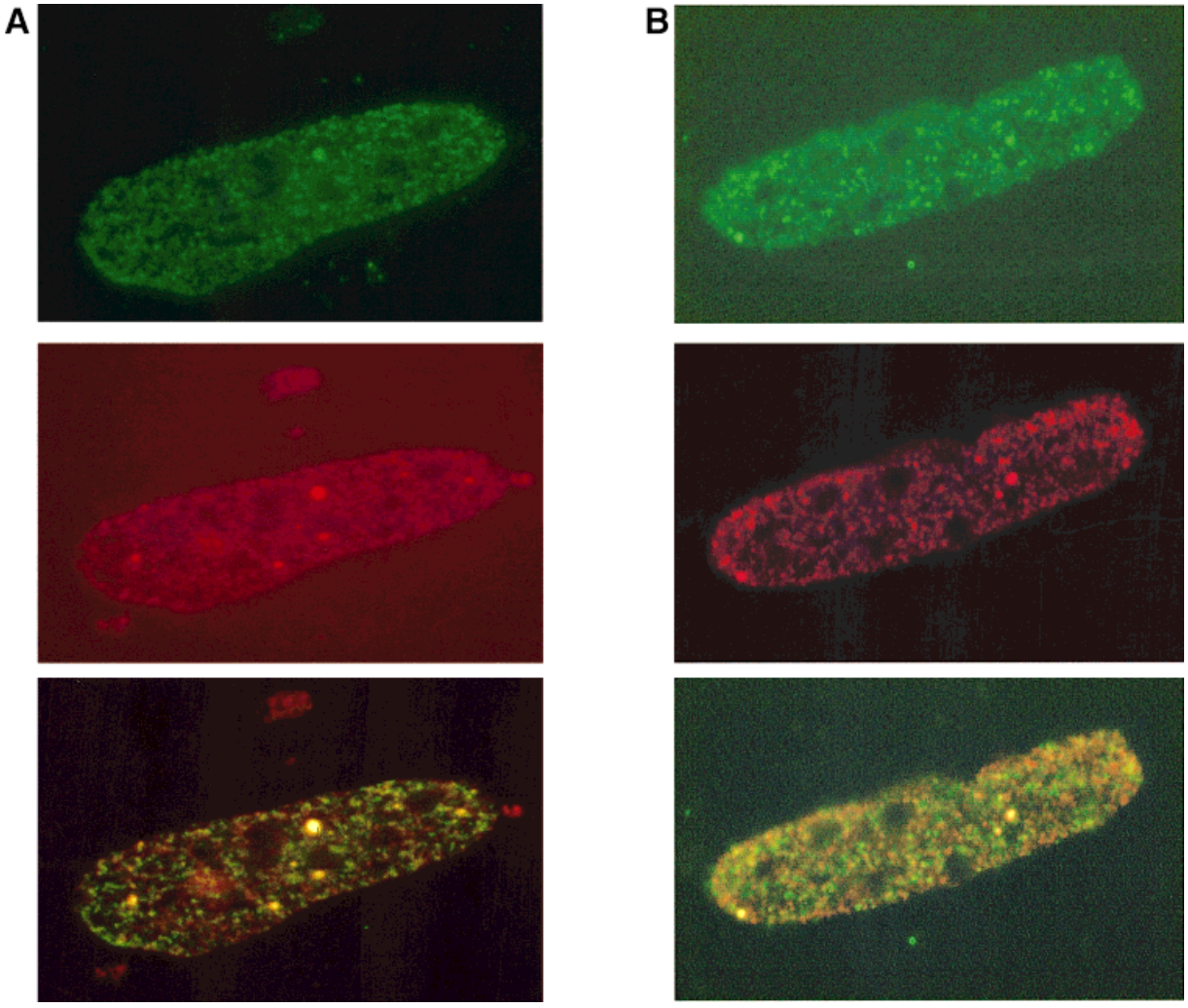

Fig. 4. CBFA2/AML-1B is directed to transcriptionally active nuclear foci that contain the hyperphosphorylated form of RNA polymerase II (pol II_0). **A** and **B** show colocalization of a subset of AML-1B with RNA pol II_0 in the nuclear matrix of human SAOS-2 osteosarcoma cells. The images were obtained 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).

bodies are nuclear structures that are associated with the nuclear matrix and modified in promyelocytic leukemia cells [Dyck et al., 1994; Nickerson et al., 1995; Weis et al., 1994]. In normal cells the PML protein resides in discrete PML bodies. However, in leukemic cells the PML protein is genetically rearranged and dispersed throughout the nucleus [Dyck et al., 1994; Weis et al., 1994]. Yet another 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 [Rogaia et al., 1997; Sobulo et al., 1997; Yano 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

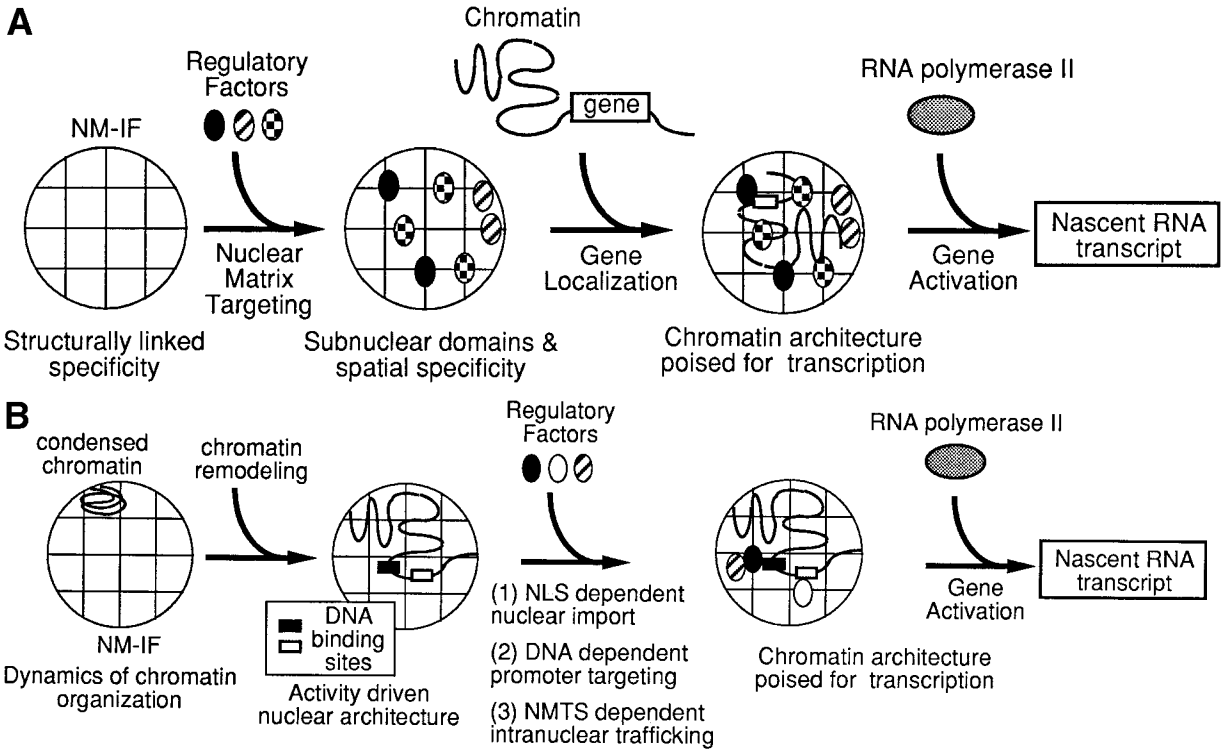

Fig. 5. Molecular mechanisms generating functional specificity of subnuclear domains that support modulations of transcriptional activity. **A:** Regional differences in the nucleus may be a direct reflection of architectural proteins of the nuclear matrix–intermediate filament scaffold. The assembly 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.

reported perturbations in the subnuclear distribution of ataxin-1 in spinocerebellar ataxia type 1. These investigators demonstrated that this neurological disorder, 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.

PROSPECTS

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 that 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 that are competent for transcription. 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.

It is difficult arbitrarily to separate nuclear structure and function or to distinguish the regulated and regulatory parameters of control. The challenges we now face are to further define the targeting of transcription factors and control that reside at the level of nuclear matrix-associated acceptor sites. The result will unquestionably be further insight into fundamental processes that 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. 5). 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 5, 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. Additionally, it will be important to define biochemically and mechanistically the checkpoints that are operative during subnuclear distribution of regulatory factors, as well as the editing steps that 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 at the right place at the right time to mediate 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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