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Subnuclear Organization and Trafficking of Regulatory Proteins: Implications for Biological Control and Cancer

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Abstract The regulated and regulatory components that interrelate nuclear structure and function must be experimentally established. A formidable challenge is to define further the control of transcription factor targeting to acceptor sites associated with the nuclear matrix. It will be important to determine whether acceptor proteins are associated with a pre-existing core-filament structural lattice or whether a compositely organized scaffold of regulatory factors is dynamically assembled. An inclusive model for all steps in the targeting of proteins to subnuclear sites cannot yet be proposed. However, this model must account for the apparent diversity of intranuclear targeting signals. It is also important to assess the extent to which regulatory discrimination is mediated by subnuclear domain-specific trafficking signals. Furthermore, the checkpoints that monitor subnuclear distribution of regulatory factors and the sorting steps that ensure both structural and functional fidelity of nuclear domains in which replication and expression of genes occur must be biochemically and mechanistically defined. There is emerging recognition that placement of regulatory components of gene expression must be temporally and spatially coordinated to facilitate biological control. The consequences of breaches in nuclear structure–function relationships are observed in an expanding series of diseases that include cancer [Weis et al., 1994; Rogaia et al., 1997; Yano et al., 1997; Rowley, 1998; Zeng et al., 1998; McNeil et al., 1999; Tao and Levine, 1999a] and neurological disorders [Skinner et al., 1997]. As the repertoire of architecture-associated regulatory factors and cofactors expands, workers in the field are becoming increasingly confident that nuclear organization contributes significantly to control of transcription. To gain increased appreciation for the complexities of subnuclear organization and gene regulation, we must continue to characterize mechanisms that direct regulatory proteins to specific transcription sites within the nucleus so that these proteins are in the right place at the right time. J. Cell. Biochem. Suppl. 35: 84–92, 2000.

Key words: Runx; nuclear matrix; nuclear organization; gene expression; transcription

Gene Expression Within the Three-Dimensional Context of Nuclear Architecture

It is becoming increasingly evident that control of gene expression must be understood within the three-dimensional context of nuclear architecture. During the past several years there have been significant advances in identification and characterization of promoter elements and cognate regulatory factors that mediate the activation and suppression of genes in response to a broad spectrum of physiological signals. However, it is necessary to mechanistically account for the integration of regulatory information and the achievement of threshold concentrations for protein–DNA and protein–protein interactions that are responsible for fidelity of gene expression.

While the mechanisms that govern the subnuclear organization of nucleic acids and regulatory proteins remain to be established, there is growing appreciation that the regulatory machinery for replication and transcription is organized in subnuclear domains that can be

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identified by biochemical, molecular, genetic, and in situ approaches.

Nuclear Microenvironments: A Structural and Functional Basis for Subnuclear Compartmentalization of Regulatory Machinery

From an historical perspective there has traditionally been a dichotomy between the pursuit of structural and functional properties of the cell nucleus. For the most part, biochemical parameters of replication and transcription have been studied independently from assignment of activities to components of nuclear architecture. Yet paradoxically, from around the turn of the last century it was recognized that there are microenvironments within the nucleus where regulatory macromolecules are compartmentalized in subnuclear domains. Chromosomes and the nucleolus provided the initial paradigms for organization of regulatory machinery within the nucleus, and during the last several decades, linkages have been established between subtleties of chromosomal anatomy and replication as well as gene expression. Regions of the nucleolus are understood in relation to ribosomal gene expression. The organization of chromosomes and chromatin are well accepted as reflections of functional properties that support competency for transcription and the extent to which genes are transcribed.

However, it is only recently that there is an appreciation for the broad-based organization of regulatory macromolecules within discrete nuclear domains [reviewed in Lamond and Earnshaw, 1998; Leonhardt et al., 1998; Ma et al., 1998, 2000; Wei et al., 1998; Zeng et al., 1998; Cook, 1999; Kimura et al., 1999; Misteli and Spector, 1999; Smith et al., 1999; Stommel et al., 1999; Verschure et al., 1999; Misteli, 2000; Scully and Livingston, 2000; Wu et al., 2000; Zhao et al., 2000; Salomoni et al., 2001]. Examples of intranuclear compartmentalization now include but by no means are restricted to SC35 RNA processing sites, PML bodies, the structural and regulatory components of nuclear pores that mediate nuclear–cytoplasmic exchange [Moir et al., 2000], coiled (Cajal) bodies, replication foci as well as defined sites where steroid hormone receptors and transcription factors reside [Glass and Rosenfeld, 2000; Leonhardt et al., 2000; McNally et al., 2000]. The integrity of these subnuclear microenvironments is indicated by structural and functional discrimination between each architecturally defined domain. Corroboration of structural and functional integrity is provided by modifications in the composition, organization, and intranuclear distribution in relation to activity [Hirose and Manley, 2000; Lemon and Tjian, 2000].

By the combined application of molecular, biochemical, genetic, and high resolution in situ analysis that permit the identification of nucleic acids and regulatory proteins in nuclei of intact cells, the intranuclear organization of nucleic acids and regulatory proteins is being further defined. We are going beyond mapping regions of the nucleus that are dedicated to replication and gene expression. We are gaining insight into interrelationships between the subnuclear organization of the regulatory and transcriptional machinery with the dynamic assembly and activity of macromolecular complexes that are required for biological control during development, differentiation, maintenance of cell and tissue specificity, homeostatic control, and tissue remodeling. Equally important, it is becoming evident that the onset and progression of cancer and neurological disorders are associated with and potentially functionally coupled with perturbations in the subnuclear organization of genes and regulatory proteins that relate to aberrant gene replication, repair, and transcription.

Trafficking to Subnuclear Destinations

The subnuclear organization of nucleic acids and regulatory proteins in discrete sites that support replication, transcription, processing of gene transcripts, mitotic apparatus assembly and activity, chromosome condensation and decondensation as well as nuclear-cytoplasmic exchange of regulatory micromolecules necessitates a mechanistic explanation for linkages of function with components of nuclear architecture. There is growing evidence that the organization of regulatory complexes within the nucleus is a multistep process. The interrelationships between nuclear morphology and the structural as well as enzymatic components of replication and gene expression are providing a basis for the assembly and physiologically responsive activity of regulatory complexes. For many years, it has been acknowledged that the enzymology for oxidative phosphorylation is architecturally organized in the mitochondria. An analogous structure–function paradigm

may be operative within the nucleus for the stringent control of nuclear regulatory events.

Necessarily, there is a requirement for mechanisms to direct regulatory proteins and/or nucleic acids to subnuclear sites. Here, several regulatory proteins, both transcription and replication factors, have been functionally dissected to reveal discrete sequences that accommodate intranuclear trafficking. The hematopoietic and bone-specific Runx/AML/CBFA transcription factors provide a viable paradigm for identification and characterization of specific sequences that direct transcription factors to subnuclear domains [Merriman et al., 1995; Banerjee et al., 1997; Zeng et al., 1997, 1998; Chen et al., 1998; Javed et al., 1999, 2000]. A 31 amino acid sequence has been identified that is necessary and sufficient to direct Runx/AML/CBF proteins to sites within the nucleus that support transcriptional regulation [Zeng et al., 1997, 1998; Javed et al., 1999] or suppression [Javed et al., 2000]. Specificity of the Runx intranuclear trafficking signal is reflected by the unique targeting sequence [Zeng et al., 1997], the X-ray crystal structure [Tang et al., 1999] and the ability to discriminate sites within the nucleus where Runx/AML/CBF proteins reside from sites where other regulatory proteins (e.g. glucocorticoid receptor [van Steensel et al., 1995; Htun et al., 1996; Tang et al., 1998], estrogen receptor (ER) [Stenoien et al., 2000, 2001], YY1 [Guo et al., 1995; McNeil et al., 1998], PIT1 [Stenoien et al., 1998], androgen receptor [van Steensel et al., 1995] and PTHRP transcription factors [Nguyen and Karaplis, 1998], nucleolar proteins [Guo et al., 1995], replication/repair factors [Wei et al., 1998]) are located. However, a comprehensive understanding of intranuclear trafficking mechanisms requires defining subtleties of subnuclear foci assembly, activity, and turnover. It is reasonable to accept that intranuclear trafficking involves multiple steps that include nuclear import and directing regulatory proteins to defined foci within the nucleus. The challenges are now to determine whether regulatory proteins are directed to scaffold-associated acceptor proteins or if intranuclear trafficking mechanisms specify macromolecular interactions that are associated with the assembly of multicomponent regulatory complexes that form a structural and functional scaffold to support both nuclear morphology as well as replication and gene expression. While the components of mechanisms that direct regulatory factors to subnuclear sites remain to be comprehensively characterized, even skeptics have difficulty in accounting for the subnuclear compartmentalization of regulatory machinery by diffusion alone. The intranuclear trafficking mechanisms that are emerging provide a basis for obtaining threshold interactions of factors involved with replication and transcription that are present at modest levels within the nucleus. However, it would be arbitrary and unrealistic to assume that a single mode of regulatory factor trafficking is operative within the nucleus or that intranuclear trafficking is required to direct all regulatory proteins to regions within the nucleus where they function. The temporal and spatial organization of the replication and transcriptional machinery is an in vivo phenomenon that must be reckoned with.

Nuclear Architecture Facilitates Integration of Regulatory Signals

We are gaining insight into the involvement of nuclear architecture in the integration of regulatory signals that control gene expression by mediating crosstalk between components of physiological signaling pathways. Mechanisms modulating chromatin remodeling require higher order nuclear structure and illustrate the requirement to direct regulatory proteins to discrete intranuclear sites [Stein et al., 1998; Jones and Kadonaga, 2000]. The human SWI/SNF and mouse BAF complexes have been shown to be punctately distributed within the nucleus and associated with the nuclear matrix [Reyes et al., 1997; Zhao et al., 1998]. Functional implications are provided by the observation that the BAF complexes are only associated with the nuclear matrix after mitogenic stimulation of T lymphocytes when gene controlling competency for proliferation and cell cycle progression are activated [Zhao et al., 1998]. In resting cells, the BAF complex is primarily present in the soluble nuclear fraction. However, immediately after the induction of proliferation, the BAF complex is principally found tightly associated with the nuclear matrix fraction [Zhao et al., 1998]. The specific parameters of chromatin remodeling that are linked to nuclear matrix binding of BAF as well as the cause and/or effect relationship between BAF activity and parameters of nuclear organization will unquestionably be informative.

Further insight into linkages between nuclear architecture, cytoarchitecture, and the regulation of chromatin structure is provided by reports that actin-related proteins are components of chromatin remodeling complexes [Cairns et al., 1998; Papoulas et al., 1998; Peterson et al., 1998; Zhao et al., 1998]. It has been suggested that these actin-related and actin-binding proteins may provide a basis for interactions between chromatin remodeling complexes and cytoskeletal structures involving actin. This suggestion is further supported by observations that both human SWI/SNF complexes and drosophila BRM complexes not only contain an actin-related protein (BAF53 in human cells and BAP55 in drosophila) but also actin [Papoulas et al., 1998; Zhao et al., 1998]. Furthermore, regions of BAF that contact myosin, proliferin, and other actin-binding proteins are similar to actin [Zhao et al., 1998]. The possibility can therefore be considered that such interactions are important for SWI/SNF function and are contributing to chromatin organization and/or remodeling. It will be important to define the mechanisms that control the recruitment and assembly of chromatin remodeling factors at subnuclear sites where the packaging of genomic DNA is modified to render specific sequences competent for interactions with transcriptional activators and suppressors.

Compromised Intranuclear Trafficking is Functionally Linked to Aberrant Subnuclear Organization of Regulatory Complexes and Compromised Gene Expression in Leukemias

Interrelationships of nuclear structure with gene expression are illustrated by the modified subnuclear organization of genes and regulatory factors in cancer (Fig. 1). Transformed and tumor cells exhibit striking alterations in nuclear morphology as well as in the representation and intranuclear distribution of nucleic acids and regulatory factors. In both leukemias and solid tumors there are modifications in components of nuclear architecture that are involved in control of gene expression. Examples include mutations of the AML [Miyoshi et al., 1991; Levanon et al., 1994; Nuchprayoon et al., 1994; Takahashi et al., 1995; Meyers et al., 1996; Rowley, 1998; McNeil et al., 1999], ALL [Rogaia et al., 1997; Sobulo et al., 1997; Yano et al., 1997] and PML [Dyck et al., 1994; Weis et al., 1994] 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 to nuclear architecture 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 that 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 tumors as well as to monitor remission, relapse, and effectiveness of treatment. There is a potential for developing therapeutics that are directed to subnuclear sites that support specific components of gene expression.

Alterations in nuclear organization are the hallmarks of leukemic cells. The gene locus encoding the Runx transcription factor that is nuclear matrix associated and is frequently the target of reciprocal chromosomal translocations in human leukemia. Replacement of the chromosome 21-encoded intranuclear trafficking signal by a targeting signal from chromosome 8 redirects the t(8;21) translocation–fusion protein to unique subnuclear sites [McNeil et al., 1999]. Thus, intranuclear targeting of the Runx/AML 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. Such an interpretation is consistent with findings [Meyers et al., 1996] that the AML/ETO t(8;21) fusion protein suppresses transcription while the chromosome 21 encoded AML protein is a transcriptional activator.

PML bodies are another example of nuclear structures that are associated with the nuclear matrix and modified in leukemic cells [Dyck et al., 1994; McNeil et al., 2000]. 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

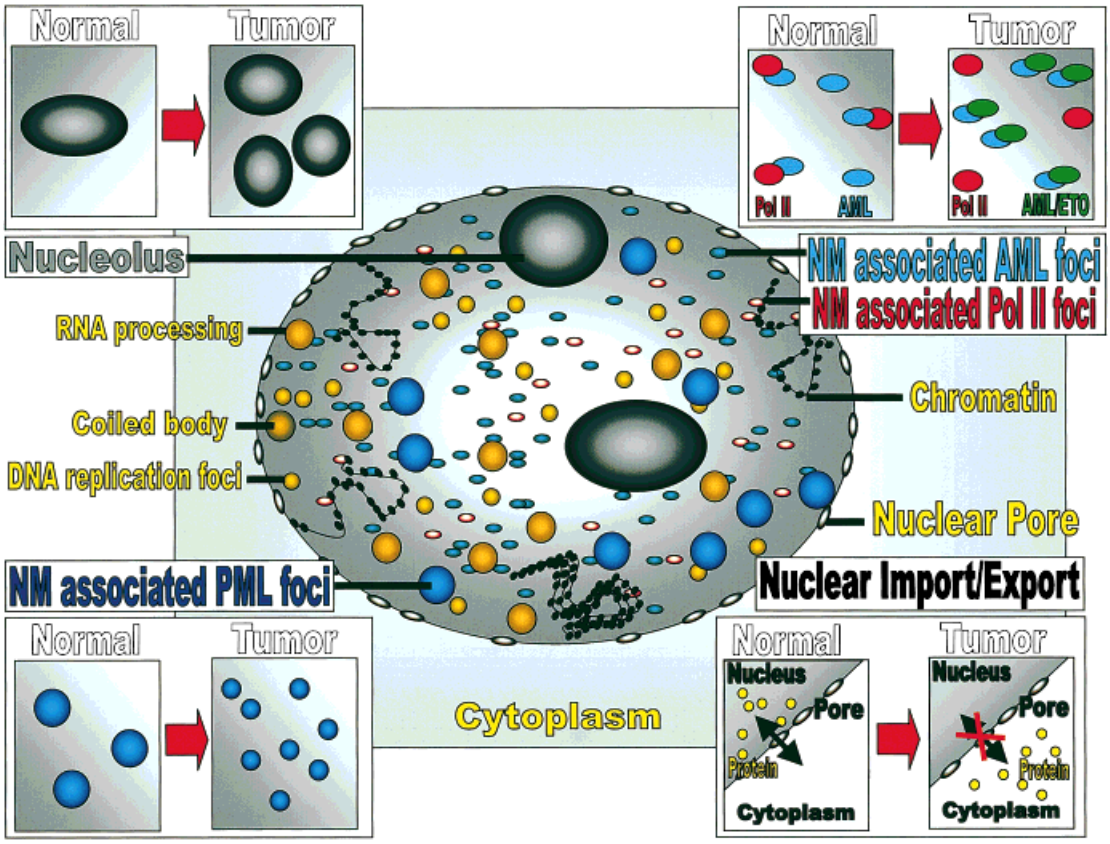

Fig. 1. Targeting of gene regulatory factors and stable compartmentalization at distinct foci within the nucleus requires multiple trafficking steps, including nuclear import and export, subnuclear targeting to specific sites, and DNA binding-dependent association of transcription factors with cognate genes. Additional regulation is required for the assembly and composition of regulatory complexes at subnuclear sites to accommodate physiological control of the machinery for gene expression. Aberrations in nuclear morphology and subnuclear organization of domains involved in gene expression and DNA replication are frequently modified during tumorigenesis. The center of the figure depicts the nucleus (gray sphere) with multiple functionally distinct subnuclear domains (colored spheres) and other pertinent nuclear features (e.g., chromatin, nucleoli, and nuclear pores). The frames in the corners indicate characteristic modifications in subnuclear organization observed in tumor cells, including PML foci (left bottom), nucleolus (left top), subnuclear targeting (AML versus AML/ETO) as well as nuclear import and export. Other tumor-linked modifications include alterations in subnuclear targeting of RNA processing factors and SWI/SNF chromatin remodeling complexes (not indicated).

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. This 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], although the chimeric transcription factors remain nuclear matrix associated [Gordon et al., 2000]. Hence, these results suggest that perturbations in subnuclear location of regulatory proteins may be related to modifications in gene expression that are linked to leukemias. Additionally, tumor-associated modifications have been observed in nuclear domains that support the processing of transcripts, the intranuclear organization of Rb and DNA replication/repair foci, and the nucleocytoplasmic shuttling of p53 that has been functionally linked to association of Mdm2 with the nucleolus [Freedman and Levine, 1998; Tao and Levine, 1999a, 1999b].

Prospects for Insight Into Directing Transcription Factors to the Right Place at the Right Time Within the Nucleus

It would be naive to anticipate a single target for tumor-related alterations in the organiza-

tion of genes, transcripts, and regulatory machinery. Rather, the mechanisms that mediate each component of gene regulation in relation to nuclear structure–function relationships must be experimentally defined. There is growing recognition that placement of regulatory components of gene expression must be temporally and spatially coordinated to optimally support biological control. The consequences of breaches in nuclear structure–function interrelationships that have been observed in an extensive series of tumors provide options for high resolution diagnoses and targeted therapies.

Therefore, one fundamental question is the mechanism by which this compartmentalization of regulatory factors is established within the nucleus. This subnuclear organization could be maintained by an underlying macromolecular framework referred to as the nuclear matrix [Berezney and Jeon, 1995; Penman, 1995; Berezney and Wei, 1998]. However, one cannot dismiss the possibility that nuclear compartmentalization is activity driven [Misteli, 2000; Pederson, 2000].

The nuclear matrix can be visualized by embedment-free electron microscopy as an anastomosing network of filaments and globular structures [Penman, 1995]. This internal nuclear matrix is surrounded by the lamina/pore complex which appears to be connected to the cytoskeletal intermediate filaments. The entire cellular architecture observed following biochemical extraction is referred to as the nuclear matrix–intermediate filament scaffold (NM–IF). Several outstanding reviews have discussed the relationship of this NM–IF structure to the in vivo organization and activities of the nucleus [Berezney and Jeon, 1995; Penman, 1995; Berezney and Wei, 1998; Misteli, 2000; Pederson, 2000]. Independent of ultrastructural definitions of the nucleus, immunofluorescence microscopy studies with nuclear matrix preparations have provided novel insights into subnuclear organization.

Consistent with a principal role for the nuclear matrix in the organization of regulatory complexes within the nucleus is the representation of proteins involved in gene expression or DNA replication, chromatin modifying enzymes, and RNA processing factors [Ciejek et al., 1983; Davie and Chadee, 1998; Wei et al., 1998; Zeng et al., 1998; Kimura et al., 1999; Javed et al., 2000]. The localization of these nuclear matrix-associated proteins to large specialized subnuclear domains further supports the architectural placement of regulatory macromolecules. These domains can be visualized by immunofluorescence microscopy in intact cells and in NM–IF preparations. Recent results have directly shown that active transcription and DNA replication occur at numerous spatially distinct foci that are also associated with the NM–IF [Wei et al., 1998, 1999]. Thus, the evidence for specific regulatory mechanisms that mediate spatial distribution of proteins within the nucleus is providing a basis for further pursuit of architecture involvement in transcriptional control for support of biological regulation and aberrations in cancer.

It is unrealistic to propose a universal mechanism for the subnuclear organization and trafficking of regulatory proteins. However, there is emerging support for both repair/replication, and transcription factors serving as a scaffold for interactions with regulatory proteins that control the assembly of multipartite complexes. The Runx/AML transcription factor provides an architectural scaffold for protein–DNA and protein–protein interactions that mediate activation, suppression, and, chromatin remodeling as well as the signals for nuclear import and intranuclear trafficking to foci within the nucleus where control of transcription resides [Javed et al., 2000]. The composite organization of foci for replication and repair appears to occur in an analogous manner. Both repair/replication and transcription domains exhibit altered composition and subnuclear distribution in tumor cells reflecting a reorganization of intranuclear regulatory machinery that is structurally and functionally related to compromised physiological control and tumorigenesis.

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