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Functional interrelationships between the intranuclear organization of nucleic acids and regulatory proteins are obligatory for fidelity of transcriptional activation and repression. In this article, using the Runx/AML/Cbfa transcription factors as a paradigm for linkage between nuclear structure and gene expression we present an overview of growing insight into the dynamic organization and assembly of regulatory machinery for gene expression at microenvironments within the nucleus. We address contributions of nuclear microenvironments to the convergence and integration of regulatory signals that mediate transcription by supporting the combinatorial assembly of regulatory complexes.

Key words: Runx/AML/Cbfa, chromatin, mitotic partitioning, gene regulation, osteocalcin, transcription complexes, BMP/TGFβ

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During the past several years our understanding of transcriptional control has evolved from independent considerations of biochemical mechanisms and concepts of nuclear morphology that are associated with genes and gene transcripts. There is growing appreciation for functional interrelationships between the intranuclear organization of nucleic acids and regulatory proteins that are instrumental for transcriptional activation and suppression in a biologically responsive manner. In this article we will focus on the dynamic organization and assembly of the regulatory machinery for gene expression at microenvironments within the nucleus from the perspective of cellular requirements for physiological control (Figure 1). Using the tissue specific Runx/AML/Cbfa transcription factors as a paradigm we will present an overview of a conceptual and experimental basis for the regulation of gene expression within the three dimensional context of nuclear architecture. We will address contributions of nuclear microenvironments to the convergence and integration of regulatory signals that mediate gene expression by supporting the combinatorial assembly of regulatory complexes. Temporal and spatial parameters of control will be emphasized.

Gene expression within the three dimensional context of nuclear architecture

Multiple levels of nuclear organization support interrelationships between nucleic acids and regulatory proteins in a manner that mediates competency for physiologically responsive expression or repression of genetically encoded information. During the past several years there has been spectacular progress in the identification and cataloguing of genes and encoded proteins. And, an understanding of the multidirectional exchange of regulatory signals between the extracellular environment and the nucleus is rapidly expanding. However, the challenge remains to gain insight into the rules that govern the

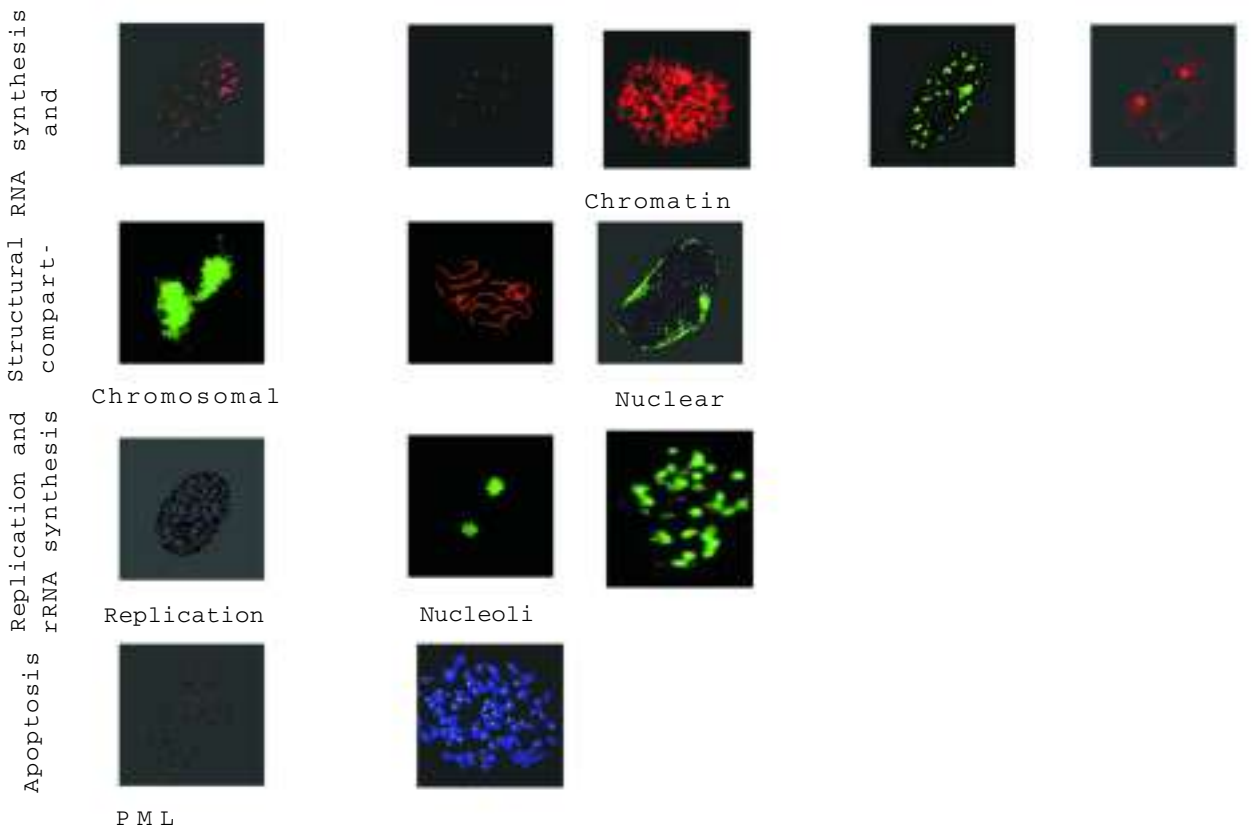

Figure 1. Components of nuclear architecture are functionally linked to the organization and sorting of regulatory information. Nuclear functions are organized into distinct, non-overlapping subnuclear domains. Nuclear matrix, the underlying network of anastomising network of filaments and fibers provides structural basis for the functional compartmentalization of nuclear functions. Immunofluorescence microscopy of the nucleus in situ has revealed the distinct subnuclear distribution of vital nuclear processes, including (but not limited to) DNA replication sites and proteins involved in replication such as BRCA I; chromatin remodeling, e.g., mediated by the SWI/SNF complex; structural parameters of the nucleus, such as the nuclear envelope, chromosomes, and chromosomal territories; Runx domains for chromatin organization and transcriptional control of tissue-specific genes; and RNA synthesis and processing involving, for example, transcription sites; SC35 domains, coiled bodies, and nucleoli as well as proteins that play a role in cell survival, for example survivin. Subnuclear PML bodies of unknown function have been examined in numerous cell types. All these domains are associated with the nuclear matrix.

integration of biological cues with the temporal and spatial parameters of cell structure and function.

There is longstanding recognition that the representation of protein coding and regulatory sequences embedded in the genome are the primary level of nuclear organization providing a blueprint for responsiveness to accommodate short term homeostatic regulation and longer term developmental and phenotype-specific requirements of cells, tissues and organs.

Chromatin structure and nucleosome organization conformationally configure DNA to establish competency for protein/DNA and protein/protein interactions at promoter elements as well as reduce distances between regulatory domains to synergistically interface the activities of transcription factors and coregulatory proteins. The dynamic ATP-dependent remodeling of chromatin organization and enzyme dependent post translational modifications of histones (e.g., acetylation, methylation and phosphorylation) are recognized as key mechanistic determinants of chromatin-mediated promoter function.

And, while compartmentalization within the nucleus is well established as reflected by the packaging of DNA as chromosomes during mitosis and the ribosomal genes as nucleoli during interphase, it is only recently that there has been a focus on contributions of higher order nuclear organization to architecturally supporting compartmentalization of regulatory machinery in subnuclear microenvironments. Consequently, the perception of a dichotomy between nuclear morphology and regulatory activity has given way to accruing appreciation that the rules governing control of gene expression; as well as those that are determinants of replication and repair, operate in a seamless manner with the temporal and

spatial organization of regulatory machinery. This architectural organization of the combinatorial components required for biological control occurs at a series of intranuclear microenvironments where multi component regulatory complexes are dynamically assembled under conditions where threshold levels of nucleic acids and proteins are present to support activity within the confines of requirements for biological responsiveness. Although the concept of focally organized microenvironments that are structurally and functionally linked to regulation is not new, the extent to which this paradigm is operative is becoming inescapably evident. There is increasing acceptance that fidelity of nuclear structure – gene expression interrelationships is necessary for integration of regulatory signals that are associated with acquisition of cues and execution of responses. The concept of checkpoints is therefore being extended to encompass surveillance of a broad spectrum of regulatory mechanisms that now include parameters of cell cycle control together with those that assess effectiveness of the organization, assembly and activity of the machinery for chromatin remodeling and the intranuclear placement of regulatory complexes. It should not be surprising that there are increasing examples of default to apoptosis when standards for fidelity of the structural and functional properties of regulatory machinery fall below expectations.

Runx/AML/Cbfa transcription factors provide a paradigm for obligatory interrelationships between subnuclear organization and gene expression

The Runx2 (AML3/Cbfa1/PEBP2_) transcription factor and the bone-specific osteocalcin gene serve as paradigms for obligatory relationships between nuclear structure and physiological control of skeletal gene expression (Banerjee et al., 1996; Ducy et al., 1997; Javed et al., 1999; Javed et al., 2000; Merriman et al., 1995). 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 (Banerjee et al., 1996; Bortell et al., 1992; Demay et al., 1990; Ducy and Karsenty, 1995; Guo et al., 1995; Hoffmann et al., 1994; Markose et al., 1990; Merriman et al., 1995; Tamura and Noda, 1994, Montecino et al. 1996; Lian et al., 1999). 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 nuclear matrix-associated Runx transcription factors contribute to the control of skeletal gene expression by sequence-specific binding to promoter elements of target genes and serve as scaffolds for the assembly and organization of coregulatory proteins that mediate biochemical and architectural control of promoter activity.

Runx-Mediated chromatin remodeling facilitates promoter accessibility and integration of regulatory activities

The concept of a central role for the architectural organization of regulatory complexes within the nucleus for physiologically responsive control of gene expression is consistent with emerging evidence for hierarchal scaffolding of regulatory complexes that include the enzymology for chromatin remodeling. The nuclear scaffold-associated RUNX1 and RUNX2 transcription factors directly interact with histone acetyltransferases and histone deacetylases as well as functionally interact with promoter regulatory elements of hematopoietic and skeletal target genes (Kitabayashi et al., 1998; Westendorf et al., 2002; Westendorf and Hiebert, 1999). This interrelationship between nuclear structure and gene expression provides a viable opportunity for characterizing the assembly of regulatory machinery for chromatin remodeling and transcriptional activation/suppression at intranuclear sites.

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 (Boyer et al., 2000; de la Serna et al., 2001; de la Serna and Imbalzano, 2002; Fischle et al., 2003a; Fischle et al., 2003b; Fischle et al., 2003c; Formosa, 2003; Hassan et al., 2001; Horn and Peterson, 2002; Jaskelioff and Peterson, 2003; Jenuwein and Allis, 2001; Peterson, 2002a; Peterson, 2002b; Peterson, 2002c; Peterson, 2003; Rice et al., 2003; Rice and

Allis, 2001) as well as the SWI/SNF-related proteins (Becker and Horz, 2002; de la Serna et al., 2001; Peterson and Workman, 2000) 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 unquestionably will provide insight into skeletal gene regulatory mechanisms from structural and functional perspectives.

The Runx-dependent 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; Gutierrez et al., 2000; Montecino et al., 1994; Montecino et al., 1996; Montecino et al., 1999; Paredes et al., 2002). 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 (Javed et al., 1999; Montecino et al., 1996; Montecino 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 (Javed et al., 1999; Montecino et al., 1996; Montecino et al., 1999; Paredes et al., 2002). 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 TFIIB transcription factor (Blanco et al., 1995; Guo et al., 1997; MacDonald et al., 1995). 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 Runx-mediated 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 (Gutierrez et al., 2000; Javed et al., 1999). 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; Shen et al., 2003). 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 Runx-responsive 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.

Runx transcription factors are scaffolds for the combinatorial organization and assembly of gene regulatory machinery

Functional interrelationships between nuclear structure and gene expression are strikingly reflected by dual recognition of regulatory proteins, such as Runx transcription factors, for interactions with both promoter elements and coregulatory proteins; such interactions modulate the structural and functional properties of targeted genes at microenvironments within the nucleus. Sequence-specific interactions with promoter elements result in placement of Runx proteins at strategic sites where they provide scaffolds for protein-protein interactions that mediate the organization of machinery for a broad spectrum of regulatory requirements. These include histone modifications and chromatin remodeling that establish competency for transcription factor binding and genomic conformations that interface activities at proximal and upstream promoter domains, as well as the integration of regulatory cues from signaling pathways that activate or suppress gene expression in a physiologically responsive manner. As a consequence, the Runx proteins are post-translationally modified (e.g., phosphorylated) to further influence the extent to which they engage in regulatory activity.

The complexity of Runx regulatory proteins that assemble as supercomplexes of transcriptional regulatory factors illustrates the potential impact on skeletal-related gene expression. Recent documentation that Runx proteins are components of a stable complex that includes basal transcription factors, chromatin remodeling factors, and histone modifying factors indicates the scope of Runx-mediated combinatorial control.

A key component of the Runx complex is the p300/CBP coactivator which functions as a transcriptional adaptor. Interactions with several transcription factors result in the formation of multimolecular complexes that regulate expression of a broad spectrum of genes (Goodman and Smolik, 2000). p300 contains a domain with intrinsic histone acetyltransferase (HAT) activity (Bannister et al., 1995; Ogryzko et al., 1996) which has been implicated in chromatin structure alterations associated with modulation of gene expression (Spencer and Davie, 1999). p300 interacts with additional proteins containing HAT activity that include P/CAF, SRC-1 and ACTR. A basis is thereby provided for formation of large multiprotein complexes that contribute multiple HAT activities with options for specificity (Chakravarti et al., 1996; Chen et al., 1997; Spencer et al., 1997; Torchia et al., 1997; Yang et al., 1996). It has been established that Runx2 and p300 are components of the same nuclear complexes in osteoblastic cells (Sierra et al., 2003). Furthermore, when recruited to the osteocalcin gene promoter by Runx2, p300 stimulates both basal and vitamin D-enhanced osteocalcin promoter activity. Thus interactions of Runx2 with p300 supports assembly of multi-subunit complexes with several HAT-containing proteins at a series of regulatory regions of the bone-specific osteocalcin gene promoter. In a parallel manner, Kitabayashi et al. (1998) have shown that in myeloid cells Runx1, a homologue of the bone-specific Runx2, interacts with p300 and together upregulate myeloid-specific genes. It was also determined that a C-terminal region of the Runt domain in both Runx1 and Runx2, is critical for their interactions with p300 (Kitabayashi et al., 1998; Sierra et al., 2003). Considering the high degree of homology between these two members of the Runx transcription factor family, it is likely that the structural determinants for Runx interactions with p300 are conserved.

p300 can also be recruited to gene promoters by the transcription factor C/EBP (Mink et al., 1997; Oelgeschlager et al., 1996). Interestingly, a C/EBP-responsive regulatory element has been identified in the proximal promoter region of the rat OC gene adjacent to the Runx2 site C (Gutierrez et al., 2002). C/EBP_ physically interacts with Runx2 and synergistically activates the osteocalcin promoter (Gutierrez et al., 2002), suggesting that both proteins form a complex with p300 and together upregulate basal tissue-specific transcription. C/EBP_ has additionally been shown to interact with ATP-dependent chromatin remodeling complexes of the SWI/SNF family (Kowenz-Leutz and Leutz, 1999), recruiting these complexes to promoter sequences and activating cell-specific expression.

In addition to functioning as transcriptional acti-

vators, Runx proteins suppress gene expression. Repression requires the recruitment of transcriptional repressors and corepressors with histone deacetylase activity (HDACs) to promoter regulatory elements of genes that are downregulated. Combinatorial control that dampens transcription is illustrated by interaction of Runx2 with the transcriptional corepressors TLE/Groucho through a conserved VWRPY domain located at the C-terminus of the protein, which represses the expression of the bone sialoprotein (BSP) gene in osteoblastic cells (Javed et al., 2000). Another example of combinatorial control that results in transcriptional suppression by Runx2 is downregulation of the $p21^{CIP/WAF}$ promoter in fibroblastic and osteoblastic cells. Here HDAC6 interacts with a second repression domain that also resides in the C-terminal region of Runx2 and is recruited to chromatin by Runx2 (Westendorf et al., 2002). Taken together, these results are consistent with combinatorial control that is mediated by Runx-dependent recruitment of coactivator and corepressors proteins that are associated with and organized as multiprotein complexes to activate or repress target genes in a physiologically responsive manner.

These findings indicate that Runx factors engage in protein-DNA and protein-protein interactions that collectively determine the composition and organization of promoter regulatory complexes. The inclusion of chromatin remodeling activity in these multi-subunit complexes provides a biochemical basis for conformational modifications of promoter elements as well as combinatorial specificity for transcription.

Transcription factors that function as scaffolds for interaction with coregulatory proteins provide an architectural basis for accommodating the combinatorial requirements of biological control. Combinatorial control supports replication, transcription and repair by two mechanisms. Context dependent combinations and permutations of regulatory proteins are assembled into multipartite complexes that increase specificity. Scaffold associated protein-DNA and protein-protein interactions permit integration of regulatory activities. Nuclear microenvironments are thereby organized, with gene promoters as focal points, where threshold concentrations of regulatory macromolecules are attained. The complexity that is achieved by these architecturally organized oligomeric factors can maximize options for responsiveness to diverse regulatory requirements for transient and long term biological control.

Intranuclear trafficking of runx regulatory proteins: a mechanism for the organization of subnuclear microenvironments that mediate gene expression

Association of osteoblast, myeloid, and lymphoid Runx transcription factors that mediate tissue-specific transcription with the nuclear matrix has permitted direct examination of mechanisms for targeting regulatory proteins to subnuclear sites where regulatory events occur (Bae et al., 1993; Banerjee et al., 1996; Banerjee et al., 1997; Ducy et al., 1997; Frank et al., 1995; Merriman et al., 1995; Meyers et al., 1993; Meyers et al., 1995; Meyers et al., 1996; Nuchprayoon et al., 1994; Satake et al., 1995; Wang et al., 1993; Zeng et al., 1997). 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., 1998; Zaidi et al., 2002a; Zeng et al., 1997; Zeng et al., 1998). 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; van Wijnen et al., 1993; 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 (Choi et al., 1999; Choi et al., 2001; Javed et al., 2000; Zaidi et al., 2002a; Zaidi et al., 2002b; Zeng et al., 1997). 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 indicates a common intranuclear targeting mechanism may be operative for the family of Runx transcription factors (Harrington et al., 2002; Javed et al., 2000; Tang et al., 1998). 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. 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 (Banerjee et al., 1996; Giese et al., 1995; Kundu et al., 2002; Mao et al., 1999; Miller et al., 2002; Ogawa et al., 1993; Xie et al., 1999) and C/EBP (Gutierrez et al., 2002; Zhang et al., 1996), Groucho/TLE (Javed et al., 2000; Javed et al., 2001; Levanon et al., 1998), HES and SMAD (Zaidi et al., 2002b; Zhang et al., 2000), Runx may facilitate recruitment of these factors to 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; Stein et al., 1996). Results from our laboratory indicate that the association of Runx transcription factors with the nuclear matrix is obligatory for activity (Choi et al., 2001; Zeng et al., 1998). 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.

Subnuclear targeting supports the integration of signaling pathways and execution of regulatory signals

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 permit 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 or repression of skeletal genes (Zaidi et al., 2002a; Zaidi et al., 2002b). Targeted mutations in the Runx nuclear matrix targeting signal have directly demonstrated that interactions of Runx with SMAD and YAP are sustained. However, execution of the BMP and Src regulatory signals are blocked. 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 Runx proteins serving as a scaffold for combinatorial interactions with coregulatory proteins that contribute to biological control and a requirement for intranuclear trafficking to complete the transduction and implementation of regulatory signals that are requisite for physiological responsiveness.

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 subnuclear localization for tissue differentiation by deleting the intranuclear targeting signal via homologous recombination. Mice homozygous for the deletion (Runx2DC) 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 Runx2DC 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) where substitution of intranuclear trafficking signals in translocation– fusion proteins redirects the regulatory factors to alternate subnuclear sites where target genes reside (Barseguian et al., 2002).

Mitotic partitioning and selective reorganization of tissue-specific transcription factor foci in progeny cells

Runx transcription factors provide a model for characterizing the distribution of regulatory proteins to progeny cells during mitosis. Runx proteins are organized as transcriptionally active subnuclear foci throughout the interphase nucleus that support Runx dependent integration of regulatory signals e.g., BMP and Src signals (Harrington et al., 2002; Zaidi et al., 2002b; Zaidi et al., 2003a). Post-mitotic gene expression requires restoration of nuclear organization and assembly of regulatory complexes. By the combined use of quantitative in situ immunofluorescence microscopy and quantitative image analysis, we have demonstrated that Runx foci persist throughout mitosis and undergo a spatio-temporal redistribution that results in equal partitioning of the protein into each of the progeny nuclei (Figure 2) (Zaidi et al., 2003b). Loss of both amount and subnuclear organization of Runx proteins is associated with genetic disorders (Choi et al., 2001; McNeil et al., 1999; Zhang et al., 2000). Equal partitioning and a complete restoration of subnuclear organization of Runx foci in telophase provides a mechanism for maintenance of cellular levels and activity of Runx proteins following mitosis. These findings are consistent with a requirement of Runx factors for post-mitotic transcriptional control and assembly of multi-component complexes to regulate Runx responsive genes. Furthermore, subnuclear organization of Runx foci precedes that of SC35 RNA processing speckles following cell division. Taken together, these findings demonstrate a spatio-temporal partitioning and reorganization of regulatory factors that render progeny cells equivalently competent for the resumption of tissue specific gene expression.

The assembly and organization of nuclear microenvironments that govern transcriptional control

Multiple lines of evidence suggest that components of nuclear architecture contribute both structurally and enzymatically to control gene expression during hematopoietic and 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 (Zaidi et al., 2003b). 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

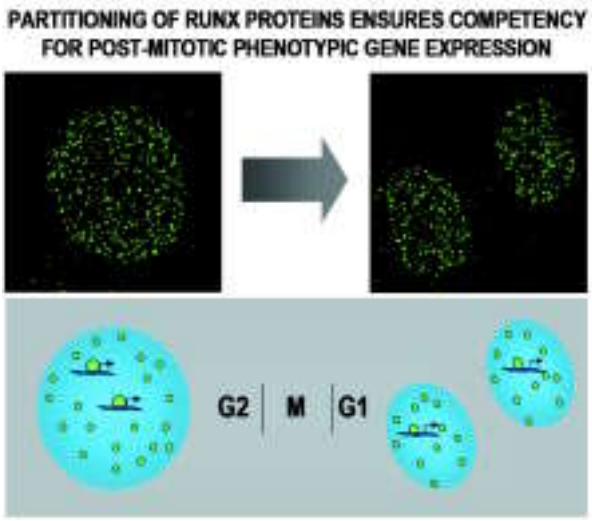

Figure 2. Partitioning of Runx proteins ensures competency for post-mitotic gene phenotypic gene expression Runx proteins are distributed at punctate subnuclear foci throughout the interphase and telophase nuclei, and partition equivalently in progeny cells following cell division. Progeny cells are equivalently competent to support phenotypic gene expression. ROS 17/2.8 osteosarcoma cells were subjected to in situ immunofluorescence microscopy. Runx2 proteins were immunolabeled with Alexa 488 fluorochrome.

sequences (Zeng et al., 1997; Zeng et al., 1998) and crystal structure for the 31 amino acid nuclear matrix targeting signal of Runx transcription factors (Tang et al., 1998) support 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 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 (Gasser, 2002; Lamond and Earnshaw, 1998; Misteli, 2000; Stein et al., 2000).

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.

The organization and assembly of regulatory machinery in subnuclear microenvironments where the combinatorial components of regulatory mechanisms are represented at threshold concentrations are not confined to AML-mediated transcriptional control. An analogous focal organization of nucleic acids and regulatory proteins within the nucleus is evident in Cajal bodies, PML bodies (Avni et al., 2003), ALL foci (Yano et al., 1997), as well as in sites of replication (Wei et al., 1998) and repair (Avni et al., 2003; Jackson, 2002; Petrini and Stracker, 2003). Recently accrued knowledge of nucleolar-mediated activities and expanded insight into the implications for intranuclear localization of chromosomal territories (Boyle et al., 2001; Croft et al., 1999; Gerlich et al., 2003; Parada and Misteli, 2002; Sun et al., 2000; Tanabe et al., 2002; Verschure et al., 1999) reinforces the relevance of nuclear organization to fidelity of regulatory mechanisms. The common denominator is that each is an example of multicomponent regulatory complexes that are functionally responsive to a broad spectrum of physiological signals. Perturbations in the composition, organization or intranuclear placement appear to be associated with aberrant biological control.

It is realistic to anticipate that further understanding of mechanisms that dynamically position genes and regulatory factors for establishment and maintenance of cell phenotypes will clarify nuclear structure-function interrelationships that are operative during differentiation and required for physiologically responsive modulation of regulatory activity.

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