Organization, Integration, and Assembly of Genetic and Epigenetic Regulatory Machinery in Nuclear Microenvironments

Implications for Biological Control in Cancer

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There is growing awareness that the fidelity of gene expression necessitates coordination of transcription factor metabolism and organization of genes and regulatory proteins within the three-dimensional context of nuclear architecture. The regulatory machinery that governs genetic and epigenetic control of gene expression is compartmentalized in nuclear microenvironments. Temporal and spatial parameters of regulatory complex organization and assembly are functionally linked to biological control and are compromised with the onset and progression of tumorigenesis. High throughput imaging of cells, tissues, and tumors, including live cell analysis, is expanding research's capabilities toward translating components of nuclear organization into novel strategies for cancer diagnosis and therapy.

Key words: **nuclear microenvironments; epigenetic control; transcription; nuclear matrix; chromatin; Runx; proteomics; genomics**

Introduction

There is growing appreciation that control of gene expression is combinatorial and related in an obligatory manner to localization within the nucleus. For several decades it has been recognized that parameters of biological control as well as the onset and progression of tumorigenesis are mediated by multicomponent regulatory complexes and cross-talk between activities that are independent gene promoter elements. And, with the development of high throughput genomic, proteomic, and bioinformatic approaches, the combinatorial underpinning of gene expression has been increasingly apparent. The emerging recognition for integrated networks where signaling pathways intersect reflects the convergence of queues that mediate the multidirectional flow and integration of regulatory information. Equally relevant from a regulatory perspective are longstanding

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observations that nuclear morphology and the content as well as arrangement of nucleic acids and proteins are modified in the nuclei of cancer cells. Such changes that are associated with transformation and tumor progression include fragmented nucleoli and chromosomal aberrations in solid tumors, as well as an increased representation of promyelocytic leukemia (PML) bodies and immature but altered nuclear structure in certain leukemias. Mechanistically, the perturbations in the architectural properties of cancer cell nuclei reflect modifications in the composition and organization of regulatory machinery for gene expression, replication, and repair that have the potential to support novel strategies for tumor diagnosis and therapy.

An Architectural Perspective of Gene Expression

Molecular Landscape of the Nucleus

Fidelity of gene expression necessitates integrating a broad spectrum of regulatory signals that govern proliferation, differentiation, and maintenance of cell and tissue phenotypes. To accommodate the requirements for short-term and sustained expression of cell growth and tissue-specific genes, it is necessary to identify and functionally characterize the promoter regulatory elements as well as cohorts of protein/DNA and protein/protein interactions that determine the extent to which genes are transcribed. However, it is becoming increasingly evident that the catalogue of regulatory elements and proteins is insufficient to support transcriptional control in the nucleus of intact cells in an *in vivo* environment. Rather, gene regulatory mechanisms must be understood in relation to the subnuclear organization of nucleic acids and regulatory proteins.

There is growing appreciation that transcriptional control requires multiple levels of nuclear organization. It is essential to package 2.5 yards of DNA as chromatin within the limited confines of the nucleus. Gene promoter elements must be rendered competent for protein/DNA and protein/protein interactions in a manner that permits binding and functional activities of primary transcription factors as well as co-activators and co-repressors. Less understood but pivotally relevant to physiologic control is the localization of the regulatory machinery for gene expression, replication, and repair at subnuclear sites where the macromolecular complexes that support DNA and RNA synthesis are localized (reviewed in Refs. 1–17).

While a formal definition of the mechanisms that govern gene expression is still absent, there is growing awareness that the fidelity of gene expression necessitates the coordination of transcription factor metabolism and the spatial organization of genes and regulatory proteins within the three-dimensional context of nuclear architecture. The components of nuclear organization include the sequence of gene regulatory elements, chromatin structure, and higher order organization of the transcriptional regulatory machinery in subnuclear domains. All of these parameters involve mechanisms that include transcription factor synthesis, nuclear import and retention (reviewed in 18–20), posttranslational modification of factors, and directing factors to subnuclear sites that support the organization[21,22] and assembly of regulatory machinery for gene expression. Remodeling of chromatin and nucleosome organization to accommodate requirements for protein/DNA and protein/protein interactions at promoter elements are essential modifications for both activation and suppression of genes and physiological control of transcription (reviewed in 23–27). This is a key component of epigenetic control that mediates competency for gene activation or suppression and conveys phenotype and lineage commitment to progeny cells during mitotic division. The reconfiguration of gene promoters and assembly of specialized subnuclear domains reflect the orchestration of both regulated and regulatory mechanisms. There are analogous and complex regulatory requirements for processing of gene transcripts. Here it has been similarly demonstrated that the regulatory components

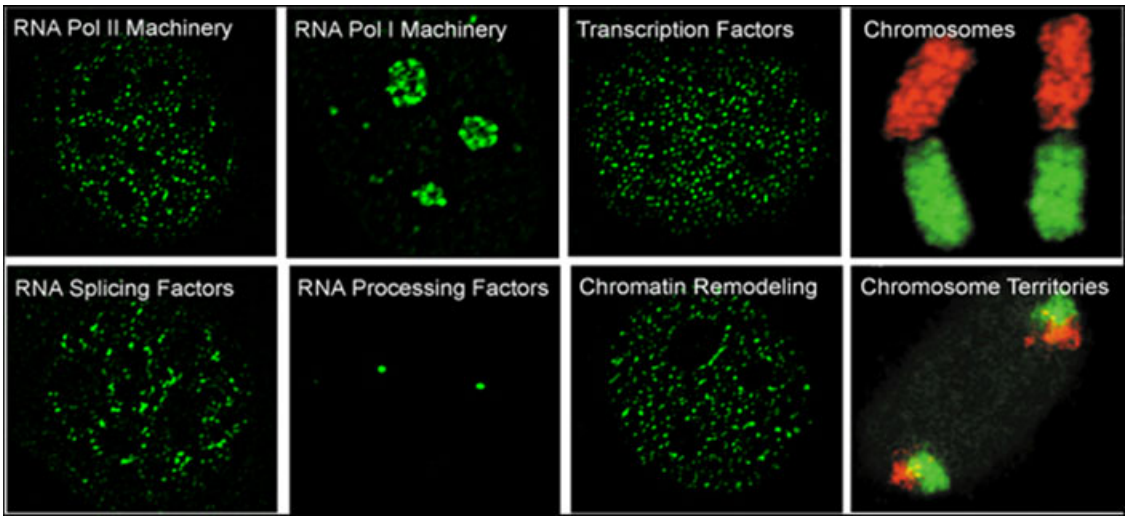

Figure 1. Nucleic acids and regulatory proteins are compartmentalized in nuclear microenvironments. Nuclear functions are organized into distinct, nonoverlapping subnuclear domains. The nuclear matrix, the underlying network of anastomizing network of filaments and fibers, provides a structural basis 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, chromatin remodeling, e.g., mediated by the SWI/SNF complex and Runx factors; structural parameters of the nucleus, such as the nuclear envelope, chromosomes, and chromosomal territories; Runx domains for transcriptional control of tissue-specific genes; and RNA synthesis and processing involving, e.g., transcription sites, SC35 domains, coiled bodies, and nucleoli. Subnuclear PML bodies of unknown function have been examined in numerous cell types. All these domains are associated with the nuclear matrix.

of splicing and export of messenger RNA to the cytoplasm are dependent on the architectural organization of nucleic acids and regulatory proteins.[28–30] There is growing evidence that the focal localization of regulatory machinery in nuclear microenvironments supports the integration of regulatory signals in a manner that facilitates competency for physiological responsiveness (reviewed in 1–15). The biological relevance of nuclear microenvironments is reflected by the punctate subnuclear localization of factors that mediate transcription, processing of gene transcripts, DNA replication, and DNA repair at discreet domains, and retention of regulatory factors with target gene promoters during mitosis to epigenetically maintain phenotype in progeny cells (Fig. 1).

From a biological perspective, each parameter of factor metabolism requires stringent control and must be linked to structure–function interrelationships that mediate transcription and processing of gene transcripts. However, rather than representing regulatory obstacles, the complexities of nuclear biochemistry and morphology provide the required specificity for physiological responsiveness to a broad spectrum of signaling pathways to modulate transcription under diverse circumstances. Equally important, evidence is accruing that modifications in nuclear architecture and nuclear structure–function interrelationships accompany and appear to be causally related to compromised gene expression under pathological conditions, particularly in cancer, providing a platform for novel dimensions to diagnosis and treatment.

Compartmentalization of Regulatory Machinery in Nuclear Microenvironments

Runx transcription factors provide a paradigm for the focal organization and

assembly of transcriptional regulatory machinery in nuclear microenvironments. These lineage-specific master regulatory proteins[31–39] control hematopoietic (Runx1), osteogenic (Runx2),[40–43] and gastrointestinal/neural (Runx3) differentiation at two levels of nuclear organization. Activity is mediated by interactions with multiple sites of target gene promoters where they strategically provide scaffolds for the recruitment and integration of regulatory signals (e.g., TGFβ, SRC), as well as the recruitment of histone-modifying enzymes and chromatin remodeling factors (e.g., HATs, HDAC, SWI/SNF) to influence promoter accessibility and placement of a broad spectrum of co-regulatory proteins that contribute to transcriptional activation and suppression. Relevance for promoter localization of Runx transcription factors has been provided by loss or decline of biological activity when promoter-binding sites of target genes are mutated or when functional domains of the Runx transcription factors are selectively mutated.[44] Gene expression within the three-dimensional context of nuclear architecture is additionally supported by the organization of Runx regulatory machinery in punctate intranuclear domains.[21,22,45] Here the necessity for fidelity of location within the nucleus is supported by the identification of a Runx-specific intranuclear targeting signal that is required for the execution of regulatory signals, Runx-dependent histone modifications, and chromatin remodeling and differentiation both *in vitro* and *in vivo*.[44,46–48]

Beyond the pivotal role for intranuclear organization of Runx regulatory complexes to support differentiation and development (e.g., osteogenesis and myeloid differentiation), there is a requirement for subnuclear localization of Runx proteins to initiate and sustain transformation and tumor progression. Localization of Runx2 within the nucleus is required for metastatic breast cancer and prostate cancer cells to form osteolytic lesions in bone,[49] and competency for Runx1 intranuclear trafficking is necessary for myeloid differentiation and mutations that prevent intranuclear localization of Runx1 in myeloid progenitor cells results in a leukemic phenotype.[50]

Despite the compelling evidence for a focal organization of regulatory machinery within the nucleus to support biological activity, as illustrated by Runx regulatory complexes, there are key parameters of control whose clarification is essential. The model for focal organization of factors to establish threshold concentrations for interactions with co-regulatory proteins and target genes remains to be formally demonstrated. Rate limiting constituents of regulatory complex formation must be determined. It is essential to discriminate between co-localization and functional interactions. Determinants for the turnover and modifications of components in regulatory complexes should be identified and characterized. The extent to which targeting and retention are the definitive determinants for focal formation and stability of regulatory domains is an open-ended question. The involvement of intranuclear trafficking and dynamic self-assembly in the organization and turnover of regulatory sites for gene expression should be further explored. Checkpoints that monitor the subnuclear distribution of regulatory factors and the sorting steps that ensure structural and functional fidelity of nuclear domains must be defined biochemically and mechanistically. However, there is growing support for informational content to organization of nuclear domains that is illustrated by the subnuclear organization of Runx regulatory machinery. Recently, mathematical algorithms designated "intranuclear informatics," have been developed to identify and assign unique quantitative signatures that define regulatory protein localization within the nucleus.[51] Quantitative parameters that can be assessed include nuclear size and variability in domain number, size, spatial randomness, and radial positioning.

The significance and implications of intranuclear informatics can be shown by three distinct biological examples: (1) Regulatory proteins with different activities can be subjected

to intranuclear informatics analysis, which assigns each protein a unique architectural signature. The overlap between the architectural signatures of different proteins is often correlated to their functional overlap. (2) Alternatively, the subnuclear organization of the protein domain can be linked with subnuclear targeting, biological function, and disease. For example, Runx2 and its subnuclear targeting defective mutant (mSTD) show distinct architectural signatures, indicating that the biological activity of a protein can be defined and quantified as subnuclear organization. (3) Finally, the data can be used to define functional conservation. For example, this technique can be used to show that the postmitotic restoration of the spatially ordered Runx subnuclear organization is functionally conserved. From the signatures that reflect regulatory protein localization within the nucleus and modifications that are associated with physiological responsiveness, transformation, and tumorigenesis, a quantitative basis is provided for defining phenotype and detection/diagnosis of disease. It is also realistic to incorporate such signatures in strategies for novel dimensions to therapy.

Transcription Factor Focal Organization and Integration of Regulatory Cues

The biological significance of focally organized regulatory complexes in nuclear microenvironments may reflect defined nuclear domains where threshold concentrations of regulatory factors for optimal formation of macromolecular complexes reside. The complexity of nuclear organization and nuclear structure–gene expression relationships ensures biological responsiveness. Each architecture-linked regulatory parameter is vulnerable to perturbations that can compromise control of cell growth, proliferation, and differentiation. However, each of these parameters is a potential target for therapy. An adjuvant therapeutic approach might be based on changes in radio- and chemosensitivity as a consequence of hypothermia-induced changes in the composition, assembly, and architectural organization of regulatory machinery within the cancer cell nucleus.[52] Challenges include: (i) methods of quantitative analysis that reproducibly capture subtle differences in subnuclear protein localization between normal and cancer cells; and (ii) development of small molecule inhibitors that specifically and selectively target components of nuclear organization that are perturbed during tumorigenesis.

These challenges can in part be overcome by an integrated biological approach. The heterogeneity of interactions that are supported by Runx transcription factors as scaffolding proteins serves as a basis for mechanisms that can accommodate diverse parameters of biological control. Architectural signatures that are derived from mathematical algorithms such as intranuclear informatics have the potential to discriminate between intranuclear localization of proteins in normal and cancer cells. Intranuclear informatics can be combined with proteomics (changes in protein–DNA and protein–protein interactions) and genomics (altered gene expression profiles) to develop a novel platform for identification and targeting of perturbed regulatory pathways in cancer cells (Fig. 2). The convergence and integration of signaling networks in nuclear microenvironments provides an architecturally based option for selectively targeting cancer-related changes in control of transcription, replication, and repair.

Architectural Parameters of Epigenetic Control

Runx Transcription Factors Contribute to Epigenetic Regulation

Runx proteins illustrate a key parameter of epigenetic control that supports physiological responsiveness. The location of Runx transcription factors at proximal and upstream sites of targeted gene promoters supports the

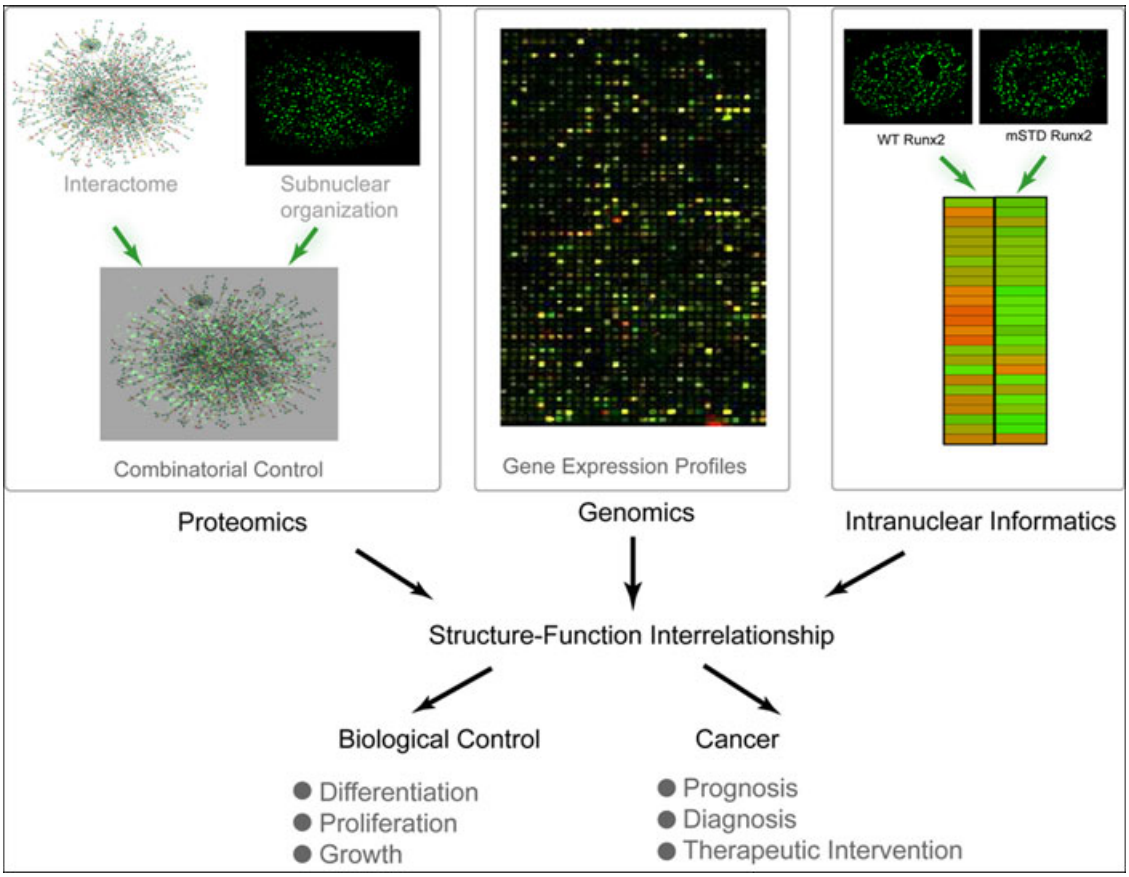

Figure 2. An integrated approach for mechanistic insights into nuclear structure–gene expression interrelationships. (*Left panel*) Knowledge of co-regulatory factors that interact with scaffolding proteins obtained by the combined application of cellular, biochemical, and molecular approaches provides a basis for developing an interactome for each scaffolding protein. Components of the interactome can be visualized, albeit to a limited extent, by *in situ* immunofluorescence microscopy. Such a "proteomics" approach provides mechanistic insight into combinatorial control of gene expression in normal and cancer cells. (*Middle panel*) In parallel, genome-wide profiles can be generated that reflect changes in gene expression during tumorigenesis ("genomics"). (*Right panel*) Importantly, newly developed mathematical algorithms can identify unique quantitative signatures for regulatory proteins ("intranuclear informatics") and apply subtle changes in these "architectural signatures" to distinguish normal proteins (e.g., WT Runx2) from subnuclear targeting deficient variants (e.g., mSTD Runx2). Such an integrated approach is necessary if we are to gain mechanistic insights into nuclear structure–gene expression interrelationships for biological control as well as for cancer diagnosis and treatment.

placement of histone-modifying and chromatin remodeling factors at regulatory domains, which control basal and enhancer-mediated activity.[44,47,48] Serving as scaffolds for assembling cohorts of regulatory factors that reconfigure chromatin organization and selectively modulate accessibility of promoter sequences to regulatory signals and proteins, an important component of biological control is provided based on a signature that does not depend on DNA sequences. This is an example of epigenetic regulatory information that establishes promoter landscape as architecturally assembled regulatory cues that can be conveyed to progeny cells during cell division. From a biological perspective such "epigenetic signatures" can sustain gene expression that establishes and ensures the persistence of phenotypes during

development and tissue remodeling. A basis is also provided to support transformation and tumor progression in a manner wherein the tumor phenotype is retained as the cell population expands and the disease progresses.

There has been an evolution in our appreciation for the informational content of epigenetic control. Initial approaches focused on the chromatin organization of candidate genes and the localization of enzymology for histone modifications in the proximity of sequences where chromatin structure supports a phenotype. Runx transcription factor interactions with basal, tissue defining, and upstream enhancer sequences of the bone-specific osteocalcin gene provide scaffolds for the placement of HATs and HDACs.[53,54] This mechanism supports epigenetic control by a master regulatory factor that is required for skeletogenesis and bone remodeling. Similarly, it is a requirement for Runx-mediated epigenetic control of skeletal genes in metastatic breast cancer and prostate cancer cells that are functionally linked to formation of osteolytic or osteoblastic lesions in bone.[55–57]

Recently, genome-wide profiling strategies have been developed that permit a global assessment of parameters for chromatin organization.[58,59] These global approaches provide complex but instructive signatures for epigenetic parameters of genome structure and organization. At the level of individual genes, the architectural context in which specific genes are embedded is revealed. Epigenetic control is not restricted to histone and chromatin signatures; DNA methylation is an additional, well-documented, component of epigenetic regulatory mechanisms.[60] As with histone modifications, genome-wide profiling has enhanced understanding of epigenetic control that is functionally linked to biological regulation as well as to a broad spectrum of diseases that include cancer. Beyond the insight into regulatory mechanisms that are supported by histone modifications and DNA methylation, these components of epigenetic control serve as a basis for tumor diagnosis. Equally as relevant, HDAC inhibitors and DNA methylation inhibitors are being effectively used for cancer chemotherapy.[60,61]

Mitotic Retention and Segregation of Transcriptional Regulatory Machinery

Postmitotic gene expression necessitates restoration of nuclear organization. Regulatory complexes must be assembled in progeny cells as they emerge from cell division. There is an immediate and stringent requirement for expression of cell cycle, cell growth, and phenotypic genes. Using the focal nuclear organization of Runx transcription factors as a paradigm, immunofluorescence microscopy has directly shown that Runx transcription factors are focally retained on mitotic chromosomes and partitioned to progeny cells.[62–65] The symmetrical localization of Runx transcription factors on mitotic chromosomes (Fig. 3) and confirmation by chromatin immunoprecipitation analysis indicate that Runx transcription factors remain associated with target genes as cells progress to mitosis.[62–64]

Consequently, the regulatory machinery for Runx control of gene expression remains in place during cell division, rendering genes competent to reinitiate a program of transcription postmitotically. The key question is the extent to which mitotic retention and segregation of regulatory proteins is a general regulatory mechanism. Several lines of evidence from gene expression profiling studies indicate mitotic retention of Runx transcription factors with more than 30 target gene promoters that are components of mechanisms that support multiple parameters of biological control.[62–64] Association of regulatory factors that include SP1,[66] C/EBP, TBP, and TTF2[67–69] with chromosomes and/or genes during mitosis establishes the generality of this mechanism as a component of epigenetic control beyond histone modifications and DNA methylation.

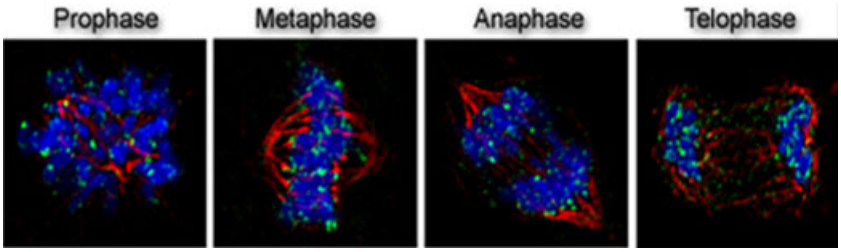

Figure 3. Phenotypic transcription factors associate with mitotic chromosomes to epigenetically convey necessary regulatory information to progeny cells for lineage maintenance and commitment. Phenotypic transcription factors that include Runx transcription factors are associated with mitotic chromosomes during cell division. This sequence-specific association of regulatory proteins with their target genes that regulate cell growth, proliferation, and differentiation bookmarks these genes for expression in the G1 phase of the cell cycle. Shown here are actively dividing human osteosarcoma SAOS-2 cells, immunostained with Tubulin (red) to identify mitotic spindle and Runx2 (green), which is a master regulator of bone formation. Cells are counterstained with DAPI (blue) to allow visualization of mitotic chromosomes. Top panels show all phases of mitosis.

Despite the compelling evidence for mitotic retention of transcription factors as a parameter of epigenetic control, there are numerous fundamental questions that must be resolved. How is association of transcription factors with target genes compatible with the global repression of genes during mitosis? Are transcription factors alone or transcription factors that are complexed with cohorts of co-regulatory proteins retained at target genes and conveyed to progeny cells? Are unique mechanisms in place to support association of transcription factors with target genes that are compatible with conformational properties of genes associated with chromatin condensation and decondensation during the entry and exit from mitosis? Are gene-associated regulatory proteins determinants for formation of interphase chromosomal territories? Resolution of these questions should reveal additional dimensions to nuclear structure–gene expression relationships that relate to epigenetic control.

Epigenetic Control Coordinates Regulation of Proliferation, Cell Growth, and Phenotype

Several lines of evidence support association of transcription factors and co-regulatory proteins with RNA polymerase I and polymerase II target genes during mitosis.[62–64] Involvement in epigenetic control of gene expression for cell fate and lineage commitment is suggested by mitotic retention of tissue-specific regulatory proteins with promoters that are functionally linked to the establishment and maintenance of cell phenotype.[62–65] In addition to mitotic retention of phenotypic genes, regulatory factors remain associated with genes that encode key components of signaling pathways, cell cycle control, and growth control.[62–64] Occupancy of ribosomal gene promoters with key regulatory factors indicates that a major component of the regulatory machinery for protein synthesis is poised for resumption of expression when cells emerge from mitosis.

Recent results implicate phenotypic transcription factors in epigenetically mediating coordinate regulation of proliferation, cell cycle, and growth control. The Runx2 skeletal transcription factor associates with promoters of genes that support tissue-specific gene expression and expression of cell cycle regulatory genes that are transcribed by RNA polymerase II.[62,70,71] In addition, Runx2 controls DNA polymerase I-mediated ribosomal gene transcription.[63] During mitosis, Runx2 resides at large discrete foci at nucleolar organizing regions where the ribosomal genes are located. The Runx2—UBF foci transition

to nucleoli at sites of ribosomal RNA synthesis during interphase (Fig. 3). Functional studies directly establish Runx control of ribosomal gene transcription and protein synthesis.[63] Similarly, the hematopoietic Runx1 and gastrointestinal/neural Runx3 transcription factors colocalize with ribosomal genes during mitosis and interphase to regulate protein synthesis. A similar mechanism is operative for control of ribosomal genes by MyoD during myogenesis and by C/EBP during adipogenesis.[65]

Interrelationships between epigenetic control of tissue-specific genes, cell cycle, and growth control appear to be operative in sustaining the transformed phenotype. The translocation of fusion protein AML/ETO associates with ribosomal genes during interphase and mitosis and contributes ribosomal gene expression and regulation of protein synthesis. Taken together, these findings are consistent with a critical molecular link between cell fate, proliferation, and growth control.

Nuclear Microenvironments in Biological Control and Cancer

The compartmentalization of regulatory machinery for gene expression is becoming increasingly evident. The focal organization of nucleic acids and regulatory proteins that support RNA polymerase I- and RNA polymerase II-mediated transcription during interphase and mitosis are consistent with an architectural basis for contributions of genetic and epigenetic control to lineage-specific coordination of cell cycle and cell growth, and phenotype regulation in nuclear microenvironments. What are the challenges and opportunities? Advances in technology for high throughput imaging of cells, tissues, and tumors, including live cell analysis, are expanding capabilities to translate components of nuclear organization to parameters of function. Temporal and spatial organization of regulatory machinery in nuclear microenvironments provides a view of mechanisms that support biological control. Equally relevant, these advances provide novel ways to understand those parameters of nuclear organization that are compromised in tumors. These resulting insights can serve as a basis for diagnosis and therapy.

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Conflicts of Interest

The authors declare no conflicts of interest.

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