MINIREVIEW / MINISYNTHÈSE

Transcription-factor-mediated epigenetic control of cell fate and lineage commitment[1]

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Abstract: Epigenetic control is required to maintain competency for the activation and suppression of genes during cell division. The association between regulatory proteins and target gene loci during mitosis is a parameter of the epigenetic control that sustains the transcriptional regulatory machinery that perpetuates gene-expression signatures in progeny cells. The mitotic retention of phenotypic regulatory factors with cell cycle, cell fate, and tissue-specific genes supports the coordinated control that governs the proliferation and differentiation of cell fate and lineage commitment.

Key words: cell cycle, nuclear microenvironment, gene expression, chromatin, Runx, intranuclear trafficking.

Résumé : Un niveau de contrôle épigénétique est requis afin de maintenir l'état d'activation ou de suppression des gènes lors de la division cellulaire. L'association de protéines régulatrices à des loci cibles lors de la mitose est un paramètre du contrôle épigénétique qui aide la machinerie régulatrice de la transcription à maintenir le profil d'expression génique chez les cellules filles. Le maintien, au cours de la mitose, des facteurs de régulation phénotypiques avec les gènes spécifiques du cycle cellulaire, de la destinée cellulaire et des tissus appuie un contrôle coordonné qui régit la prolifération et la différenciation en lien avec la destinée cellulaire et l'engagement vers une lignée.

Mots-clés : cycle cellulaire, microenvironnement nucléaire, expression génique, chromatine, Runx, trafic intranucléaire.

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Introduction

A fundamental process in biological control is the passage of regulatory information from parental to progeny cells, during mitosis, to support cell fate and lineage commitment. While the mitotic distribution of genes is mechanistically understood, there is a requirement for epigenetic control to establish and sustain the activation and (or) suppression of phenotypic genes during cell division. The traditional parameters of epigenetic control are DNA methylation and the post-translational modifications of histones. These non-genomic mechanisms convey regulatory cues that are configured as signatures, based on specificity dictated by DNA structure and (or) accessibility for protein–DNA and protein–protein interactions. MicroRNAs potentially provide another dimension to the epigenetic modulation of biological control. Recent results suggest that the association between regulatory proteins and target gene loci during mitosis is a parameter of epigenetic control that retains the regulatory machinery for transcriptional control to perpetuate the expression of genes that determine cell specialization and identity. We will focus on several lines of support for the mitotic persistence of phenotypic regulatory factors with cell cycle, cell growth, and tissue-specific genes within the context of the coordinated control of parameters that are required to govern proliferation and differentiation during development and tissue remodeling.

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Organization and association of regulatory machinery in nuclear microenvironments

Runt-related (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 (Zaidi et al. 2004, 2005, 2006, 2007; Stein et al. 2004, 2006; Li et al. 2005; Speck and Gilliland 2002; Galindo et al. 2005; Barseguian et al. 2002; McNeil et al. 1999; Ito et al. 2005; Durst and Hiebert 2004; Huang et al. 2008; Lian et al. 2004; Westendorf and Hiebert 1999) control hematopoietic (Runx1), osteogenic (Runx2), and gastrointestinal–neural (Runx3) differentiation at 2 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., transforming growth factor (TGF)β, SRC), and for the recruitment of histone-modifying enzymes and chromatin-remodeling factors (e.g., histone acetylases (HATs), histone deacetylases (HDACs), SWI/SNF), which influence promoter accessibility and the placement of a broad spectrum of coregulatory proteins that contribute to transcriptional activation and suppression. The relevance of the promoter localization of Runx transcription factors has been provided by the 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 (Gutierrez et al. 2004). Gene expression within the 3-dimensional context of nuclear architecture is additionally supported by the organization of Runx regulatory machinery in punctate intranuclear domains (Zeng et al. 1997, 1998; Zaidi et al. 2001). Here, the necessity for fidelity of location within the nucleus is supported by the identification of a Runx-specific intranuclear targeting signal, which is required for the execution of regulatory signals, Runx-dependent histone modifications and chromatin remodeling and differentiation both in vitro and in vivo (Gutierrez et al. 2004, 2007; Choi et al. 2001; Javed et al. 1999).

Beyond the pivotal role of the intranuclear organization of Runx regulatory complexes in supporting differentiation and development (e.g., osteogenesis and myeloid differentiation), subnuclear localization of Runx proteins is required 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 (Javed et al. 2005). Competency for Runx1 intranuclear trafficking is necessary for myeloid differentiation, and mutations that prevent intranuclear localization of Runx1 in myeloid progenitor cells result in a leukemic phenotype (Vradii et al. 2005).

Despite the compelling evidence of a focal organization of regulatory machinery within the nucleus that supports biological activity, as illustrated by Runx regulatory complexes, there are key parameters of control that must be clarified. The model of focal organization of factors that establish threshold concentrations for interactions with coregulatory 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 colocalization and functional interactions. Determinants of the turnover and modifications of the components of regulatory complexes should be identified and characterized. The extent to which targeting and retention are the definitive determinants of the focal formation and stability of regulatory domains is open ended. The involvement of intranuclear trafficking and dynamic self-assembly in the organization and turnover of the regulatory sites of 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 that organizes nuclear domains, illustrated by the subnuclear organization of Runx regulatory machinery.

Quantitative signatures for nuclear localization of 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 (Young et al. 2004). Quantitative parameters that can be assessed include nuclear size, and the variability in domain number, size, spatial randomness, and radial positioning. The significance and implications of intranuclear informatics can be shown with 3 distinct biological examples. First, 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. Second, the subnuclear organization of the protein domain can be linked to subnuclear targeting, biological function, and disease. For example, Runx2 and its subnuclear targeting defective mutant show distinct architectural signatures, indicating that the biological activity of a protein can be defined and quantified as subnuclear organization. Finally, the data can be used to define functional conservation. 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 and diagnosis of disease. It is also realistic to incorporate such signatures in strategies for novel dimensions of therapy.

The significance of focally organized regulatory complexes in nuclear microenvironments may reflect defined nuclear domains where threshold concentrations of regulatory factors for the optimal formation of macromolecular complexes reside. The complexity of nuclear organization can support biological responsiveness by mediating the convergence and integration of signaling networks. Architectural signatures that are derived from mathematical algorithms, such as intranuclear informatics, have the potential to discriminate the intranuclear localization of proteins that are associated with subtle changes in biological control. Intra-

nuclear informatics can be combined with proteomics (changes in protein–DNA and protein–protein interactions) and genomics (altered gene expression profiles) to attain comprehensive insight into the nuclear structure–gene expression relationships that relate to both biology and pathology.

Architectural parameters of epigenetic control

Runx proteins are a key parameter of epigenetic control that supports physiological responsiveness. The location of Runx transcription factors at the proximal and upstream sites of targeted gene promoters places histone-modifying and chromatin-remodeling factors at regulatory domains that control basal and enhancer-mediated activity (Gutierrez et al. 2004, 2007; Javed et al. 1999). An important component of biological control, which serves as a scaffold from which to assemble cohorts of regulatory factors that reconfigure chromatin organization and selectively modulate the accessibility of promoter sequences to regulatory signals and proteins, is based on a signature that does not depend on DNA sequences. This is an example of epigenetic regulatory information that establishes the promoter landscape as architecturally assembled regulatory cues, which can be conveyed to progeny cells during cell division. From a biological perspective, such "epigenetic signatures" can sustain the gene expression that establishes and ensures the persistence of phenotypes during development and tissue remodeling. Support is provided for transformation and tumor progression in a manner in which 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 (Westendorf et al. 2002; Yang et al. 2007). This mechanism supports epigenetic control by a master regulatory factor, which is required for skeletogenesis and bone remodeling. Similarly, there is a requirement for Runx-mediated epigenetic control of skeletal genes in metastatic breast cancer and prostate cancer cells, which are functionally linked to the formation of osteolytic or osteoblastic lesions in bone (Barnes et al. 2003, 2004; Pratap et al. 2005).

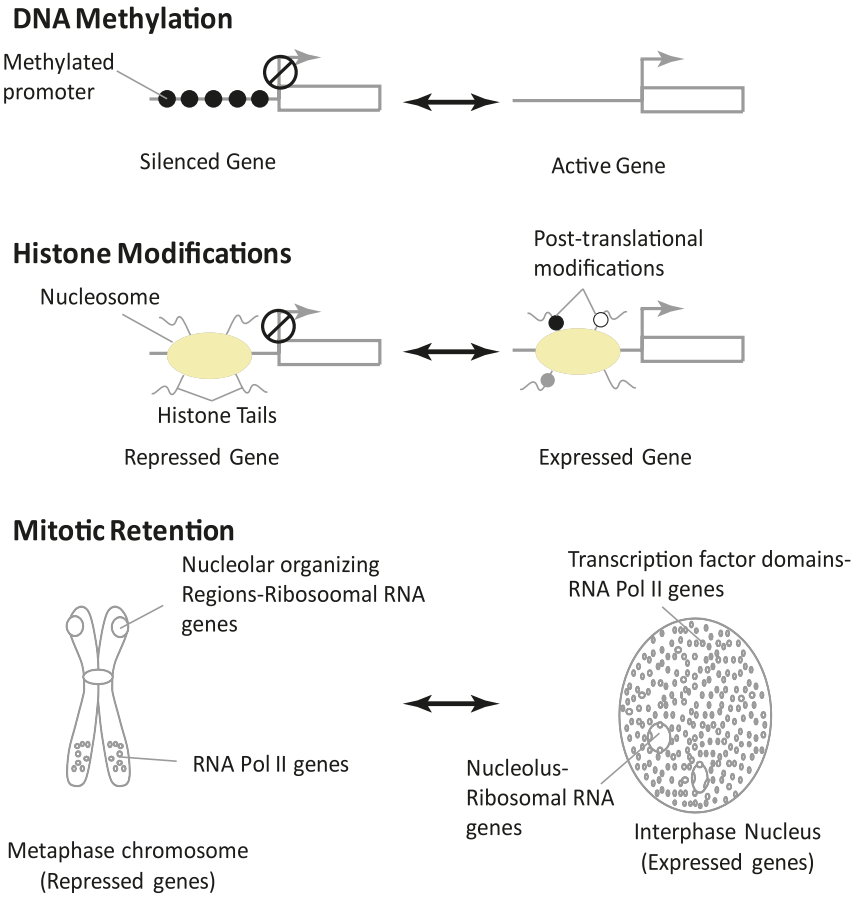
Recently, genome-wide profiling strategies have been developed, which permit a global assessment of parameters for chromatin organization (Liu et al. 2005; Hajkova et al. 2008). These global approaches provide complex but instructive signatures for the epigenetic parameters of genome structure and organization. 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 (Yoo and Jones 2006). As with histone modifications, genome-wide profiling has enhanced our understanding of the epigenetic control that is functionally linked to biological regulation, as well as to a broad spectrum of diseases that includes 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 relevant are the HDAC inhibitors and DNA methylation inhibitors that are being effectively used for cancer chemotherapy (Marks et al. 2004; Yoo and Jones 2006).

Mitotic retention and segregation of transcriptional regulatory machinery

Postmitotic gene expression necessitates the 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 the expression of cell cycle, cell growth, and phenotypic genes. Using the focal nuclear organization of Runx transcription factors as a "proof of principle," immunofluorescence microscopy has directly shown that Runx transcription factors are focally retained on mitotic chromosomes and partitioned to progeny cells (Zaidi et al. 2003; Young et al. 2007*a*, 2007*b*; Ali et al. 2008). The symmetrical localization of Runx transcription factors on mitotic chromosomes, confirmed by chromatin immunoprecipitation analysis, indicates that Runx transcription factors remain associated with target genes as cells progress to mitosis (Young et al. 2007*a*, 2007*b*). Consequently, the regulatory machinery for Runx control of gene expression remains in place during cell division, rendering genes competent to reinitiate a postmitotic program of transcription. 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 the mitotic retention of Runx transcription factors, with more than 30 target gene promoters, which are components of mechanisms that support multiple parameters of biological control (Young et al. 2007*b*). The association between regulatory factors, which include SP1 (He and Davie 2006), C/EBP, TBP, and TTF2 (Jiang et al. 2004; Segil et al. 1996; Tang et al. 2003), and chromosomes and (or) genes during mitosis establishes the generality of this mechanism as a component of epigenetic control, beyond histone modifications and DNA methylation.

Despite the compelling evidence that mitotic retention of transcription factors is a parameter of epigenetic control, there are numerous fundamental questions that must be resolved. How is the 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 coregulatory proteins retained at target genes and conveyed to progeny cells? Are unique mechanisms in place to support the association of transcription factors with target genes that are compatible with the conformational properties of genes, which are associated with chromatin condensation and decondensation during the entry and exit from mitosis? Are gene-associated regulatory proteins determinants of the formation of interphase chromosomal territories? Resolution of

Fig. 1. Mechanisms of the epigenetic maintenance of gene expression. Cells have adapted several mechanisms for epigenetically transmitting regulatory information from one to the next progeny. The DNA methylation of CpG islands that is present in several gene promoters is a well-studied and well-understood mechanism by which cells silence developmental genes, often irreversibly. Several tumor-suppressor genes have also been shown to have methylated promoter regulatory regions that result in the silencing of these genes and lead to cellular transformation. Similarly, post-translational modifications of the amino-terminal tails of nucleosomal histones play a pivotal role in the controlled regulation of gene expression. The "histone code" defines the transcriptional state of a gene, and allows the expression or suppression of the locus in a physiological manner. Recently, phenotypic transcription factors on nucleolar organizing regions (NORs–RNA Pol I-responsive genes) and elsewhere (RNA Pol II-responsive genes) on the mitotic chromosomes have presented a novel mechanism through which to epigenetically convey regulatory information from one progeny to the next for lineage commitment and maintenance.



these questions should reveal additional dimensions to the nuclear structure–gene expression relationships that relate to epigenetic control.

Transcription-factor-mediated epigenetic control coordinates regulation of proliferation, cell growth, and phenotype

Several lines of evidence support the association of transcription factors and coregulatory proteins with RNA polymerase I and RNA polymerase II target genes during mitosis (Zaidi et al. 2003; Young et al. 2007*a*, 2007*b*). Involvement in the epigenetic control of gene expression for cell fate and lineage commitment is suggested by the mitotic retention of tissue-specific regulatory proteins with promoters that are functionally linked to the establishment and maintenance of cell phenotype (Young et al. 2007*b*; Ali et al. 2008). In addition to the mitotic retention of phenotypic genes, regulatory factors remain associated with genes that encode key components of signaling pathways, cell cycle control, and growth control (Young et al. 2007*b*). The association between the occupancy of ribosomal gene promoters and key regulatory factors indicates that a major component of the regulatory machinery for protein synthesis is poised to resume expression when cells emerge from mitosis.

Recent results implicate phenotypic transcription factors in the epigenetically mediated coordination of the regulation of proliferation, cell cycle, and growth control. Runx2 skeletal transcription factors associates with promoters of genes that support tissue-specific gene expression and the expression of cell cycle regulatory genes, which are transcribed by RNA polymerase II (Zaidi et al. 2003). In addition, Runx2 controls DNA polymerase I-mediated ribosomal gene transcription (Young et al. 2007*a*). During mitosis, Runx2 resides at large discrete foci in 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. 1). Functional studies directly establish Runx control of ribosomal gene transcription and protein synthesis (Young et al. 2007*a*). Similarly, the hema-

topoietic Runx1 and gastrointestinal–neural Runx3 transcription factors colocalize with ribosomal genes during mitosis and interphase to regulate protein synthesis. A similar mechanism operates in the control of ribosomal genes by MyoD during myogenesis, and by C/EBP during adipogenesis (Ali et al. 2008).

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

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