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Nuclear Microenvironments Support Assembly and Organization of the Transcriptional Regulatory Machinery for Cell Proliferation and Differentiation

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Abstract The temporal and spatial organization of transcriptional regulatory machinery provides microenvironments within the nucleus where threshold concentrations of genes and cognate factors facilitate functional interactions. Conventional biochemical, molecular, and in vivo genetic approaches, together with high throughput genomic and proteomic analysis are rapidly expanding our database of regulatory macromolecules and signaling pathways that are requisite for control of genes that govern proliferation and differentiation. There is accruing insight into the architectural organization of regulatory machinery for gene expression that suggests signatures for biological control. Localized scaffolding of regulatory macromolecules at strategic promoter sites and focal compartmentalization of genes, transcripts, and regulatory factors within intranuclear microenvironments provides an infrastructure for combinatorial control of transcription that is operative within the three dimensional context of nuclear architecture. J. Cell. Biochem. 91: 287–302, 2004.

Key words: transcription; nuclear architecture; regulatory factors; gene expression; proliferation/differentiation; signaling pathways

Development and tissue remodeling require stringently regulated expression of cell growth and phenotypic genes to support proliferation and differentiation. Consequently, there is a necessity for the physiologically responsive activation and suppression of target genes to sustain biological control. As complexities of the regulatory mechanisms for gene expression rapidly accrue through genomic and proteomic approaches, the importance to understand the assembly and organization of regulatory complexes within the nucleus emerges. Using cell cycle regulated histone genes [Stein et al., 1975; Lichtler et al., 1982; Pauli et al., 1987; van Wijnen et al., 1988, 1992, 1994, 1996; Holthuis et al., 1990; Ramsey-Ewing et al., 1994; Vaughan et al., 1995, 1998; Xie et al., 2002a] and the bone specific osteocalcin gene [Lian et al., 1989; Stein and Lian, 1995; Lian and Stein, 2003a,b] as paradigms for transcription that occurs sequentially and mutually exclusively during development and tissue renewal (Fig. 1), we will present an overview of evidence that the regulatory machinery for combinatorial control of gene expression is organized in architecturally associated nuclear microenvironments (reviewed in Stein et al., 2000a,b, 2003).

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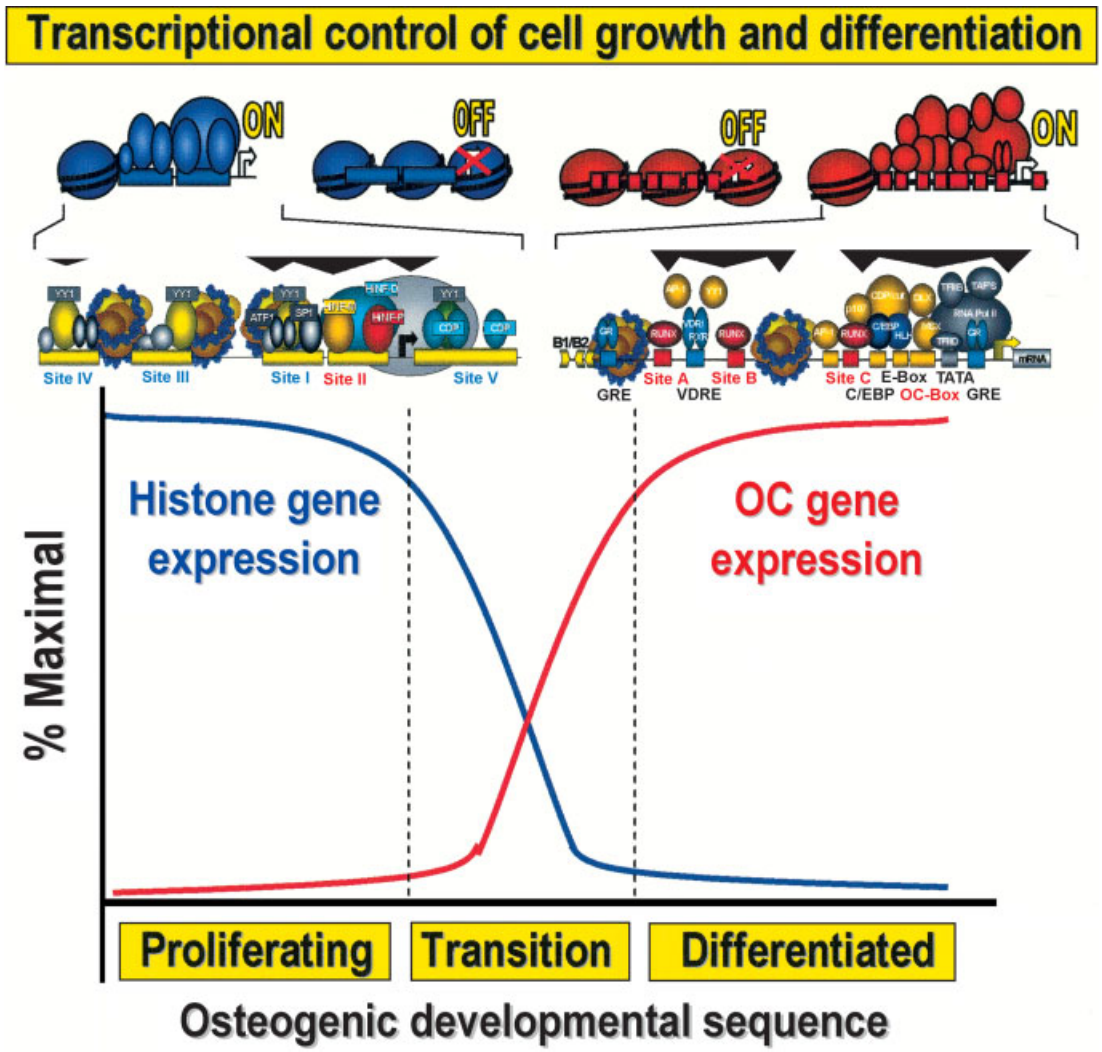

Fig. 1. Transcriptional control during cell growth and differentiation of osteoblasts. Osteoblast differentiation is characterized by a developmental switch (**top portion**) in which transcription of proliferation-specific genes (e.g., histones) is silenced and bone-tissue specific genes (e.g., osteocalcin) are activated. The histone and osteocalcin genes are each reversibly associated with a distinct set of gene-selective transcription factors (**middle portion**). The sequential expression of genes during osteoblast phenotype development is reflected by the reciprocal regulation of histone and osteocalcin gene expression as osteoblasts progress through the proliferative phase, the post-proliferative transition, and the differentiated stage (**bottom portion**). This developmental sequence has been well-characterized in primary osteogenic progenitor cells isolated from rodent calvaria. These calvarial osteoblasts undergo a multi-stage developmental sequence that recapitulates cellular events, that normally occur during the formation of the membranous bones of the cranium.

exploring novel options for pursuing regulatory parameters that mediate subcellular localization of components for transcriptional control.

COMPARTMENTALIZATION OF GENE REGULATORY MACHINERY

There is growing recognition that the temporal/spatial organization of nucleic acids and regulatory proteins within the nucleus provides a structural and functional infrastructure for transcriptional activation and suppression (reviewed in Penman, 1995; Htun et al., 1996; Lamond and Earnshaw, 1998; Wei et al., 1998; Misteli, 2000; Stein et al., 2000a,b, 2003; Gasser, 2002; DeFranco, 2002a).

Orchestration of regulatory mechanisms that are essential for biological control of gene expression requires complex enzymology that is functionally assembled at strategic promoter sites. The common denominators of combinatorial mechanisms that govern transcription

under a broad spectrum of biological circumstances include: (1) dynamic modifications in the composition, representation, and organization of multiprotein complexes which mediate acetylation, methylation, phosphorylation, poly ADP ribosylation, and ubiquitination of histones as well as other structural and regulatory proteins together with DNA methylation and protein turnover; (2) promoter regulatory element accessibility that is associated with competency for protein–DNA interactions; (3) scaffold proteins that interact with promoter sequences to architecturally organize regulatory factors that mediate nucleosome placement, chromatin organization as well as transcriptional activation, enhancement, and repression; (4) threshold concentrations of regulatory proteins for modulation of gene expression that are focally localized at intranuclear domains; and (5) the intranuclear trafficking of regulatory proteins to sites within the nucleus where the machinery for gene expression is assembled.

Threshold and combinatorial requirements of transcriptional control are accommodated by intranuclear compartmentalization of genes, and regulatory complexes. Such subnuclear microenvironments contain the machinery for transcription [Grande et al., 1997; Jackson, 1997; Zeng et al., 1997, 1998; Wei et al., 1998; McNeil et al., 1999; Javed et al., 2000; Harrington et al., 2002; Johnson et al., 2003] as well as DNA replication [Leonhardt and Cardoso, 1995; Cardoso et al., 1997; Cardoso and Leonhardt, 1998; Ma et al., 1998; Wei et al., 1998] and repair [Avni et al., 2003]. From an historical perspective, compartmentalization of the regulatory machinery for ribosomal genes in nucleoli and the organization of chromosomes during mitosis have provided platforms for conceptually and experimentally addressing intranuclear localization of genes and transcription complexes (recently reviewed by Stein et al., 2003).

During the past several years there has been growing recognition that the organization of nucleic acids and regulatory proteins is functionally linked to the assembly, organization, and activity of gene regulatory machinery (reviewed in Berezney et al., 1996; Htun et al., 1996; Stein and Berezney, 1996; Misteli, 2000; Stein et al., 2000a,b, 2003; DeFranco, 2002b). Cellular, molecular, biochemical, and genetic evidence indicate an obligatory relationship between sites within the nucleus where regulatory complexes reside and fidelity of transcriptional control. The biological relevance for the intranuclear distribution of regulatory complexes is directly reflected by aberrant nuclear structure-gene expression interrelationships that are associated with perturbations in skeletal development [Choi et al., 2001] and leukemia [Rogaia et al., 1997; Yano et al., 1997; McNeil et al., 1999; Stein et al., 2000a, 2003; Gordon et al., 2000; Barseguian et al., 2002; Nakamura et al., 2002], breast cancer [Townson et al., 2003], and colon cancer [Joslyn et al., 1993].

SCAFFOLDING OF REGULATORY COMPONENTS FOR COMBINATORIAL CONTROL OF TISSUE-SPECIFIC GENE EXPRESSION

Functional interrelationships between nuclear structure and gene expression are strikingly reflected by dual recognition of regulatory proteins, such as the hematopoietic and osteogenic 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 [Guo et al., 1997; Lindenmuth et al., 1997; Westendorf and Hiebert, 1999; Yagi et al., 1999a; Javed et al., 2000; Jakubowiak et al., 2000a; Hiebert et al., 2001; Wang and Hiebert, 2001; Zaidi et al., 2001, 2002; Gutierrez et al., 2002; Westendorf et al., 2002]. 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 [Westendorf and Hiebert, 1999; Javed et al., 2000; Shen et al., 2002, 2003; Westendorf et al., 2002] and genomic conformations that interface activities at proximal and upstream promoter domains [Guo et al., 1995, 1997], as well as the integration of regulatory cues from signaling pathways that activate or suppress gene expression in a physiologically responsive manner [Yagi et al., 1999b; Jakubowiak et al., 2000b; Zaidi et al., 2001, 2002]. 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.

Contributions of p300/CBP to 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 results 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; Yang et al., 1996; Chen et al., 1997; Spencer et al., 1997; Torchia et al., 1997]. 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 support assembly of multi-subunit complexes with several HAT-containing proteins at a series of regulatory regions of the bone-specific osteocalcin gene promoter. Figure 2 schematically illustrates the dynamic RUNX-mediated modifications in chromatin organization of the osteocalcin gene promoter during osteoblast differentiation that are functionally linked to developmental and steroid hormone-responsive control of expression. 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

Fig. 2. **A**: Dynamic integration of physiological signals at the bone-related osteocalcin gene promoter. The chromatin organization and molecular architecture of the osteocalcin (OC) promoter are dynamically adjusted in response to physiological cues as the endpoint of transduction cascades, that control the activities of transcription factors. When the OC gene is inactive in immature proliferating osteoblasts, regulatory elements that control transcription of the OC gene are sequestered in randomly positioned nucleosomes in a conformation that restricts access to transcription factors and coregulatory proteins (first row). During the post-proliferative transition period, the chromatin structure of the OC locus changes into a transcriptionally competent, open state by the recruitment of Runx2 and associated chromatin remodeling factors (not indicated). This open chromatin state is reflected by two nuclease hypersensitive sites that flank a positioned nucleosome and a low level of histone acetylation (Ac). However, the gene remains in a latent, attenuated transcriptional state through a series of inhibitory gene regulators (glucocorticoid receptor [GR], YY1, the Msx2, and CDP/cut homeodomain proteins, as well as the retinoblastoma-related protein p107) (second row). The tissue-specific induction of OC gene transcription in mature osteoblasts is mediated by bone-related synergistic transcriptional activation events involving Runx2 proteins with AP1 (Fra2/JunD), C/EBP (β/δ), Dlx5, and HLH proteins) (Third row, left). These events occur concomitant with the recruitment of general transcription factors (TFIID, TFIIB, and TAFs) and histone acetyl transferases, as well as with increased nuclease hypersensitivity and acetylation of histones. Extracellular cues that signal a reduced physiological demand for osteocalcin biosynthesis (e.g., c-scr signaling) results in the recruitment of co-repressors (e.g, YAP) that interact with Runx2 and support the activities of histone deacetylases (HDACs) which reduce acetylation of histone at the OC locus (third row, right). Maximal activation of OC gene expression is observed in differentiated osteoblasts upon induction of VDR/RXR heterodimers that synergize with VDR dependent co-activators and Runx2 (fourth row). Vitamin D enhanced transcription is reflected by increased nuclease hypersensitivity and hyperacetylation of histones. **B**: Spatial integration of physiological signals and cross-talk between distal vitamin D responsive and proximal bone-tissue related elements. The presence of a positioned nucleosome in the OC promoter supports the formation of protein/protein bridges between bone tissue-specific proximal promoter elements and distal vitamin D responsive enhancer elements. Under basal conditions (**top portion**), transient protein/protein interactions may be formed between YY1 (distal) and TFIIB (proximal) proteins, while YY1 forms complexes with HDACs to maintain a hypo-methylated chromatin conformation. When osteoblasts respond to vitamin D, distal and proximal protein/protein bridges are altered, the OC locus becomes hyper-acetylated, and VDR/RXR heterodimers (including the cognate co-factors and distinct HATs) are recruited to the OC promoter to increase transcription (**bottom portion**). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

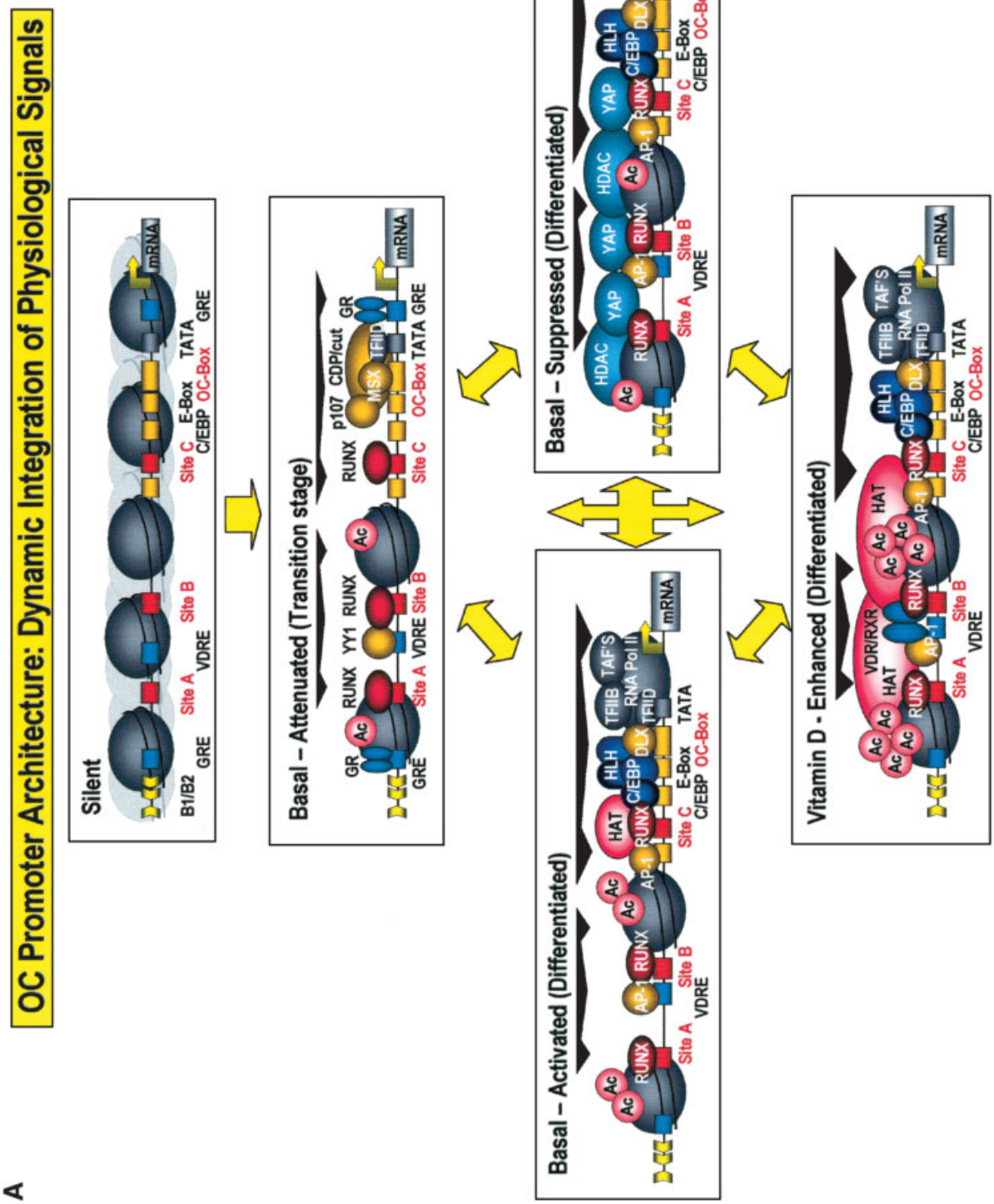
A
OC Promoter Architecture: Dynamic Integration of Physiological Signals
Silent
B1/B2
GRE
Site A
VDRE
Site B
Site C
C/EBP
E-Box
OC-Box
TATA
GRE
mRNA
Basal – Attenuated (Transition stage)
GR
RUNX
YY1
RUNX
p107
CDP/cut
RUNX
MSX
GR
TFIID
Ac
GRE
Site A
VDRE
Site B
Site C
OC-Box
TATA
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Fig. 2.

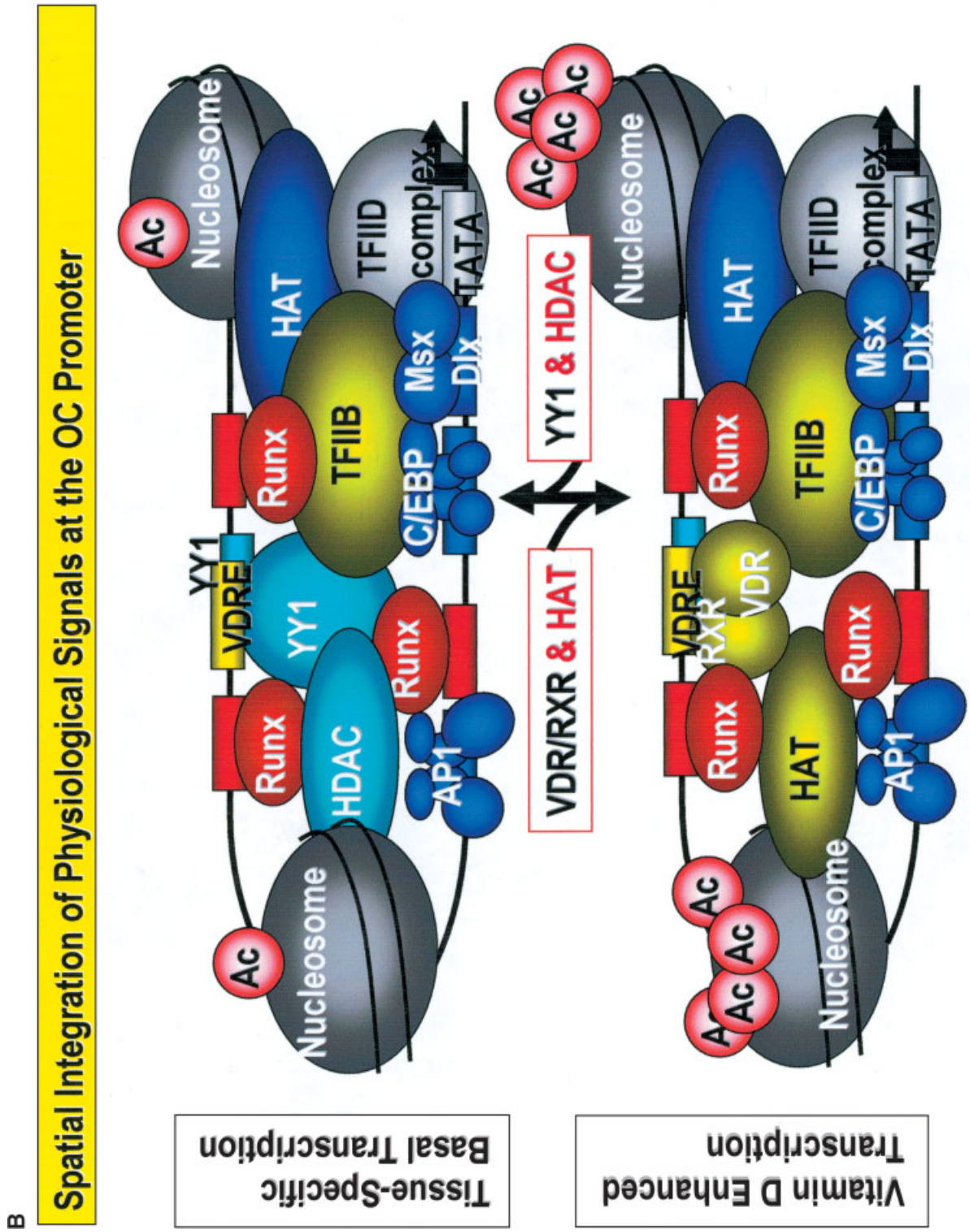

Fig. 2. (*Continued*)

[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.

Runx-Mediated Combinatorial Transcriptional Suppression

In addition to functioning as transcriptional activators, RUNX proteins suppress gene expression. Repression requires the recruitment of transcriptional repressors and corepressors with histone deacetylase activity (HDACs) to promoter 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 osteocalcin 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 corepressor proteins that are associated with and organized as multiprotein complexes to activate or repress target genes in a physiologically responsive manner.

Contributions of C/EBP to Runx-Mediated Combinatorial Control

p300 can also be recruited to gene promoters by the transcription factor C/EBP [Oelgeschlager et al., 1996; Mink et al., 1997]. 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/EBPβ 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/EBPβ 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.

Runx Proteins are Architectural Scaffolds That Provide a Biochemical Basis for Physiologically Responsive Configuration of Promoter Elements and Combinatorial Specificity for Transcription

Taken together 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 multisubunit 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 architecturally mediated mechanism 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 REGULATORY FACTORS TO SUBNUCLEAR SITES THAT SUPPORT TRANSCRIPTION

There is a need to gain insight into mechanisms that direct factors to subnuclear sites where regulatory events occur. 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 transcriptionally active subnuclear domains [Bae et al.,

1993; Meyers et al., 1993, 1995, 1996; Wang et al., 1993; Nuchprayoon et al., 1994; Frank et al., 1995; Merriman et al., 1995; Satake et al., 1995; Banerjee et al., 1996, 1997; Ducy et al., 1997; 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., 2002; Zeng et al., 1997, 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 [Dworetzky et al., 1992; Bidwell et al., 1993; van Wijnen et al., 1993; Blencowe et al., 1994; Mancini et al., 1994; Nickerson et al., 1995; 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 [Zeng et al., 1997; Choi et al., 1999; Javed et al., 2000; Zaidi et al., 2002]. 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 [Tang et al., 1998; Javed et al., 2000; Harrington et al., 2002]. 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. A series of deletions and internal mutations was constructed and assayed for competency to associate with the nuclear matrix by Western blot analysis of biochemically prepared nuclear fractions and by in situ immuno staining following transfection into intact cells. 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 [Ogawa et al., 1993; Giese et al., 1995; Banerjee et al., 1996; Mao et al., 1999; Xie et al., 1999; Kundu et al., 2002; Miller et al., 2002] and C/EBP [Zhang et al., 1996; Gutierrez et al., 2002], Groucho/TLE [Levanon et al., 1998; Javed et al., 2000, 2001], HES, and SMAD [Zhang et al., 2000; Zaidi et al., 2002], RUNX may facilitate recruitment of these factors to the nuclear matrix.

Properties of Transcriptionally Active Subnuclear Compartments

Association of genes and cognate factors with the nuclear matrix may support the formation and/or activities of nuclear domains that facilitate transcriptional control [Guo et al., 1995; Merriman et al., 1995; Nickerson et al., 1995; Berezney et al., 1996; Chen et al., 1996; Nardozza et al., 1996; Stein et al., 1996; Alvarez et al., 1997; Davie, 1997; Grande et al., 1997; Jackson, 1997; Lindenmuth et al., 1997]. Results from our laboratory indicate that the association of RUNX transcription factors with the nuclear matrix is obligatory for activity [Zeng et al., 1998; Choi et al., 2001]. 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. An initial indication of transcription factor interactions with the nuclear matrix is provided by crystal structure of the RUNX nuclear matrix targeting signal that was determined by X-ray diffraction analysis at 2.7 A [Tang et al., 1998, 1999].

Subnuclear Targeting and Integration of Signaling Pathways

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/TGFb-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 of skeletal genes [Zaidi et al., 2002]. 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 proteins serving as a scaffold for interactions with coregulatory proteins that contribute to biological control.

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 fidelity of subnuclear localization for tissue differentiating activity by deleting the intranuclear targeting signal via homologous recombination. Mice homozygous for the deletion (RUNX2ΔC) 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 RUNX2ΔC 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].

ARCHITECTURAL ORGANIZATION OF REGULATORY PROTEINS FOR COMBINATORIAL CONTROL OF CELL CYCLE PROGRESSION

Histone gene expression at the G1/S phase cell cycle transition is obligatory for DNA replication. Expression is responsive to regulatory signals that are initiated at the R point by CDK2/cyclin E activation of NPAT and culminates in NPAT upregulation of histone gene

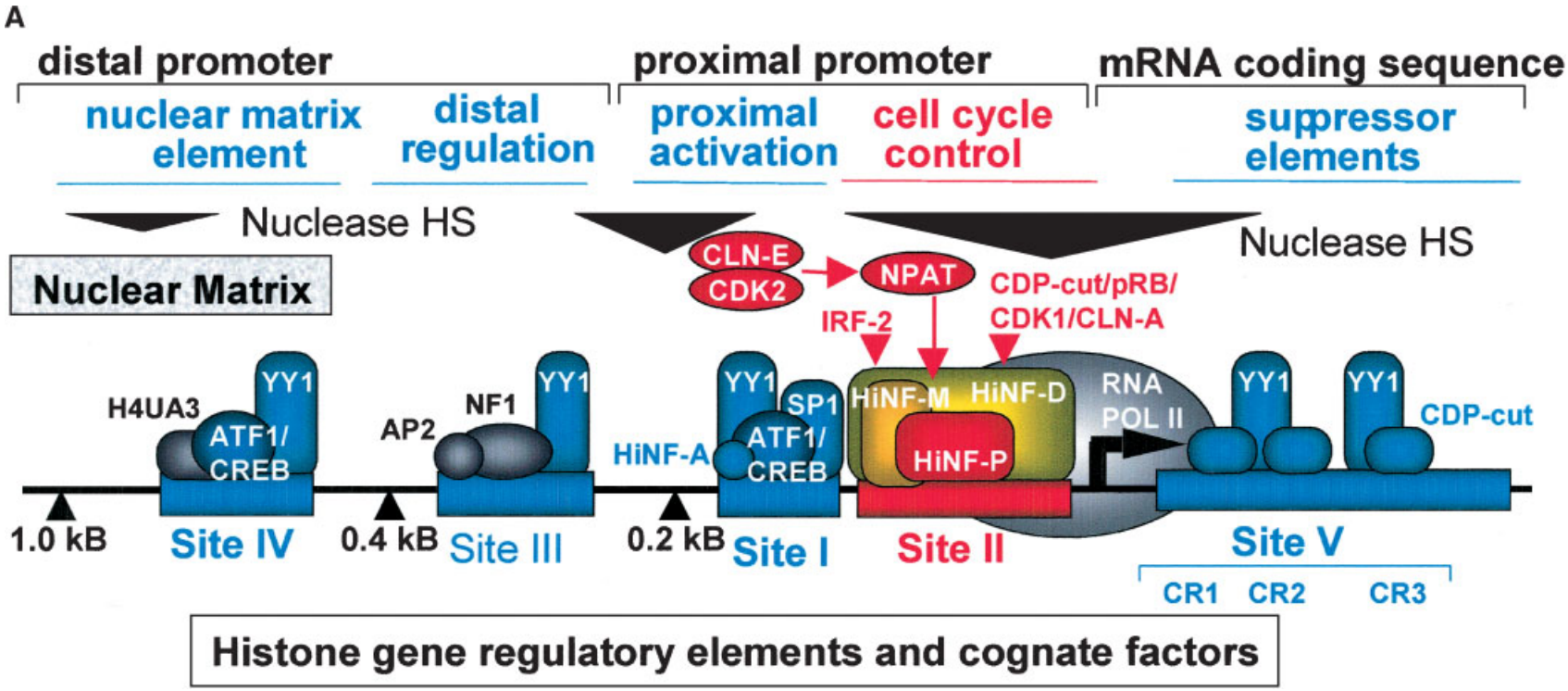

Fig. 3. **A**: Organization of the histone H4 gene promoter. H4 gene transcription is controlled by multiple regulatory factors and cognate elements (e.g., SP1, YY1, ATF1). The principal regulatory domain that mediates H4 gene activation at the G1/S phase transition is Site II, which interacts with factors HiNF-P, HiNF-D (CDP/pRB/CDK1/CLN-A comlex) and HiNF-M (IRF-2). HiNF-P activates H4 gene transcription together with its co-activator NPAT in response to the CDK2/cyclin E dependent phosphorylation of NPAT. **B**: Transcriptional control during the cell cycle. Control of cell growth requires the cell cycle stage specific expression of genes. At the Restriction (R) point, the growth factor-dependent activation of cyclin-dependent kinases (e.g., cyclin E/CDK2), which disrupt the inhibitory interaction between newly phosphorylated pRB and E2F, culminates in the activation of E2F responsive target genes. At the G1/S phase transition, cyclin E/CDK2 phosphorylates NPAT, another principal CDK2 substrate that functions as a co-activator of histone gene transcription factor HiNF-P to control H4 gene transcription. Concomitantly, hyperphosphorylated pRB remains competent to form a complex with the CDP-cut homeodomain protein to attenuate S phase specific transcription. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

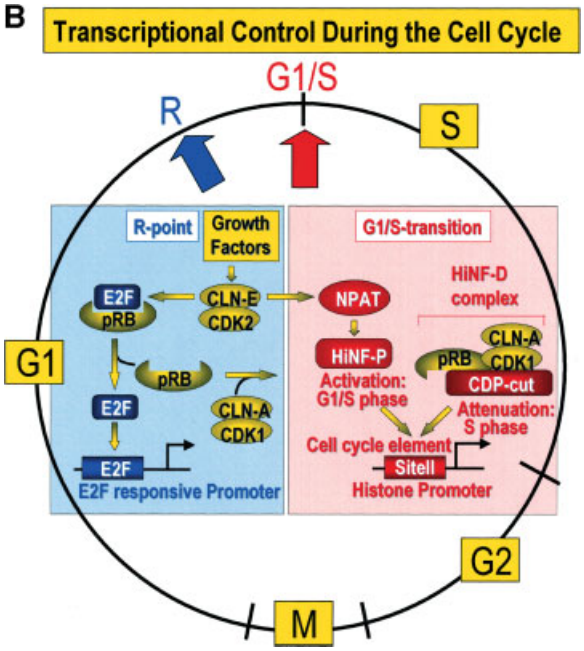

Fig. 3. (*Continued*)

transcription that is coupled with DNA replication by an E2F-independent, cyclin A/CDK1 mechanism [Mitra et al., 2003]. The element of the H4 histone gene promoter that controls transcription at the onset of S phase [Lichtler et al., 1982; Green et al., 1984; Pauli et al., 1987; Ramsey-Ewing et al., 1994; Stein et al., 2000a] is regulated by three multipartite complexes (designated HiNF-D [van Wijnen et al., 1992, 1994], HiNF-P [Mitra et al., 2003], and HiNF-M [Vaughan et al., 1995; Xie et al., 2002b; Stein et al., 2003]) that collectively, through combinatorial mechanisms, determine the extent to which the H4 histone gene is transcribed (Fig. 3). Consistent with functional interrelationships between nuclear organization and regulation of gene expression, the chromatin organization of the histone gene is remodeled in a cell cycle and cell growth-dependent manner to render promoter elements competent for protein–DNA and protein–protein interactions that support the integration and execution of signals associated with licensing of cell cycle progression. Histone genes are associated with coiled bodies (Cajal bodies) [Shopland et al., 2001] during S phase providing an architectural basis for cell cycle-dependent organization of the transcriptional regulatory machinery for localization of sites within the nucleus where microenvironments facilitate temporal as well as spatial responsiveness that is requisite for fidelity of expression at the G1/S phase boundary.

NUCLEAR MICROENVIRONMENTS ACCOMMODATE THE RULES THAT GOVERN COMBINATORIAL CONTROL

Demands on the genome to accommodate biological control in vivo during development, to sustain phenotype-restricted function and to support tissue remodeling, require competency for selective expression of cell growth and phenotypic genes in a physiologically responsive manner. The temporal and spatial organization of the transcriptional regulatory machinery provides microenvironments within the nucleus where threshold concentrations of genes and cognate factors facilitate functional interactions. The conservation and partitioning of Runx proteins and foci during mitosis is consistent with competency for post mitotic assembly of regulatory complexes for immediate resumption of phenotype-specific gene expression in progeny cells [Zaidi et al., 2003]. Conventional biochemical, molecular, and in vivo genetic approaches, together with high throughput genomic and proteomic analyses are rapidly expanding our database of regulatory macromolecules and signaling pathways that are requisite for control of genes that govern proliferation and differentiation. However, there is accruing insight into the architectural organization of regulatory machinery for gene expression that suggests signatures for biological control. Localized scaffolding of regulatory macromolecules at strategic promoter sites and focal compartmentalization of genes, transcripts and regulatory factors within intranuclear microenvironments provides an infrastructure for combinatorial control of transcription that is operative within the three dimensional context of nuclear architecture.

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