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Histone Gene Transcription: A Model for Responsiveness to an Integrated Series of Regulatory Signals Mediating Cell Cycle Control and Proliferation/Differentiation Interrelationships

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Abstract Histone gene expression is restricted to the S-phase of the cell cycle. Control is at multiple levels and is mediated by the integration of regulatory signals in response to cell cycle progression and the onset of differentiation. The H4 gene promoter is organized into a series of independent and overlapping regulatory elements which exhibit selective, phosphorylation-dependent interactions with multiple transactivation factors. The three-dimensional organization of the promoter and, in particular, its chromatin structure, nucleosome organization, and interactions with the nuclear matrix may contribute to interrelationships of activities at multiple promoter elements. Molecular mechanisms are discussed that may participate in the coordinate expression of S-phase-specific core and H1 histone genes, together with other genes functionally coupled with DNA replication. © 1994 Wiley-Liss, Inc.

Key words: cell cycle control, histone gene expression, S-phase, regulatory signals, differentiation

INTRODUCTION

Histone protein synthesis is restricted to the S-phase of the cell cycle supporting the requirement of histone proteins for the ordered assembly of newly replicated DNA into nucleosomes and higher order chromatin structure within the nucleus. Molecular mechanisms associated with regulation of histone gene expression cannot be defined as isolated components of gene regulation. Rather, when understood within the context of the integrated cascade of events that are associated with growth control, subtle parameters operative in transcription of histone genes provide insight into the diverse physiological regulatory mechanisms mediating responsiveness to a broad spectrum of cellular requirements for stringent control of the proliferative process. [Pardee, 1989; Stein and Lian, 1992; Hofbauer and Denhardt, 1991; Zambetti et al., 1991; Baserga and Rubin, 1993].

This prospect is not inclusive and for a more comprehensive treatment of histone gene regulation the reader is directed towards several excellent reviews [Stein et al., 1984; Marzluff et al., 1988; Osley et al., 1991; Heintz et al., 1991]. Instead, we will focus on the contributions of activities at a series of promoter regulatory elements to transcription of the cell cycle regulated histone genes. Emphasis will be on the relationships between gene organization and biological function, where sequence-specific interactions of cognate transcription factors with multiple regulatory elements of the modularly organized histone gene promoter accompany and/or are functionally related to cell cycle progression and the onset of differentiation. Additionally, the participation of nuclear architecture in regulating the transcriptional properties of the histone genes will be considered. We will address the involvement of modifications in chromatin structure, nucleosome organization, and association of histone genes with the nuclear matrix, as a means of integrating activities at independent promoter domains. Fundamental properties of histone gene transcriptional regulation will be discussed in relation to consequences of abrogated growth control in tumor cells.

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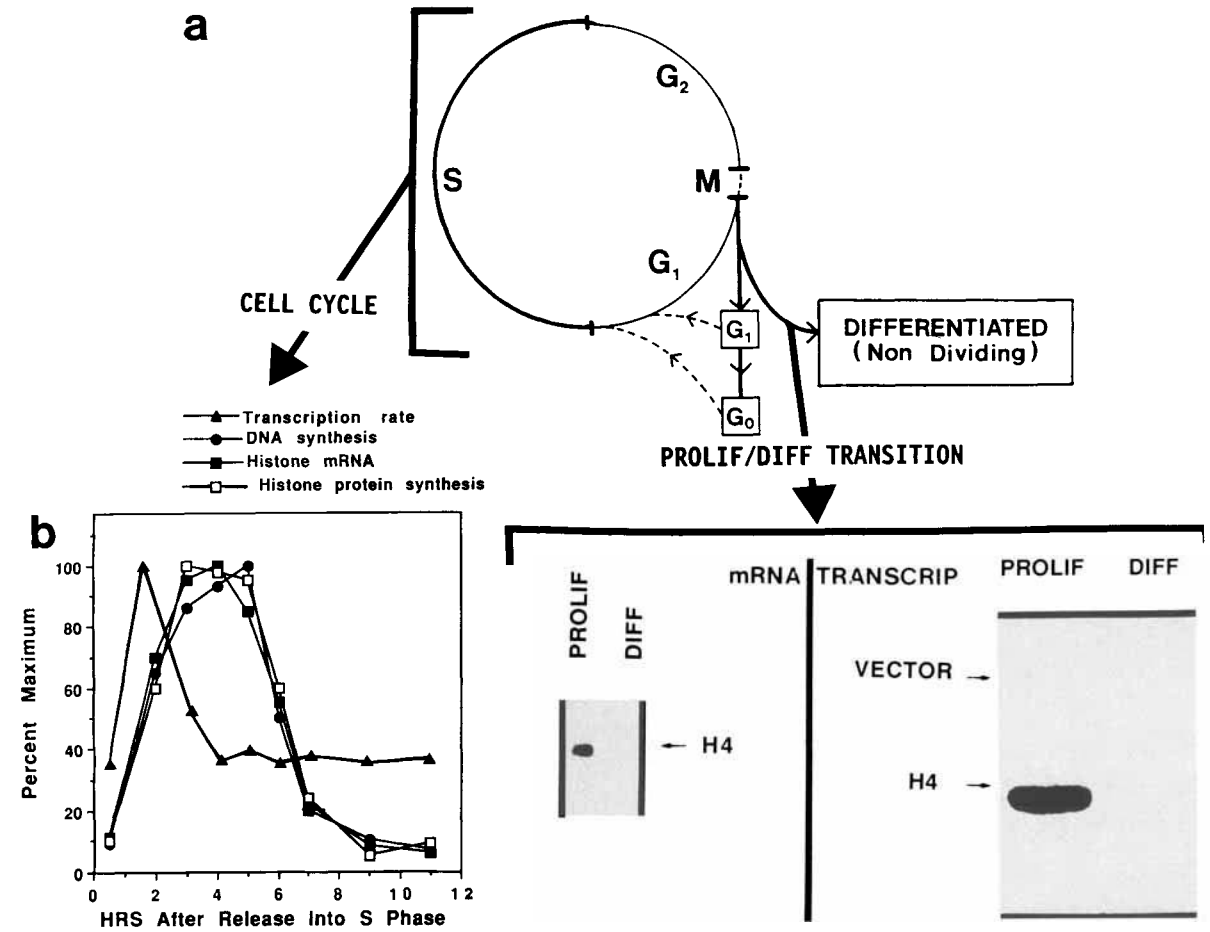


Fig. 1. Regulation of a cell-growth-controlled histone gene *during* growth and differentiation. **a:** Schematic representation of the cell cycle (G_1, S, G_2, and mitosis), indicating how the pathway is associated with the post-proliferative onset of differentiation (proliferation-differentiation transition). **b:** Definition of the principal biochemical parameters of histone gene expression, indicating the restriction of histone protein synthesis and the representation of histone mRNA to S-phase cells (DNA synthesis). Constitutive transcription of histone genes is evident throughout the cell cycle with an enhanced transcriptional level during the early part of S-phase. These results establish the combined contribution of transcription and mRNA stability to the S-phase-specific regulation of histone biosynthesis in proliferating cells, with histone mRNA levels as the rate-limiting step. In contrast, the completion of proliferative activity at the onset of differentiation is mediated by transcriptional down-regulation of histone gene expression. Evidence for this is the parallel decline in rate of H4 transcription and cellular mRNA levels (not shown). ▲, transcription (histone); ○, histone mRNA; ●, DNA synthesis; □, histone protein synthesis.

HISTONE GENE EXPRESSION IS CONTROLLED AT MULTIPLE LEVELS

Biological requirements for histone proteins are reflected by two functional classes of genes in the multi-gene family of histone coding sequences. The cell cycle regulated histone genes are expressed during S-phase to support DNA replication [Sierra et al., 1983; Kroeger et al., 1987; Pauli et al., 1987; van Wijnen et al., 1989; Dailey et al., 1988; Lee et al., 1991; Ramsey-Ewing et al., in press]. In contrast the cell cycle independent histone genes, also referred to as "variant" or "differentiation specific," are expressed throughout the cell cycle or are transiently expressed post-proliferatively at the onset of differentiation [Hatch et al., 1990; Wells et al., 1987; Shalhoub et al., 1989; Collart et al., 1991; Kardalinou et al., 1993]. Competency for expression of the variant histone genes in non S-phase cells is at least in part due to promoter regulatory sequences which are controlled by cell cycle independent transcription factors. Additionally, stability of transcripts from these genes is not dependent on ongoing DNA synthesis. The role of these variants may be to allow biosynthesis of histone proteins in the absence of DNA synthesis, thus enabling modifications in chromatin structure to be made, which sup-

port the initial expression of tissue-specific phenotypic properties post-proliferatively.

We will initially confine our considerations to a cell cycle regulated human H4 histone gene designated H4-FO108 which has been studied extensively with respect to expression and mechanisms that are operative in control [Ramsey-Ewing et al., in press; Wright et al., submitted; Sierra et al., 1983; Kroeger et al., 1987; Pauli et al., 1987; van Wijnen et al., 1989]. Later, we will assess the potential for coordinate control of cell cycle regulated core (H4, H3, H2a, and H2b) and H1 histone genes, together with other genes functionally coupled to DNA synthesis.

Post-Transcriptional Control

The five principal classes of cell cycle regulated histone genes are controlled at multiple levels. Cellular levels of histone mRNA are the rate limiting parameter for histone biosynthesis (Fig. 1). In proliferating normal diploid and transformed cells, histone mRNA and protein synthesis are confined to the S-phase of the cell cycle, with a parallel relationship between cellular histone mRNA content, the rate of histone protein synthesis, and DNA replication [Plumb et al., 1983a,b]. Half-life determinations of histone mRNA induced at different times during the cell cycle directly indicate increased stability during S-phase [Morris et al., 1991]. Histone mRNA is destabilized selectively at the natural completion of S-phase and following inhibition of DNA synthesis [Stein et al., 1984; Marzluff et al., 1988; Osley et al., 1991; Heintz et al., 1991], possibly by autonomous regulation [Stein and Stein, 1984; Peltz et al., 1991; Morris et al., 1991]. Interestingly, the coupling of histone mRNA stability with DNA synthesis appears, at least in part, to be regulated by association of histone mRNA-containing polyribosomes with the cytoskeleton [Zambetti et al., 1987, 1990]. The non-random distribution of histone mRNA in the cytoplasm may contribute to stability and/or translatability of histone mRNA [Pauli et al., 1989]. Rate of histone mRNA processing within the nucleus may contribute further to cellular histone mRNA levels during the cell cycle [Harris et al., 1991].

Transcriptional Control

Histone gene transcription occurs throughout the cell cycle in proliferating cells, with an up-regulation at the onset of S-phase. Cell cycle dependent transcriptional and post-transcriptional control are both involved in determining the level of expression. The extent to which transcriptional and post-transcriptional control contribute to histone gene expression in response to biological activity of cells can be modified. A dramatic illustration is the switch in regulatory mechanisms that are operative during differentiation. This is particularly evident at a key transition point early in the developmental sequence of genes expressed during osteoblast, monocyte and adipocyte differentiation (Fig. 1) when proliferation is completed and key components of tissue-specific gene expression are initiated. During proliferation, both transcription and mRNA stability modulate histone gene expression, whereas post-proliferatively, down-regulation of the histone genes is transcriptionally controlled [Shalhoub et al., 1989; Owen et al., 1990a,b; Collart et al., 1988; Stein et al., 1989, 1990].

INDEPENDENT AND OVERLAPPING PROMOTER ELEMENTS INTERACT WITH MULTI-PARTITE FACTORS TO REGULATE HISTONE GENE TRANSCRIPTION

Transcriptional control of histone gene expression is mediated by a modularly organized series of 5′-regulatory sequences that interact with multiple sequence-specific promoter binding factors [reviewed in Stein et al., 1992; Sierra et al., 1983; Kroeger et al., 1987; Pauli et al., 1987; van Wijnen et al., 1989; Dailey et al., 1988; Lee et al., 1991; van Wijnen et al., 1991a; Sharma et al., van Wijnen et al., 1992; 1989; LaBella et al., 1989; Harvey et al., 1982; Hinkley and Perry, 1991]. Further evidence for this complex regulation is provided by the observations that several promoter elements interact with heteromeric factors and/or represent composite protein-DNA interaction sites, and that both positive and negative elements may contribute to levels of transcription. Competence for transcription and the extent to which the histone genes are transcribed are determined by selective occupancy of these positive and negative promoter elements by cognate transcription factors. The interaction of these factors with regulatory sequences varies under different biological circumstances. Occupancy of histone gene promoter elements may be regulated partly by biosynthesis of transcription factors. Additionally, recruitment of these factors involves synergistic and mutually exclusive interactions that are partly regulated by phosphorylation and protein-

protein complex formation [Roberts et al., 1991; van Wijnen et al., 1991b; van der Houven van Oordt et al., 1992]. Here, cooperativity at the level of protein-DNA interactions involving multiple promoter elements may contribute to transcriptional control.

Focusing on the H4 gene (designated FO108), several classes of regulatory sequences, that each reflect a different component of control, independently and collectively establish the extent to which the gene is transcribed in response to a series of cellular transcriptional parameters associated with growth control. Regulation of transcription can therefore be viewed as being rate-limited by the cellular representation of any one of several factors or by the availability of specific complexes involved in activities at multiple regulatory elements. Transcriptional activity of the H4-FO108 gene depends on cellular levels of ubiquitous transcription factors (e.g., Sp1 and ATF) reflecting overall cellular transcriptional capacity, factors that interact with all cell-cycle-regulated core and H1 histone genes, and factors that exhibit recognition specificity for individual histone genes or subsets of the histone multigene family.

Figure 2 is a schematic representation of the regulatory organization of the initial 1.0 kb of the H4-FO108 gene promoter. Whereas this region, which is devoid of repetitive elements [Collart et al., 1985], contains the minimal sequences required for regulated expression, the functional limits of the H4-FO108 gene appear to extend considerably upstream. Indeed, *cis*-acting elements up to −6.5 kb may influence developmental expression of the H4-FO108 gene in vivo in transgenic animals and in cell lines competent for differentiation isolated from transgenic mice [Helms et al., 1987; van Wijnen et al., 1991c; Gerbaulet et al., 1992]. We have established two domains of in vivo protein-DNA interactions for the H4-FO108 gene [Pauli et al., 1987] in the intact cell at single-nucleotide resolution. These have been designated H4-Site I (nt −156 to −113) and H4-Site II (nt −97 to −47).

The H4-Site I promoter domain is a bipartite *cis*-activating element that interacts distally with a member of the ATF/AP-1 family of transcription factors, and proximally with a GC-box-binding protein, histone nuclear factor-C (HiNF-C) [van Wijnen et al., 1989; Wright et al., submitted], most likely identical to Sp1. Both factors are capable of synergistically mediating a 5-fold stimulation of transcription.

The H4-Site II domain represents a mosaic of functional recognition sequences that contribute to H4 gene transcription. H4-Site II is a multi-partite protein-DNA interaction site for sequence-specific factors HiNF-D, HiNF-M, and HiNF-P [van Wijnen et al., 1991b], with HiNF-P being similar to H4-TF2 [Dailey et al., 1986]. The proximal region of H4-Site II spans a TATA motif and is sufficient to mediate accurate transcription initiation in vitro, but not in vivo [Sierra et al., 1983], presumably by interaction with initiation factor TFII-D. However, the distal region of H4-Site II influences transcriptional competency, as well as the timing and extent of H4 histone mRNA synthesis in vivo [Ramsey-Ewing et al., in press; Kroeger et al., 1987]. This Site II distal region contains several distinct sequence motifs that either stimulate the basal level of H4 gene transcription (C-box) or influence periodic levels of transcription (M-box) [Ramsey-Ewing et al., in press].

The distal-activating elements, H4-Sites III and IV, encompass regions that stimulate transcription in vivo and interact with the heteromeric nuclear factors H4UA-1 [van der Houven van Oordt et al., 1992] and H4UA-3 (Wright, unpublished data), respectively. Additionally, H4-Site IV overlaps with a putative nuclear matrix attachment site spanning nucleotides −730 to −589 [Dworetzky et al., 1992]. This element interacts with a sequence-specific nuclear matrix protein (NMP-1), an ATF transcription factor, and may influence expression of the H4 gene promoter by transient anchorage to the nuclear matrix.

PROTEIN-DNA AND PROTEIN-PROTEIN INTERACTIONS AT HISTONE GENE PROMOTER ELEMENTS DURING THE CELL CYCLE AND POST-PROLIFERATIVELY AT THE ONSET OF DIFFERENTIATION

Insight into transcriptional control of histone gene expression is provided by modifications in the interactions of promoter-binding factors with the initial 1.0 kb of the H4-FO108 gene promoter at Sites I, II, III, and IV. Here, the extent of histone gene transcription can be related to responsiveness of multiple physiologic regulatory parameters governing cell cycle progression and differentiation.

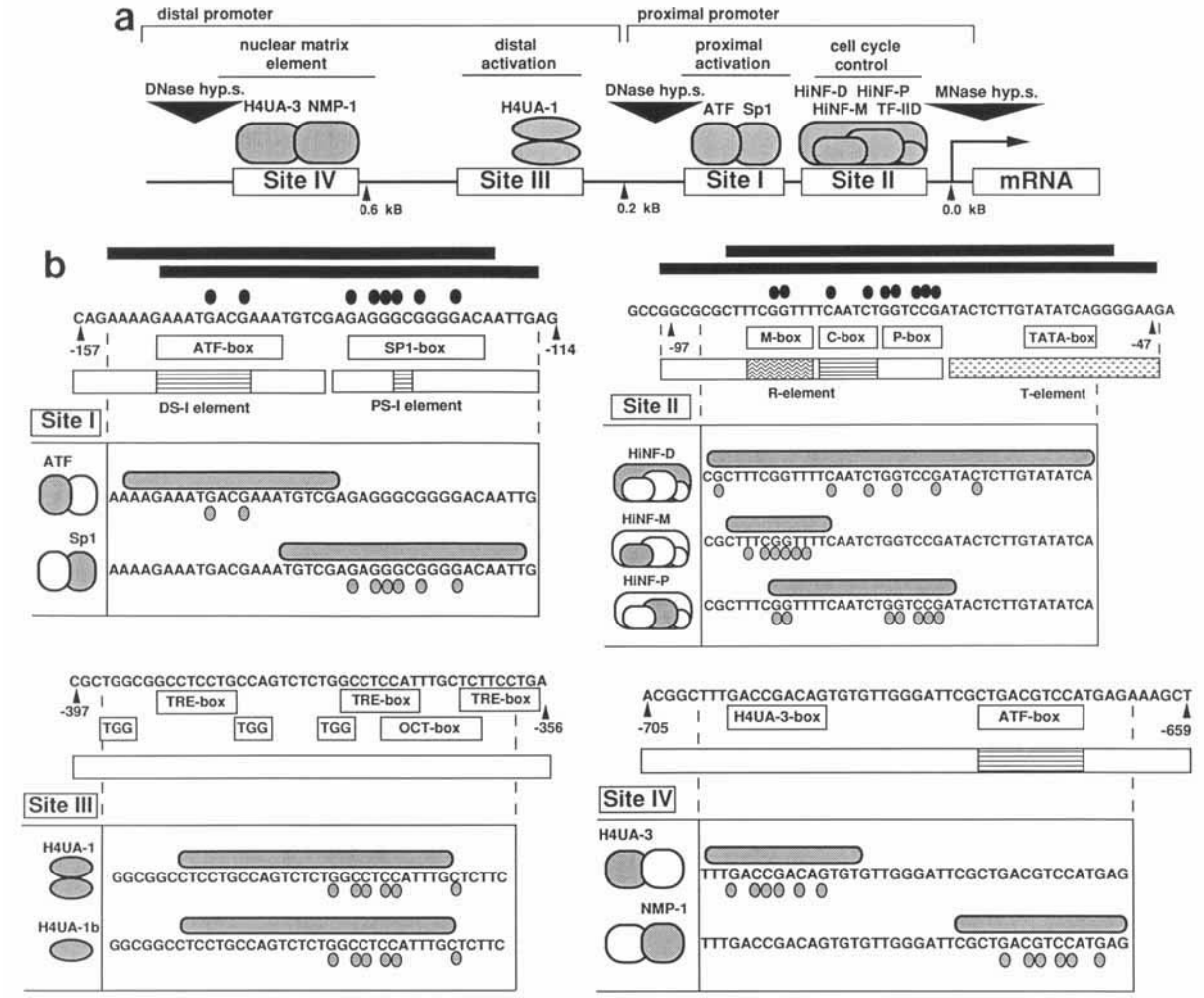


Fig. 2. Regulatory organization of the H4-FO108 gene promoter. The overall modular organization of the promoter (**a**) is reflected by distal (Sites IV and III) and proximal (Sites I and II) domains (boxes) that are interspersed with several nuclease hypersensitive regions (inverted triangles). Multiple promoter binding proteins interact with each site (e.g., HiNF-D, HiNF-M, HiNF-P, and TF-IID at Site II) and determine the extent and timing of histone gene transcription (arrow indicates transcriptional start site). **b:** Sites I and II (middle panels) are protein/DNA interaction domains established in the intact cell in vivo (black bars: genomic DNaseI footprint; black dots genomic dimethyl sulphate fingerprint) which each span a series of sequence-motifs (e.g., M-, C- and P-boxes underneath sequence) and coincide with several in vivo and in vitro transcriptional elements (e.g., R- and T-elements). The parameters influenced by these elements are as follows: cap-site initiation of mRNA (box with caret marks), basal levels of transcription (striped boxes), and/or the cell cycle periodicity of histone gene transcription (■). Interactions with promoter-binding proteins also occur within these elements; minimal recognition sequences and methylation-interference protein-DNA contacts (shaded vertical ovals) are shown. **c:** Sites III and IV are distal-activating protein-DNA interaction domains (same symbols as for Sites I and II), with Site IV being a component of a putative nuclear matrix attachment site. ATF, activating transcription factor; HiNF, histone nuclear factor; NMP, nuclear matrix protein.

Cell Cycle Control

Cell cycle regulation of histone gene transcription resides at Site II. Holthuis et al. [1990] have established that HiNF-D is cell-cycle-regulated, restricted to S-phase in normal diploid rat osteoblasts and human diploid fibroblasts. However, HiNF-D binding activity is only modulated during the cell cycle of cells with normal, untransformed cell growth characteristics [Holthuis et al., 1990]. This is consistent with deregulation of cell-cycle-dependent binding activity of this factor during neoplastic transformation in tumor cells where stringent growth control is abrogated. Further insight into the molecular mechanisms underlying the protein-DNA interactions responsible for cell-cycle control is provided by

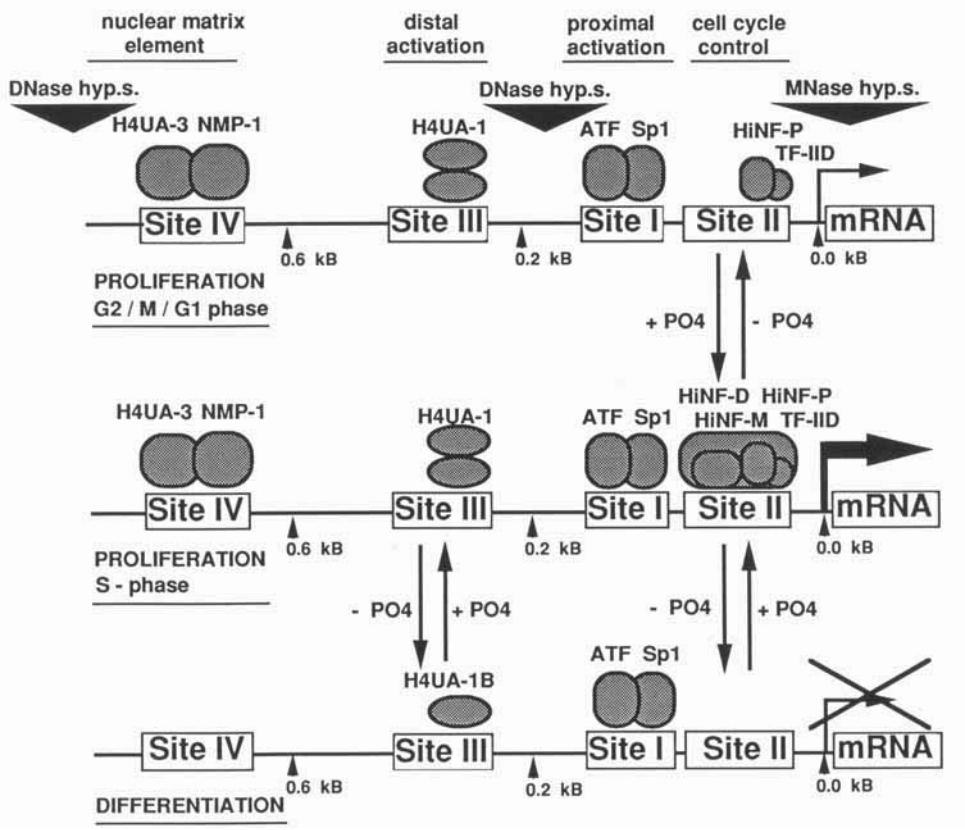


Fig. 3. Modifications in phosphorylation-dependent protein/protein and protein/DNA interactions influencing H4-FO108 gene transcription during the cell cycle and cessation of proliferation at the onset of differentiation. Shown here is the postulated model for the regulation of H4-FO108 gene transcription involving differential occupancy of Site II by histone nuclear factor-D (HiNF-D), HiNF-M, HiNF-P, and TF-IID during the S and G2/M/G1 phases of the cell cycle resulting in basal levels (thin arrow), or maximal levels (thick arrow) of transcription. Shutdown of H4-FO108 gene transcription at the cessation of proliferation (thin arrow covered by X) coincides with modifications of protein/protein interactions at Site III and downregulation of Site II occupancy by its cognate factors. The contribution of phosphorylation to protein/DNA interactions at Site II and protein/protein interactions at Site III are indicated. ATF, activating transcription factor; NMP, nuclear matrix protein.

results from in vivo analysis of deletion and site-directed mutations. In vivo competition studies further demonstrate that sequences responsible for cell-cycle-dependent H4 transcription reside within Site II [Ramsey-Ewing et al., in press].

Proliferation-Differentiation Interrelationships

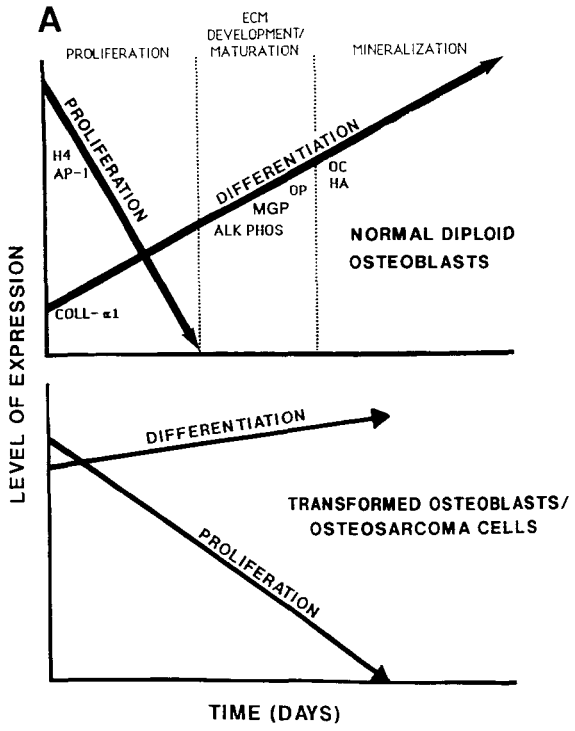
A remodeling of histone gene promoter occupancy by transcription factors is evident post-proliferatively in cells initiating expression of genes supporting differentiation. Striking examples are development of the monocytic, osteoblastic, and adipocytic phenotypes.

As indicated in Figures 2 and 3, with the down-regulation of proliferation during phorbol ester-induced differentiation of HL-60 promyelocytic leukemia cells into monocytes, a coordinate transcriptional repression of cell-cycle controlled H4 [Stein et al., 1989], H2B [Collart et al., 1991] and H1 [Collart et al., 1988] genes is observed. Cessation of H4-FO108 gene transcription coincides with selective down-regulation of protein-DNA interactions at H4-Site II in vivo and persistent occupancy of H4-Site I [Stein et al., 1989]. The vacancy of H4-Site II coincides with loss of in vitro binding of nuclear factor HiNF-D, with at least two factors (HiNF-C/Sp1 and HiNF-A/high mobility group-I) maintaining constitutive DNA-binding activity post-proliferatively during the onset of differentiation [van Wijnen et al., 1989]. The correlation between down-regulation of cell proliferative activity, transcriptional down-regulation of histone gene expression, and loss of HiNF-D binding activity has been confirmed in numerous cell culture systems, including differentiating primary rat calvarial osteoblasts and mouse 3T3

pre-adipocytes [Stein et al., 1990], serum-deprived CF-9 fibroblasts [Wright et al., 1992], as well as density-inhibited rat osteosarcoma cells [van den Ent et al., 1993; van den Ent et al., 1994]. Moreover, HiNF-D decreases in parallel with down-regulated expression of the cell-cycle controlled mouse H4 genes and at cessation of chimeric FO108-reportergene gene transcription in transgenic mice during hepatic development [van Wijnen et al., 1991c].

Transcription Factor Phosphorylation

A functional relationship between activity of DNA binding proteins and the extent to which they are phosphorylated provides the potential basis for integrating cellular signaling mechanisms involving protein kinases and phosphatases with cell cycle-dependent control of transcription. Dephosphorylation affects protein-DNA interactions at both H4-Site II [van Wijnen et al., 1991b] and Site III [van der Houven van Oordt et al., 1992]. At Site II, dephosphorylation appears to modify interaction of both HiNF-D and HiNF-M binding to DNA, whereas at Site III protein-protein interactions may be involved. It remains to be formally established whether phosphorylation events are involved in H4 cell cycle regulation.



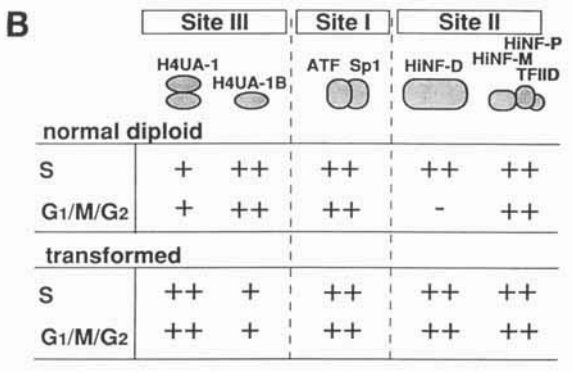


Fig. 4.

ABROGATION OF GROWTH CONTROL IN TRANSFORMED AND TUMOR CELLS IS REFLECTED BY DEREGULATION OF CELL GROWTH-DEPENDENT TRANSCRIPTION FACTOR INTERACTIONS WITH HISTONE GENE PROMOTER ELEMENTS

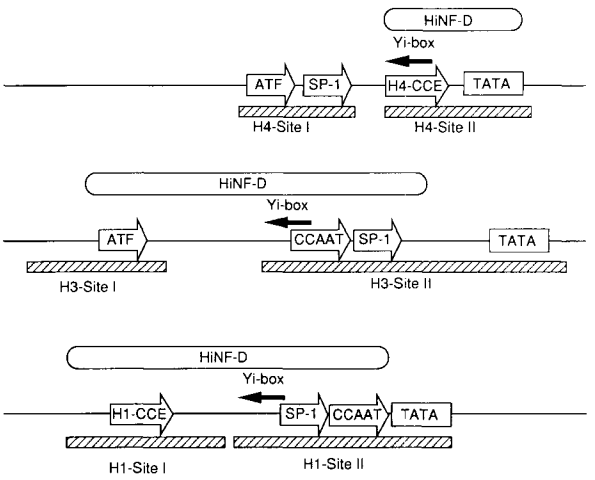
In transformed and tumor cells the abrogation of growth control and proliferation-differentiation interrelationships is accompanied by modifications in protein-DNA interactions at two proximal histone gene promoter regulatory elements (Fig. 4). The first is observed at Site II. While in normal diploid cells HiNF-D binding at

Fig. 4. **A:** Model of the relationship between proliferation and differentiation in normal diploid cells during the rat osteoblast developmental sequence and in osteosarcoma (transformed) cells. The top panel illustrates the reciprocal growth-differentiation relationship operative in normal diploid osteoblasts. The three principal developmental periods are designated (proliferation, extracellular matrix maturation, and extracellular matrix mineralization). The lower panel schematically illustrates the deregulation of the relationship between growth and differentiation in transformed osteoblasts or osteosarcoma cells. The proliferation and differentiation vectors reflect the co-expression of cell growth and bone tissue-specific genes. In contrast to normal diploid cells where expression of osteoblast phenotype markers is post-proliferative in transformed cells these genes are expressed constitutively. There is an absence of the two developmentally important transition points observed in normal diploid cells: designated by broken vertical lines when proliferation is downregulated and at the onset of extracellular matrix mineralization. In osteosarcoma cells the concomitant expression of cell growth and tissue-specific genes reflects abrogation of growth control and the consequential deregulation of interrelationships between growth and differentiation. Several genes which are developmentally expressed during osteoblast differentiation are designated [Owen et al., 1990b; Lian et al., 1992; Stein and Lian, 1993]. **B:** Cellular levels of transcription factors during the cell cycle. The switch from stringent cell-cycle regulated (S-phase restricted) occupancy at Site II of the histone gene promoter in normal diploid cells is schematically compared to constitutive occupancy at Site II during the cell cycle in transformed and tumor cells [Holthuis et al., 1991; Pauli et al., 1987].

Site II is restricted to S-phase, protein-DNA interactions at Site II are constitutive during the cell cycle in rat osteosarcoma cells and in SV40 transformed human diploid fibroblasts [Holthuis et al., 1990]. A similar requirement of the normal diploid phenotype for cell cycle-dependent occupancy of Site II by cognate transcription factors is observed in the analogous domains of the H3 and H1 histone gene promoters [van Wijnen et al., 1991d]. This suggests that the coordinate regulation of HiNF-D interactions with cell cycle regulatory domains of *histone gene promoters* is operative in normal diploid cells. Despite the observed cell cycle regulation of HiNF-D activity in normal diploid cells, compared with constitutive activity in tumor cells, both exhibit downregulation with the cessation of proliferation [van den Ent et al., 1993, 1994; Owen et al., 1990a,b]. A principal variation in promoter factor binding, reflecting the normal diploid versus transformed phenotypes, is the phosphorylation-dependent representation of H4UA1 or H4UA1B at Site III. Here the observed differences in transcription factor complexes are accounted for by protein-protein interactions [van der Houven van Oordt et al., 1992].

COORDINATE TRANSCRIPTIONAL CONTROL OF HISTONE AND OTHER S-PHASE RELATED GENES

From a restricted perspective the concomitant expression during S-phase of core and H1 histone genes necessitates an understanding of analogous regulatory mechanisms. The integration of mechanisms controlling the coordinately regulated transcription of multiple histone genes involves several shared promoter-binding activities, including both ubiquitous and histone gene-specific transcription factors [van Wijnen et al., 1991a; Ito et al., 1989; Dalton and Wells, 1988; Gallinari et al., 1989; van Wijnen et al., 1991d; van den Ent et al., in press]. HiNF-D-related protein-DNA interactions are also represented



HiNF-D binding sites	H1 : nt -139/-152	CG	CCg	CCG	CT	Cg	CG
	H3 : nt -126/-139	GT	CCa	TCT	CT	gT	GC
	H4 : nt -101/-88	CG	CCg	GCG	CG	CT	TT
	Yi motif		CCC	- C -	- -	CT	
factor Yi binding sites	mTK (MT-1): nt -98/-85	GT	CCC	GCC	CC	CT	TT
	mTK (MT-2): nt -71/-84	TG	CCC	GCG	AA	CT	CA
	mTK (MT-3): nt -43/-30	CA	CCC	ACG	GA	CT	CT
	hTK: nt -99/-86	CT	CCC	GCC	AG	gT	CA
	hTK: nt -79/-92	CG	gCC	GCT	GA	Cc	TG

Fig. 5. Coordinate transcriptional control of three distinct histone (H4, H3, and H1) gene classes. The top panel is a schematic representation of the promoters of three typical H4 (top), H3 (middle), and H1 (bottom) genes. Each promoter contains ubiquitous transcriptional elements and cognate factors (e.g., ATF, SP-1, and CCAAT; the orientation of elements within the promoters are indicated by the open arrows) that are shared to various degrees between distinct histone genes, as well as gene-specific cell cycle control elements (e.g., H4-CCE and H1-CCE) [Dalton and Wells, 1988; LaBella et al., 1989; van Wijnen et al., 1991a,b; Ramsey et al., 1994]. These promoter elements are contained within larger multi-partite gene-regulatory protein/DNA interaction domains (e.g., in vivo genomic protein/DNA interaction domains H4-Sites I and II, and H3-Sites I and II) [Pauli et al., 1987, 1989]. Apart from histone promoter factors recognizing short defined elements, the three histone H4, H3, and H1 gene promoters each interact with the cell cycle regulated nuclear factor HiNF-D (elongated rounded box); factor HiNF-D simultaneously recognizes heterogeneous arrangements of at least two sequence motifs (CA- and AG-boxes; not indicated) [van Wijnen et al., 1991a,c; van den Ent et al., 1994]. A subset of the AG-motifs strongly resemble the consensus sequence of the thymidine kinase nuclear factor Yi (Yi-box, black arrows) [Dou et al., 1991, 1992]. The bottom panel represents an alignment of three AG-type recognition motifs of HiNF-D located in, respectively, H1, H3, and H4 promoters [the large black dots represent specific methylation-contacts for HiNF-D; van den Ent et al., 1994]. The three HiNF-D recognition elements strongly resemble the consensus Yi-motif [Dou et al., 1991] providing evidence for coordinate regulation of S-phase related genes by a shared factor. The Yi motif is based on alignment of three in vitro protein/DNA interaction elements for factor Yi in the murine thymidine kinase promoter (MT-1, MT-2, and MT-3) [Dou et al., 1991]. Similar motifs are also observed in the human thymidine kinase promoter and bind a Yi related factor [Li et al., 1993].

Fig. 5.

in H3 and H1 histone gene promoters [van Wijnen et al., 1991d], suggesting the possibility of coordinate transcription factor interactions regulating several histone gene classes (Fig. 5).

In a broader biological context there are similarities of histone gene Site II sequences with those in the proximal promoter of the thymidine kinase gene which exhibits enhanced transcription during S-phase (Fig. 5) [Coppock and Pardee, 1987; Sherley and Kelly, 1988; Knight et al., 1989; Dou et al., 1991]. The possibility may therefore be considered that genes functionally related to DNA replication may at least be in part coordinately controlled. Support for such a mechanism is provided by recent observations that both the cell cycle-regulated H4 histone gene (van Wijnen A, Grana-Amat X, Giordano A, Lian J, Stein J, Stein G, unpublished results) and the analogous promoter domain of the thymidine kinase gene [Dou et al., 1992; Li et al., 1993] supports sequence-specific interactions with cdc2 and Rb-related protein complexes. Here, we should not dismiss the possibility that a mechanism may be operative where cell cycle control (transitions from $G_1 \rightarrow S \rightarrow G_2$) and mitotic control are interrelated at histone and thymidine kinase promoters by cyclin-related phosphorylation (Fig. 6).

NUCLEAR STRUCTURE-HISTONE GENE TRANSCRIPTION INTERRELATIONSHIPS:

A synergistic contribution of activities by Sites I, II, III, and IV H4 histone gene promoter elements to the timing and extent of H4-FO108 gene transcription has been established experimentally [Kroeger et al., 1987; Wright et al., 1992; Ramsey-Ewing et al., in press; Wright et al., submitted]. The integration of intracellular signals that act independently upon these multiple elements may partly reside in the three-dimensional organization of the promoter within the spatial context of nuclear architecture (Fig. 7). A nuclear matrix attachment site has been identified in the upstream region (−0.8 kB) of the H4-FO108 gene promoter [Dworetzky et al., 1992], which may serve two functions: imposing constraints on chromatin structure, and concentrating and localizing transcription factors. Such a role for the nuclear matrix in regulation of histone gene expression is supported by distinct modifications in the composition of nuclear matrix proteins observed when proliferation-specific genes are down-regulated during differentiation [Dworetzky et al., 1990] and, more directly, by the isolation of a unique ATF transcription factor from the nuclear matrix [Dworetzky et al., 1992], which interacts with Site IV of the H4-FO108 gene promoter. The presence of nucleosomes in the H4 promoter [Moreno et al., 1986; Chrysogelos et al., 1985] (Fig. 7) may serve to increase the proximity of independent regulatory elements, and supports synergistic and/or antagonistic cooperative interactions between histone gene DNA binding activities. In addition, chromatin structure and nucleosomal organization varies as a function of the cell cycle [Moreno et al., 1986; Chrysogelos et al., 1989], which may enhance and restrict accessibility of transcription factors, and modulate the extent to which DNA-bound factors are phosphorylated. The specific mechanisms by which the 5′ histone gene promoter regulatory elements and sequence-specific transactivating factors partici-

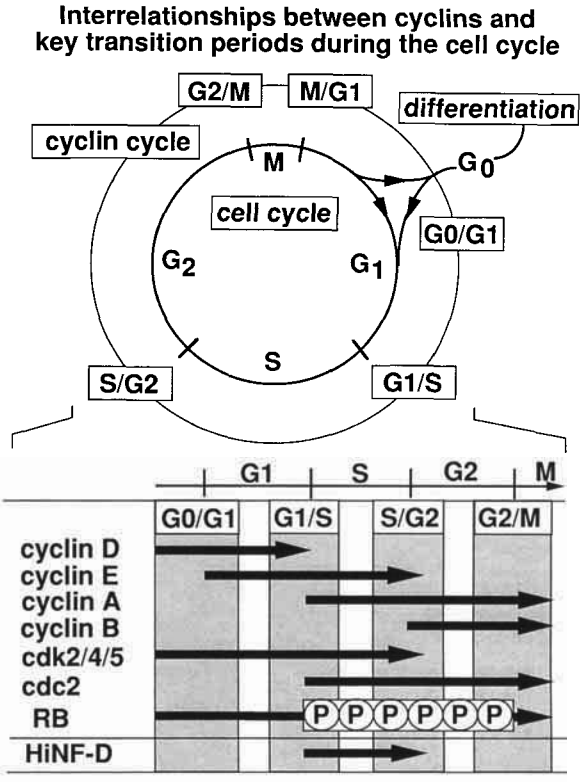


Fig. 6. Interrelationships between cell cycle progression (inner circle) and periodicity of cyclin association with cyclin-dependent kinases (outer circle). The bottom component of the figure is a linear representation of the cell cycle with the G_0/G_1, G_1/S, S/G_2, and G_2/M transition points indicated. The arrows reflect cell cycle stages when the principal cyclins and cyclin-dependent kinases are observed at maximum levels. Also indicated are changes in the phosphorylation state of RB and the cell cycle dependent representation of HiNF-D binding activity.

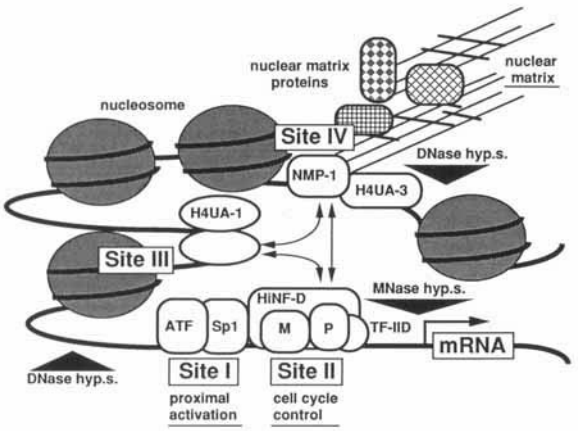


Fig. 7. Spatial integration of intra- and extra-cellular signals modulating H4-FO108 gene transcription by reversible alterations in chromatin structure and nucleosomal organization of the promoter. Shown is a schematic representation of a model for the three dimensional organization of the H4-F0108 promoter depicting a relationship of distal and proximal protein/DNA interaction sites when H4-FO108 promoter DNA (solid black line) is packaged into nucleosomes (ovals). Indicated are possible cooperative and/or mutually exclusive higher-order nucleoprotein interactions (arrows) between various DNA bound trans-acting factors (see Fig. 2 for symbols). The presence of a putative attachment site (Site IV) with the nuclear matrix (network of lines) containing matrix associated sequence-specific DNA binding proteins (rounded boxes filled with alternative symbols), provides a basis for restricted mobility of the promoter to a confined position within the nucleus as well as for the concentration, localization, and targeting of transcription factors. ATF, activating transcription factor; HiNF, histone nuclear factor; NMP, nuclear matrix protein.

pate in regulating transcription of the histone H4-FO108 gene remain to be determined. However, regulation is unquestionably operative within the context of the complex series of spatial interactions, which are responsive to a broad spectrum of biological signals.

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