Coordinate Control and Selective Expression of the Full Complement of Replication-dependent Histone H4 Genes in Normal and Cancer Cells*

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The replication of eukaryotic genomes necessitates the coordination of histone biosynthesis with DNA replication at the onset of S phase. The multiple histone H4 genes encode identical proteins, but their regulatory sequences differ. The contributions of these individual genes to histone H4 mRNA expression have not been described. We have determined, by real-time quantitative PCR and RNase protection, that the human histone H4 genes are not equally expressed and that a subset contributes disproportionately to the total pool of H4 mRNA. Differences in histone H4 gene expression can be attributed to observed unequal activities of the H4 gene promoters, which exhibit variations in gene regulatory elements. The overall expression pattern of the histone H4 gene complement is similar in normal and cancer cells. However, H4 genes that are moderately expressed in normal cells are sporadically silenced in tumor cells with compensation of expression by other H4 gene copies. Chromatin immunoprecipitation analyses and in vitro DNA binding assays indicated that 11 of the 15 histone H4 genes interact with the cell cycle regulatory histone nuclear factor P, which forms a complex with the cyclin E/CDK2-responsive co-regulator p220NPAT. These 11 H4 genes account for 95% of the histone H4 mRNA pool. We conclude that the cyclin E/CDK2/p220NPAT histone nuclear factor P signaling pathway is the principal regulator of histone H4 biosynthesis.

Histones have crucial roles in replication, transcription, repair, and recombination (1–3). There is a fundamental requirement for coordinated de novo synthesis of the core histone proteins H2A, H2B, H3, and H4 as well as the linker H1 protein during S phase to package nascent genomic DNA (1, 2). Replication of a complete mammalian genome requires 108 of each of the individual histone proteins. Efficient production of this vast quantity of proteins necessitates that transcription of multiple histone genes at multiple loci be coordinately regulated with the onset and progression of genome replication during the cell cycle (4).

Histone biosynthesis is a unique process involving transcription initiation from compact promoters to form primary transcripts that lack introns and that contain a highly conserved stem-loop structure that forms the 3′-end of the mature non-polyadenylated mRNA (4, 5). Histone genes are organized into clusters, and this organization has persisted throughout the course of evolution from yeast to human (2, 4). The majority of the 74 known and characterized human histone genes is located in two major clusters at chromosomes 6p21 and 1q21, respectively (Table One) (1, 6–8). It is now known that the human genome contains 15 histone H4 genes that encode identical proteins. H4 genes in lower eukaryotes (i.e. sea urchin and Drosophila) are organized with the other histone gene types (i.e. H2A, H2B, H3, and H1) into units that are tandemly repeated, and all H4 genes in these organisms have virtually identical promoters and coding regions. Although the coding regions of the human histone H4 genes are translated into identical proteins, there is surprising variation in the organization of the proximal promoters. Based on the availability of the complete human genome sequence, it is now possible to definitively assess the expression and regulation of the full complement of histone H4 genes.

Previous studies on a limited number of H4 genes have suggested that the expression of many (if not all) of these H4 copies is coordinately controlled during the cell cycle (2, 9–11). The human histone H4/n gene, which is temporally and functionally linked to DNA synthesis, provides a paradigm for cell cycle-dependent coordinate control of gene expression at the G1/S phase transition. This H4 gene is regulated by multiple elements and cognate DNA-binding activities, and one proximal promoter element (Site II) mediates cell cycle-dependent transcription (12–17). A phylogenetically conserved H4 subtype-specific element is typically present in H4 genes within Site II. Site II is not responsive to the E2F class of transcription factors. Thus, Site II cell cycle regulatory mechanisms at the onset of S phase function independently of E2F (2, 15, 18). Three histone nuclear factors (HiNF-M, -D, and -P)3 interact with Site II to mediate cell cycle control of transcription at the onset of S phase (4, 12–24). Recently, HiNF-P has been identified as the protein that, in conjunction with the cyclin E/CDK2-responsive protein p220NPAT, controls H4 gene transcription via binding to its cognate H4 subtype-specific element (25). It is necessary to assess whether all human histone H4 genes are coordinately controlled and whether HiNF-P is the key factor that synchronizes transcription of the histone H4 gene family with DNA replication.

Because of the extensive similarity of the histone H4 sequences, it has been difficult to determine the relative contributions of the 15 individual H4 genes to the total histone H4 mRNA pool by molecular

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§ The abbreviations used are: HiNF, histone nuclear factor; qPCR, quantitative PCR; ChIP, chromatin immunoprecipitation; qRT-PCR, quantitative reverse transcription-PCR; RPAs, RNase protection assays; PBS, phosphate-buffered saline; EMSA, electrophoretic mobility shift assay.
Quantitative Reverse Transcription-PCR (qRT-PCR) Analysis of H4 Gene Expression—RNA was extracted from specified cell lines using TRIzol reagent (Invitrogen) according to the manufacturer’s protocol. Purified total RNA was subjected to DNase I digestion, followed by column purification using the DNA Free RNA Kit™ (Zymo Research Corp., Orange, CA). Eluted total DNA-free RNA was quantitated by spectrophotometry, and 1 μg was added to a reverse transcription reaction using the iScript™ cDNA synthesis kit (Bio-Rad) with a mixture of random hexamers and oligo(dT) primers. Varying dilutions of cDNA were used as templates in qPCRs with oligonucleotides specific to the different histone H4 gene 5′-untranslated regions (see TABLE TWO). Relative quantitation was determined using an ABI PRISM 7000 sequence detection system (Applied Biosystems) measuring real-time SYBR Green (Bio-Rad) fluorescence and calculated by the ΔΔCT method as described recently (26). Overall efficiencies of qPCR were calculated from the slopes of the standard curves of serial dilutions in steps of 2 (log(2) scale) and found to be nearly identical for each primer set. Expression profiles for H4/a mRNA were extrapolated by comparing qPCRs with H4/a fluorescent minor groove binder probe to qPCRs with both SYBR Green and minor groove binder probes specific for H4/a mRNA.

**RNAse Protection Assays (RPAs)—**RPAs were performed as described (27). Briefly, 32P-labeled antisense RNA was transcribed in vitro from linearized template DNA using the MAXIscript T7/T3 kit (Ambion, Inc., Austin, TX) in the presence of 3.3 mM [α-32P]UTP (3000 Ci/mmol, 50 mCi) and 50 mM unlabeled UTP for use in RPAs (RPA I kit from Ambion, Inc.). [α-32P]UTP-labeled β-actin antisense RNA was added to each hybridization reaction to normalize RNA quantities. Protected RNA fragments were resolved on a denaturing 8 M urea and 5% (v/v) polyacrylamide gel. A 32P-end-labeled Sau3AI digest of pUC19 DNA was used as a size marker. A PhosphorImager (Amersham Biosciences) was used to quantitatively evaluate RNA fragments. The relative expression of the individual histone H4 genes was calculated as the ratio of signal intensity standardized by β-actin. To compare RPA data with qRT-PCR data, the relative expression of H4/d was set to a value of 100% (maximum), and the relative expression levels of the other H4 mRNAs were determined as a percent of this maximal level.

**Reporter Gene Analysis—**In all experiments, promoter activity was measured in whole cell extracts using a luciferase assay kit (Promega Corp., Madison WI), and the results were normalized by cotransfection with 1 μg of pCMV-β-gal. To determine the expression of histone H4 as a function of the HiNF-P-p220NPAT complex, cells were transiently transfected with either 150 ng of wild-type HiNF-P or 300 ng of an expression vector containing wild-type p220NPAT or both with 200 ng of one of the wild-type histone H4 promoter-luciferase reporter constructs. All cDNAs and reporter constructs were mixed with FuGENE 6 (Roche Applied Science) in 100 μl of serum-free medium for 20 min at room temperature and then applied to cells. Cells were incubated over-night with the DNA mixture in a final volume of 2 ml of medium and then harvested 24 h later. Luciferase activity was measured using a Monolight 2010 luminometer (Analytical Luminescence Laboratory, San Diego, CA).

ChIP—Synchronously or asynchronously growing adherent cells were washed twice with ice-cold 1× phosphate-buffered saline (PBS) on the plate. Cells were immediately treated with 5 ml of 1% formaldehyde cross-linker in 1× PBS at room temperature for 10 min with gentle rotation. The cross-linking reaction was quenched by the addition of 5 ml of 0.25 M glycine in 1× PBS at room temperature for 5 min. Cells were then washed twice more with ice-cold 1× PBS, scraped into 5 ml of 1× PBS on ice, and harvested by centrifugation at 1000 × g for 5 min at

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**EXPERIMENTAL PROCEDURES**

**Cell Lines and Culture Conditions—**COS-7, HeLa, HCT116, IMR90, SaOS, and T98G cells were obtained from American Type Culture Collection (Manassas, VA). The COS-7, HeLa, and T98G stock cultures were maintained in Dulbecco’s modified Eagle’s medium (Invitrogen) supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 units/ml penicillin, and 100 μg/ml streptomycin. HCT116 and SaOS cells were maintained in McCoy’s 5A medium (Invitrogen) supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 units/ml penicillin, and 100 μg/ml streptomycin. IMR90 cells were maintained in Eagle’s basal medium (Invitrogen) supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 units/ml penicillin, and 100 μg/ml streptomycin. All cell lines were split at a ratio of 1:1 when 70% confluent in 100-mm plates (Corning Inc., Corning, NY). Culture conditions were 37°C in a 98% humidified and 5% CO₂ incubator.
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4 °C. Cross-linked cell pellets were resuspended in 50 mM Tris-Cl (pH 8.1), 150 mM NaCl, 1% (v/v) Nonidet P-40, and 2× Complete protease inhibitor mixture (Roche Applied Science) and incubated on ice for 20 min. Lysates were then sonicated to an average DNA size of 500–100 bp by agarose gel electrophoresis, and the extracts were cleared by centrifugation at 14,000 × g for 15 min at 4 °C. Cleared extracts were divided into sample and input aliquots to allow subsequent quantitation. Sample aliquots were subjected to primary immunoprecipitation with 2 µg of purified immunoglobulin or 3 µl of crude serum for each respective antibody. Following primary antibody incubations, a 0.1 volume of protein A/G-agarose bead slurry (Santa Cruz Biotechnology, Inc., Santa Cruz, CA) was added and allowed to bind for 1–4 h. Immunocomplexes bound to the beads were harvested by centrifugation at 1000 × g for 3 min. The beads were then washed consecutively with the following buffers: a low salt buffer containing 20 mM Tris-Cl (pH 8.1), 150 mM NaCl, 1% Triton X-100, and 2× EDTA; and a high salt buffer containing 20 mM Tris-Cl (pH 8.1), 500 mM NaCl, 1% Triton X-100, and 2× EDTA; 10 mM Tris-Cl (pH 8.1), 250 mM LiCl, 1% deoxycholate, 1% Nonidet P-40, and 1× EDTA; and three washes with 10 mM Tris-Cl (pH 8.1) and 1× EDTA. Cross-linked protein-DNA complexes were eluted from antibodies and beads twice with 100 µl of 1% SDS and 100 mM NaHCO3. The pooled eluates were supplemented with 0.1 volume of 3 M sodium acetate (pH 5.2), and the cross-links were reversed at 65 °C overnight. DNA was then purified via phenol/chloroform extraction and isopropyl alcohol precipitation with 5–20 µg of glycogen carrier. Precipitated DNA was allowed to rehydrate in 10 mM Tris-Cl (pH 8.1), and material ratios between samples and inputs were carefully documented to allow subsequent quantitation of locus immunoprecipitation. ChIP samples were then subjected to qPCR analysis using the ABI PRISM 7000 sequence detection system with locus-specific primers and probes.

Electrophoretic Mobility Shift Assay (EMSA)—In vitro DNA binding of HiNF-P to selected histone H4 genes was analyzed as described (13, 25). The radiolabeled probe we used represents an optimized HiNF-P-binding site that is based on the HiNF-P recognition site in Site II of the H4/n gene (5′-CTT CAG GTT TTC AAT CTG GTC CGA TAC T). The probe was incubated with HiNF-P-enriched nuclear extract from HeLa cells. Competition experiments were carried out with a 50-, 100-, 200-, or 400-fold excess of unlabeled oligonucleotides spanning the analogous regions in the promoters of distinct H4 genes.

RESULTS

mRNAs of Multiple Human Replication-dependent Histone H4 Genes Are Coordinately Induced in S Phase Cells—We predicted temporal and/or quantitative differences in expression of the 15 human histone H4 genes because of the heterogeneity in 5′-flanking sequences of the individual genes. Therefore, we initially examined the relative levels of expression of distinct H4 mRNAs during the cell cycle. Human T98G glioblastoma cells were serum-deprived for 72 h to induce quiescence and then stimulated to re-enter the cell cycle with serum. Synchronously cycling cells were harvested at 4, 21, and 25 h post-stimulation, corresponding to the G1, S, and G2 phases of the cell cycle, respectively, as determined by fluorescence-activated cell sorter analysis (Fig. 1A). Total RNA was examined by qRT-PCR. The relative contributions of individual histone H4 genes to the total H4 mRNA pool were determined with primer pairs specific to the unique 5′-untranslated region of each H4 gene (TABLE TWO). Primer pairs were tested against dilutions of genomic DNA to verify that the amplification efficiencies were similar. The mRNA levels of each histone H4 gene were up-regulated in S phase between 5- and 20-fold above the levels in G1 and G2 phases (Fig. 1B). This result is the first demonstration that the complement of H4 genes in the human genome is temporally and coordinately controlled at the G1/S phase transition and is consistent with previous studies that examined a limited number of representative human histone H4 genes (9–11, 13, 20, 22, 25, 28).

Our results also indicate that there are differences in the extent to which distinct H4 mRNA species are maximally expressed. We found that the H4/b, H4/d, H4/e, H4/j, H4/n, and H4/o genes account for >76% of the total histone H4 mRNA during S phase. H4/g, H4/h, and H4/i are moderately expressed, together representing 20% of the total histone H4 mRNA, whereas H4/a, H4/c, H4/k, H4/m, and H4/p contribute minimally to the total H4 mRNA pool (<5%). Thus, nine of the histone H4 genes generate the majority of mRNAs that are required to accommodate DNA replication and nascent chromatin assembly.

Differential Regulation of Histone H4 Gene Expression in Normal and Transformed Human Cells—The promoter elements of H4 genes interact with a multiplicity of transcription factors that respond to cell growth-related signaling pathways that may be differentially activated in normal versus tumor cells. Thus, we assessed whether the deregulation of signaling pathways in transformed and tumor cells affects the composition of H4 mRNA pools. We compared the expression patterns of individual human histone H4 genes in fetal liver, fetal colon, and IMR90 (normal diploid lung fibroblast) cells with those in four different tumor-derived human cell lines (i.e. HCT116 colorectal carcinoma cells, T98G glioblastoma cells, SaOS osteosarcoma cells, and HeLa cervical carcinoma cells).

We initially compared the RPA data with the qRT-PCR data in two human carcinoma lines (data not shown). The RPA and the qRT-PCR data were performed in completely independent experiments. The two assays showed consistent quantitative differences in the relative expression of individual histone H4 genes in asynchronous HeLa and SaOS cells lines. In additional studies, we used qRT-PCR as the primary method to determine the relative contributions of the individual histone H4 genes in various cell lines and tissues.

The most striking result is that the expression levels of the individual H4 genes are substantially different regardless of the cell type and that the relative levels are distinct from the theoretically expected contribution of ~7% per gene copy (expression of 15 genes is 100%) (Fig. 2, A and B, dashed lines). In three normal and four tumor-derived cell types, we found that the five most highly expressed genes (H4/d, H4/e, H4/j, H4/n, and H4/o) contribute the majority of H4 mRNAs to the total pool.
Differential Expression of Histone H4 Genes

The only quantitative difference is that these highly expressed genes contribute a disproportionate percentage of the total H4 mRNAs in tumor-derived cells compared with normal cells. For example, the above five highly expressed genes and six modestly expressed H4 genes (e.g. H4/a, H4/b, H4/c, H4/k, H4/m, and H4/p) contribute 55 and 18% in normal cells, whereas these contributions are 80 and 9% in tumor-derived cells, respectively. These differences can be attributed primarily to undetectable mRNA levels of a subset of H4 genes in tumor-derived cells (i.e. H4/b, H4/c, H4/k, and/or H4/m). These results suggest that modestly expressed H4 genes are silenced in tumor cells with concomitant compensatory responses by other more highly expressed H4 genes.

Basal H4 Promoter Activity Dictates H4 mRNA Accumulation—It is well documented that all histone H4 mRNAs contain a 3′-stem-loop structure (hairpin) that is necessary for 3′-end processing and mRNA stability (8). Because the hairpin sequences are completely conserved among all H4 genes, it is unlikely that the differential expression of H4 gene mRNAs is due to these post-transcriptional mechanisms. Rather, quantitative differences and temporal similarities in the expression of H4 gene copies may reflect the divergence and conservation of distinct 5′-transcriptional regulatory elements within H4 promoters. Therefore, we tested whether the observed changes in expression of H4 gene copies are related to differences in H4 gene promoter activity. We monitored luciferase reporter gene expression using promoters of all H4 genes located within the two major histone gene superclusters (human chromosomes 1 and 6) in multiple cell types. The promoters with the highest transcriptional activity in normal and tumor-derived cells include H4/d, H4/i, and H4/n and, by inference, also H4/e and H4/o, as their corresponding promoters are essentially duplicates of H4/d and H4/n, respectively (Fig. 3). These are the same H4 genes that exhibit high and low expression in normal cells compared with tumor-derived cells, respectively (Fig. 2). Furthermore, the activities of individual H4 promoters are more similar to the average in normal cells than in tumor-derived cells, mirroring the endogenous H4 mRNA expression patterns (Figs. 2 and 3). Thus, differential expression of H4 genes is transcriptionally mediated, as expected from the divergence in the overall organization of regulatory elements within H4 promoters.

The H4/n gene has been shown to be regulated by a highly conserved cell cycle regulatory domain (Site II) and an auxiliary module (Site I) that augments the transcription rate. Regions analogous to Site II of the H4/n gene are present in the other H4 genes and can easily be aligned (Fig. 4). However, there is considerable heterogeneity in promoter organization beyond Site II. Alignment of the 15 human Site II sequences revealed that there are four H4 genes (i.e. H4/a, H4/c, H4/k, and H4/l) that exhibit clear mismatches with the H4 subtype-specific consensus element, whereas a fifth H4 gene (i.e. H4/m) exhibits a single nucleotide deviation in the TATA box. These five genes generally have lower than
levels, although the presence of HiNF-P and/or RNA polymerase II does not guarantee high transcript levels. The genomic promoters of all H4 genes examined, with the exception of H4/a, H4/c, and H4/m, interact with HiNF-P in vivo. The genes that do not interact with HiNF-P are minimally expressed and do not contribute appreciably (<3%) to the overall H4 mRNA pool. This finding supports our previous (25) and present results that HiNF-P occupancy of H4 gene loci is necessary for optimal expression and that HiNF-P is a primary regulator of H4 gene transcription.

Highly Expressed H4 Genes Are Responsive to the Cyclin E/CDK2/p220NPAT/HiNF-P Signaling Pathway—HiNF-P activation of the H4/n gene depends critically on coactivation by the cyclin E/CDK2-responsive p220NPAT protein, and endogenous HiNF-P and p220NPAT levels are limiting for H4/n gene transcription (25). We assessed which of the multiple histone H4 promoters are regulated by this signaling pathway using reporter gene assays in which HiNF-P and p220NPAT were coexpressed (Fig. 7). For example, the H4/n gene is up-regulated 3-fold by HiNF-P or p220NPAT alone (data not shown) and is synergistically activated 10-fold or more when both proteins are coexpressed (Fig. 7), consistent with our previous observations (25). As expected, the H4/a, H4/c, and H4/m genes, which do not bind HiNF-P as determined by EMSA and ChIP-qPCR analysis (Figs. 5 and 6), do not respond to the HiNF-P/p220NPAT signaling pathway in HCT116, T98G, and SaOS cells. H4/a and H4/m genes respond modestly in IMR90 cells, which appear to be the exception. However, the induced activities of these two promoters remain quite low in IMR90 cells, perhaps reflecting an indirect effect of HiNF-P/p220NPAT. More importantly, of the 11 HiNF-P-dependent genes that we analyzed, seven are robustly up-regulated by p220NPAT signaling in all cell types and three in at least two cell types. The 11th gene (H4/g) responds qualitatively, but exhibits very low induced promoter activity. We conclude that all 11 HiNF-P-responsive H4 genes are also co-responsive to p220NPAT. Because these genes contribute to >95% of the total H4 mRNA pool (Fig. 2), it appears that the HiNF-P/p220NPAT signaling pathway is essential for coordinate control of histone H4 gene expression.

DISCUSSION

In this study, we have examined the similarities and differences among the 15 human histone H4 genes in cell cycle control, expression in normal and tumor-derived cells, proximal promoter activity, and promoter binding of transcription factors. Our results firmly establish coordinate cell cycle control of the majority of histone H4 gene expression as a result of the cyclin E/CDK2/p220NPAT/HiNF-P signaling pathway. Furthermore, a subset of these genes are sporadically silenced in tumor cell lines in comparison with normal cells. The unequal expression of H4 genes may reflect differences in position within nuclear architecture and/or distinctive promoter element organization. We have observed that histone H4 proximal promoter activities are consistent with the expression levels of endogenous H4 mRNAs. Finally, we have established that all highly expressed H4 genes are regulated by the cell cycle-dependent transcription factor HiNF-P.

Coordinate Induction of H4 Gene Expression during the G1/S Phase Transition—Many previous studies have focused on the cell cycle-dependent expression of total histone mRNAs as determined by northern blot analysis. A subset of these studies included assays capable of distinguishing one or more histone gene copies. However, as these studies were performed before the human genome project was completed, none of the previous findings permitted a complete analysis of the individual contribution of all members of the histone gene family.
It has been well established that the biosynthesis of histone H4 is mediated by multiple functionally expressed H4 genes (2, 9–11). By comprehensive analysis of the expression of the human histone H4 gene family in synchronized cells, one major finding of this study is that all mRNAs derived from the multiple human histone H4 genes are indeed simultaneously up-regulated when cells progress into S phase. Because post-transcriptional mechanisms operating on distinct H4 mRNAs are expected to be identical, this coordinate regulation is directly attributable to transcriptional mechanisms. Although our data now conclusively establish that coordinate regulation of the full complement of histone H4 genes does indeed occur, the results indicate that a surprisingly small number of genes account for the majority of H4 gene expression. We found that the promoters of the histone H4 genes are not regulated in an equivalent manner by cell cycle-driven signaling events. Interestingly, two of the histone H4 genes (H4/n and H4/o) that are most responsive to signals at the G1/S phase transition are recent duplicates.
Differential Expression of Histone H4 Genes

The Promoters of Histone H4 Genes Exhibit Variations in Promoter Element Organization—The burden of coordinate control of mRNA levels is shared by two main mechanisms, transcriptional induction and increased mRNA stability by 3'-endonucleolytic processing. However, it is well documented that all histone H4 3'-stem-loop structures are completely conserved. Therefore, the variation in the endogenous expression of histone H4 mRNAs in normal and carcinoma cells is directly attributable to the relative activities of the individual H4 gene promoters. Alignment of the H4 subtype-specific cell cycle elements revealed that some residues are necessary for HiNF-P binding and consequently cell cycle coordinate control of H4 expression at the G1/S phase transition. Furthermore, variation in the H4 subtype-specific element sequences causes some H4 genes to be poorly responsive to HiNF-P and results in lower contribution to the total pool of H4 mRNA. Thus, the variation in the contributions of the individual H4 genes is not likely due to mRNA stability, but rather to divergence of promoter elements and/or overall chromatin organization of histone gene clusters.

Highly Expressed H4 Genes Are Responsive to the HiNF-P Signaling Pathway—HiNF-P and RNA polymerase II occupancy of histone H4 promoters does not completely correlate with H4 gene mRNA contribution to the total H4 mRNA pool. Recruitment of RNA polymerase II to low expressing genes may indicate a stalled RNA polymerase II complex. Lack of promoter occupancy by HiNF-P is typical for low expressing H4 genes, albeit that Site II occupancy by HiNF-P does not assure high transcription rates. This finding suggests that HiNF-P is required for high level expression, but insufficient for maximal activation of cell cycle-dependent H4 gene transcription. HiNF-P activation of these genes depends on coactivation by the cyclin E/CDK2-responsive p220NPAT protein (25, 28), and we found that the H4 genes that are most responsive to the HiNF-P-p220NPAT complex are also the most highly up-regulated at the G1/S phase transition. Furthermore, our study shows that there is a correlation between HiNF-P occupancy, responsiveness of the histone H4 promoter, and maximal induction at the G1/S phase transition. Taken together, these findings support the concept that the HiNF-P-p220NPAT complex is the principal regulatory module that functionally links the coordinate regulation of histone H4 genes with the onset of DNA replication at the G1/S phase transition.

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