The Human H1 Histone Gene FNC16 Is Functionally Expressed in Proliferating HeLa S3 Cells and Is Down-regulated during Terminal Differentiation in HL60 Cells*  

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The human H1 histone gene FNC16 resides in a 2.7-kb EcoRI fragment present in a histone gene cluster that also contains one copy of each of the core (H2A, H2B, H3, and H4) histone genes. The cap site for FNC16 H1 mRNA is located 58 nucleotides upstream of the ATG translational start codon, and S1 nuclease protection analysis clearly distinguishes between correctly initiated FNC16 transcripts and transcripts from other nonidentical H1 histone genes. We have observed, using S1 analysis, that the FNC16 H1 histone gene is expressed in a replication-dependent manner in HeLa cells and is expressed in proliferating, but down-regulated in differentiated, HL60 cells. Similar results were found in HeLa S3 and HL60 cells for the cell cycle-dependent human H4 histone gene FO108. Nuclear extracts derived from HeLa S3 cells are capable of directing FNC16 H1 histone gene transcription in vitro. This finding is consistent with previous work that established at least two sites for protein-DNA interaction in vitro in the proximal promoter region of this gene. We have observed a difference in the extent to which the FNC16 H1 histone gene is expressed in HeLa S3 and proliferating HL60 cells, which suggests that this H1 gene is differentially regulated in various cell types. Although results reported for a potentially identical human H1 histone gene designated Hh8C (La Bella, F., Zhong, R., and Heintz, N. (1988) J. Biol. Chem. 263, 2115–2118) support differential regulation of human H1 genes in various cell types, their observations that the Hh8C gene is not expressed in HeLa cells and that the restriction patterns differ indicate that FNC16 and Hh8C are different H1 genes.

Histones are a complex family of highly conserved basic proteins responsible for packaging chromosomal DNA into nucleosomes (1-3). It is well established that the synthesis of replication-dependent histone proteins is functionally and temporally coupled to DNA replication (4, 5), unlike the synthesis of replication-independent variants (4, 6, 7). Human histone genes are a family of moderately reiterated sequences located on several chromosomes and arranged in polymorphic clusters (8-13). Replication-dependent human histone genes are coordinately expressed during S phase of the cell cycle, and this expression is coupled with DNA synthesis (14-19). Although the processes governing replication-independent human histone mRNA levels are not well understood, replication-dependent human histone mRNAs have been studied in detail, and their abundance is regulated at both the transcriptional and post-transcriptional levels (20, 21). Transcriptional regulation of several replication-dependent human histone genes studied thus far involves a 3-5-fold enhancement in the rate of transcription at the G1/S phase boundary with a return to basal level by mid to late S phase (17, 19, 22). Post-transcriptional regulation of these histone genes involves a rapid destabilization of histone transcripts toward the end of S phase or upon inhibition of DNA synthesis (15, 23).

In this study, we have established that a previously cloned human histone H1 gene designated FNC16 (11) is preferentially expressed during the S phase of the cell cycle in proliferating HeLa S3 and HL60 cells but is down-regulated in differentiated HL60 cells. S1 analysis clearly distinguishes between correctly initiated transcripts from the FNC16 H1 histone gene and transcripts of other H1 histone genes. Nuclear extracts derived from proliferating HeLa S3 cells are capable of directing FNC16 H1 histone mRNA synthesis in vitro. We have also observed a higher level of expression of the FNC16 H1 histone gene in proliferating HL60 cells than in HeLa S3 cells.

MATERIALS AND METHODS

Preparation of Hybridization Probes and Southern Analysis—Nick-translated probes (32P-labeled) were prepared as described by Maniatis et al. (24). DNA was transferred from agarose gels to nitrocellulose essentially as described by Southern (25) and hybridized to nick-translated probes as described previously (26).

HeLa S3 Cell Culture and Synchronization—HeLa S3 cells were grown in suspension culture in Joklik-modified Eagle’s minimal essential medium supplemented with 7% calf serum and synchronized by two successive treatments with 2 mM thymidine (27). The two 14-h 2 mM thymidine treatments were spaced 23 h apart (start to start). Rates of DNA synthesis were monitored by measuring the incorporation of [3H]thymidine into acid-precipitable material in a 30-min pulse (27). DNA synthesis inhibition was as previously described (17), using 1 mM hydroxyurea for 1 h, beginning 4 h after release from the second thymidine block. S phase cells (both hydroxyurea-treated and untreated) were harvested 5 h after release and G2 phase cells 11 h after release from the second thymidine block (28).

HL60 Cell Culture and Differentiation—HL60 cells were plated at a cell density of 2 × 103/3, grown for 24 h to a cell density of 3 × 109/mL, and harvested. HL60 cells to be differentiated were treated with 12-O-tetradecanoylphorbol-13-acetate at a final concentration of 16 nM, grown for 3 days, and harvested.

RNA Isolation and Analysis—RNA isolation was carried out as previously described (17). S1 analysis was carried out according to Berk and Sharp (29) as modified by Haegeman et al. (30).

Preparation of Sequencing Markers—Sequencing reactions were
carried out essentially as described by Maxam and Gilbert (31).

Nuclear Extract Preparation and in Vitro Transcriptions—Nuclear extracts were made from HeLa S3 cells 75 min after release from the double thymidine synchronization. The protocol used was essentially as described by Dignam et al. (32) except 0.6 M KCl was used in the nuclear extraction buffer. Protein concentrations were assayed by the Bradford method (33). In vitro transcription reactions were performed in a final volume of 25 μl containing 12 mM Hepes (pH 7.9), 12% glycerol, 0.3 mM dithiothreitol, 0.12 mM EDTA, 8 mM MgCl₂, 280 μM ATP, CTP, GTP, and UTP (Sigma), 0.5 μg of supercoiled plasmid template DNA, and approximately 54 μg of nuclear extract. Reactions proceeded for 30 min at 30°C and were stopped by the addition of 50 mM sodium acetate, 0.5% sodium dodecyl sulfate, and 50 μg/ml yeast tRNA. The nucleic acids were purified by repeated phenol/chloroform extractions. DNase I digestion was carried out to remove the template DNA, and the RNA was again purified by phenol/chloroform extractions. Analysis of the product RNA was by S1 protection as described by Berk and Sharp (29) as modified by Haegeman et al. (30).

RESULTS

The FNC16 Human Histone H1 Gene—The FNC16 human histone H1 gene used in this study was derived from a histone gene cluster that also contains one copy each of the core (H2A, H2B, H3, and H4) histone genes (11). A 1.46-kb EcoRI/PvuII fragment from this cluster was subcloned into pBR322 to generate the plasmid FNC16A (12). Southern blot analysis was carried out on the histone gene cluster containing the FNC16 H1 gene. Restriction with EcoRI demonstrates that the FNC16 H1 histone gene resides in a 2.7-kb EcoRI fragment whereas upon restriction with HindIII FNC16 sequences were observed in the 3.3-kb HindIII fragment (Fig. 1A). Portions of the H1 protein coding region and over 500 bp of the 5′-flanking region have been analyzed by dideoxy (34) and/or Maxam and Gilbert (31) sequencing (11, 35). Fig. 1B illustrates the FNC16 promoter region which contains several consensus promoter elements such as a highly symmetrical TATA box (bp -92 to -79) and a PuCCAAT box (bp -112 to -107) (36). The latter has been shown to act as a binding site for a two-component CCAAT box binding protein designated HNF-B (37). La Bella et al. (38) have reported that the FNC16 H1 histone gene may be identical to the Hh8C H1 histone gene; however, there are discrepancies in the Southern analysis.

The FNC16 Human Histone H1 Gene Is Expressed in a DNA Replication-dependent Manner in HeLa S3 Cells—S1 protection analysis was carried out using a 731-nucleotide Stul/PvuII probe, derived from pFNC16 (Fig. 1B), 5′-end labeled at the Stul site. HeLa S3 RNA, isolated from exponentially growing cultures and cultures at the peak of S phase, protected a 260-nucleotide fragment representing correctly initiated FNC16 H1 histone transcripts (Fig. 2A). Very low levels of transcripts were detected in RNA isolated from cells outside of S phase or after inhibition of DNA synthesis with hydroxyurea (Fig. 2A). The cap site for FNC16 H1 mRNA is located 58 bp upstream of the ATG translational start codon. S1 analysis clearly distinguishes between correctly initiated FNC16 transcripts and transcripts from other nonidentical H1 histone genes. This analysis (Fig. 2A) demonstrates that the FNC16 human H1 histone gene is expressed in proliferating HeLa S3 cells in a replication-dependent manner. In addition, S1 protection analysis was carried out using a 760-nucleotide Neol/HindIII probe, derived from a known replication-dependent human H4 histone gene FO108 (39), 5′-end-labeled at the Neol site. The 280-nucleotide protected fragment represents properly initiated transcripts from FO108 and shows a cell cycle expression pattern similar to that of the FNC16 H1 gene (Fig. 2B).

In addition the FNC16 H1 gene was analyzed using an in vitro transcription system. We observed that nuclear extracts derived from S phase HeLa S3 cells are capable of directing proper FNC16 H1 histone gene transcription in vitro (Fig. 3). Similar results were observed for the FO108 H4 gene (Fig. 3). The S1 nuclease analysis and in vitro transcription results are consistent with and supportive of previous work carried out in this laboratory indicating that this H1 gene is actively transcribed in HeLa S3 cells (35, 37).

Expression of the FNC16 Human H1 Histone Gene in Proliferating and Differentiated HL60 Cells—In order to address the levels of FNC16 human H1 histone gene expression in other cell types, we examined a human promyelocytic leukemia cell line, HL60. HL60 is a pluripotent cell line that can be induced to differentiate, by a variety of agents, to mono-

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1 The abbreviations used are: Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; kb, kilobase(s); bp base pair(s); Pu, purine.

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FIG. 1. Southern blot analysis, restriction and sequence data for the XHHG 415 histone gene cluster. A, Southern blot analysis of XHHG 415 (45). DNA from X415 was digested with the enzymes indicated, electrophoretically separated on a 1% agarose gel, transferred to nitrocellulose, and hybridized to 32P-labeled H1 cDNA-pBR322 (a gift from J. R. E. Wells, University of Adelaide). Kbp, kilobase pairs. B, restriction map of X415 and partial sequence data for the H1 histone subclone FNC16. The X415 restriction map shows the position of the EcoRI and HindIII restriction sites (see A) surrounding the H1 histone gene FNC16. Depicted below the map is the PvuII/Stul fragment used for S1 analysis (Fig. 2A) and the DNA sequence of the first 127 bp upstream of the translational start codon of the gene (+1) showing the start of mRNA synthesis (CAP).
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Fig. 2. S1 mapping of total RNA from HeLa and HL60 cell lines. 14 μg of total RNA from log HeLa cells (L) and 25 μg of total RNA from S phase HeLa cells (both hydroxyurea-treated (H) and untreated (U)), G1 phase HeLa cells (Gl), proliferating HL60 cells (P), and differentiated HL60 cells (D) were used for S1 analysis. A, S1 analysis was carried out using a 731-nucleotide (nt) StuI/PvuII probe derived from the human H1 histone gene FNC16 (Fig. 1A). 5'-end labeled at the StuI site. G + A, C + T, and C sequencing reactions were performed on the S1 probe to function as markers. The size of the H1-protected fragment is indicated to the right of the autoradiogram.

In vitro transcription of the FNC16 H1 gene and FO108 H4 gene. In vitro transcription reactions were carried out with 54 μg of HeLa S3 phase-specific nuclear extract on equal amounts of supercoiled plasmid templates, FNC16 and FO108. Transcripts were analyzed by nuclease S1 mapping. Arrows refer to properly initiated transcripts.

cyte- or granulocyte-like cells (40-42). We have isolated RNA from proliferating HL60 cells and from HL60 cells that have been induced to differentiate to monocyte-like cells by the phorbol ester 12-O-tetradecanoylphorbol-13-acetate (har- vested 4 days after induction). We observed that FNC16 mRNAs were present in proliferating cells, whereas barely detectable levels of FNC16 mRNAs were present when RNA from differentiated HL60 cells was used for S1 protection analysis (Fig. 2A, lanes P and D). As observed in HeLa cells, the cap site for FNC16 H1 mRNA is located 58 nucleotides upstream of the ATG translational start codon (Figs. 1B and 2A). Similar results were observed in proliferating and differentiated HL60 cells when the H4 histone probe, described in the preceding section, was used in an S1 protection analysis experiment (Fig. 2B, lanes P and D).

Densitometric analysis, adjusted for the amount of RNA loaded per lane (Fig. 1A and B), reveals that although the FO108 H4 histone gene is expressed to the same extent in both HeLa and proliferating HL60 cells, the FNC16 H1 histone gene shows a differential expression in the two cell lines. Our results demonstrate that the FNC16 H1 histone gene is expressed at a two-fold higher level in proliferating HL60 cells than in proliferating HeLa cells (Fig. 2A). These results are consistent with the observation by La Bella et al. (38) that the Hh8C H1 histone gene is differentially expressed in KB and HeLa cells. However, our results show that the FNC16 H1 histone gene is expressed in HeLa S3 cells (although at a lower level than in proliferating HL60 cells) whereas La Bella et al. (38) observed no Hh8C expression in HeLa cells.

DISCUSSION

The FNC16 and Hh8C H1 histone genes, as indicated by La Bella et al. (38), may be identical. The published restriction sites in the Hh8C H1 gene are quite homologous with the known restriction sites in the FNC16 H1 histone gene (11, 35) although some differences do exist. La Bella et al. (38) report a 135-nucleotide protected fragment when total RNA extracted from KB cells (a human adenocarcinoma-derived cell line) was hybridized to a 5'-end labeled NcoI fragment of pHh8C and subjected to S1 protection analysis, indicating the presence of a NcoI restriction site 135 bp downstream from the Hh8C cap site. Our own S1 protection analysis (Figs. 1B and 2A) together with our published sequence analyses (11, 35) places the cap site of the FNC16 H1 histone gene 143 nucleotides upstream from the NcoI restriction site, only 8 nucleotides more than that reported for the Hh8C H1 gene (38). When taking into account that labeled restriction fragments were used as markers to size the Hh8C H1-protected fragment discussed above (38), an 8-nucleotide difference in the placement of the FNC16 and Hh8C H1 cap sites is well within experimental error. However, while many reported similarities do exist between the structures of the FNC16 and Hh8C H1 histone genes, discrepancies are present between the Southern blot data of the two genes (38) and Fig. 1A). The Hh8C H1 gene promoter sequence has been reported to hybridize with 3.3- and 1.8-kb EcoRI and 2.7-kb HindIII genomic fragments (38) whereas Southern blot analysis (Fig. 1A) demonstrates that the FNC16 H1 gene resides within 2.7-kb EcoRI and 3.3-kb HindIII cloned genomic fragments. The FNC16 and Hh8C H1 histone genes may each be derived from similar but independent clusters, which could explain both their differences and similarities.

We demonstrated, using S1 analysis, that the FNC16 H1 histone gene is expressed in a replication-dependent manner in HeLa S3 cells. When hydroxyurea was used to inhibit DNA synthesis during S phase we observed a reduction of FNC16 H1 message to barely detectable levels. It should be noted that S1 analysis reflects FNC16 mRNA levels and does not determine to what extent transcriptional or post-transcrip-
tional regulatory events contribute to these mRNA levels. However, in studies on several other replication-dependent human histone genes (15, 23), it has been shown that the rapid loss of histone message upon inhibition of DNA synthesis can be attributed mainly to post-transcriptional regulatory control. The reduction in FNC16 H1 mRNA levels in HeLa cells upon inhibition of DNA synthesis would be consistent with the post-transcriptional regulatory control observed for other human histone genes (15, 23). Transcriptional regulation, in HeLa cells, of several replication-dependent human histone genes studied thus far involves a 3–5-fold enhancement in the rate of transcription at the G1/S phase boundary with a return to a basal level by mid to late S phase (17, 19).

Nuclear extracts derived from HeLa S3 cells are capable of directing FNC16 H1 histone mRNA synthesis in vitro. This finding not only supports our S1 analysis data but is consistent with the presence of a mosaic H1 histone CCAAT box binding protein (HiNF-B) in these extracts (35, 37). HiNF-B was seen by La Bella et al. (38) between KB cells and HeLa cells. However, in studies on several other replication-dependent histone genes, the differences in the structures of the FNC16 and Hh8C genes and the structural differences discussed earlier support the possibility that the FNC16 and Hh8C H1 histone genes are allelic. However, we have clearly demonstrated that the human H1 gene FNC16 is functionally expressed in proliferating HeLa S3 cells and down-regulated upon terminal differentiation in HL60 cells.

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