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A Human Histone H2B.1 Variant Gene, Located on Chromosome 1, Utilizes Alternative 3′ End Processing

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Abstract A variant human H2B histone gene (GL105), previously shown to encode a 2300 nt replication *independent mRNA*, has been cloned. We demonstrate this gene expresses alternative mRNAs regulated differentially during the HeLa S_3 cell cycle. The H2B-GL105 gene encodes both a 500 nt cell cycle dependent mRNA and a 2300 nt constitutively expressed mRNA. The 3′ end of the cell cycle regulated mRNA terminates immediately following the region of hyphenated dyad symmetry typical of most histone mRNAs, whereas the constitutively expressed mRNA has a 1798 nt non-translated trailer that contains the same region of hyphenated dyad symmetry but is polyadenylated. The cap site for the H2B-GL105 mRNAs is located 42 nt upstream of the protein coding region. The H2B-GL105 histone gene was localized to chromosome region 1q21-1q23 by chromosomal in situ hybridization and by analysis of rodent-human somatic cell hybrids using an H2B-GL105 specific probe. The H2B-GL105 gene is paired with a functional H2A histone gene and this H2A/H2B gene pair is separated by a bidirectionally transcribed intergenic promoter region containing consensus TATA and CCAAT boxes and an OTF-1 element. These results demonstrate that cell cycle regulated and constitutively expressed histone mRNAs can be encoded by the same gene, and indicate that alternative 3′ end processing may be an important mechanism for regulation of histone mRNA. Such control further increases the versatility by which cells can modulate the synthesis of replication-dependent as well as variant histone proteins during the cell cycle and at the onset of differentiation.

Key words: histone variant, alternative 3′ processing, growth regulation

Human histone genes are a family of moderately reiterated sequences arranged in polymorphic clusters on several chromosomes [Green et al., 1984; Sierra et al., 1982; Stein et al., 1984; Tripputi et al., 1986; Zhong et al., 1983; Heintz et al., 1981]. Replication-dependent histone genes do not contain introns and are transcribed into non-polyadenylated mRNAs having a short 5′ leader sequence and a short 3′ trailer sequence containing a characteristic 3′ stem-loop motif [reviewed in Stein et al., 1984]. In contrast, replication independent histone genes are structurally more complex. They may contain introns [Brush et al., 1985; Wells and Kedes, 1985; Wells et al., 1987], and their mRNAs, which are polyadenylated, frequently contain longer 5′ leaders or 3′ trailers than their replication-dependent counterparts [Brush et al., 1985; Wells and Kedes, 1985; Wells et al., 1987; Chaubet et al., 1988; Ernst et al., 1987; Harvey et al., 1983; Hatch and Bonner, 1988, 1990; Mannironi et al., 1989; Hochhuth and Doenecke, 1990].

The expression of replication-dependent histone genes is coupled with DNA synthesis and the abundance of these mRNAs is regulated at both the transcriptional and post-transcriptional levels [reviewed in Schümperli, 1988; Marzluff and Pandey, 1988; Stein and Stein, 1984]. Transcriptional regulation involves a two- to fivefold increase in the rate of transcription at the G1/S phase boundary, with a return to basal level by mid to late S phase [Schümperli, 1988; Marzluff and Pandey, 1988; Stein and Stein, 1984]. Post-transcriptional regulation of these histone genes involves a rapid and selective destabilization of histone mRNAs toward the end of S phase or in response to inhibition of DNA

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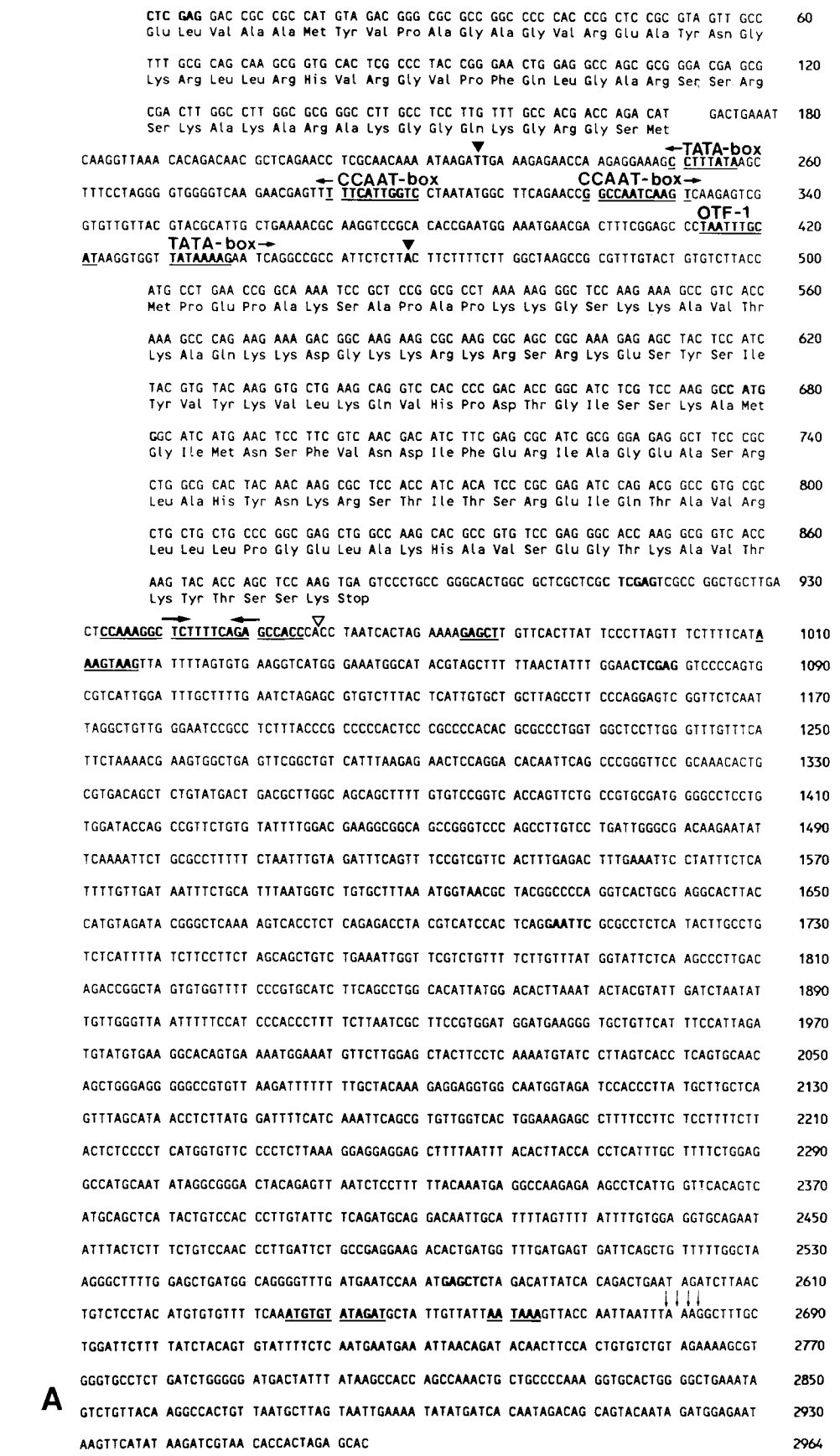

Fig. 1. (Figure continued on page 376.)

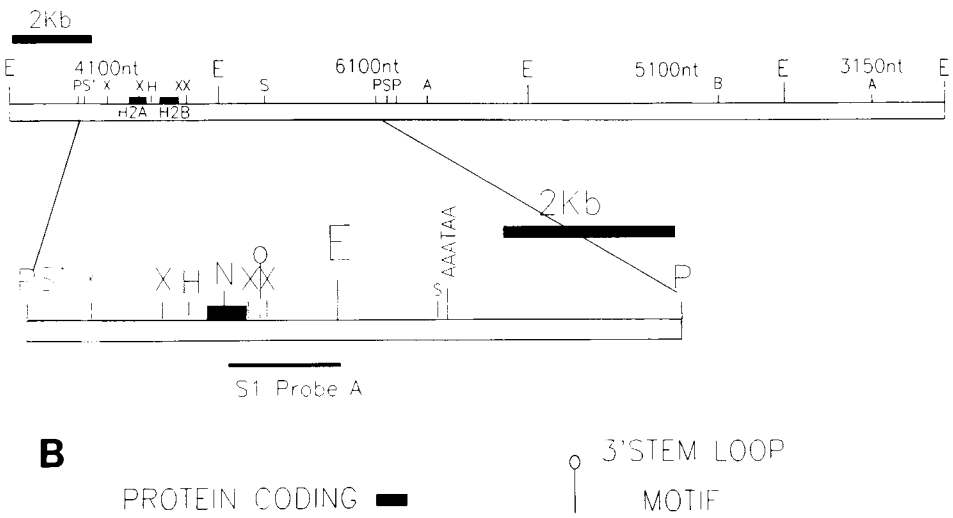

Fig. 1. **A:** *Nucleotide* and deduced amino acid sequences of the human H2B and H2A histone genes from the genomic clone λHHG5E. Nucleotides are numbered continuously from Glu^{56} of the H2A gene through to the 3′ trailer region of the H2B gene. The locations of the H2A and H2B cap sites are marked by *filled triangles* at nt 227 and nt 459, respectively. The TATA-boxes and CCAAT-boxes for the H2A and H2B genes, as well as an OTF-1 site, are displayed in underlined bold font. The 3′ untranslated region of the H2B gene contains the conserved histone 3′ stem-loop motif, indicated by arrows and displayed in underlined bold font, as well as a poly (A) addition sequence located 1778 nt downstream from the protein stop codon, also displayed in underlined bold font. The 3′ terminus of the 500 nt cell cycle dependent H2B mRNA is indicated by an empty triangle at nt 958 and the 3′ terminus region of the 2222 nt cell cycle independent H2B mRNA is indicated by arrows at nt 2679–nt 2682. Consensus splice site sequences at nt 1010 and nt 2635 are illustrated by underlined bold font. **B:** Restriction map of the genomic clone λHHG5E. The locations of the H2A and H2B protein coding regions within the λHHG5E insert are illustrated by hatched boxes located within the 4100 nt *Eco* RI fragment. The 6100 nt *Pst* I fragment containing the entire H2B mRNA coding region and the conserved histone 3′ stem-loop motif is expanded below the λHHG5E insert. Probe A used for S1 nuclease protection analysis is represented below the *Pst* I fragment. Restriction endonuclease sites are as follows: A, *Asp* 718; B, *Bam* HI; E, *Eco* RI; H, *Hind* III; N, *Nco* I; P, *Pst* I; S, *Sac* I; S′, *Sph* I; X, *Xho* I.

synthesis [Morris et al., 1991; Ross et al., 1986]. In contrast, the mRNA levels of replication independent histone genes are not regulated in the same cell cycle dependent manner as their replication-dependent counterparts [Ernst et al., 1987; Harvey et al., 1983; Bird et al., 1985; Brown et al., 1985; Carrino et al., 1987; Jarvis and Marzluff, 1989; Shalhoub et al., 1989; Collart et al., 1991].

Although several replication-dependent human histone H2B genes and one pseudogene have been described [Sierra et al., 1982; Zhong et al., 1983; Marashi et al., 1984; Plumb et al., 1983; Prokopp, 1984], no replication independent human H2B genes have been reported. However, we have recently isolated and characterized a human H2B histone cDNA which is reciprocally expressed in relation to replication-dependent H2B histone genes during HL60 cell differentiation [Collart et al., 1991]. Here we describe the cloning and characterization of this variant human H2B histone gene (GL105). This gene expresses alternative mRNAs that are regulated differentially during the HeLa S_3 cell cycle. The structural and organizational components of this H2B histone gene include consensus elements for regulation of DNA synthesis dependent histone genes as well as elements typical of constitutively expressed histone genes. The complex expression pattern of this H2B histone gene may be of functional importance in providing H2B.1 histone proteins in response to modifications of chromatin architecture that accompany alterations in gene expression.

MATERIALS AND METHODS

DNAs and Cloning

The 6100 nt *Pst* I fragment, 4100 nt *Eco* RI fragment, and 6100 nt *Eco* RI fragment from λHHG5E (Fig. 1B) were isolated and cloned into pUC19 and the resulting subclones termed pGL105, pGL101, and pGL102, respectively. The genomic clone λHHG5E was isolated using a unique sequence probe from the 3′ non-translated region of a human H2B histone cDNA which we have recently isolated and characterized [Collart et al., 1991].

Preparation of Radiolabeled DNA

Random oligonucleotide primed labeling was carried out using the PRIME-IT random primer kit purchased from Stratagene, according to conditions described by the manufacturer. DNA fragments used as probes for S1 hybridization analysis were treated with calf intestinal alkaline phosphatase [Maniatis et al., 1982] followed by 5′ end labeling with T4 polynucleotide kinase and γ-^{32}P-ATP [Maniatis et al., 1982]. Alternatively, DNA fragments were 3′ end labeled with T4 DNA polymerase and α-^{32}P-dCTP [O'Farrell, 1981].

EMBL4 Genomic DNA Library Screening

An adult human lymphocyte genomic library, containing *Sau3A* I partially digested inserts of between 15 and 20 Kb in λ EMBL4, was generously provided by Dr. Paul Dobner (University of Massachusetts Medical Center, Department of Molecular Genetics and Microbiology). A complete equivalent of this library (8×10^5 phage) was hybridized with a ^{32}P-labeled H2B 3′ nontranslated probe (871 nt *Eco* RI/*Sac* I fragment isolated from λHHC289) [Collart et al., 1991].

DNA Sequencing

Dideoxy sequencing [Sanger et al., 1977] was carried out using a Sequenase 2.0 DNA sequencing kit, purchased from United Stated Biochemical Corp., according to conditions described by the manufacturer.

Isolation and Purification of Eukaryotic Genomic DNA

Eukaryotic genomic DNA was isolated and purified as described by Blin and Stafford [1976]. Cellular DNAs used for chromosome localization were isolated as described [Huebner et al., 1985, 1986].

Southern Blot Analysis

Restriction endonuclease digested DNAs were separated by electrophoresis through agarose gels and transferred to Zeta-Probe blotting membrane (Bio-Rad) as described by Reed and Mann [1985]. Hybridization conditions were as previously described [Collart et al., 1991]. The hybridized membranes were then washed three times as follows: 1) in 100 ml 100 mM potassium phosphate (pH 7.4) for 30 min at room temperature; 2) in 100 ml 100 mM potassium phosphate (pH 7.4) for 30 min at 60°C; 3) in 100 ml 1 × SSC and 0.2% SDS (w/v) for 30 min at 60°C. ^{32}P-labeled probes used for Southern analysis of the genomic H2B clone λHHG5E were as follows: H1, 1445 nt *Pst* I/*Eco* RI fragment from pFNC16 [Plumb et al., 1983]; H2A, 980 nt *Sst* I fragment from pFF435 [Marashi et al., 1984]; H2B, 340 nt *Eco* RI/*Xho* I fragment from λHHC289 [Collart et al., 1991]; H3, 2100 nt *Eco* RI fragment from pST519 [Marashi et al., 1986]; H4, 1740 nt *Hind* III/*Eco* RI fragment from pFO108A [Plumb et al., 1983].

S1 Nuclease Protection Analysis

Isolation of total cellular mammalian RNA was carried out as previously described [Plumb et al., 1983]. Poly A$^+$ RNA was selected using a single pass over oligo dT-cellulose (Sigma) as described by Maniatis et al. [1982]. S1 analysis was carried out according to Berk and Sharp [1978] as modified by Haegeman et al. [1979]. S1 analysis was carried out under conditions which do not favor probe reannealing. Autoradiography was performed using pre-flashed Kodak XAR-5 X-ray film and a Cronex Lightning Plus screen at −70°C.

Chromosomal In Situ Hybridization

Chromosomal in situ hybridization was performed as described [Cannizzaro et al., 1990]. Probe DNA (871 nt *Eco* RI/*Sac* I fragment isolated from λHHC289 [Collart et al., 1991]) was labeled with ^{3}H to a specific activity of $2–4 \times 10^7$ dpm/μg. Slides containing metaphase chromosomes from normal male (46 XY) peripheral blood lymphocytes were aged at 4°C for 7–10 days and pretreated with ribonuclease A (Sigma) for 1 h at 37°C. The chromosomal DNA was denatured at 70°C for 2 min in a 70% formamide:2 × SSC mixture (pH 7.0). The probe DNA was denatured in a hybridization mixture containing 50% formamide, 2 × SSC, and 10% dextran sulfate (pH 7.0). Hybridization was carried out at 37°C overnight. After rinsing at 39°C in 3 changes of 50% formamide: 2 × SSC and 5 changes of 2 × SSC, slides were dehydrated, air dried, subjected to autoradiography, and banded with Wright's-Giemsa stain solution mixed with 1–3 parts of a pH 9.2 borate buffer [Cannizzaro et al., 1990].

Cell Culture and Synchronization

HeLa S_3 cell culture and synchronization were carried out as previously described [Collart et al., 1991; Stein and Borun, 1972]. Isolation, propagation, and characterization of parental human and murine cells as well as the human-mouse somatic cell hybrids were carried out as previously described [Huebner et al., 1985, 1986]. The human chromosomes (either partial or complete chromosomes) retained in the rodent-human hybrids used in determining the chromosomal location of the H2B-GL105 gene are schematically illustrated in Figure 5.

RESULTS

Cloning and Nucleotide Sequence of the H2B-GL105 Gene

To investigate the relationship between gene structure and the regulation of variant H2B mRNA levels, we used a non-coding 3′ fragment from an H2B cDNA clone (λHHC289) previously isolated in our laboratory [Collart et al., 1991] to probe a λ human genomic library. A positive clone (λHHG5E) corresponding to the λHHC289 cDNA was selected for further analysis. The sequence of a 2964 nt portion of λHHG5E was determined by dideoxy sequence analysis and revealed the presence of an H2A gene upstream of the H2B gene. The nucleotide and deduced amino acid sequences of the human histone H2B-GL105 and a portion of the adjacent H2A histone GL101 are presented in Figure 1A. The λHHG5E H2B gene encodes a protein identical to the somatic H2B.1 mouse protein in key amino acid positions (Ser^{14}, Ala^{21}, Asp^{25}, Lys^{27}, Gly^{60}, Asp^{68}, Gly^{75}, Glu^{76}), as reviewed by Zweidler [1980]. A restriction map of the genomic clone λHHG5E demonstrating the locations of the H2A and H2B protein coding regions is shown in Figure 1B. The λHHG5E H2A/H2B gene pair share an intergenic region with a spacing of 329 nt between their protein coding regions and 231 nt between their cap sites (Fig. 1A). The H2A and H2B genes each contain consensus TATA and CCAAT boxes with the H2B promoter also including an OTF-1 element (Fig. 1A). The H2B-GL105 gene includes a 3′ non-translated region containing the conserved histone 3′ stem-loop motif as well as a poly (A) addition sequence located 1778 nt downstream from the protein stop codon and consensus splice site sequences at nt 1010 and nt 2635 (Fig. 1A,B). Thus, this H2B.1 histone gene contains structural elements commonly found in cell cycle dependent histone genes as well as those reported for constitutively expressed histone genes. The H2B-GL105 gene is completely identical to the λHHC289 cDNA [Collart et al., 1991] and apparently contains no intervening sequences.

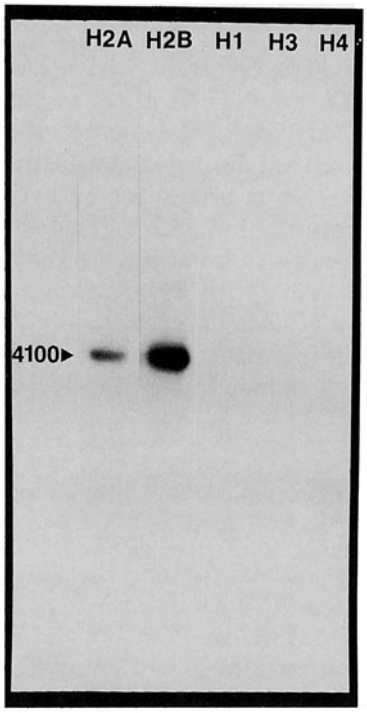

Fig. 2. Southern blot analysis of the genomic clone λHHG5E with ^{32}P-labeled probes for histones H1, H2A, H2B, H3, and H4. Five lanes of a 0.8% agarose gel were loaded with 3.0 μg *Eco* RI restricted λHHG5E DNA each and the fragments separated electrophoretically. The DNA was transferred to Zeta-probe nylon membrane and each lane individually hybridized to either a histone H2A, H2B, H1, H3, or H4 ^{32}P-labeled DNA probe as indicated at the top of the figure (see Materials and Methods for a detailed description of the probes). The filled triangle indicates the position of the 4100 nt λHHG5E fragment containing the H2A and H2B genes.

Genomic Organization of λHHG5E

To determine if apart from the H2A/H2B pair additional histone genes are located within the λHHG5E genomic segment, we carried out Southern blot analysis of *Eco* RI digested λHHG5E DNA (Fig. 2) using ^{32}P-labeled probes for histones H1, H2A, H2B, H3, and H4. The results confirmed the presence of an H2A and an H2B histone gene within the 4100 nt *Eco* RI fragment of λHHG5E, but no additional histone genes were detected (Fig. 2). However, this does not exclude the possibility that additional his-

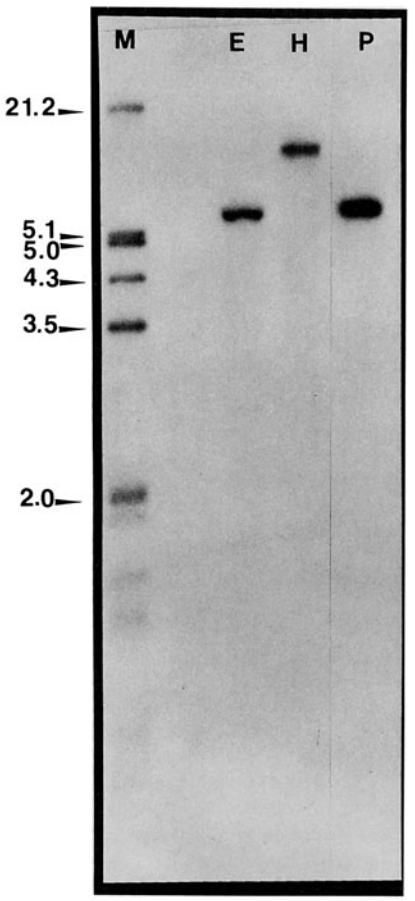

Fig. 3. Southern blot analysis of HeLa genomic DNA with a H2B-GL105 probe. Each lane of a 0.8% agarose gel was loaded with 10 μg of HeLa genomic DNA restricted with *Eco* RI **(E)** *Hind* III **(H)** or *Pst* I **(P)**. **Lane M** was loaded with 3′-labeled λ *Eco* RI/*Hind* III marker DNA. Following separation of the DNA fragments electrophoretically, the DNA was transferred to Zeta-probe nylon membrane and hybridized to a ^{32}P-labeled probe (871 nt *Eco* RI/*Sac* I fragment isolated from λHHC289) that spans the distal half of the H2B-GL105 3′ non-translated trailer (see Fig. 1, nt 1706–nt 2577 and Collart et al. [1991]). The sizes of the marker fragments are indicated to the left of the autoradiogram in Kb.

tone genes may be within proximity but outside the boundaries of the λHHG5E genomic segment.

To evaluate if the H2B-GL105 gene is present as a single copy within the genome, we carried out Southern blot analysis of HeLa genomic DNA restricted with either *Eco* RI, *Hind* III, or *Pst* I (Fig. 3) using a ^{32}P-labeled probe (871 nt *Eco* RI/*Sac* I fragment isolated from λHHC289 [Collart et al., 1991]) that spans the distal half of the H2B-GL105 3′ non-translated trailer (Fig. 1; nt 1706–nt 2577). As can be seen in Figure 3, the 3′ non-translated probe hybridized to a single restriction fragment from each digest, indicating that it is present in a single copy in the human genome.

Chromosomal Localization of the H2B-GL105 Gene

To determine the chromosomal position of the H2B-GL105 gene, a panel of DNAs from rodent-human somatic cell hybrids carrying overlapping subsets of human chromosomes was tested for the presence of the H2B-GL105 locus by Southern blot analysis as shown in Figure 4. Hybridization of the *Pst* I digested human DNA (Fig. 4, lane 2) revealed a 6100 nt *Pst* I fragment which was also detected in hybrids 77–31, Ph124, and BD3 (Fig. 4, lanes 8, 10, 18); mouse DNA (Fig. 4, lane 1) and all other hybrid DNAs were negative for the H2B-GL105 locus. The three positive hybrids carry only human chromosomes 1 and X in common; many of the negative hybrids retain the human X chromosome; additionally all H2B-GL105 negative hybrids were also negative for chromosome 1 (see Fig. 5 for the chromosomal content of the hybrids). Thus, the presence of the H2B-GL105 histone gene in

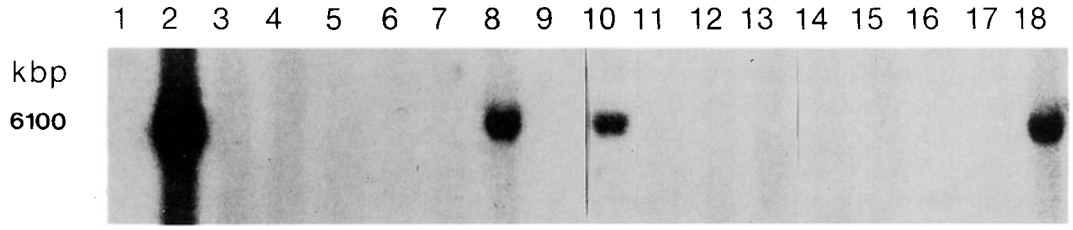

Fig. 4. Southern blot analysis of DNAs from mouse-human somatic cell hybrids probed with a ^{32}P-labeled DNA fragment from the human histone gene H2B-GL105. An 0.8% agarose gel was loaded with ~ 10 μg/lane DNA from mouse **(lane 1)**, human **(lane 2)**, hybrid 8c **(lane 3)**, hybrid PB5-1 **(lane 4)**, hybrid 442S **(lane 5)**, hybrid cl31 **(lane 6)**, hybrid c121 **(lane 7)**, hybrid 77-31 **(lane 8)**, hybrid cl17 **(lane 9)**, hybrid Phl24 **(lane 10)**, hybrid AA3 **(lane 11)**, hybrid 3a **(lane 12)**, hybrid N9 **(lane 13)**, hybrid S3 **(lane 14)**, hybrid AB3 **(lane 15)**, hybrid C4 **(lane 16)**, hybrid G5 **(lane 17)**, and hybrid BD3 **(lane 18)** cleaved with *Pst* I, separated electrophoretically, transferred to nylon membrane, and hybridized to the same H2B-GL105 probe used in Fig. 3. Chromosomes retained by the hybrids are depicted in Fig. 5.

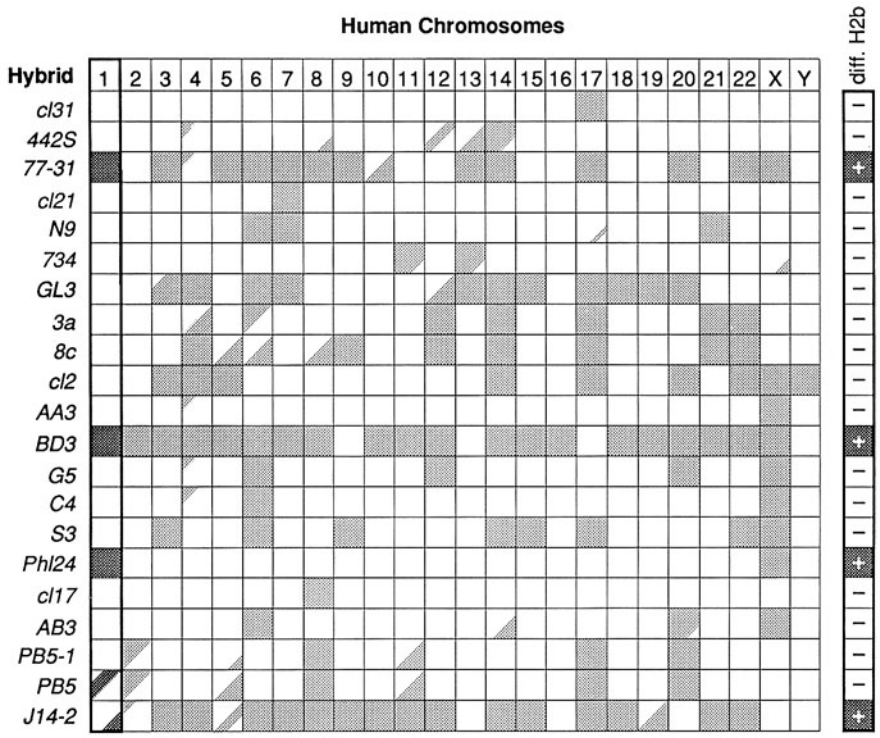

Fig. 5. Presence of the human histone gene H2B-GL105 in a panel of 21 rodent-human hybrids. A shaded box indicates that the hybrid named in the left column contains the chromosome indicated in the upper row; a shaded triangle in the lower right corner of a box indicates the presence of the long arm of the chromosome (or part of the long arm represented by an even smaller fraction of shading); a shaded triangle in the upper left corner of a box indicates the presence of the short arm (or partial short arm) of the chromosome; and an empty box indicates the absence of the chromosome listed above the column. The column for chromosome 1 is boldly outlined and stippled to highlight correlation of the presence of this chromosome with presence of the H2B-GL105 gene. The pattern of retention of the H2B-GL105 gene in the panel is shown in the column to the right of the figure where the presence of the gene in the hybrid is indicated by a stippled box enclosing a plus sign and absence of the gene is indicated by an open box enclosing a minus sign.

hybrid cells correlates with the presence of human chromosome 1 as summarized in Figure 5.

Additionally, the H2B-GL105 locus was absent in a hybrid, PB5, which retains most of the short arm [Haluska et al., 1988] of chromosome 1 and was present in a hybrid, J14-2, which retains portions of the long arm of chromosome 1 but not the short arm (data not shown, but summarized in Fig. 5). Thus, somatic cell hybrid analyses suggest localization of H2B-GL105 on the long arm of chromosome 1.

To confirm and refine the location of the H2B-GL105 gene on chromosome 1, in situ hybridization was carried out on metaphase chromosomes from a normal male subject (46, XY). A total of 100 metaphases were analyzed and 281 grains were hybridized to chromosomes. Of these, 11% (31/281) were hybridized selectively to the long arm of chromosome 1 (1q). The majority of grains on 1q were found within the q21–q23 subregion containing 61.3% (19/31) of the grains hybridized to 1q. Figure 6 illustrates the hybridization of the H2B-GL105 gene on chromosome 1 with the primary site of localization to 1q21–1q23. The in situ hybridization has, therefore, regionally localized the H2B-GL105 gene to the proximal region of 1q within q21–q23. Interestingly, the human histone cluster HHG41, containing several histone genes including an H4 (FO108) which is cell cycle dependent and regulated in a reciprocal fashion to the H2B-GL105 gene [Stein et al., 1984; Shalhoub et al., 1989; Collart et al., 1991], has also been localized to the same region of chromosome 1 [Green et al., 1984].

Mapping of the H2B-GL105 mRNA 3′ Termini

We have previously demonstrated that the H2B-GL105 gene encodes a 2300 nt cell cycle independent poly A^+ mRNA containing consen-

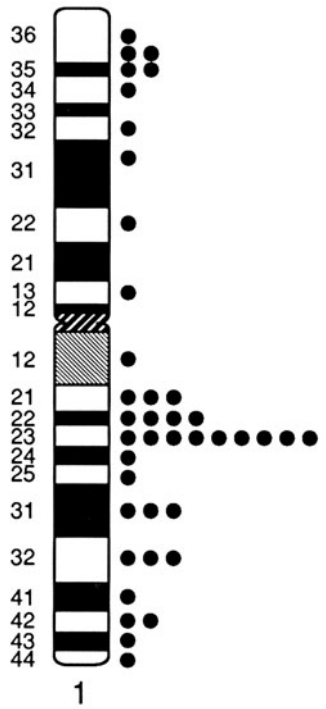

Fig. 6. Regional localization of the human histone gene H2B-GL105 on chromosome 1. The in situ hybridization of the H2B-GL105 probe to chromosome 1 is illustrated diagrammatically. The results indicate that 1q21–1q23 is the location of this gene.

sus splice site sequences [Collart et al., 1991]. These splice site sequences provide the cell with a potential mechanism by which alternative mRNAs may be expressed from a single gene. Alternatively, the conserved histone 3′ stem-loop motif, present in the 3′ non-translated region of the H2B-GL105 gene (Fig. 1A,B), provides an additional avenue for the production of alternative mRNAs. To investigate the 3′ terminus of the H2B-GL105 mRNA, S1 nuclease protection analysis was carried out on total cellular (Fig. 7, lane 1) and poly A^+ (Fig. 7, lane 2) RNA isolated from proliferating HeLa cells using a 3′ end labeled probe derived from the λHHG5E H2B gene (Fig. 1B, S1 probe A). A 195 nt protected fragment mapping approximately to the H2B-GL105 stop codon and a 278 nt protected fragment mapping to the consensus histone mRNA terminus [Strub et al., 1984; Birnstiel et al., 1985; Strub and Birnstiel, 1986] were detected in the total cellular RNA sample (Fig. 7, lane 1). In the poly A^+ RNA sample we observed a protected fragment corresponding to the 1028 nt probe, indicating protection to the end of S1 probe A (Fig. 7, lane 2) which would be consistent with the presence of a 2300 nt poly A^+ mRNA produced from the

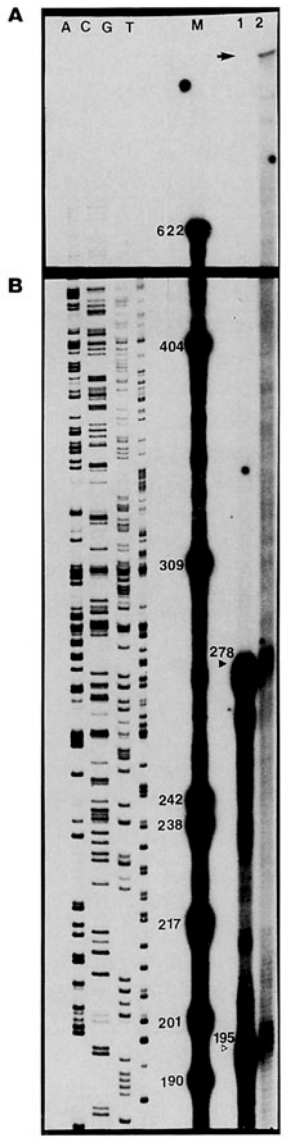

Fig. 7. S1 nuclease protection analysis of the 3′ termini of the H2B-GL105 mRNA. Total cellular (**lane 1**) and poly A^+ (**lane 2**) RNAs were isolated from proliferating HeLa cells. Total cellular RNA, 150 μg, and 4 μg poly A^+ RNA were used for S1 analysis. S1 analysis was carried out using a 1028 nt *Nco* I/*Eco* RI probe derived from the H2B-GL105 gene (Fig. 1A, nt 681–nt 1028; Fig. 1B, S1 probe A) and 3′ end labeled. **Lane M** was loaded with 3′-labeled pBR322 *Hpa* II markers. The sizes of various marker bands are given on the left side of lane M. **Lanes A, C, G,** and **T** were loaded with the products of dideoxy sequencing reactions on M13 mp18 single stranded template DNA utilizing a −40 primer (5′-GTTTTCCCAGTCACGAC-3′). The 278 nt H2B-GL105 protected fragment is indicated by a filled triangle and the 195 nt H2B "stop codon" protected fragment is indicated by an empty triangle. The arrowhead at the top of the autoradiogram indicates protection to the end of S1 probe A (1028).

H2B-GL105 gene [see Collart et al., 1991]. Taken together these results verify the presence of two H2B-GL105 mRNA species and demonstrate that the 3′ terminus of the shorter H2B-GL105 mRNA maps to the consensus histone mRNA terminus which is characteristic of cell cycle dependent histone mRNAs.

Mapping of the H2B-GL105 5′ mRNA Start Site in Synchronized HeLa Cells

To evaluate the cell cycle dependent nature and 5′ start site of the alternative H2B-GL105 mRNA we carried out analysis on total cellular RNA, isolated from synchronized cells after release *from* a double thymidine block, and on samples enriched for poly A^+ RNA, using a single pass over oligo dT-cellulose. A single oligo dT-cellulose selection greatly enriches for poly A^+ RNA but does not totally remove all of the poly A^- RNA. S1 nuclease protection analysis was carried out using a 419 nt *Hind* III/*Nco* I probe derived from the H2B-GL105 gene (Fig. 1A; nt 258–nt 676) and 5′ end labeled at the *Nco* I site (Fig. 8). A 217 nt H2B protected fragment was detected, indicating the cap site for the H2B-GL105 mRNAs is located 42 nt upstream of the ATG translational start codon (Fig. 8). The H2B fragment was detected primarily at the peak of S phase (4 h) in the total cellular RNA sample (Fig. 8). We only detect a protected fragment in the poly A^+ RNA sample with long exposure times, which is consistent with the very low levels of the 2300 nt cell cycle independent poly A^+ mRNA present in proliferating cells [Collart et al., 1991]. Based upon the 3′ terminus of the shorter H2B-GL105 mRNA and its 5′ cap site, these results demonstrate that the H2B-GL105 gene encodes a 500 nt H2B mRNA in addition to the 2300 nt mRNA previously described [Collart et al., 1991], with the 500 nt mRNA being more abundant than the 2300 nt species. In addition, these results establish that the 500 nt H2B-GL105 mRNA species is regulated in a cell cycle dependent manner, unlike the 2300 nt species [Collart et al., 1991].

In addition, S1 nuclease protection analysis carried out on HeLa RNA, using a 257 nt *Xho* I/*Hind* III probe derived from the H2A-GL101 gene (Fig. 1A, nt 2–nt 258) and 5′ end labeled at the *Xho* I site, produced a 225–226 nt H2A protected fragment, indicating that the cap site for the H2A-GL101 mRNAs is located 46 nt upstream of the ATG translational start codon (Fig. 1A). The S1 nuclease protection analysis

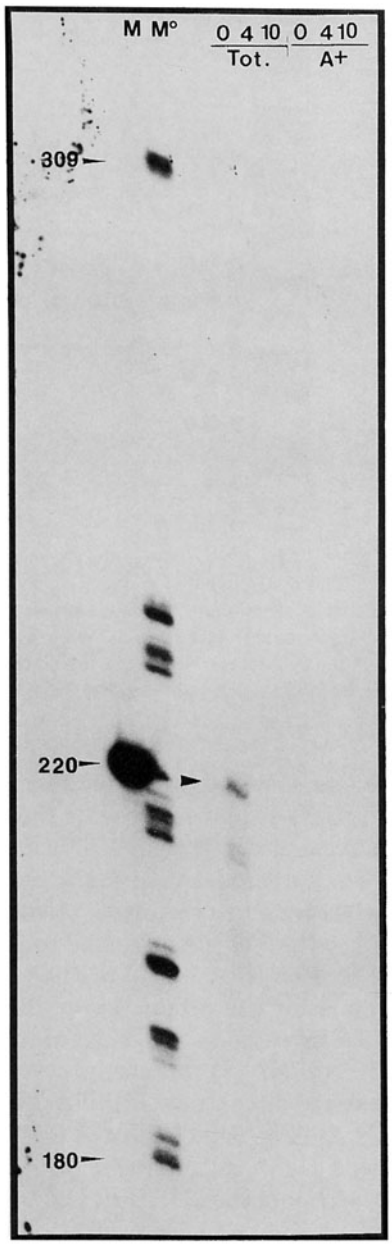

Fig. 8. S1 nuclease mapping of the H2B-GL105 5′ mRNA start site. Total cellular and poly A^+ RNAs were isolated from synchronized HeLa cells prior to (0) and after (4 and 10 h) release from a double thymidine block. Total cellular RNA (**Tot.**), 50 μg, and 2 μg poly A^+ RNA (**A^+**) were used for S1 analysis. S1 analysis was carried out using a 419 nt *Hind* III/*Nco* I probe derived from the H2B-GL105 gene (Fig. 1A, nt 258–nt 676) and 5′ end labeled at the *Nco* I site. **Lanes M** and **M°** were loaded with 3′-labeled pBR322 *Hinf* I and pBR322 *Hpa* II markers, respectively. The sizes of various marker bands are given on the left side of the figure. The 217 nt H2B protected fragment is indicated by an arrowhead in the center of the autoradiogram.

clearly distinguished between correctly initiated H2A-GL101 transcripts and transcripts from other non-identical H2A histone genes (data not shown). The H2A protected fragment was detected primarily at the peak of S phase.

DISCUSSION

We have isolated and sequenced a variant human H2B histone gene (GL105) which expresses alternative mRNAs regulated differentially during the HeLa cell cycle. Our previous studies utilizing the H2B-GL105 cDNA (λHHC289) demonstrated that the 2300 nt H2B-GL105 mRNA species is reciprocally expressed in relation to replication-dependent H2B histone genes during HL60 cell differentiation [Collart et al., 1991]. The nucleotide and deduced amino acid sequences reveal that the human H2B-GL105 gene encodes a protein identical to the somatic H2B.1 mouse protein in key amino acid positions [Zweidler, 1980]. The H2B.1 histone variant is present in small amounts in rapidly growing tissues [Zweidler, 1980]. However, in those tissues with low growth rates in the adult, H2B.1 increases dramatically in relative amount during and especially just after the postnatal growth phase to reach a plateau at about 6 months [Zweidler, 1980]. The cell must utilize complex transcriptional and post-transcriptional regulatory mechanisms to control cellular levels of mRNAs from various human H2B histone genes during progression through the cell cycle and at the onset of differentiation in order to modulate the ratios of H2B variant proteins in response to the growth state of the cell.

Histone gene transcriptional regulation involves a transient increase in the rate of transcription at the G1/S phase boundary [Schümperli, 1988; Marzluff and Pandey, 1988; Stein and Stein, 1984]. Previously, nuclear run-on transcription analysis revealed a twofold increase in transcription of the H2B-GL105 gene during S phase [Collart et al., 1991]. Sequence analysis shows that the H2B-GL105 gene contains, apart from a consensus TATA-box, a histone promoter specific CCAAT box [van Wijnen et al., 1988], as well as an OTF-1 element [Fletcher et al., 1987] which are all promoter elements typical of cell cycle dependent H2B histone genes. The cell cycle transcription pattern of the H2B-GL105 gene and the apparent similarities of its promoter elements to those of cell cycle dependent H2B genes suggest that transcriptional regulation is not the sole modulator in controlling H2B variant ratios.

We have observed that the H2B-GL105 gene expresses a 500 nt cell cycle dependent mRNA in addition to the 2300 nt cell cycle independent mRNA previously described [Collart et al., 1991]. The 3′ end of the cell cycle dependent mRNA terminates immediately following the region of hyphenated dyad symmetry, whereas the cell cycle independent mRNA has a 1798 nt nontranslated trailer that contains the region of hyphenated dyad symmetry and a poly (A) addition sequence, followed by a poly (A) tail. The ability of the cell to utilize two mechanisms for mRNA 3′ end formation may allow the H2B-GL105 gene to regulate the relative levels of H2B.1 protein in relation to cell cycle dependent histone protein during various growth states of the cell.

In proliferating cells we could detect very little H2B-GL105 mRNA in the poly A^+ fraction, suggesting that the majority is processed to the 500 nt mature mRNA species using the endonucleolytic cleavage reaction observed by Birnstiel et al. [1985]. However, the level of the 2300 nt H2B-GL105 species increases approximately tenfold during HL60 cell differentiation, whereas the levels of cell cycle dependent H2B mRNAs decrease to less than 1% of those in proliferating cells [Collart et al., 1991]. This result would suggest that at the onset of differentiation the U7 mediated [Strub et al., 1984; Strub and Birnstiel, 1986; Melin et al., 1992] histone maturation process is slowed or shut down, allowing additional time for polyadenylation of the histone pre-mRNA and resulting in an increase of the 2300 nt H2B-GL105 mRNA level.

In support of this suggestion, Hoffmann and Birnstiel [1990] have observed that 5′ sequences of the U7 snRNP, which hybridize with the histone downstream spacer motif during 3′ processing [Schaufele et al., 1986], are occluded in the G_0 stage of the cell cycle, and Vasserot et al. [1990] have observed that during the transition from a proliferative to a non-dividing state while rat myoblasts differentiate to myotubes the 5′ ends of U7 snRNPs are protected against micrococcal nuclease attack and presumably unavailable for histone 3′ end processing. In addition, Lüscher and Schümperli [1987] (and personal communication with Urs Albrecht) have shown a histone 3′ processing factor becomes limiting in G1-arrested cells, Stauber and Schümperli [1988] have demonstrated 3′ processing of pre-

mRNA plays a major role in proliferation-dependent regulation of histone mRNA levels, and Liu et al. [1989] have shown that the efficiency of 3′ end formation contributes to the relative levels of different histone mRNAs. This mechanism may be operative for other histone genes which have been observed to produce multiple species of mRNA by alternative 3′ end processing [Mannironi et al., 1989; Challoner et al., 1989; Cheng et al., 1989; Kirsh et al., 1989].

In conclusion, we have characterized an H2B.1 gene which encodes both a 500 nt cell cycle dependent mRNA species and a 2300 nt constitutively expressed mRNA species which are regulated differentially in response to alterations in the proliferative state of the cell. These results suggest that 3′ processing of histone H2B mRNA precursors may play an important role in the modulation of H2B.1 mRNA and subsequently H2B.1 protein levels during the transition from a proliferative to a non-dividing state.

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