Isolation and Characterization of a cDNA from a Human Histone H2B Gene Which Is Reciprocally Expressed in Relation to Replication-Dependent H2B Histone Genes during HL60 Cell Differentiation†,‡

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ABSTRACT: A variant human histone H2B cDNA (HHC289) has been cloned and characterized and shown to have a complex pattern of regulation with respect to the HeLa S_3 cell cycle and HL60 cell differentiation. The H2B protein coding region of HHC289 is flanked at the 3′ end by a 1798-nt nontranslated trailer that contains a region of hyphenated dyad symmetry and a poly(A) addition sequence, followed by a poly(A) tail. Nuclear run-on transcription analysis revealed a 2-fold increase in transcription of the HHC289 gene during S phase, in comparison to replication-dependent human histone genes which exhibit a 2–3-fold increase in transcription during S phase. Northern blot analysis indicated that the levels of the 2300-nt HHC289 mRNA species did not vary significantly during the HeLa S_3 cell cycle, in comparison to replication-dependent H2B mRNAs which are elevated 15-fold during S phase. Northern blot analysis also revealed a reciprocal relationship during the onset of HL60 differentiation between the expression of the HHC289 H2B gene and the replication-dependent H2B genes. The levels of the 2300-nt HHC289 H2B species increased approximately 10-fold during HL60 cell differentiation whereas the levels of cell cycle dependent H2B mRNAs decreased to less than 1% of those in proliferating cells. These results suggest that complex transcriptional and posttranscriptional regulatory mechanisms control cellular levels of mRNAs from various human H2B histone genes during progression through the cell cycle and at the onset of differentiation.

Histones are a complex family of highly conserved basic proteins responsible for packaging chromosomal DNA into nucleosomes [reviewed in Igo-Kemenes et al. (1982) and Isenberg (1979)]. Histone proteins exhibit two levels of diversity: (i) evolutionary diversity between species and (ii) subtype diversity in a class (H1, H2A, H2B, H3, or H4) within a species (Stein et al., 1984). The subtypes within a species are often referred to as variants (Wu & Bonner, 1981; Zweidler, 1980). Replication-dependent histone variants represent the majority of the histone proteins synthesized in proliferating cells, and it is well established that their synthesis is functionally and temporally coupled to DNA replication (Stein et al., 1987). Replacement variants are expressed in nonproliferating cells and are often expressed in proliferating cells, although normally at a lower level than their replication-dependent counterparts (Stein et al., 1989; Zweidler, 1980). The third type of variant within a species is the tissue-specific variant (Branson et al., 1975; Dalton et al., 1986; Hwang & Chae, 1989; Tsai & Hnilica, 1975). Tissue-specific variants are generally not synthesized in a replication-dependent manner (Dalton et al., 1986; Hwang & Chae, 1989).

Histone H2B proteins have been studied in a variety of species—including chicken, mouse, rat, and human—with respect to their subtype diversity [Branson et al., 1975; Jackson, 1985; reviewed in Stein et al. (1984) and Zweidler (1980)]. Although the replication-dependent and tissue-specific H2B proteins are easily detected in most species, the replacement histone H2B proteins are not always observed in humans, possibly due to the lack of sensitivity of the assay (Stein et al., 1984).

Human histone genes are a family of moderately reiterated sequences arranged in polymorphic clusters on several chromosomes (Green et al., 1984; Stein et al., 1984; Tripputi et al., 1986). Replication-dependent histone genes do not contain introns (Stein et al., 1985), and their mRNAs are structurally simple; they are not polyadenylylated and have short 5′-leader and 3′-trailer sequences (Hentschel & Birnstiel, 1981). However, replication-dependent histone mRNAs do have a characteristic 3′ stem–loop motif (Stein et al., 1984). Replication-dependent histone genes are coordinately expressed during the cell cycle, and their expression is coupled with DNA synthesis (Baumbach et al., 1984; Heintz et al., 1983; Pauli et al., 1987; Plumb et al., 1983a,b; van Wijnen et al., 1988). The abundance of the replication-dependent human histone mRNAs is regulated at both the transcriptional and posttranscriptional levels [reviewed in Schümperli (1986) and Stein et al. (1984)]. Transcriptional regulation involves a 2–5-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 (Baumbach et al., 1987; Heintz et al., 1983). Posttranscriptional regulation of these histone genes involves a rapid and selective destabilization of histone mRNAs toward the end of S phase or upon inhibition of DNA synthesis (Baumbach et al., 1984; Helms et al., 1984; Morris et al., 1987).

In contrast, replacement histone genes may contain introns (Brush et al., 1985; Wells & Kedes, 1985; Wells et al., 1987), and their mRNAs, which are polyadenylylated and frequently contain long 5′ leaders and 3′ trailers, are structurally more complex than their replication-dependent counterparts (Brush et al., 1985; Chaubet et al., 1988; Ernst et al., 1987; Harvey et al., 1983; Hatch & Bonner, 1988; Wells & Kedes, 1985; Wells et al., 1987). Although the processes governing replacement histone mRNA levels are not well understood, it

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is clear that their mRNAs (Bird et al., 1985; Brown et al., 1985; Carrino et al., 1987; Ernst et al., 1987; Harvey et al., 1983; Jarvis & Marzluff, 1989; Shalhoub et al., 1989; Sittman et al., 1983) as well as tissue-specific histone mRNAs (Challoner et al., 1989; Cole et al., 1986; Grimes et al., 1987; Hwang & Chae, 1989; Kim et al., 1987) are not always regulated in a replication-dependent manner.

To date, H2B histone gene organization and expression have been studied most thoroughly in the chicken; five replication-dependent, one partially replication-dependent, one uncharacterized, and one testis-specific chicken H2B genes have been described (Challoner et al., 1989; Grandy et al., 1982; Grandy & Dodgson, 1987; Hwang & Chae, 1989). Human H2B histone gene expression has not been reported in such detail. Although several human histone H2B replication-dependent genes and one pseudogene have been described (Heintz et al., 1983; Marashi et al., 1984; Plumb et al., 1983a,b; Prokopp, 1984; Sierra et al., 1982; Stein et al., 1984; Zhong et al., 1983), no human replacement or tissue-specific H2B genes have been reported.

In this paper, we describe the cloning and characterization of a variant human histone H2B cDNA. This gene is expressed in a reciprocal relationship with replication-dependent H2B genes during the onset of HL60 cell differentiation, exhibiting a 10-fold increase in expression of a 2300-nucleotide (nt)[1] mRNA species as cellular levels of transcripts from the replication-dependent H2B histone genes decline. On the basis of the structure of this cDNA and on Northern blot analysis, we conclude that the HHG289 gene encodes a replication-independent H2B mRNA that contains an unusually long 3′ trailer with a region of hyphenated dyad symmetry as well as a poly(A) tail.

Materials and Methods

Plasmid and Recombinant Phage DNA. Plasmid growth and purification were carried out as previously described (Collart et al., 1985). Recombinant phage DNA was isolated and purified as described by Maniatis et al. (1982). The plasmid RGAPDH-13 (Fort et al., 1985) was generously provided by Dr. Ph. Jeanteur and the plasmid Gβ2m (Cosenza et al., 1988) by Dr. Kenneth Soprano after preparation from a 550 bp *Pst*I fragment of human β_2-microglobulin kindly provided by Dr. K. Itakura, Harvard University.

Preparation of Radioactive Probes. A random oligonucleotide priming technique (Feinberg & Vogelstein, 1983; Taylor et al., 1976), simplified by Roberts and Wilson (1985), was used to prepare ^{32}P-labeled DNA probes.

λgt11 Library Screening. A λgt11 human liver cDNA library, made from total cellular poly(A+) RNA and generously provided by Dr. S. L. C. Woo (Baylor College of Medicine, Department of Cell Biology), was screened with a ^{32}P-labeled H2B probe [550-nt *Sst*II fragment isolated from pFF435B (Marashi et al., 1984)] according to the procedure described by Benton and Davis (1977) with the following modifications in the hybridization conditions. The filters were prehybridized at 60 °C for 2 h in a solution containing 5 × Denhardt's [100 × is 2% ficoll/2% poly(vinylpyrrolidone)], 5 × SSPE (20 × is 3.6 M sodium chloride, 0.2 M sodium phosphate, and 0.02 M EDTA, pH 7.4), 0.2% SDS, 0.1% w/v BSA, and 500 μg/mL double-stranded *Escherichia coli* DNA. Hybridization was carried out at 60 °C for 24 h in a solution containing 0.5 × Denhardt's, 5 × SSPE, 0.2% SDS, 0.01% w/v BSA, 500 μg/mL *E. coli* DNA, and 2 × 10^6 dpm of heat-denatured probe DNA fragment. Hybridized filters were then washed at 24 °C for 0.5 h in 100 mM potassium phosphate (pH 7.4) followed by two 0.5-h washes at 60 °C in 2 × SSPE/0.3% SDS. Autoradiography was performed with Kodak XAR-5 X-ray film and a Cronex Lightning Plus screen at −70 °C.

Sequencing. Dideoxy sequencing (Sanger et al., 1977) was carried out by using a United States Biochemical Corp. Sequenase DNA sequencing kit with conditions as described by the manufacturer. After the sequencing reactions were completed, loading buffer (95% formamide, 20 mM EDTA, 0.05% bromophenol blue, and 0.05% xylene cyanol) was added, and the samples were heat-denatured and loaded onto 6% polyacrylamide sequencing gels containing 7.8 M urea (Maniatis et al., 1982). After electrophoresis, the gels were soaked in 10% acetic acid/12% methanol for 15–30 min to remove urea, dried under vacuum at 80 °C, and placed against preflashed Kodak XAR-5 or Cronex X-ray film at room temperature. In order to obtain accurate sequence data, the HHC289 cDNA was sequenced in both directions and at least 2 times in one direction.

HeLa S_3 Cell Culture and Synchronization. HeLa S_3 cells were grown in suspension culture in Joklik-modified Eagle's minimal essential medium supplemented with 7% calf serum and were synchronized by two successive treatments with 2 mM thymidine (Stein & Borun, 1972). The two 14-h, 2 mM thymidine treatments were spaced 9 h apart. Rates of DNA synthesis were monitored by measuring the incorporation of [^{3}H]thymidine into acid-precipitable material in a 30-min pulse (Stein & Borun, 1972). DNA synthesis was inhibited by treating the cell culture with 1 mM hydroxyurea for 1 h, beginning 4 h after release from the second thymidine block. Cells (hydroxyurea treated or untreated) were harvested at various times after release from the second thymidine block.

HL60 Cell Culture and Differentiation. HL60 cells were plated at a cell density of 2 × 10^5/mL, grown for 24 h to a cell density of 3 × 10^5/mL, and harvested by centrifugation. For differentiation of HL60 cells, cultures at a density of 3 × 10^5/mL were treated with TPA at a final concentration of 16 nM, and harvested after periods of 1, 2, 3, 4, and 5 days.

RNA Isolation and Analysis. RNA isolation was carried out as previously described (Plumb et al., 1983a). Poly(A+) RNA was then selected by using a single pass over oligo(dT)–cellulose (Sigma) as described by Maniatis et al. (1982). A single oligo(dT) selection greatly enriches for poly(A+) RNA but does not totally remove all of the poly(A−) RNA. Northern blot analysis was carried out as follows: the RNA was size-fractionated in a 1.5% agarose/6% formaldehyde gel, a modification of the method used by Lehrach et al. (1977), and transferred as described by Thomas (1980) to Zeta-Probe blotting membrane (Bio-Rad) using 20 × SSC (3 M NaCl/0.3 M sodium citrate, pH 7.4). Following transfer, the membrane was baked in a vacuum oven at 80 °C for 1 h, prewashed in 0.5% SDS/0.1 × SSC for 1 h at 65 °C, and then prehybridized and hybridized by using a modification of the method described by Wahl et al. (1979). Prehybridization was carried out for 2 h in 0.05 M sodium phosphate (pH 6.5), 0.45% SDS, 47% formaldehyde, 9% dextran sulfate, and 500 μg/mL double-stranded *E. coli* DNA. After prehybridization, ^{32}P-labeled probe was added to the mix to a concentration of 1 × 10^6 dpm/mL and hybridized for 6–18 h at 48 °C. The hybridized Zeta-Probe membrane was then washed 5 times as follows: (1) 10 min at room temperature with 5 × SSC/1 × Denhardt's; (2) 30 min at 65 °C with 5 × SSC/1 × Den-

[1] Abbreviations: SDS, sodium dodecyl sulfate; BSA, bovine serum albumin; TPA, 12-*O*-tetradecanoylphorbol 13-acetate; TCA, trichloroacetic acid; tH2B, testis-specific H2B; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; nt nucleotide(s).

hardt's; (3) 30 min at 65 °C with 2 × SSC/0.1% SDS; (4) 30 min at 65 °C with 1 × SSC/0.1% SDS; (5) 30 min at 65 °C with 0.1 × SSC/0.1% SDS. Autoradiography was performed by using preflashed Kodak XAR-5 X-ray film and a Cronex Lightning Plus screen at −70 °C. The developed X-ray films were analyzed by laser densitometry using an LKB 2400 GelScan XL densitometer.

In Vitro Nuclear Run-On Transcription Analysis. The in vitro nuclear run-on transcription assays were performed by using a modification (Baumbach et al., 1987) of the method used by Flint et al. (1984). Cells were harvested by centrifugation, and the cell pellet was washed twice in cold isotonic buffer [125 mM potassium phosphate, 30 mM Tris-HCl (pH 7.9), 5 mM $MgCl_2$, and 10 mM β-mercaptoethanol]. Cells were disrupted by homogenization (6–12 strokes) with a Dounce homogenizer, Wheaton type A pestle. After >90% of the cells had been lysed, nuclei were pelleted by centrifugation at 2000 rpm for 10 min in an IEC centrifuge at 4 °C and resuspended in nuclei storage buffer containing 40% glycerol [50 mM Tris-HCl (pH 8.3), 5 mM $MgCl_2$, and 0.1 mM EDTA]. Nuclei were aliquoted and used fresh in the in vitro transcription reactions. Reactions typically contained 10^7 nuclei, 100 μCi of [α-^{32}P]UTP (3000 Ci/mmol), 1 mM ATP, and 0.25 mM GTP and CTP in a final volume of 130 μL and were incubated for 30 min with intermittent shaking at 30 °C. Radiolabeled RNAs were isolated by treatment of nuclei with DNase I (100 μg/mL) in the presence of 0.6 M NaCl, 50 mM Tris-HCl (pH 7.5), and 20 mM $MgCl_2$ for 15 min at room temperature. The mixture was then incubated with proteinase K (200 μg/mL) for 30–60 min at 37 °C in the presence of 150 mM NaCl, 12.5 mM EDTA, 100 mM Tris-HCl (pH 7.5), and 20 mM $MgCl_2$. Sodium acetate (pH 5.5) was added to 0.2 M, and nucleic acids were extracted several times by the hot phenol method (Clayton et al., 1983; Scherrer & Darnell, 1962). To the aqueous solution of ^{32}P-labeled RNAs were added 150 μg of yeast RNA and 2.5 volumes of 95% ethanol. Precipitation was overnight at −20 °C. Radiolabeled transcripts were resuspended in 1 mL of 10 mM Tris-HCl (pH 8.0)/1 mM EDTA, and an aliquot of each sample was precipitated with 150 μg of yeast RNA and cold 10% TCA. TCA-precipitable counts were determined by liquid scintillation spectrometry.

Following preparation of Southern blots (Southern, 1975) of electrophoretically separated restriction endonuclease digested plasmid DNAs or slot blots of linearized plasmid DNAs, DNA excess hybridizations were carried out. Southern or slot blots on nitrocellulose were prehybridized in 1 M NaCl, 20 mM Tris-HCl (pH 7.4), 2 mM EDTA, 0.1% SDS, 5 × Denhardt's, 25 μg/mL denatured *E. coli* DNA, and 12.5 mM sodium pyrophosphate at 65 °C for a least 6 h. Hybridizations were conducted at 65 °C for 72 h in 1 M NaCl, 20 mM Tris-HCl (pH 7.4), 2 mM EDTA, 0.1% SDS, 2.5 × Denhardt's, and 25 μg/mL *E. coli* DNA with ^{32}P-labeled transcripts at (5 × 10^5)–(1 × 10^6) TCA-precipitable counts per milliliter of hybridization solution. Blots were washed at 65 °C for 15 min in fresh prehybridization solution: 1 h in 2 × SSC/0.1% SDS, overnight in 2 × SSC/0.1% SDS, and 1 h in 0.2 × SSC/0.1% SDS. Air-dried filters were autoradiographed with preflashed XAR-5 or Cronex film and Cronex Lightning Plus Screens at −70 °C. The developed X-ray films were analyzed by laser densitometry using an LKB 2400 GelScan XL densitometer.

Results

Sequencing of a Poly(A+) H2B cDNA. Twenty-two cDNA clones containing H2B histone sequences were obtained from the initial screening of the λ library; the clone with the largest insert that also contained a poly(A) tract (λHHC289, 2119 nt) was then selected for further analysis. The nucleotide sequence of HHC289 was determined by dideoxy sequence analysis and is presented in Figure 1A. The HHC289 cDNA encodes H2B amino acids 25–125 and the translation loop codon. Also contained in the cDNA sequence is the poly(A) addition sequence, AATAAA, located 1776 nt downstream from the translation stop codon, and a poly(A) stretch located 16 nt downstream of the poly(A) addition sequence. The protein-coding region is GC rich (63%) whereas the 3′-nontranslated trailer is relatively GC poor (45%). Shown in Figure 1B are pertinent restriction sites and the HHC289 fragments used as probes for Northern blot analysis.

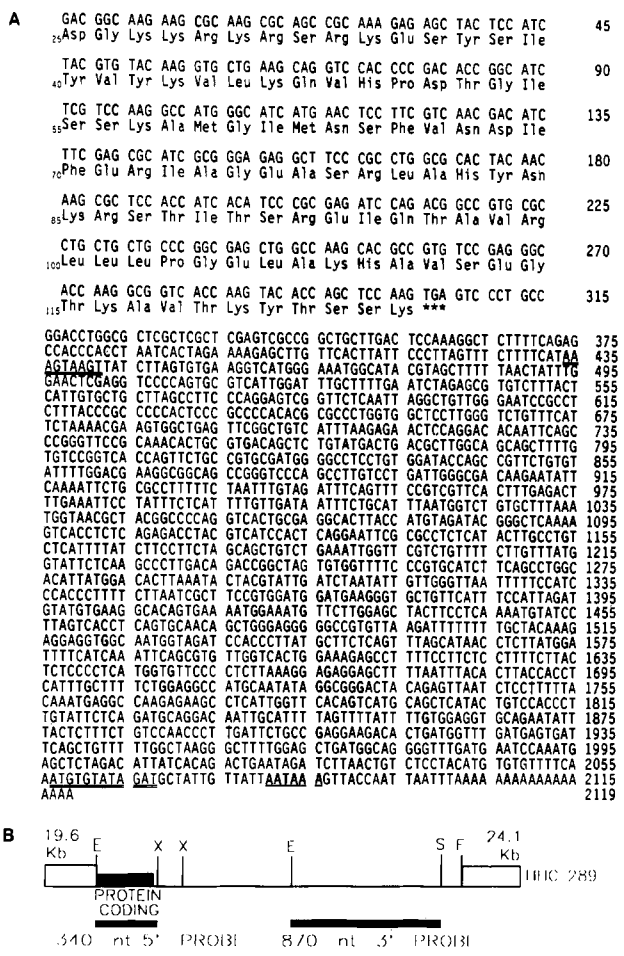

FIGURE 1: (A) Nucleotide and deduced amino acid sequence of the human H2B histone cDNA HHC289. The HHC289 cDNA encodes H2B amino acids 25–125 and the translation stop codon. Also contained in the HHC289 cDNA sequence is the poly(A+) addition sequence, *AATAAA*, located 1776 nt downstream from the protein stop codon and a poly(A+) stretch located 16 nt downstream from the last nucleotide of the poly(A+) addition sequence. Solid double underlined sequences represent potential splice sites. (B) Restriction map of the cDNA HHC289. The H2B protein-coding region is illustrated by a closed box, and the λgt11 arms are represented by the open boxes. Solid bars below the restriction map illustrate HHC289 probes used for Northern analysis. Restriction enzyme abbreviations are as follows: E = *Eco*RI; X = *Xho*I; S = *Sst*I.

Comparison of the HHC289 Predicted Amino Acid Sequence with Other H2B Amino Acid Sequences. In Figure 2A is shown a comparison of selected amino acids from the predicted histone H2B sequence of HHC289 with a multispecies consensus H2B protein sequence (Wells, 1986), a human genomic sequence (Prokopp, 1984), and several chicken genomic sequences (Challoner et al., 1989; Grandy & Dodgson, 1987; Harvey et al., 1982). The amino acids shown vary

A

	18	21	25	26	30	31	32	39	60	94	122	124	
A.	---	---	Asp	Gly	Lys	Arg	Ser	Ile	Gly	Ile	Thr	Ser	Human
B.	Leu	Ala	*	*	*	*	*	•	*	*	*	*	Multi
C.	Val	Ala	*	*	*	*	*	Val	*	Val	*	Lys	Human
D.	Val	Thr	Gly	Asp	*	*	Ala	*	Ser	*	*	*	Chicken
E.	---	---	---	---	---	---	---	*	*	*	Ile	*	Chicken
F.	Val	Thr	Gly	Asp	Arg	Lys	•	*	*	*	*	*	Chicken
G.	Val	Thr	Gly	Asp	*	Lys	*	*	*	*	*	*	Chicken
H.	Val	Thr	Gly	Asp	Arg	*	Thr	*	*	*	*	*	Chicken

B

	TGCA	CCCAAA	GGCTCTTTTCAGAGCCACC	GAGCT	AATAAA	
1.	TGA..28..	TGCA..17..	TCCAAA	GGCTCTTTTCAGAGCCACC..18..	GAGCT..1800..	AATAAA..15..+
2.	TAG...5..	TGCG..15..	CCCAAA	GGCTCTTTTCGGAGCCACC..16..	GGGCT..????	.....?
3.	TAA...5..	TTCT...9..	CCCAAACGGCTCTTTTCGGAGCCCAA..13..	GGGCT..????		.....?
4.	TTA...9..	TGCA..14..	CCCAAA	GGCTCTTTTCAGAGCCACT..16..	GAGCTATATACTGACATGTGAAGAT	
5.	TAA..13..	TGCA...9..	CCCTAACGGCTCTTTTAAGAGCCACC..16..	GAGCTGGTGCTTGTATTTCCTCCTC		
6.	TGA..15..	TGCA..36..	CAGCAA	GGCTCTTTTCAGAGCCACC..17..	GAGCTGTTGTGCTTTGGATTATGCC	
7.	TAA...1..	TGTA..26..	CCCAAA	GGCTCTTTTCAGAGCCACC..18..	TAGCTGTGATAATTTTTTGTTGTCT	
8.	TAA..23..	TTCA..	TTCTCAAAAAAAAAAAAAAAATTTCTCTTCTTCCTGTTAT...422..	ATTAAA..15..+		
9.	TAA...8..	TGGA..	TTCCTTGTTATCTCAGGACTCTAAATACTCTAACAGCTGT...302..	AATAAA..16..+		
0.	TAG...5..	TGCG..15..	CCCAAA	GGCTCTTTTCAGAGCCACC..16..	GAGTG..????	.....+

FIGURE 2: (A) Comparison of the HHC289 histone H2B amino acid sequence with the sequences of other H2Bs: (A) HHC289 human cDNA; (B) multispecies consensus H2B protein sequence (Wells, 1986); (C) HHG39 human genomic sequence (Prokopp, 1984); (D) pBBA-3.0 chicken genomic sequence (Grandy & Dodgson, 1987); (E) pPP2d-4.0 chicken genomic sequence (Grandy & Dodgson, 1987); (F) pKR1a-1.3, pPP2d-2.3, pRR3c-3.5, pRR2c-3.5, and pBRA-5.4 chicken genomic sequences (Grandy & Dodgson, 1987); (G) λCH-02 and λCH-01/λCH-05 chicken genomic sequences (Harvey et al., 1982); (H) pStH2B-16 chicken genomic sequence (Challoner et al., 1989). Dashed lines indicate undetermined amino acids due to incomplete nucleotide sequence information, and an asterisk indicates an amino acid which is identical with the HHC289 predicted amino acid in the same position. The amino acid position is indicated by the boldface number at the top of each column, and the origin of each sequence is indicated in the right-hand column. Amino acids which do not vary among the genes reported above are not illustrated. (B) DNA sequence comparison of the 3′-untranslated region of the following histone sequences: (1) HHC289 human H2B cDNA; (2) pKR1a-1.3 chicken H2B genomic sequence (Grandy & Dodgson, 1987); (3) pBBA-3.0 chicken H2B genomic sequence (Grandy & Dodgson, 1987); (4) HHG39 human H2B genomic sequence (Prokopp, 1984); (5) pHh4A/pHh4C human H2B genomic sequence (Zhong et al., 1983); (6) pHh5G human H2A genomic sequence (Zhong et al., 1983); (7) pST519 human H3 genomic sequence (Marashi et al., 1986); (8) human H3.3 genomic sequence (Wells et al., 1987); (9) human H2A.Z cDNA sequence (Hatch & Bonner, 1988); (0) pStH2B-16 chicken genomic sequence (Challoner et al., 1989). Numbers flanked by dots indicate the number of bases contained between the two adjacent reported sequences. A blank space is a gap created to facilitate alignment. A plus sign symbolizes the presence of a poly(A+) tail in the mRNA, and a question mark indicates the lack of data concerning polyadenylylation or the number of bases contained between two adjacent reported sequences. Arrows indicate the conserved 3′ stem–loop motif sequence. The conserved sequences are displayed in the top line, in boldface print.

at the indicated positions among the genes reported in Figure 2A. These amino acids are either species-specific [that is, they are conserved among known chicken H2B genes but may differ between the chicken and other species reported in Figure 2A (amino acids 18, 21, 25, and 26)] or subtype-specific, which may vary among H2B protein variants within a species or between species (amino acids 30, 31, 32, 39, 60, 94, 122, and 124). The amino acid sequence data for human H2B histones are too incomplete to permit classification of the HHC289 H2B protein as a replication-dependent, replacement, or tissue-specific variant. However, the predicted protein is 97% similar to the replication-dependent HHG39 H2B protein (Prokopp, 1984) with amino acid differences at positions 39, 94, and 124 (Figure 2A) and identical with the multispecies consensus H2B sequence compiled by Wells (1986). The HHC289 predicted amino acid sequence is also similar to the pHh4A/pHh4C H2B sequence reported by Zhong et al. (1983), except in the region of amino acids 27 through 33.

Comparison of 3′-Nontranslated Sequences from HHC289 and Other Histone cDNAs and Genes. The 3′-nontranslated sequences from HHC289 and other histone cDNAs and genes are illustrated in Figure 2B. The histone 3′ stem–loop element (Hentschel & Birnstiel, 1981) is present in all of the poly(A−) histone sequences (Grandy & Dodson, 1987; Marashi et al., 1986; Prokopp, 1984; Zhong et al., 1983). Included in this comparison are three poly(A+) human histone sequences (Hatch & Bonner, 1988; Wells et al., 1987) and one poly(A+) chicken tH2B sequence (Challoner et al., 1989); of these, only HHC289 and the chicken tH2B sequence contain a 3′ stem–loop motif. The HHC289 H2B and the H2A.Z sequences (Hatch & Bonner, 1988) contain the AATAAA poly(A) addition element (Fitzgerald & Shenk, 1981; Proudfoot & Brownlee, 1976) while the H3.3 sequence (Wells et al., 1987) contains an ATTAAA element, also implicated in polyadenylylation (Hagenbuchle et al., 1980; Jung et al., 1980) (see Figure 2B). However, the tH2B sequence does not appear to contain either polyadenylylation element (Challoner et al., 1989). In addition to containing a 3′ stem–loop motif, HHC289 is distinct from other known human poly(A+) histone sequences as it contains the longest 3′ trailer—1798 nt as compared to 510 nt for H3.3 (Wells et al., 1987) and 376 nt for H2A.Z (Hatch & Bonner, 1988).

HHC289 mRNA Is Expressed throughout the HeLa S_3 Cell Cycle and during Inhibition of DNA Synthesis. To determine the extent of cell cycle regulation of the HHC289 H2B histone gene, we first measured the level of HHC289 mRNA throughout the HeLa S_3 cell cycle. Presented in Figures 3 and 4 are the results of Northern blot analysis of poly(A+) RNA isolated from synchronized HeLa S_3 cells, prior to and after release from a double thymidine block, as well as from unsynchronized HeLa cells in logarithmic growth and after treatment with the DNA synthesis inhibitor hydroxyurea. The peak of S phase, as determined by [^{3}H]thymidine incorporation, occurred at 4–5 h after release from the second thymidine block (data not shown).

To follow the mRNA levels of all H2B species, two different probes from HHC289 were used. One probe was the 340-nt *Eco*RI/*Xho*I fragment (Figure 1B) which spans the H2B protein-coding region and has strong sequence similarity with other reported H2B sequences. The second probe was the 870-nt *Eco*RI/*Sst*I fragment (Figure 1B) that spans the distal half of the 3′-nontranslated trailer. Northern blot analysis using the 3′-specific probe revealed a 2300-nt band that was present throughout the HeLa S_3 cell cycle (Figure 3A). As an internal control for the amount of mRNA per lane, this blot was reprobed with the ^{32}P-labeled plasmid RGAPDH-13

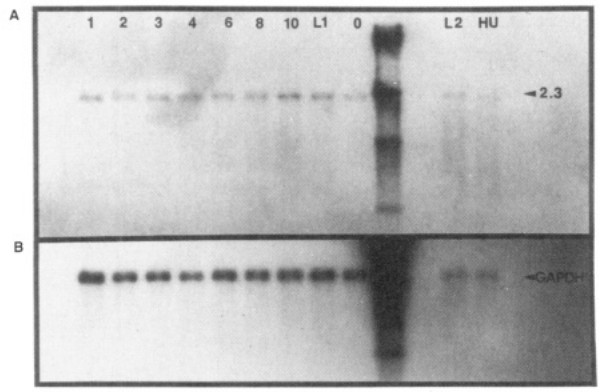

FIGURE 3: HHC289 mRNA levels during the HeLa cell cycle. Poly(A+) RNA was isolated from synchronized HeLa S_3 cells, prior to (0) and after (1, 2, 3, 4, 6, 8, and 10 h) release from a double thymidine block, as well as from unsynchronized HeLa cells in logarithmic (L1) growth. In a separate experiment, poly(A+) RNA was isolated from unsynchronized HeLa cells, in logarithmic growth, which had been treated with the DNA synthesis inhibitor hydroxyurea (HU) as well as from cells which were not treated (L2). Two micrograms of each RNA sample was size-fractionated, passively transferred to Zeta-Probe nylon membrane, prewashed, prehybridized, and hybridized to the following ^{32}P-labeled probes: 870-nt *Eco*RI/*Sst*I 3′ fragment from HHC289 (panel A); plasmid RGAPDH-13 (Fort et al., 1985) containing GAPDH sequences from rat (panel B). The filters were then washed, and autoradiography was performed with Kodak XAR-5 film. Lane M contains RNA markers purchased from Bethesda Research Labs. The sizes of the various bands are indicated, in kilobases, by the numbers on the right side of the figure.

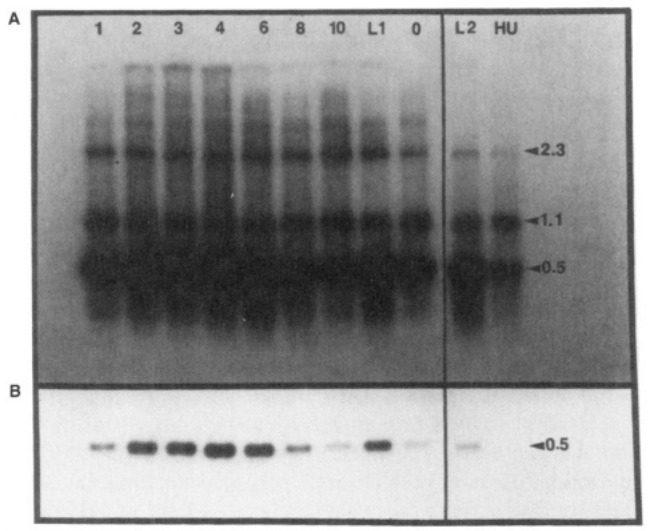

FIGURE 4: H2B mRNA levels during the cell cycle. Northern blot analysis was carried out on the same HeLa S_3 samples used in Figure 3 with the ^{32}P-labeled 340-nt *Eco*RI/*Xho*I fragment from HHC289 as probe (panel A). Panel B contains a shorter exposure of the 0.5-kb bands from panel A. The sizes of the various bands are indicated, in kilobases, on the right side of the figure.

(Fort et al., 1985), which contains rat cDNA sequences coding for GAPDH (Figure 3B). GAPDH mRNA levels have been shown in our lab to remain constant throughout the HeLa S_3 cell cycle. In cells in which DNA synthesis was inhibited with hydroxyurea, the level of the 2300-nt HHC289 mRNA species decreased by approximately 50% (Figure 3A), as indicated by densitometry and normalization to GAPDH mRNA levels (Figure 3B).

When Northern blot analysis was carried out using the protein-coding fragment from HHC289 (Figure 4A,B), H2B mRNAs were observed at 2300 and 1100 nt, in addition to low molecular weight H2B species migrating at approximately 500 nt. It should be noted that a single round of selection with oligo(dT)–cellulose greatly enriches for poly(A+) RNA but

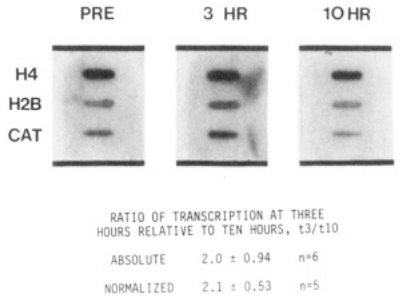

FIGURE 5: Transcription of the HHC289 histone gene during the HeLa S_3 cell cycle. The polyclonal HeLa S_3 cell line 201p1, which contains an H4 histone–CAT fusion gene, was synchronized via double thymidine block. Cells were harvested at the G1/S border (PRE), in mid S phase (3 h), and in G2/M (10 h). Radiolabeled transcripts (10^7 dpm) from in vitro nuclear run-on transcription were hybridized to slot blots containing linearized plasmid DNAs: H4, replication-dependent human H4 histone gene (Plumb et al., 1983a); H2B, 1020-nt 3′ *Eco*RI fragment from the HHC289 replication-independent H2B cDNA; CAT, H4 promoter/CAT fusion gene. The hybridized filters were then washed, and autoradiography was performed by using XAR-5 or Cronex film and Cronex Lightning Plus screens at −70 °C. Densitometric analysis was performed, and the resultant values for transcription of the histone genes and H4/CAT fusion gene were normalized to those of the 18S ribosomal RNA gene (not shown). The results obtained in six similar experiments are represented in the table.

does not totally remove all the poly(A−) RNA. The 500-nt H2B mRNAs were detected thoughout the HeLa S_3 cell cycle, but their levels clearly fluctuated as a function of the cell cycle (Figure 4A,B). Levels of the 1100- and 2300-nt mRNAs remained relatively constant throughout the cell cycle, in comparison to the 500-nt replication-dependent mRNAs (Figure 4A). Densitometric analysis revealed that after treatment of proliferating HeLa S_3 cells with hydroxyurea the levels of the HHC289 mRNA (2300 nt), as well as the 1100-nt mRNA, dropped to approximately 50% of their noninhibiting levels, whereas the 500-nt replication-dependent mRNAs decreased by 95% (Figure 4A).

Transcription of the HHC289 Histone Gene during the HeLa S_3 Cell Cycle. To determine the extent to which transcription contributes to the cell cycle regulation of HHC289 gene expression, we carried out in vitro run-on transcription analysis of nuclei isolated from synchronized HeLa S_3 cells after release from a double thymidine block (Figure 5). During S phase (3 h), the transcription from the HHC289 gene was 2-fold higher than its level outside of S phase (10 h). In comparison, we observed that during S phase total H4 histone and H4/CAT fusion gene transcription increased 2–3-fold over the levels outside of S phase (Figure 5).

HHC289 mRNA Is Expressed in a Reciprocal Relationship with Replication-Dependent H2B mRNAs during the Onset of HL60 Cell Differentiation. We examined the level of HHC289 mRNa following induction of HL60 promyelocytic leukemia cells along the monocytic pathway, as proliferation ceases and monocyte-specific gene expression is initiated. RNA was isolated from proliferating HL60 cells and from cells at various times after induction of differentiation with TPA. Northern blot analyses, using the 3′-specific probe (Figure 6A) or the protein-coding fragment (Figure 7A), revealed that the 1100- and 2300-nt H2B histone mRNAs, present in very low levels in the proliferating HL60 sample, were induced upon TPA treatment. On the basis of densitometric analysis, the 2300-nt HHC289 mRNA was present at a 10-fold higher level by day 2 than in the proliferating sample (Figure 7B). The 1100-nt mRNA, present at a very low level in the proliferating HL60 cells, also increased until day 2 (Figure 7A). The 500-nt H2B mRNAs were detected at a high level in the proliferating

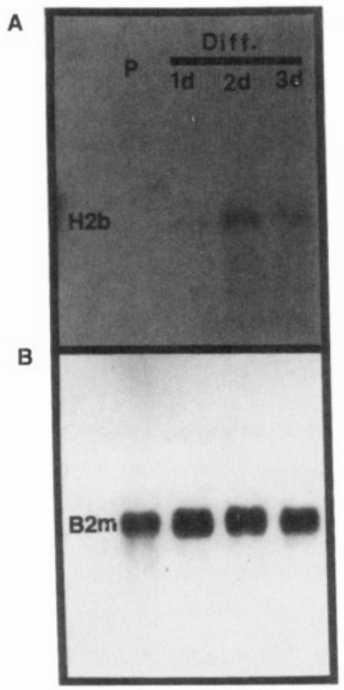

FIGURE 6: HHC289 mRNA levels during proliferation and differentiation of HL60 cells. One microgram of poly(A+) RNA from proliferating (P) and differentiated (diff.) HL60 cells was used for Northern blot analysis. RNA was isolated from differentiated HL60 cells at 1 (1d), 2 (2d), and 3 days (3d) after treatment with TPA. The ^{32}P-labeled probes were the 870-nt *Eco*RI/*Sst*I 3′-noncoding fragment from HHC289 (panel A) and the 550-nt *Pst*I insert from pGβ2m (Cosenza et al., 1988) (panel B).

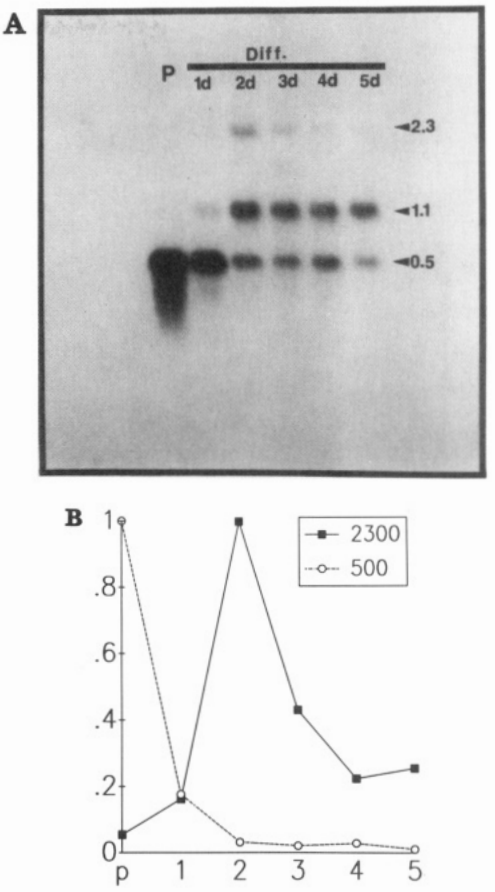

FIGURE 7: H2B mRNA levels during proliferation and differentiation of HL60 cells. (A) One microgram of poly(A+) RNA from proliferating (P) and differentiated HL60 cells was used for Northern blot analysis with the ^{32}P-labeled *Eco*RI/*Xho*I H2B coding-region fragment of HHC289 as probe. RNA was isolated from differentiated HL60 cells at 1 (1d), 2 (2d), 3 (3d), 4 (4d), and 5 days (5d) after treatment with TPA. The sizes of the various bands are indicated, in kilobases, on the right side of the figure. (B) Densitometric analysis of the 500-nt replication-dependent H2B mRNA (○, dashed line) and the 2300-nt HHC289 mRNA (■, solid line) levels during differentiation of HL60 cells. The mRNA levels are presented as a fraction of the maximum for each mRNA (verticle axis).

HL60 sample but rapidly declined until at day 5 they had decreased approximately 100-fold from their level in the proliferating sample. As illustrated in Figure 7B, a reciprocal relationship exists during HL60 differentiation between the high molecular weight H2B mRNAs, which increase 10-fold, and the 500-nt H2B mRNAs, which decrease to less than 1% of their level in proliferating HL60 cells.

DISCUSSION

Structural Analysis of the HHC289 H2B cDNA. Although comparison of the nucleotide sequence of HHC289 with several other H2B histone sequences revealed that the protein-coding region is highly conserved, the striking differences in the 3′-trailing sequences suggest that the HHC289 cDNA is unique among H2B histone sequences described thus far. The HHC289 H2B cDNA is the only H2B human histone sequence reported which contains both the highly conserved histone 3′ stem–loop structure (Hentschel & Birnstiel, 1981) and a poly(A) addition sequence (Fitzgerald & Shenk, 1981; Proudfoot & Brownlee, 1976) followed by a poly(A) stretch, although Mannironi et al. (1989) have reported an H2A histone isoprotein (H2A.X) which is encoded by a poly(A+) mRNA containing the 3′ stem–loop structure and a poly(A) addition sequence. HHC289 is also distinct from the other poly(A+) histone sequences reported in that it contains the longest 3′ trailer. The structural elements described for HHC289 may play a role in conferring both replication-dependent and replication-independent properties.

Cell Cycle Regulation of the HHC289 H2B Histone Gene. When mRNA levels were measured throughout the HeLa S_3 cell cycle, the 2300- and 1100-nt mRNAs did not undergo cell cycle fluctuations as did the replication-dependent histone mRNAs (Baumbach et al., 1984; Heintz et al., 1983; Plumb et al., 1983a,b). In addition, the level of the 2300-nt HHC289 mRNA species in proliferating HeLa S_3 cells dropped only 50% after treatment with hydroxyurea, suggesting incomplete coupling to DNA synthesis. This is consistent with results on the rat testis-specific H4 and sea urchin α-histone H4 mRNAs containing the 3′ stem–loop motif, which have been observed to be stable in the presence of hydroxyurea (Grimes et al., 1987; Jarvis & Marzluff, 1989). In vitro run-on transcription analysis of nuclei isolated from HeLa S_3 cells revealed that transcription from the HHC289 gene is 2-fold higher during S phase than outside of S phase, whereas during S phase total H4 histone and H4/CAT fusion gene transcription increased 2–3-fold over the levels outside of S phase (Figure 5). These results suggest that transcriptional regulation of HHC289 expression is similar to that of replication-dependent histone genes.

Our HeLa S_3 cell cycle data raise the following question: Does the internal 3′ stem–loop motif influence HHC289 mRNA processing and/or stability, or are these controlled primarily by other 3′-trailer elements, such as the large size of the 3′-trailer sequence as well as its poly(A+) tail? It is also possible that nucleotide sequences in protein-coding regions are involved in histone mRNA degradation (Jarvis & Marzluff, 1989). The level of the 2300-nt HHC289 mRNA species is very low in proliferating HeLa S_3 and HL60 cells; this observation raises the possibility of a dual processing pathway in which the majority of the HHC289 transcripts are

cleaved to the internal 3′ stem–loop motif, leaving only a minority in the 2300-nt form. This is consistent with reports of histone genes with alternative pathways of mRNA 3′-end processing that produce independently regulated mRNAs from a single gene (Challoner et al., 1989; Cheng et al., 1989; Chodchoy et al., 1987). The possibility of a dual processing pathway for the HHC289 mRNA is further supported by the presence of the sequence AAAGTAAGT (position 434–442 in Figure 1A), located 51 nt 3′ to the stem–loop motif, which is nearly identical with the 5′-splice consensus (Breathnach & Chambon, 1981). The HHC289 sequence also contains a potential 3′-splice site (Breathnach & Chambon, 1981), TGTGTATAGAT (positions 2057–2068), that if spliced to the AAAGTAAGT 5′-splice site would result in a poly(A+) mRNA similar in size to the bulk of H2B mRNA observed in cycling cells. This potential splice junction conforms to the "GT-AG" rule (Breathnach & Chambon, 1981) and is immediately preceded by a pyrimidine-rich region and a branchpoint sequence (Green, 1986), TCTTAAC, 33 nt upstream. Stauber et al. (1986) observed possible processing of an H4 mRNA at a similar 5′-splice site consensus located 41 nt 3′ to the stem–loop motif to an undefined 3′-splice site further downstream. The 1100-nt H2B mRNA observed when Northern blot analysis was carried out using the protein-coding fragment from HHC289 (Figure 4A,B) could result from expression from another H2B gene or may represent dual processing of transcripts from the HHC289 gene.

Regulation of HHC289 mRNA during Differentiation in HL60 Cells. Northern blot analysis of poly(A+) RNA isolated from proliferating and differentiated HL60 cells demonstrated that the HHC289 mRNA is regulated in a very different manner than the mRNAs for most other H2B histones. The 2300-nt HHC289 mRNA and replication-dependent H2B histone mRNA levels appear to be regulated in a reciprocal fashion when HL60 cells are terminally differentiated. These results confirm and extend our previous studies demonstrating the down-regulation of replication-dependent histones and their reciprocal relationship with a replication-independent H2B gene during the onset of HL60 cell differentiation (Collart et al., 1988; Shalhoub et al., 1989; Stein et al., 1989). The increase in 2300-nt HHC289 mRNA levels during differentiation may provide H2B histone proteins required for the reorganization and condensation of chromatin taking place during differentiation.

A striking similarity exists between the up-regulation of the 2300-nt HHC289 H2B mRNA during differentiation in HL60 cells and the up-regulation of the chicken tH2B mRNA in pachytene spermatocytes (Challoner et al., 1989). Both the HHC289 mRNA and the tH2B mRNA contain the 3′ stem–loop motif in addition to a poly(A) tail (Challoner et al., 1989). Replication-dependent H2B mRNAs are also transcribed from the same gene as the tH2B mRNA but are processed by an alternate pathway of mRNA 3′-end formation (Challoner et al., 1989). The existence of alternate 3′-processing pathways for this chicken H2B gene raises the possibility that a similar dual processing may be operative for the HHC289 gene, which would be consistent with the presence of potential splice sites in the HHC289 sequence (Figure 1).

We speculate that the HHC289 H2B histone gene encodes H2B proteins which are used in three situations: (i) during DNA synthesis when deposition of new histone proteins is required for packaging DNA into chromatin; (ii) for replacement of old histones on active genes or of damaged histones in the genome; (iii) during differentiation when the cell's chromatin is being reorganized or condensed and when deposition of new histone proteins may be required. We suggest that the primary role for variant histone genes such as HHC289 is to provide histones for replacement and reorganization or condensation when the bulk of replication-dependent histone mRNAs have been degraded and the majority of histone protein synthesis has been shut down. Although the HHC289 mRNA has various structural features that make it unique among human H2B histone mRNAs, such as a large 3′-flanking region and a polyadenylylation sequence, it also shares elements with replication-dependent histone mRNAs, such as the 3′ stem–loop motif. These elements may provide the means for alternative mRNA processing as the metabolic requirements of the cell are altered. However, it is also possible that the dual processing pathways are constitutive and that the mRNAs are differentially or selectively degraded.

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REFERENCES

Baumbach, L. L., Marachi, F., Plumb, M., Stein, G., & Stein, J. (1984) *Biochemistry 23*, 1618–1625.

Baumbach, L. L., Stein, G. S., & Stein, J. L. (1987) *Biochemistry 26*, 6178–6187.

Benton, W. D., & Davis, R. W. (1977) *Science 196*, 180–182.

Bird, R. C., Jacobs, F. A., Stein, G., Stein, J., & Sells, B. S. (1985) *Biochim. Biophys. Acta 824*, 209–217.

Branson, R. E., Grimes, S. R., Yonuschot, G., & Irvin, J. L. (1975) *Arch. Biochem. Biophys. 168*, 403–412.

Breathnach, R., & Chambon, P. (1981) *Annu. Rev. Biochem. 50*, 349–383.

Brown, D. T., Wellman, S. E., & Sittman, D. B. (1985) *Mol. Cell. Biol. 5*, 2879–2886.

Brush, D., Dodgson, J. B., Choi, O.-R., Stevens, P. W., & Engel, J. D. (1985) *Mol. Cell. Biol. 5*, 1307–1317.

Carrino, J. J., Kueng, V., Braun, R., & Laffler, T. G. (1987) *Mol. Cell. Biol. 7*, 1933–1937.

Challoner, P. B., Moss, S. B., & Groudine, M. (1989) *Mol. Cell. Biol. 9*, 902–913.

Chaubet, N., Chaboute, M., Clement, B., Ehling, M., Philipps, G., & Gigot, C. (1988) *Nucleic Acids Res. 16*, 1295–1304.

Cheng, G., Nandi, A., Clerk, S., & Skoultchi, A. I. (1989) *Proc. Natl. Acad. Sci. U.S.A. 86*, 7002–7006.

Chodchoy, N., Levine, B. J., Sprecher, C., Skoultchi, A. I., & Marzluff, W. F. (1987) *Mol. Cell. Biol. 7*, 1039–1047.

Clayton, D., & Darnell Jr., J. (1983) *Mol. Cell. Biol. 3*, 1552–1561.

Cole, K. D., Kandala, J. C., & Kristler, W. S. (1986) *J. Biol. Chem. 261*, 7178–7183.

Collart, D., Stein, G. S., & Stein, J. L. (1985) *Mol. Cell. Biochem. 67*, 161–170.

Collart, D. G., Wright, K. L., van Wijnen, A. J., Ramsey, A. L., Lian, J., Stein, J. L., & Stein, G. S. (1988) *J. Biol. Chem. 263*, 15860–15863.

Cosenza, S. C., Owen, T. A., Soprano, D. R., & Soprano, K. J. (1988) *J. Biol. Chem. 263*, 12751–12758.

Dalton, S., Coleman, J. R., & Wells, J. R. E. (1986) *Mol. Cell. Biol. 6*, 601–606.

Ernst, S. G., Miller, H., Brenner, C. A., Nocente-McGrath, C., Francis, S., & McIsaac, R. (1987) *Nucleic Acids Res. 15*, 4629–4644.

Feinberg, A. P., & Vogelstein, B. (1983) *Anal. Biochem. 132*, 6–13.

Fitzgerald, M., & Shenk, T. (1981) *Cell 24*, 251–260.

Flint, S. J., Plumb, M. A., Yang, U.-C., Stein, G., & Stein, J. L. (1984) *Mol. Cell. Biol. 4*, 1363–1371.
Fort, Ph., Marty, L., Piechaczyk, M., Sabrouty, S. E., Dani, Ch., Jeanteur, Ph., & Blanchard, J. M. (1985) *Nucleic Acids Res. 13*, 1431–1442.
Grandy, D. K., & Dodgson, J. B. (1987) *Nucleic Acids Res. 15*, 1063–1080.
Grandy, D. K., Engel, J. D., & Dodgson, J. B. (1982) *J. Biol. Chem. 257*, 8577–8580.
Green, L., van Antwerpen, R., Stein, J., Stein, G., Tripputi, P., Emanuel, B., Selden, J., & Croce, C. (1984) *Science 226*, 838–840.
Green, M. R. (1986) *Annu. Rev. Genet. 20*, 671–708.
Grimes, S., Weisz-Carrington, P., Daum III, H., Smith, J., Green, L., Wright, K., Stein, G., & Stein, J. (1987) *Exp. Cell Res. 173*, 534–545.
Hagenbuchle, O., Bovey, R., & Young, R. A. (1980) *Cell 21*, 179–187.
Harvey, R. P., Robins, A. J., & Wells, J. R. E. (1982) *Nucleic Acids Res. 10*, 7851–7863.
Harvey, R. P., Whiting, J. A., Coles, L. S., Krieg, P. A., & Wells, J. R. E. (1983) *Proc. Natl. Acad. Sci. U.S.A. 80*, 2819–2823.
Hatch, C. L., & Bonner, W. B. (1988) *Nucleic Acids Res. 16*, 1113–1124.
Heintz, N., Sive, H. L., & Roeder, R. G. (1983) *Mol. Cell. Biol. 3*, 539–550.
Helms, S., Baumbach, L., Stein, G., & Stein, J. (1984) *FEBS Lett. 168*, 65–69.
Hentschel, C. C., & Birnstiel, M. L. (1981) *Cell 25*, 301–313.
Hwang, I., & Chae, C.-B. (1989) *Mol. Cell. Biol. 9*, 1005–1013.
Igo-Kemenes, T., Horz, W., & Zachau, H. G. (1982) *Annu. Rev. Biochem. 51*, 89–121.
Isenberg, I. (1979) *Annu. Rev. Biochem. 48*, 159–191.
Jackson, V. (1985) *Biochemistry 24*, 6921–6930.
Jarvis, J. W., & Marzluff, W. F. (1989) *Dev. Biol. 132*, 325–330.
Jung, A., Sippel, A. E., Grez, M., & Schütz, G. (1980) *Proc. Natl. Acad. Sci. U.S.A. 77*, 5759–5763.
Kim, Y.-J., Hwang, I., Tres, L. L., Kierszenbaum, A. L., & Chae, C.-B. (1987) *Dev. Biol. 124*, 23–34.
Lehrach, H., Diamond, D., Wozney, J. M., & Boedtker, H. (1977) *Biochemistry 16*, 4743–4751.
Maniatis, T., Fritsch, E. F., & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
Mannironi, C., Bonner, W. M., & Hatch, C. L. (1989) *Nucleic Acids Res. 17*, 9113–9126.
Marashi, F., Prokopp, K., Stein, J., & Stein, G. (1984) *Proc. Natl. Acad. Sci. U.S.A. 81*, 1936–1940.
Marashi, F., Helms, S., Shiels, A., Silverstein, S., Greenspan, D. S., Stein, G., & Stein, J. (1986) *Biochem. Cell Biol. 64*, 277–289.
Morris, T. (1990) *Mol. Cell. Biol.* (in press).
Pauli, U., Chrysogelos, S., Stein, G., Stein, J., & Nick, H. (1987) *Science 236*, 1308–1311.
Plumb, M., Stein, J., & Stein, G. (1983a) *Nucleic Acids Res. 11*, 2391–2410.
Plumb, M., Stein, J., & Stein, G. (1983b) *Nucleic Acids Res. 11*, 7927–7945.
Prokopp, K. M. (1984) M.S. Thesis, University of Florida.
Proudfoot, N. J., & Brownlee, G. G. (1976) *Nature 263*, 211–214.
Roberts, C., & Wilson, G. L. (1985) *Focus (BRL) 7(3)*, 16.
Sanger, F., Nicklen, S., & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. U.S.A. 74*, 5463–5467.
Scherrer, K., & Darnell, J. (1962) *Biochem. Biophys. Res. Commun. 7*, 486–490.
Schümperli, D. (1986) *Cell 45*, 471–472.
Shalhoub, V., Gerstenfeld, L. C., Collart, D., Lian, J. B., & Stein, G. S. (1989) *Biochemistry 28*, 5318–5322.
Sierra, F., Lichtler, A., Marashi, F., Rickles, R., van Dyke, T., Clark, S., Wells, J., Stein, G., & Stein, J. (1982) *Proc. Natl. Acad. Sci. U.S.A. 79*, 1795–1799.
Sittman, D. B., Graves, R. A., & Marzluff, W. F. (1983) *Proc. Natl. Acad. Sci. U.S.A. 80*, 1849–1853.
Southern, E. M. (1975) *J. Mol. Biol. 158*, 157–179.
Stauber, C., Lüscher, B., Eckner, R., Lotscher, E., & Schümperli, D. (1986) *EMBO J. 5*, 3297–3303.
Stein, G. S., & Borun, T. W. (1972) *J. Cell. Biol. 52*, 292–307.
Stein, G. S., Stein, J. L., & Marzluff, W. F., Eds. (1984) *Histone Genes*, John Wiley & Sons, New York.
Stein, G., Lian, J., Stein, J., Briggs, R., Shalhoub, V., Wright, K., Pauli, U., & van Wijnen, A. (1989) *Proc. Natl. Acad. Sci. U.S.A. 86*, 1865–1869.
Taylor, J. M., Illmensee, R., & Summers, J. (1976) *Biochim. Biophys. Acta 442*, 324–330.
Thomas, P. (1980) *Proc. Natl. Acad. Sci. U.S.A. 77*, 5201–5205.
Tripputi, P., Emanuel, B. S., Croce, C. M., Green, L. G., Stein, G. S., & Stein, J. L. (1986) *Proc. Natl. Acad. Sci. U.S.A. 83*, 3185–3188.
Tsai, Y. H., & Hnilica, L. S. (1975) *Exp. Cell Res. 91*, 107–112.
van Wijnen, A. J., Massung, R. F., Stein, J. L., & Stein, G. S. (1988) *Biochemistry 27*, 6534–6541.
Wahl, G. M., Stern, M., & Stark, G. R. (1979) *Proc. Natl. Acad. Sci. U.S.A. 76*, 3683–3687.
Wells, D. E. (1986) *Nucleic Acids Res. 14*, r119–r149.
Wells, D., & Kedes, L. (1985) *Proc. Natl. Acad. Sci. U.S.A. 82*, 2834–2838.
Wells, D., Hoffman, D., & Kedes, L. (1987) *Nucleic Acids Res. 15*, 2871–2889.
Wu, R. S., & Bonner, W. M. (1981) *Cell 27*, 321–330.
Zhong, R., Roeder, R. G., & Heintz, N. (1983) *Nucleic Acids Res. 11*, 7409–7425.
Zweidler, A. (1980) *Dev. Biochem. 15*, 47–56.