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The primary structure and expression of four cloned human histone genes

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ABSTRACT

The complete nucleotide sequence of four human histone genes has been determined. Each gene codes for a core histone protein which is very homologous with the corresponding calf thymus or rat histones. The 5' and 3' flanking regions of the human histone genes contain previously identified concensus sequences: the TATA box, the GACTTC element; the CCAAT sequence; the 3' terminal dyad symmetry element thought to be involved in transcription termination; and a recently identified H2b specific upstream sequence. A putative H2a specific upstream sequence 5'-TTCTTGGACTCCTCTTTTC-3' is present approximately 40 base pairs upstream from the TATA box in the human H2a gene promoter. Nuclease S1 analysis of the human histone mRNAs encoded within each of these clones demonstrates that the mRNA terminii map to the expected positions relative to the known concensus sequences, and that the abundance of each mRNA is regulated during the HeLa cell cycle. Finally, in contrast to the H2b, H3 and H4 mRNAs encoded within clones pHh4A/pHh4C, pHh5B and pHu4A, respectively, the H2a mRNA encoded by Hh5G is not present in human placental RNA.

INTRODUCTION

Recent studies of the structure and expression of histone genes from a variety of higher enharyotes (reviewed in 1) have revealed an interesting complexity within this gene family. It is now apparent that the highly structured tandem repetition of invertebrate histone gene clusters (1) is not characteristic of the genomes of several vertebrates, including frog (6, 7), chicken (8, 9), human (10, 11) and mouse (12, 13). One consequence of the less ordered topological arrangement of vertebrate histone gene families is that each gene may comprise an independent regulatory unit, having no functional relationship to other nearby histone genes. This idea is in contrast to Zweidler's proposal, based on the existence of variant histone proteins which are expressed in a particular tissue (2, 3) or growth state (4, 5) that functionally related histone proteins might be coordinately regulated as an operon (2). In the simplest form of this proposal, neighboring histone genes could be coordinately expressed due to their particular position in the genome. To determine the relationship between the organization and expression of

particular copies of the human histone genes, and to define the functional unit for several of these genes, we have begun a detailed analysis of their fine structure and expression.

In this paper, we report the complete nucleotide sequence of four previously mapped (10) human histone genes. In addition, we have used the S1 nucelase mapping procedure (14) to determine the 5' and 3' ends of the transcripts encoded by these genes, and to examine their abundance during the HeLa cell cycle and in total RNA from human placenta. Each of these genes is expressed and regulated during the HeLa cell cycle and, with the exception of the H2a gene encoded within pHh5G, is also expressed in human placenta.

EXPERIMENTAL PROCEDURES

Aphichcolin was supplied by the Natural Products Branch Division of Cancer Treatment, Naitonal Cancer Institute. Restriction enzymes were purchased from Bethesda Research Laboratories or New England Biolabs. All radioactive precursors were from New England Nuclear. Cell culture, synchronization and isolation of HeLa cell RNA were exactly as described in (18).

Placental RNA was obtained from R. Darnell and I. Boime, Department of Pharmacology, Washington University School of Medicine, St. Louis, Missouri. Total cellular RNA was prepared from fresh term placenta as previously described by those investigators (30).

5' and 3' end labelling of DNA fragments were done as described by Maniatis et al. (15). DNA sequencing was by method of Maxam and Gilbert (16).

Nuclease S1 mapping was performed by the method of Berk and Sharp (14), as modified by Weaver and Weissman (17). The S1 protected DNA probes were resolved on 6% polyacrylamide: urea gels prepared according to the recipe of Maxam and Gilbert (16). The dried gels were exposed to Kodak XAR-5 film at -80° using Dupont Cronex Lightning Plus intensifying screens.

RESULTS

The histone genes analyzed in this study were subcloned from three distinct human genomic DNA fragments contained within a Charon 4A human DNA library. The isolation and characterization of these phages has been previously described (10). We chose to analyze these particular subclones for two reasons. First, their positions within the genomic clones are accurately known. Second, they had recently been employed as hybridization probes to study the rates of synthesis and decay of each of the four core histone mRNA

populations during the HeLa cell cycle (18) which thereby, justifies a detailed analysis of the fine structure and expression of these genes.

The nucleotide sequence of each subclone, and the strategy employed for sequence analysis are presented in Figures 1-4. Several features of these sequences deserve consideration and are outlined below. However, it is probable that additional interesting information will become evident as many copies of each of the individual core histone gene types are studied.

The histone H2a gene within pHh5G

The nucleotide sequence of an 866 b.p. DNA fragment containing the entire H2a coding region and several hundred nucleotides of 5' and 3' flanking sequence is presented in Figure 1. The amino acid sequence of the H2a protein encoded by this gene is closely related to the calf thymus protein (19) and corresponds to the rat replicative variant H2a.1 (3). The flanking sequences of this gene contain several sequences common to other histone genes (reviewed in 1). A TATA box (2) is located approximately 30 base pairs upstream from the H2a mRNA CAP site (21: see below). The conserved element GATCC located approximately 10 base pairs prior to the TATA box in sea urchin histone genes (22) is not present at that location. Rather, in this and several other human histone genes analyzed here, sequences identical to, or closely related to GACTTC are found between 30 and 50 nucleotides upstream from the TATA box (Figures 1-4). The sequence CAACT is present 70 nucleotides upstream from the CAP site, and may represent the functional equivalent of the CCAAT sequence (23) which is commonly found at that position in other protein coding eukaryotic genes. Finally, the 3' flanking region of this human H2a gene is peculiar because it contains two of the dyad symmetry elements which are found near the 3' ends of most histone genes (1), and which have been implicated in the termination of transcription (24).

The histone H2b gnee encoded pHh4C/pHh4A

The nucleotide sequence of a 840 b.p. DNA fragment containing a human histone H2b gene is presented in Figure 2. As suggested previously from hybridization selection assays, this H2b gene spans the junction between the Hh4A and Hh4C Eco R1 fragments in the genomic clone Hh4 (10). The histone H2b protein encoded at this position differs in only one amino acid from the calf thymus H2b (19). The presence of a Gly rather than Ser residue at position 76 in this sequence indicates that this gene encodes a protein corresponding to the rate replacement variant H2b.1 (3). The pHh4A/pHh4C H2b gene is flanked by the expected 5' consensus sequences, including the TATA box (20), the CCAAT sequence (23), and the GACTTC element referred to above. In addition, a

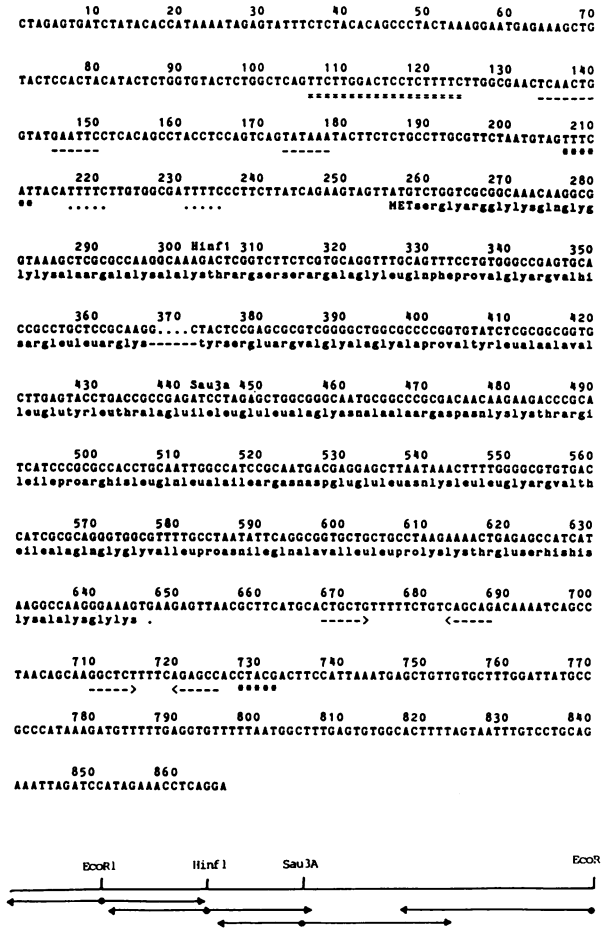

FIGURE 1. A) Sequence of the pHh5G human histone H2a gene and flanking regions. Symbols are as follows: sequences related to previously identified consensus sequences are underlined; potential mRNA terminii (S1 mapping, see Figure 5) are indicated by asterisks; TTTC element is denoted by a dot; restriction sites used to generate probes for S1 mapping are labelled; and amino acid residues in the corresponding calf thymus histone which differ from the human histone encoded herein are shown at that position in the nucleotide sequence. Nucleotide residues which were not identified are indicated as a dash.

B) Nucleotide sequencing strategy for sequence presented in (A).

recently described histone H2b gene specific element 5'CTCATTTGCATC3' (25), is found very close to the TATA box. The 3' flanking region contains dyad symmetry sequence surrounding a cluster of T residues and followed by an A rich sequence similar to that first identified approximately 10 nucleotides beyond

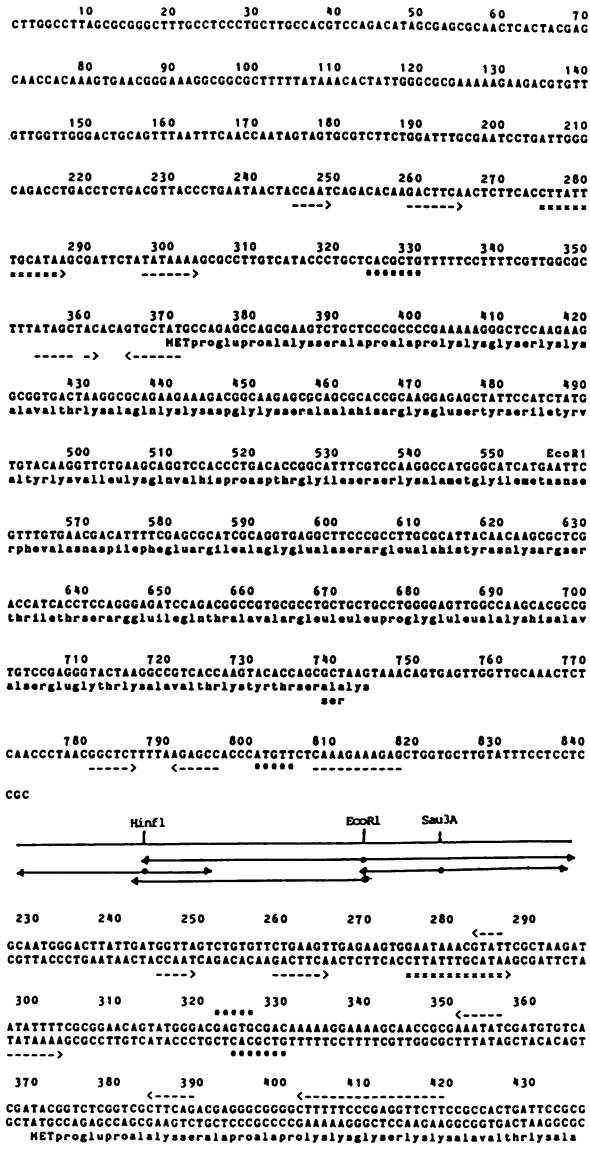

FIGURE 2. A) Sequence of the pHh4A/pHh4C human histone H2b gene and flanking regions. Symbols are as in Figure 1. A recently described H2b specific sequence element (25) is indicated by double underlining.

B) Nucleotide sequencing strategy.

C) Nucleotide sequence of divergently transcribed promoter elements within pHh4C.

the 3' end of sea urchin histone genes (24). It may be significant that there is a dyad symmetry element at the AUG initiation codon of the H2b mRNA, which could be involved in translational control of the H2b mRNA.

A particularly interesting feature of this H2b gene is that it appears to be a member of an extremely tightly linked divergently transcribed gene pair. The following characteristics of the H2b nucleotide sequence (Figure 2c) suggest that the closely linked gene is an H2a gene: the opposite strand from the H2b promoter contains a TATA box and GACTTC element symmetrically arranged with respect to the H2b mRNA CAP site and corresponding promoter elements; approximately 40 base pairs upstream from the TATA box there is the sequence 5'TTCTTGGAGCCCTTTTTC3' which is almost identical to a sequence in the same position of the independently evolving pHh5G H2a gene (see below); immediately downstream from the TATA box there is an A rich sequence which is also present in the chicken H2a gene present in clone CH01 (25). In spite of these characteristics, we have not identified an H2a protein coding region within the first 200 nucleotides downstream from the TATA box. This may not be surprising, since the chicken H2a gene mentioned above (25) also appears to encode a very long 5' transcribed but not translated leader sequence. If this is, in fact, an H2a promoter element, then this H2a mRNA provides another example of a eukaryotic mRNA in which the first AUG triplet is not used for translation initiation (6).

The histone H3 gene encoded within pHh5B

The nucleotide sequence of an 698 b.p. DNA fragment containing a human histone H3 gene is presented in Figure 3. This H3 gene differs from the calf thymus H3 histone only in a valine to glutamine change at position 125 of the mature protein (19). This gene also contains the expected consensus sequences at its 5' and 3' ends: the TATA box; two CCAAT sequences approximately 20 and 60 nucleotides upstream from the TATA box and the 3' dyad symmetry element approximately 40 nucleotides downstream from two in phase translation stop codons. Following the dyad symmetry, there is an A rich region which is quite similar in position and sequence to the corresponding sea urchin sequence element (24). In contrast to the three other human histone sequences reported here, the sequence GACTCC does not appear in the 5' flanking region on this gene.

The human H4 histone gene encoded within pHu4A

The nucleotide sequence of a 710 b.p. DNA fragment containing a human histone H4 gene is shown in Figure 4. A partial nucleotide sequence for this gene was previously reported (10). In this study, we have resequenced this

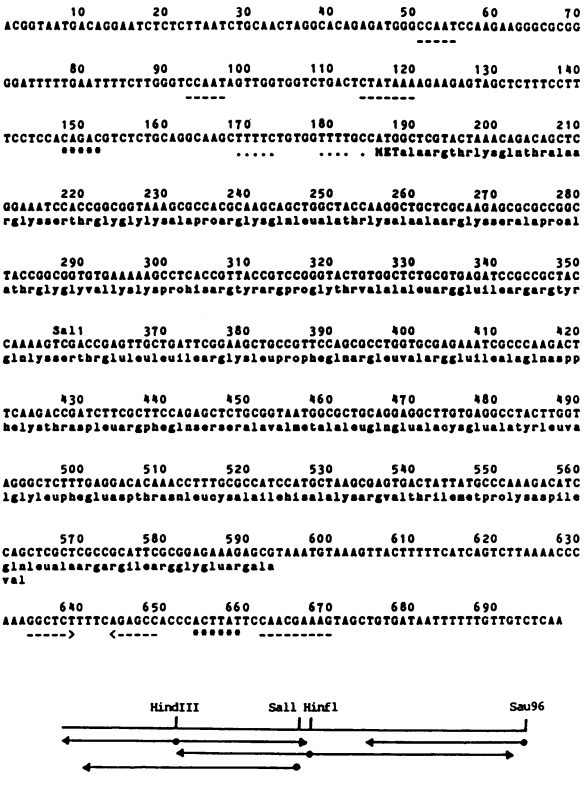

FIGURE 3. Sequence of the pHh5B human histone H3 gene. Symbols are as in Figure 1.
B) Nucleotide sequencing strategy for pHh5B.

gene and found that the previous sequence contained two errors which led us to the incorrect statement that this H4 protein is different from the calf histone H4 at two positions (10). The present data indicate that this is not the case: the H4 protein encoded by pHu4A is, in fact, identical to the calf thymus H4 (19). As previously noted (19), this gene also contains a TATA box, the GACTTC sequence elements in the 5' flanking region and the dyad symmetry element characteristic of the 3' ends of most histone genes.

As detailed above, the human histone genes share many of the nucleotide sequence elements that have been noted in other organisms (1). It is perhaps important that the presence and disposition of these elements near the coding regions of the four genes we have analyzed can vary, although it is difficult to assess the significance of this fact in the absence of sequence data for

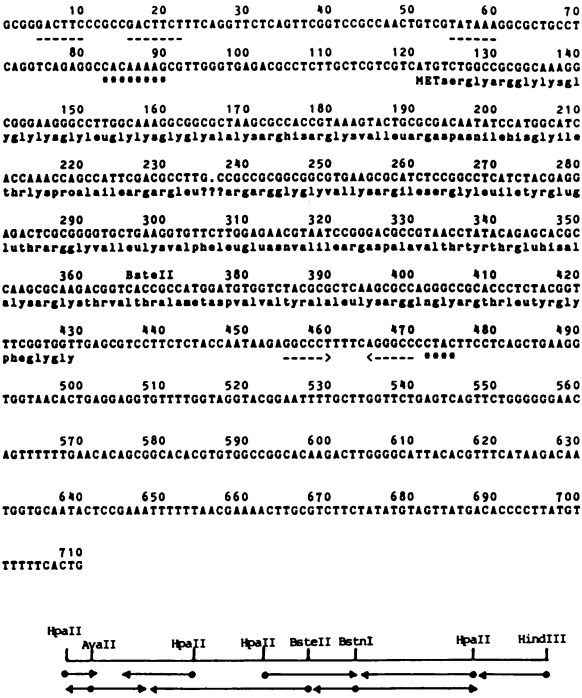

FIGURE 4. Sequence of the pHu4A human histone H4 gene (see also ref. 10). Symbols are as in Figure 1.
B) Nucleotide sequencing strategy for pHu4A.

several genes of each histone subtype. We wish to note, however, the presence of the simple sequence element--TTTTC--between the CAP site and the AUG initiator codon in three of the four genes analyzed (see Figures 1-4). While it is not clear that this sequence is important for histone gene regulation, the recent evidence supporting posttranscriptional control of histone protein production (18, 27) focuses additional interest on this region on the mRNA and has drawn our attention to this sequence.

Expression of Four Human Histone Genes

In our previous work, the histone gene subclones whose sequences are presented above (Fig. 1-4) were utilized in hybridization studies to measure the increase in steady state concentration of histone mRNA during the HeLa S phase, and to assess the contributions of transcriptional and post-transcriptional events to that increase (18). The techniques utilized (i.e., Northern blot, DNA excess filter hybridization) measured any histone mRNA

species capable of hybridizing to the subclones and, as a result, probably reflect the behavior of a number of individual histone mRNAs. In this study, we wish to examine the terminii of the particular histone mRNA encoded by pHh5G, pHh4A/4C, pH45B and pHu4A, and to study their expression in vivo. The technique most appropriate for this objective is the S1 nuclease mapping procedure first described by Berk and Sharp (14). In the following study we have used DNA fragments containing a single labelled end located within the coding region of each of the histone genes, and extending beyond the expected mRNA terminii (Figure 1-4).

The 5' terminus of the H2a mRNA encoded within pHh5G was mapped using a DNA fragment which was 5'-end labelled in the Hinf 1 site at position 306 of the sequence shown in Figure 1, whereas the 3' probe was labelled in the Sau3A site at position 443. After denaturation, each fragment was hybridized to total HeLa cell RNA and the hybrids treated with S1 nuclease to digest unhybridized nucleic acids. The S1 protected fragment from the 5' probe (Fig. 5) is 94-98 nucleotides in length, placing the H2a mRNA CAP site within the sequence -TTCAT- at position 208-212 of the sequence shown in Figure 1. Considerations from consensus sequences of other histone mRNA 5' terminii (1) suggest that this H2a mRNA is probably initiated with the A residue at position 211, 33 nucleotides downstream from the TATA box (Fig. 1). The S1 protected fragment from the 3' labelled Sau3A probe is approximately 286 nucleotides long (Fig. 5), indicating the 3' terminus of this H2a mRNA is near position 729 of the sequence shown in Figure 1, immediately downstream from the second dyad symmetry element. Since this type of experiment has never resulted in an S1 protected fragment 250-260 nucleotides in length, it appears that the first dyad symmetry element is not used to generate H2a 3' terminii in HeLa cells. Finally, definition of these mRNA terminii shows that the H2a mRNA encoded by pHh5G is approximately 520 nucleotides long.

The 5' and 3' ends of the H2b mRNA encoded by subclones pHh4A/pHh4C were also mapped using the S1 nuclease technique (14). In this case, labelled DNA probes were prepared using the Eco RI site which separates pHh4A and pHh4C such that both of the DNA probes terminated at position 560 of the H2b sequence shown in Figure 2. As shown in Figure 5, DNA fragments approximately 235 and 244 nucleotides long were generated using the 5' and 3' DNA probes, respectively. This places the mRNA cap site within the sequence -CACGCT-, 20-25 nucleotides downstream from the TATA box. The 3' terminus of the H2b mRNA maps at or near position 659, as expected from the position of the consensus sequence elements present in the 3' region of this H2b gene. The

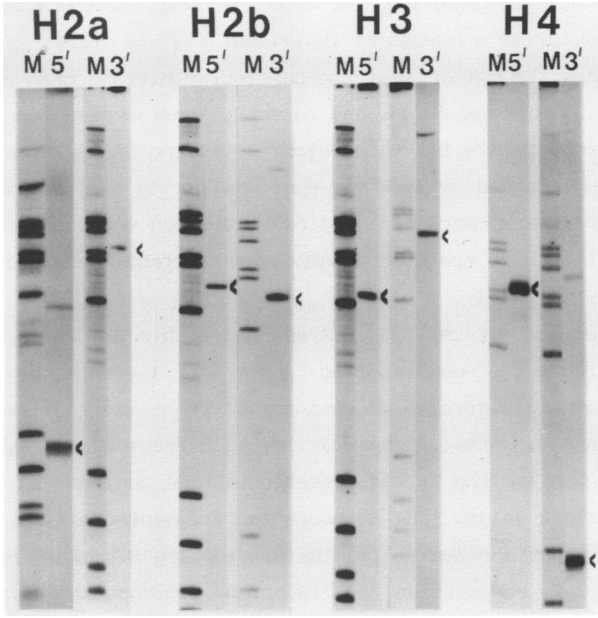

FIGURE 5. S1 mapping of 5' and 3' mRNA terminii of human histone mRNA terminii. DNA probes were prepared by 5' or 3' end labelling of restriction sites indicated in Figures 1-4 (see also text). 5-25ug of S phase HeLa cell total RNA were hybridized with 5000 cpm of probe at 48°C for three hours in a 20ul volume. S1 trimming was as in (17) for 30 minutes at 30°C. Protected DNA fragments were resolved on 8M urea, 6% polyacrylamide gels (60:3, acrylamide:bisacrylamide). Markers are pBr322/Sau3A DNA fragments which have been 3' end labelled with Klenow DNA polymerase (15). The marker DNA fragments shown on this gel are 1374, 665, 358, 341, 317, 272, 258, 207, 105, 91, 78 and 75 nucleotides in length.

mRNA encoded by this gene is, therefore, about 480 nucleotides in length.

In an experiment exactly analogous to those described above, the terminii of the H3 mRNA encoded by pHh5B were loated using DNA probes labelled within the Sal 1 site and terminating at positon 360 (Fig. 3). DNA fragments 210 and 297 nucleotides were protected from S1 digestion using the 5' and 3' probes, respectively (Fig. 5). The 5' end of the H3 mRNA is, therefore, near position 150 of the sequence shown in Figure 3, approximately 30 nucleotides from the TATA sequence. The 3 terminus of the H3 mRNA, on the other hand, maps at or near position 657 of the nucleotide sequence in the expected position immediately downstream from the dyad symmetry. THe H3 mRNA encoded by this gene is about 500 nucleotides long.

The DNA probes used to map the H4 mRNA terminii from the pHu4A subclone

were prepared using the BSTEII site in the middle of the H4 coding region and terminated at position 368 of the sequence shown in Figure 4. In this case, the 5' DNA fragment protected from nuclease digestion by total cellular RNA was 280 nucleotides in length, and that protected by the 3' probe is 104 nucleotides long (Fig. 5). The 5' terminus of this H4 mRNA is, therefore, 30 nucleotides downstream from the TATA sequence at about position 90 (Fig. 4). The 3' end is again found in the expected position immediately 3' to the dyad symmetry element. The pHu4A mRNA contains approximately 382 nucleotides.

There are several points which can be made from these data. The facts that the mRNA terminii analyzed above map to these positions expected from the consensus sequences, and that 5' and 3' transcribed but noncoding regions of these genes are protected from S1 nuclease, suggest that these particular human histone genes (or other encoding mRNAs of identical sequence) are expressed in cultured human (HeLa) cells. A second point is that a perfect stem loop structure is not always sufficient to generate 3' terminii of human histone mRNAs. Hence, the first perfect dyad symmetry element beyond the coding sequences of the pHh5G H2a gene may not be used to generate 3' ends, whereas the second dyad symmetry element is this H2a gene is apparently utilized. The fact that only the most distal of these dyad symmetry elements is utilized to generate 3' terminii *in vivo* may indicate that the positional context of these elements, in addition to their structure, is important. Finally, the fine structure mapping of these transcripts indicate lengths for the human histone mRNAs which are uniformly somewhat shorter than those previously determined by Northern blot hybridization (18), illustrating the importance of this sort of analysis to obtain accurate mRNA sizes.

Cell Cycle Regulation of Histone mRNAs Encoded by pHh5G, pHh4A/pHh4C, pHh5B, pHu4a.

The next question addressed was whether the abundance of each of these particular histone mRNAs was regulated during the HeLa cell cycle. To detect specific mRNA sequences, the S1 nuclease mapping procedure was again employed with two important considerations: the end-labelled DNA probe must be in excess in the hybridization reaction; and the S1 nuclease digestion conditions must be titrated so that we observe the band of interest, but not so rigorous that true hybrids are digested. Hence, each freshly prepared DNA probe was titrated against 5 micrograms of total cellular RNA prepared from S phase HeLa cells and in each experiment several different S1 nuclease concentrations were used.

In the experiment shown in Figure 6, the 5' DNA probes used in the

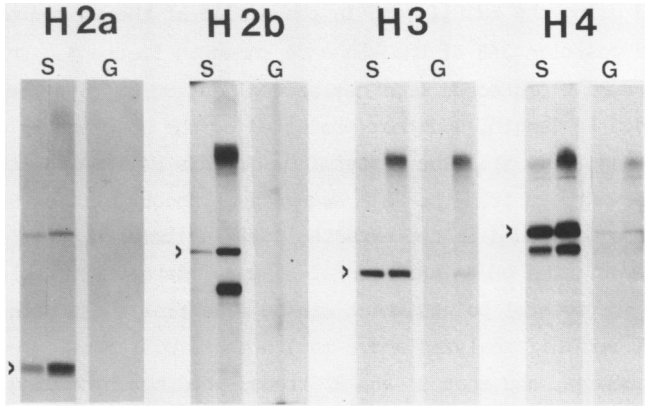

FIGURE 6. Cell cycle regulation of human histone mRNAs. S1 mapping analysis of the abundance of human histone mRNAs in total cellular RNA prepared from synchronized HeLa cells three hours after release into S phase (S) or ten hours past release (G). Hybridization and S1 trimming conditions are as in Figure 5, except that in each case hybrids were digested with both 100 and 30 units of S1 nuclease (left and right lanes, respectively, of each pair) in a 200ul reaction volume, and that 5ug of total RNA was used (see also text).

mapping studies (see Figure 5) were hybridized to 5 micrograms of total cellular RNA prepared from S phase or G1 phase HeLa cells (see Methods), the hybrids were treated with two different concentrations of S1 nuclease and the resulting protected DNA fragments resolved by gel electrophoresis. It is immediately apparent that each of these histone mRNAs is very much more abundant in S phase than in G1 phase total cellular RNA. They are, therefore, cell cycle regulated.

At the lowest S1 nuclease concentrations (right lanes), we consistently observe a slightly lower molecular weight band for the histone H2b and H4 DNA probes. The sizes of these bands are those expected for DNA fragments protected by other H2b and H4 mRNA species between the labelled DNA end and the end of the protein coding sequence. We presume that in these hybrids the transcribed but not translated mRNA sequences are sufficiently divergent so that they are not protected from S1 digestion. The ratio of the full length DNA band to the coding region band varies for the different probes utilized (Fig. 6). It is tempting to speculate that this difference in ratio of full length to coding region S1 protected bands reflects the relative abundance of the transcripts fully complementary to the probe being used versus the remaining mRNAs for that histone type. In this case, for example, the H2b gene on pHh4A/pHh4C would represent a relatively minor H2b mRNA species. There is,

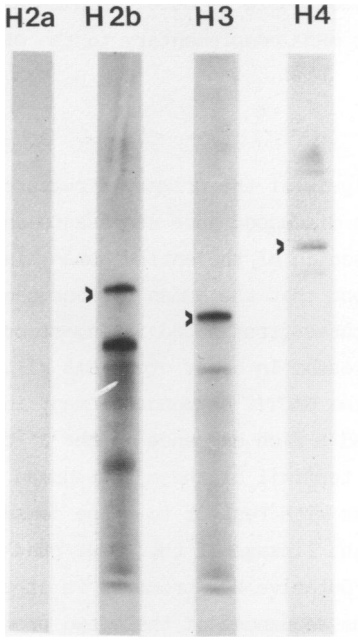

FIGURE 7. Presence of human histone mRNAs encoded by pHh5G, pHh4C/pHh4A, pHh5B and pHh4A in human placental RNA. S1 nuclease analysis was as described for Figures 5 and 6, except 50ug of total RNA from human placenta was used in each experiment, and that the resulting gel was exposed to film for 2-3 weeks instead of the more typical exposure of 1-2 days when HeLa cell RNA was used.

however, no convincing way to demonstrate that this correlation actually reflects the quantitative ratios of the specific histone mRNA species.

Representation of Histone mRNA Sequences in Human Placenta

Since each of the histone genes thus far analyzed is expressed and regulated in human cultured cells, we next wished to determine whether they were also expressed in human tissue. For this purpose, we again employed the 5' end labelled DNA probes (see above) in the S1 nuclease assay to detect the presence of these histone mRNAs in human placental RNA (the generous gift of R. Darnell and I. Boime). As shown in Figure 7, mRNAs complementary to the H2b, H3 and H4 histone gene probes could be detected in placental RNA, whereas many attempts to detect mRNA complementary to the H2a gene encoded within pHh5G failed. Since we could routinely generate end labelled probes of 10 fold higher specific activity using the procedure for 3' end labelling than 5' end labelling, the assay employing the 3' probes is significantly more sensitive.

The fact that we were also unable to detect H2a mRNA using the 3' end labelled DNA probe indicates that mRNA complementary to the pHh5G gene is not present in human placental RNA.

DISCUSSION

The detailed analysis of the primary structure and expression of the four human histone genes discussed here represents another step in our analysis of the structure and function of the entire human histone gene family. From these data, it is apparent that the human histone genes share many of the nucleotide sequence features first noted in invertebrate histone gene clusters, and later shown to be present in other organisms (1). These include the TATA box, the CCAAT box and the GACTTC sequence element in the 5' flanking region; and the dyad symmetry and A rich sequence in the 3' flanking sequence. S1 nuclease mapping of the terminii of these four human histone mRNAs place them in the expected positions with respect to these consensus sequences.

The extremely tight linkage of the pHh4C/pHh4A H2b promoter and the divergently transcribed putative H2a promoter is of considerable interest. The location of the consensus sequences of these two promoters suggests that the CAP sites for these two genes are coincident or overlapping. Because of this close apposition, the consensus sequences for these two promoters may be present within the transcribed regions of this gene pair. Furthermore, the conserved H2a upstream sequence is entirely within the coding region of the H2b protein coding sequence on the opposite DNA strand. If both of these promoters function *in vivo*, this gene pair represents a unique example of a tight compression of transcriptional regulatory sequence elements within the eukaryotic genome.

We have suggested that a sequence similar to -TTCTTGGACTCCTCTTTTC- may represent a histone H2a specific upstream sequence because it is present in the same position in the putative H2a promoter in the pHh4C subclone, and in the pHh5G H2a gene. The fact that the sequences surrounding the various conserved elements of these two promoters have diverged, and the fact that there is no histone H2b gene in proximity with the pHh5G H2a gene, indicate that these two genes are evolving independently from one another and support the conclusion that this sequence element is significant. The H2a specific element previously described in sea urchin genes (28) provides a precedent for, but is not homologous with, the human H2a specific sequence. As is evident from the position of this element within the coding region of the opposing H2b histone gene (Figure 2b), any closely linked and divergent histone H2a/H2b gene pair

will contain this sequence upstream from the H2a promoter. This is, in fact, the case in a recently characterized chicken histone clone CH01 (25). It is also relevant that Bendig and Hentschel (31) have recently observed significant transcription initiation from a "pseudopromoter" within the sea urchin H2b gene after transfection into HeLa cells. A role for a sequence homologous to the H2a specific element reported here (and located within the sea urchin H2b coding region) in generating these erroneous transcripts seems possible.

A more important point to be considered is the relevance of this putative H2a specific sequence element, and the previously identified H2b specific element reported by Harvey, Robins and Wells (25), to histone gene expression in vivo. These data raise the possibility that each type of core histone gene may have different and significant transcriptional regulatory sequences, and that these sequences are important for coordinating the expression of relevant members of the corresponding gene family. If this were indeed the case, one might expect that each histone subtype would have a distinct transcriptional effector protein. This speculation is, of course, experimentally accessible through further sequence analysis, in vitro mutagenesis and, ultimately, purification of those proteins necessary for histone gene transcription.

In discussing our results concerning the expression of the pHh5G, pHh4A/4C, pHh5B and pHu4A human histone genes in vivo, it is important to consider two limitations of the nuclease mapping technique. First, although it is possible to establish that the cell contains mRNA that is fully complementary to the cloned gene being studied, it is not possible to prove that the mRNA is expressed from that particular histone gene. There remains the formal possibility that there are two histone genes encoding an identical mRNA but that their program of expression differs as a result of either their positions in the genome or nontranscribed flanking sequences. A second point is that while the absence of a nuclease resistant band in the S1 mapping experiment may result from the lack of expression of a particular gene in the cell from which the RNA was extracted, the same result would be obtained if there were a very limited number of nucleotide changes in that particular histone gene allele between the two individuals who were the sources of the nucleic acid. The data in Figures 5 and 6 clearly indicate that the mRNAs encoded by each of the histone subclones (pHh5G, pHh54A/pHh4C, pHh5B, pHu4A) are expressed in HeLa cells, and that their steady state concentration dramatically increases during S phase. Although quantitation of this data has not been provided, it is apparent from inspection of the autoradiograph in

Figure 6 that the magnitude of the increase during S phase can differ for each specific mRNA. This is consistent with the idea that each individual histone mRNA species functions independently, and that its modulation via those mechanisms which regulate histone mRNA abundance is a consequence of its particular nucleotide sequence, position in the genome, etc. A thorough and quantitative analysis of several mRNAs encoding a given histone type will be necessary to test the validity of this idea.

A most interesting finding of this study, which must be qualified by the genetic consideration mentioned above, is that the pHh5G histone H2a mRNA, in contrast to the H2b, H3 and H4 mRNAs, is not present in total cellular RNA from human placenta. It is possible that this results from a cell type specific mode of expression for this histone H2a gene. If this is indeed the case, then the fact that the active H3 gene (pHh5B) and the inactive H2a gene (pHh5G) were isolated from the same genomic clone (Hh5; ref. 10) strongly suggests the independent regulation of these two closely linked human histone genes.

The inherent limitations of the S1 nuclease mapping procedure as a method for determining the *in vivo* expression of a specific cloned human histone gene requires further tests of the potential activity of each cloned gene. In this regard, each of the cloned human histone gene promoters is currently being tested for promoter activity in a soluble HeLa cell transcription system (19). In most instances, these genes will also be assayed for the ability to promote gene expression by transfection into cultured mammalian cells (30). In this way, we hope to generate definitive data concerning *in vivo* expression of many cloned histone genes.

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