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ARTICLES

# Association of a Human H1 Histone Gene With an H2A Pseudogene and Genes Encoding H2B.1 and H3.1 Histones

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**Abstract** A cluster of human histone genes was found on three overlapping clones isolated from cosmid and bacteriophage libraries. These three overlapping segments of the human genome comprise genes coding for H3.1, an H2A pseudogene, and an H2B.1 gene downstream of the previously characterized H1.2 gene. The cosmid clone covers 30 kb upstream of the H1.2 gene and overlaps with two phage clones covering the core histone genes and the pseudogene. The same arrangement of an H3 gene, an H2A pseudogene and an H2B gene downstream of an H1 gene has been described within a mouse histone gene cluster [Yang et al.: *J Biol Chem* 262:17118–17125, 1987; Gruber et al.: *Gene* 95:303–304, 1990]. © 1993 Wiley-Liss, Inc.

**Key words:** histone H1, histone H2A, histone H2B, histone H3, histone genes, histone pseudogene

The coordinate synthesis of histones during the S phase of the cell cycle requires the expression of sets of genes coding for H1 and the four core histones. These genes are grouped in clusters, which are organized in some lower eukaryotes as tandem repeats of histone gene quintets [Hentschel and Birnstiel, 1981; Maxson et al., 1983]. In contrast, varied patterns of histone genes have been observed in vertebrates. Tandem repeats of identical clusters have neither been found in avian [D'Andrea et al., 1985; Tönjes et al., 1989] nor in mammalian genomes. D'Andrea et al. [1985] have presented the most detailed description of the chromosomal organization of vertebrate histone genes: 40 histone genes are clustered in three major groups in the chicken genome in addition to smaller groups of histone genes or solitary genes [Engel et al., 1982; Krieg et al., 1983]. Besides some preferred associations (H2A with H2B genes, H3 with H4 genes), the five histone genes were irregularly distributed, and no tandem repeats of gene quintets were observed.

In mammals, several authors have described parts of mouse and human histone gene clusters with varied numbers of the five main histone classes [Clark et al., 1981; Sittman et al., 1981; Heintz et al., 1981; Sierra et al., 1982; Zhong et al., 1983; Stein et al., 1984; Yang et al., 1987; Gruber et al., 1990; Albig et al., 1991]. In four cases, human H1 histone genes were associated with core histone genes [Carozzi et al., 1984; Zwollo et al., 1984; Albig et al., 1991], whereas the majority of published segments of human histone gene clusters showed nothing but core histone genes [for review, see Stein et al., 1984].

In contrast to the main type H1 genes, the H1° histone gene is a solitary gene [Doenecke and Tönjes, 1986] in analogy to its avian counterpart H5 [Krieg et al., 1982; Doenecke and Tönjes, 1984].

In addition to the initial publication of a human H1 histone gene partial sequence by Carozzi et al. [1984], we have published four sequences of human main type H1 histone genes [Eick et al., 1989; Albig et al., 1991] and we have termed these genes H1.1, H1.2, H1.3, and H1.4. Here we show that the H1.2 gene [Eick et al., 1989] is associated with two core histone genes and a H2A histone pseudogene. A similar overall arrangement of genes including an H2A pseudo-

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The sequence data presented in this article have been deposited with the EMBL sequence data library under the accession numbers X57127 (H2B gene), X57128 (H3 gene), X57131 (H2A pseudogene).

gene has been described in the mouse genome [Yang et al., 1987; Gruber et al., 1990].

## MATERIALS AND METHODS

### Screening of Two Human Genomic Libraries

A human DNA library constructed in EMBL3 bacteriophage (Clontech, Palo Alto, CA) and a cosmid library in pcos2EMBL as a cloning vector (kindly provided by Dr. A. Zimmer, Max-Planck-Institute for Experimental Medicine, Göttingen, Germany) were screened using the human H1.2 gene [Eick et al., 1989] as a probe. The screening of the phage library was done using the in situ plaque hybridization technique [Benton and Davis, 1977]. The DNA probe was a 0.7 kb AvaII fragment from the H1.2 clone covering most of the coding region and part of the 3′ flanking part of the gene. The probe was labeled with [$^{32}$P] by the method of Feinberg and Vogelstein [1983].

The human cosmid library in pcos2EMBL as a vector [Ehrich et al., 1987] was screened as described by Herrmann et al. [1987] with the same H1.2 probe as the EMBL3 library (see above).

### Mapping and Subcloning of the Phage and Cosmid Insert DNA

DNA was prepared from the respective phage [Sambrook et al., 1989] and cosmid [Herrmann et al., 1987] clones, cut with several restriction enzymes and analyzed by agarose gel electrophoresis. The gels were blotted onto nylon membranes and hybridized [Southern, 1975] with human H1.2 [Eick et al., 1989] and duck core histone gene probes [Tönjes et al., 1989] labeled by nick translation [Rigby et al., 1977] or random priming [Feinberg and Vogelstein, 1983].

Individual segments of the phage and cosmid inserts were subcloned and further characterized by restriction analysis and Southern blot hybridizations. DNA fragments were cloned in pUC19 plasmid, DH5α was used for transformation and plasmid DNA was prepared as described [Birnboim and Doly, 1979].

### Nucleotide Sequence Analysis

The subcloned fragments derived from hybridizing DNA segments were sequenced initially by partial chemical degradation [Maxam and Gilbert, 1977] and then by the dideoxy method [Sanger et al., 1977] using the T7 DNA polymerase sequencing kit provided by Pharmacia (Uppsala, Sweden). The [$^{32}$P]- or [$^{35}$S]-labeled sequencing reaction products were separated on 6% polyacrylamide gels [Maxam and Gilbert, 1977] and analyzed by autoradiography. Each of the sequences presented were obtained by analyzing both DNA strands.

### Materials and Enzymes

Restriction enzymes and other nucleic acid modifying enzymes were from Boehringer (Mannheim, Germany), BRL (Gaithersburg, MD), and New England Biolabs (Beverly, MA). Chemicals for electrophoresis were from Serva (Heidelberg, Germany), the Maxam-Gilbert sequencing reagents were from Fluka (Buchs, Switzerland). Microbiological growth media were obtained from Difco (Detroit, MI) and Gibco (Gaithersburg, MD). All other chemicals were from Merck (Darmstadt, Germany).

Nitrocellulose and nylon filter membranes were from Schleicher and Schuell (Dassel, Germany) and Amersham-Buchler (Braunschweig, Germany), respectively. Radioactively labeled compounds were supplied by Amersham-Buchler.

## RESULTS AND DISCUSSION

### Three Overlapping Segments of Human Histone Gene Clusters

A human genomic DNA library and a cosmid library were screened using a human H1 histone gene [H1.2, Eick et al., 1989] as a hybridization probe. Both DNA inserts overlapped with the previously isolated clone 53 [Eick et al., 1989]. A detailed restriction enzyme cleavage and Southern blot hybridization analysis with the respective probes indicated the presence of three core histone genes downstream of the H1.2 gene (see Fig. 1 for composite drawing of the three clones).

Sequence analysis of the H1.2 gene on the cosmid clone did not reveal any differences compared with the H1.2 gene obtained with phage 53 [Eick et al., 1989]. Similarly, restriction sites within that region were identical with the results of the phage 53 insert analysis. As shown in Figure 1, the cosmid clone covers primarily the DNA region upstream of the H1.2 gene. Hybridization analysis of that part of the cosmid insert DNA with human core histone probes did not indicate any presence of core histone genes within a region 30 kb upstream of the H1.2 gene.

The second bacteriophage clone (C3.1, see Fig. 1) overlapped with both the cosmid insert and with the phage 53 insert containing H1 and H3

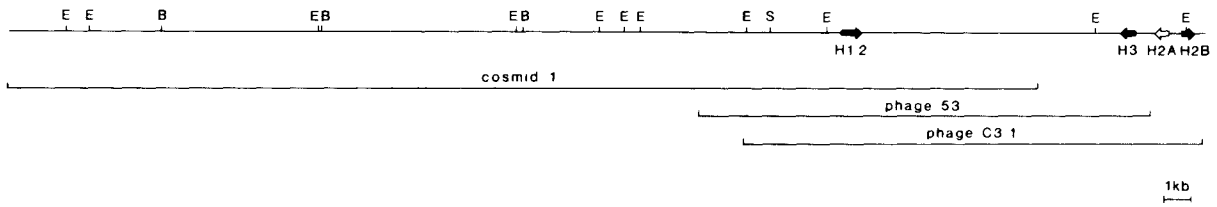


**Fig. 1.** Organization of a human histone gene cluster. Composite drawing of three overlapping clones isolated from a cosmid library and two independent genomic libraries in EMBL3 bacteriophage [phage 53 had been described before: Eick et al., 1989]. Coding portions of genes are indicated by arrows (direction of transcription). The open arrow (H2A) indicates the H2A pseudogene. A detailed map of the H3/H2A/H2B gene/pseudogene region is presented in Figure 2. Restriction enzyme cleavage sites: *EcoRI* (E), *BamHI* (B), *SalI* (S).

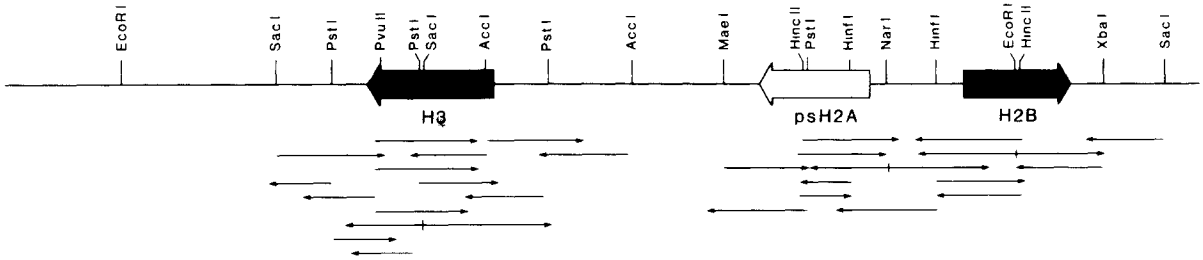


**Fig. 2.** Sequencing of three human histone genes. Restriction enzyme cleavage site distribution and sequencing strategy used for the analysis of a cluster of two functional histone genes and a pseudogene (psH2A, open arrow) as indicated in Figure 1.

genes. The overlapping parts were identical as to restriction sites and sequence details. In addition, this clone covered two additional sites hybridizing with H2A and H2B gene probes.

## Sequences of Human H2B and H3 Core Histone Genes

Three DNA segments showing sequence homologies with the core histone H3, H2A, and H2B genes were found downstream of the H1.2 gene (Figs. 1, 2). Each of the core histone genes within that cluster was sequenced (strategies given in Fig. 2) and the results are described in Figures 3–5.

The primary structure of the H2B derived from the gene sequence differs at three positions from previously published human H2B sequences, whereas the H3 translation product fits the mammalian H3.1 consensus sequence [Wells and McBride, 1989].

Several nonallelic variants of core histones have been defined by Franklin and Zweidler [1977] on the basis of few (2 or 3) amino acid exchanges. This allows to term the genes presented here as H2B.1 ($gly^{75}$ and $glu^{76}$) and H3.1 ($cys^{96}$).

The total agreement of the H3.1 amino acid sequence with other mammalian H3.1 proteins reflects the evolutionary conservation of H3 [Isenberg, 1979]. As to H2B.1, our finding of several amino acid changes in addition to those at the crucial positions 75 and 76 [Franklin and Zweidler, 1977] suggests, however, that the numbering of H2B subtypes may need further additions.

## Human H2A Pseudogene Within a Histone Gene Cluster

In contrast to the H1.2, H2B.1, and H3.1 genes, which form part of the cluster, the H2A data (Fig. 5) considerably vary from consensus sequences. Several deletions and insertions interrupt the H2A-related reading frame. It does not begin with an ATG but it begins with a sequence coding for amino acids 2 to 5 (when compared with consensus H2A primary structures). It then continues after a two base deletion as an open reading frame (with one G inserted at position 27) until it reaches a TAA triplet (at the position of codon 119). The reading frame then continues for another 9 triplets and reaches a second TAA at the same position as expected in consensus H2A sequences [Wells and McBride, 1989].

In addition to the deletions and insertions mentioned above, the most striking differences with a H2A consensus sequence are two twelve

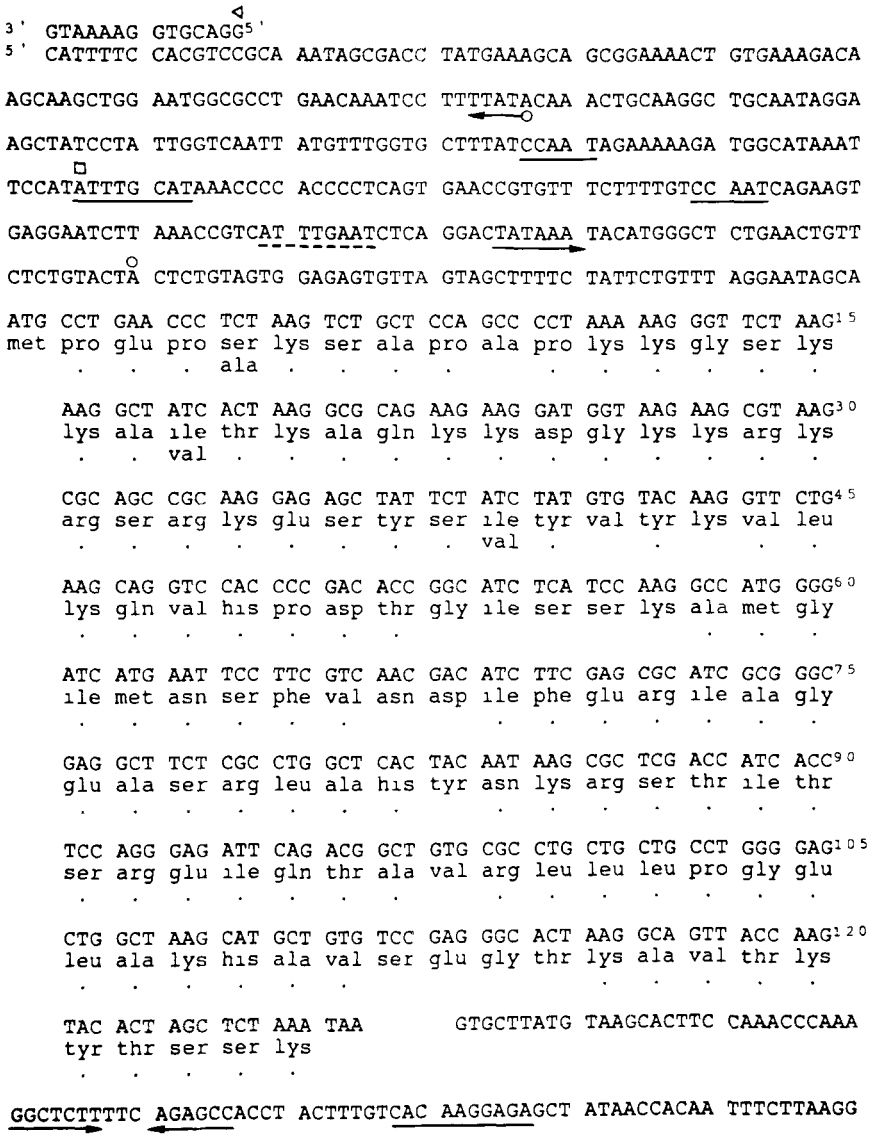

Fig. 3. Sequence of a human H2B 1 histone gene and its flanking nucleotides The mRNA like strand is shown in 5′ → 3′ orientation The amino acid sequence is compared with human H2B 1 [third line, Ohe et al , 1979], identical amino acids are indicated by dots The protein is characterized as H2B 1 by the dipeptide gly/glu at position 75/76 The TATA-box is underlined by an arrow, the TATA element upstream of the neighbouring H2A pseudogene (see Fig 5) is indicated by a circle/arrow symbol CCAAT- and octanucleotide (square symbol) elements are underlined in the 5′ flanking region The dotted line indicates a modified octanucleotide element A potential cap site [Hentschel and Birnstiel, 1981] is marked by a circle 3′ dyad symmetry structure and purine rich element are indicated in the 3′ noncoding region The top line shows the complementary strand as the beginning of the H2A pseudogene sequence as described in Figure 5 The arrowhead marks the same G as indicated in the H2A pseudogene sequence

base pair deletions which result in the loss of the coding capacity for amino acids 11–14 and 51–54 without shifting the reading frame.

The insertions and deletions in the coding part of the gene as well as the missing ATG may suffice to classify this H2A gene as a pseudogene. The other three histone genes (H1.2, H2B.1, and H3.1) appear to be functional. As to the H1.2 gene, its expression has been proven by S1 nuclease mapping [Eick et al., 1989]. Thus, we can conclude that functional (H1.2) and nonfunctional (H2A) genes contribute to this cluster.

Marashi et al. [1984] described a human histone gene cluster with the gene order H2B, H2A, H3, and H4. In that case, the H2A and H2B genes were interpreted as pseudogenes, whereas the sequence and functionality of the H3 and H4 genes was not analyzed. A complete comparison of our H2A pseudogene data with

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TGGGGCAACT CATCCAATAA GATTGTCTAG TAATGAACCA ATCAGTCTGG TCACTCTTCA

GCCAATGATT TTATCGCGCG GGACTTTTGA AATATTACAG GACCAATCAG AATGTTTCTC

                                  o
ACTATATTTA AAGGCCACTT GCTCTCAGTT CACTACACTT TGTGTGTGCT CTCATTGCAA
          ---->

ATG GCT CGT ACG AAG CAA ACA GCT CGC AAG TCT ACC GGC GGC AAA GCT
    ala arg thr lys gln thr ala arg lys ser thr gly gly lys ala15

    CCG CGC AAG CAG CTT GCT ACT AAA GCA GCC CGT AAG AGC GCT CCG
    pro arg lys gln leu ala thr lys ala ala arg lys ser ala pro30

    GCC ACC GGT GGC GTG AAG AAA CCT CAT CGC TAC CGC CCG GGC ACC
    ala thr gly gly val lys lys pro his arg tyr arg pro gly thr45

    GTG GCC TTG CGC GAA ATC CGT CGC TAC CAG AAG TCC ACC GAG CTG
    val ala leu arg glu ile arg arg tyr gln lys ser thr glu leu60

    CTG ATC CGG AAG CTG CCG TTC CAG CGC CTG GTG CGA GAA ATC GCC
    leu ile arg lys leu pro phe gln arg leu val arg glu ile ala75

    CAG GAC TTC AAA ACC GAC CTG CGT TTC CAG AGC TCT GCG GTG ATG
    gln asp phe lys thr asp leu arg phe gln ser ser ala val met90

                      □
    GCG CTG CAG GAG GCT TGC GAG GCC TAC CTG GTG GGA CTC TTC GAA
    ala leu gln glu ala cys glu ala tyr leu val gly leu phe glu105

    GAC ACC AAT CTG TGC GCT ATT CAC GCT AAA CGC GTC ACC ATC ATG
    asp thr asn leu cys ala ile his ala lys arg val thr ile met120

    CCC AAA GAT ATC CAG CTG GCA CGT CGC ATC CGT GGG GAA AGG GCA
    pro lys asp ile gln leu ala arg arg ile arg gly glu arg ala135

    TAA    GTCTGCCCGT TTCTTCCTCA TTGAAAAGGC TCTTTTCAGA GCCACTCACA
                                  ---->      <----

ATTTCACTTA AAAACAGTTG TAACCCATTC GGTTGTCTAT GTTAGTTTCC AGGAGATATA

AACGTGATAA CTACACACAA GTTTTGTAAC TGCAGACAAG TCTATCAGGC CTTTTCAACC
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**Fig. 4.** Sequence of a human H3.1 histone gene and its flanking nucleotides. The mRNA-like strand is shown in 5′ → 3′ orientation. The amino acid sequence is identical to the mammalian consensus H3.1 sequence (characterized as subtype H3.1 by $cys^{96}$, indicated by a square symbol). CCAAT- and TATA-elements (arrow) are indicated in the 5′ noncoding region (underlined). A potential cap site is marked by a circle. Arrows indicate the dyad symmetry structure in the 5′ flanking sequence (purine rich box underlined).

these results is impossible, since Marashi et al. did not sequence the H2A pseudogene beyond amino acid 50 [Marashi et al., 1984]. In their case, despite frequent third base exchanges, the amino acid sequence was completely conserved compared with other H2A species except for a deletion of amino acids 11–14. Since this site was highly conserved in all H2A species described until then, Marashi et al. suggested that the absence of these four amino acids may render this histone gene product unfunctional. Interestingly, the H2A pseudogene described here shows a deletion of four amino acids exactly at the same position. In contrast to the pseudogene described by Marashi et al., additional deletions and insertions and the missing ATG render this gene unfunctional by all means. The absence of the amino acids 11–14 in both H2A pseudogenes suggests, however, that both originated from a common precursor gene. Marashi et al. [1984] did not exclude the possibility that the otherwise conserved H2A subtype gene which they described, may in fact encode a minor H2A subtype. In that case, the pseudogene described here may have evolved from this variant H2A gene.

The overall arrangement of histone genes described here including the H2A pseudogene shows the same composition and an identical orientation of its constituent genes as the mouse histone gene cluster described by Yang et al. [1987] and Gruber et al. [1990]. This may suggest that the two clusters are homologous to each other. On the other hand, H2A-H2B gene pairs in the vicinity of H3 genes have been observed several times within histone gene clusters [see, e.g., compilation in Stein et al., 1984]. Rearrangements in the joint promoter region between the H2A and H2B genes may have resulted in both cases (mouse and human) in unfunctional H2A genes.

In addition, we should consider that an H4 gene was mapped in the mouse system about 4 kb upstream of the H1 gene [Yang et al., 1987],

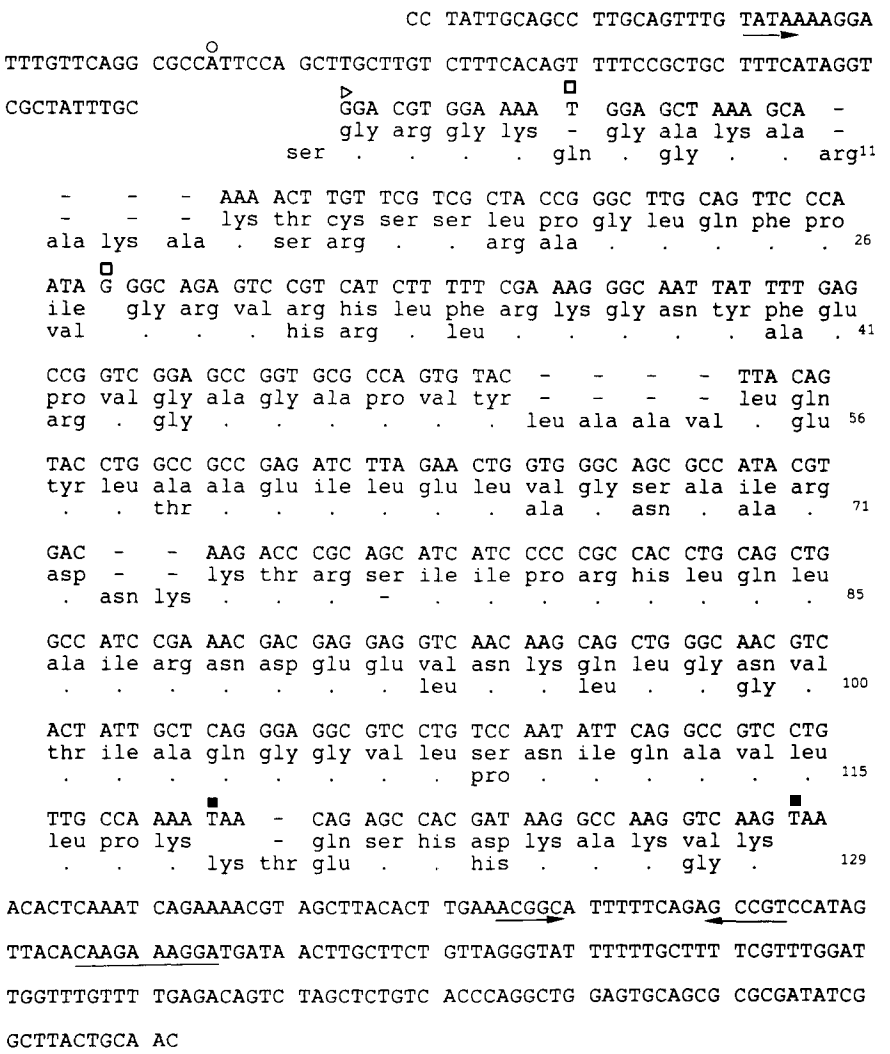


**Fig. 5.** Sequence of a human H2A histone pseudogene. The deduced protein primary structure is compared with a H2A consensus sequence [Wells and McBride, 1989]. The mRNA-like strand is shown in 5′ → 3′ orientation. Nucleotides and first amino acid line: H2A pseudogene and aligned translation product; second amino acid line: H2A consensus sequence. Numbering refers to consensus sequence. Dots indicate identical amino acids, hyphens describe gaps; open squares: frame-shift positions, two stop codons are labeled by filled squares. A consensus cap site motif [Hentschel and Birnstiel, 1981] is indicated by a circle. TATA-box and inverted repeat 3′ element are marked by arrows (see also Fig. 5), the purine rich 3′-element is underlined. Arrowhead symbol above G indicates the beginning of the complementary sequence presented as the H2B upstream region in Figure 3.

whereas no histone gene was found within 30 kb upstream of the human H1.2 gene (Fig. 1). Since no H2A pseudogene sequence data were presented by Gruber et al. [1990], we cannot compare the mouse and human clusters in detail.

### 3′ and 5′ Flanking Portions of the Core Histone Genes

Core and H1 histone genes, which are expressed during the S-phase of the cell cycle, generally show a palindromic motif within their 3′ flanking sequence [Birnstiel et al., 1985]. This element, which corresponds to a stem and loop structure at the mRNA level, is followed by ACCA (or minor variations thereof, such as ACCCA) and a purine-rich element. This is the site of 3′ processing of the respective mRNA. This consensus motif is found in the H2B.1 and H3.1 histone gene sequences described here as well as in the H1.2 gene forming part of this cluster [Eick et al., 1989]. Even the H2A pseudogene features slightly modified stem-and-loop-, ACCA-, and purine-rich elements [Birnstiel et al., 1985] at its 3′ end (Fig. 6).

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H1.2  TAG..27....GGCTCTTTTCAGAGCCACCAC..6..CAATAAAAGA
H3    TAA..27....GGCTCTTTTCAGAGCCACTCA..6..CACTTAAAAA
H2B   TAA..29....GGCTCTTTTCAGAGCCACCTA..6..CACAAGGAGA
psH2A TAA..34..ACGGCATTTTTCAGAGCCGTCCA..6..CACAAGAAAG
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**Fig. 6.** Comparison of 3′ dyad symmetry elements. Even the H2A pseudogene (psH2A) 3′ flanking region shows remnants of the consensus element terminating the transcribed region of most histone genes [Hentschel and Birnstiel, 1981; Birnstiel et al., 1985]. The H1.2 data are taken from Eick et al. [1989]. Inverted symmetry is indicated by arrows, followed by purine rich boxes. Figures indicate numbers of nucleotides between termination codon and dyad symmetry element and between the latter element and the purine rich motif, respectively.

TATA elements [Breathnach and Chambon, 1981] are found in the 5′ region of each of the core histone genes, even upstream of the H2A pseudogene. CCAAT motifs are present 5′ of the H3.1 and H2B.1 genes, the element is repeated four times in the flanking portion of the H3.1 gene.

The efficient transcription of H2B histone genes during the S-phase of the cell cycle depends on the specific octanucleotide ATTTGCAT [Sive and Roeder, 1986], which is also found in several genes coding for proteins other than histones [Falkner and Zachau, 1984; Ephrussi et al., 1985]. Figure 3 shows that the H2B.1 gene promotor described here contains this regulatory element upstream of the CCAAT motif. A slight modification of that octanucleotide element, ATTTGAAT, occurs between the CCAAT and TATA elements at a position where the octanucleotide had been originally described by Zhong et al. [1983].

Even the H2A pseudogene, which shares its 5′ flanking portion with the H2B.1 gene upstream region, carries a TATA element 20 nucleotides upstream of a potential "cap box" CCATTC [Hentschel and Birnstiel, 1981]. A CCAAT element with the orientation of the H2A pseudogene is, however, missing upstream of this pseudogene.

A divergent transcriptional orientation of H2A and H2B genes as found here in the gene/pseudogene pair has been described in several systems [D'Andrea et al., 1985; Hwang and Chae, 1989]. With two functional genes, it may suggest a joint regulation of expression of both genes, since the 5′ consensus elements of both genes are located near each other and a transcriptional activation at this site may affect both genes. For example, Hwang and Chae [1989] described the common upstream region of the rat testis-specific H2A and H2B genes, which spans 300 nucleotides (from ATG to ATG). As shown above, the distance between the functional H2B.1 gene and the H2A pseudogene is about 480 nucleotides in length and the H2A pseudogene shows just remnants of promotor elements. Thus, the generation of this pseudogene, which may have arisen initially by an unequal crossing-over event between histone gene clusters, may have resulted in an altered sequence and an increased distance between the two genes. In contrast to the H2B.1 gene, which maintained its basal promotor elements, the H2A promotor function was abolished and the resulting unfunctional H2A gene subsequently may have accumulated mutations in its coding part as well.

## CONCLUSIONS

The clustered arrangement of the histone genes described here and the 3′ flanking consensus sequences are typical features of S-phase regulated histone genes [Hentschel and Birnstiel, 1975]. This contrasts with basally expressed histone genes, which are solitary genes and their mRNA is polyadenylated [Molgaard et al., 1980; Wells and Kedes, 1985; Kress et al., 1986].

Even the H2A pseudogene, which is located between apparently functional H3.1 and H2B.1 genes, has maintained most of the 3′ stem-and-loop structure. This suggests that it is derived from a cell cycle dependent gene just as the neighboring functional histone genes.

The H3 and H2B sequences derived from the genes described here closely fit the consensus primary structures of H3.1 and H2B.1 histones. The H1.2 gene, which is located about 9 kb downstream of the H3.1 gene appears to mark the end or a major interruption of a histone gene cluster, since no further histone genes were found within 30 kb upstream of the H1.2 gene.

## ACKNOWLEDGMENTS

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