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Characterization of two types of histone H2B genes from macronuclei of *Tetrahymena thermophila*

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ABSTRACT

Two histone H2B gene clones were isolated from macronuclei of Tetrahymena thermophila. Nucleotide sequences of the two clones were highly homologous within the coding region but not in the noncoding region. Comparison of the deduced amino acid sequences between the two clones showed three differences in a total of 121 amino acids. Each of the two clones contained a TAA triplet within the coding region, which appeared to code for a glutamine residue. To demonstrate the existence of histone mRNA containing UAA triplet, nuclease P1 protection mapping using total cellular RNA and nucleotide sequencing of primer extension products were carried out. The results clearly indicated that two cloned histone H2B genes were transcribed, giving rise to the major histone H2B mRNAs with a UAA triplet sequence in frame. The tentative 5'- and 3'-ends of histone H2B mRNAs were determined.

INTRODUCTION

Tetrahymena thermophila, a ciliated protozoa, has two functionally different nuclei, macronucleus and micronucleus. It is known that under the conditions of vegetative growth, macronucleus is transcriptionally active as a vegetative nucleus, while micronucleus is thought to be dormant. On the other hand, when cells undergo sexual reproduction through the conjugation process, old macronucleus is disintegrated while micronucleus undergoes meiosis and mitosis, and one of the daughter micronuclei becomes a new macronucleus (1). During formation of macronucleus, the DNA content increases to about 20-fold of that of the micronucleus (2,3) and at the same time 10 - 20 % of micronuclear DNA sequences are eliminated (3). This elimination is accompanied by the specific breakage and the rejoining of the remaining sequences (4-6).

The first step toward elucidating the mechanism for activation of genes through rearrangement and endoreplication (or polyploidization) during macronuclear differentiation should be to analyze structural differences between the macronucleus and the micronucleus of a given gene. Among the proteins of Tetrahymena, histones are the most well-characterized proteins (7). Five histone protein species (H1, H2A, H2B, H3 and H4) have been purified from T. pyriformis (an amicronucleate strain, GL) and their amino acid sequences have been determined (8-13). In the primary structures, Tetrahymena histones are unexpectedly different from other eucaryotic histones, though histone proteins are generally thought to be highly conserved among eucaryotic organisms. For example, in both histone H3 and H4, known as well-conserved histone species, there are remarkable differences between Tetrahymena and higher eucaryotes (12,13). Furthermore, histone H1 of Tetrahymena lacks most of the central globular structure conserved in other histone H1 proteins so far studied (8,14-16). On the other hand, little was known about genes of histone proteins of Tetrahymena until Gorovsky and his co-workers recently isolated a micronuclear histone H4 gene and macronuclear histone H3, H4 and H1 genes from T. thermophila (16-20).

We have isolated two histone H2B clones from macronuclei of T. thermophila. Both clones were found to contain a TAA triplet which appeared to be a glutamine (Gln) codon instead of a termination codon. Most of the several genes cloned from ciliates also contain TAA triplets, possibly as a glutamine codon, in their coding regions (16,20-24). The presence of TAA triplet as a glutamine codon in ciliates has recently been supported by the finding that a glutamine tRNA species with a UUA anticodon was isolated from Tetrahymena (25). However, a more detailed analysis is essential to prove that the two clones are active histone H2B genes. In this paper, we demonstrate that both cloned histone H2B genes were in fact transcribed and that their transcripts containing a UAA triplet were the major histone H2B mRNAs in T. thermophila.

MATERIALS AND METHODS

Cell culture

Tetrahymena thermophila was grown at 28 °C in an aseptic medium composed of 2 % (w/v) proteose peptone (Difco No.3), 1 % (w/v) yeast extract (Difco), and 0.6 % glucose (Nakarai, Japan).

Preparation of macronuclear DNA

Nuclei were prepared as described previously (26). The nuclear suspension in 0.25 M sucrose - 10 mM $MgCl_2$ was applied to a membrane filter (polycarbonate membrane with 5.0 μm pore size, Nuclepore Corp.) to separate macronuclei from micronuclei. After extensive washing, macronuclei were washed off from the upper surface of the membrane with the same solution and were collected by centrifugation at 1500 x g for 10 min.

Macronuclei were lysed with 0.5 % SDS - SET (0.15 M NaCl - 50 mM Tris-HCl, pH 8.0 - 1 mM EDTA) containing 0.3 mg/ml of proteinase K (Merck) and were incubated for two hours at 37 °C. The mixture was extracted three times with an equal volume of distilled phenol and once with chloroform. After an addition of two volumes of ethanol, DNA fibers were spooled and taken out and then redissolved in SET. After incubation with 50 μg/ml of RNase A for 60 min at 37 °C, DNA was treated with proteinase K in the presence of 0.5 % SDS and was extracted as described above. Ethanol precipitated DNA was dissolved in TE (10 mM Tris-HCl, pH 8.0 - 1 mM EDTA).

Oligonucleotide Probes

Two different oligonucleotides as hybridization probes were synthesized by the phosphoramide method (27,28). Probe A and B correspond to the amino acid residues 51 - 57 and 41 - 46 of histone H2B of *T. pyriformis*, respectively (10,11). Two groups of "Probe A" were synthesized independently as seen below.

Group I : 5'-T-T-C-A-T-**U**-A-T-U-T-T-C-A-T-N-G-C-Y-T-T

Group II : 5'-T-T-C-A-T-**T**-A-T-U-T-T-C-A-T-N-G-C-Y-T-T

Similarly, four groups of "Probe B" were synthesized independently as follows.

Group I : 5'-A-C-U-T-C-**U**-G-G-U-T-G-**U**-A-C-Y-T

Group II : 5'-A-C-U-T-C-**U**-G-G-U-T-G-**Y**-A-C-Y-T

Group III: 5'-A-C-U-T-C-**Y**-G-G-U-T-G-**U**-A-C-Y-T

Group IV : 5'-A-C-U-T-C-**Y**-G-G-U-T-G-**Y**-A-C-Y-T

(N denotes A,G,C or T, U; A or G, Y; T or C)

Southern Hybridization and Colony Hybridization

Southern hybridization analysis was performed according to the method of Maniatis et al.(29) with some modifications. The oligonucleotides were labelled at the 5'-ends. Hybridization with "Probe A" was carried out at 37°C, and with "Probe B" at 30°C. After hybridization for 16 hr, the filters were washed with 6 x SSC (1 x SSC; 0.15 M NaCl - 0.015 M sodium citrate) twice at room temperature and three times at hybridization temperature. The same hybridization procedure was applied to colony hybridization.

Plasmid Cloning

When the macronuclear DNA was digested with several different restriction enzymes and was hybridized with the synthetic oligonucleotide probes, several distinct bands were detected with only group I of probe A or probe B (data not shown). Some of these were hybridized to both probes. For example, about 2.6 kb and 2.7 kb bands, or about 5.6 kb and 3.8 kb bands were detected in the Hind III or in the EcoR I digest of macronuclear DNA, respectively (data not shown). The Hind III digest was used as a starting material and was separated on a 1 % agarose gel. The DNA fragments from 2.2 to 3.0 kb in length (termed Hind III 2.65 kb fragments) were recovered by electroelution and were digested with Sca I followed by separation on a 1 % agarose gel. Since about 2.4 kb of the DNA fragments were identified as containing histone gene sequences after the Sca I digestion, the DNA fragments from 2.2 to 2.6 kb in length were recovered. However, since only a small portion of the Hind III 2.65 kb fragments were digestable with Sca I, after the ligation of the Sca I digests to the vector (2.3 kb of Hind III - Pvu II fragment of pBR322 DNA), the ligation products were digested with Hind III and separated on a 1 % agarose gel. About 4.7 kb of the DNA fragments were recovered, self-ligated and then used for transformation of E. coli HB 101 (29). One positive clone, pTH2B-1, was obtained from a few thousand colonies.

To isolate other histone gene sequences, the Hind III 2.65 kb fragments were digested with EcoR I. The digests were separated on a 1 % agarose gel and about 1.9 kb of the DNA fragments were recovered. These DNA fragments were cloned as described above except for the use of the Hind III - EcoR I fragment of pBR322 DNA as a vector. One positive clone, pTH2B-2, was obtained from a few thousand colonies.

DNA Sequencing

DNA sequencing was performed according to the method of Maxam and Gilbert (30).

Preparation of RNA

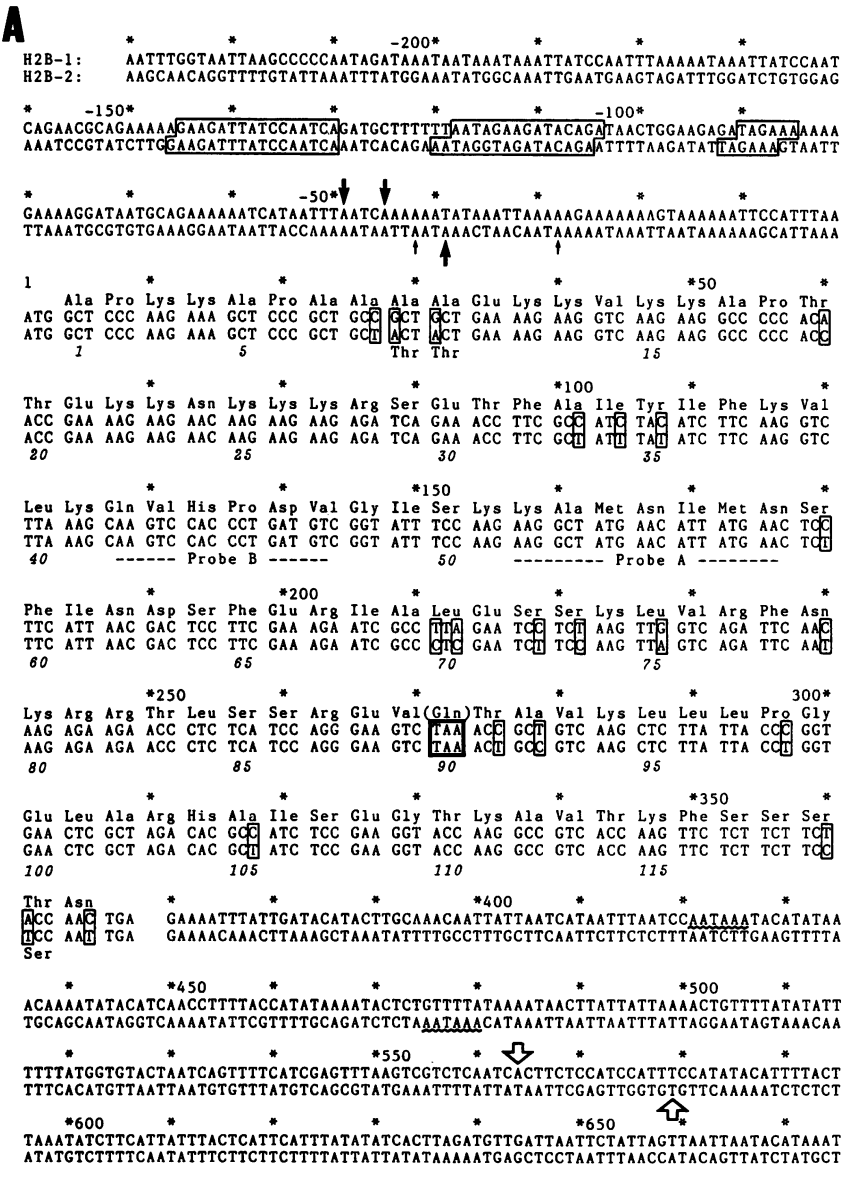
Exponentially growing Tetrahymena cells were harvested, and washed with 10 mM Tris-HCl, pH 7.8. The cell pellet was lysed with 0.1 M Tris-HCl, pH 8.0 - 40 mM EDTA - 1 % SDS. Nucleic acids were extracted twice with an equal volume of distilled phenol, once with a 1:1 mixture of phenol and chloroform, and twice with chloroform. After an addition of two volumes of ethanol, the bulk of DNA fiber was spooled and then removed. RNA was collected by centrifugation at 1500 x g, for 15 min, and used as total cellular RNA.

Primer Extension and Sequencing of Extension Products

The Alu I - Alu I fragment (100 bp) from pTH2B-2 was labelled at the 5'-ends. The single stranded fragment was used as a primer. The primer was hybridized with 1 mg of total cellular RNA in 200 μl of 80 % formamide - 40 mM Pipes, pH 6.4 - 0.4 M NaCl - 1 mM EDTA for 16 hr at 37 °C. The nucleic acids were precipitated with ethanol and dissolved in 500 μl of 50 mM Tris-HCl, pH 8.3 - 100 mM KCl - 10 mM $MgCl_2$ - 10 mM DTT - 1 mM of each dNTP. Reverse transcription was carried out with 100 units of reverse transcriptase (TAKARA Shuzo, Japan., derived from Rous associated virus 2). After incubation for 1 hr at 42 °C, the reaction products were precipitated with ethanol and separated by electrophoresis on a 5% polyacrylamide gel containing 6 M urea. Fully extended complementary DNA fragments were recovered from the gel and sequenced by the method of Maxam and Gilbert (30).

P1 nuclease Protection Mapping

For 3'-end analysis, 359 bp of Ban I - Acc II fragment


A
H2B-1:
H2B-2:
-200*
-150*
-100*
-50*
Probe B
Probe A
*200
*250
*300
*350
*400
*450
*500
*550
*600
*650
B
H2B Protein:
H2B-1 :
H2B-2 :
-Ala-Ala-Ala-( )-
-Ala-Ala-Thr-Thr-
-Thr-
-Ser-

prepared from pTH2B-1 or 409 bp of Ban I - Dra I fragment from pTH2B-2 was labelled at the 3'-end by the filling-in reaction. In the case of 5'-end analysis, 138 bp of Mbo II - Sau96 I fragment prepared from pTH2B-1 or 131 bp of Dra I - Sau96 I fragment from pTH2B-2 was labelled at the 5'-ends. The single stranded probe was hybridized with 100 μg of total cellular RNA. Hybridization was carried out in 20 μl of a low concentration of formamide - 40 mM Pipes, pH 6.4 - 0.4 M NaCl - 1 mM EDTA for 16 hr at 37 °C. The conditions (20 % formamide for 5'-end analyses, and 30 % for 3'-end analyses) were determined empirically in each case. P1 nuclease digestion and gel electrophoresis were as described previously (31).

RESULTS

Nucleotide Sequences of pTH2B-1 and pTH2B-2 Clones

The cloned DNA fragments were 2456 bp and about 2.0 kb in length for pTH2B-1 and pTH2B-2, respectively. The structural gene region of each clone was located near the end of the insert (Fig. 2), and the nucleotide sequences used as the probes were found in the expected regions (Fig. 1). The deduced amino acid sequences matched exactly that of histone H2B protein from T. pyriformis (10,11) except for a single alanine insertion in pTH2B-1 clone, and one threonine insertion and two amino acid replacements (Ala to Thr and Thr to Ser) in pTH2B-2 (Fig.1).

When the nucleotide sequences of the structural gene regions (369 nucleotides) of the two clones were compared, 21

Figure 1. (A) Nucleotide sequences of cloned histone H2B genes and deduced amino acid sequences. Thin boxes within the structural gene region indicate nucleotide differences between the two clones, and thick box TAA triplets. Nucleotides are numbered starting with the first nucleotide of the coding sequence, and amino acid residues are numbered with italic letters. Closed thick arrows indicate tentative major initiation sites, and closed thin arrows minor ones. Tentative 3'-ends of mRNA are marked with open arrows, and AATAAA sequences with wavy lines. Three conserved sequence blocks in 5'-flanking region, are boxed with solid lines. (B) Schematic comparison of histone H2B protein sequence from T. pyriformis and deduced amino acid sequences from T. thermophila histone H2B genes. The dots represent matching of amino acids.

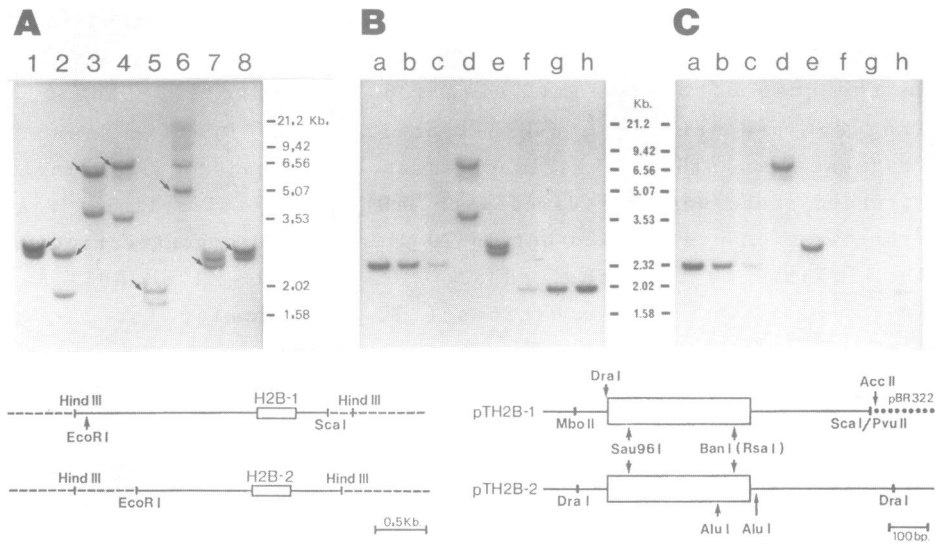


Figure 2. (A) Southern blot analysis of genomic DNA. Macronuclear DNA (10 μg) was digested with restriction enzymes. Nick-translated Dra I - Rsa I fragment from pTH2B-1 was used as a probe. Filters were washed with 0.5 x SSC at 68 °C. Lanes 1 and 8: Hind III, lanes 2: Hind III and EcoR I, lane 3: EcoR I, lane 4: Hinc II, lane 5: Rsa I, lane 6: Sca I, lane 7: Hind III and Sca I. Bands with arrow were retained after washing with 0.1 x SSC at 68°C. Some faint bands in lanes 6 and 7 resulted from imcomplete digestion with Sca I. (B,C) Estimation of copy number. Various amounts of 2.35 kb of EcoR I - Acc II fragment of pTH2B-1 (lanes a,b and c) or 2.0 kb of EcoR I - Hind III fragment of pTH2B-2 (lanes h, g and f) were mixed with 10 μg of E. coli DNA as carrier and were electrophoresed. Lanes d and e represent macronuclear DNA (10 μg) digested with Hinc II and Hind III, respectively. Lanes a and h: 200 pg, lanes b and g: 100 pg, lanes c and f: 20 pg. The filter was washed first with 2 x SSC at 68 °C, autoradiographed (B) and further washed with 0.1 x SSC at 68 °C (C). Genomic organization of histone H2B genes in T. thermophila is shown in the lower left hand corner. Solid lines indicate cloned segments and solid boxes denote structural gene regions. Restriction maps of pTH2B-1 and pTH2B-2 are also shown in the lower right hand corner. Only the sites used for preparation of probes and primer are shown. Dotted line represents pBR322 DNA.

nucleotides were found to be different, 17 out of which were at the third letter of the codons. Residual 4 were seen at the first letter of the codons, 3 of which were responsible for amino acid replacements. Within the structural gene region of

each clone, a TAA triplet, which is normally used as a termination codon, was found. By comparison with the amino acid sequence of T. pyriformis histone H2B, this TAA triplet appeared to be used as a glutamine (Gln) codon.

In contrast to the high homology (94.3 %) within the structural gene regions between the two clones, both 5'- and 3'-flanking regions were highly diverse, although several conserved sequences were found in the 5'-flanking regions (Fig. 1). Moreover, the G+C contents of the cloned DNA fragments were about 26 %, which is similar to that of genomic DNA in Tetrahymena (32). In contrast, higher G+C contents of 47 % for pTH2B-1 and 45 % for pTH2B-2 were observed within the structural gene regions.

Southern Blot Analyses with Cloned Histone H2B DNA Fragments

The Southern blot analysis of the macronuclear DNA using the synthetic oligonucleotide probes showed that the two clones seemed to be two major histone H2B genes (data not shown). To confirm this, Southern blotting experiments were carried out using DNA fragments of the cloned histone H2B genes as probes. As shown in Fig. 2A, when the Dra I - Rsa I fragment of the coding region of pTH2B-1 was used as a hybridization probe, two major bands were detected under the standard washing conditions. The size of each band was exactly the same as that obtained from the experiments using the oligonucleotide probes. When stringent washing conditions were employed, only one of the two bands was retained, which corresponded to the origin of pTH2B-1 clone. Under the same hybridization conditions, the probe hybridized with pTH2B-1 DNA but not with pTH2B-2 DNA as shown in Fig. 2C (lanes a-c and f-h). The situation was much the same in the experiment in which the pTH2B-1 probe was replaced by a probe from the coding region of pTH2B-2. In this case, only the other band, corresponding to the origin of pTH2B-2, was detected (data not shown). These results showed that a small difference of 21 out of 369 nucleotides between the two DNA sequences was detected under the hybridization conditions used here, which in turn indicates that two clones are highly likely to be representatives of two major histone H2B genes of this organism. Assuming that the DNA content of macronucleus is 10 pg (ref. 2),

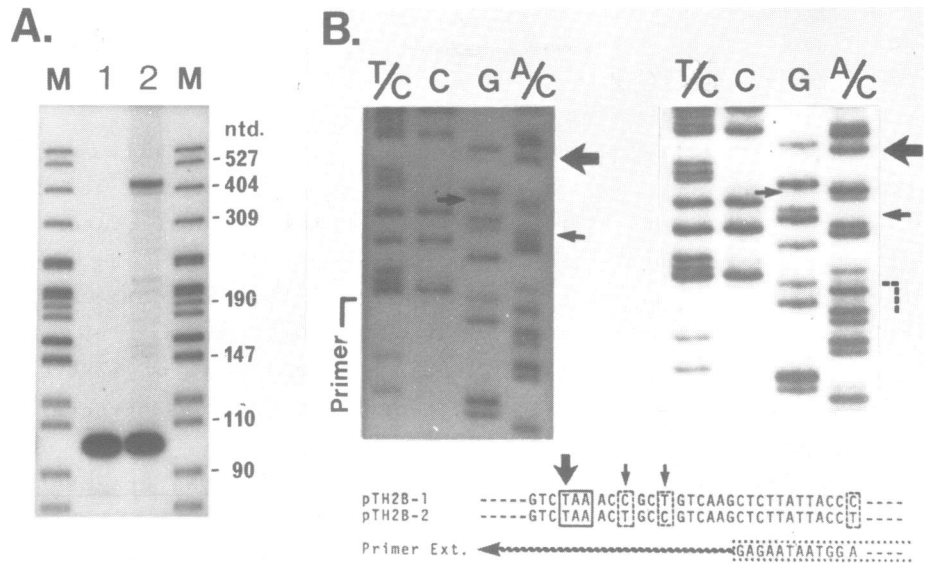


Figure 3. Primer extension and nucleotide sequences of extension products. (A) 100 μg of tRNA (lane 1) or 1 mg of total cellular RNA (lane 2) was hybridized with primer and reverse transcribed as in Materials and Methods. An aliquot of extension products was applied on a 10 % polyacrylamide - 8 M urea gel along with size marker (Hpa II and Bam HI digest of pBR322 DNA, lane M). (B) The same sample as in (A) was sequenced (left panel). The corresponding region of pTH2B-2 DNA is shown in the right panel. The position corresponding to the first letter of TAA triplet is marked with a large arrow. Differences in nucleotides between the two clones are marked with small arrows. The schematic representation of the primer extension experiment is shown at the bottom.

the copy number of each histone H2B gene was estimated to be about 50 per macronucleus (Fig.2B). This value was unchanged irrespective of washing conditions (compare Fig. 2B with 2C). Although some minor bands were also detected as in Fig. 2 (at most several copies), we do not know at present whether these sequences were other minor types of histone H2B genes.

Primer Extension Analysis

As noted above, each of the two histone H2B gene clones contained a TAA triplet within the structural gene region. To examine whether these genes were transcribed *in vivo* and whether histone H2B mRNA contained the UAA but not CAA or CAG sequence

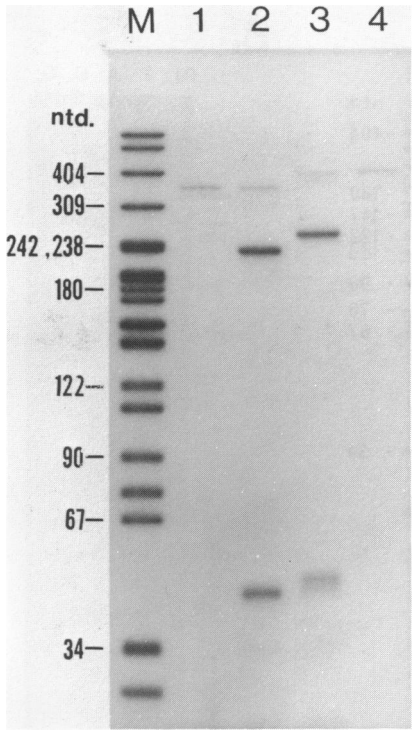


Figure 4. 3'-end analysis of histone H2B mRNA by nuclease P1 protection mapping. Ban I - Acc II fragment of pTH2B-1 and Ban I - Dra I fragment of pTH2B-2 were labelled at the 3'-ends, strand-separated, and used as probes. Probes of pTH2B-1 (lanes 1 and 2) and pTH2B-2 (lanes 3 and 4) were hybridized with total cellular RNA (lanes 2 and 3) and tRNA (lanes 1 and 4), respectively. The same size marker as in Fig. 3 was used.

(normal glutamine codons), primer extension experiments were carried out, in which we sequenced the complementary DNA synthesized *in vitro*. As shown in Fig. 3A, about 420 nucleotides of cDNA was obtained as a major extended product, whose length was consistent with the 5'-end mapping data shown in the next section. The results shown in Fig. 3B revealed that the nucleotide sequences of the fully extended cDNA matched almost perfectly those of pTH2B-2 clone. The nucleotide sequences of the region in question were indeed ATT (antisense of TAA). Neither GTT (antisense of CAA) nor GTC (antisense of CAG)

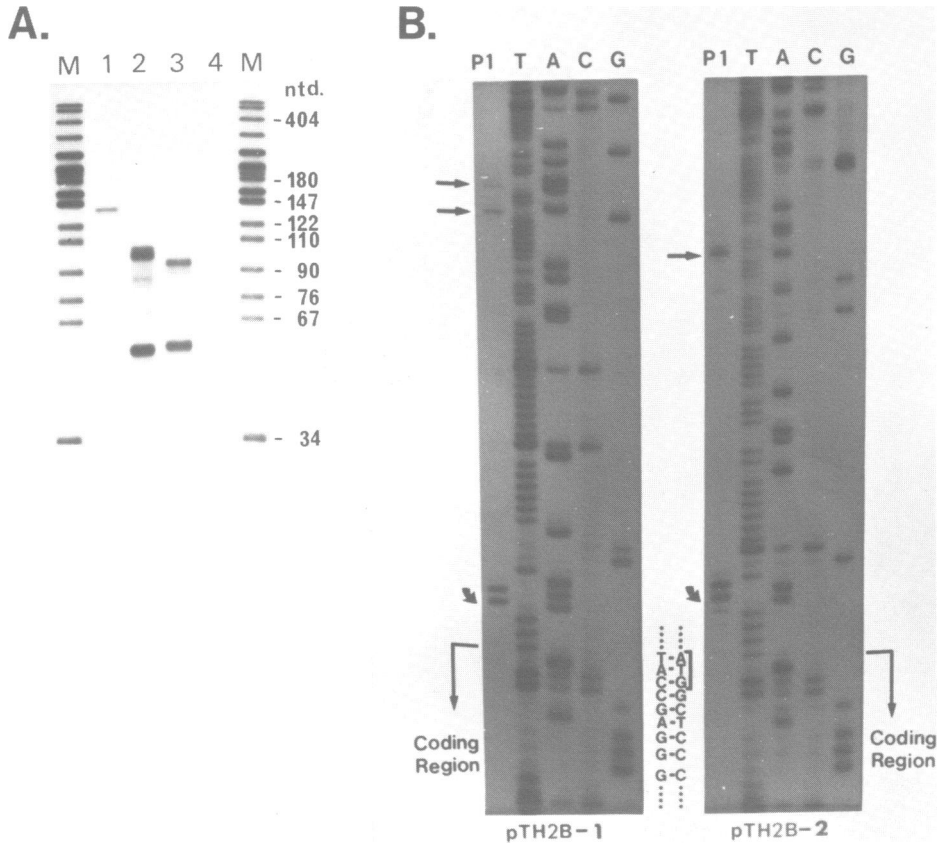


Figure 5. 5'-end analysis of histone H2B mRNA by nuclease P1 protection mapping. (A) Mbo II - Sau96 I fragment of pTH2B-1 and Dra I - Sau96 I fragment of pTH2B-2 were labelled at the 5'-ends, strand-separated and used as probes. Probes of pTH2B-1 (lanes 1 and 2) and pTH2B-2 (lanes 3 and 4) were hybridized with total cellular RNA (lanes 2 and 3) and tRNA (lanes 1 and 4), respectively. The same size marker as in Fig. 3 was used. (B) Localization of the 5'-ends of histone H2B mRNAs. The same sample in lane 2 or 3 of (A) was electrophoresed on a 12 % polyacrylamide - 8M urea gel along with chemically cleaved pTH2B-1 probe DNA (left panel) or pTH2B-2 probe DNA (right panel), respectively. Arrows indicate the positions of the protected fragments. The tentative initiation sites are indicated with straight arrows.

were detected. Additionally, it was shown that the in vitro product was a mixture of two similar cDNAs, since two different nucleotides were simultaneously observed at the positions 3 and

6 nucleotides downstream from the ATT sequence. Their positions and kinds of nucleotide indicated that the cDNA had the sequences of both pTH2B-1 and pTH2B-2 clones. This was not surprising since a fragment containing 3'-coding region was used as the primer.

P1 nuclease Analysis

Finally, to map the ends of histone H2B mRNA, we performed single strand specific nuclease protection analysis using total cellular RNA. In the 3'-end analysis (Fig. 4), two major protection bands (about 240 and 50 nucleotides) were obtained in each experiment using a probe of pTH2B-1 or pTH2B-2 clone. Judging from the size of the longer band, the 3'-end of histone H2B mRNA from pTH2B-1 or pTH2B-2 gene was mapped about 195 or 210 bp downstream from the TGA stop codon, respectively. The shorter band in each case was mapped at the end of the coding region. Considering the nucleotide sequence homology in the coding region between the two clones, it was thought that the shorter band implied the protection by mRNA from either of the two histone H2B genes, pTH2B-2 or pTH2B-1.

On the other hand, as shown in Fig. 5A, when the 5'-end analysis of mRNA from pTH2B-1 type gene was carried out, two major protection bands (about 100 and 55 nucleotides) were detected as in the case of the 3'-end analysis. To precisely locate the 5'-ends, the protected fragments were electrophoresed together with a corresponding DNA sequencing ladder. As shown in Fig. 5B, the longer bands in Fig. 5A were mapped at 49 and 45 nucleotides upstream from the ATG start codon. The shorter one was mapped at 2 and 3 nucleotides upstream from the ATG start codon. As in the case of 3'-ends, the shorter band appeared to be produced by the mRNA from the pTH2B-2 gene. Similar results were obtained in the experiment using a probe from the pTH2B-2 clone, in which the 5'-end of mRNA was mapped at 39 nucleotides upstream from the ATG start codon.

DISCUSSION

In this paper we have described the cloning and characterization of the two histone H2B genes from macronuclei of Tetrahymena thermophila. The nucleotide sequences in the coding

regions of the two histone H2B genes were highly conserved. Only three differences in a total of 121 amino acids were observed between the two deduced amino acid sequences. Two of them were located at N-terminal region which is known to be variable both in sequence and in length among histone H2B proteins (14,15).

The two histone H2B genes cloned here contained a TAA triplet as the 90th residue. Studies on the structure of protein coding genes cloned from ciliates have revealed that a TAA triplet appears to be used as a codon for glutamine instead of a stop codon (19-24). On the other hand, Kuchino et al. have recently isolated a glutamine tRNA containing UUA sequence as an anticodon and further have cloned the corresponding gene from T. thermophila (25). Furthermore, TGA triplet seems to be used exclusively as a stop codon in ciliates (32). It is known that the amino acid, corresponding to the 90th residue in the deduced sequence of pTH2B-1 or pTH2B-2, is exclusively conserved as glutamine among all histone H2B proteins so far studied including that from T. pyriformis (14,15). Histone H2B protein from T. thermophila showed relatively identical mobility to that from T. pyriformis (total 120 amino acid residues; 10,11), when electrophoresed on a SDS polyacrylamide gel, and any smaller proteins truncated at the 89th amino acid residue were not observed (unpublished observation). In the present study, we showed that both cloned genes are actively transcribed and that the majority of histone H2B mRNAs in T. thermophila contain UAA sequence at the 90th amino acid residue. These results give further support to the concept that a TAA triplet is used for a glutamine codon in ciliates.

When the nucleotide sequences of 3'-flanking regions of the two genes were compared with those of histone genes from other species, some interesting features were observed. For example, the sequence of "hyphenated dyad symmetry" known in the histone genes of sea urchin and higher eucaryotes was not found (34,35), and further, AATAAA sequence was not observed at ordinary positions either, which was at about 140 bp (pTH2B-1) or 100 bp (pTH2B-2) upstream from the tentative 3'-end of each histone H2B gene. In higher eucaryotes, the AATAAA sequence which plays a

role in polyadenylation is located commonly at 15 - 30 bp upstream from the cleavage site (36-38). Bannon et al. have suggested that histone H3 and H4 mRNAs in Tetrahymena have poly A tails at the 3'-ends (18). We also observed that histone H2B mRNA was recovered in poly A^+ fraction through oligo dT column chromatography (data not shown). The following two possibilities are raised to explain the inconsistent observations; first, signal(s) for polyadenylation of histone mRNA in Tetrahymena might be different from that generally seen in other eucaryotic mRNA. Second, histone mRNA in Tetrahymena might possess no poly A tract at its 3'-end. In this case, the recovery of the histone mRNA in poly A^+ fraction might be due to the presence of a cluster of adenine residues in the 5'-untranslated region (see Fig. 1). Recently, similar observations have been made regarding the macronuclear histone H4 genes of Tetrahymena (17). Therefore, a more careful examination will be required to conclude that histone mRNAs in Tetrahymena are indeed polyadenylated.

Within the 5'-flanking regions of the two histone H2B genes, three conserved sequence blocks were observed (Fig. 1). The first one, the most proximal to the tentative initiation site, was TAGAAA sequence, the second was AATAGN(N)AGATACAGA, and the most distal to the initiation site was GAAGATTATCCAATCA. Some of these sequence blocks are also found in other genes of Tetrahymena (16,17,19 and 23), and somewhat resemble the regulatory elements known in higher eucaryotic genes (34,37). However, further analysis is necessary to make clear whether these elements function to regulate histone H2B gene expression.

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