Polyadenylated and 3′ processed mRNAs are transcribed from the mouse histone H2A.X gene

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Received December 31, 1990; Revised and Accepted April 8, 1991 EMBL accession no. X58069

ABSTRACT

We have isolated a cDNA clone encoding a mouse histone H2A.X from a cDNA library of teratocarcinoma F9 cells. The predicted amino acid sequence of this clone is 97% identical to human histone H2A.X. The first 119 residues of the mouse H2A.X were very similar (96 – 97%) to those of the major H2A histones (H2A.1 and H2A.2) of mouse and the long carboxy terminal sequence of H2A.X was homologous with those of several lower eukaryotes. Northern blot analysis revealed that this cDNA hybridized with two mRNAs in different sizes, 0.5 kb and 1.4 kb. The two mRNAs were present in tissue culture cells, and in spleen, thymus and testes of mice, but the ratio of abundance of the two transcripts differed in different cells and tissues. The shorter mRNA contained the highly conserved palindromic sequence typical of the 3′ end of replication-dependent histone genes. The amount of this transcript was coupled to DNA synthesis and rapidly decreased in culture cells. It was synthesized just after the beginning of S-phase and degraded just after the end of S-phase. On the other hand, the longer mRNA was polyadenylated at 0.9 kb downstream from the palindromic sequence. This transcript was very stable when compared with the shorter one. These results indicate that these two mRNAs are transcribed from a single gene and maintained differently during the cell cycle, perhaps to maintain a partially replication-dependent level of histone H2A.X.

INTRODUCTION

Histones are small, highly basic proteins that associate with the DNA to form nucleosome, and play a fundamental role in organizing chromatin architecture by compacting DNA. There are several nonallelic variants for each histone in mammalian cells and their respective roles are still controversial (1).

As for Histone H2A, one of the core histones, at least four species of isoproteins are detected; H2A.1, H2A.2, H2A.X and H2A.Z (2,3). The former two are the predominating forms and synthesized in concert with DNA replication, while the latter two are minor form and synthesized through the cell cycle (4,5).

The expression of the replication-dependent type histone genes is regulated at the transcriptional and post-transcriptional levels and the mRNAs are increased 15–20 fold in S-phase (6–8). Their transcripts are processed just 3′ to a region of hyphenated dyad symmetry and just before a purine-rich sequence (9). At the end of S-phase, this type of histone mRNA is specifically degraded through a translation-dependent mechanism that does not affect polyadenylated mRNAs (10–12). In contrast to the replication-dependent histones, the expression of replacement type histone genes do not fluctuate through the cell cycle. Replacement type histone mRNA lacks the 3′ terminal stem-loop typical of replication-dependent histone mRNA and most of them are polyadenylated (13). The replacement type mRNA accumulates in quiescent and/or differentiated cells (5).

Recently, Mannironi *et al.* (14) have isolated and characterized the cDNA coding for human H2A.X histone isotype. They have shown that two forms of H2A.X mRNA were found and that they have unique C-terminal sequence which is homologous to those of several lower eukaryotes.

Here we report the isolation of a cDNA encoding a mouse histone H2A.X. We demonstrate that this gene also can produce simultaneously two types of mRNAs mentioned above and that these two transcripts are maintained differently during the cell cycle. Thus the minor histone H2A.X is produced in a partially replication-dependent manner during the cell cycle by a unique mechanism. Contribution of this kind of regulation of mRNAs and physiological role of the minor histone H2A.X is discussed.

MATERIALS AND METHODS

Cells and Animals

Teratocarcinoma F9 cell, PYS-2 cell and a cell line from mouse placenta were cultured in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal calf serum (Whittaker Bioproducts, Inc.). Differentiation of F9 cell was induced by adding retinoic acid (2×10^{-7} M) in the medium. Actinomycin D (5 μg/ml) was added in the medium to inhibit transcription.

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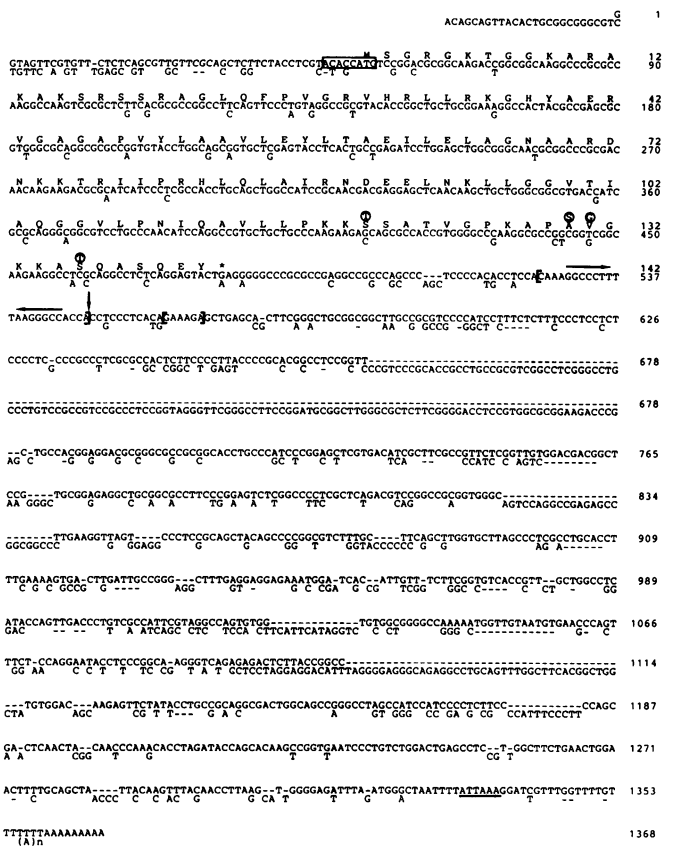

Figure 1. Nucleotide sequence and deduced amino acid sequence of the mouse H2A.X cDNA. The sequence was determined as described in Materials and Methods. Nucleotide numbering begins at the base G next to the *Eco*RI-*Bam*HI linker DNA. Amino acid numbering begins from the next codon after the initiation codon (Met). The amino acid sequence is indicated above the nucleotide sequence. Putative translation initiation sequence (Kozak's sequence) is boxed. Two conserved sequence motifs for 3' processing are denoted by frames; the first motif contains a hyphenated dyad symmetry (horizontal arrows) and vertical arrow marks the major cleavage site, and the second motif contains a purine-rich spacer sequences. Putative polyadenylation signal is underlined. The nucleotide sequence of mouse H2A.X cDNA is compared with human H2A.X (14) and different nucleotides are indicated below the mouse sequence. Sequences are aligned to obtain maximal homology; deletions introduced for this purpose are denoted by dashes. The different amino acids of human H2A.X from mouse homologue are denoted above the amino acid sequence of mouse H2A.X and circled.

BALB/c 3T3 (clone A-31) cell was cultured in DMEM supplemented with 10% calf serum (Whittaker Bioproducts, Inc.). BALB/c 3T3 cell was synchronized in G_1 phase by the serum deprivation method (15). After 48 hours, the medium was replaced with the medium containing 10% calf serum. DNA synthesis was monitored by pulse-labeling the culture with [^{3}H]-thymidine (1 μCi/ml) for 1 hour and determining the total trichloroacetic acid-precipitable radioactivity (16).

Laboratory mice 129/Sv were sacrificed and their organs were collected.

DNAs and RNAs

Genomic DNA was extracted from the liver of 129/Sv mice. Synthetic oligonucleotides were synthesized by cyanoethylphosphoamidite method on an Applied Biosystems 381A DNA synthesizer. The sequence of the synthetic probes are as follows;(1) 5'-CTGAGAGGCCTGCGAGGCCTTCTT-

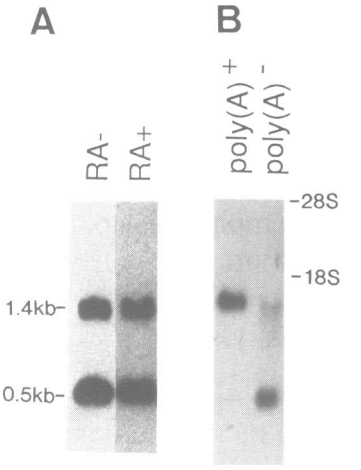

Figure 2. Northern blot analysis of H2A.X in F9 cells. (A) RA- indicates that the RNA (10 μg) was prepared from undifferentiated F9 cells and RA+ indicates that the RNA (10 μg) was prepared from F9 cells cultured in the presence of retinoic acid for 120 hours. (B) Total cellular RNA (10 μg) prepared from undifferentiated F9 cells was separated by oligo(dT)-cellulose chromatography. Resulting poly(A)$^+$ fraction and flow-through fraction described as poly(A)$^-$ in the figure, were analyzed by Northern blot analysis. The hybridization was probed with the synthetic oligonucleotide encoding the unique C-terminal of H2A.X cDNA labeled with polynucleotide kinase. The longer 1.4 kb H2A.X transcript is detected in the poly(A)$^+$ fraction, while the smaller 0.5 kb transcript is detected in poly(A)$^-$ fraction. The positions of the 28S and 18S ribosomal RNA markers are indicated.

GCCGACCGCCGGCGCCTTGGGCCCCACGGTGGCGC-T-3', a specific sequence to mouse H2A.X (see the text),(2) 5'-CAGGCGCACGGCCGTCTGGATCTCCCGGGACGTG-ATGGTCGAGCGCTTGTTGTAATGCGC-3', a part of the sequence of histone H2B reported by Sittman *et al.* (17), (3) 5'-CTTTGAGACAAGCATATGCTACTGGCAGGATCAAC-CAGGTA-3', a complementary sequence of 5' part of mouse 18S rRNA (18).

Total RNA was extracted with guanidine isothiocyanate followed by centrifugation in CsCl solution (19). Poly(A)$^+$ RNA was prepared with oligo(dT)-cellulose column.

Construction and screening of cDNA libraries

cDNA library of mouse 8-cell stage embryos was constructed as previously reported (20). cDNA library of F9 cells was prepared by the procedure as follows. cDNA was prepared from 1 μg of poly(A)$^+$ RNA by the method of Gubler and Hoffman (21), and methylated with *Eco*RI methylase. *Bam*HI-*Eco*RI-*Bam*HI synthetic linker (5'-GGATCCGAATTCGGATCC-3') was ligated to this cDNA and digested with *Eco*RI. From resulting digests, cDNA molecules were separated from the linker by electrophoresis on low-melting-point agarose gel and ligated with *Eco*RI digested lambda ZAP (Stratagene). Ligated DNA was packaged with packaging extract and introduced into host *E. coli* cell. A cDNA library consisting of 9.5×10^4 independent recombinants was obtained.

Sequencing

DNA sequencing was performed by the dideoxynucleotide chain termination method (22). The analysis of DNA sequences and predicted amino acid sequence was performed using the programs of IDEAS (23) and UWGCG (24) in micro VAX II computer.

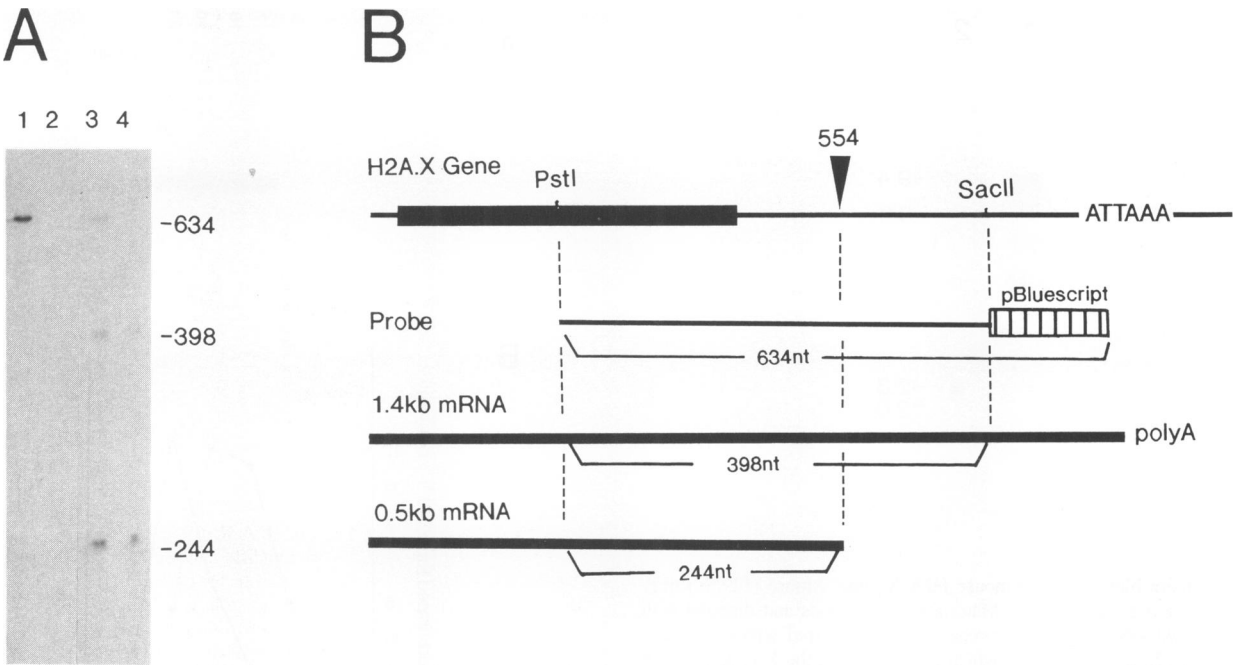

Figure 3. S1-nuclease analysis of the two mouse H2A.X transcripts. Total RNA was prepared from undifferentiated F9 cells and used for analysis. (A) The RNA samples were hybridized with ^{32}P labeled probe. The lane 1 shows the migration of labeled intact probe. In the lane 2, *E. coli* tRNA was added in place of F9 RNA to the reaction. F9 RNA was hybridized with the probe at 55°C (lane 3) and 60°C (lane 4). Two bands corresponding to 398 and 244 nucleotide lengths were observed. (B) Scheme of the putative protected bands in S1-nuclease mapping. Intact probe used in this assay consists of the *PstI-SacII* 398 bp cDNA segment of H2A.X linked with *SacII-HinfI* 236 bp fragment derived from pBluescript SK(−) (see Materials and Methods). The expected length of each fragment protected is indicated in the scheme.

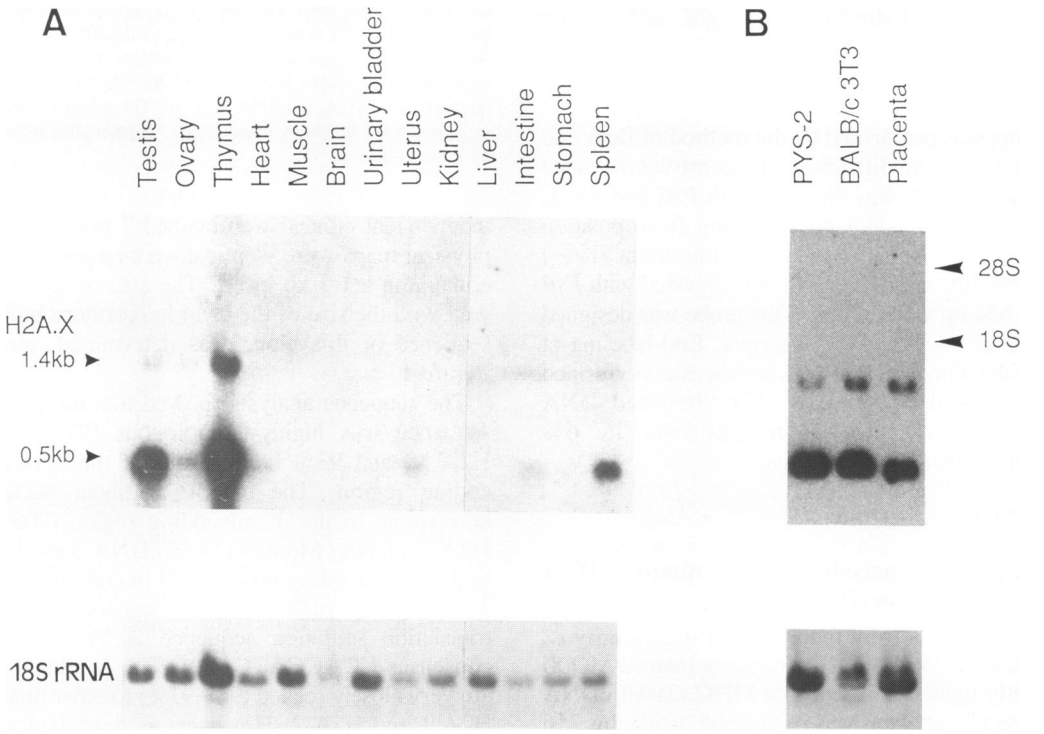

Figure 4. Northern blot analysis of mouse H2A.X in adult tissues (A) and culture cells (B). Total cellular RNA was prepared as described in Materials and Methods, and 10 μg of each was used for hybidization analysis. The hybridization was probed with the synthetic probe (for H2A.X) as described in Figure 2. The positions of the 28S and 18S rRNA markers are indicated at the left. As a control the same blots were hybridized with synthetic probe for mouse 18S rRNA.

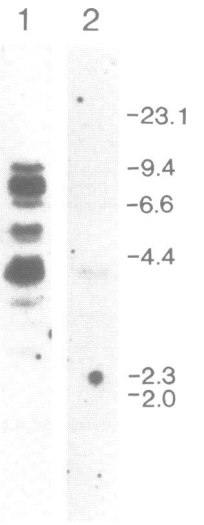

Figure 5. Southern blot anlysis of mouse H2A.X gene. Mouse (129/Sv) liver DNA was prepared as described in Materials and Methods and digested with *Eco*RI. Duplicated DNA blots were prepared and hybridized with the *Eco*RI-*Pst*I 305 bp H2A.X cDNA fragment which corresponds to the 5′ coding region (lane 1) and MO.8 cDNA which corresponds to 3′ noncoding region of Mouse H2A.X (lane 2). Numbers indicated at the right show the size in kilo-base-pairs of marker DNA prepared by the *Hind*III digest of lambda phage DNA.

Southern and Northern analysis

*Eco*RI digested mouse liver DNA was electrophoresed on 0.6% agarose gels and was transferred to nitrocellulose filters. Hybridization was performed overnight, then the filters were washed with 2×SSC/0.1% SDS at 65°C.

Northern blot analysis was performed as described by Lehrach *et al.* (25) The filters were hybridized overnight, and washed with 2×SSC at 55°C.

S1-mapping

S1-nuclease mapping was performed by the method of Berk and Sharp (26) except for some modifications. To construct the intact probe, mouse H2A.X cDNA was digested with *Pst*I and *Sac*II, and the resulting fragment of 398 bp extending from position 306 to 704 was subcloned into *Pst*I-*Sac*II site of Bluescript SK(−) (Stratagene). This construct, pBH2A-PS, was digested with *Pst*I and *Hinf*I to obtain 634 bp intact probe. This probe was designed to assay the 3′ end of the H2A.X transcripts. End-labeling at the 3′ end of the probe with T4 DNA polymerase was performed according to the protocol of Maniatis (27). Protected DNA fragments were analyzed by electrophoresis in 6% polyacrylamide gels containing 7 M urea.

RESULTS

Isolation and sequence analysis of the mouse cDNA homologous to human histone H2A.X

While characterizing cDNAs from mouse 8-cell stage embryos, we coincidently isolated a 256 bp cDNA fragment (named MO.8) which was artificially ligated adjacent to a MHC class I cDNA (28). When Northern blot analysis was performed, using this 256 bp fragment as a probe, a mRNA of 1.4 kb could be detected in undifferentiated teratocarcinoma F9 cells . To further characterize this fragment, we prepared a cDNA library from poly(A)$^+$ mRNA of undifferentiated F9 cells and screened it for cDNAs corresponding to the MO.8 cDNA. From 9.5×10^4 independent clones, we obtained 7 positive clones. Since their physical maps were identical we characterized the largest clone containing a 1.4 kb insert. The size of the insert corresponded well with the size of the band in Northern blots. The nucleotide sequence of this clone was determined, and is presented in Figure 1.

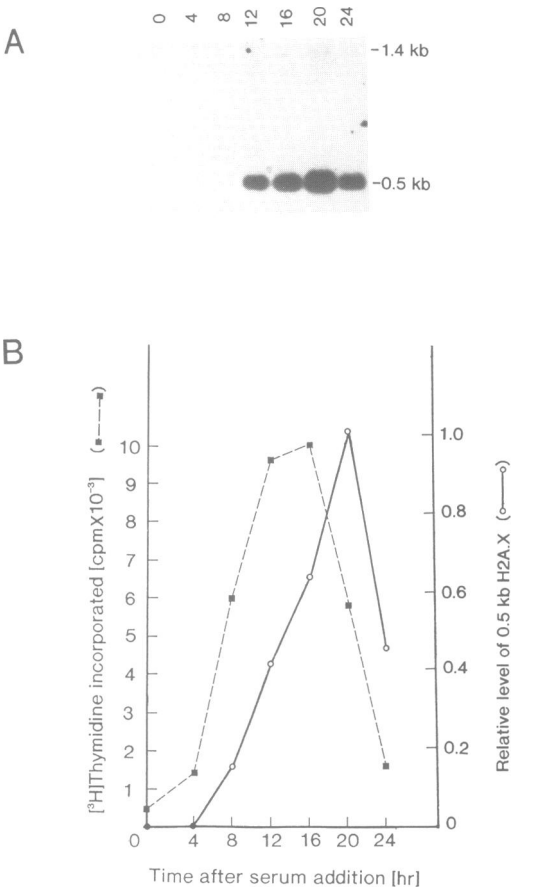

Figure 6. Kinetics of accumulation of the two H2A.X transcripts in the cell cycle. (A) Total cellular RNA was prepared at various times indicated in the cell cycle from BALB/c 3T3 cells synchronized as described in Materials and Methods. Samples (3 μg) were assayed by Northern blot analysis, using the synthetic oligonucleotide probes for H2A.X as described in Figure 2. (B) Densitometric quantitation of H2A.X mRNA (0.5 kb). The relative values comparing with that in 20 hours are shown by open circles. ^{3}H-thymidine incorporation are presented by closed squares.

The sequence analysis showed that the predicted amino acid sequence was highly homologous (97%) to human histone H2A.X, and 93% homologous at the nucleotide level in the coding region. The cDNA fragment MO.8 was found to correspond to the 3′ noncoding region (705−960) of histone H2A.X cDNA. Mouse H2A.X cDNA consists of 51 nucleotides in the 5′ noncoding region, 429 in coding region and 888 in the 3′ noncoding region. The 5′ noncoding region contains the translation initiation sequence ACACCATG similar to the consensus CCACCATG (29). The N-teminal 119 amino acids are very closely related (96−97%) to other mouse H2A subtypes, H2A.1 and H2A.2. However, as described in human H2A.X, mouse H2A.X has a long and unique C-terminal sequence of 23 amino acids (3). The unique C-terminal sequence of mouse H2A.X has 4 amino acid substitutions compared with that of human histone H2A.X. (Figure 1). The nucleotide sequence homology between mouse H2A.X and human H2A.X extend into

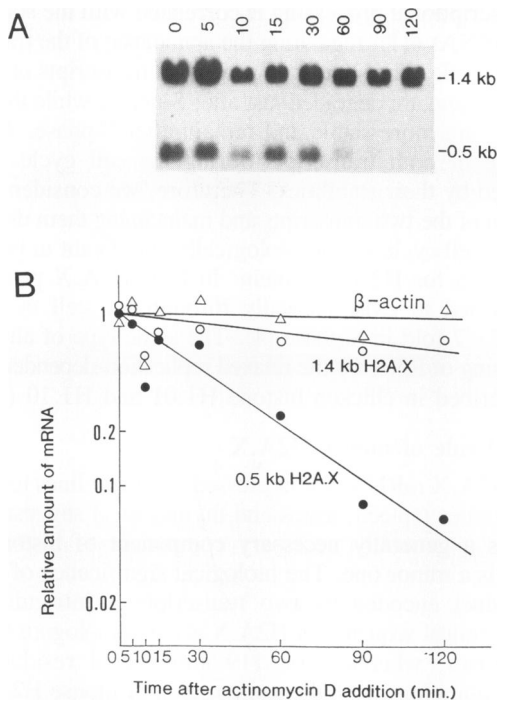

Figure 7. Stability of the two H2A.X transcripts. (A) Total cellular RNA was prepared from the F9 cells treated with 5 μg/ml actinomycin D for indicated times (minutes), and 10 μg of each samples were assayed by Northern blot analysis. The hybridization was probed with the H2A.X cDNA fragment. (B) Densitometric quantitation of autoradiograms. The logarithym of the relative amounts of accumulated H2A.X was plotted against the time after actinomycin treatment. The points where the extrapolating lines cross with the vertical coordinate are taken as unity.

the 3′ noncoding region (Figure 1). Just downstream from the translation termination codon, there exists highly conserved sequences composed of the dyad symmetry element, the purine-rich spacer, and the putative 3′ processing site (554 nucleotide in Figure 1), that are typical of replication-dependent type histone genes (6). Twenty three nucleotides upstream from the poly(A) tract there exist a polyadenylation signal, ATTAAA. Both the 3′ processing region (481−677) and polyadenylation signal region (1183−1359) are well conserved between human and mouse H2A.X, 71% and 79%, respectively.

Two forms of transcripts of mouse histone H2A.X are produced in F9 cells

As histone H2A have many variants, we synthesized an oligodeoxy nucleotide 60-mer corresponding to the C-terminal part (412−473) of H2A.X cDNA as a probe to detect only H2A.X variant DNA or mRNA.

The Northern blot analysis using H2A.X specific probe detected 1.4 kb and 0.5 kb mRNAs in F9 cells (Figure 2A). The 1.4 kb mRNA bound to an oligo(dT)-cellulose column, while the shorter 0.5 kb mRNA flowed through the column (Figure 2B). This indicates that the 1.4 kb mRNA is polyadenylated at the 3′ end, but that the 0.5 kb mRNA is not polyadenylated. To analyze the 3′ end of the mRNA produced from the H2A.X gene, we performed S1 nuclease mapping. 3′ End labeled probe in Figure 3 was hybridized with total RNA derived from undifferentiated F9 cells, and digested with S1 nuclease. Two protected DNA fragments corresponding to 244 and 398 nucleotides long were observed (Figure 3A). As shown in the scheme, the shorter protected band is consistent with a 0.5 kb mRNA cut at position 554 (Figure 1 and Figure 3B) very close to the major 3′ cleavage site for vertebrate replication-dependent histone mRNAs (9). On the other hand, the longer protected band corresponds to the complete length of the H2A.X fragment cloned in the vector, thought to be derived from the longer polyadenylated transcript (Figure 3B).

In F9 cells, the longer poly(A)$^+$ transcript is very abundant, but this abundance is not specific to embryonal carcinoma cells, because other EC cells, PCC4 and PCC3 showed no abundance of the longer mRNA (data not shown). F9 cells are known to differentiate *in vitro* by the treatment with retinoic acid to become parietal endoderm (30,31). The amount of H1 histone subtype $H1_0$ mRNA has been reported to increase during differentiation of F9 cells to parietal endoderm (32). We studied the abundance of the H2A.X mRNAs during differentiation of F9 cells (Figure 2A). The abundance of the longer transcript did not change after retinoic acid treatment for 120 hours, when the cells became flat and mRNA of endodermal cytoskeletal protein (EndoA) was detected (data not shown). We do not know why F9 cells accumulate high levels of the longer transcript. Histone H2B was processed normally, indicating that the 3′ processing machinery is functioning normally. In any case, the chromatin structure of F9 cells may be unique, if it is enriched in the H2A.X variant. The relation between the chromatin structure and gene activity of F9 cells should be elucidated.

Two transcripts are also detected in other cells and tissues

Although we isolated histone H2A.X cDNA from libraries of 8-cell stage embryos and undifferentiated F9 teratocarcinoma cells, its expression was not restricted to embryonic cells. Figure 4 shows the tissue distribution of H2A.X transcripts in adult mice. In thymus, testis, and spleen high levels of both the 1.4 kb and 0.5 kb transcripts were detected. They were also detected in ovary, uterus and intestine in lesser amounts. In other tissues, H2A.X transcripts were not detected. These two mRNAs were also found in several tissue culture cell lines (PYS-2 cells, BALB/c 3T3 cells and Placenta cells), though the longer mRNA is much smaller fraction than the shorter one (Figure 4).

The mouse H2A.X gene

Histone genes are known to form a multigene family with 5−20 genes for each type of histone (33,34). To investigate whether the two transcripts of H2A.X were derived from a single gene, Southern blot analysis of mouse genomic DNA was performed. When the whole cDNA fragment including the coding region was used as a probe, more than ten bands were detected (Figure 5, lane 1), presumably caused by the homology between the coding regions of the H2A.X and other major and minor histone H2A variant genes. However, when the 3′ noncoding region of H2A.X (MO.8) was used as a probe, only a single band could be detected (Figure 5, lane 2), indicating that the two forms of mRNA are produced from a single gene and alternatively processed at the 3′ end.

Different regulation of the two mouse H2A.X mRNAs in the cell cycle

We analyzed the amount of the two transcripts during the cell cycle. As shown in Figure 6, the cell cycles of 3T3 cells were synchronized by the starvation and stimulation of the serum. The amount of 0.5 kb poly(A)$^-$ H2A.X mRNA changes in parallel with [^{3}H]-thymidine incorporation, which represents the rate of

DNA synthesis. The same kinetics were also observed in H2B mRNA (data not shown). Although the bands were faint, the 1.4 kb poly(A)$^+$ H2A.X mRNA was detected 20 hours after serum addition. The lesser amount of the long form in this experiment comparing with that of BALB/c 3T3 in Figure 4B might be due to some different cell condition by serum starvation. Then we measured the half-life of these mRNAs. As shown in Figure 7, the amount of 0.5 kb poly(A)$^-$ H2A.X mRNA decreased rapidly after inhibiting the transcription by actinomycin D, whereas the 1.4 kb poly(A)$^+$ mRNA was stable. The half-life of 0.5 kb poly(A)$^-$ mRNA was calculated to be about 30 minutes. This value is in good agreement with the average half-life of replication-dependent type histones, about 45−60 minutes in S-phase and 10−15 minutes at the end of S-phase (9). On the other hand, the 1.5 kb poly(A)$^+$ mRNA was very stable with a half-life of about 8 hours. This result indicates that the two H2A.X mRNAs accumulate differently during the cell cycle according to their rate of degradation.

DISCUSSION

Both 3′ processed and polyadenylated transcripts are simultaneously produced from mouse H2A.X gene

Sequence analysis revealed that the H2A.X cDNA contains not only the motifs that are involved in processing and post-transcriptional control of replication-dependent type histone mRNAs, but also the polyadenylation signal, ATTAAA (9). Challoner *et al.* (35) have reported polyadenylated mRNA of replication-dependent histone H2B are detected in avian spermatids. However, in histone H2A.X, the polyadenylated mRNA is expressed in many tissues and not restricted to spermatids. Furthermore, the length of the 3′ noncoding region of mouse histone H2A.X is much longer (0.9 kb) than that of avian spermatid histone H2B (26−28 nucleotides). Recently a proliferation-dependent histone H2A.1 mRNA enriched in poly(A)$^+$ fraction has been described (36), but it has a nonterminal oligo(A) tract directly upstream from the typical 3′ terminal hairpin loop of replication-dependent histone mRNAs. Genomic sequencing of the mouse histone H2A.X gene revealed that the 3′ poly(A) sequence is not present in the genome (our unpublished result). We have shown that the mouse histone H2A.X gene can produce two forms of mRNA by the two different mechanisms for 3′ end formation. The processing of mRNA in this manner has also been found in human H2A.X cDNAs (14), findings which indicate that this type of alternate processing may be a generally important mechanism to produce H2A.X histone in mammals. Interestingly, the region covering the 3′ processing site and the region upstream of polyadenylation site were highly conserved between human and mouse histone H2A.X cDNAs.

Typical replication-dependent histone mRNAs increase 30−50 fold during S-phase in the cell cycle and are rapidly degraded thereafter. The metabolism of these type of histone mRNAs are known to be regulated at multiple levels ; during transcription initiation, 3′ processing of mRNA, transport of the mature mRNA and the rate of degradation (10), while the replacement type histone genes are transcribed through out the cell cycle. In the case of mouse histone H2A.X, transcription initiation seems to be the same for both poly(A)$^+$ and poly(A)$^-$ transcripts of H2A.X, because both appeared at the same time just before S-phase. In addition, RNAase protection assays suggest that transcription of H2A.X starts at one site (our unpublished result).

Post-transcriptional processing is correlated with the stability of histone mRNAs (11), regulating the abundance of the transcripts in the cell cycle. The shorter 3′ processed transcripts of H2A.X are unstable and are degraded just after S-phase, while the longer transcripts are more stable and remain after S-phase. Thus the abundance of both transcripts during the cell cycle may be determined by their stabilities. Therefore, we consider that the production of the two transcripts and maintaining them differently during the cell cycle are physiologically significant in providing the templates for H2A.X protein. In fact, H2A.X protein has been reported to exist basically through the cell cycle, and increase 3−7 fold in S-phase (4). The same type of alternative 3′ processing of RNA for the relaxed replication-dependence have been described in chicken histone H1.01 and H1.10 (37).

Biological role of mouse H2A.X

Histone H2A.X mRNA was expressed in all cell lines tested and in adult tissues (spleen, testes and thymus *etc.*) suggesting that H2A.X is a generally necessary component of histone even though it is a minor one. The biological significance of H2A.X gene product encoded by two transcripts is intriguing. The carboxy-terminal sequence of H2A.X is not homologous to major H2A histones, whereas first 119 amino acid residues have 96−97% homology with the same region of mouse H2A.1 and H2A.2, respectively (3). As reported for human H2A.X (14), the carboxy-terminal amino acid sequence (Ser-Gln-Glu) of H2A.X is homologous with those of several species of lower eukaryotes, *e.g. Saccharomyces cervisiae* H2A.1 and H2A.2 (38), *Aspergillus nidulans* H2A (39), Tetrahymena H2A.1 and *Schizosaccharomyces pombe* (40). In lower eukaryotes, this type of histone is estimated to compose a large fraction of H2A proteins and their chromatin is largely composed of transcriptionally active region. On the other hand, chromatin in mammalian cells is predominantly nontranscribed; only 10−20% is thought to be active (41). Should the carboxy-terminal region of H2A.X type histones play some role in active chromatin, it seems reasonable to suggest that the mouse H2A.X compose about 11% of H2A histones (2,4). If the H2A.X is associated with active chromatin, it must be synthesized in S-phase in order to be positioned at newly synthesized active chromatin. Furthermore, it might also be necessary in other phases of the cell cycle to replace those H2A.X histones of active chromatin during transcription. In order to regulate the levels of histone H2A.X and ensure a supply of cell-cycle-stable RNA, two different 3′ processed transcripts would be produced. The micrococcal nuclease digestion of chromatin revealed that H2A.X is fractionated in soluble mononucleosomes with active immunoglobulin κ chain gene and high mobility group proteins HMG 14 or 17 (42). The amount of the HMG 14, which is supposed to be associated with active chromatin is regulated in a partially replication-dependent manner as is histone H2A.X (4). The other minor variant H2A.Z is also found associated with chromatin that is enriched for transcriptional activity (43). So, both H2A.X and H2A.Z might be associated with the active chromatin simultaneously with other non-histone proteins (40).

ACKNOWLEDGEMENTS

We thank Drs. W.M.Bonner and K.Ozato (National Institutes of Health, USA) for helpful discussions and critical reading of the manuscript. This work was supported by Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan (No.02260207).

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