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ABSTRACT

We have isolated and sequenced a mouse replacement variant histone H3.3 cDNA. It corresponds to the most abundant mRNA expressed from a unique gene by the use of one out of three polyadenylation sites. The 3′ non coding region of H3.3 is very long (~1100 nt) and highly conserved throughout evolution since it is about 95% homologous to the 3′ non coding region of the chicken H3.3B gene (1). We studied the expression of the H3.3 gene during SV40- and polyoma-induced mitotic host reaction in confluent, Go-arrested primary mouse kidney cell cultures. H3.3 replacement variant mRNA steady state levels increased during the Go to S-phase transition, apparently as the result of two mechanisms: one related to cell growth, whereas the other was linked to cellular DNA synthesis. The latter mechanism was however far less pronounced than with replication histone variant mRNAs. The biological implications of these results are discussed.

INTRODUCTION

Histones in higher eucaryotes are divided in three major classes: a) the replication dependent variants the expression of which is tightly coupled to S-phase (e.g. H3.1 and H3.2); b) replication independent, or replacement variants which are synthesized throughout the cell cycle and in non dividing differentiated cells (e.g. H3.3); and c) tissue-specific variants, such as the erythroid-cell specific histone H5 found in birds and amphibia. The replacement variants, in contrast to the replication-dependent histones, are encoded by apparently single genes which contain introns and their mRNAs are polyadenylated (2).

To characterize cellular genes differentially expressed during cell proliferation or cell transformation induced by SV40, polyoma virus or by non viral mitogens, several research groups have isolated the corresponding cDNAs by differential screening of a cDNA library (3−16). We used a similar approach and constructed a cDNA library corresponding to cytoplasmic polyA$^+$ mRNAs isolated shortly after infection of primary mouse kidney cell cultures with polyoma virus (16a).

Polyoma virus and SV40 are closely related tumor viruses endemic in mouse and monkey, respectively. In permissive tissue culture cells both viruses induce a lytic infection leading to the production of progeny virus and cell lysis; in non permissive cells, unable to initiate replication of viral DNA, infection remains abortive and leads to mitosis; a low percentage of the abortively infected cells becomes stably transformed (17−19). The early events of lytic and abortive infection are very similar; in primary mouse kidney cell cultures arrested in phase Go of the mitotic cycle SV40 and polyoma induce a mitotic host reaction comprising the sequential activation of cellular RNA and protein synthesis and S-phase (20, 21).

By differential screening of our cDNA library we have isolated, among others (16a),

cDNA recombinant pmH3.3. Using pmH3.3 cDNA as probe, we studied the differential expression of the H3.3 gene during SV40- and polyoma-induced Go to S-phase transition. Although stimulation of H3.3 gene expression during SV40- and polyoma-induced mitotic reaction remained rather modest, we selected pmH3.3 for further studies, since DNA sequence analysis revealed that pmH3.3 codes a mouse histone replacement variant H3.3 mRNA with a strikingly long and highly conserved 3′ non coding region.

MATERIALS AND METHODS

Cell cultures and virus infection.

Confluent primary mouse kidney cell cultures (about 10^7 cells per 85 mm dish) were infected (20 PFU/cell) either with polyoma A2 wild type strain (22), or wild type SV40 (23); parallel cultures were mock-infected (24). After virus adsorption for 90 minutes at 37°C, the cultures were incubated at 37°C in serum-free medium.

Construction of a cDNA library in λgt10 and differential screening.

Cytoplasmic polyA$^+$ mRNA from polyoma-infected cultures was isolated by chromatography on oligo-dT-cellulose (Pharmacia, type 7; 25). Double-stranded cDNA (26) was subcloned in the EcoRI site of λgt10 (27). The library, which contains about 70000 independent recombinants with inserts of 1−2 kb, was subjected to two rounds of differential screening: duplicate nitrocellulose filters bearing recombinant phage plaques were hybridized with 10^6 cpm/ml (specific activity 10^8 cpm/μg) ^{32}P-labeled single stranded cDNAs corresponding to cytoplasmic polyA$^+$ mRNAs isolated from polyoma- or mock-infected cultures, respectively. cDNAs of differential recombinants were subcloned in pUC8, M13, or its derivative Bluescribe$^+$ (28).

Northern and Southern blotting.

Total RNA was extracted from 2 dishes per experimental point and aliquots of 5 μg were subjected to Northern blotting on Gene Screen nylon membranes (29); RNA on the membranes was revealed and quantitated by staining with methylene blue. For size determination we used the BRL RNA ladder. Genomic DNA was extracted from primary mouse kidney cell cultures (30) and aliquots of 5 μg were subjected to digestion with

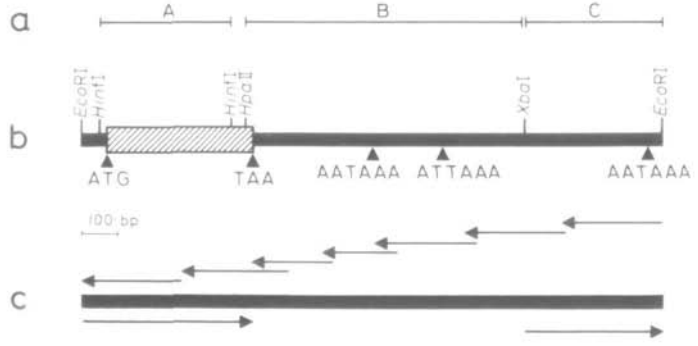

Figure 1 *Restriction map and sequencing strategy of the pmH3.3 cDNA insert.* a) Location of the subclones used for Northern blot analyses. b) The shaded box represents the coding region. The location of several restriction enzyme recognition sites, the initiation and stop codons and the polyadenylation signal sequences are indicated c) Subclones prepared by exonuclease III (34) used for sequence analysis.

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                                                                    -70
                                                                    GAAT
       -60         -50         -40         -30         -20         -10         -1
TCCGTTCGAG  CGCTTCCAGC TCGCCTCGGT CTCAGCAAGG GCCCCAGACT CGGCTTTGGG TGAAAAAAA
           10          20          30          40          50          60
ATG GCC CGA ACC AAG CAG ACC GCT AGG AAG TCC ACC GGT GGG AAA GCC CCC CGC AAA CAG
Met Ala Arg Thr Lys Gln Thr Ala Arg Lys Ser Thr Gly Gly Lys Ala Pro Arg Lys Gln
       70          80          90          100         110         120
CTG GCC ACC AAG GCG GCT CGG AAA AGC GCG CCC TCT ACC GGC GGG GTG AAG AAG CCT CAC
Leu Ala Thr Lys Ala Ala Arg Lys Ser Ala Pro Ser Thr Gly Gly Val Lys Lys Pro His
      130         140         150         160         170         180
CGC TAC AGG CCA GGG ACC GTG GCT CTG AGA GAG ATC CGT CGT TAC CAG AAA TCG ACT GAC
Arg Tyr Arg Pro Gly Thr Val Ala Leu Arg Glu Ile Arg Arg Tyr Gln Lys Ser Thr Glu
      190         200         210         220         230         240
CTG CTC ATC CGG AAG CTG CCA TTC CAG AGA TTG GTG AGG GAG ATC GCC CAG GAT TTC AAA
Leu Leu Ile Arg Lys Leu Pro Phe Gln Arg Leu Val Arg Glu Ile Ala Gln Asp Phe Lys
      250         260         270         280         291         300
ACC GAC TTG AGG TTT CAA AGT GCA GCC ATC GGT GCC CTT CAG GAG GCT AGC GAA GCA TAC
Thr Asp Leu Arg Phe Gln Ser Ala Ala Ile Gly Ala Leu Gln Glu Ala Ser Glu Ala Tyr
      310         320         330         340         350         360
CTG GTG GGG TTG TTT GAA GAT ACC AAT CTG TGT GCC ATC CAC GCC AAG AGA GTC ACC ATC
Leu Val Gly Leu Phe Glu Asp Thr Asn Leu Cys Ala Ile His Ala Lys Arg Val Thr Ile
      370         380         390         400         410         420
ATG CCC AAA GAC ATC CAG TTG GCT CGC CGG ATA CGG GGG GAG AGA GCT TAA   GTTGAAGCG
Met Pro Lys Asp Ile Gln Leu Ala Arg Arg Ile Arg Gly Glu Arg Ala ***
      430         440         450         460         470         480         490
GTTTTTATGG CATTTTGTAG TAAATTCTGT AAAATACTTT GGTTTAATTT GTGACTTTTT TTGTAAGAAA
      500         510         520         530         540         550         560
TTGTTTATAA TATGTTGCAT TTGTACTTAA GTCATTCCAT CTTTCACTCA GGATGAATGG GAAGAGTGAC
      570         580         590         600         610         620         630
TGACTGTTCA CAGACCTCAG TGATGTGAGC ACTGTGGCTC AGGAGTGACA AGTTGCTAAT ACGCAGAAGG
      640         650         660         670         680         690         700
GATGGGTGAT ACTTCTTGCT TTTCATGATG CATGTTTCTG TATGTTAATG ACTTGTTGGG TAGCTATTAA
      710         720         730         740         750         760         770
GGTACTAGAA TTGATAAATG TGTACAGGGT CCTTTTGCAA TAAAACTGGT TATGACTTGA TCCAAGTGTT
      780         790         800         810         820         830         840
TAACCATACA TCACTGTGAT AGAATGTGGG CTTTTTCAAA GGTTGAAGAT ACAAGTTTTA GCCACAGTGT
      850         860         870         880         890         900         910
AACAGTTTCC TTTAAAAAAA AAAAGTAAAC CTGGCAGCTA TAGAATACAC TATGTGCATT TATAATAGCT
      920         930         940         950         960         970         980
ATTTTATATA TTGTAGTGTT CAACATTTTT AAATTAAATG TTTTACATTC ACAAGTGGTG GGGAGTCTTG
      990        1000        1010        1020        1030        1040        1050
TCATTAAGGT GTGTGTAATT TAGTCCAGTT GGTATTTCCT GACTAGACTG CATTTGTTTT TAACAGTAGA
     1060        1070        1080        1090        1100        1110        1120
AAAATGCTAT GCGTATTAAA CCTTGCATAA GTCCTCATTC TACCACATGT TCACTAACCC CTGACCCTCT
     1130        1140        1150        1160        1170        1180        1190
GGCTGGTAAC ACAACACTAA CGGGGATTTT ATTTATAAGG GCTCTAGAGT AAAATAACAA GCTATTCACA
     1200        1210        1220        1230        1240        1250        1260
CCAGCATCAT CTATTACTAA TCTAGTTAGT GCAGCTTTTC ATTGTGTTTG GTCTTAGGCC TAGATTGAGT
     1270        1280        1290        1300        1310        1320        1330
TCGGTTTCGT TTCTTTTTAA TCTTTAAGGG GGAAAACTGT ACAAGGTTTG TTTTTCCTTG TAGAGTTTAT
     1340        1350        1360        1370        1380        1390        1400
AAACATGGGA GTAAGAACAC AGCTTCAACT TCTTGGACCT CTGCCAATGA AGATGGGTTA GGTTACACCT
     1410        1420        1430        1440        1450        1460        1470
GGTTACTATA CTGGCAAAAT CCCTTTTATA GAGATGGCCT TCAAGTGGTT TTTAAAAGTA TCCTATTGAA
     1480        1490        1500        1510        1520        1530        1540
GTTTTTAGGT CAATTATGTA TGTTGACTAA ATTTACAAAT AAACTTGTTT ATTCAAAAAA AAAAAAAGGG
     1550
AATTCC
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Figure 2 *DNA sequence and predicted amino acid sequence of the pmH3.3 cDNA insert.* The AATAAA and ATTAAA polyadenylation signal sequences are underlined and in italics/underlined, respectively. The internal polyA stretch is in bold.

Table 1 Percentage of homology between the coding region of pmH3.3 and mouse (M) human (H) and chicken (C) H3 genes. M H3-6 (36), M H3-1, M H3-2 (37); H H3-5B (38); H H3B-2 (39, 40); C H3-G (41); C H3-d (42), C H3.3B, C H3.3A (1).

	DNA	Protein
M H3-6	71.53	86.86
M H3-1	81.02	96.35
M H3-2	81.27	97.08
H H3-5B	76 50	96.35
H H3B-2	75.67	100.00
C H3-G	80.29	97 08
C H3-d	76.40	96.35
C H3.3B	87.10	100.00
C H3.3A	79.56	99 27

restriction endonucleases (28). Southern blotting was performed electrophoretically according to the GeneScreen (NEN) protocol using GeneScreen membranes. After transfer the membrane was irradiated with U.V. for 2 minutes (31) and the DNA was revealed with methylene blue. The fragments A, B and C (Fig. 1) were either subcloned in Bluescribe$^+$ (B and C) or directly isolated from the pmH3.3 cDNA (A; 28). The pmH3.3 cDNA insert and the fragments A, B, C were ^{32}P-labeled using the random primer labeling kit (Boehringer), yielding a specific activity of about 10^9 cpm/μg. Hybridization was for 20 hours with 10^6 cpm/ml (Northern: GeneScreen protocol Method I; Southern: GeneScreen protocol Method III). Autoradiography with intensifying screens was at -70°C, using Kodak X-Omat AR films. The relative amounts of hybridized RNA were determined by scanning the autoradiographs at 630 nm with a Shimadzu CS930 scanning densitometer, followed by integration of the absorption curves.

DNA sequence analysis.

Nucleotide sequence determination was performed by the dideoxy chain termination method (32) using ^{35}S-thio dATP (33). The reaction products were separated by electrophoresis through buffer gradient gels (33). Exonuclease III digestion (34) was used to introduce progressive deletions into the cDNA sequence to allow sequencing of the entire clone. The nucleotide sequence was compared to sequences registered in GenBank and EMBL data bases, using the Goad and Kanehisa DNA sequence similarity program (35).

RESULTS

pmH3.3 cDNA codes a mouse histone replacement variant.

Figure 1 shows the restriction map of the pmH3.3 insert and the sequencing strategy; figure 2 gives the DNA sequence (1619 bp) with the predicted amino acid sequence. Comparison of the pmH3.3 nucleotide sequence with DNA sequences registered in the GenBank and EMBL data bases, using the Goad and Kanehisa DNA sequence similarity program (35), revealed about 80% homology of the coding region with mouse, human and chicken histone H3 genes (Table 1) and with all other H3 histone genes registered in the data bases (unpublished results). Furthermore, at the amino acid level (Table 1), a homology higher than 90% was detected between pmH3.3 and the mouse replication variant histones H3-1 and H3-2 (37); surprisingly, the amino acid sequence of pmH3.3 is identical to the chicken H3.3B (1) and to the human H3.3 (39, 40) replacement variant histones. Since the amino acid sequence of H3.3 is highly conserved throughout evolution, this indicates that most of the divergence at the nucleotide level corresponds to silent mutations.

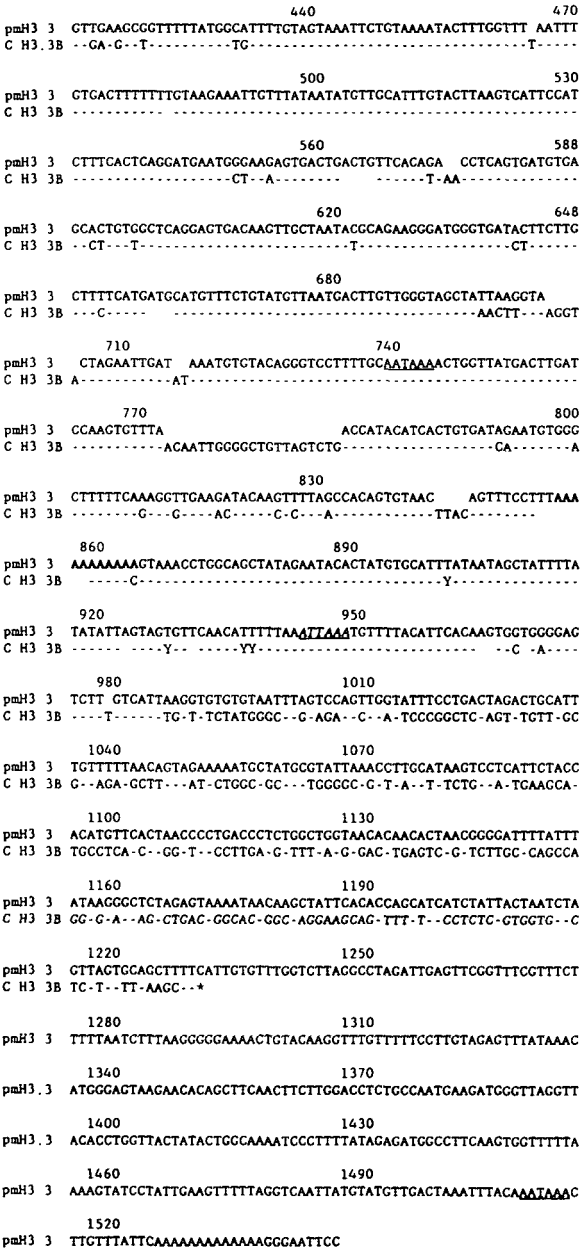

Figure 3 *Comparison of the 3' non coding sequence of pmH3.3 to the chicken H3.3B 3' non coding sequence (1).* The 3' non coding sequence of pmH3.3 was positioned to yield the maximum homology between the two clones. The AATAAA and ATTAAA polyadenylation signal sequences are underlined and in italics/underlined, respectively. The internal polyA stretch is in bold.

Figure 4 *Northern blot analysis using different subclones of the pmH3.3 cDNA as probes.* Aliquots of 5 μg total RNA extracted 24 hours after infection with SV40 were subjected to Northern blotting (29). As probes, we used the ^{32}P-labeled pmH3.3 cDNA (A), subclone B (B), and subclone C (C) respectively (Fig. 1). Autoradiography was for 1 day. For size determination we used the BRL RNA ladder.

While no significant homology was observed in the 3′ non coding region between pmH3.3 and the mouse H3-1 and H3-2 genes (not shown), about 95% homology was detected between the 3′ non coding region of pmH3.3 and the 3′ non coding region of the chicken H3.3B gene (Fig. 3). The mouse and chicken H3.3 non coding 3′ regions are particularly long for histone mRNAs (~1100 and ~500 nt, respectively) and exhibit some unusual sequences: an internal polyA stretch and, in addition to consensus polyadenylation signals AATAAA (positions +739, +1507 in mouse and +1493 in chicken), an ATTAAA polyadenylation signal (position +943 in mouse, +1714 in chicken; Fig. 3). The homology between the mouse and the chicken sequences extends further downstream from the initially presumed 3′ end of chicken H3.3B mRNA (1) and ends after the ATTAAA polyadenylation signal (Fig. 3); this result confirms the more recent observation that the chicken mRNA ends at position +1732 (43).

All 3 polyadenylation signals are used for mouse H3.3 mRNA production.

Northern blot analysis using ^{32}P-labeled pmH3.3 cDNA as probe revealed in total RNA from mouse kidney cell cultures 4 bands of about 1.8, 1.2, 1.0, and 0.58 kb, respectively (Fig. 4); furthermore, one or more faint bands of about 0.7 kb could be detected. To characterize the different mRNAs, we performed Northern blot analyses using specific subclones of the pmH3.3 cDNA (Fig. 1) as probes. Subclone A, which corresponds to the major part of the coding region (positions −21 to +351) revealed the same 5 bands (not shown) as the entire cDNA; subclone B, which comprises the proximal part of the 3′ non coding region (positions +390 to +1163) detected only the large mRNAs of 1.8, 1.2 and 1.0 kb (Fig. 4). Since the 3′ non coding region, which does not exhibit extensive

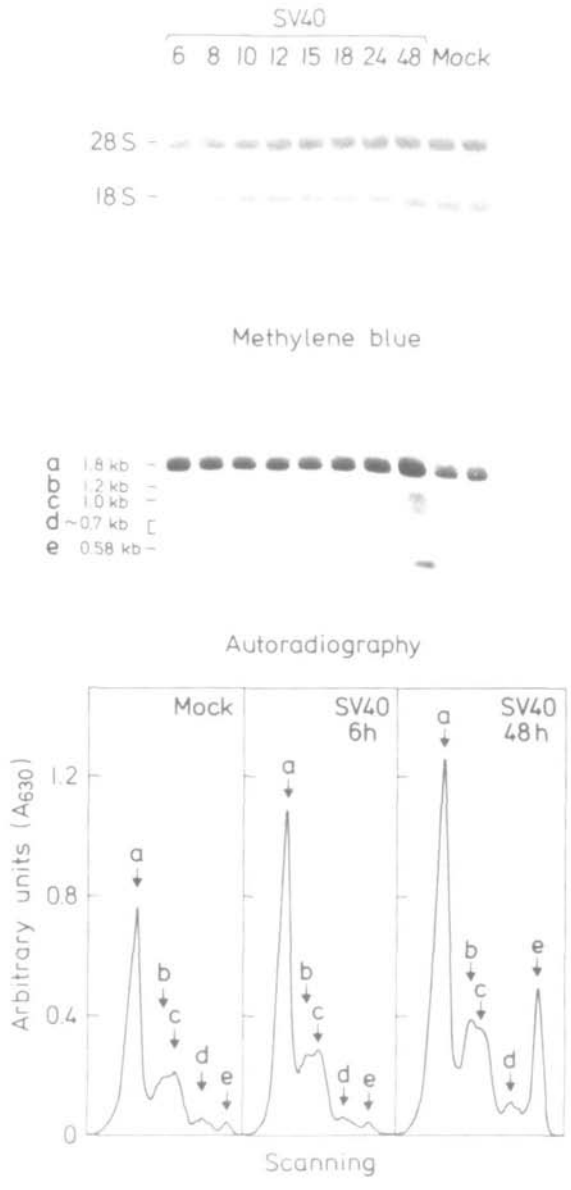

Figure 5 *pmH3.3 mRNA levels in SV40- and mock-infected cultures.* Northern blot analysis of 5 μg total RNA using ^{32}P-labeled pmH3.3 cDNA as probe was performed as described (29). The membrane was stained with methylene blue before hybridization. Autoradiography was for 3 days. The relative amounts of pmH3 3 mRNAs were determined by scanning of the autoradiograph, followed by integration of the absorption curves with a Shimadzu scanning densitometer CS930.

homology to any other known mouse histone gene, revealed only these 3 mRNAs, the latter correspond to the histone replacement variant mRNAs encoded by the pmH3.3 cDNA. Northern blot analysis using subclone C comprising the 3′ end of the cDNA (positions +1163 to +1544) as probe, revealed only the 1.8 kb mRNA. This mRNA therefore most probably results from the use of the polyadenylation signal at position +1507, whereas the mRNAs of 1.2 and 1.0 kb correspond to the use of polyadenylation signals at positions +943 and +739, respectively, taking into account a polyA$^+$ tail of about 200 nucleotides. As determined by scanning of the autoradiographs (Fig. 5), the 1.8 kb mRNA is the most abundant, representing about 70% of the 3 replacement mRNAs, whereas the 1.2 and the 1.0 kb mRNAs correspond to about 15% each.

In addition to the large mRNAs, the coding region also recognizes the small mRNAs of 0.58 and ~0.7 kb; they therefore probably correspond to mRNAs which crosshybridize with the coding region because of a high homology at the nucleotide level (see below).

Replacement and replication H3.3 mRNA steady state levels increase during SV40- and polyoma- induced transition from Go to S-phase.

Total RNA was extracted between 6 and 48 hours from mock-, SV40- and polyoma-infected (not shown) cultures and aliquots of 5 μg were subjected to Northern blot analysis, using ^{32}P-labeled pmH3.3 cDNA as probe. As determined by scanning of the autoradiographs, in mock-infected cultures the steady state levels of all 5 mRNAs remained constant (Fig. 5). In SV40-infected cultures the 1.8, 1.2, 1.0 kb replacement histone mRNA levels were increased by a factor of about 1.5 already by 6 hours after infection, i. e. coincident with the onset of SV40 T-antigen synthesis and before detectable begin of SV40-induced DNA synthesis (44, 45); thereafter, they slightly increased in parallel with the SV40-induced cellular DNA synthesis, while by 48 hours SV40-infected cultures contained about twice the amount of 1.8, 1.2 and 1.0 kb replacement mRNAs than the mock-infected controls. The amount of nuclei containing SV40 T-antigen reached a maximum plateau of about 50% around 24 hours and remained unchanged during the next 2−3 days (44, 45). Similarly, in polyoma-infected cultures H3.3 replacement variant mRNAs increased before and then in parallel with the virus-induced cellular DNA synthesis; when DNA synthesis was inhibited with 5-fluorodeoxyuridine, the DNA synthesis-associated increase was prevented (not shown).

The following results indicate that the 0.58 kb mRNA probably corresponds to a replication variant histone mRNA (H3.1 and/or H3.2) detected by pmH3.3 because of their high homology in the coding region: in SV40-infected cultures the 0.58 kb mRNA steady state levels remained very low and constant until about 15 hours after infection; they then increased in parallel with the onset of SV40-induced cellular DNA synthesis (44, 45), while by 48 hours the amounts of the 0.58 mRNA were 7−8 times higher than in mock-infected controls. A similar increase in the 0.58 kb mRNA levels, correlated with the virus-induced cellular DNA synthesis was observed in polyoma-infected cultures and was completely prevented if the latter was inhibited by 5-fluorodeoxyuridine (not shown).

In conclusion, the replacement histone mRNA levels slightly increased before and in parallel with the virus-induced DNA synthesis, whereas the accumulation of replication histone mRNA was much more pronounced and strongly correlated with the latter.

Since the 0.7 kb mRNA did not exhibit the marked, characteristic increase of replication variant mRNAs, it might therefore rather be coded by another member of a replacement H3.3 multigene family, exhibiting some homology in the coding region to pmH3.3.

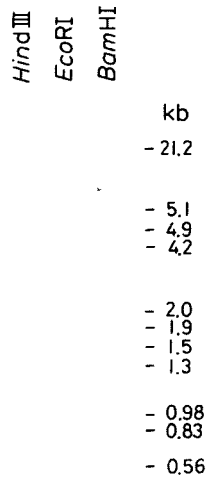

Figure 6 *Southern blot analysis of mouse genomic DNA.* 5 μg of DNA were digested with HindIII, EcoRI and BamHI, respectively. As probe we used the ^{32}P-labeled subclone C (Fig. 1). Lane kb: molecular weight markers. Autoradiography was for 7 days.

pmH3.3 seems to be encoded by a single gene.

Southern blot analysis (Fig. 6) of mouse kidney DNA using the 3′ end of pmH3.3 (clone C) as probe, revealed single EcoRI (~9.5 kb), Bam HI (~7 kb) and Hind III (~6 kb) bands, suggesting that pmH3.3 is encoded by a single gene. This result, however, does not exclude the possibility that the pmH3.3 gene is member of a replacement H3.3 multigene family, highly conserved in the coding region, but divergent in the 3′ non coding region.

DISCUSSION

We have isolated and sequenced mouse cDNA pmH3.3 (Figs. 1 and 2) exhibiting more than 80% homology in the coding region with histone H3 genes from a variety of species (Table 1 and unpublished results). The amino acid sequence differs only in 4 and 3 amino acids from the mouse replication variants H3-1 and H3-2, respectively (37) and is identical to the chicken H3.3B (1) and human H3.3 (40) replacement variant histones.

Similarly, H2A.Z, a replacement histone H2A isoprotein, is very well conserved in mammals (46) and mammalian H2A.Z is highly homologous to Drosophila H2AvD (47), chicken H2A.F, sea urchin H2A.F/Z and Tetrahymena hv1 (48). These replacement variants differ substantially from their replication counterparts. Together with their evolutionary conservatism, this observation argues strongly for an important function of these H2A replacement histone variants, probably different from their replication counterparts. Although this function remains largely unknown, the replacement variant hv1 of Tetrahymena has been found associated with the transcriptionally active macronucleus, suggesting a role in transcriptional activity (49, 50). On the other hand, mouse H1 replacement variant H1°, which is highly homologous to human H1° and avian H5, seems to be functionally correlated with the differentiated state (51).

Since the amino acid sequence of the replacement variant H3.3 is very similar to its

replication counterparts, they may not have fundamentally different functions. Rather, the particularity of the H3 replacement variant might reside in the regulation of its accumulation during the cell cycle, since H3.3 is the major non allelic H3 variant synthesized in quiescent cells and in growing cells outside the S-phase (52). In this context, it is of particular interest that the silent mutations observed in the replacement variant histone coding sequences seem of functional importance, either at the level of transcriptional efficiency of the corresponding mRNAs, or at a structural level (53).

Regulation of replication variant histone mRNA accumulation occurs, in addition to histone gene transcription, at the level of 3′ end processing and mRNA stability involving the 3′ non coding region, (2, 54, 55); it is therefore of particular interest that the 3′ non coding region of the mouse replacement H3.3 cDNA is extremely well conserved throughout evolution; whereas no homology has been detected between the mouse H3.3 3′ sequence and the two replication counterparts H3-1 and H3-2, H3.3 3′sequence is 95% homologous to the 3′ non coding region of the chicken H3.3B gene (Fig. 3; 1). This homology extends about 300 nucleotides downstream from the originally assigned 3′ end of chicken mRNA and ends after the recently determined end, downstream from a non consensus polyadenylation signal (ATTAAA; 43). In mouse, the same unusual ATTAAA polyadenylation signal is present at position +943, in addition to two consensus polyadenylation signals AATAAA (positions +739 and +1507; Fig. 3). Furthermore, like in the chicken gene, the mouse 3′ non coding region is strikingly long (~1100 nt in mouse, ~500 nt in chicken) and contains an internal polyA stretch, the function of which remains unknown. No highly conserved inverted repeat capable of forming a hairpin structure, typical of all histone genes that encode nonpolyadenylated messages (55) is present in the pmH3.3 cDNA. However, numerous GT-rich sequences, usually lying downstream from the polyadenylation sites, are detected after each polyadenylation signal in the pmH3.3 cDNA (Fig. 2; 56, 57).

A similar high degree of 3′ sequence homology has been observed between the 3′ non coding region of human replacement variant H3.3 and the chicken H3.3A gene (40). Like the chicken and the mouse 3′ non coding regions, the human sequence is long (more than 500 nt), contains unusual polyadenylation signals and internal polyA stretches. Furthermore, mammalian H2A.Z (46) and mouse and human H1° mRNAs (51) have strikingly long and well conserved 3′ non coding sequences.

This conservation of sequence, length, polyadenylation signals and unusual structures during evolution suggests a fundamental role of the 3′ non coding region, possibly in the differential regulation of replacement histone mRNA accumulation during the cell cycle as compared to the histone replication variants.

We studied the expression of the H3.3 gene during SV40- and polyoma-induced Go to S-phase transition in primary mouse kidney cell cultures. Northern blot analysis using pmH3.3 as probe revealed 5 bands of 1.8, 1.2, 1.0, about 0.7 and 0.58 kb, respectively (Fig. 4). The use of specific subclones (Fig. 1) of the pmH3.3 cDNA as probes (Fig. 4) allowed to determine that the 3 largest bands correspond to mRNAs resulting from the use of the 3 polyadenylation signals (Figs. 1 and 2), taking into account a polyA^+ tail of about 200 nucleotides. The 1.8 kb mRNA (consensus signal AATAAA) is the most abundant (about 70%), whereas the 1.2 and 1.0 kb mRNAs (signals AATAAA and ATTAAA, respectively) correspond to about 15% each. The mechanism which determines the preferential use of the downstream polyadenylation signal remains unknown.

Since the coding region of pmH3.3 exhibits about 80% homology at the nucleotide level

with the mouse replication variants H3-1 and H3-2 (37), the small 0.58 mRNA revealed by the pmH3.3 cDNA might correspond to a replication variant mRNA. This hypothesis is actually supported by the observation that the steady state levels of the 0.58 kb mRNA increased in parallel with the onset of SV40- and polyoma-induced cellular DNA synthesis, while by 48 hours the amounts of the 0.58 mRNA were 7–8 times higher than in mock-infected controls (44, 45; Fig. 5 and unpublished results); furthermore, this increase did not take place if DNA synthesis was inhibited with 5-fluorodeoxyuridine (unpublished results). Similar results have been observed with the mouse replication variant histone H4 mRNA (45, 58).

On the other hand, the 3 replacement variant H3.3 mRNAs followed a different time course of induction: the steady state levels of the 1.8, 1.2 and 1.0 kb mRNAs were increased by a factor of about 1.5 already by 6 hours after infection with SV40, i.e. in parallel with the onset of SV40 T-antigen synthesis and before detectable onset of virus-induced cellular DNA synthesis (Fig. 5; 44, 45). Thereafter, the steady state levels increased in parallel with cellular DNA synthesis (about 2-fold), an increase which was prevented when DNA synthesis was inhibited (not shown).

These results suggest that the increase in steady state levels of replacement histone H3.3 mRNAs is correlated with the induction of the proliferative state and, furthermore, with the onset of cellular DNA synthesis. In this context it is of interest that in non confluent proliferating primary mouse kidney cell cultures (70–80% of the cells are in S-phase) the amount of the three replacement histone mRNAs is about 3 times higher than in the quiescent counterparts (not shown). Similarly, it was reported that in non proliferating cells such as quiescent hamster ovary cells (CHO), quiescent mouse 3T3 fibroblasts and non stimulated human lymphocytes, synthesis of H3.3 replacement histone protein is low as compared to the stimulated, growing state (52); in Tetrahymena, ten times more hv1 message is present in growing than in starved cells (48). Furthermore, in CHO cells the amount of H3.3 protein increased about 5-fold during transition from G1 to S-phase and this increase was inhibited to about 30% of its control value when DNA synthesis was blocked (59).

Usually, replacement histone protein synthesis accounts for about 10% of total histone synthesis during S-phase (59); it is therefore rather surprising that the mouse replacement H3.3 mRNAs still represent about 60% of the replacement and replication H3.3 histone mRNAs, even when replication H3.3 mRNA level is at its maximum (Fig. 5). This might be due to the use of a probe which is not fully homologous to the replication variant, thus lowering the signal of hybridization. However, it seems that the H3.3 variant, in contrast to H2 replacement variants, may be the major H3 histone protein in mouse liver (60), mouse kidney and other mouse tissues (61). One should also keep in mind that the amounts of histone mRNAs may not reflect the amounts of the corresponding proteins (1), although a correlation between mRNA and protein levels has been reported in some instances (62, 63).

Southern blot analysis indicates that pmH3.3 is encoded by a single gene with respect to its 3′ non coding sequence (Fig. 6). The Drosophila H2AvD (47), Tetrahymena hv1 (48) and mouse H1° (51) genes are also unique, a situation which differs from the replication histone genes, which are repeated about 5–20 times in the genome (2, 55). Our results, however, do not exclude the possibility that the gene encoding pmH3.3 is part of a multigene family with divergent non coding sequences. In this context it is of interest to note that the human H3.3 is encoded by a gene, which is member of a multigene family, mainly

composed of processed pseudogenes (40). However, as pointed out by Wells et al. (40), a second human H3.3 gene, highly homologous in its 3′ region to the chicken H3.3B gene (1), may remain to be isolated. Similarly, in mouse, another H3.3 gene might be homologous to the chicken H3.3A gene. The 0.7 kb mRNA detected in our Northern blot analyses could actually correspond to a H3.3 mRNA encoded by another member of the mouse H3.3 multigene family.

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