Butyrate induced accumulation of a 2.3 kb polyadenylated H1^o histone mRNA in HeLa cells

Hubert Kress, Ralf Tönjes and Detlef Doenecke

Physiologisch-Chemisches Institut I, Philipps-Universität, Emil-Mannkopff-Strasse, D-3550 Marburg, FRG

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ABSTRACT

Sodium butyrate was used to induce the accumulation of human H1^o mRNA in HeLa cells. The length of this mRNA (2,300 nucleotides) was determined by Northern blot hybridization and S₁ nuclease analysis using a human H1^o gene probe. The mRNA shows long 5' and 3' non coding segments and it is polyadenylated. The signal for this step of mRNA maturation (cleavage and polyadenylation) appears to be the hexanucleotide AAUAAA in analogy to most (other than histone) mRNA species. Thus, the mode of maturation of H1^o mRNA differs, on one hand, from that of the cell cycle dependent mRNA species, where it is based on a specific stem-and-loop structure. On the other hand, the 3' end of H1^o mRNA varies from H5 mRNA, which is characterized by two unique dyad symmetry structures at its 3' end.

INTRODUCTION

The structure of the mammalian H1^o histones is highly related to that of the avian H5 species. The homology between these members of the H1 histone family has been established by immunological (1-5) as well as partial protein sequencing (6,7) data and by the recent analysis of a human H1^o gene (8). Neither the synthesis of H5 nor the accumulation of H1^o, which is found in terminally differentiated cells (9,10), is strictly related to DNA replication (for review, see Ref.6). This variant mode of expression may be reflected in differences between transcripts of H5 genes and cell cycle regulated histone genes (11-13), whereas the structure of any H1^o mRNA remained unknown until now. We show here that H1^o mRNA is polyadenylated like its H5 counterpart (14), but in contrast to the H5 system, the signal for this step appears to be the consensus AAUAAA hexanucleotide (15,16). Sequences flanking H1^o and H5 genes show some homologies, but the 5'

and 3' ends of H1^o mRNA are extended and add up with the coding segment and the poly(A) tail to a size of about 2,300 nucleotides.

MATERIALS AND METHODS

Butyrate treatment of HeLa cells; RNA and histone preparations.

HeLa S3 cells (kindly provided by Frauke Seifart) were grown in Dulbecco's modified Eagle's medium, supplemented with 5% newborn calf serum to a density of about $5 \cdot 10^5$ cells/ml. Sodium butyrate (5 mM final concentration) was added and 300 ml aliquots of the cell culture were taken for RNA preparations at the times indicated in Fig.1. Cytoplasmic RNA was prepared according to the protocol of Gallwitz et al.(17) and the RNA was then chromatographed on oligo(dT) cellulose as described by Aviv and Leder (18). For the preparation of histones, nuclei from aliquots of the various cell preparations were isolated (19) and were subsequently extracted with 0.25 N HCl, precipitated with acetone and analyzed on sodium dodecyl sulfate (SDS) polyacrylamide gels (20).

S₁ nuclease mapping of the 5' and 3' ends of the transcribed sequence.

DNA fragments for S₁ nuclease mapping were prepared from the plasmid pBHu5, which contains a BamHI flanked insert out of the H1^o DNA containing recombinant phage described in Ref.8. The analysis of the DNA sequence described previously (8) had to be extended further downstream at the 3' end of the H1^o gene and this was done according to the strategy described in Fig.1 using the Maxam-Gilbert technique (21). Appropriate DNA fragments (indicated in Fig.1) were labeled at their 5' or 3' ends, respectively (22), the anti-mRNA strands were separated from their complementary strands (21) and hybridized to the respective RNA preparation (5 µg RNA). Hybridization was done for 60 min at 65°C in 4x SSC (SSC is 0.15 M NaCl, 0.015 M sodium citrate) and 0.4% (w/v) SDS. After hybridization, the samples were divided into two equal aliquots and were digested in 100 µl S₁ nuclease buffer (23) with 250 and 750 units of S₁ nuclease per ml. After 60 min at 30°C, the digests were precipitated with ethanol and analyzed on DNA sequencing gels (21) by comparison with selected DNA fragment ladders.

Electrophoresis and blotting of RNA.

Cytoplasmic RNA preparations (10 µg), either polyadenylated or poly(A) free, from control and butyrate treated HeLa cells were denatured with glyoxal and dimethylsulfoxide (24) and blotted on to nitrocellulose filters after electrophoresis on a 1.5% (w/v) agarose gel (25). AluI-digested pBR322 and HinfI-digested pSP64 plasmid DNA were treated in the same way as the RNA preparations and were coelectrophoresed as markers. Hybridization of the blotted RNA was done with a nick translated (26) H1^o probe, which covered the segment between the EcoRI site at the third codon and the AluI site at codon 149 in the human H1^o gene (8). Hybridization was done with 4xSSC in 5xDenhardt solution (27), 0.1% (w/v) SDS, at 68°C for 18 hours. After repeated washing in 2xSSC, 0.1% SDS, the filters were dried and exposed to Kodak X-ray film.

Materials and Enzymes.

Cell culture media were from Gibco and substances for bacterial growth media from Difco. The ultrapure reagents for DNA sequencing and RNA denaturation were from Fluka, chemicals for the electrophoretic procedures were from Serva and Bethesda Research Laboratories, respectively. S₁ nuclease was from Bethesda Research Laboratories and the other enzymes were either from Boehringer, New England Biolabs or Bethesda Research Laboratories. Filters were from Schleicher & Schuell and all other chemicals from Merck.

RESULTS AND DISCUSSION

Induction of H1^o accumulation in HeLa cells with sodium butyrate.

The first description of H1^o histones by Panyim and Chalkley (9) demonstrated already that this histone is confined to mammalian cells with little cell division. Consequently, several protocols were developed for the induced accumulation of the H1^o histone protein in cell cultures upon inhibition of cell proliferation or induction of cell differentiation (28-31). One of these inducers is sodium butyrate (32), which leads to an erythroid differentiation of Friend virus transformed cells and causes an accumulation of H1^o histones in this system (30,33) as well as in other cell lines (34-36). We have adapted these protocols to HeLa cells and show in Figure 1 that addition of 5 mM sodium butyrate

Figure 1. H1⁰ mRNA in HeLa cells. Induction of H1⁰ accumulation and S₁ nuclease mapping of the transcribed sequence. (a) Northern blot hybridization of poly(A)⁺-RNA from HeLa cells (butyrate present for 0, 6 and 24 hours) with a human H1⁰ probe (8). Marker sizes are given in kb. (b) SDS polyacrylamide gel electrophoresis (20) of H1 histones (arrow: H1⁰) extracted from the same cells as used for RNA isolation. (c) S₁ nuclease analysis of 5' end of the human H1⁰ gene. From left to right: hybrids treated with 250 and 750 units S₁ nuclease/ml, then G and A/G reaction ladders (21). (d) S₁ analysis of 3' end, from left: C reaction ladder, then 250 and 750 units S₁ nuclease/ml. The poly(A)⁻ RNA fraction did not protect the DNA against S₁ nuclease nor did it hybridize to the H1⁰ probe in the Northern blot (except some slight cross-reaction with rRNA, data not shown). The map at the bottom shows the H1⁰ gene region (8). The coding portion is shaded, open triangles delimit the transcribed sequence, arrows at the bottom right show the sequencing strategy used for the analysis shown in the lower panel of Fig.2.

leads to a relative increase of a H1⁰ histone band migrating slightly ahead of the main type H1 bands in SDS polyacrylamide gels. This gradual accumulation of H1⁰ agrees with the Northern

blot data shown in Fig.1a. Poly(A)⁺-RNA from HeLa cells (isolated 0, 6 and 24 hours after addition of butyrate) was separated electrophoretically and hybridized to a DNA probe from the coding section of the H1^o gene (8). The autoradiograph shows a single band of RNA, which is 2.3 kb in length. A low level is present even at zero time and the synthesis is stimulated upon addition of butyrate. These data conform with previous reports, which showed in cells other than HeLa (35,36) a small basal level of H1^o protein and about a tenfold increase upon addition of sodium butyrate.

Size determination of the polyadenylated human H1^o histone mRNA.

Only polyadenylated RNA (as defined by its retention on oligo-(dT) cellulose (18)) hybridized to the H1^o gene probe. It thus shares this type of posttranscriptional modification with the H5 gene transcript (11-14). We therefore asked whether the H5 and H1^o mRNAs had common nucleotide sequences in such flanking DNA sites, which are highly conserved in H5 chicken (11) and duck (13) mRNA and may be involved in the H5 mRNA processing. A prerequisite for this analysis was an extended sequencing of the H1^o gene (8) and a mapping of the DNA segment which is transcribed into the H1^o mRNA.

The size of the H1^o mRNA which accumulated upon addition of butyrate was about 2.3 kb (Fig.1a). If we assume a poly(A) tail of about 100 nucleotides (in analogy to the H5 mRNA (14)), this relates to a transcribed region of about 2,200 nucleotides. This fits the results of the S₁ nuclease analysis of both the 5' and 3' ends of the human H1^o mRNA as shown in Figures 1c and 1d. Again, only poly(A)⁺-RNA hybridized to the H1^o gene and, as shown in Figure 1c, the mRNA cap site maps 303 nucleotides upstream of the ATG start site of the coding region and 20 nucleotides downstream of a TATA promotor structure (37).

Sequence comparison with the duck H5 gene (13) shows a partial homology around the start site of transcription (Fig.2, upper panel) and immediately upstream of the coding region, but the intermediate segment differs in size and sequence organization.

Figure 1d, which describes the S₁ nuclease mapping of the 3' end of the gene, shows a strong signal around position +1893 (i.e. 1310 nucleotides downstream of the termination codon). Thus,

Figure 2. Human H1⁰ gene: flanking sequences and homologies to the duck H5 gene. Sequences of human H1⁰ (8) and duck H5(13) genes are aligned for maximal homology in the upper (analysis of 5' end) and middle (3' homologies) panels. Dots indicate homologies, ATA boxes underlined, asterisks indicate cap sites, and the inverted arrows in the H5 3' flanking sequence describe conserved dyad symmetry structures (11,13). Numbers refer to positions upstream (labeled minus) or downstream of ATG to allow comparison with Reference 8. The lower panel shows the sequence around the 3' end of the mRNA (complementing previous (8) sequence information). The vertical arrows (around +1893) describe the site of polyadenylation, and the putative signal for this step of maturation is underlined. In each of the three panels, the 5'→3' sequences of the non-coding (i.e.mRNA-like) strands are presented.

the distance between the cap and polyadenylation sites adds up to about 2,200 nucleotides. Some much less intense signals were seen at intermediate sites with clustered A-T base pairs, such as around +650 (not shown, see Ref.8), which may be slightly labile and accessible to high concentrations of S₁ nuclease. In conclusion, the only prominent signals of hybrid protection against the

single strand specific nuclease map to positions, which are 2.2 kb apart and fit the mRNA size determination (Fig.1a).

Sequence analysis of the non coding 3' segment of the polyadenylated H1^o histone mRNA.

Since we (13) and others (11) had shown that the 3' non coding parts of H5 mRNAs from duck and chicken both feature potential hairpin structures (one immediately after the termination codon and one just upstream of the site of H5 mRNA polyadenylation), we searched for homologies between H1^o and H5 mRNAs in this respect. No homologue for the first hairpin was found, but some remnants of the second dyad symmetry structure (shortly upstream of the polyadenylation site in H5 mRNA) may be traced in the H1^o gene (Fig.2, middle panel). This fits the idea of a close relationship between H5 and H1^o, but the Northern blot data and the S₁ nuclease digestion had indicated a further extension of the 3' non translated region in H1^o mRNA. Hence, a different site and mode of H1^o mRNA 3' processing had to be expected.

As shown in Fig.2 (lower panel), the downstream extension of the previous (8) sequence determination demonstrates that a AATAAA hexanucleotide maps around 20 nucleotides upstream of the polyadenylation site as deduced from the S₁ nuclease assay (Fig. 1d). This is the consensus sequence for the cleavage and polyadenylation of most mRNAs (15,16), whereas the non polyadenylated mRNAs coding for the primary types of histones are characterized by a highly conserved stem-and-loop structure at the ends of their respective mRNAs (38,39). As stated above, H5 histones feature a third type of 3' end organization, since their mRNA neither shows a conventional polyadenylation signal nor a histone mRNA consensus sequence (11,13). On the other hand, both the polyadenylated H5 and H1^o mRNAs contain G/T-rich sequences shortly after the site of polyadenylation. The correlation of such downstream sequences with a faithful cleavage and polyadenylation has been established in several systems (39).

In conclusion, our data show that the differential mode of histone gene expression is reflected in a varied organization of the respective mRNAs. In this respect, H1^o mRNA contrasts with other histone mRNAs in its size and inducibility, but it retains structural and functional homologies to H5, its counterpart in nucleated erythroid cells.

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