

Cloning and analysis of cDNA for rat histone H1°

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In most eukaryotes, two classes of histone genes are present: i) replication-dependent, the expression of which is restricted to the S phase of cell cycle and ii) replication- independent or basal genes, expressed at low but quite constant level throughout the cell cycle (for review see ref. 1). Histone genes in the first class are characterized by lack of introns; corresponding mRNAs are not polyadenylated; histone genes in the second class, on the other hand, are similar to most genes transcribed by RNA polymerase II (they can have introns and the corresponding mRNAs are polyadenylated (1).

At the end of mitotic divisions that fix their number, cortical neurons stop dividing (they become blocked in G°/G1 phase of cell cycle) and start terminal differentiation; the latter event is characterized by dramatic rearrangements of the overall structural organization of chromatin (2–5); these rearrangements are accompanied *in vivo* by accumulation of histone H1°, although the class H1 decreases as a whole (6, 7); by using a synthetic medium to culture rat cortical neurons, an active synthesis of histones (in the absence of cell division) has been confirmed (8). These findings suggest the existence of replication- independent genes, active in neurons during terminal differentiation and involved in chromatin rearrangements.

In looking for genes of this class we have cloned, in λgt10, cDNAs corresponding to the polyadenylated fraction of RNA from rat brain at the embryonal day 16 and we have screened the library with probes corresponding to conserved regions of different histone classes. Here we report some findings concerning a cDNA for histone H1° (subclone pMH1°). Northern analysis of total and poly(A)$^+$ RNAs (not shown) indicates that the sequence corresponds to an mRNA of about 2.0 Kb, highly enriched in the polyadenylated fraction. This finding and comparison with other H5/H1° sequences (done using MacMolly Tetra program by Gene Soft) reveals that our clone lacks the short 5′ leader sequence and the first 90 nucleotides in the coding region; it contains, on the other hand the whole 3′-untranslated portion. The alignment of our clone with other H5/H1° clones revealed (in addition to obvious homology in the coding region) the presence of highly conserved motifs in the long 3′-untranslated region. Of particular interest seems a conserved palindrome (Figure 1) with potential to form a hairpin-loop structure; as indicated in the figure, most nucleotides in the palindrome are conserved in the human 3′-untranslated region.

Since H1° synthesis seems to be regulated both at transcriptional and posttranscriptional levels (9–13), we suggest that conserved sequences in the 3′-region are involved in secondary structures important for translational regulation of H1° mRNA.

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REFERENCES

1. Osley M.A. (1991) *Annu. Rev. Biochem.* **60**, 827–861.
2. Brown,I.R. (1978) *Biochem. Biophys. Res. Commun.* **84**, 285–292.
3. Jaeger,A.W. and Kuenzle,C.C. (1982) *EMBO J.* **1**, 811–816.
4. Cestelli,A., Di Liegro,I., Castiglia,D., Gristina,R., Ferraro,D., Salemi,G. and Savettieri,G. (1987) *J. Neurochem.* **48**, 1053–1059.
5. Ivanov,T.R. and Brown,I.R. (1989) *Neurochem. Res.* **14**, 129–137.
6. Cestelli,A., Castiglia,D., Di Liegro,C. and Di Liegro,I. (1992) *Cell Mol. Neurobiol.* **12**, 33–43.
7. Dominguez,V., Pina,B. and Suau,P. (1992) *Development* **115**, 181–185.
8. Cestelli,A., Gristina,R., Castiglia,D., Di Liegro,C., Savettieri,G., Salemi,G. and Di Liegro,I. (1992) *Neurochem. Res.* **17**, 1049–1055.
9. Alonso,A., Breuer,B., Bouterfa,H. and Doenecke,D. (1988) *EMBO J.* **7**, 3003–3008.
10. Khochbin,S., Gorka,C. and Lawrence,J.J. (1991) *FEBS Lett.* **283**, 65–67.
11. Steuer,B., Breuer,B. and Alonso,A. (1992) *Biochem. Biophys. Res. Commun.* **188**, 1153–1160.
12. Rousseau,D., Khochbin,S., Gorka,C. and Lawrence,J.J. (1992) *Eur. J. Biochem.* **208**, 775–779.
13. Boix,J. and Ruiz-Castillo,A. (1992) *Exp. Cell Res.* **201**, 531–534.
14. Doenecke,D. and Tonjes,R. (1986) *J. Mol. Biol.* **187**, 461–464.

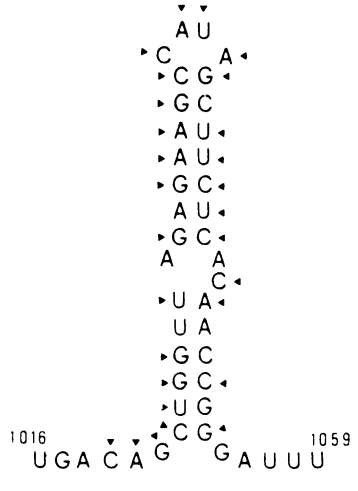

Figure 1. Potential hairpin-loop structure in the 3′ region of rat H1° mRNA. Triangles indicate nucleotides conserved in the human sequence (from 14). Numbers refer to nucleotide position in the sequence of cDNA.

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