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Construction of chimeric plasmids containing histone H5 cDNA from hen erythrocyte. DNA sequence of a fragment derived from the 5' region of H5 mRNA

Rosa Ruiz-Vazquez[1] and Adolfo Ruiz-Carrillo[2]

Max-Planck-Institut für Molekulare Genetik, 1000 Berlin 33, Ihnestrasse 63-73, FRG

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ABSTRACT

We report the construction and characterization of chicken erythrocyte histone H5 cDNA recombinant plasmids. cDNA was synthesized from poly(A)$^+$ polysomal RNA enriched in H5 mRNA and inserted into the PstI site of pBR322. Several clones containing H5 cDNA sequences were obtained and one of them (p541), expressing H5 antigenic determinants, was sequenced. The DNA insert of p541 contains 118 nucleotides from the 5' non-translated region of H5 mRNA and sequences coding for up to residue 46 of the N-terminus of the arginine (position 15) H5 variant. There is a strikingly high number of repeated sequences both in the leader and coding region; among these, the octanucleotide 5' GCG GCG GC 3' is found five times along the sequence. Although the H5 mRNA 5' leader is GC-rich (66%), there is an AT-rich region, about 16 nucleotides long, which shares strong homology with the leaders of sea urchin histone H1 mRNAs.

INTRODUCTION

H5, a histone functionally and structurally related to the histone H1 family, is found only in the nucleated red blood cells of birds, amphibians, and reptiles (1,2), and its occurrence has been correlated with the inactivity of the erythrocyte chromatin.

Although in some respects H5 could be considered a histone H1 variant, its expression has unique features. Thus, while synthesis of the main histone classes is coupled to DNA replication (3-5), H5 is synthesized after cessation of DNA synthesis (6,7) resulting in the partial replacement of H5 for H1 in the chromatin (8,9). Synthesis of H5 is, however, also observed concurrently with that of the other histones in erythroblasts (8,9) and even in proerythroblasts transformed by avian erythroblastosis virus (AEV) (10,11) (Fig.1). The nature of this uncoupling and the levels at which the differential expression of H5 is regulated are

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as yet unknown. However, the fact that H5 mRNA is polyadenylated (12), a feature not shared in common with the other histone mRNA from most systems (13,14), may be related to this question.

In this paper we describe the construction and characterization of recombinant plasmids containing complementary DNA copies of H5 mRNA. These molecular probes will allow us to study the relationship between histone gene organization and regulation of histone expression in the nucleated erythrocyte.

MATERIALS AND METHODS

Cells

Immature hen erythrocytes were obtained and purified as described (15). AEV (RAV-2) transformed chicken erythroblast precursor cells (5) were kindly provided by Dr. T.Graf. Cells were labeled with 10-30 μCi of [^{3}H]lysine (75 Ci/mol, Amersham) as decribed previously (16).

RNA

Immature hen erythrocyte polysomes were obtained as described (15). Poly(A)$^+$ polysomal RNA was prepared by poly(U)-Sepharose (Pharmacia) chromatography (17). Poly(A)$^+$ RNA was fractionated by sedimentation in 10-40% sucrose gradients containing 10 mM Tris-HCl (pH 7.5), 5 mM EDTA, 0.5% NaDodSO$_4$ in a Beckman SW40 rotor at 37,000 rpm for 16 hr at 20°C.

Poly(A)$^+$ RNA was also prepared from polysomes enriched in H5 mRNA after indirect immunoprecipitation of H5-synthesizing polysomes (18). mRNA activity was determined by translation in a rabbit reticulocyte lysate (New England Nuclear) as described (15). In some assays 30 μCi of [^{3}H]lysine were also included. Histone H5 was selectively extracted from the translation assays and immunoprecipitated with affinity chromatography purified rabbit anti-H5 antibodies (12).

Construction of recombinant DNA molecules

Single-stranded cDNA (sscDNA) was synthesized by AMV-reverse transcriptase (the generous gift of Dr. J.W.Beard) after priming the poly(A)$^+$ RNA with oligo(dT)$_{12-18}$ (Collaborative Research) (19). sscDNA made in the presence of Actinomycin D (100 μg/ml) was tailed at the 3' end with dCTP and terminal deoxyribonucleotidyl

transferase (20) (P-L Biochemicals). The complementary strand was synthesized by reverse transcriptase after priming the template with oligo(dG)$_{12-18}$ (Collaborative Research) (21).

Double-stranded cDNA was also synthesized by reverse transcriptase from sscDNA made in the absence of Actinomycin D (22).

The procedure used for the construction of recombinant plasmids was that of cDNA insertion in the PstI site of pBR322 after homopolymer tailing (23). Aliquots of the annealed DNAs were used to transform E.coli 5K made competent according to Dagert and Ehrlich (24).

Colony screening and characterization of recombinant plasmids

Tc^r Ap^s colonies were screened for expression of H5 antigenic determinants according to a modification of published methods (25,26). Colonies were grown on nitrocellulose filters, fixed with 18% trichloroacetic acid and neutralized with 1 M Tris-HCl (pH 7.5). Filters were pre-treated with 60 µl/cm^2 of 3% bovine serum albumin in 150 mM NaCl, 5 mM EDTA, 50 mM Tris-HCl (pH 7.5), 0.05% Nonidet P-40, 0.02% NaN_3 (buffer A) for 2 hr at 37°C and incubated under the same conditions with 35 µg/ml of affinity-chromatography purified rabbit anti-H5 antibodies for 16 hr. Filters were washed with excess buffer A and reacted with 0.5 µCi of ^{125}I-protein A (0.43 µCi/µg) in buffer A for 1.5 hr at 37°C. Filters were quickly rinsed once in water and washed twice in 1 M NaCl, 5 mM EDTA, 50 mM Tris-HCl (pH 7.5), 0.4% Sarkosyl, 0.02% NaN_3 for 1 hr each and autoradiographed using Kodak X-Omatic XR-5 film. Colonies were also screened as described by Grunstein and Hogness (27).

Plasmid DNA from positive clones was linearized with EcoRI (Boehringer), denatured, and coupled to DBM paper (28) (5 µg/cm^2). Paper-bound DNA was hybridized to 150 µg/ml of total poly(A)$^+$ RNA (20 µl/cm^2) as described (28). Hybridized RNA was eluted by three consecutive incubations at 65°C for 1 min each with 25 µl/cm^2 of 99% formamide, 1 mM EDTA, 10 mM Tris-HCl (pH 7.5), 25 µg/ml calf liver tRNA. RNA was recovered from the combined supernatants by ethanol precipitation and its activity was determined by cell-free translation.

DNA sequence analysis

The cloned DNA insert of p541 (see Results section) was sequenced as described by Maxam and Gilbert (29) using 0.4 cm thick gels (30). The sequence of approximately 230 nucleotides from each strand was determined from two independent experiments. Sequence homologies and other features were analyzed with the computer-assisted programs developed by Staden (31,32) and Brutlag et al. (33).

Other analytical techniques

$NaDodSO_4$ polyacrylamide and agarose gel electrophoresis were carried out as described previously (34,35). Polyacrylamide gels were impregnated in Enhance (New England Nuclear) and fluorographed (36) according to the instructions of the manufacturer.

DNA was transferred to nitrocellulose filters (BA 85, Schleicher & Schüll) (37). Hybridization was as described (38) except that poly(G) (100 µg/ml) was included in the pre-hybridization and hybridization mixtures, and E.coli DNA (30 µg/ml) was also included in the Grunstein-Hogness (27) colony hybridizations. The probe used was DNA-labeled with [α-^{32}P]dCTP (400 Ci/mmole, Amersham) by nick-translation (39) to a specific activity of 1 - 2 x 10^8 cpm/µg.

DNA fragments were purified by electrophoresis in Sea-Plaque agarose (Marine Colloids) gels. The DNA was bound to DE-52 cellulose, eluted in 1.5 M NaCl, 1 mM EDTA, 10 mM Tris-HCl (pH 8.0) and precipitated with 0.5 vol of isopropanol.

Biosafety conditions

All recombinant DNA experiments were carried out under L3/B1 conditions as specified by the Zentrale Kommission für Biologische Sicherheit of the Federal Republic of Germany.

RESULTS

Histone H5 is the major histone synthesized in the peripheral red blood cells of phenylhydrazine-treated hens (Fig.1c). The background synthesis of the other histones reflects the small percentage of erythroblasts (1-4%) found in the anemic blood (8). However, since H5 mRNA represents at most 0.3 - 0.5% of all cellular mRNAs in the immature erythrocyte (18), we decided to parti-

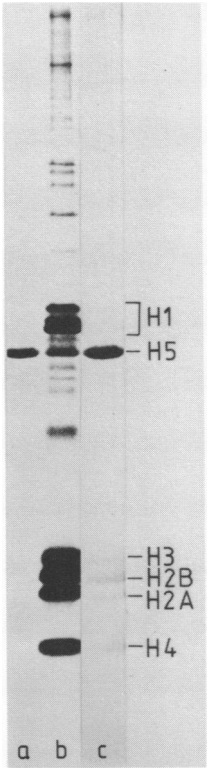

Fig.1 Histone H5 expression in erythroblasts and chicken immature erythrocytes. Fluorogram of a NaDodSO$_4$ polyacrylamide gel electrophoresis of [^{3}H]lysine labeled nuclear proteins from AEV transformed erythroblasts (b), and peripheral anemic red blood cells (c). Lane a: Fluorogram of HCl-soluble nuclear proteins from AEV transformed erythroblasts that precipitated with rabbit anti-H5 antibodies.

ally purify it prior to cDNA synthesis.

Since H5 mRNA is polyadenylated (12), poly(A)$^+$ RNA was first selected from polysomal RNA by poly(U)-Sepharose chromatography. It was further fractionated by sedimentation in sucrose gradients (Fig.2A) and equal amounts of RNA from the gradient fractions were translated in a rabbit reticulocyte lysate. The products of the cell-free translation were analyzed by NaDodSO$_4$-polyacrylamide gel electrophoresis and fluorography (Fig.2B) and also by selective extraction of H5 with 1 M NaCl, 5% HClO$_4$ (12) and precipitation with affinity-chromatography purified anti-H5 anti-

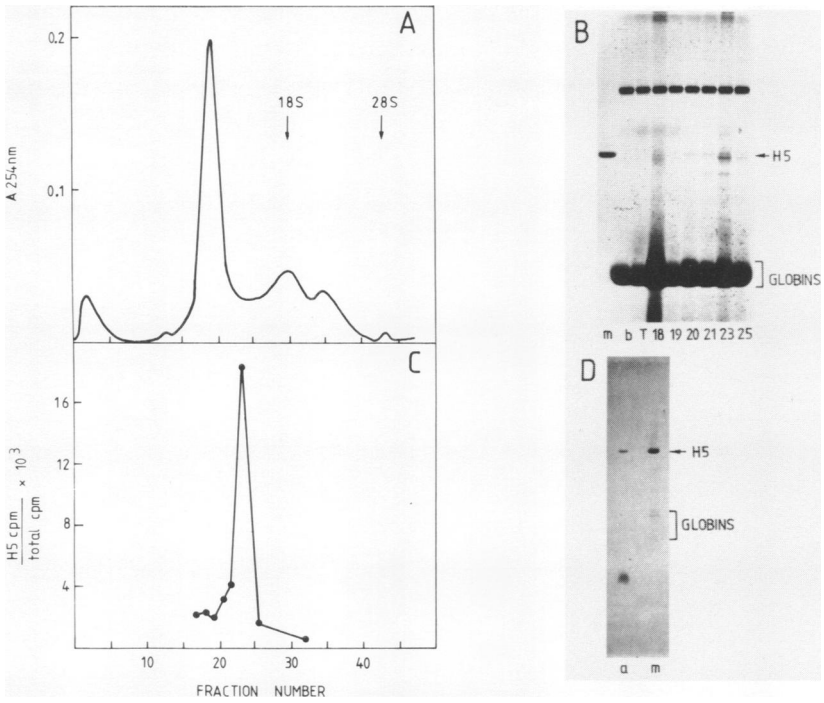

Fig.2 Fractionation of mRNA.
(A) Sedimentation profile of total poly(A)$^+$ polysomal RNA in sucrose gradients.
(B) Fluorogram of the cell-free translation products directed by 25 ng of RNA from the indicated fractions from the gradient shown in (A) after electrophoresis in $NaDodSO_4$ polyacrylamide gels. Lane m: H5 labeled by reductive methylation with [^{3}H]-CH_2O (45); lanes b and T: endogenous activity of the reticulocyte lysate after no addition, or addition of 1 µg of calf liver tRNA, respectively. H5 is the slowest band of the doublet seen in the H5 region.
(C) Fraction of the total protein-bound radioactivity from the cell-free assays that was soluble in 1 M NaCl, 5% $HClO_4$ and was immunoprecipitated by anti-H5 antibodies.
(D) Lane a: Fluorogram of the proteins precipitated by the anti-H5 antibodies (see C) after electrophoresis in $NaDodSO_4$ polyacrylamide gels; lane m: [^{3}H]lysine H5 labeled *in vivo*.

bodies. The main A_{254nm} peak (sedimenting at about 9-10S) corresponded to globin mRNAs (Fig.2B fractions 17-21). H5 mRNA sedimented at 11-12S (Fig.2C, 2B fractions 21-23). Fig.2D shows that the protein-bound radioactivity precipitated by anti-H5 antibodies was indeed H5. The small peptides which precipitated toge-

ther with H5 may represent aborted products of H5 mRNA translation. H5 mRNA was enriched about tenfold after this step although it was still highly contaminated with other mRNAs, mainly globin mRNAs and that of a cytoplasmic protein (18) that has a slightly higher electrophoretic mobility in $NaDodSO_4$ gels than H5 (Fig.2B, fractions 23-25).

sscDNA was synthesized (in the presence of Actinomycin D) from poly(A)$^+$ RNA, corresponding to the peak of H5 mRNA activity (Fig. 2B,C, fractions 21-24) (19). After alkaline hydrolysis of the RNA, the 3' end of the sscDNA was tailed with dCTP and terminal deoxyribonucleotidyl transferase (20). For synthesis of the complementary chain, the template was primed with oligo(dG)$_{12-18}$ hybridized to the 3' homopolymer tail and extended by reverse transcriptase (21). After a second round of 3' end tailing with dCTP, the double-stranded cDNA was annealed to PstI-linearized, oligo (dG)-tailed, pBR322 DNA (23) and used to transform competent bacteria.

cDNA inserted in the PstI site of pBR322 is under the control of the ß-lactamase promotor and has therefore the possibility of being expressed in the host cell, insofar as the orientation and reading frame is the proper one (23). Because of the difficulties involved in purifying H5 mRNA, we resorted to anti-H5 antibodies for the screening of the Tc^r Ap^s transformants. From 66 clones analyzed we found three which after reaction with anti-H5 antibodies and ^{125}I-protein A (see Materials and Methods) gave signals consistently stronger than background. To illustrate this, Fig.3 shows an autoradiograph of the putative positive clones: p507, p541, and p558 grown amidst colonies of the bacterial host. Methylene blue staining of the colonies on the filter indicated that the stronger signals of these clones were not due to overgrowth (not shown).

To find out whether or not these clones contained H5 cDNA, p541 recombinant plasmid DNA was linearized with EcoRI, denatured and bound to DBM paper (28). The immobilized DNA was then hybridized to total poly(A)$^+$ polysomal RNA under stringent conditions. After melting, the hybridized RNA was translated in a rabbit reticulocyte lysate. Fig.4 shows the translation products of total poly(A)$^+$ polysomal RNA and of RNA hybridized to p541 and salmon

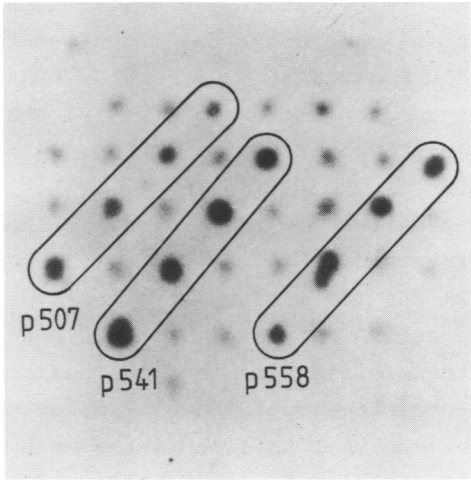

Fig.3 Expression of putative H5 antigenic determinants. Clones p507, p541, and p558 that appeared to express H5 determinants were grown among colonies of E.coli 5K as indicated. The picture shows an autoradiogram of the colonies after reaction with anti-H5 antibodies and ^{125}I-protein A.

sperm DNA. The major band in the H5 region from the translation of poly(A)$^+$ polysomal RNA corresponds to a cytoplasmic protein (see Fig.2B) which sometimes is not well resolved from H5. Fig. 4 b and d shows the electrophoretic analysis of the cell-free products solubilized by 1 M NaCl, 5% $HClO_4$ from the corresponding assays. Clearly p541 DNA hybridized to H5 mRNA (Fig.4c, d) and, therefore, was likely to contain a H5 cDNA insertion.

To verify this, the inserted DNA was released from the vector by digestion with PstI and its two complementary strands were sequenced. Fig.5 shows the sequence of the cDNA strand that has the polarity of the mRNA (i.e. non-coding strand). The fragment of cDNA cloned is 259 base pairs long and has an open reading frame, following the only AUG triplet, which corresponds to the codons for up to residue 46 of the H5 variant containing an arginyl residue at position 15 (40,41). In addition, it contains 118 nucleotides from the 5' non-translated region.

The lengths of the cloned cDNAs of p541, p507, and p558 were in all cases short, and no additional H5 cDNA clones were found after screening the transformants by colony hybridization (27)

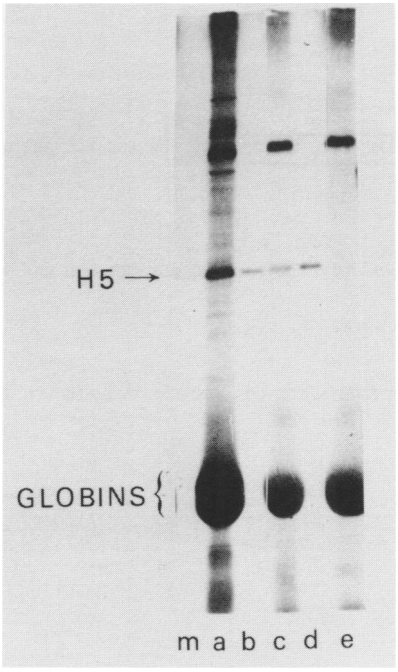

Fig.4 Characterization of RNA complementary to recombinant plasmid DNA. Total poly(A)$^+$ polysomal RNA was hybridized to p541 DNA bound to DBM-paper (28). Hybridized RNA was translated in a rabbit reticulocyte lysate and the products were analyzed by fluorography after electrophoresis in NaDodSO$_4$ polyacrylamide gels. Lane a: total poly(A)$^+$ polysomal RNA. In this electrophoresis H5 comigrated with a major cytoplasmic protein. Lane c: RNA hybridized to p541 DNA. Lanes b and d: cell-free products soluble in 1 M NaCl, 5% $HClO_4$ and precipitated by anti-H5 antibodies from the assays shown in a and c. Lane e: proteins directed by RNA selected by hybridization to salmon sperm DNA. Lane m as in Fig. 2.

using nick-translated p541 cDNA as probe.

Since long cDNAs constitute better probes for the study of the sequence organization of a gene, we carried out an independent cloning experiment in which cDNA was made from poly(A)$^+$ RNA enriched in H5 mRNA, obtained by indirect immunoadsorption of H5-synthesizing polysomes (18). This fractionation resulted in twenty to thirty-fold purification of the H5 mRNA as judged from cell-free translation assays (not shown). In this case sscDNA was synthesized by reverse transcriptase in the absence of Actinomycin D; the complementary strand was synthesized by making use

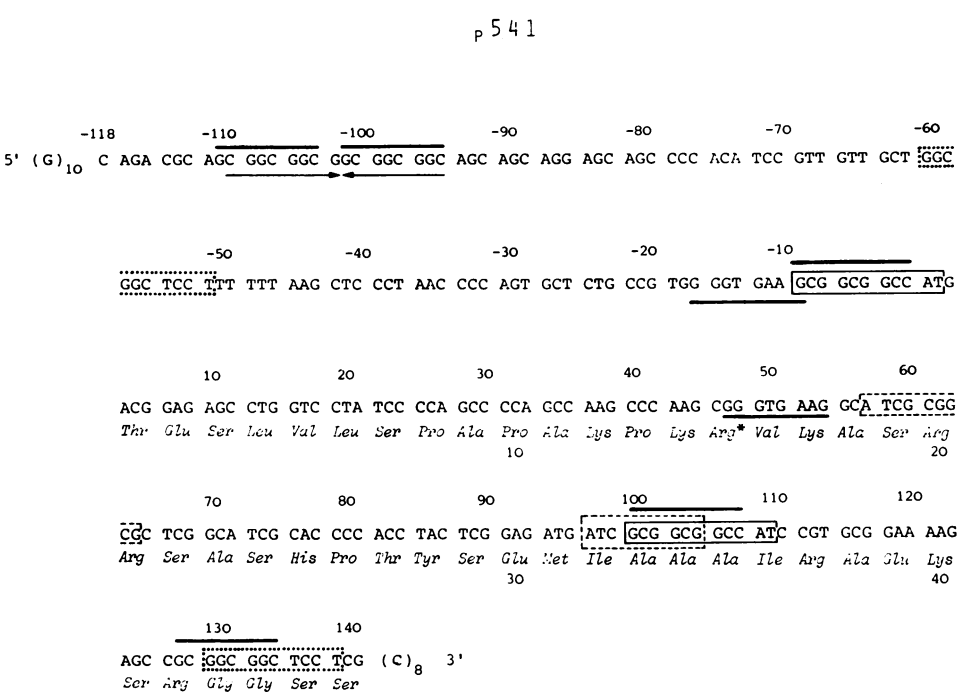

Fig.5 Nucleotide sequence of the cDNA insert of p541. The sequence of the DNA strand that has the polarity corresponding to that of the mRNA is shown. The oligo(dG)$_{10}$ and oligo(dC)$_8$ stretches at the 5' and 3' ends are the result of the homopolymer-tailing step involved in the construction of the recombinant plasmid. The sequence upstream of the ATG initiation codon is numbered with negative digits. The amino acid sequence deduced from the DNA sequence indicates that the cloned DNA corresponds to a fragment of the mRNA coding for the Arg (position 15) variant of histone H5 (41). The main types of direct repeats are indicated by bars over or underlining the nucleotide sequence, and by boxes. A symmetrical sequence near the 5' end is indicated by arrows.

of the self-primed template, and the resulting double-stranded cDNA was digested with S1 nuclease (22). Recombinant plasmids were then constructed as indicated above and the Tc^r Ap^s transformants were screened by colony filter hybridization using as probe the cDNA insert of p541 labeled by nick-translation.

Twelve strongly positive clones, from about 1300 screened, were found (i.e. about 1% of the transformants). The frequency of positive clones was lower than expected from the relative proportion of H5 cDNA (i.e. about 10% of the total sscDNA was of the

size expected for H5 sscDNA, unpublished observations). This could be partly due to the small size and 5' nature of the probe used to identify them. Two of the transformed clones, p551 and p554, were shown to harbor long cDNA inserts: 1350 and 860 base pairs, respectively, and an internal PstI site (Fig.6A, b-e). Southern blot analysis (37) using nick-translated p541 as probe indicated that the 720 base pairs fragment from p551 and the 280 base pairs fragment of p554 were derived from the 5' portion of H5 mRNA (Fig.6B).

DISCUSSION

We have reported the construction and characterization of chimeric plasmids containing hen erythrocyte sequences complementary

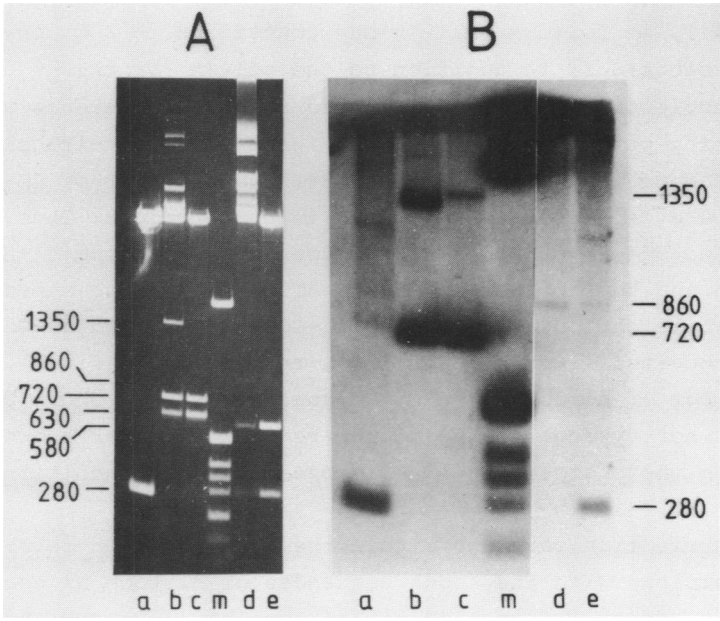

Fig.6 Characterization of long H5 cDNA recombinants. DNA from p551 and p554 was digested with PstI and electrophorized in a 1.2% agarose gel. (A) Ethidium bromide staining. Lane m: pBR322 DNA digested with HinfI. Lanes a-e: recombinant DNA digested with PstI. Lane a: p541; lanes b and c: p551 digested for 20 and 60 min, respectively; lanes d and e: p554 digested for 20 and 60 min, respectively. (B) Autoradiogram of a blot of gel (A) (37) after hybridization with p541 DNA labeled with [α-^{32}P]dGTP by nick-translation. Only the portion of the gel containing the inserted DNA was transferred to the nitrocellulose filter.

to histone H5 mRNA, a relatively minor cellular mRNA species (18). For their construction we have started with partially purified H5 mRNA and have been able to use standard oligo(dT) priming for sscDNA synthesis in agreement with our previous observations that the H5 mRNA is polyadenylated at its 3' end (12).

Two of the recombinant plasmids, obtained from H5 mRNA enriched by immunoprecipitation of H5-synthesizing polysomes, contain very long H5 cDNA fragments, in particular p551 (i.e. 1350 base pairs). This cDNA is longer than our estimate of the length of the bulk H5 mRNA (about 1100 nucleotides, ref.12 and unpublished observations). We do not know, as yet, whether the basis for this apparent discrepancy is due to an anomalous mobility of the DNA in agarose gels, to long homopolymer tails, or to an unexplained cloning artifact. However, since H5 mRNA has 567 coding nucleotides (41), it follows that it may contain up to 400 non-translated nucleotides, in addition to the poly(A) track.

The nucleotide sequence of the H5 cDNA clone reveals several interesting points. The GC content in both the non-translated and translated regions is very high (66%) and a number of GC-rich sequences are repeated several times in both regions. Thus, the undecanucleotide 5'-GCGGCGGCCAT-3', the decanucleotide 5'-GGCGGCT CCT-3', and the nonanucleotide 5'-ATCGCGGCG-3' are repeated twice, while the octanucleotides 5'-GCGGCGGC-3' and 5'-GGCTGAAG-3' are repeated five and two times, respectively (Fig.5). This high number of direct repeats, unusual for such a short DNA fragment, is not typical of the leader regions of other known mRNAs including those of sea urchin histones which, in addition, are AT-rich (42,43).

The nucleotide sequence within the coding region reveals little or no homology with the known sequences of histone H1 genes (44) as may be expected from the lack of homology in the N-terminus of the proteins. It is also evident from Table 1 that sea urchin histone mRNA and H5 mRNA have a tendency to use different codons. There is a clear bias against using U and a preference of G over A as the third letter of the codons in H5 as compared to sea urchin mRNAs.

Despite these differences, there is an AT-rich stretch of 16 nucleotides in the H5 mRNA leader (Fig.7) that shares strong ho-

Table 1 Codon usage in histone mRNA*

		H5	SU			H5	SU
Ser	U C U	0	13	Ala	G C U	0	29
	U C C	2	11		G C C	3	50
	U C A	0	7		G C A	2	25
	U C G	5	2		G C G	3	7
	A G U	0	10				
	A G C	2	15				
				Pro	C C U	0	13
					C C C	2	12
Lys	A A A	0	33		C C A	2	10
	A A G	4	87		C C G	0	1

* Data from H5 was derived from amino acids appearing four or more times (see Fig.5). Data from sea urchin histone (SU) was derived from the sequenced genes of S.purpuratus (45), P.miliaris (44) and L.pictus H4 mRNA (47) as reviewed by Kedes (48).

mology (82%) with the leaders of sea urchin histone H1 mRNAs (42, 43). This homology is significative statistically since the expectation to find the sequence in any two of the species compared is 0.5×10^{-4} (33). It is notable that the homology of this leader sequence between H5 and sea urchin H1 mRNAs is as high as that found between the H1 mRNAs of two sea urchin species. Whether this sequence originated in a common ancestor to H1 and H5 genes or it was introduced into these genes later during evolution, it is clear that it has been a high selective pressure to conserve it. It is possible that this sequence may play a common functio-

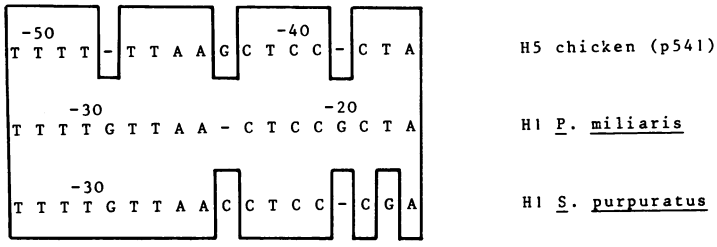

Fig.7 Sequence homology in the 5' non-translated regions of mRNA of functionally related histones. DNA sequence data of the leader of S.purpuratus embryo H1 mRNA is taken from Sures et al. (42), and that of P.miliaris H1 mRNA from Hentschel et al. (43). The region of homology is boxed. Hyphens represent loop outs for maximal alignment. The sequences have been numbered from the common ATG that precedes the translated region of the mRNAs.

nal role in the regulation of the expression of H5 and H1.

Experiments aimed at characterizing the structure of the H5 gene(s) and its relationship to the genes for the other histones are currently underway.

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[1]Present address: Departamento de Genetica, Facultad de Ciencias, Universidad de Murcia, Murcia, Spain.
[2]To whom correspondence and reprint requests should be addressed: Biologie Molèculaire, Unité des Virus Oncogênes, Institut Pasteur, 25 Rue du Dr. Roux, Paris 75015, France.

ABBREVIATIONS: sscDNA: single-stranded cDNA. Buffer A: 150 mM NaCl, 5 mM EDTA, 50 mM Tris-HCl (pH 7.5), 0.05% Nonidet P-40, 0.02% NaN_3.

REFERENCES

1. Neelin, J.M., Callahan, P.X., Lamb, D.C. and Murray, K. (1964) Can. J. Biochem. 42, 1743-1752.
2. Hnilica, L.S. (1964) Experientia 20, 13-14.
3. Borum, T.W., Scharff, M.D. and Robbins, E. (1967) Proc. Natl. Acad. Sci. USA 58, 1977-1983.
4. Perry, R.P. and Kelly, D.E. (1973) J. Mol. Biol. 79, 681-696.
5. Stahl, H. and Gallwitz, D. (1977) Eur. J. Biochem. 72, 385-392.
6. Appels, R. and Wells, J.R.E. (1972) J. Mol. Biol. 70, 425-434.
7. Ruiz-Carrillo, A., Wangh, L.J. and Allfrey, V.G. (1976) Archs. Biochem. Biophys. 174, 273-290.
8. Ruiz-Carrillo, A., Wangh, L.J., Littau, V.C. and Allfrey, V.G.

(1974) J. Biol. Chem. 249, 7358-7368.
9. Billet, M.A. and Hindley, J. (1972) Eur. J. Biochem. 28, 451-462.
10. Beug, H., Von Kirchbach, A., Döderlein, G., Conscience, J.F. and Graf, T. (1979) Cell 18, 375-390.
11. Gazzolo, L., Samarut, J., Bouabdelli, M. and Blanchet, J.P. (1980) Cell 22, 683-691.
12. Molgaard, H.V., Perucho, M. and Ruiz-Carrillo, A. (1980) Nature 283, 502-504.
13. Adesnick, M. and Darnell, J.P. (1972) J. Mol. Biol. 67, 397-406.
14. Grunstein, M., Levy, S., Schedl, P. and Kedes, L.H. (1973) Cold Spring Harbor Symp. Quant. Biol. 38, 717-724.
15. Perucho, M., Molgaard, H.V., Pataryas, T., Shevack, A. and Ruiz-Carrillo, A. (1979) Analyt. Biochem. 98, 464-471.
16. Ruiz-Carrillo, A., Wangh, L.J. and Allfrey, V.G. (1975) Science 190, 117-128.
17. Payvar, F. and Schimcke, R.T. (1979) Eur. J. Biochem. 101, 271-282.
18. Perucho, M., Molgaard, H.V. and Ruiz-Carrillo, A. J. Biol. Chem., in press.
19. Kacian, D.L. and Myers, J.C. (1976) Proc. Natl. Acad. Sci. USA 73, 2191-2195.
20. Roychoudhury, R., Jay, E. and Wu, R. (1976) Nucleic Acids Res. 3, 863-877.
21. Land, H., Grez, M., Hauser, H., Lindenmaier, W. and Schütz,G. (1981) Nucleic Acids Res. 9, 2251-2266.
22. Maniatis, T., Kee, S.G., Efstratiadis, A. and Kafatos, F.C. (1976) Cell 8, 163-182.
23. Villa-Komaroff, L., Efstratiadis, A., Broome, S., Lomedico,P. Tizard, R., Naber, S.P., Chick, W.L. and Gilbert, W. (1978) Proc. Natl. Acad. Sci. USA 75, 3727-3731.
24. Dagert, M. and Ehrlich, S.D. (1979) Gene 6, 23-28.
25. Renart, J., Reiser, J. and Stark, G.R. (1979) Proc. Natl. Acad. Sci. USA 76, 3116-3120.
26. Towbin, H., Staehelin, T. and Gordon, J. (1979) Proc. Natl. Acad. Sci. USA 76, 4350-4354.
27. Grunstein, M. and Hogness, D.S. (1975) Proc. Natl. Acad. Sci. USA 72, 3961-3965.
28. Alwine, J.C., Kemp, D.J. and Stark, G.R. (1977) Proc. Natl. Acad. Sci. USA 74, 5350-5354.
29. Maxam, A.M. and Gilbert, G. (1977) Proc. Natl. Acad. Sci. USA 74, 560-564.
30. Sanger, F. and Coulson, A.R. (1978) FEBS Lett. 87, 107-110.
31. Staden, R. (1977) Nucleic Acids Res. 4, 4037-4051.
32. Staden, R. (1978) Nucleic Acids Res. 5, 1013-1015.
33. Brutlag, D.L., Clayton, J., Friedland, P. and Kedes, L.H. Nucleic Acids Res., in press.
34. Ruiz-Carrillo, A. and Jorcano, J.L. (1979) Biochemistry 18, 760-768.
35. Ruiz-Carrillo, A., Puigdomenech, P., Eder, G. and Lurz, R. (1980) Biochemistry 19, 2544-2554.
36. Laskey, R.A. and Mills, A.D. (1975) Eur. J. Biochem. 56, 335-341.
37. Southern, E.M. (1975) J. Mol. Biol. 98, 503-517.
38. Jeffreys, A.J. and Flavell, R.A. (1977) Cell 12, 429-439.

39. Maniatis, T., Jeffrey, A. and Kleid, D.G. (1975) Proc. Natl. Acad. Sci. USA 72, 1184-1188.
40. Greenaway, P.J. and Murray, K. (1971) Nature New Biol. 229, 233-238.
41. Briand, G. Kniecik, D., Sautiere, P., Wouters, D., Borie-Loy, O., Biserte, G., Mazen, A. and Champagne, M. (1980) FEBS Lett. 112, 147-151.
42. Sures, I., Levy, S. and Kedes, L.H. (1980) Proc. Natl. Acad. Sci. USA 77, 1265-1269.
43. Hentschel, C., Irminger, J.-C., Bucher, P. and Birnstiel,M.L. (1980) Nature 285, 147-151.
44. Schaffner, W., Kunz, G., Daetwyler, H., Telford, J., Smith, H.O. and Birnstiel, M.L. (1978) Cell 14, 655-671.
45. Sures, I., Maxam, A., Cohn, R.H. and Kedes, L.H. (1976) Cell 9, 495-502.
46. Rice, R.H. and Means, G.E. (1971) J. Biol. Chem. 246, 831-832.
47. Grunstein, M. and Grunstein, J. (1977) Cold Spring Harbor Symp. Quant. Biol. 42, 1083-1092.
48. Kedes, L.H. (1979) Ann. Rev. Biochem. 48, 837-870.