10. M. W. Kieran and B. M. Longnecker, *Cancer Metastasis Rev.* **2**, 165 (1983).
11. A preliminary report of this work was presented at the meeting of the American Society for Cell Biology on 30 November 1983 [P. A. Netland and B. R. Zetter, *J. Cell Biol.* **97**, 90a (1983)]. Supported by PHS grant R01 CA 28540 P.A.N. is a recipient of NIH fellowship 5 T32 GM 07258 and B.R.Z. is a Glick-Francheim cancer research scholar and holds a faculty research award (263) from the American Cancer Society. The B16-F10 (clone 11) melanoma cells were provided by T. Koestler and the reticulum cell sarcoma cells were from Arthur D. Little, Inc. We thank P. D'Amore and J. Glowacki for critical comments.

29 September 1983; accepted 18 November 1983

## Clustering of Human H1 and Core Histone Genes

Abstract. *An H1 histone gene was isolated from a 15-kilobase human DNA genomic sequence. The presence of H2A, H2B, H3, and H4 genes in this same 15-kilobase fragment indicates that mammalian core and H1 histone genes are clustered.*

The core histones, H2A, H2B, H3, and H4, are fundamental parts of the primary structural unit of chromatin, the nucleosome (*1, 2*). Also there is compelling evidence that H1 histones are involved in internucleosomal interactions and higher order structures (*1–5*). While the synthesis of core histone polypeptides is confined predominantly to the S phase of the cell cycle (*6–14*), two H1 histone subspecies, $H1^0$ and H1e, accumulate mainly in quiescent cells (*15, 16*). Analysis of cloned genomic histone sequences has shown that the genes encoding mammalian core histones are clustered but not organized as simple tandem repeats (*17–22*). The inability to identify H1 histone genes in human and murine histone gene clusters indicates that there may be functional significance to the apparent separation of H1 and core histone coding sequences. We now report the isolation of a human H1 histone gene from a λCharon 4A human genomic library. We present evidence that this human H1 histone gene resides in a 15-kilobase segment of DNA that also contains H2A, H2B, H3, and H4 histone genes.

A λCharon 4A human gene library (*23*) was screened with the use of a hybridization probe, the chicken H1 histone gene from pCH 4.8E (*24*). The 1.8-kb Sma I fragment containing the chicken H1 sequence was isolated from a low gelling temperature agarose gel and nick-translated in the presence of $\alpha$-$^{32}$P-labeled deoxycytidine triphosphate (dCTP). Of the 12 recombinant phage originally isolated and plaque-purified, only one (λHHG 415) was eventually definitively shown to contain a human H1 histone gene.

A restriction map of λHHG 415 was developed by standard procedures with the use of restriction endonucleases Eco RI, Hind III, Xba I, Sma I, and Bam HI (Fig. 1A). The representation and localization of histone coding sequences were determined by hybridizing Southern blots (*25*) of restriction endonuclease–digested DNA with a series of homologous and heterologous histone gene probes. The core histone genes were identified by hybridization with human H2A, H2B, H3, and H4 histone genes (*18, 26, 27*) while the H1 gene was identified by hybridization with a plasmid containing a 650-base-pair (bp) chicken H1 complementary DNA (cDNA) (Fig. 1B). Confirmation of the representation and organization of human histone coding sequences was by hybrid selection and in vitro translation with either the entire λHHG 415 phage DNA or various subcloned fragments used to select polysomal messenger RNA's (mRNA) from HeLa cells. Each of the core histone genes as well as an H1 histone gene was represented in the genomic fragment in λHHG 415 (Fig. 1C).

Unequivocal identification of the H1 gene in λHHG 415 was provided by DNA sequence analysis of the 5′ region of the gene encoding the first 58 amino acids of an H1 histone protein (Fig. 2A). The amino terminal sequence determined for the human H1 histone protein was compared (Fig. 2B) with the amino acid sequences of H1 histone proteins from rabbit, *Xenopus*, and trout (*28, 29*). There was extensive cross-species sequence homology even in the amino terminal region of H1 histone protein, which is generally considered to be poorly conserved (*28*).

Hybridization of a restriction fragment containing the cloned H1 gene ($^{32}$P-labeled) to total genomic human DNA (restriction endonuclease–digested, fractionated electrophoretically, and immobilized on nitrocellulose) verified that this H1 containing histone gene cluster is represented in the genome of intact cells (compare Fig. 3A with Fig. 1A). Similar analyses, with the H1 gene having different lengths of 3′ and 5′ flanking sequences as $^{32}$P-labeled hybridization probes, indicated that one (or more) reit-

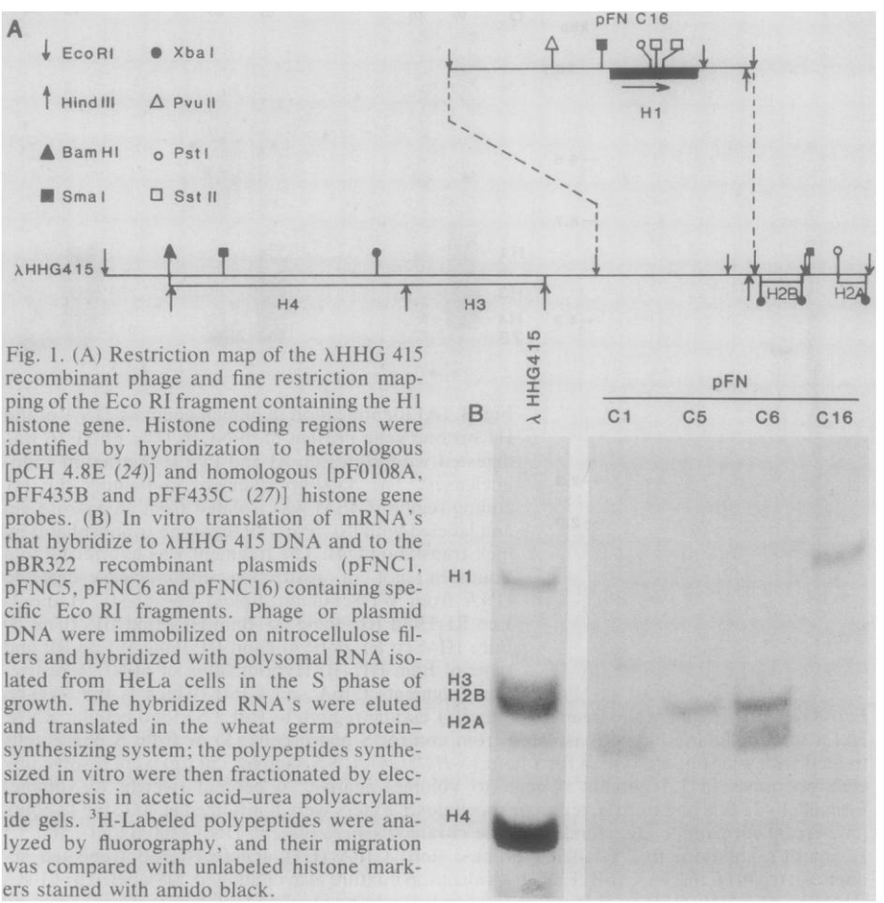


Fig. 1. (A) Restriction map of the λHHG 415 recombinant phage and fine restriction mapping of the Eco RI fragment containing the H1 histone gene. Histone coding regions were identified by hybridization to heterologous [pCH 4.8E (*24*)] and homologous [pF0108A, pFF435B and pFF435C (*27*)] histone gene probes. (B) In vitro translation of mRNA's that hybridize to λHHG 415 DNA and to the pBR322 recombinant plasmids (pFNC1, pFNC5, pFNC6 and pFNC16) containing specific Eco RI fragments. Phage or plasmid DNA were immobilized on nitrocellulose filters and hybridized with polysomal RNA isolated from HeLa cells in the S phase of growth. The hybridized RNA's were eluted and translated in the wheat germ protein-synthesizing system; the polypeptides synthesized in vitro were then fractionated by electrophoresis in acetic acid–urea polyacrylamide gels. $^3$H-Labeled polypeptides were analyzed by fluorography, and their migration was compared with unlabeled histone markers stained with amido black.

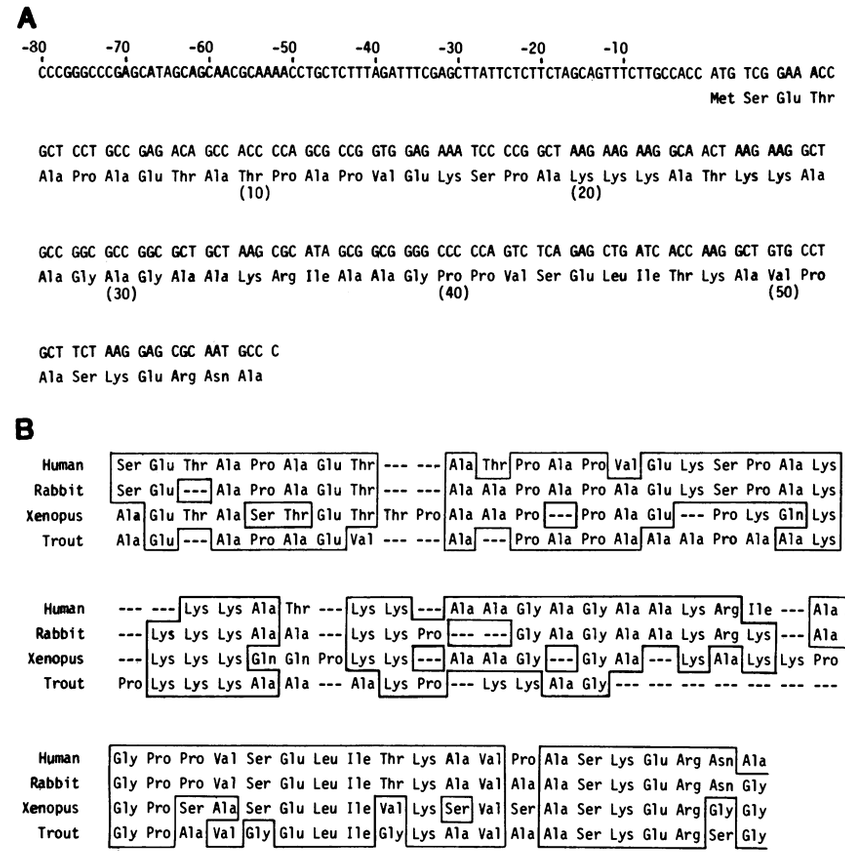


Fig. 2. (A) Nucleotide sequence data for the NH2-terminal region of the H1 histone gene. The Sma I–Sst II restriction fragment of pFNC16 (Fig. 1A) was 5′-end labeled at the Sma I site with polynucleotide kinase and α-$^{32}$P-labeled adenosine triphosphate (ATP), after treatment of the DNA with alkaline phosphatase; it was then sequenced (*33*). (B) Comparison of the human H1 amino acid sequence encoded in pFNC16 with H1 amino acid sequences reported for rabbit (*28*), trout (*28*), and *Xenopus* (*29*).

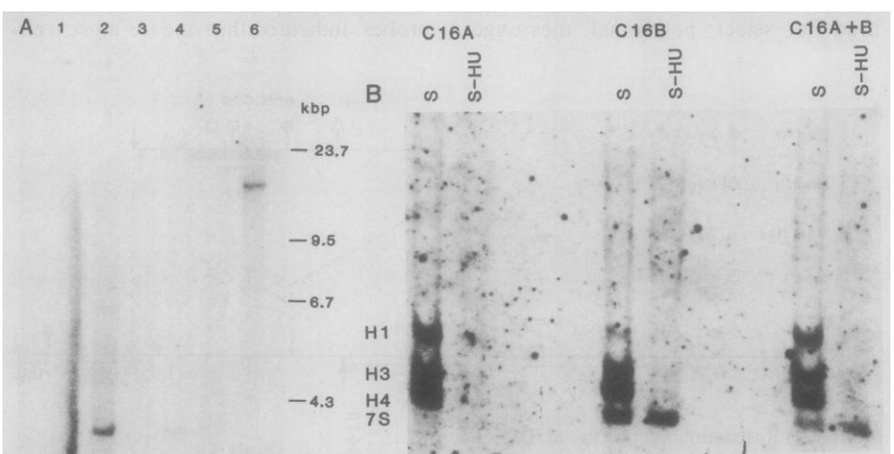


Fig. 3. (A) Identification in human genomic DNA of the H1 histone gene present in pFNC16. The pFNC16 was digested with both Eco RI and Pvu II restriction endonucleases. The 1500-bp fragment containing the H1 coding region (C16A) was isolated from an agarose gel and labeled with [α-$^{32}$P]dCTP (cytidine triphosphate) by nick translation (*34*). The fragment was hybridized to a Southern blot of restriction endonuclease–digested DNA from WI38 human diploid fibroblasts. (Lane 1) Eco RI–Hind III; (lane 2) Bam HI–Hind III; (lane 3) Bam HI–Eco RI; (lane 4) Hind III; (lane 5) Eco RI; and (lane 6) Bam HI. (B) Hybridization of pFNC16 restriction fragments C16A and C16B (which is the 1000-bp Eco RI–Pvu II fragment upstream from the H1 coding region) to HeLa S3 total cellular RNA. HeLa total cellular RNA was isolated from control S phase cells (*S*) or from S phase cells treated with 1 m*M* hydroxyurea for 1 hour (*S-HU*) (*26*). RNA samples (50 μg) were resolved by electrophoresis in 1.5 percent (weight to volume) agarose, 6 percent (weight to volume) formaldehyde gels and transferred to nitrocellulose. Filters were hybridized at 40°C for 48 hours [50 percent formamide, 3× standard saline citrate, *Escherichia coli* DNA (100 μg/ml), and 5× Denhardt's solution] to $^{32}$P-labeled probes: (left) C16A (H1); (center) C16B (repeated sequence); (right) C16A + C16B. Each hybridization mixture also contained $^{32}$P-labeled pF0108A (H4) and pFF435C (H3) so that reference bands could be identified. Filters were analyzed by autoradiography.

erated sequences resides within the 1500 nucleotides adjacent to the 5′ end of the H1 mRNA coding region (data not shown). This repeated sequence (or sequences) hybridizes to a 7*S* RNA species that does not vary in its cellular representation during the cell cycle or after inhibition of DNA synthesis [Fig. 3B and (*30*)].

The absence of H1 histone genes in human or murine histone-containing DNA sequences previously isolated is difficult to explain, although this may in part reflect the hybridization probes used in gene library screening. In most cases either homologous or heterologous H3 and H4 sequences were formerly used. In our present experiments, we found a human H1 histone gene after screening a gene library with chicken H1 histone sequences and analyzing numerous potentially positive H1 clones. Our ability to obtain only a single isolate containing an H1 gene, coupled with the location of the gene in close proximity to core histone sequences, suggests that clustering of core with H1 human histone genes may not be the predominant type of organization.

This cluster containing human core and H1 histone genes appears to be the only mammalian histone gene cluster thus far isolated that resembles the organization found in the early sea urchin histone genes (*17, 31, 32*). However, it remains to be established whether there are any functional analogies between this human histone gene cluster and the sea urchin histone genes expressed early in development. One striking difference is that, while the early sea urchin histone genes are organized into a simple tandem repeat with several hundred copies of the basic unit containing one each of the core and H1 histone genes, the human cluster containing core and H1 histone genes is not highly repeated.

Using this cloned human H1 histone gene as a hybridization probe, we have shown that H1 histone mRNA's are present in the cell predominantly during the S phase of the cell cycle, both in continuously dividing HeLa S3 cells and in quiescent human diploid fibroblasts

stimulated to proliferate (*30*). Expression of the gene appears to be coupled with DNA replication since inhibition of DNA synthesis results in a rapid loss of hybridizable cellular H1 histone mRNA's. However, because of the heterogeneity of H1 histone proteins and the degeneracy of the genetic code, not all H1 histone mRNA's may have been detected by this H1 histone gene hybridization probe. Therefore, it would be premature to conclude that the pattern of expression observed for this gene is a general feature of all human H1 sequences. A more complete understanding of the structural and functional properties of human H1 histone genes and their relation to the organization and expression of core histone sequences must await isolation and characterization of additional H1 genes.

NADINE CAROZZI*
FARHAD MARASHI
MARK PLUMB
SELMA ZIMMERMAN†
ARTHUR ZIMMERMAN‡
*University of Florida College of Medicine, Gainesville 32610*
L. S. COLES
J. R. E. WELLS
*University of Adelaide, Adelaide, South Australia*
GARY STEIN
JANET STEIN
*University of Florida College of Medicine*

### References and Notes

1. G. Felsenfeld, *Nature (London)* **271**, 115 (1978).
2. R. D. Kornberg, *Ann. Rev. Biochem.* **46**, 931 (1977).
3. F. Thoma, T. H. Koller, A. Klug, *J. Cell. Biol.* **83**, 403 (1979).
4. J. Allan, G. J. Cowling, N. Harborne, P. Cattini, R. Craigie, H. Gould, *J. Cell. Biol.* **90**, 279 (1981).
5. P. Labhart, T. Koller, H. Wunderli, *Cell* **30**, 115 (1982).
6. F. Marashi, L. Baumbach, R. Rickles, F. Sierra, J. L. Stein, G. S. Stein, *Science* **215**, 683 (1982).
7. A. M. Delegeane and A. S. Lee, *ibid.*, p. 79.
8. J. Spalding, K. Kajiwara, G. L. Mueller, *Proc. Natl. Acad. Sci. U.S.A.* **56**, 1535 (1966).
9. E. Robbins and T. W. Borun, *ibid.*, **57**, 409 (1967).
10. G. S. Stein and T. W. Borun, *J. Cell. Biol.* **52**, 292 (1972).
11. R. Moll and E. Wintersberger, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 1863 (1976).
12. R. Wu and W. M. Bonner, *Cell* **27**, 321 (1981).
13. M. A. Tarnowka, C. Baglioni, C. Basilico, *ibid.* **15**, 163 (1978).
14. W. I. Waith, J. Renaud, P. Nadeau, D. Pallotta, *Biochemistry* **22**, 1778 (1983).
15. R. W. Lennox and L. W. Cohen, *J. Biol. Chem.* **258**, 262 (1983).
16. J. R. Pehrson and R. D. Cole, *Biochemistry* **21**, 456 (1982).
17. C. C. Hentschel and M. L. Birnstiel, *Cell* **25**, 301 (1981).
18. F. Sierra *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 1795 (1982).
19. N. Heintz *et al.*, *Cell* **24**, 661 (1981).
20. A. Seiler-Tuyns and M. Birnstiel, *J. Mol. Biol.* **151**, 607 (1981).
21. D. B. Sittman, I.-M. Chiu, C.-J. Pan, R. H. Cohn, L. H. Kedes, W. F. Marzluff, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 4078 (1981).
22. S. J. Clark, thesis, University of Adelaide, South Australia (1982).
23. R. M. Lawn, E. F. Fritsch, R. C. Parker, G. Blake, T. Maniatis, *Cell* **15**, 1157 (1978).
24. S. J. Clark, P. A. Krieg, J. R. E. Wells, *Nucleic Acids Res.* **9**, 1583 (1981).
25. E. M. Southern, *J. Mol. Biol.* **98**, 503 (1975).
26. M. Plumb, J. Stein, G. Stein, *Nucleic Acids Res.* **11**, 2391 (1983).
27. G. S. Stein *et al.*, in *Histone Genes*, G. Stein, J. L. Stein, W. F. Marzluff, Eds. (Wiley, New York, in press).
28. I. Isenberg, *Annu. Rev. Biochem.* **48**, 159 (1979).
29. P. C. Turner, T. C. Aldridge, H. R. Woodland, R. W. Old, *Nucleic Acids. Res.* **11**, 4093 (1983).
30. M. Plumb, F. Marashi, L. Green, A. Zimmerman, S. Zimmerman, J. Stein, G. Stein, *Proc. Natl. Acad. Sci. U.S.A.* **81**, 434 (1984).
31. L. H. Kedes, *Annu. Rev. Biochem.* **48**, 837 (1979).
32. R. Maxson, R. Cohn, L. Kedes, T. Mohan, *Annu. Rev. Genet.* **17**, 239 (1983).
33. A. M. Maxam and W. Gilbert, *Methods Enzymol.* **65**, 499 (1980).
34. T. Maniatis, A. Jeffrey, D. Kleid, *Proc. Natl. Acad. Sci. U.S.A.* **72**, 1184 (1975).
35. Supported by grants PCM 80-18075 and PCM 83-18177 from the National Science Foundation, grant GM 32010 from the National Institutes of Health, and grant 1-813 from the March of Dimes Birth Defects Foundation.
* Present address: Abbott Laboratories, Department of Molecular Biology, North Chicago, Ill. 60064.
† Present address: Division of Natural Science, Glenden College, York University, Toronto, Canada.
‡ Present address: Department of Zoology, University of Toronto, Toronto, Canada M5S-1A1.

27 December 1983; accepted 8 March 1983

## Amplification of the c-*myb* Oncogene in a Case of Human Acute Myelogenous Leukemia

Abstract. *Amplification is one of the mechanisms by which cellular oncogenes may be altered in their function, possibly leading to neoplastic transformation. The oncogenes* c-myc, c-abl, *and* c-*Ki*-ras *are amplified in several different human neoplasias. The oncogene* c-myb, *which is specifically expressed and regulated in hematopoietic cells, was found to be amplified in cell lines ML-1, ML-2, and ML-3, which were separately cultured from cells of a patient with acute myelogenous leukemia (AML). A five- to tenfold amplification was correlated with high levels of expression of normal size* c-myb *messenger RNA and with chromosomal abnormalities in the region 6q22–24, where the* c-myb *locus is normally located. Amplification and cytogenetic abnormalities were detected in DNA's from primary and secondary cultures of ML cells, suggesting that they may have contributed to leukemogenesis. The similar AML cell lines HL-60 and ML's contain different amplified oncogenes:* c-myc *and* c-myb, *respectively. Alternative activation of structurally and possibly functionally similar oncogenes may distinguish—at the pathogenetic level—phenotypically similar tumors.*

Structural or regulatory alterations of cellular oncogenes appear to be associated with several human tumors (*1, 2*). Qualitative changes—that is, point mutations—have been found by isolating oncogene alleles that act in transforming NIH/3T3 fibroblasts in transfection assays in vitro (*2*). Alternatively, chromosomal translocations involving cellular oncogenes have been found in a number of different tumors and are likely to cause changes in normal oncogene regulation (*3*). Gene amplification represents an additional event that could influence oncogene expression. This mechanism has been shown for various genetic loci (*4*) and more recently has been reported to involve different oncogenes in several human or mouse tumors (*5–11*). Examples of oncogene amplification include c-*myc* amplification in an acute promyelocytic leukemia cell line (*5, 6*), in neuroendocrine tumors (*7*), and in small cell lung cancer cell lines (*8*); c-Ki-*ras* amplification in a neuroendocrine tumor (*9*); c-*abl* amplification in an erythroleukemia cell line (*10*); and c-N-*myc* amplification in neuroblastoma cell lines (*11*).

Although a causative link between oncogene amplification and the pathogenesis of these neoplasias has not been conclusively shown, the same genes that are found amplified are clearly associated with transformation when activated by mechanisms other than amplification. For example, the c-*myc* oncogene is activated by promoter-enhancer insertions in chicken B-cell lymphomas (*12*) and by chromosomal translocations in murine and human B-cell lymphomas (*13*). Different mechanisms, including amplification, may result in analogous regulatory alterations in development or maintenance (or both) of the malignant phenotype. Determining which oncogenes can regularly or occasionally be amplified in certain tumors may aid in the development of a functional classification of oncogenes and of molecular pathways in different tumors.

In view of the finding of c-*myc* oncogene amplification in a few cases of human myelogenous leukemia (*6*), we investigated another oncogene, c-*myb*, for amplification in human hematopoietic neoplasias. This gene has structural and possibly functional similarities to the c-*myc* gene (*14*), is specifically expressed in hematopoietic cells (*15*), and appears to be tightly regulated during myeloid cell differentiation and proliferation (*15*). We now report that the c-*myb* oncogene is amplified in a case of human acute myelogenous leukemia, both in the independently derived cell lines ML-1, ML-2, and ML-3 and in the primary cells