15. O. P. Hamill, A. Marty, E. Neher, B. Sackmann, F. J. Sigworth, *Pfluegers Arch.* **391**, 85 (1981).
16. H. Lecar and R. Nossal, *Biophys. J.* **11**, 1068 (1971).
17. V. Dionne and M. D. Leibovitz, *ibid.* **39**, 253 (1982).
18. W. Almers, P. R. Stanfield, W. Stuhmer, *J. Physiol. (London)* **336**, 261 (1983).
19. H. Ohmori, S. Yoshida, S. Hagiwara, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 4960 (1981).
20. M. M. Boucek and R. Snyderman, *Science* **193**, 905 (1976).
21. We are grateful to H. Lecar for his helpful comments and to A. Chang for technical assistance.

2 July 1984; accepted 20 August 1984

A Major Human Histone Gene Cluster on the Long Arm of Chromosome 1

Abstract. *A human histone gene cluster was assigned to chromosome 1 by Southern blot analysis of DNA's from a series of mouse-human somatic cell hybrids with ^{32}P-labeled cloned human H4 and H3 histone DNA as probes. Localization of this histone gene cluster on the long arm of chromosome 1 was confirmed by in situ hybridization of this DNA probe to metaphase chromosomes.*

Human histone genes constitute a multigene family of moderately repeated sequences with variations in the structure, organization, and regulation of different copies (*1–4*). Yet our understanding of human histone gene organization is restricted to the observations that both core and H1 histone genes are clustered but not represented as simple tandem repeats (*1, 2, 4*) and that a limited number of histone sequences are nonfunctional pseudogenes (*3*). To further address the organization of human histone genes, we have focused on a histone gene cluster designated λHHG41 containing H4 and H3 histone coding sequences, the transcripts of which are predominant histone messenger RNA (mRNA) species associated with polysomes of cells undergoing DNA replication (*5, 6*).

Somatic cell hybrids with limited numbers of human chromosomes combined with probes for specific copies of the human histone genes provide a high resolution approach for chromosomal assignment of the various members of the histone gene family. DNA's from a series of mouse-human hybrid cells were digested with restriction endonucleases, fractionated electrophoretically, and, after transfer to nitrocellulose, were hybridized with ^{32}P-labeled (nick-translated) human H4 and H3 histone gene fragments subcloned from the λHHG41 genomic DNA cluster (Fig. 1A). The probes included flanking sequences to facilitate identification of these specific copies of the histone genes in Southern blots of total genomic DNA, and particularly, to distinguish between human and murine histone coding sequences.

Our initial studies, which were restricted to hybrid cells containing human chromosome 7, were based on reports that human histone genes are located on this chromosome (*7, 8*). Figure 1, B and C, clearly indicates that both the H4 and H3 histone genes that reside in the human DNA segment cloned in λHHG41 can be identified in restriction endonuclease–digested DNA from human cells and that a comparable gene is not observed in mouse cells. The absence of the H4 and H3 λHHG41 histone genes in the well-characterized hybrid cell line Nu9, which contains only human chromosomes 6 and 7 (*9*), was unexpected and provided the first indication that this gene cluster resides on another human chromosome (Fig. 1, B and C). The absence of this human histone gene cluster on chromosome 7 was substantiated by similar analysis of other human chromosome 7–containing hybrids. We then systematically analyzed Southern blots of genomic DNA's from an extensive series of mouse-human somatic cell hybrids that collectively contain all human chromosomes (*9, 10*). The hybrid cell lines used in these studies were derived from several different sets of human and mouse parentals, but gave consistent results establishing the location of the λHHG41 human histone gene cluster on chromosome 1 (Fig. 1D and Table 1). The H4 and H3 histone genes of λHHG41 have previously been mapped to 2.0- (H4) and 7.0-kilobase (H3) Hind III fragments of genomic DNA's from a number of human cells, including diploid, transformed, and tumor cell lines

Table 1. Representation of human histone genes in DNA's from a panel of mouse-human somatic cell hybrids representing the complete complement of human chromosomes. DNA (15 μg) from the designated hybrid cell lines were digested to completion with the restriction endonuclease Hind III, fractionated electrophoretically, and transferred to nitrocellulose. DNA's from human and mouse cell lines were included in all experiments as controls along with Hind III–digested λ phage as molecular weight markers. The DNA's immobilized on the filter were hybridized with ^{32}P-labeled human H4 or H3 histone genes representing the genomic DNA cluster λHHG41 and analyzed by autoradiography. Each DNA preparation was subjected to Southern blot analysis at least twice, and two or more DNA preparations from each hybrid cell line were analyzed. The construction and characterization of the various somatic cell hybrids have been reported (*9, 10*).

Hybrids	Chromosomes																						H3	H4
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X		
PT47C/3																	X					X		−	−
1P1							X			X				X			X						X	−	−
Nu9						X	X																	−	−
CMC2859																					X			−	−
53-83-3C/10							X																	−	−
M44295														X										−	−
DSK13II2A5C/2			X					X						X		X	X					X		−	−
DSK13II2A5C/20														X	X		X	X			X	X		−	−
77B10C/30TK$^+$			X		X					X			X	X			X			X			X	−	−
77B10C/31TK$^+$	X		X		X			X	X	X			X	X			X	X		X			X	+	+
53-83-3C/21							X																	−	−
D2C16S3			X	X		X					X			X	X		X					X		−	−
CSK-N9-51C1-C/11	X			X				X		X	X	X	X	X	X	X	X	X	X	X			X	+	+
57-77-F7DC7					X								X	X	X								X	−	−
PAFBalbIVC/5		X			X			X				X							X					−	−
GM119XLMTK$^-$C/3				X											X		X			X				−	−
GM119XLMTK$^-$C/5				X				X				X			X					X		X		−	−

and leukocytes from individuals of various genetic backgrounds. As shown in Fig. 1D and Table 1, the only hybrids which yielded positive signals at 2.0 kb with the H4 probe and at 7.0 kb with the H3 probe were those containing chromosome 1. Furthermore, all hybrids containing chromosome 1 gave positive results in Southern blot analysis with λHHG41-derived probes.

To confirm that the λHHG41 H4 histone gene is located on chromosome 1, we carried out in situ hybridization analysis, using as a probe a ^{3}H-labeled plasmid containing the H4 fragment from λHHG41. Metaphase chromosome preparations from peripheral blood cultures

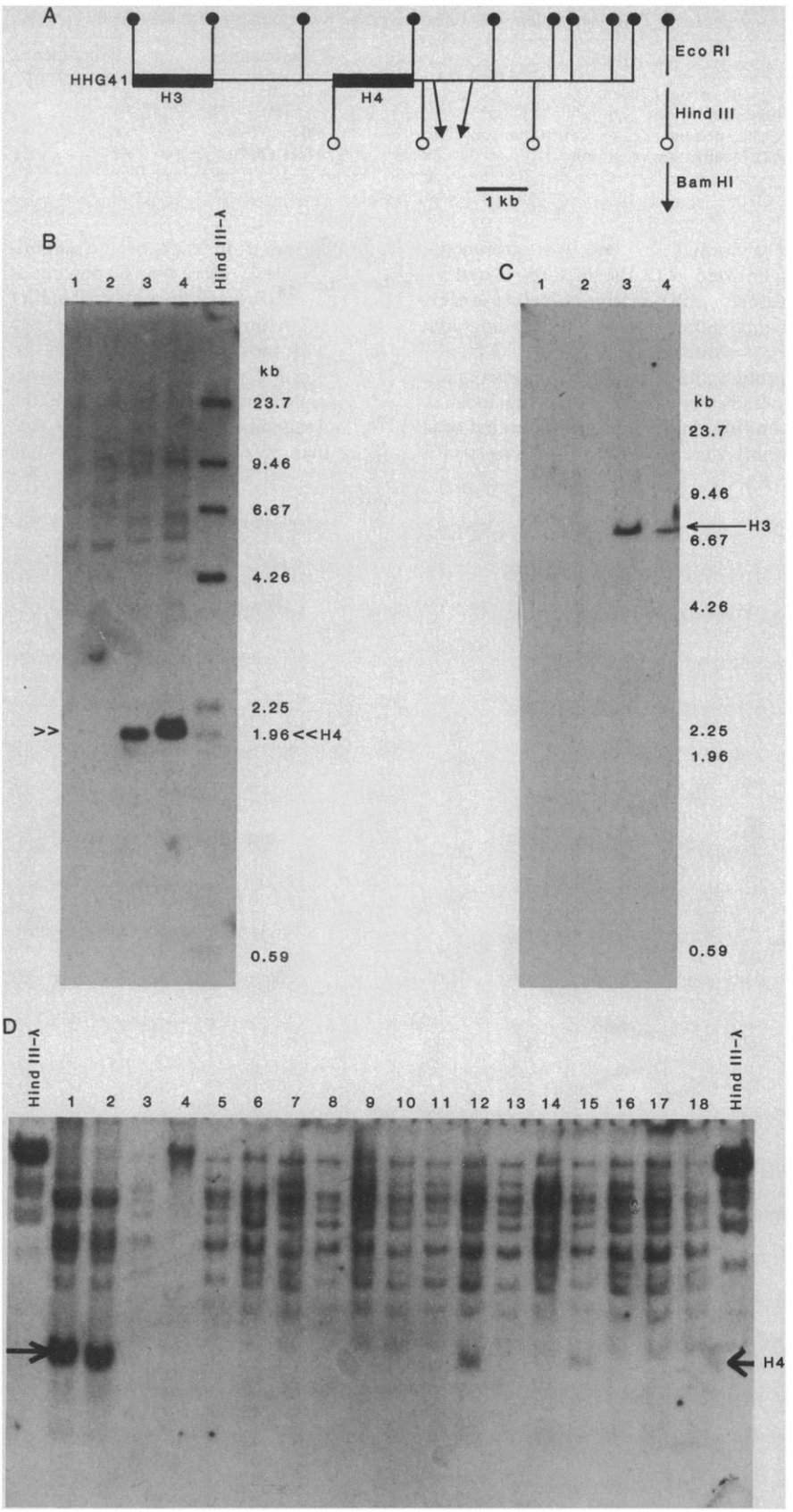

Fig. 1. (A) Restriction endonuclease map of a cloned human genomic DNA sequence containing an H4 and H3 histone gene. The isolation and characterization of the human histone genes have been reported (*1–3, 5*). (B) Southern blot analysis of genomic DNA from the mouse-human hybrid cell line Nu9. DNA (15 μg) from the hybrid Nu9, which contains only human chromosomes 6 and 7, were digested to completion with the restriction endonuclease Hind III, fractionated electrophoretically, and transferred to nitrocellulose. DNA's from a mouse cell line and two human cell lines were similarly analyzed. The filter-immobilized DNA's were hybridized with a ^{32}P-labeled human H4 histone gene and analyzed by autoradiography. ^{32}P-labeled Hind III–digested λ phage was subjected to electrophoresis simultaneously in the same gel and transferred to nitrocellulose to serve as a size marker. (Lane 1) Nu9 DNA (mouse-human hybrid); (lane 2) NP3 DNA (mouse control); (lane 3) cell line LNSV DNA (human control); (lane 4) HT1080 DNA (human control). (C) Southern blot analysis of Hind III–digested genomic DNA from the mouse-human hybrid cell line Nu9 with a ^{32}P-labeled human H3 histone gene probe. (Lane 1) Nu9 DNA (mouse-human hybrid); (lane 2) NP3 DNA (mouse control); (lane 3) LNSV DNA (human control); (lane 4) HT1080 DNA (human control). (D) Southern blot analysis of genomic DNA's from mouse-human cells, with a ^{32}P-labeled human H4 histone gene used as the probe. DNA (15 μg) from a series of mouse-human hybrid cell lines, each hybrid containing the complete complement of murine chromosomes and a limited number of human chromosomes, were digested to completion with the restriction endonuclease Hind III, fractionated electrophoretically, and transferred to nitrocellulose. The filter-immobilized DNA's were hybridized with a ^{32}P-labeled human H4 histone gene and analyzed by autoradiography (lanes 3 to 16). DNA's from two human (lanes 1 and 2) and two mouse (lanes 17 and 18) cell lines are included as controls. ^{32}P-labeled Hind III–digested λ phage was subjected to electrophoresis simultaneously in the same gel and transferred to nitrocellulose to serve as a size marker. (Lane 1) ML3 human cell DNA; (lane 2) K562 (human cell) DNA; (lane 3) PT47C/3 DNA; (lane 4) 1P1 DNA; (lane 5) Nu9 DNA; (lane 6) CMC2859 DNA; (lane 7) 53-83-3C/10 DNA; (lane 8) M44295 DNA; (lane 9) DSK13II2A5C/2 DNA; (lane 10) DSK13II2A5C/20 DNA; (lane 11) 77B10C/30 DNA; (lane 12) 77B10C/31 DNA; (lane 13) 53-83-3C/21; (lane 14) D2 C16S3 DNA; (lane 15) CSK-N9-51C1-C/11 DNA; (lane 16) 57-77-F7DC7 DNA; (lane 17) NP3 mouse cell DNA; and (lane 18) IT22 mouse cell DNA. The same DNA samples were also analyzed by hybridization with a ^{32}P-labeled human H3 histone gene probe and the data are included in Table 1, which also indicates which human chromosomes are present in these hybrid cell lines. Construction of the hybrid cell lines has been reported (*9, 10*). Representation of human and murine chromosomes has been confirmed by karyotype analysis following Giemsa staining and by isozyme analysis for markers assigned to human and murine chromosomes.

Table 2. Hybridization of H4 histone probe to metaphase chromosomes.

Item	Experiment 1	Experiment 2
Number of metaphases	29	71
Number of grains	45	189
1q chromosomal grains (centromere to q25)	14/45 (31%)	64/189 (34%)
1q21 localization of grains	11/14 (79%)	57/64 (89%)

of a normal male were denatured and hybridized with the nick-translated H4 histone probe in two separate experiments. Subsequent to autoradiography the chromosomes were G-banded through the emulsion, and metaphase spreads were analyzed for grain localization (Fig. 2). More than 30 percent of all grains were located on the long arm of chromosome 1 in each of the experiments (Table 2). More than 80 percent of the 1q grains were in the proximal half of 1q, in the region from the centromere to 1q25, with most grains at 1q21 (Fig. 3). The long arm of chromosome 1 represents approximately 4.5 percent of the haploid genome. Our observation that more than 30 percent of the H4 histone probe hybridization is located in the proximal half of this region is highly significant ($P << 0.01$) and suggests that the regional location of this H4 histone gene is the q2 region of chromosome 1.

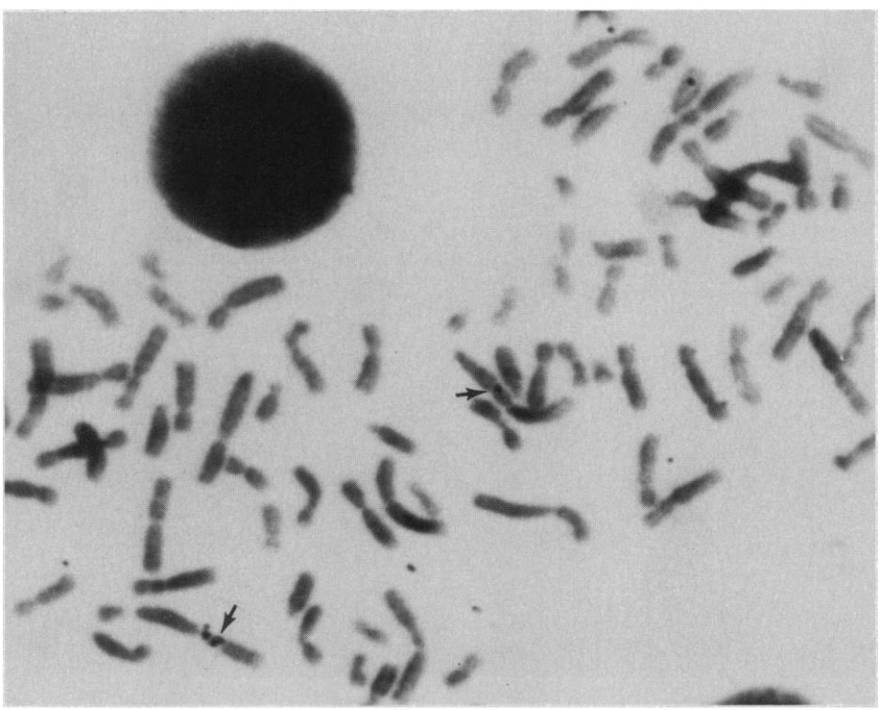

Fig. 2. Representative autoradiograph from in situ hybridization of tritium-labeled H4 human histone probe to metaphase chromosomes from a normal male (46,XY). Arrows indicate grains located on the 1q21 region in two adjacent metaphase spreads.

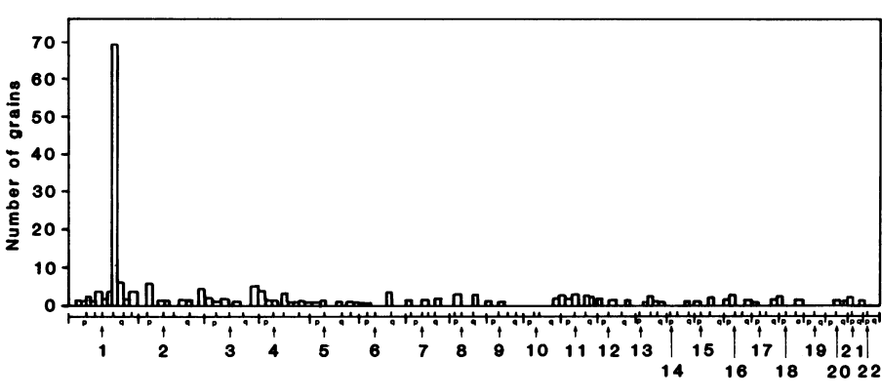

Fig. 3. Histogram showing distribution of silver grains over human autosomes. The grain distribution indicates that the locus of this H4 human histone gene is the long arm of human chromosome 1. No grains were detected over the X and Y chromosomes.

Thus, our results appear to be inconsistent with the previous assignment of human histone genes to chromosome 7. However, the original assignments were based on in situ hybridization in which either a histone mRNA–enriched RNA fraction from human cells (*7*) or sea urchin histone complementary RNA (cRNA) was used (*8*). Neither probe permits recognition of specific histone gene copies, and the mRNA's and heterologous cRNA probes may not recognize all histone coding sequences with equal effectiveness. The possible presence in the histone mRNA–enriched fraction of 7*S* RNA's, which share sequence homology with highly repeated human DNA sequences, further complicates the interpretation of hybridization signals obtained in previous studies. Nevertheless, our results do not preclude the possibility that other human histone gene clusters are on chromosome 7. In fact our preliminary results from analysis of another series of human histone gene clusters suggest that human histone coding sequences are located on more than one chromosome.

L. Green, R. Van Antwerpen
J. Stein, G. Stein
University of Florida, College of Medicine, Gainesville 32610
P. Tripputi, B. Emanuel
J. Selden, C. Croce
Department of Pathology and Human Genetics, University of Pennsylvania School of Medicine, and *Wistar Institute, Philadelphia 19104*

References and Notes

1. F. Sierra *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 1795 (1982).
2. N. Carozzi *et al.*, *Science* **224**, 1115 (1984).
3. F. Marashi, K. Prokopp, J. Stein, G. Stein, *Proc. Natl. Acad. Sci. U.S.A.* **81**, 1936 (1984).
4. N. Heintz, M. Zernik, R. G. Roeder, *Cell* **24**, 661 (1981).
5. A. C. Lichtler, F. Sierra, S. Clark, J. R. E. Wells, G. S. Stein, J. L. Stein, *Nature (London)* **298**, 195 (1982).
6. F. Sierra, G. Stein, J. Stein, *Nucleic Acids Res.* **11**, 7069 (1983).
7. L. C. Yu, P. Szabo, T. W. Borun, W. Prensky, *Cold Spring Harbor Symp. Quant. Biol.* **42**, 1101 (1977).
8. M. E. Chandler, L. H. Kedes, R. H. Cohn, J. J. Yunis, *Science* **205**, 908 (1979).
9. R. Dalla-Favera, M. Bregni, J. Erikson, D. Patterson, R. C. Gallo, C. R. Croce, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 7824 (1982).
10. R. Dalla-Favera, G. Franchini, S. Martinotti, F. Wong-Staal, R. C. Gallo, C. M. Croce, *ibid.*, p. 4714.
11. Supported by NSF grant PCM83-18177, by NIH grants GM32010, GM20138, and GM20700, and by grant 1-813 from the March of Dimes Birth Defects Foundation.

11 June 1984; accepted 19 July 1984