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Human histone genes map to multiple chromosomes

(*in situ* hybridization/Southern blot analysis/human chromosomes)

P. TRIPPUTI*†, B. S. EMANUEL*†, C. M. CROCE*†, L. G. GREEN*‡, G. S. STEIN*‡, AND J. L. STEIN*‡

*Wistar Institute and †Departments of Human Genetics and Pediatrics, University of Pennsylvania School of Medicine, Philadelphia, PA 19104; and ‡University of Florida College of Medicine, Gainesville, FL 32610

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ABSTRACT **Histone genes were mapped to at least three human chromosomes by Southern blot analysis of DNAs from a series of mouse–human somatic cell hybrids (using ^{32}P-labeled cloned human histone DNA as probes). Chromosome assignment was confirmed by *in situ* hybridization of radiolabeled histone gene probes (^{3}H-labeled) to metaphase chromosomes. One human histone gene cluster (λHHG41) containing an *H3* and *H4* gene resides only on chromosome 1, whereas other clusters containing core (*H3*, *H4*, *H2A*, and *H2B*) alone (λHHG17) or core together with *H1* histone genes (λHHG415) have been assigned to chromosomes 1, 6, and 12. These results suggest that the multigene family of histone coding sequences that reside in a series of clusters may be derived from a single cluster containing one each of the genes for the five principal classes of histone proteins. During the course of evolution, a set of events, probably involving reduplication, sequence modification, and recombination, resulted in the present pattern of human histone gene distribution among several chromosomes.**

Human histone genes are represented as a family of moderately repeated sequences with variations in the structure, organization, and regulation of the different copies (1–5). Functionally, there are three classes of histone genes, with the expression of most of the genes coupled with DNA replication (6–8), ≈10% expressed in a non-cell-cycle-dependent manner (9, 10), and several nonexpressed pseudogenes (3). Our present understanding of human histone gene organization is based on analysis of a series of cloned 15- to 20-kilobase (kb) human genomic DNA segments containing one to five core or core plus *H1* histone genes (1–5, 11). The human histone genes are represented by at least 11 different types of these cloned clusters, and there is no evidence of a simple tandem repeat as observed for the histone genes expressed early during development in several lower eukaryotes (12, 13). Other mammalian histone genes appear to be organized in a manner similar to that in humans (14, 15). Yet, to date, the structural relationship between these histone gene clusters remains to be resolved.

MATERIALS AND METHODS

Somatic Cell Hybrids. The construction and characterization of the various somatic cell hybrids have been reported (16, 17). Representation of human and murine chromosomes has been confirmed by karyotype analysis following Giemsa staining and isozyme analysis from markers assigned to human and murine chromosomes.

Southern Blot Analysis. DNAs (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 restriction endonucleases, fractionated electrophoretically, and transferred to nitrocellulose. Hybridization was carried out in 6× SSC (1× SSC = 0.15 M NaCl/0.015 M sodium citrate, pH 7.0)/5× Denhardt's buffer [0.1% Ficoll 400 (Pharmacia)/0.1% polyvinylpyrrolidone (Sigma)] *Escherichia coli* DNA (0.1 mg/ml)/bovine serum albumin (20 μg/ml)/^{32}P-labeled probe (2 × 10^6 cpm/ml). Hybridization was for 24 hr at 65°C. Blots were washed for 30 min in 0.1 M potassium phosphate (pH 7.2) at 65°C followed by two 30-min washes at 65°C in 1× SSC/0.6% $NaDodSO_4$. DNAs from human and mouse cell lines were included in all experiments as controls. The DNAs immobilized on the filter were hybridized with ^{32}P-labeled human *H1*, *H4*, or *H3* histone genes representing the genomic DNA clusters λHHG41, λHHG415, or λHHG17 (1, 2) and were 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.

***In Situ* Hybridization.** *In situ* hybridization was carried out by the method of L. Cannizzaro and B.S.E. (unpublished). Human metaphase chromosome preparations on glass slides were air-dried, treated with RNase to remove chromosome-associated RNA, and dehydrated by a series of ethanol washes. Chromosomal DNA was denatured in 2× SSC containing 70% formamide at 70°C, followed by another series of ethanol washes. ^{3}H-labeled human histone gene probes were prepared by nick-translation in the presence of [^{3}H]dCTP, [^{3}H]dGTP, and [^{3}H]dATP to a specific activity of 2.0 × 10^7 cpm/μg. Hybridization was in 2× SSC/50% formamide/10% dextran sulfate, pH 7.0, in the presence of a 1000-fold excess of salmon sperm DNA. The radiolabeled histone gene probes were denatured for 5 min at 70°C and rapidly chilled prior to hybridization at a concentration of 50 ng/ml. Hybridization was for 18 hr at 37°C, followed by extensive washing in hybridization buffer at 39°C to remove nonspecifically bound probe. The preparations were dehydrated by a series of ethanol washes, dipped in Kodak NTP-2 liquid nuclear track emulsion, and stored in the dark at 4°C for 1–4 weeks. Chromosome banding was carried out with Wright's Giemsa stain, and the preparations were analyzed by light microscopy.

RESULTS AND DISCUSSION

We have previously shown, by Southern blot analysis and *in situ* hybridization, that a human histone gene cluster containing an *H3* and *H4* histone gene is located on the long arm of chromosome 1 (18). To examine further the overall organization of human histone genes, we investigated whether components of this multigene family reside on one or more chromosomes. Our experimental approach to mapping specific histone gene clusters to human chromosomes involved subcloning DNA fragments containing histone genes from three classes of genomic DNA segments (Fig. 1) (1, 2). These DNA fragments were used as ^{32}P-labeled (nick-translated)

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Abbreviation: kb, kilobase(s).

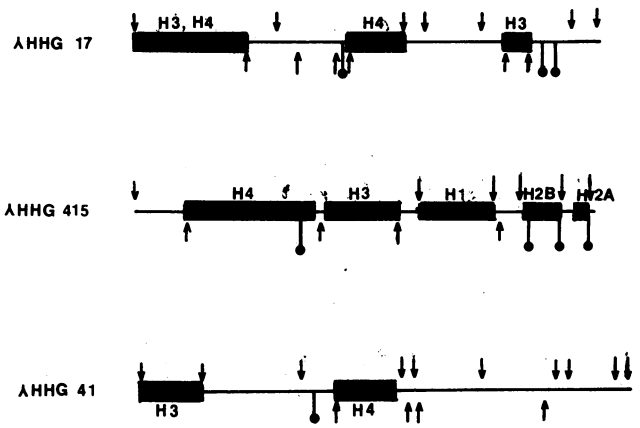

FIG. 1. Restriction endonuclease maps of cloned human genomic DNA sequences containing various histone genes used as probes in these experiments. The isolation and characterization of the human histone genes have been reported (1–5). Restriction sites are indicated as follows: *Hin*dIII, ↑; *Eco*RI, ↓; and *Xba* I, ●.

probes for Southern blot analysis of DNAs from an extensive series of mouse–human somatic cell hybrids with limited numbers of human chromosomes and the complete complement of murine chromosomes (16, 17). Restriction digests of DNAs from hybrid cells were selected so that the resulting fragments containing the human and murine histone sequences could be distinguished, and at the same time the specific copy of the human histone gene used as the hybridization probe could be identified. *In situ* hybridization of ^{3}H-labeled human histone gene probes to human metaphase chromosome spreads provided results that are consistent with chromosome assignments by Southern blot analysis of somatic cell hybrid DNAs.

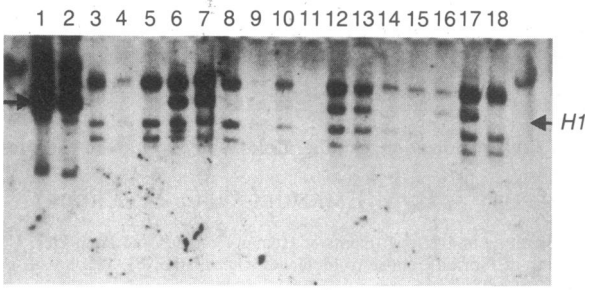

FIG. 2. Southern blot analysis of genomic DNAs from human, mouse, and mouse–human somatic hybrid cells with a ^{32}P-labeled human *H1* histone gene used as the probe. DNAs (15 μg) were digested to completion with the restriction endonuclease *Xba* I, fractionated electrophoretically, and transferred to nitrocellulose. The filter-immobilized DNAs were hybridized with a human *H1* histone gene (pFNCl6A) and analyzed by autoradiography. Lanes: 1, PAF human DNA; 2, ML3 human DNA; 3, PT47Cl1; 4, DSK1B2A5Cl2; 5, DSK1B2A5Cl20; 6, Nu9; 7, PAF×BalbIVCl5; 8, 77B10Cl8; 9, 77B10Cl26 (very little DNA in this lane); 10, 77B10Cl30; 11, 77B10Cl28 (very little DNA in this lane); 12, 77B10Cl31; 13, 77B10Cl33; 14, GM×LMTK-Cl3; 15, GM×LMTK-Cl5; 16, D2Cl6S3; 17, CSK-NS5-1Cl-Cl11; 18, IT22 mouse DNA. The same DNA samples as well as several other hybrids were also analyzed by hybridization with other ^{32}P-labeled histone gene probes and the data are included in Table 1. The *Xba* I restriction fragment containing the human pFNC16A human histone gene is indicated by an arrow.

An H1 histone gene probe, pFNC16A, derived from λHHG415, a human histone gene cluster containing one copy of each core histone gene and an *H1* gene (2), was used to systematically examine Southern blots from a panel of mouse–human hybrid cells (16, 17) collectively representing the complete population of human chromosomes. As indicated in Fig. 2 and Table 1, positive signals were obtained for

Table 1. Panel of human–mouse somatic cell hybrids containing different chromosome complements

Hybrids	Chromosomes																						Genes			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X	*H1**	*H4*†	*H3*†	*H4*‡
PT47Cl4																	X					X		−	−	−	−
PAF×BalbIVCl5		X			X							X							X					+	−	−	+
77B10Cl30			X		X				X	X			X	X						X		X	X	−	−	−	−
77B10Cl31	X		X		X			X	X	X			X	X				X		X		X	X	+	+	+	+
D2Cl6S3			X	X		X					X			X	X		X					X		+	−	−	+
CSKNS51C1Cl11	X			X				X		X	X	X	X	X	X	X	X	X	X	X			X	+	+	+	+
5468F$_2$Cl15	X											X												+			
54-68F$_1$Cl16	X			X				X		X	X	X	X	X	X	X	X							+			
54-68F$_1$Cl11	X											X												+			
54-68F$_1$Cl15												X												+			
1P1							X			X					X		X					X	X	−	−	−	
CML2759Cl5																					X			−			
Nu9						X	X																	+	−	−	+
GMXLMTK-Cl3				X			X								X		X			X				−	−	−	−
DSK1B2A5Cl2A			X											X	X	X	X					X		−			−
DSK1B2A5Cl20A								X						X	X		X	X			X	X	X	−			−
77B10Cl8	X		X		X			X	X				X	X				X		X		X	X	+			+
77B10Cl26	X		X		X			X	X	X				X				X		X		X	X	+			+
77B10Cl28	X		X		X									X				X					X	+			+
77B10Cl33	X		X		X			X	X	X			X	X				X		X		X	X	+			+
57-87-1F1Cl21							X																	−			−
77B10Cl12	X				X		X	X	X					X				X		X			X	+			+
M44Cl2S5														X										−	−	−	−
57-77-F-7DC7					X								X	X	X								X	−	−	−	−
53-87-3Cl10							X																		−	−	

Representation of human histone genes in DNAs from a panel of mouse–human somatic cell hybrids representing the complete complement of human chromosomes. DNAs (15 μg) were digested to completion with restriction endonucleases *Hin*dIII or *Xba* I, fractionated electrophoretically, and transferred to nitrocellulose. The DNAs immobilized on the filter were hybridized with ^{32}P-labeled human *H1*, *H4*, or *H3* histone genes representing the genomic DNA clusters λHHG41†, λHHG415*, and λHHG17‡, and analyzed by autoradiography.

all hybrids containing human chromosomes 1, 6, or 12. Hybridization with an 8.3-kb band of *Xba* I-digested DNA from the hybrid cells was consistent with mapping of the *H1* histone gene in λHHG415 to an 8.3-kb *Xba* I fragment of human genomic DNAs isolated from >30 normal diploid and transformed human cell lines. It should be noted that our results do not completely exclude the possibility that the histone *H1* gene fragment may also be located on chromosomes 2, 11, or 19 because in any hybrids in which 2, 11, or 19 occurred, one of the chromosomes 1, 6, or 12 was also present. However, for each of the chromosomes 1, 6, and 12, the pFNC16A H1 probe could be unequivocally assigned, because somatic cell hybrids were identified that contained one of these chromosomes and not the others or 2, 11, or 19. For example, five probe-positive hybrids contained chromosome 1, but not 2, 6, 11, 12, or 19. Also the line Nu9 had a positive signal and contained only chromosomes 6 and 7, but chromosome 7 was excluded by analysis of other cell lines. Finally, one probe-positive cell line contained only chromosome 12 and there were two positive lines that contained only chromosomes 1 and 12. In agreement with our earlier observation (18), a similar series of Southern blot analyses using *Hin*dIII-digested cellular DNA from hybrid cells and probes for both *H3* and *H4* λHHG41 histone genes (Table 1) indicated that the λHHG41 histone gene cluster resides only on chromosome 1.

The chromosomal localization of the λHHG415 histone gene cluster is supported by *in situ* hybridization studies, using as a probe the pFNC16A H1 histone gene fragment subcloned from λHHG415, ^{3}H-labeled by nick-translation. Metaphase chromosome preparations from peripheral blood cultures of a normal male were denatured and hybridized with the radiolabeled H1 histone probe. After autoradiography, the chromosomes were G-banded through the emulsion. One hundred metaphase spreads were analyzed for grain location, and 162 grains were identified over chromosomes. Several chromosomal sites of hybridization were noted: 21 grains (13%) were over chromosome 6 (p12 → q16), 16 grains (10%) were over chromosome 12 (p11.2 → q21), and 12 grains (7.4%) were over chromosome 1 (cent → q31) (Fig. 3*A*). Each of these regions is approximately the length of chromosome 18, which for the sake of comparison had only 4 grains or 2.5% hybridization. This signal is considered to be nonspecific background. These results, represented in a histogram (Fig. 3*A*) and illustrated schematically in chromosomal idiograms (Fig. 3*B*), are consistent with the somatic cell hybrid data, which suggest that there are multiple loci for this H1 histone-containing gene cluster, including sites on chromosomes 1, 6, and 12. It is possible that the pFNC16A *H1* histone gene probe will hybridize *in situ* with other human *H1* histone genes. However, the presence of ≈800 nucleotides of flanking sequences in the probe and the amino acid sequence differences among various members of the H1 histone family would suggest that the probe will detect primarily the λHHG415 *H1* histone gene.

The next most frequently represented site of hybridization was chromosome 7 (pter → q11), which had nine grains (5.6%) over a region similar in length to chromosome 18. However, the Southern blot analysis of DNAs from hybrid cells containing either human chromosome 7 alone or with other chromosomes clearly indicated that none of the three distinct human histone gene clusters that we have thus far examined (λHHG17, λHHG41, and λHHG415, together containing 11 different human histone genes) are located on this chromosome. The absence of a positive signal on blots of DNA from the hybrid cells 57-87-1FC121 and 53-83-3 C110, where 7 is the only human chromosome present as confirmed by isoenzyme analysis with β-glucuronidase and by Southern blot analysis with an *erbB* genomic probe (P.T., unpublished data), strongly supports this conclusion. However, the *in situ*

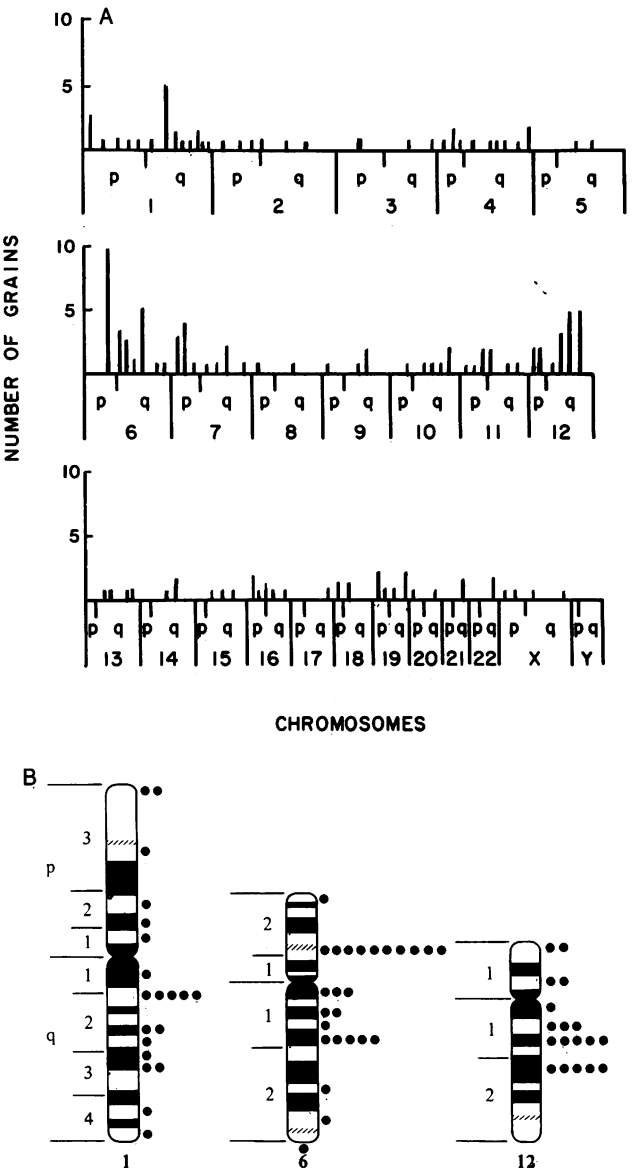

FIG. 3. *In situ* hybridization of the λHHG415 H1 histone probe (pFNC16A) to normal metaphase chromosomes. pFNC16A contains a 1.5-kb genomic DNA fragment that includes ≈700 base pairs of H1 coding sequences (2). (*A*) The histogram shows the grain distribution in 100 metaphase spreads hybridized with the ^{3}H-labeled H1 histone probe. The abscissa represents the chromosomes in their relative size proportion; the ordinate indicates the number of silver grains. (*B*) The idiogram schematically indicates the intrachromosomal localization of grains.

hybridization studies and previous studies using either a histone mRNA-enriched RNA fraction from human cells (19) or sea urchin complementary RNA (20) suggest that other histone genes may be located on chromosome 7. Possibly, the low grain count over chromosome 7 represents cross-hybridization of the pFNC16A H1 probe with other members of the *H1* gene family.

Another histone cluster, λHHG17, containing two *H4* and two *H3* genes was similarly mapped by Southern blot analysis of DNAs from the mouse–human hybrid panel. Despite the marked differences in the representation and organization of histone genes in the λHHG17 and λHHG415 clusters, the hybrids that gave positive signals using a ^{32}P-labeled *H4* gene probe from the λHHG17 cluster (pST523B) were identical to those that were positive for the H1 histone probe derived from λHHG415.

From these studies, a picture of the overall organization of

human histone genes emerges, which suggests that some segments of DNA containing histone sequences, such as those in the λHHG41 clone, are present only on a single chromosome, whereas other segments of DNA containing histone genes, such as those in the λHHG415 and λHHG17 clones, map to at least three chromosomes. These results provide the first indication of the overall genomic organization of the moderately repeated human histone genes. The distribution of these genes may offer insight into the functional properties of the various components of this multigene family. The physical proximity of histone genes for which expression is coupled with DNA replication, and likewise the extent to which cell cycle-independent histone genes are distributed, may be related to molecular mechanisms operative in regulation of expression. Coordinate control and shared structural elements of broad functional domains are two possibilities within this context.

The pattern of histone gene distribution among various chromosomes may also be related to the evolution of this multigene family. It seems reasonable to postulate that the present representation and organization of human histone genes could have arisen from a cluster containing one each of the five classes of histone coding sequences by a series of events involving reduplication, sequence modifications, and recombination. The observation that two histone gene-containing DNA segments with core and *H1* histone genes are both present on chromosomes 1, 6, and 12, may offer clues to the origin of this arrangement of histone genes and to other genes in their proximity. Note that additional human histone genes may reside on these and other chromosomes. Further assignment of other copies of the 100–200 human histone genes to chromosomes should clarify the relationship between their organization, representation, and expression. Such information will also indicate the extent to which our present results reflect the general organization of human histone genes. In this regard, it has recently been shown that murine histone genes, which are similarly represented as a moderately repeated family of genes, are also distributed among at least 3 chromosomes (D. Cox and W. Marzluff, personal communication).

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