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HUMAN HISTONE GENES ARE INTERSPERSED WITH MEMBERS OF THE ALU FAMILY AND WITH OTHER TRANSCRIBED SEQUENCES

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SUMMARY. We have isolated a series of recombinant λCh4A phages containing human histone genes. Histone H2A, H2B, H3 and H4 genes have been found to be clustered, but are not present in any simple repeat pattern. Hybridization of a blot containing phage DNA with S phase polysomal cDNA indicates the presence of additional sequences complementary to HeLa polysomal RNA sequences. Northern blot analysis using these clones as probes has also shown the presence of sequences complementary to non-histone-coding RNAs, some of which accumulate differentially in different stages of the cell cycle. We have also found, by hybridization with appropriate probes, that histone genes are interspersed with several copies of the Alu DNA family; however, not all of the histone genes are associated with an Alu DNA sequence.

We have isolated a series of recombinant λCh4A phages containing genomic human histone genes (1). Histone coding regions were located by hybridization to heterologous histone DNA probes, northern blot analysis and hybrid selection-*in vitro* translation. From these studies we concluded that in humans, histone genes are clustered, but no tandem repeat comparable to those of sea urchin and *Drosophila* was readily apparent. During the course of these studies, it became evident that our clones contained transcribed sequences other than histone genes, detectable by northern blot analysis, as well as by hybridization to cDNA prepared to 7-11S polysomal RNA from HeLa cells. In a previous report (2), we have shown that one of our clones, λHHG 41, contains in addition to histone H3 and H4 coding regions, a sequence that hybridizes strongly to an RNA species of about 330 nucleotides, present predominantly during the G1 phase of the HeLa cell cycle.

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Tashima *et al* have reported that over 95% of the recombinants present in a human genomic library cloned into λCh4A hybridize to a probe containing Alu DNA sequences (3). In most eukaryotes, repeated sequences approximately 300 nucleotides long have been found interspersed with single copy sequences throughout the genome (4). In humans, the Alu DNA family is predominant among these reiterated sequences, its repetition number being 300,000 per haploid genome (5). Although Alu DNA sequences have been found to be interspersed with single copy sequences, as well as dispersed multigene families (6), no data are available concerning their presence among families of clustered, repeated sequences, such as the histone genes. In this communication, we present evidence suggesting that human histone genes also are interspersed with members of the Alu DNA family.

MATERIALS AND METHODS

Microorganisms and DNA Isolation: Histone clones were obtained by screening a human genomic DNA library containing fetal liver DNA cloned into the Eco RI sites of the vector λCh4A (7). The library was generously provided by Dr. T. Maniatis. Bacteriophage were grown in *E. coli* strain DP50*supF* (obtained from Dr. F. Blattner) in NZCYM•DT broth as suggested by Blattner *et al* (8). Phage DNA was isolated by a modification of the method described by Blattner (8).

The Alu DNA containing clone pCDF2 (9) was a gift from Dr. S. Weissman and was grown in L broth in the presence of 50 µg/ml of ampicillin. Plasmid DNA was isolated by the cleared lysate procedure (10), followed by CsCl-ethidium bromide density gradient centrifugation.

DNAs were digested with restriction endonucleases, electrophoresed on 0.8% or 1.2 % (w/v) agarose gels, and transferred to nitrocellulose essentially as described by Southern (11). Nick-translated probes were prepared as described by Maniatis *et al* (12). All experiments involving viable bacteriophage and bacteria containing recombinant DNA were performed under conditions specified by the NIH Guidelines for Research Involving Recombinant DNA Molecules.

Hybrid Selection-*in vitro* Translation: 30-120 µg of each λHHG phage DNA were immobilized on nitrocellulose filters and hybridized at 43° for 6 hours in 50% formamide, 20 mM Hepes pH 6.8, 0.5 M NaCl, 10 mM EDTA and 0.5% SDS. Filters were washed with buffers of decreasing ionic strength in the absence of formamide, followed by several washes in 10 mM Hepes, pH 6.8 to remove SDS. Hybridized RNAs were eluted in 90% formamide, 20 mM Hepes, pH 6.8, 0.5 M NaCl and 10 mM EDTA. RNAs were precipitated by ethanol and translated as described (13). *In vitro* translated proteins were analyzed on a slab acid-urea gel to display histone polypeptides (14).

Preparation of cDNA: 7-11S RNA from S phase HeLa S3 cells was polyadenylated using ATP-polynucleotidyl exotransferase from maize (15) in a reaction containing 70 mM Tris•HCl pH 8.8, 1 mM ATP, 10 mM dithiothreitol, 1 mM $MnCl_2$. Polyadenylated RNA was reverse transcribed by AMV reverse transcriptase (kindly provided by Dr. J. Beard) in the presence of [α-^{32}P]dCTP in a reaction containing 40 µg/ml RNA, 50 mM Tris•HCl pH 8.3, 20 mM β-mercaptoethanol, 10 mM $MgCl_2$, 30 mM NaCl, 20 µg/ml oligo (dT), 50 µg/ml actinomycin D, 1 mM each of *dATP, dGTP* and dTTP, 30 mM dCTP, and 200 units/ml reverse transcriptase.

Hybridization of Southern blots to [^{32}P]cDNA was carried out as described by Lawn *et al* (7). Hybridization of Southern blots to nick-translated plasmid DNA was done as described by Southern (11).

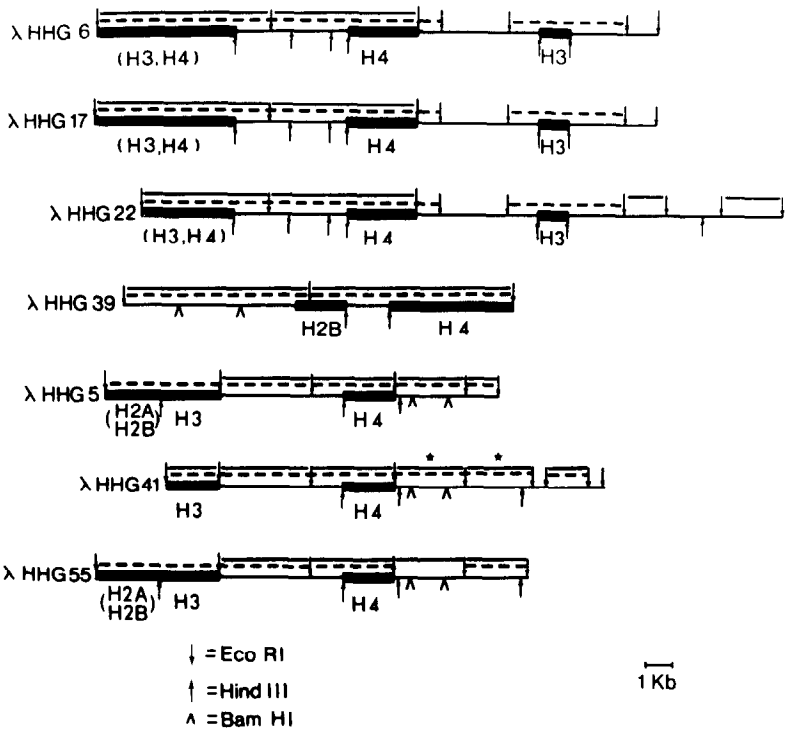

Figure 1. Maps of λHHG recombinant phage. Eco RI, Hind III and Bam HI restriction sites were determined by single and double digestions; histone coding regions were assigned as described in the text. Clone λHHG 22 is shown in an orientation (with respect to λ) opposite to that of the other λHHG clones. Boxes indicate restriction fragments to which histone coding sequences have been assigned; full lines above each clone indicate restriction fragments that hybridize to an Alu DNA probe; broken lines above each clone indicate restriction fragments that hybridize to cDNA to HeLa 7-IIS S phase polysomal RNA. The two fragments denoted with an asterisk (*) migrate as a doublet, and therefore the hybridization results with respect to these two fragments are inconclusive.

RESULTS AND DISCUSSION

Isolation of Clones Containing Human Histone Genes: A human genomic library cloned into λCh4A was screened for histone genes by using as a probe the insert from p2.6, a plasmid containing chicken genomic histone H3 and H4 coding sequences. Fifteen clones (designated λHHG) were isolated and seven of these were further characterized by restriction mapping, using restriction endonucleases Eco RI, Hind III and Bam HI (Figure 1). Histone coding regions within these clones were initially located by hybridization of Southern blots of restricted λHHG DNAs with several specific heterologous histone probes. H3 and H4 coding regions were determined by hybridization with defined fragments obtained from p2.6 (not shown). H2B was determined by hybridization with yet

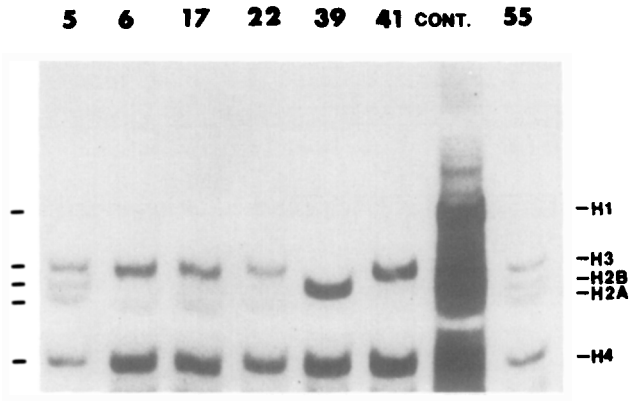

Figure 2. Hybrid selection-*in vitro* translation. RNAs were selected by using filter-bound λHHG DNAs and was analyzed as described in Materials and Methods.

another chicken genomic clone, p4.8, as well as by hybridization with an H2B fragment derived from cloned sea urchin (*S. purpuratus*) histone genes (not shown). The presence of H2A coding regions in the λHHG clones was determined by hybrid selection and *in vitro* translation of HeLa polysomal RNA. Figure 2 shows the polypeptides translated *in vitro* using RNA selected by hybridization to filter-bound λHHG DNAs. These experiments showed H2A genes to be present in clones λHHG 5 and λHHG 55, and also allowed us to confirm all the histone gene assignments obtained by hybridization to heterologous probes. Assignment of H2A coding regions to specific restriction fragments was based on hybrid selection-*in vitro* translation data obtained with subclones of the λHHG inserts.

Taken together, studies on the structure and organization of the human histone genes have shown these genes to be clustered, but no simple repeat pattern has been found. Only the core histone genes have been detected so far in clusters while H1 has not been localized in any of our clones, either by hybrid selection-*in vitro* translation or by Northern blot analysis, techniques which in our hands have been successfully used to detect the presence of an H1 histone gene in a chicken genomic clone (unpublished results). Six of the clones under study fall into either one of two arrangements with respect to coding regions, as well as restriction endonuclease sites; the seventh clone, λHHG 39, is apparently unique. Preliminary data obtained from clones λHHG 5, 41 and 55 have shown some degree of microheterogeneity with respect to other

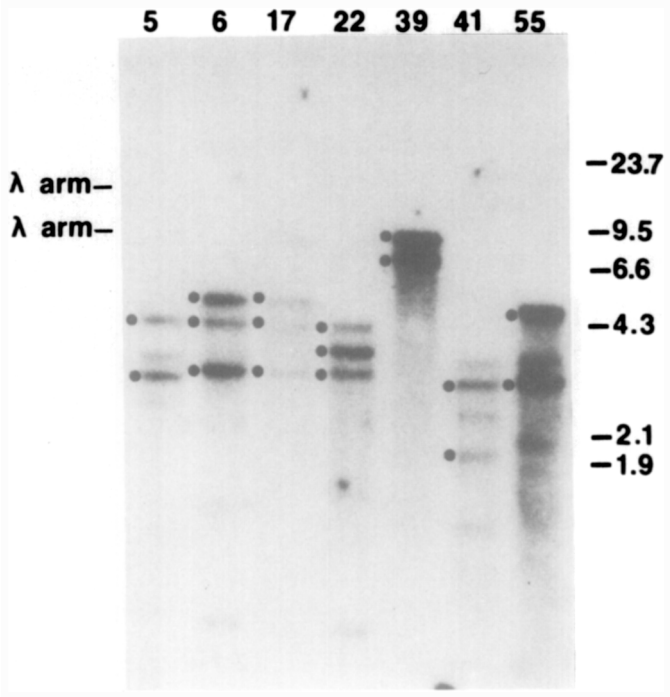

Figure 3. Hybridization of λHHG clones to HeLa cDNA. [^{32}P]cDNA to 7-11S polysomal RNA from S phase HeLa cells was prepared as described in Materials and Methods and hybridized for 20 hrs to a Southern blot containing Eco RI-digested λHHG DNAs. Numbers on the right indicate the size (in Kbp) of λ Hind III markers electrophoresed in the same gel. Black dots at the left of hybridizing bands indicate Eco RI fragments to which histone genes have been assigned by other methods (see text).

restriction endonucleases. These results suggest that seemingly overlapping clones may have arisen from different areas of the genome.

Human Histone Genes Are Interspersed with Other Sequences: When Eco RI-digested λHHG DNAs were hybridized to a cDNA made to *in vitro* polyadenylated HeLa cell 7-11S polysomal RNA (Figure 3), Eco RI fragments known to contain histone genes gave the strongest hybridization signals; weaker, although specific, hybridization signals were obtained with Eco RI fragments that do not contain any histone coding sequences. These weaker signals could be due to hybridization with sequences that are under-represented in the cDNA preparation, or alternatively, bear only partial homology with the genomic sequences present in the recombinant phages. Hybridizing fragments are indicated by parallel broken lines in the maps shown in Figure 1.

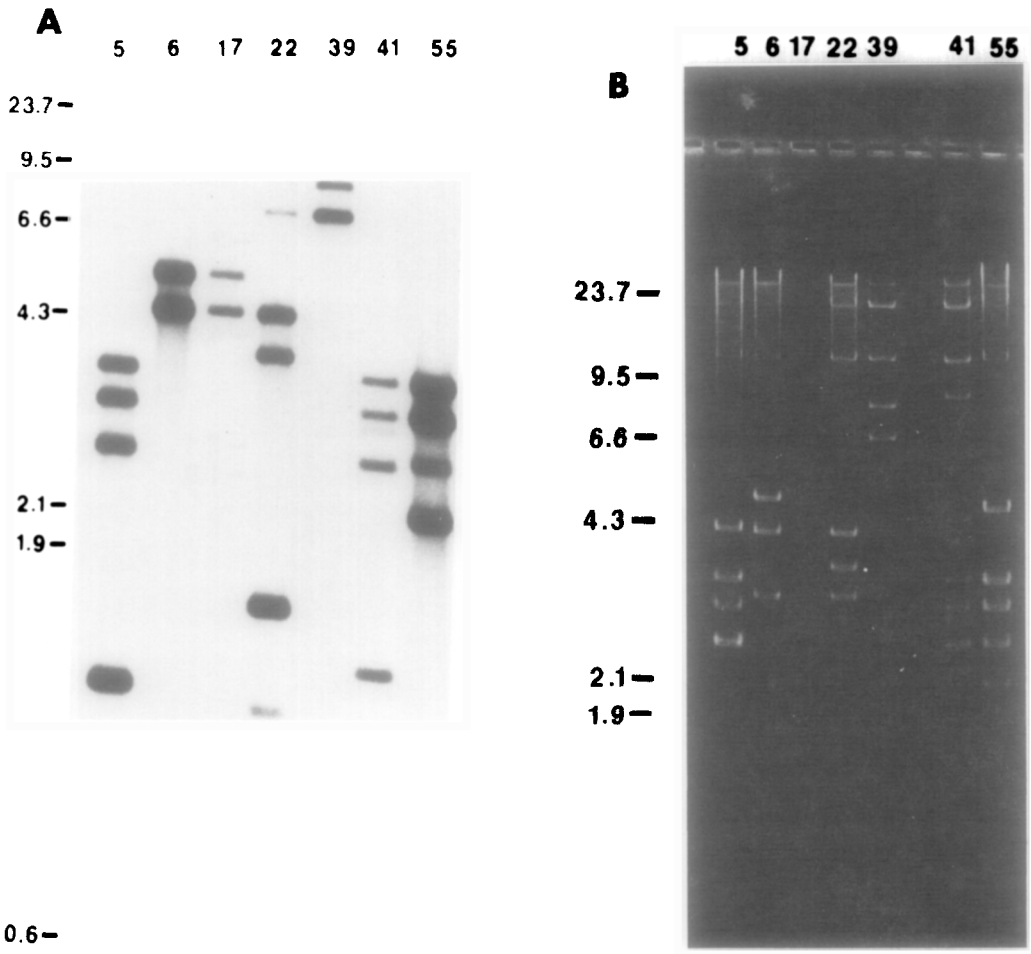

Figure 4. Hybridization of λHHG clones to Alu DNA sequences. A. Hybridization of a Southern blot containing Eco RI-digested λHHG DNA with [^{32}P]-labeled, nick-translated pCDF2, a plasmid containing an Alu DNA sequence. B. The ethidium bromide staining pattern of a gel similar to that shown in A. Numbers at the left of each panel indicate the size (in Kbp) of λHind III markers electrophoresed in the same gel.

In view of this observation and the fact that most, but not all, of the recombinant phages present in the genomic library from which the λHHG clones were selected have been found to contain members of the Alu family of DNA sequences (3), it was of interest to look directly at the representation of Alu DNA sequences in our histone gene containing clones. The probe used in these experiments was pCDF2, a recombinant plasmid containing a 482 bp sequence inserted into pBR322 (9). Most of this insert (300 bp) corresponds to a member of the Alu family, with some flanking regions corresponding to a single copy sequence found in the human β-globin gene cluster. Figure 4 shows the hybrid-

ization pattern obtained when a Southern blot containing Eco RI-digested λHHG DNAs was hybridized to nick-translated pCDF2. Hybridizing fragments are indicated by parallel solid lines in the maps shown in Figure 1. It is interesting to note that clones λHHG 5 and λHHG 55 have an Eco RI fragment in which three histone mRNA coding regions have been detected, but no Alu sequences have been identified by hybridization to pCDF2. These results suggest that at least one of these histone genes is not associated with Alu DNA sequences.

Comparison of the Eco RI fragments that hybridize to the Alu DNA sequences with those that hybridize to the 7-11S HeLa polysomal cDNA show that both sets are not completely superimposable. It is possible that both results are independent, and some of the cDNA hybridization may be due to sequences other than Alu or histone, which are transcribed and present in the polysomes of HeLa S3 cells. This possibility is supported by the observation that, although the Alu sequences are present in HnRNA and in the cytoplasm of human K562 cells, little or none of these sequneces have been found associated with polysomes (16). On the other hand, Calabretta *et al* (17) have reported that in CCRF-CEM, another human cell line, Alu sequences are represented in poly A^+ polysomal RNA.

We have already reported the presence in clone λHHG 41 of a sequence (or sequences) that hybridizes by northern blot analysis to an RNA of approximately 330 nucleotides that is present in the cytoplasm of HeLa cells preferentially during the G1 phase of the cell cycle (2). This sequence appears to be unrelated to the repeated Alu family of DNA sequences, since other clones containing Alu DNA sequences fail to hybridize to this RNA under the conditions used in our experiments. Additionally, northern blots have indicated that clone λHHG 39 may also contain a non-histone-coding sequence. In this case, however, the RNA appears to be present in the cytoplasm of HeLa cells throughout the cell cycle.

Taken together, our results indicate that the moderately reiterated human histone genes are interspersed with members of the Alu DNA family and with other transcribed sequences.

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