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Chromosomal Localization of the Human Placental Lactogen-Growth Hormone Gene Cluster to 17q22-24

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SUMMARY

Recombinant plasmid HCS-pBR322 containing a 550-base-pair (bp) insert of cDNA to human placental lactogen (hPL) mRNA was ^{3}H-labeled by nick translation and hybridized in situ to human chromosome preparations in the presence of 10% dextran sulfate. A high percentage of cells (80%) were found to exhibit label on the distal end of the long arm of chromosome 17. Silver grains on this region constituted 25.5% of all labeled sites, allowing assignment of the hPL and growth hormone (hGH) genes, which have over 90% nucleotide homology in their coding sequences, to 17q22-24. A gene copy number experiment showed that both genes are present in ~ three copies per haploid genome.

INTRODUCTION

Localization of genes on human mitotic chromosomes provides a physical basis for the human genetic map, which has important implications in clinical medicine as well as in characterizing the organization and regulation of the human genome. Thus far, of the 50,000 or so unique structural genes estimated to be present in the human genome [1, 2], over 100 gene loci have been assigned to the X chromosome and over 240 have been assigned to specific autosomes [3]. About 60% of the autosomal assignments have been made by analysis of somatic cell hybrids, either

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by determining concordance between the presence of a specific chromosome and the expressed phenotype in the hybrid cells [4] or, more recently, by probing DNA isolated from hybrid clones with specific DNA or RNA sequences [5, 6]. Although this method allows regional localization by use of somatic cell hybrids containing various partial chromosome deletions [7], this approach is tedious and lengthy. In addition, refined mapping using this method requires, for practical purposes, that a chromosomal assignment be already known.

In situ hybridization provides a method for the direct visualization of genes on chromosomes. Although the method has proved useful in the past for localizing repeated genes in eukaryotic genomes, such as the repetitive sequences that are transcribed into poly A+ RNA [8] and the 40-fold repetitive histone genes [9, 10], attempts to locate specific single-copy genes on mitotic chromosomes by in situ hybridization have until recently been unsuccessful. This failure was due to a lack of adequate signal obtained when probing with short segments of DNA. Recently, however, it has been found that the sensitivity of detection can be increased manyfold by hybridizing a nick-translated cloned DNA in the presence of 10% dextran sulfate [11].

The human growth hormone (hGH), placental lactogen (hPL, also called chorionic somatomammotropin), and growth hormone-like genes have recently been assigned to chromosome 17. This was accomplished by probing a panel of somatic cell hybrids with cloned cDNA to hGH mRNA [12]. In our study, in situ hybridization of a cloned cDNA specific for hPL mRNA to human chromosome preparations resulted in substantial labeling of bands q22-24 of chromosome 17. Since the hPL and hGH genes exhibit over 90% homology in their coding sequences [13] and are separated by only 11 kilobase (kb) of DNA (V. J. Kidd and G. F. Saunders, personal communication, 1981), we conclude that the hPL-hGH gene cluster is located on chromosome segment 17q22-24.

MATERIALS AND METHODS

Preparation of Plasmid DNA

Recombinant plasmid HCS-pBR322, containing a 550-bp hPL cDNA sequence [14] inserted in the Hind III site of pBR322 using synthetic Hind III linker molecules, was kindly provided by Dr. P. Seeburg. Plasmid DNA was isolated from transformed cells as described by Katz et al. [15]. An approved EKI host-vector system was used with P2 physical containment in accordance with the National Institutes of Health Guidelines for Recombinant DNA Research.

In Situ Hybridization

Radiolabeling of probe. HCS-pBR322 was labeled by nick translation using [^{3}H]dCTP (60.4 Ci/mmol), [^{3}H]dATP (41.2 Ci/mmol), and [^{3}H]dTTP (78.1 Ci/mmol) (New England Nuclear, Boston, Mass.) as described by Lai et al. [16]. The DNA (specific activity 2×10^7 cpm/μg) was separated from free ^{3}H-labeled nucleotides by centrifugation through 1 ml of hydrated Sephadex G-100 at 1800 *g*.

Hybridization. In situ hybridization was carried out essentially as described by Harper and Saunders [11]. Human mitotic chromosome preparations, prepared from amethopterin-synchronized peripheral blood lymphocyte cultures [17], were ribonuclease-treated and denatured. ^{3}H-labeled HCS-pBR322 DNA at a concentration of 0.05–0.5 μg/ml in 50% formamide-2XSSC-10% dextran sulfate (Pharmacia, Uppsala, Sweden), pH 7.0, and with 500-fold excess sonicated salmon sperm DNA as carrier, was hybridized for 8–16 hrs at 37°C. Slides were rinsed well in 50% formamide-2XSSC at 39°C and exposed to Kodak NTB2 nuclear track emulsion (Eastman, Rochester, N.Y.) for 5–22 days at −80°C, followed by development with Kodak Dektol and G-banding of chromosomes.

Determination of Gene Copy Number

Preparation of genomic DNAs. Nuclei from human term placenta were isolated as described [18]. Nuclei were also isolated from chicken erythrocytes kindly provided by Dr. M. T. Kuo [19]. High molecular weight nuclear DNA was isolated from both preparations by proteinase K and sodium dodecyl sulfate treatment, followed by phenol and Sevag extraction. After the addition of 2 vol of ethanol, the DNA was collected on a glass rod, washed with ethanol, and redissolved in 10 mM Tris, 1 mM EDTA.

Preparation of probe. HCS-pBR322 DNA was digested with Hind III and subjected to electrophoresis on a horizontal 0.3% agarose gel. The band corresponding to the 550-bp insert was excised, frozen, and thawed. After the sample was spun at 10,000 rpm for 10 min, the supernatant was collected and the DNA extracted with an equal volume of SSC-saturated phenol [20]. The DNA was precipitated with ethanol and then dissolved in 10 mM Tris, 1 mM EDTA. The insert (hPL cDNA) was ^{32}P-labeled by nick translation to specific activity up to 10^{8} cpm/μg [16] and separated from free [^{32}P]dCTP by Sephadex G-50 column chromatography.

Southern blotting and hybridization. HCS-pBR322 DNA was linearized by digestion with restriction endonuclease Pst I. Two lanes of Eco R1-digested human placental DNA (20 μg each) were separated by electrophoresis on a 1% agarose gel along with various amounts of linearized HCS-pBR322 DNA corresponding to one, two, four, or six copies of insert per haploid genome in 20 μg of total human DNA. These amounts were calculated as follows. Assuming a human haploid genome size of 3×10^{9} bp, the 550-bp hPL cDNA insert from HCS-pBR322 constitutes 1.8×10^{-7} of the human haploid genome. Therefore, the amount of insert in 20 μg of total human DNA, if present in one copy per haploid genome, is 3.7×10^{-6} μg. However, since use is made of the total recombinant plasmid HCS-pBR322, of which only 12% is comprised of the lactogen cDNA insert, 3.1×10^{-5} μg of plasmid DNA was loaded as the equivalent of one copy of insert per haploid genome in 20 μg of total human DNA. Lanes corresponding to two, four, or six copies of insert contained the appropriate multiples of this amount. Plasmid lanes also contained 20 μg of Eco R1-digested chicken DNA as carrier, unless otherwise noted. Following electrophoresis, the DNA was transferred to nitrocellulose paper and hybridized [11] with the ^{32}P-labeled hPL cDNA probe described above. The filter was autoradiographed with X-ray film in the presence of an intensifying screen at −70°C for 3 days. The intensity of hybridization in the total human DNA lanes was compared with that in the plasmid lanes by densitometric scanning of the autoradiogram.

RESULTS

In situ hybridization of ^{3}H-labeled HCS-pBR322 DNA to human chromosome preparations was carried out in the presence of 10% dextran sulfate. Metaphases hybridized with a probe concentration of 0.5 μg/ml for 12 hrs and exposed to autoradiographic emulsion for 22 days exhibited an average of 4.9 labeled chromosomal sites per cell. Because of the relatively long exposure, many of these labeled sites were composed of several silver grains. The distribution of labeled sites in 20 metaphase cells from this hybridization is shown in figure 1. Sixteen, or 80%, of the cells exhibited label on the distal portion (bands q21-25) of the long arm of one

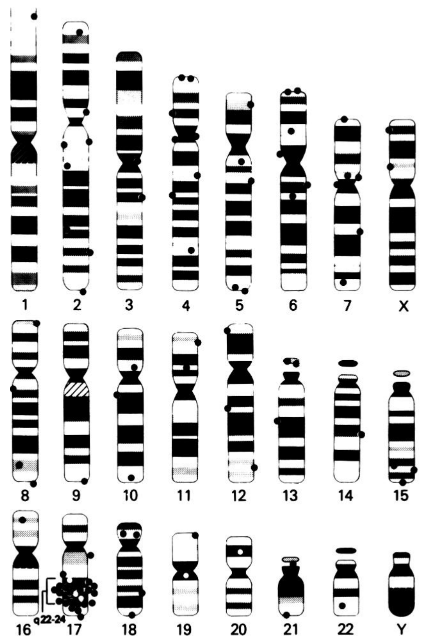

FIG. 1.—Schematic representation of human G-banded mid-metaphase chromosomes, illustrating positions of labeled sites in 20 cells hybridized with [^{3}H]HCS-pBR322 at a concentration of 0.5 μg/ml for 12 hrs and exposed for 22 days. Of these cells, 16 (80%) exhibit label on region q2 of one or both chromosomes 17. These sites represent 25.5% (25/98) of the total labeled sites throughout the chromosome complement. Chromosome idiogram from Yunis et al. [27].

or both chromosomes 17. Of 98 total chromosomal sites labeled in the 20 cells, 25, or 25.5%, were located in this region of the long arm of chromosome 17. Furthermore, it can be seen that a large percentage (> 70%) of labeled sites on chromosome 17 were within bands q22-24.

Cells hybridized with [^{3}H]HCS-pBR322 at 0.1 μg/ml for 12 hrs and exposed for 9 days exhibited less chromosomal label, composed almost exclusively of single grains. Of 146 metaphases analyzed, which exhibited an average of 2.1 chromosomal grains, 20.5% exhibited label on region 2 of one or both chromosomes 17. Again, over 70% of grains on chromosome 17, which represented 23% of the total labeled chromosomal sites, were located within bands q22-24. A representative metaphase cell that illustrates the typical pattern of labeling over the distal end of the long arm of one chromosome 17 is shown in figure 2. The remainder of the chromosomes show few grains, and background is low.

To quantitate the number of gene copies for hPL as well as for hGH in the human genome, a gene copy number experiment was carried out. Figure 3 shows the results of hybridizing [^{32}P]HCS-pBR322 insert to a Southern blot of Eco R1-digested human DNA (lanes H). The 2.8- and 2.6-kb bands contain large portions of the hPL and hGH genes, respectively [13]. The hybridization intensities of these bands, when compared with those of the various concentrations of

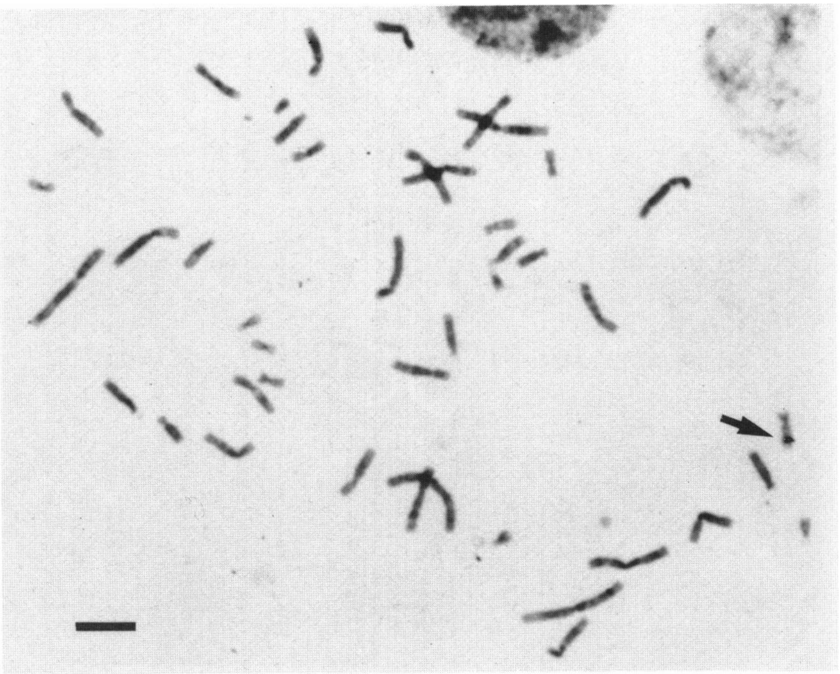

FIG. 2.—Representative chromosome spread hybridized with [^{3}H]HCS-pBR322 at a concentration of 0.1 μg/ml for 12 hrs and exposed for 9 days, showing label on the distal end (bands q22-24) of the long arm of chromosome 17 (*arrow*). Bar: 5 μm.

linearized HCS-pBR322, indicate that the genes for hPL and hGH are present in ~ three copies per haploid genome.

DISCUSSION

The hPL and hGH genes are two members of the prolactin multigene family that encode growth-promoting and lactogenic hormones. Although they vary in potency in their overlapping biological actions and are expressed in a tissue-specific manner (i.e., lactogen in the placenta and growth hormone in the pituitary), the two hormones are closely related. Both are composed of 191 amino acid residues that show 85% homology [21], and their mRNAs are more than 90% homologous [13]. These two genes, along with that for the pituitary hormone prolactin, which has much lower nucleotide sequence homology with hPL and hGH [22], are believed to have originated from a common ancestral gene by duplication [21].

Recently, analysis of three overlapping genomic fragments cloned in λCh4A [23] that hybridize to the HCS-pBR322 recombinant plasmid showed that the hPL and hGH genes are linked in genomic DNA and separated by only 11 kb (V. J. Kidd and G. F. Saunders, personal communication, 1981). Both genes were identified since HCS-pBR322 hybridizes with about equal intensity to hPL- and hGH-

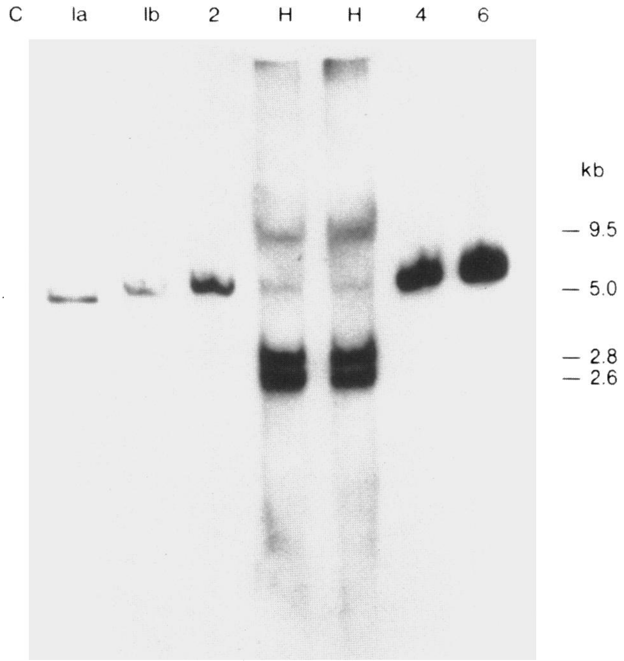

FIG. 3.—Analysis of number of genomic sequences complementary to cloned 550-bp hPL cDNA. *Lanes H* and *C* were loaded with 20 μg Eco R1-digested total human and chicken DNA, respectively. The *other five lanes* were loaded with linearized HCS-pBR322 DNA in amounts corresponding to one, two, three, four, or six copies of insert per haploid genome in 20 μg of total human DNA. *These lanes* also contained 20 μg of Eco R1-digested chicken DNA, except for *1a*, which contained no chicken DNA carrier. Electrophoresis, Southern transfer, hybridization with ^{32}P-labeled hPL cDNA insert, and autoradiography were carried out as described in MATERIALS AND METHODS. Intensities of the autoradiographic bands indicate that ~ three copies of the hPL and hGH genes per haploid genome were present. The less intense 9.5- and 5.0-kb bands are believed to represent Eco R1 fragments containing hPL and hGH gene variants (V. J. Kidd and G. F. Saunders, personal communication, 1981).

specific sequences, which was confirmed by the Southern blot hybridization in figure 3. Therefore, in situ hybridization to human chromosomes using this probe was expected to detect both the hPL and hGH genes. As shown in figure 1, a significant number of grains were observed on the long arm of chromosome 17, particularly throughout bands q22-24. The observation of only one site of heavy labeling can be explained by the fact that, based on percentage of the total chromosome complement length, chromosome segment 17q22-24 contains several thousand kb of DNA.

It is also likely that several variants of the hPL and hGH genes are present in this chromosomal region and hybridize to the probe. Analysis of the number of genomic sequences complementary to the cloned 550-bp hPL cDNA inserted in HCS-pBR322 indicates that the hPL and hGH genes are present in ~ three copies per haploid genome (fig. 3). Similar results regarding copy number of hPL genes have been obtained in independent experiments using solution hybridization of labeled hPL cDNA to total placental DNA [24]. In agreement with these experiments, three types of hPL gene sequences, all contained within 2.8-kb Eco R1 restriction fragments, and three types of hGH genes, all contained within 2.6-kb fragments, have been described [25].

Prolactin, the third member of this multigene family, has recently been assigned to chromosome 6 by DNA analysis of somatic cell hybrids [26]. Experiments to regionally localize the human prolactin gene by in situ hybridization are currently in progress. These studies have important implications in understanding the structure, evolution, and regulation of the prolactin multigene family.

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