Linkage Arrangement of Human Placental Lactogen and Growth Hormone Genes*

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Human placental lactogen (hPL) and growth hormone (hGH) are two hormones thought to have evolved from a common ancestral gene (along with prolactin), yet they have quite different functions and specificities. The nucleic acid sequences of the respective cDNAs of the two genes share considerable homology, as well as the existence of multiple forms of each gene within the genome. In this study we report on the linkage arrangement of several genes from this group. Two hPL-like genes as well as an hGH gene are shown to be linked within a 38-kilobase pair region of DNA. Linkage between a variant hGH gene and an hPL gene is also shown. The orientation and structural organization of these genes was previously established using 5'- and 3'-specific probes from a placental lactogen cDNA clone and detailed restriction endonuclease mapping. Restriction fragments from the overlapping clones were verified by comparison to digests of high molecular weight genomic DNA. In addition, the location of a specific class of repetitive DNA sequences, the Alu family, was mapped on these clones using the recombinant clone BLUR 8. All members of this multigene family have Alu repeat sequences either immediately flanking their 3' or 5' untranslated regions or within their intervening sequences.

Human placental lactogen and growth hormone are polypeptide hormones closely related in structure but different in both function and expression (1, 2). Placental lactogen is expressed in increasing amounts during pregnancy, accounting for almost 5% of the total poly(A)+ RNA in the placenta during the third trimester of pregnancy (3, 4). This hormone's function is not completely understood, but it is believed to be involved in supplying maternal nutrients to the fetus (5). Growth hormone is expressed in the anterior lobe of the pituitary and is involved in the regulation of growth and metabolism during development (6). Both of these hormones belong to a polypeptide hormone family that also includes the pituitary hormone prolactin and comprises the so-called "PRL" set of genes (7). This set of genes is believed to have evolved from a common ancestral gene by gene duplication (1, 2, 7) and recently, it has been demonstrated that the hPL and hGH genes are located on human chromosome 17 at band q22-24 (8, 9). Recombinant DNA techniques facilitate the isolation of large genomic DNA fragments containing genes, their flanking regions, and regulatory elements that may be evolved in their differential and tissue-specific expression.

We have previously isolated and characterized seven recombinant clones containing four hPL- and three hGH-like genes. In this article, we report on the close physical linkage of the hGH- and hPL-like genes within a single recombinant phage and between a series of overlapping phage clones. Detailed analysis of two closely linked hPL-like genes is presented, including the presence of an Alu family sequence within the first intron of an hPL-like gene. The nature of the Alu family sequences present in these recombinant clones has been examined by differential hybridization experiments. In addition, a conserved region of DNA found 2–3 kb from the 3' end of several members of this multigene family was identified.

**EXPERIMENTAL PROCEDURES**

A human chromosomal library (11) created by partial digestion with restriction endonucleases Hae III and Alu I and inserted into the Charon 4A vector using synthetic Eco RI linkers was screened using the hPL cDNA probe HCS-pBR322 (3) according to the method of Woo (12). Escherichia coli strain LE 392 was used in propagation of the plasmids. All procedures were carried out in compliance with National Institutes of Health guidelines.

**Recombinant Probes and Nick Translation**—The recombinant clones HCS-pBR322, BLUR 2, and BLUR 8 (13) were used in these experiments. HCS-pBR322 contains a 560-bp insert complementary to lactogen mRNA and has previously been described (3). 5'- and 3'-specific probes were prepared by digestion of HCS-pBR322 with HindIII and Xba I. This allowed isolation of 150-bp 5' coding region and 400-bp 3' coding region fragments from a 6% polyacrylamide gel following elution by diffusion overnight at 37 °C (3). Labeling of DNA by nick translation was carried out according to the procedure of Lai et al. (14) using Amersham radiolabels (specific activity of 2000–3000 Ci/mmol).

**Phage Growth and Isolation**—After plaque purification, recombinant phage were grown according to the procedure of Lai et al. (15). Recombinant DNA Analysis of Clones—The DNA was digested with various restriction endonucleases (BRL, according to manufacturer's specifications), applied to 1 or 1.5% agarose horizontal gels in regular strength TAE buffer (40 mM Tris-acetate, pH 8.0, and 2 mM EDTA), and subjected to electrophoresis for 12 h at 40 V. The gel was stained with ethidium bromide and transferred to nitrocellulose paper according to the Southern method (16) or by bidirectional transfer (17). Before hybridization the filter was treated with 2-fold strength Denhardt's solution (6 × SSC, 0.04% Ficoll, 0.04% polyvinylpyrrolidone, 0.04% bovine serum albumin) for 24 h at 68 °C. Filters were then hybridized at 68 °C overnight in 2-fold strength Denhardt's solution plus 0.5% sodium dodecyl sulfate and 1 mM EDTA. Genomic DNA digests were hybridized in the presence of the same solution plus 10% dextran sulfate. The filters were then washed two times each for 2 h at 68 °C in SSC (0.15 × NaCl and 0.015 ×

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1 The abbreviations used are: hPL, human placental lactogen; hGH, human growth hormones; IVS, intervening sequence; kb, kilobase pairs; bp, base pairs.

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sodium citrate) plus 0.5% SDS. Labeled DNA bands were detected by exposure to x-ray film overnight in the presence of an intensifying screen (DuPont Instruments) at -80 °C.

**Plasmid Subclones and Transformation—**Phage DNA was restricted with Eco RI, and separate bands were isolated by 1% agarose gel electrophoresis and electrophoretic elution of the particular fragment (18). The Eco RI fragments of interest were then cloned into the Eco RI site of pBR322, which had been treated with bacterial alkaline phosphatase (Worthington), using T4 DNA ligase (BRL). E. coli strain RR1 was transformed with the ligated DNA samples. Ampicillin-tetracycline-resistant colonies were selected and analyzed by the minilysate procedure (19). Specific plasmid subclones were then chosen and grown.

**Human Genomic Blot—**The Southern blot of genomic DNA was done using 20 µg of high molecular weight human placental DNA in each restriction digest. Digestion conditions were the same as the phage digests, except that 2 units of enzyme/µg of DNA and 2-h incubations were used. The digested DNAs were run on an 0.8% agarose (TAE) gel at 40 V overnight. The gel was transferred according to the method of Southern (16), and the resulting filter was treated as described above. The filter was hybridized with 2 x 10^6 cpm of HCS-pBR322 (10^5 cpm/µg of DNA) in the presence of 10% dextran sulfate (w/v). The filter was then washed sequentially in CHCl3, 1 x SSC, and 0.5% sodium dodecyl sulfate at 68 °C.

**End Labeling of DNA and DNA Sequence Analysis—**Plasmid recombinant DNA, 40-80 µg, was digested with an appropriate restriction endonuclease at 1 unit/µg of DNA for 2 h at 37 °C in a 200-µl reaction volume. The DNA was precipitated with 2.5 volumes of 95% EtOH in the presence of 0.3 M NaOAC, and the pellet was vacuum dried. The DNA fragments were treated with bacterial alkaline phosphatase and the 5' termini labeled with [γ-32P]ATP (Amersham, 2000-3000 Ci/mmol), ATP, and T4 polynucleotide kinase (BRL) in a reaction volume of 30 µl (20). Briefly, 300-400 µCi of [γ-32P]ATP were taken to dryness in a 1.5-ml Eppendorf tube. DNA was resuspended in 25 µl of 1 x kinase buffer (21) (stored as a 2 x kinase buffer stock at -20 °C) and placed in a 0.5-ml Eppendorf tube containing the [γ-32P]ATP, vortexed, 5 µl of T4 polynucleotide kinase was added, and the mixture was incubated at 37 °C for 1 h. Terminally labeled DNA was then purified by polyacrylamide gel electrophoresis as described above.

Labeled DNA fragments were cleaved asymmetrically with a restriction endonuclease and isolated by preparative polyacrylamide gel electrophoresis. Fragments were eluted by placing the crushed gel slices in 0.1 x SSC at 37 °C overnight and then subjecting it to chemical degradation according to the procedure of Maxam and Gilbert (21). Electrophoresis of degraded DNA fragments on thin polyacrylamide gels (25 and 10% or 20%) was carried out as described by Saenger and Coulson (22).

**RESULTS AND DISCUSSION**

**Screening of Genomic Library and Identification of Positive Clones—**A total of 900,000 recombinant phages were screened using 32P-labeled hPL cDNA as a probe. Previous evidence indicated 95% sequence homology between the hPL and hGH cDNA (23), enabling detection of both genes with this probe. Seven clones were identified containing hPL, hGH, or hPL- and hGH-like gene sequences complementary to the lactogen probe.2 Three of these clones were found to contain more than one member of the hGH-hPL multigene family. In addition, three of these clones were found to have overlapping DNA sequences and span a 38-kb region.

When the restriction endonuclease Eco RI was used, the four clones displayed different types of hybridization patterns with the lactogen probe (13). XH17 contained two hybridizing bands at 2.9 kb and 8.6 kb, while yH18 contained two hybridizing bands at 1.6 kb and 9.5 kb. Fiddes et al. (23) reported that large portions of the hPL and hGH gene sequences are contained in 2.8-kb and 2.6-kb Eco RI fragments, respectively. The two genes can be further identified by the presence of a Bgl II restriction site but no Xba I site in the 2.6-kb fragment (hGH), whereas the 2.9-kb (hPL) fragment contains an Xba I site (23). By these criteria, clone AH16 contains an hGH-like chromosomal gene and clone AH17 contains two hPL-like chromosomal gene sequences (Fig. 1). Additional restriction mapping and hybridization data for the 2.4-kb fragment contained in AH16 indicate it is similar to both hGH1, the expressed gene, and hGH2, the hGH variant gene (23, 24), but contains a different regional restriction map indicating the presence of a third hGH gene.2 hGH2 differs in nucleotide sequence from the hGH1 gene by about 3%, including a Bam HI restriction site found in IVS D of the gene (Ref. 25, Fig. 2).

The third recombinant phage, yH18, contains a 17.5-kb human genomic DNA insert. Extensive overlapping between XH17 and AH18 is shown by several restriction endonuclease

![Fig. 1. Regional restriction endonuclease maps of the hGH- and hPL-recombinant clones. The regional restriction maps for each recombinant clone are given in this map. Structural gene regions are indicated by the open blocks, and their transcriptional directions are indicated by the arrows.2](http://www.jbc.org/)

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fragments. A 4.2-kb Xba I fragment that hybridizes strongly to the lactogen probe is contained in two of the clones, AH17 and AH18. This 4.2-kb Xba I fragment extends from approximately 200 bp to the right of the Eco RI restriction site that constitutes the 5’ end of the 2.9-kb hPLc fragment to 4 kb to the left of the same Eco RI site and contains a major portion of the hPLc gene. Digests of AH17 and AH18 with restriction endonucleases Eco RI and Xba I result in a common 3.8-kb hybridizing band. This fragment is part of the larger 4.2-kb Xba I fragment mapping from the left of the same Eco RI restriction site (Fig. 1). The majority of both the hPLc and hPLc chromosomal genes are contained in similar 4.2-kb Eco RI fragments. The 9.5-kb Eco RI fragment extends from the 5’ end of the 2.9-kb hPLc gene sequence and contains the entire hPLc chromosomal gene. It extends to the left toward the hGH3 gene sequence. An Eco RI linker site 8.5 kb from the 5’ end of the 2.9-kb hPLc sequence interrupts the 9.5-kb Eco RI fragment in AH17.

AH19 also contained two Eco RI fragments that hybridized to the lactogen cDNA (Fig. 1). When this phage was digested with both Eco RI and Bam HI the 2.9-kb Eco RI fragment was reduced to 2.4 kb, and the 1.9-kb Eco RI fragment split into 0.9-kb and 1.0-kb fragments that hybridized to the probe. This indicated a Bam HI site near the 5’ end of the 2.9 Eco RI fragment and a Bam HI site near the 3’ end of the 1.9-kb Eco RI fragment (Fig. 2). It tentatively identifies the 1.9-kb Eco RI fragment as the hGH3 gene sequence previously described (23, 24).

**Linkage Arrangement of hPL and hGH Genes Within Recombinant Clones**—Many multigene families with common evolutionary histories are found in linked groups. Initial experiments showed physical linkage of different family members within a single clone. The mapping and hybridization data presented previously indicate linkage within several recombinant clones of hGH- and hPL-like genes. AH17 contains two gene sequences, the hPLc and hPLc genes. These two genes are separated by 2.0 kb of DNA and are in opposite orientations with respect to one another (Fig. 1).

The second recombinant clone, AH18, also contains two linked genes of this family. AH18 contains 1.5 kb of the 2.6-kb Eco RI fragment containing the hGH3 gene sequence. This gene does not contain the previously reported 3’ Bam HI restriction site characteristic of the hGH3 gene (22–25), but the regional map near its 3’ end, as well as that shown for AH16, differs from AH20 and AH21, believed to be the “true” hGH gene (Fig. 1; Ref. 26). This 1.5-kb Eco RI fragment containing the sequence termed hGH3 is linked to the 9.5-kb Eco RI fragment containing the hPLc gene. The hGH3 gene is in the same transcriptional orientation as the hPLc gene; therefore, the hPLc gene transcriptional orientation is opposite the hGH3 orientation. The two genes are separated by 12.5 kb of DNA (Fig. 1).

Clone AH19 also contains two linked genes. This clone contains a 1.9-kb Eco RI fragment with both a 3’ Bam HI and a 3’ Bgl II restriction site (Figs. 1 and 2), characteristics of the hGH3 gene (23–25). The second gene is contained in a 2.9-kb Eco RI fragment and has a characteristic Xba I site in the coding region that corresponds to the cDNA sequence of lactogen mRNA. The two genes are in the same transcriptional orientation with respect to one another, as indicated by 5’- and 3’-specific probe hybridization experiments. The two genes are separated by 6.5 kb of DNA (Fig. 3).

**Linkage Arrangement of hPL and hGH Genes from Overlapping Clones and Comparison with Genomic Blot Data**—Linkage between several genes spanning greater than 20–30 kb is usually accomplished by isolating overlapping phage clones; an example is the human β-globin gene family (27). The matching of numerous restriction sites and gene sequences is necessary to establish this type of linkage. Location of other DNA sequences, such as Alu family sequences, may also be used to establish linkage between distant genes. Examination of the various restriction maps of the hPL-hGH genomic clones revealed that three of the recombinant clones overlapped one another. This was subsequently confirmed by comparison with hybridizing fragments present in high molecular weight genomic DNA. The restriction maps of AH17 and AH16 have an 8.5-kb Eco RI fragment in common. This fragment contains six restriction endonuclease sites that are identical in both AH17 and AH18 (Fig. 3). The restriction fragments generated by cleavage at these sites hybridize to the same lactogen-specific probes (10). The positions of specific Alu family member repeats also map to the same location in both recombinant clones. Therefore, it is apparent that AH17 and AH18 overlap in an 8.5-kb region (Figs. 1 and 3).

The 1.6-kb Eco RI fragment in AH18 containing the hGH3 sequence also appears to overlap a similar sequence found at the 3’ end of AH16 (Fig. 3). The two genes have restriction sites common to the hGH3 gene. Establishing overlap between AH16 and AH18 is difficult, since the region they share in common is a gene sequence. A better method is to compare...
overlapping restriction fragments to bands present in total genomic DNA digests.

The linkage of several of the phage clones was indicated by the restriction maps and hybridization patterns obtained in previous experiments. Several of the phage have genomic DNA inserts that, when aligned with one another, overlap with respect to restriction endonuclease sites. Digests of high molecular weight genomic DNA from placenta indicate the linkage found in these recombinant clones exists in the genome and is not generated in the cloning process.

Genomic DNA was restricted with various restriction endonucleases and hybridized with the lactogen cDNA probe (Fig. 4). The gene cluster containing the two hPL and hGH genes was verified by comparison of the regional map restriction sites and their fragments with bands seen in the genomic blot. This cluster of genes has very few HindIII restriction sites. In fact, hGH and hPL are contained within a single 21-kb HindIII fragment. A 21-kb HindIII fragment can also be found on the genomic blot (Fig. 4). Similarly, the 6.8-kb Bam HI fragment containing hPL, the 4.2-kb Xba I fragments containing hPL and hPL, the 8-kb Bgl II fragment containing hPL, the 4.5-kb Bgl II fragment containing hGH, and the 12-kb Xba I fragment containing hGH, are all found in the genomic blot (Figs. 3 and 4). The finding of restriction fragments in genomic DNA that correspond to fragments found in a set of overlapping clones confirms their linkage arrangement.

Analysis of Middle Repetitive DNA with the Alu Family Clones BLUR 2 and BLUR 8—In many eukaryotic genomes, including man, less than 5% of the single copy DNA codes for proteins (28). Human single copy DNA is interspersed with inverted repeat sequences (29, 30) and middle repetitive sequences that average 300 bp in size (30). The predominant middle repetitive sequence family in man contains a common Alu I restriction endonuclease site and comprises at least 6% of the human genome (31, 32). Members of the Alu repeat family have been proposed to have cellular functions on the basis of various characteristics, including their widely dispersed arrangement, the conservation of a 40-bp sequence of the Alu repeat in mammalian evolution, the homology of a 14-bp sequence with a sequence near the replication origin in several DNA viruses, and the observation that the Alu repeat is represented in transcripts generated by both RNA polymerase II and III (33, 34). Similar interspersed repetitive sequences have also been found in mice (35, 36), Chinese hamsters (33), and chickens (37).

The presence of Alu repeats in recombinant clones λH19 and λH20 was demonstrated by hybridization with the Alu family clones BLUR 2 and BLUR 8 (Fig. 5). The nucleotide sequences of these Alu family members diverge by 20% from one another as well as the Alu consensus sequence (12). A comparison of the hybridizing bands to the restriction site locations was made, and their positions were mapped (Fig. 6). A differential hybridization pattern (Fig. 5) was obtained by using the BLUR 2 and BLUR 8 clones. Although many of the bands hybridize to both probes, several bands are shown to

Fig. 3. Restriction map of part of the hPL and hGH gene cluster. This figure shows the regional map spanning 38 kb of the genome that is generated when the three clones are overlapped and the linkage arrangement of the three genes present in these clones. The map establishes the arrangement 5'-hGH2-hPL1-hPL2-3' for the genes. The transcriptional direction of the hPL2 gene is opposite the transcriptional direction of the hGH2 and hPL1 genes.

Fig. 4. Human genomic DNA hybridized with the lactogen cDNA probe. Twenty micrograms of high molecular weight human placental DNA were restricted with various enzymes, subjected to electrophoresis on a 0.8% agarose (TAE) gel, and transferred to nitrocellulose according to the method of Southern (16). The blot was hybridized with [32P]5S rDNA pBR322 DNA in the presence of 10% (w/v) dextran sulfate. The following restriction enzymes were used: (a) Eco RI, (b) Hind III, (c) Bgl II, (d) Bam HI, and (e) Xba I.
hybridize almost exclusively with either BLUR 2 or BLUR 8. The presence of different family members of Alu repeats in and around the hGH and hPL genes could imply that these sequences have different functions. It is possible that some Alu family sequences were involved in the duplication of these genes while others may represent recently acquired elements that regulate gene expression. Recombination involving two similar repetitive sequences has been suggested as a mechanism involved in deletions of the human α-globin gene region (38).

The subcloned hPLα gene was also examined using the BLUR 8 clone insert (Fig. 7). The 0.72-kb Bam HI fragment that hybridizes with the probe corresponds to the 5' coding region and IVS A of the hPLα gene (Figs. 2, 6, and 7). The larger 6.7-kb Bam HI fragment also contains an Alu repeat. Digestion with Eco RI and Pvu II indicated that this second Alu family sequence was flanking the 3' end of the hPLα gene. The hPLα gene is the only gene of the seven-member hGH-hPL gene family that contains an Alu repeat in an intron.

It can be argued that insertion of DNA sequences, such as the Alu family member in IVS A of hPLα, into intervening sequences modifies or adapts these introns into new sequences capable of altering the structure or function of the gene. Perhaps introns function not only as a means of separating functional domains of proteins (39, 40) but also as regions actively involved in evolution or the expression of genes. Studies of immunoglobulin gene systems indicate that rearrangements of the germline DNA are responsible for the diversity of immunoglobulins expressed in somatic tissues (41–43). Genetic recombination in the germline DNA involving repetitive noncoding regions appears to be the mechanism for expression of antibody diversity. Some Alu family sequences might be involved in mediating the expression of members of the hGH-hPL multigene family. Other Alu family members might mediate recombination events that could not only maintain the structural similarity of the multigene family but also result in hybrid genes. The hPLα gene is an excellent example of such a hybrid gene structure. These hybrid genes could eventually assume new functions within the hGH-hPL multigene family.

**Detailed Analysis of Subcloned Genes**—The three genes present in λH17 and λH18 were subcloned so that a more
extensive analysis of their structure could be accomplished. The two hPL genes were examined using 5' and 3' lactogen probes to determine more clearly their structure and orientation. The results of this analysis with the hPL gene are shown in Fig. 8. Confirmation of earlier restriction mapping data was made by a comparison to the fragments found in Fig. 6. The gene was almost entirely included in a 6.8-kb Bam HI fragment and a 1.2-kb Pvu II fragment (Fig. 8, A and B, lanes a and h). However, only the 3' probe hybridized to the 2.6-kb Pvu II fragment (Fig. 8, lane a), indicating that the gene extended from the 1.2-kb Pvu II fragment into the 2.6-kb fragment. The 5' end of the gene was almost entirely contained in a 0.8-kb Pst I fragment and a 1.9-kb Pvu II fragment adjacent to the Eco RI site bordering the 2.9-kb Eco RI hPL gene in λH17 (Fig. 8A, lanes a, f, and g; Fig. 1). The Ava I digest showed that the 5' lactogen probe hybridized to a 1.9-kb Ava I fragment that was also flanked on the right side by the natural Eco RI site (Fig. 8, C and D, lanes a and b, and Fig. 2). These data, along with the Eco RI-Xba I fragment in λH17 and λH18, indicated the transcriptional orientation of the hPL gene was opposite that of the hGH gene and the hPLv gene (Figs. 1 and 4). The data indicated the 5' Pst I site found in the lactogen cDNA sequence (4) was adjacent to a 5' Bam HI site, but an additional Bam HI site was present in λV6 A of this gene (Fig. 2). The size of the natural gene was determined to be approximately 2.4 kb. Comparison of mapping data from the subcloned gene and that of the lactogen cDNA also confirmed the presence of four intervening sequences and five coding regions. The hPLv gene sequence contained in the 2.9-kb Eco RI fragment of λH17 was also subcloned into pBR322. The insert was analyzed by restriction endonuclease digestion and hybridization with the 5' and 3' lactogen cDNA sequences (Fig. 8, C and D). The results indicated a Bam HI site, 0.5 kb from the 5' Eco RI site of the fragment (Fig. 8, C and D, lane j), was the 5' boundary of the gene. Unlike other hPL and hGH genes, hPLv has only a single Peu II site near the 3' end of the gene (Fig. 8, C and D, lanes l and m). The gene does, however, have a Pst I site as in the lactogen cDNA (Fig. 8, C and D, lanes n and o). The 5' lactogen probe hybridizes to a 1.6-kb Pst I fragment (Fig. 8, C and D, lanes n and o). An additional 0.4-kb Pst I fragment that does not hybridize to the lactogen cDNA sequence (a 550-bp fragment lacking the 5' end of the coding sequence) maps to the 5' end of the fragment (Fig. 8, C and D, lanes n and o).

The hPLv gene also contains Bgl II sites in IVS A, IVS C, flanking the 3' end of the gene, and in the 3' untranslated region (Fig. 9). This is quite different from the other hPL and hGH genes analyzed (Fig. 2). Hybridization with the 5' specific probe is unusually weak (Fig. 2). Hybridization with the 5' specific probe is unusually weak (Fig. 9) for a homologous gene probe. In addition, the Xba I site found in the 2.9-kb Eco RI fragment is not found in the gene coding region; it is present in the 5' untranslated region near the Bam HI site (Fig. 2). Restriction and hybridization analyses indicate that the 0.4-kb Pst I fragment contains the 5' untranslated region, but no clear indication of the leader sequence was detected; this has been confirmed by preliminary DNA sequence analysis. The 0.8-kb Pst I fragment extends from the 5' untranslated region to IVS B (Fig. 2). The cDNA sequence found in this fragment extends to what would normally be the Xba I site, approximately 280 bp in the cDNA sequence. However, exon II must contain most of this coding sequence, while IVS C is approximately 700 bp long. Two Hae III restriction sites are found in the cDNA sequence (25). The first Hae III site is found near the location of the 5' Pvu II site while the second is found in the 3' untranslated region. Hybridization data from the 5' and 3' probes used to analyze hPLv (Fig. 9, lane a) indicated a third Hae III site approximately 250 bp from the 3' end of the gene. Therefore, IVS D is approximately 120-150 bp in size. The hPLv gene is approximately 1.9 kb long and contains four intervening sequences and five exons (Fig. 2).

Conserved DNA Regions Occur 3' to the hGH and hPL Genes—Examination of the restriction maps of the various recombinant clones containing hGH and hPL genes reveals the presence of structurally similar regions containing conserved restriction sites 3' to many of the genes (Fig. 10). Two Bam HI restriction fragments, 1.2 kb and 0.6 kb in size, are found in tandem and in the same orientation with respect to the 3' end of hPLv, hPLa, hGH1, hGH2, and hGH3 genes. These conserved regions are located 2-3 kb from the genes and contain Alu family members. Two of these conserved regions, the 1.2-kb Bam HI fragment flanking hPLv and hPLa, were isolated. Both contained Alu I restriction sites near the 5' Bam HI restriction site of the 1.2-kb fragment. Preliminary DNA sequence analysis of 40 bp of this region revealed greater than 84% sequence homology between the two regions. The primate globin gene region clusters also have intergenic DNA regions with very low rates of divergence (44). A comparison of early embryonic and oocyte actin gene expression in sea urchins shows that expression of different members of this gene family is influenced by the 3' untranslated flanking regions (45). Perhaps the expression of the genes within the hGH and hPL gene family is also related to conserved 3' untranslated sequences. The presence of conserved regions 3' to the various genes may also represent the remnants of DNA sequences involved.
Fig. 8. Hybridization of 5′ and 3′ Specific Probes to pHPL1:8.5. A more detailed restriction map of the hPLs and hPL2 was generated by detailed restriction endonuclease analysis of the genes. Eco RI fragments containing these genes were subcloned into the plasmid vector pBR322. Recombinant plasmids were renamed pHPL1:8.5 and pHPL2:2.9 (initially pHPL1:8.5 was called pHI7a). To establish the orientation of these genes within the vector and to confirm data from previous experiments, pHPL1:8.5 and pHPL2:2.9 were restricted with various enzymes, underwent electrophoresis on 1.5% agarose gels, and transferred bi-directionally to nitrocellulose. These blots were then hybridized to the 5′ and 3′ lactogen cDNA probes. This figure shows pHPL1:8.5 hybridized to the 5′ probe (A and C) and to the 3′ probe (B and D). Restriction enzymes used in the digests in A and B were: (a) Eco RI and Pvu II, (b) Bgl II, (c) Bgl II and Eco RI, (d) Xba I, (e) Xba I and Eco RI, (f) Pst I, (g) Pst I and Eco RI, (h) Bam HI, (i) Eco RI. The following restriction enzymes were used in C and D: (a) Aat II, (b) Aat II and Eco RI, (c) Acc I and Eco RI, (d) Acc I and Eco RI, (e) Acc I and Aat II, (f) Dde I, (g) Dde I and Eco RI, (h) Hae II, (i) Hae II and Bam HI, (j) Hae II and Bgl II, (k) Hpa II, (l) Hpa II and Bgl II, (m) Rsa I, (n) Rsa I and Pvu II, (o) Sac II, (p) Sac II and Hae II.

Fig. 9. Hybridization of 5′ and 3′ lactogen cDNA probes to restriction digests of pHPL2:2.9. The left panel shows the ethidium bromide-stained gel. The other panels indicate the lactogen probe used in hybridization. Restriction enzymes used were as follows: (a) Hae III, (b) Hae III and Alu I, (c) Aat I and Xba I, (d) Xba I and Eco RI, (e) Hha I and Eco RI, (f) Alu I and Eco RI, (g) Alu I, (h) Aat I, (i) Aat I and Eco RI, (j) Bam HI and Eco RI, (k) Bgl II and Eco RI, (l) Pvu II, (m) Pvu II and Eco RI, (n) Pst I, (o) Pst I and Eco RI.
in the duplication of the hGH and hPL family members. Therefore, a detailed examination of the flanking conserved sequences should provide insight into the evolutionary history of this gene family.

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