The human growth hormone (hGH) cluster contains five genes. The hGH-N gene is predominantly expressed in pituitary somatotropes, whereas the remaining four genes, the chorionic somatomammotropin genes (hCS-L, hCS-A, and hCS-B) and hGH-V, are expressed selectively in the placenta. In contrast, the mouse genome contains a single pituitary-specific GH gene and lacks any GH-related CS genes. Activation of the hGH transgene in the mouse is dependent on its linkage to a previously described locus control region (LCR) located −15 to −32 kilobases upstream of the hGH cluster. The sporadic, nonreproducible expression of hCS transgenes lacking the LCR suggests that they may be dependent on hGH LCR activity as well. To determine whether the hCS genes could be expressed with appropriate placental specificity, a series of five transgenic mouse lines carrying an 87-kilobase human genomic insert encompassing the majority of the hGH gene cluster and the entire contiguous LCR was established. All of the hGH cluster genes were appropriately expressed in each of these lines. High level expression of hGH was restricted to the pituitary and hCS to the labyrinthine layer of the placenta. The expression of the GH cluster genes in their respective tissues paralleled transgene copy numbers irrespective of the transgene insertion site in the host mouse genome. These studies have extended the utility of the transgenic mouse model for the analysis of the full spectrum of hGH gene cluster activation. Further, they support a role for the hGH LCR in placental hCS, as well as pituitary hGH gene activation, and expression.

The human growth hormone (hGH) gene family is encoded within a 48-kilobase (kb) cluster on chromosome 17q22–24. It contains five genes that share greater than 94% sequence identity (1, 2). hGH-N is selectively expressed in the somatotrope and lactosomatotrope cells of the anterior pituitary, whereas the other four genes, hCS-L, hCS-A, hGH-V, and hCS-B, are expressed in the syncytiotrophoblastic layer of the mid- to late gestational placenta (3–5). Among the placental genes, hCS-A and hCS-B are highly expressed, whereas hCS-L and hGH-V are expressed at trace levels (6). The mutually exclusive tissue specificities and distinctive developmental controls of the closely spaced and structurally related hGH and hCS genes make the hGH gene cluster a highly informative model system for the study of gene expression in mammalian development.

Activation and expression of genes in their native chromatin context reflect the combined actions of multiple determinants. The most well characterized cis-acting determinants of gene expression are those located in close proximity to the respective promoter. There are a number of well defined and functionally characterized promoter proximal determinants critical to the expression of hGH-N. Among these are a pair of binding sites for the tissue-restricted POU-homeodomain protein, Pit-1/ GHI-F-1 (7), and a binding site for the more widely expressed zinc-finger protein factor (Zn-15) (8). In the case of the hGH-N gene these proximal promoter elements, necessary for gene expression in cell transfection assays, are insufficient to mediate in vivo, tissue-specific expression in transgenic mouse lines (9–11). This observation points to the existence of determinants located at a greater distance from the hGH gene that are essential to the establishment of a transcriptionally active chromatin domain. A set of such determinants, located from 14.5 to 32 kb 5’ to the hGH-N gene promoter, were identified by DNaseI hypersensitivity (HS) mapping of chromatin from expressing tissues. The closely spaced HS1 and HSIII are specific to pituitary chromatin, HSIII and HSIV are shared by pituitary and placental chromatin, and HSIV is specific to placental chromatin. When linked to the hGH-N gene, the full set of determinants directs high level, position-independent, somatotrope-specific expression of the hGH-N gene in transgenic mice in a consistent and predictable manner (10, 11). The ability of these determinants to overcome site of integration effects on the hGH-N gene fulfills the functional criteria for a locus control region (LCR). These criteria were initially defined for the human β-globin LCR (12–15) and subsequently for a small group of additional gene systems including the chicken lysozyme LCR (16, 17) and the LCR for the human red and green visual pigment genes (18, 19). A similar dependence of the placental genes in the hGH gene cluster on LCR activation has not been demonstrated.

The hCS-A transgene, with extensive 5’- and 3’-flanking sequences, is either not expressed at all or is expressed at low and unpredictable levels in the mouse placenta (10). These observations might reflect two alternative, or coexisting, mechanisms: 1) the mouse, which lacks CS-like genes, is unable to support hCS transgene expression in the placenta because of a species-specific array of placental transcription factors poorly suited for hCS gene activation or 2) the consistent activation of the hCS transgene is dependent on linkage to the hGH LCR. This second possibility is supported by our previous observation that a specific subset of hGH LCR HS form in the chord
mating of primary human placental syncytiotrophoblast nuclei (10). In the present report we have isolated a human genomic clone that contains the entire LCR as well as much of the contiguous gene cluster encompassing both hGH-N and hCS-A. By introducing this 87-kb transgene into the mouse genome we are able to document the competence of the mouse transgenic model to reliably support appropriate expression of the hGH gene cluster in both the pituitary and the placenta. These data support the role of the hGH LCR in activation of both hGH and hCS gene expression.

EXPERIMENTAL PROCEDURES

Enzymes, Probes, and Primers

Restriction and modification enzymes were purchased from New England Biolabs, Promega, Life Technologies, Inc., and Roche Molecular Biochemicals. RNAzol B RNA isolation solvent was purchased from Tel-Test, Inc; the Oligotex mRNA kit was from Qiagen Inc; [α-32P]dCTP, [γ-32P]ATP with a Random-Primer DNA Labeling Kit (Roche Molecular Biochemicals), and 0.1% SDS, then 0.1 SSC, 0.1% SDS, and finally 0.1 SSC, 0.1% SDS) at room temperature to 65 °C.

Reverse Transcription-Polymerase Chain Reaction (RT-PCR)

hGH-N expression in the pituitary was documented and quantified relative to endogenous mGH mRNA by the RT-PCR approach generating an hGH-N to mGH mRNA ratio as described previously (10). Briefly, reverse transcription and subsequent PCR were carried out beginning with 0.2–0.5 μg of total RNA using primers corresponding to regions perfectly conserved between mGH and hGH (mGH primer set, Table I). After 30 cycles of PCR the 3′-end-labeled cDNA products were digested with BstNI to generate fragments specific to hGH-N and mGH mRNAs. Intensities of the two bands were compared by phosphorimager analysis.

The RT-PCR analysis of hCS-A expression was performed with the hGHNCsA I primer set (Table I). These primers annealed to both hCS-A and hGH-N mRNAs. The 5′-end-labeled cDNA products were cut with BstNI to generate fragments specific to hGH-N and mGH mRNAs. The membrane was subsequently hybridized by adding 1–2 × 106 cpm/ml of the indicated probe at 42 °C overnight and washed (2 × SSC, 0.1% SDS). The washed blots were analyzed using a Storm Phosphorimager System, DNA was transferred to Zetabind, and the membranes were incubated with a [32P]-labeled oligomer corresponding to the T7 site in the P1 vector or to a probe corresponding to the 3′-end of linearized P1 6057. This mapping served to screen for any deletions or rearrangements in the insert compared with the sequence (GenBank® AC005803) or the known restriction map of the LCR and gene cluster (1, 10).

Generation and Analysis of P1 Transgenic Mice

P1 6057 plasmid DNA was linearized at the NotI site (Fig. 1), purified by Etulip (Schleicher & Schuell), adjusted to 2 ng/μl in 10 mM Tris-HCl, pH 7.6, 0.1 mM EDTA, and microinjected into the male pronucleus of fertilized mouse eggs (University of Pennsylvania Transgenic and Chimeric Mouse Core). Positive founders were identified by the blot analysis of tail DNA. All 3′-end-labeled fragments were mapped in P1 progeny, and the transgene copy number for each line was determined by Southern blot analysis (described in "Results").

Hybridization Analyses

Southern Blotting—Total RNA was extracted from various organs with RNAzol, and poly(A)+ RNA was extracted from placenta with an Oligotex mRNA Mini Kit. 5–10 μg of total RNA or poly(A)+ mRNA were denatured at 65 °C, fractionated on 1% agarose gels containing 2.2 mM formaldehyde in 0.1 M MOPS, pH 7.0, 0.6 mM sodium acetate, 0.1 mM EDTA, and 500 μg/ml denatured salmon sperm DNA at 65 °C overnight. The membrane was subsequently hybridized at 65 °C overnight with 2 × 106 cpm/ml of probe, washed twice with 0.5 × SSC, 0.1% SDS, and washed a third time with 0.1 × SSC, 0.1% SDS. The washed blots were exposed to XAR-5 film (Kodak), and signals were quantified by phosphorimager analysis.

Northern Blotting—Total RNA was extracted from various organs with RNAzol, and poly(A)+ RNA was extracted from placenta with an Oligotex mRNA Mini Kit. 5–10 μg of total RNA or poly(A)+ mRNA were denatured at 65 °C, fractionated on 1% agarose gels containing 2.2 mM formaldehyde in 0.1 M MOPS, pH 7.0, 0.6 mM sodium acetate, 0.1 mM EDTA, and 500 μg/ml denatured salmon sperm DNA at 65 °C overnight. The membrane was subsequently hybridized by adding 1–2 × 106 cpm/ml of the indicated probe at 42 °C overnight and washed (2 × SSC, 0.1% SDS, then 1 × SSC, 0.1% SDS, and finally 0.1 × SSC, 0.1% SDS) at room temperature to 65 °C.

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In Situ Hybridization

Generation of hCS-A cDNA—A pair of primers (hcs cDNA 5′ and hcs cDNA 3′; Table I) were used to amplify a 614-bp hCS-A cDNA fragment extending from coordinates 114 to 738 of the hCS-A cDNA clone (pG12F) extends from 102 to 654 nucleotides of hCS-A cDNA plus the 3′-UTR and 77 base poly(A) tail (6). PCR products were purified by recovery from the gel and ligation into pGEM-T-easy vector (Promega).

Generation of Riboprobes—The subcloned hCS-A cDNA plasmid (641 pGEM-T-A) was linearized with SpeI (for T7 transcription) or XolI (for Sp6 transcription). The linearized plasmids were transcribed in vitro using MEGAScript T7 Kit (for the antisense probe) or Sp6 Kit (for sense probe), in the presence of [α-32P]UTP.

In Situ Hybridization—Placentas were collected from mouse embryos generated by crossing an hGH P1 line 813I male with a CD-1 female. The mother was euthanized at embryonic day 16 and the placentas were fixed in 4% paraformaldehyde in PBS (pH 7.2) at 4 °C overnight. Genotypes were determined by dot blot analyses of embryonic tissues. Placentas of the transgenic and wild-type mice were embedded in paraffin, and 5-μm sections were prepared and treated for in situ hybridization as described previously with minor modifications (22). Briefly, wax was removed, and the sections were rehydrated, fixed with 4% paraformaldehyde in PBS (pH 7.2) for 20 min, and washed twice with PBS for 5 min each. The sections were treated with 20 μg/ml proteinase K in 50 mM Tris-HCl, pH 8.0, 5 mM EDTA for 7.5 min. After washing with PBS, the slides were treated in 0.1 M triethanolamine (TEA) for 30 min at room temperature, washed with PBS, and fixed again in 4% paraformaldehyde for 5 min. After washing with PBS, the slides were treated in 0.1 M triethanolamine for 5 min followed by incubation in 0.25% acetic anhydride in 0.1 M triethanolamine for 5 min. Following washing with 2× SSC, the slides were incubated overnight with 4 × 10^6 cpm of probe in 100 μl of hybridization buffer (40% formamide, 0.48 M NaCl, 8 mM Tris-HCl, pH 7.5, 1.6 mM EDTA, 0.8× Denhardt’s solution, 0.4 mg/ml yeast tRNA, 80 μg/ml poly(A) RNA, and 10% dextran sulfate) overnight at 65 °C. The slides were washed with 5× SSC for 10 min at 50 °C, then in 50% formamide, 2× SSC for 20 min at 65 °C, next in RNase buffer (0.3 M NaCl, 10 mM Tris-HCl, pH 8.0, 5 mM EDTA) for 30 min at 37 °C, and then digested with RNase A (50 μg/ml) in the same buffer for 30 min at 37 °C. Finally the slides were washed with RNase buffer, 50% formamide in 2× SSC, 2× SSC alone, and 0.1× SSC. The slides were dehydrated and exposed to emulsion for about 2 weeks and then developed and stained with hematoxylin and eosin. Bright field and dark field micrographs were generated using a Nikon Microphot-FX microscope.

RESULTS

Isolation and Mapping of a P1 Clone Encompassing the hGH Locus and Its Contiguous LCR—A genomic fragment containing the entire hGH LCR and the majority of the contiguous hGH gene cluster was isolated from a human genomic P1 library. The library was screened with amplimers specific for the region immediately 5′ to HSV of the LCR and a second primer set specific for a site 3 kb 3′ to the hCS-A gene. A clone positive with both probes, P1 6057, was isolated and named “hGH/P1.” The restriction map of this clone was compared with the known genomic structure (1, 10). The hGH-N probe, which cross-hybridizes to each of the genes in the cluster, identified unique 10.5 and 7.8 kb BglII bands corresponding to hCS-A and hCS-L and a more intense 2.6-kb band containing the two identical sized fragments with the hGH-N and hGH-V genes, respectively (Fig. 1B, right). Missing was the most 3′-gene in the cluster, hCS-B, which would have been present on a 3.9-kb BglII fragment. The HSI and -II probe detected the expected 1.6-kb BglII fragment (Fig. 1B, middle), and the HSIV probe hybridized to the expected 22-kb EcoRI band encompassing HSV, HSIII, and HSIV (Fig. 1B, left). Additional mapping of the hGH/P1 insert DNA by complete and partial digestion failed to reveal any rearrangements or deletions when compared with the genomic structure (data not shown). The orientation of the insert relative to the vector (Fig. 1C) was established by demonstrating that the vector-derived T7 polymerase segment and the HSIV site were located on the same ClaI restriction fragment (data not shown). Thus the 87-kb P1 6057 (hGH/P1) insert spanned coordinates ~45.8 to 40.6 kb of the hGH locus (hGH-N transcription start site as coordinate 1) and contained all five HS sites of the LCR and the contiguous hGH-N, hCS-L, hCS-A, and hGH-V genes (Fig. 1A and C).

Generation of hGH/P1 Transgenic Mouse Lines—The hGH/P1 plasmid was mapped for rare cutting endonucleases sites to identify a unique site for linearization prior to zygotic microinjection. NotI, SalI, and SfiI were tested because each cleaves a single site adjacent to the P1 vector cloning site (23). The banding pattern of the restriction digestions on 1% agarose pulsed field gels demonstrated that the insert contained two SfiI sites and one SalI site but lacked a NotI site (data not shown).

Fig. 1. Characterization of a P1 clone, hGH/P1, containing the hGH gene cluster and contiguous LCR. A, hGH/P1 map. A linear diagram of the human genomic insert in the hGH/P1 clone is shown. The positions of each of the four genes of the hGH gene cluster encompassed by the insert (hGH-N, hCS-L, hCS-A, and hGH-V) are represented as black boxes, and the positions of each of the HS constituting the hGH LCR are indicated as above the line (labeled vertical arrows). The coordinates (kb) are referenced to the 5′-terminus of the cluster (hGH-N transcription initiation site = 0). The P1 vector is shown schematically as a solid black line. The positions of the 5′- and 3′-amplicons used to identify the hGH/P1 clone are indicated (HSV and CSAS-EF in Table I) as the EcoRI (E) and BglII (B) sites used in mapping (labeled arrows below the line). Restriction fragment sizes are also indicated. B, Southern blot analysis of the hGH/P1 insert. The hGH/P1 plasmid was digested with EcoRI or BglII (for diagram of restriction sites see A). Hybridization with an hGH probe that hybridizes to all four genes in the cluster resulted in BglII bands corresponding to hCS-A (10.53 kb), hCS-L (7.79 kb), hGH-N (2.58 kb), and hGH-V (2.50 kb). Hybridization with an HSI probe revealed the expected 1.6-kb BglII fragment. An appropriately sized 22-kb fragment containing HSIII, HSIV, and HSV was detected following hybridization of the EcoRI digestion with a probe corresponding to HSIV. C, map of the hGH/P1 plasmid. The orientation of the hGH cluster in the P1-based plasmid and the position of the NotI site used for linearization (“Linearization site”) are shown. The positions of the four genes of the hGH cluster in the hGH/P1 insert and the positions of the five LCR HSs are indicated.
Transgenic Expression of the hGH Gene Cluster

Expression of the hGH/P1 Transgene in the Mouse Pituitary—

In previous studies we documented that the hGH-N gene is appropriately expressed in the pituitaries of transgenic mice when linked to its contiguous LCR (10). To confirm and extend this observation, expression of the hGH cluster containing the hGH-N gene as well as the linked and placentally expressed hCS-L, hCS-A, and hGH-V genes was analyzed. RNA was isolated from the pituitaries of adult F1 transgenic mice from each of the five hGH/P1 lines. These samples were analyzed for hGH-N, hCS, and hGH-V mRNAs. hGH-N mRNA was identified and quantified relative to endogenous mGH mRNA using an RT-PCR co-amplification assay (10) (Fig. 3A). This approach co-amplifies hGH and mGH as identically sized fragments that can be subsequently differentiated and quantified by restriction analysis (the primers do not amplify hGH-V or hCS). The analyses of all five transgenic mouse lines revealed the expected three cDNA fragments; two (170 and 125 bp) corresponding to the two major splice products of the hGH transcript and the third (110 bp) corresponding to mGH cDNA. The ratios of the hGH to mGH signals were quantified and normalized to transgene copy number. The pituitary expression from the hGH-N gene was consistent from line to line, varying less than 3-fold (0.34–0.96) (Fig. 3A). This range of hGH-N transgene expression was remarkably similar to that observed previously for the LCR linked to the isolated hGH-N gene (10). This result demonstrated reproducible, copy number-dependent expression of the hGH-N gene from the 87-kb hGH P1 transgene.

The genes in the hGH gene cluster are highly similar in structure and yet are expressed in mutually exclusive tissue distributions, predominantly in the pituitary or the placenta. To determine whether this tissue specificity of expression from the hGH gene cluster is maintained in the mouse model, the expression of hCS and hGH-V was tested in the pituitary. RT-PCR was performed on transgenic pituitary RNA using a pair of primers that were complementary to both hGH-N and hCS-A mRNAs; the co-amplified cDNAs were then distinguished by restriction digestions (Fig. 3B). In contrast to the strong hGH-N signals in each sample, there was a complete absence of hCS-A expression in all lines. The total lack of hCS-A expression was confirmed using a technically independent hCS-specific nested RT-PCR assay (data not shown). A similar RT-PCR approach designed to detect ectopic expression of the placent al hGH-V revealed total absence of expression in transgenic mouse pituitaries from each of the five transgenic lines (Fig. 3C). Expression of the hCS-L gene was not tested, because the function of this gene has been lost during the evolution of the cluster via multiple alternative splicing errors (24). The high levels of hGH-N and absence of detectable expression of hGH-V or hCS-A indicated that the genes in the hGH gene cluster were expressed with appropriate tissue specificity in the pituitaries of all five of the hGH/P1 lines.

Phenotype of the hGH/P1 Mouse Lines—All five of the hGH/P1 transgenic mouse lines displayed normal fertility, fecundity, and lifespan. Males and females from each line were weighed at intervals starting at 3 weeks of age. Although there was minor variations in body weight from line to line, only line 813I displayed a growth curve that differed significantly from sex-matched wild-type littersmates. The 813I transgenic mice (both males and females) were approximately 10% larger than the matched controls after 7 weeks of age. This difference did not increase with time. The normal body growth rate of all the remaining groups indicated that hGH expression from the P1 transgene was under normal physiologic control and that no significant ectopic and/or unregulated transgene expression was occurring in other tissues.

Transgene Expression in the Placenta—In a previous study, we had generated six mouse lines transgenic for a 15-kb HindIII fragment encompassing the hCS-A gene along with 5.4 kb of contiguous 5′-flanking region and 7.2 kb of contiguous 3′-flanking region (10). When assessed for expression in the placenta, only two of these lines expressed hCS mRNA at clearly detectable levels, two at trace levels, and two not at all (10). This lack of consistent transgene expression suggested that the transgene was subject to site of integration effects. To test the hypothesis that hGH LCR element(s) might be necessary for consistent locus activation in the placenta, gene expression was assessed in the placentas of fetuses from each of the five lines carrying the hGH/P1 transgene. F1 transgenic mice from each of the lines were crossed with wild-type mates to generate embryonic day 18 and 19 fetuses. The genotype of each embryo was determined by DNA dot blotting, and the corresponding placentas were assessed for hCS-A transgene expression by an
RT-PCR restriction endonuclease assay as well as by Northern blot analyses (Fig. 4, A and B, respectively). By RT-PCR, all five lines were positive for placental expression of hCS-A mRNA. Of note, low levels of hGH-N mRNA were also detected in all samples, but in each case these levels were approximately 1% that of the hCS-A mRNA. The levels of hCS-A mRNA detected in placental samples were consistent for each of the five lines by Northern blot analysis. An hCS-A mRNA band was detected in placental RNA from each of the five lines (Fig. 4B). The signal intensity was normalized by phosphorimagery and divided by the transgene copy number. These normalized hCS-A expression values were remarkably consistent among four of the mouse lines (2-fold range). A fifth line, 809C line, expressed hCS at a 10-fold level. Thus the hCS-A gene in the hGH/P1 transgene was expressed in all lines irrespective of its site of integration, and with the exception of line 809C, the levels of expression were closely related to transgene copy number.

The Ectopic Expression of the Transgenes in the Different Tissues—The above data demonstrated that hGH-N was selectively expressed in the pituitary and hCS-A in the placentas of hGH/P1 mice. The possibility of ectopic expression of the GH cluster outside of the pituitary or placenta was next investigated. By Northern blotting a strong hGH mRNA signal was detected in the pituitary and a weaker hCS mRNA signal in the placenta. In contrast, no signals were obtained in any of multiple other tissues surveyed (a single exception was a very weak signal in the testis of line 813I, the highest transgene copy line) (data not shown). To assess ectopic expression at a higher level of sensitivity, we carried out RT-PCR analyses with three sets of primers specific for hGH-N, hCS-A, or β-actin. A representative study using line 809C is shown in Fig. 5. Strong signals were seen for hGH-N in the pituitary. Low level expression of hGH-N was detected in the testis, ovary, and spleen in a subset of lines. A strong hCS-A signal was generated from placental RNA and very low levels of expression were observed in the brain in all lines and in the testis, ovary, and spleen in a subset of lines. A strong hCS-A signal was generated from placental RNA and very low levels of expression were observed in the brain, testis, and ovary of several lines. These data support specific and mutually exclusive tissue specificities for the hGH and hCS loci in the context of the hGH/P1 transgene.

Sublocalization of hCS Transgene Expression within the hGH/P1 Mouse Placenta—In prior studies we have demonstrated by immunohistochemical staining the co-localization of hGH and hMG expression in pituitary somatotrope cells of transgenic lines carrying the hGH-N gene linked to the LCR (11). A similar study was carried out to identify the site of hCS expression in the placenta of hGH/P1 transgenic mice. The mouse placenta is composed of a fetal portion and a maternal portion. The fetal portion can be divided three regions: labyrinth, spongiotrophoblast, and trophoblast giant cells (Figs. 6, A and B). In situ histohybridization with 32P-labeled antisense RNA probes demonstrated that hCS-A mRNA was confined to the labyrinth of P1 transgenic mouse placentas. Control studies with the 32P-labeled sense probes and stains of wild type placentas were all negative (Figs. 6, C and D). By high power...
magnification, the positive cells in the labyrinth were mononuclear, of moderate size, elongated, and usually located adjacent to the maternal blood sinuses (Figs. 6, E and F). There was some heterogeneity of expression levels among positive cells. This was best seen by variation of signal strength on dark field analyses. The morphology, location, and distribution of the positive cells all suggested that they represented mononuclear labyrinthine trophoblast cells.

DISCUSSION

Previous studies have demonstrated that expression of the hGH-N transgene is dependent on remote regulatory elements. These elements were originally identified as a set of DNaseI HS in pituitary chromatin located from 14.5 to 32 kb 5’ to the hGH gene cluster. Subsequent functional analyses demonstrated that these elements were able to overcome site of integration position effects and establish autonomously regulated chromatin domains in mouse pituitary chromatin. The activated hGH-N transgene was expressed at consistent and uniform levels that were comparable to that of endogenous mouse GH. Based on their ability to impart copy number dependence on the hGN-N transgene, these elements fulfilled the criteria for LCR action (14, 25).

Genes in a cluster can compete with each other for LCR elements, and such competition can constitute an essential aspect of their normal developmental control. This is well illustrated in the human β-globin gene cluster in which the normal developmental silencing of the fetal γ-globin gene is dependent on the presence of the adult-specific β-globin gene in cis (reviewed in Ref. 24) (25). Thus an essential step in the characterization of expression patterns of genes and establishing their mechanisms of control is to determine their expression profiles when they are in the native configuration of their family cluster. In the present studies, we introduced a large human DNA fragment containing four members of the hGH gene family along with an extensive segment of 5’-flanking region encompassing all five HS of the LCR into the mouse genome. Analysis of hGH-N expression revealed that five of five hGH/P1 lines expressed hGH-N in the pituitary at levels correlating with transgene copy number. The levels of expression/transgene copy for these lines were tightly grouped and were comparable (34–96%) to the level of endogenous mGH. This is quite similar to the levels of expression observed when the hGH-N gene with contiguous LCR elements is introduced into the mouse genome in the absence of the rest of the cluster (10). In both cases the transgenic mice grew normally (i.e. no
gigantism), suggesting that there was no significant ectopic, unregulated hGH-N expression and that the pituitary expression was under normal physiologic regulation. Thus comparison of the present report with our prior studies allows us to conclude that the expression of the hGH-N transgene linked to the LCR is insertion site-independent, copy number-dependent, and pituitary-specific whether isolated or in its native gene cluster environment.

The hGH gene cluster contains a set of placentally expressed genes whose expression patterns are distinct and mutually exclusive from the linked and structurally related hGH-N gene. When a 15-kb transgene fragment containing the hCS-A gene with 5.4 kb of 5′-flanking sequences and 7.2 kb of 3′-flanking sequences were tested for expression in a transgenic assay, the placental expression of hCS-A was sporadic and variable from line to line because of site of integration effects. Of the six lines carrying this transgene, two lines expressed hCS-A at low levels, two at trace levels, and two did not express hCS-A at all (10). This weak and erratic expression profile was reminiscent of hGH-N transgene expression in the absence of the LCR (10). A common dependence on LCR function was further suggested by the observation that HSIII and HSV of the GH LCR HS are present in both placental and pituitary chromatin. Taken together these data suggested that the hCS-A gene, like the linked and closely related hGH-N, was dependent upon LCR function for activation.

In contrast to the erratic expression profile of the hCS-A transgene lacking LCR elements seen in prior studies (10), five of five mouse lines carrying the hGH/P1 transgene in the present study expressed hCS-A in the placenta. Furthermore, the levels of hCS-A mRNA expression/transgene copy number were highly reproducible (2-fold range) for four of the five lines. The remarkably consistent expression of the hCS-A gene in the context of the hGH/P1 transgene supports a role for the remote 5′-flanking region LCR elements in establishing a transcriptionally productive domain for the hCS-A gene in the placenta.

The consistent activation of the hCS-A gene in the context of the hGH/P1 transgene is surprising from an evolutionary viewpoint. Both the structure and composition of the GH gene family and the structure and function of the placenta have diverged between mouse and man. The multiple evolutionary rounds of gene duplication that have given rise to a five member GH/CS cluster in humans have not occurred in the rodent lineage. Instead, the GH-related but much more divergent prolactin gene (26) has duplicated in the mouse, and most of these multiple prolactin-related genes are expressed in the mouse placenta (27, 28). It is not clear whether the function(s) of hCS in primates and these prolactin-related hormones in rodents are similar. There is also, at this time, no evidence to support an LCR linked to the single mGH gene locus. Thus, whatever transcriptional factors are acting on the hGH/P1 transgene to

\[ Y. Ho, S. A. Liebhaber, and N. E. Cooke, unpublished data. \]
activate the hCS locus in a consistent manner in the mouse placenta but have been conserved despite this divergence between mouse and primate at the GH gene locus.

The structures of the mouse and human placenta present fundamental similarities and important distinctions (29). The placentas in both species belong to the deciduate group in that they both contain fetal and maternal portions. However, the details of their structures and endocrine functions differ significantly. The mouse placenta is labyrinthine, whereas the human placenta is villous (30). The murine labyrinthine and human villous areas of the respective placentas are the sites at which nutrients and gases exchange occur between fetal and maternal blood. The maze-like labyrinthine layer of the mouse placenta is composed of anastomosing cords or plates of trophoblasts. These trophoblasts are in direct contact with the maternal blood circulating in the maternal blood sinuses. In this respect the murine labyrinthine trophoblasts are analogous to the human syncytiotrophoblasts that line the outer layer of the fetal placental villi and are also in direct contact with the maternal circulation. In the mouse, the labyrinthine trophoblasts form three layers separating the maternal blood from the fetal blood, a single mononuclear cellular layer and two layers of a trophoblast syncytium. The mononuclear cellular layer comes in direct contact with maternal blood (31). In human placentas, hCS-A is expressed in syncytiotrophoblast cells that line the surface of the villi and contact maternal blood. In the hGH/P1 transgenic mouse, hCS-A-positive cells were mononuclear and lined the maternal blood sinuses within the labyrinth. Thus we conclude that the site of hCS-A gene expression in the trophoblasts cells lining the maternal blood sinuses in the labyrinth of the mouse placenta is functionally analogous to its natural site of expression in the human placenta.

Overlying the murine labyrinth is the spongiotrophoblast layer (Fig. 6A). The outermost cells in this layer are the trophoblast giant cells that form the interface with maternal blood sinuses in the placenta. In the present studies, in situ hybridization showed that hCS-A expression was limited to the labyrinth of transgenic mouse placenta. In contrast, the mouse prolactin-related genes, PL-1 and PL-2, are expressed in the trophoblast giant cells (27, 28). The prolactin-like proteins, prolferin and prolferin-related protein, are expressed in giant cells in the mouse placenta (32–37). These data indicate that the cell-specific expression of hCS-A in hGH/P1 transgenic mice may functionally parallel the site of hCS-A expression in human placenta and is clearly distinct from the sites of expression of the prolactin gene family in mouse placenta.

An extensive survey of tissue RNA samples from hGH/P1 mice was carried out to identify tissues in addition to pituitary and placenta that express the transgene. Northern blot analysis demonstrated only pituitary and placental expression of the transgenes. However, a more sensitive RT-PCR approach revealed several additional sites. hGH-N was expressed at trace levels in the brain in all hGH/P1 transgenic lines and in testis, ovary, or spleen in a subset of lines. hCS-A expression was detectable at trace levels in brain, testis, ovary, and kidney. Recent studies have reported low levels of GH gene expression in the rat brain (38) and GH and CS gene expression in human testis (39) and ovary (40). These observations suggest that the low level “ectopic” expression from the hGH/P1 transgene that we have observed may instead reflect normal physiologic patterns of low level gene expression.

In conclusion, the present study established a remarkable parallel between the expression of the hGH gene cluster in situ and its expression from a large continuous transgene encom-
The Human Growth Hormone Gene Cluster Locus Control Region Supports Position-independent Pituitary- and Placenta-specific Expression in the Transgenic Mouse

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