SUPPLEMENTAL MATERIALS

METHODS

Chromatin immunoprecipitation assays. Single-cell suspensions of pituitary and liver cells were prepared from the indicated transgenic mice, as described (Ho et al, 2002). H3K4me3 chromatin immunoprecipitation assays (ChIP) were performed as previously described (Ho et al, 2002; Ho et al, 2006). Antibody against H3K4me3 was purchased from Abcam (ab8580; Cambridge, UK) and normal rabbit serum from Santa Cruz (Santa Cruz, CA). The sequences and positions of the primer sets are as listed previously (Ho et al., 2006). Quantifications of amplified DNA fragments were carried out by Southern blotting using the PCR products as probes.

Chromatin conformation capture assay

The 3C procedure was performed as described (Tolhuis et al, 2002) with modifications. All primers used for this 3C analysis were designed in the same orientation so that amplified junctions were limited to restriction fragments ligated 'head-to-head' (**Suppl. Table I**). Briefly, dissociated pituitary cells and hepatocytes were fixed as in the ChIP assays. The fixed nuclei were washed twice in 1X digestion buffer (100 mM NaCl, 50 mM Tris-HCl pH 7.9, 10 mM $MgCl_2$, and 1 mM DTT) and 20 μg were incubated with shaking in 1X digestion buffer containing 0.095% SDS for 30 min at 37°C. Triton X-100 was added to 1% final concentration to sequester SDS. The samples were incubated at 37°C for 30 min, then 800 U of *Bgl*II (New England Biolabs, Beverly,

MA) was added to the reactions and the samples were incubated at 37ºC overnight with shaking. The digestion reactions were stopped by adding SDS (2%, final concentration) and heating at 65ºC for 30 min. Half of the digested chromatin was removed and the crosslinks were reversed by heating at 65ºC overnight. DNA was purified from the sample and an aliquot was analyzed by Southern blots to document digestion efficiency (**Suppl. Fig. 1**). Only samples digested to greater than 85% were used. The remainder of the digested chromatin sample was diluted into 4 ml of 1X ligation buffer (1% Triton X-100, 50 mM Tris-HCl pH 7.5, 10 mM $MgCl_2$, 10 mM DTT, 0.1 mg/ml BSA, and 1 mM ATP) and incubated at 16ºC for 2 hr in the presence of 4000 U of T4 DNA ligase (New England Biolabs). A control was performed under identical conditions, but without T4 DNA ligase. The ligation reaction was stopped by adding EDTA (10 mM, final concentration). Crosslinks were reversed by incubating the sample at 65ºC overnight in the presence of 100 μg proteinase K. The samples were extracted with phenol and chloroform, precipitated with ethanol, and treated with 1 μg RNase A. The RNase A was removed by phenol/chloroform extraction and the DNA was ethanol precipitated. The random ligation control comprised *Bgl*II-digested *hGH/P1* plasmid ligated with T4 DNA ligase in the same manner as for the DNA isolated from the digested chromatin preparations.

PCR analysis of the ligation products

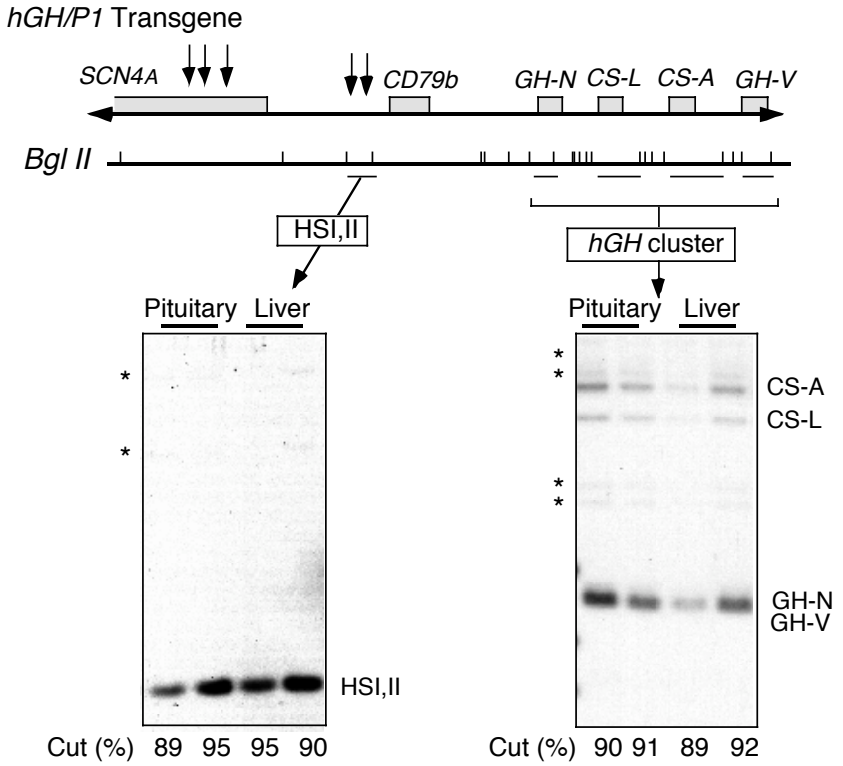
The PCR was performed using 1/50th of the purified DNA from each ligation reaction. One nanogram of the ligated *hGH/P1* plasmid DNA was used for the random ligation control (see above). The primers for the PCR are listed in **Suppl. Table 1**. The

annealing temperatures were specifically adjusted for each primer set to optimize PCR efficiency. The PCR products were separated on 1.5% agarose gels. In a set of control studies, each of the PCR products was purified, sequenced, and confirmed to represent the predicted ligation product (**not shown**). The intensities of the bands were quantified using GelDoc software (BioVision Technologies, Inc. Exton, PA). The intensities of all bands were in linear range.

The ligation efficiency was determined either as a ratio of products ligated to the *hGH-N* promoter relative to the *hCS/hGH-V* promoter fragments when the GH/CS primer served as the anchor primer (**Figs. 2, 4B**). When the HSII primer served as the anchor primer the endogenous *ercc3* locus was used as internal control (**Figs. 3, 4C**); *ercc3* encodes a basal transcription factor expressed in all tissues (Patrinos et al, 2004). A pair of identically-oriented primers located within two adjacent *Bgl*II fragments of the *ercc3* locus were used to control for the consistency and efficiency of the *Bgl*II digestion and ligation during each 3C assay. An additional primer pair, located within a single *ercc3* *Bgl*II fragment, was used as loading control for the PCR in the calculation of the relative cross-linking efficiency. The ligation efficiency was calculated as

$(\text{Signal}_{\text{tissue}}/\text{Signal}_{\text{random control}})/(\text{Signal}_{\text{ercc3 ligation}}/\text{Signal}_{\text{ercc3 loading}})$.

FIGURES

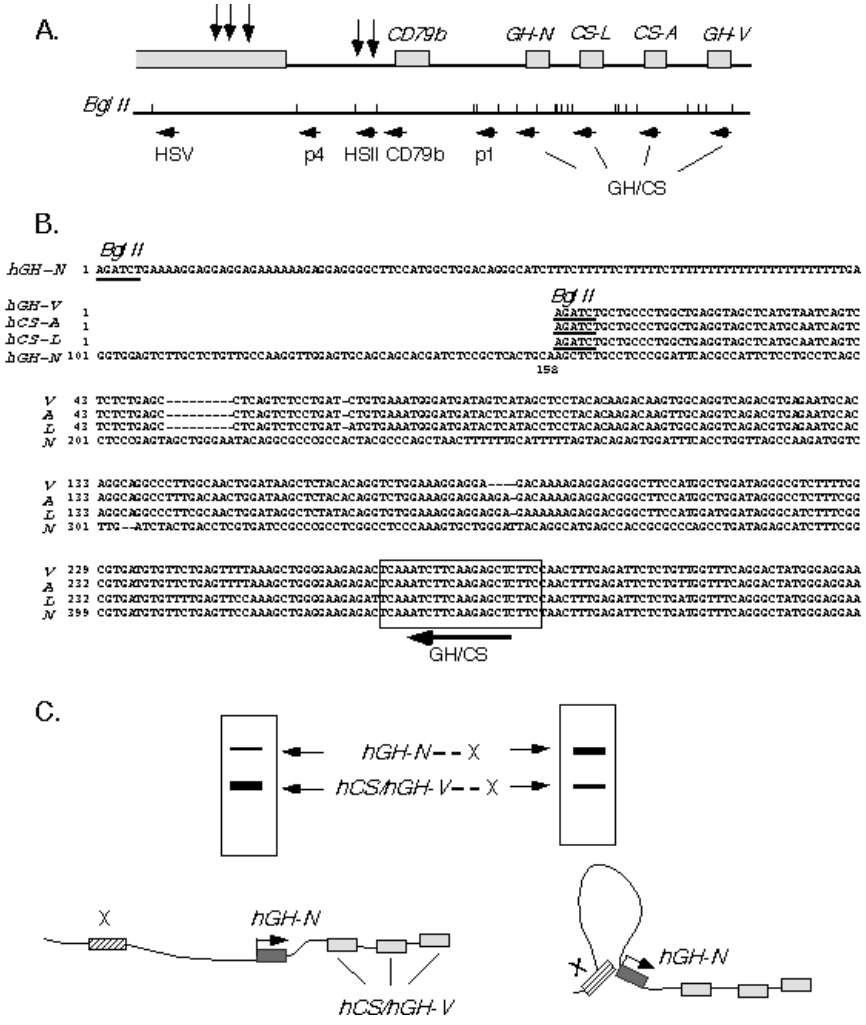


Supplemental Figure 1. Formalin-fixed chromatin isolated from transgenic pituitary and liver samples is efficiently digested by *Bgl*II.

Top: Map of the hGH/P1 transgene and Bgl*II *sites. The locus map is as described (**Fig. 1**). The position of each *Bgl*II site is shown below the map.

Bottom: Southern hybridization analysis confirms that Bgl*II *can effectively digest formaldehyde cross-linked chromatin samples from transgenic pituitaries and livers. Cross-linked chromatin from disaggregated pituitary or liver cells of an *hGH/P1* transgenic mouse was digested with *Bgl*II. Samples from individually cross-linked pituitary or liver chromatin preparations are shown in each panel. Southern blot analysis of the *Bgl*II-digested chromatin samples using the HSI,II specific probe (-15, as indicated under the *Bgl*II map) (left panel) and using a probe common to *hGH* and *hCS* genes (right

panel) are shown. The asterisks to the left of each panel indicate partial digestion products. The percentage of the chromatin sample that was digested to completion is shown at the bottom (calculated as described in **Methods**). Each digestion was performed in duplicate as shown.



Supplemental Figure 2. The *Bgl*II fragments encompassing the *hGH-N* promoter and the placental-specific *hGH-V*/*hCS* promoters can be distinguished by size.

A. The positions of amplification primers used for the chromatin conformation capture PCR analyses. The position and orientation of each primer (arrow) is indicated below the map of the genomic locus (also see **Suppl. Table 1**). The *Bgl*II sites are indicated by the tick marks. The primers are all designed in a uniform orientation to assure selective detection of head-to-head ligation products. The 'GH/CS' primer anneals to a conserved sequence within the *Bgl*II fragments encompassing each of *hGH* cluster gene promoters.

B. Alignment of *Bgl*II restriction fragments containing *hGH-N* and each of the *hGH-V*/*hCS* gene promoters. The *Bgl*II sites that define the 5' termini of the four fragments recognized by the *GH*/*CS* probe are underlined. A single nucleotide unique to the *hGH-N* sequence results in the selective loss of the *Bgl*II site at position 159 to 164. Thus the 5' terminus of the *hGH-N* promoter fragment extends an additional 158 bases to the next 5' *Bgl*II site (underlined). The sequence of the '*GH*/*CS*' universal primer, common to all four genes in the transgene cluster, is boxed and an arrow indicates its orientation. The sequences shown are located approximately 0.7 to 1.2 kb 5' of the promoter of each gene.

C. A PCR co-amplification assay that directly compares ligation products containing either the *hGH-N* promoter or the placental *hGH-V*/*hCS* promoters. The left panel diagrams the pattern of PCR products in the setting of a linear conformation of the locus, *i.e.,* the ligation of randomly arranged fragments. The greater intensity of the *hCS*/*hGH-V* products in the linear/random arrangement reflects the presence of the three placental-specific fragments that would be co-detected. The right panel diagrams the predicted result of a PCR reflecting a chromatin conformation with the *hGH-N* promoter

looped into close proximity to the remote X fragment. Note that greater intensity of the *hGH-N* band compared to the *hCS*/*hGH-V* band can be predicted if a relatively stable loop forms.

SUPPLEMENTAL TABLES

Supplemental Table 1. Primers for the chromatin conformation capture assay

Name	Sequence
HSV	5'-GAACGGCAGGGTGGAGAGGC-3'
p4	5'-CAGGGTCGAAATATCTAACC-3'
HSII	5'-ACAGCACCCATGAGGCAACA-3'
CD79b	5'-TCCGTTTTCCAGTCTGTGCT-3'
p1	5'-TCCTATGTCCAACCTCGACT-3'
GH/CS	5'-GAAGAGCTCTTGAAGATTTGA-3'
ercc3 3-1	5'-TCTCTCAGCTTCCCAGGACC-3'
ercc3 3-2	5'-TGGTGATACACATGGCCAAA-3'
ercc3 3C	5'-TCTGCCGCTTCACCTCAGAT-3'

SUPPLEMENTAL REFERENCES

Ho Y, Elefant F, Cooke N, Liebhaber S (2002) A defined locus control region determinant links chromatin domain acetylation with long-range gene activation. *Mol Cell* **9**(2)**:** 291-302

Ho Y, Elefant F, Liebhaber SA, Cooke NE (2006) Locus control region transcription plays an active role in long-range gene activation. *Mol Cell* **23**(3)**:** 365-375

Tolhuis B, Palstra RJ, Splinter E, Grosveld F, de Laat W (2002) Looping and interaction between hypersensitive sites in the active beta-globin locus. *Mol Cell* **10**(6)**:** 1453-1465