Tissue-Specific Chromatin Modifications at a Multigene Locus Generate Asymmetric Transcriptional Interactions

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Random assortment within mammalian genomes juxtaposes genes with distinct expression profiles. This organization, along with the prevalence of long-range regulatory controls, generates a potential for aberrant transcriptional interactions. The human CD79b/GH locus contains six tightly linked genes with three mutually exclusive tissue specificities and interdigitated control elements. One consequence of this compact organization is that the pituitary cell-specific transcriptional events that activate hGH-N also trigger ectopic activation of CD79b. However, the B-cell-specific events that activate CD79b do not trigger reciprocal activation of hGH-N. Here we utilized DNase I hypersensitive site mapping, chromatin immunoprecipitation, and transgenic models to explore the basis for this asymmetric relationship. The results reveal tissue-specific patterns of chromatin structures and transcriptional controls at the CD79b/GH locus in B cells distinct from those in the pituitary gland and placenta. These three unique transcriptional environments suggest a set of corresponding gene expression pathways and transcriptional interactions that are likely to be found juxtaposed at multiple sites within the eukaryotic genome.

The random assortment of structural loci in higher eukaryotic genomes brings with it a need to functionally “insulate” genes that are closely packed and yet must maintain distinct tissue and developmental specificities. While such insulation can be mediated by fixed chromatin structures (2, 21), not all unwanted transcriptional interactions can be blocked in this simple linear manner. For example, genes in close proximity can be under the control of remote control elements that are interdigitated and/or situated within chromatin domains or transcription units of neighboring genes (30). Thus, establishing and maintaining specificity of gene expression in the context of varied locus organizations is likely to demand an array of mechanistic solutions. In certain cases, problems of transcriptional insulation may not be fully resolved and may result in gene expression in the “wrong” tissue (5). Such ectopic expression or “leakiness” within the transcripome may be the price that the cell pays for the random distribution of its genetic elements.

The CD79b multigene cluster represents an informative model for the study of developmental control (17, 27). This cluster, located on chromosome 17q22-24, encompasses five growth hormone paralogs (Fig. 1A). hGH-N is expressed in pituitary somatotrope cells; the four remaining genes, hCS-L, hCS-A, hGH-V, and hCS-B, are expressed in the syncytiotrophoblast (STB) layer of the placental villi (6, 22). Activation of the pituitary and the placental genes is dependent on distinct ac-

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specific determinants and chromatin modifications interface with the adjacent hGH cluster to result in the overall pattern of transcriptional interactions within this multigene cluster.

**MATERIALS AND METHODS**

**Cell lines and purification of B cells from mouse spleen.** The following cell lines were used in this study: K562 (erythroleukemia cell line); JM1 and REH (pro-B-cell lines); Nalm6 and 697 (pre-B-cell lines); CA46, 15A, and 25A (mature B-cell lines); U266, CRL9068, CRL1621, and CRL1484 (plasma B-cell lines); and JEG3 (human choriocarcinoma cell line). Several of the cell lines have specific ATCC designations: JM1 is designated CRL10423; REH, CRL8286; CA46, CRL1648; and U266, TIB196. B-cell lines were maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum, 100 U/ml penicillin, and 100 mg/ml streptomycin; JEG3 cells were cultured in minimal essential medium. All cells were grown at 37°C and 5% CO2 and were maintained at a maximum cell density of 1 × 10^6 cells/ml.

To purify mouse lymphocyte cells, spleens removed from anesthetized mice were dispersed through a 30-μm nylon mesh to generate a single-cell suspension. Lymphocytes were isolated by Ficoll gradient using Ficoll-Plaque plus (Amersham Biosciences, Piscataway, NJ) by centrifugation at 650 g for 30 min without braking. After centrifugation, the cells at the interface between Ficoll and the aqueous phase were collected and washed twice with phosphate-buffered saline (PBS).

For primary B-cell chromatin immunoprecipitation (ChIP) assays, lymphocytes were further purified by negative selection using MACS CD43 (Lys48) microbeads (Miltenyi Biotec, Germany). B cells were magnetically enriched (excluding pro-B cells) using MACS LD depletion columns and Mini-MACS cell sorting kits (Miltenyi Biotec, Germany) according to the manufacturer’s instructions.

**Transgenic constructs and generation of transgenic mouse lines.** All transgenic mouse lines used in this study are listed in Table 1 (also see the diagram in Fig. 3A). The hGH/P1 line (87-kb hCD79b/GH transgene), −1.3CD79b (5.6-kb hCD79b transgene), and −0.2CD79b (4.6-kb hCD79b transgene) have been previously reported (5, 27). −0.5CD79b contains a 4.9-kb genomic fragment generated by BspMI and EcoRI digestions of a 5.6-kb hCD79b genomic DNA fragment (5). This fragment was gel isolated, extracted with a Qiagen gel extraction column (Qiagen, Germany), purified through an Elutip D column (Schleicher & Schuell, Germany), and adjusted to a concentration of 2 ng/μl in TE buffer (10 mM Tris-HCl [pH 7.6], 0.1 mM Na2EDTA) prior to microinjections into fertilized mouse oocytes (C57BL/6 × SJL). A BAC genome clone encompassing the hCD79b/GH locus was screened by Invitrogen Life Technol-
A 123-kb insert was released by NotI digestion. The NotI sites and resuspended at 10^8 cells/ml in ice-cold sucrose buffer I (0.32 M sucrose, 3 mM MgCl_2, 2 mM magnesium acetate, 10 mM Tris-HCl [pH 8.0], 1 mM dithiotheritol, 0.5 mM phenylmethylsulfonyl fluoride [PMSF], 0.05% Triton X-100). Cells from the various B-cell lines were collected, washed with PBS, and resuspended again at 10^8 cells/ml of RB buffer, and a baseline (at 0 s) aliquot was withdrawn. RB buffer containing 3 mM CaCl_2 and 300 U of DNase I (Invitrogen, Carlsbad, CA) was added to the cell line (K562). The nuclei were digested with 120 U of DNase I (50 μM NaHPO_4, [pH 7.2]), 0.1 mM EDTA, 1% bovine serum albumin, 7% sodium dodecyl sulfate [SDS] and then washed (1× SSC, 0.15 M NaCl, 0.015 M NaCl, 0.1% SDS, and finally 0.1× SSC, 0.1% SDS [1× SSC is 0.1 M NaCl plus 0.015 M sodium citrate]) at room temperature to 65°C, and signals were detected by exposure to a PhosphorImager screen. Signals were quantified by PhosphorImager analysis with a Storm 840 PhosphorImager (Molecular Dynamics, Sunnyvale, CA) analyses of Southern blots. The transgenic mouse founders were generated by routine procedures by the University of Pennsylvania Transgenic and Chimeric Mouse Core Facility. Positive founders were identified by Southern blot analyses of NcoI-digested tail genomic DNA (Table 2). The forward primer was 5′-GCCAAAAACGTTGAGTCAC-3′, and the reverse primer was 5′-GCGAACCTGTCTCTGAGTTG-3′. The resulting released BAC (named mhIg) was used for microinjection as described above.

### TABLE 2. Primer sets used for ChIP, HS mapping, and coamplified reverse transcription-PCR assays

<table>
<thead>
<tr>
<th>Name</th>
<th>Forward</th>
<th>Reverse</th>
<th>Location (kb)</th>
</tr>
</thead>
<tbody>
<tr>
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<td>GCTCACATAGATCTATGGCA</td>
<td>AATTGTGAACACTACGGG</td>
<td>-25.2</td>
</tr>
<tr>
<td>HSV</td>
<td>CTGGCAGCTTGGTCACATT</td>
<td>CTGAGGCTTGTCTCCCTT</td>
<td>-18.8</td>
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<td>TATCATGACAGATGTCACA</td>
<td>-16.5</td>
</tr>
<tr>
<td>P6</td>
<td>CGGCGGTCGCGAGTATGAG</td>
<td>GACCCCTGTTGCTAGTGTG</td>
<td>-13.4</td>
</tr>
<tr>
<td>P5</td>
<td>GCCCTAAGACTCTGAGTG</td>
<td>GGAGCTCCTGAGGCTG</td>
<td>-7.88</td>
</tr>
<tr>
<td>HSI/II</td>
<td>CCAAGCCCTCTCCAGCTTAC</td>
<td>GAYTCTGGCAGGCCCCCTT</td>
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</tr>
<tr>
<td>Cdp</td>
<td>GCCACTACAGCTTCTTCTTGTG</td>
<td>GACCCCAGGGTACAC</td>
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</tr>
<tr>
<td>P3</td>
<td>CTTCCCCACAGGAGACTGA</td>
<td>GGAGGGAACTGTTAACAGTCG</td>
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</tr>
<tr>
<td>P2</td>
<td>TGTCAGACACCCTATGCA</td>
<td>TCAACAGGAGTTGAGACCA</td>
<td>+3.90</td>
</tr>
<tr>
<td>P1</td>
<td>GATTACAAGGCCGGCCTACCT</td>
<td>GAGAGAATAGCGAGGAGGTTG</td>
<td>+7.90</td>
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<tr>
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<td>CTTCCTCTCAGTGTTGCC</td>
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<tr>
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<tr>
<td>Prb2</td>
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<td>ATTCAGAGGCTCCCTCCTCCTAC</td>
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<td>GCCAAAAAGGCTTCATCCTC</td>
<td>CTGCTTCACACCTTTCGTGA</td>
<td>ATCCCCAGAGGACCTC</td>
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</table>

*a* The first ATG of hCD79b is located at +1 kb. 
*b* —, forward on exon 5 (+2.8 kb), reverse on exon 6 (+3.2 kb).

### Colimated reverse transcription-PCR endonuclease cleavage assay.

To quantify the expression of each transgene in each B-cell preparation, reverse transcription, PCR, and restriction enzyme digestion methods were performed as previously described (5). All assays were carried out on lymphocytes purified from transgenic mouse spleens (as described above). In all cases, 0.5 μg of total RNA, extracted from tissues or cell lines using RNA-Bee (Tel-Test Inc., Friendswood, TX), was reverse transcribed with an oligo(dT) primer in the presence of avian myeloblastosis virus reverse transcriptase. Human and mouse CD79b mRNAs were then coamplified using a primer set corresponding to regions perfectly conserved between hCD79b and mCD79b (mhGAPDH) forward and reverse; Table 2). The forward primer was 5′-end-labeled with [γ-32P]ATP and T4 polynucleotide kinase. This primer set spans intron 5 to distinguish cDNA from amplified genomic DNA. PCR products at 25 cycles were digested; HinfI specifically cleaves mCD79b cDNA, and SflI specifically cleaves hCD79b cDNA. Fragments were separated on 6% polyacrylamide gels, and bands were quantified by PhosphorImager analyses. The ratios of hCD79b to mCD79b cDNA products were normalized to the transgene copy numbers, and the final hCD79b mRNA expression value was calculated as a percentage of the expression from a single endogenous mCD79b gene. All PCR results were confirmed to be within the linear range of amplification.

### Chromatin immunoprecipitation (ChIP) assay for modified histones.

ChIP studies were performed with human B-cell lines (1484 and U266), a human B-cell-like cell line (K562), and the resulting RNA was amplified using primer sets listed in Table 2. Parallel dilutions of 1/300, 1/600, and 1/1,200 were made from 15% of the input sample and from the entire immunoprecipitated sample pellet. Aliquots containing a final concentration of 0.16%, 0.08%, and 0.04% of each immunoprecipitated sample were analyzed by PCR to confirm that each assay was within the linear range of amplification. Each product was analyzed by electrophoresis on a 1% agarose gel followed by Southern blotting. The blots were hybridized with a Microzen centrifugal filter (Amicon Inc., Bedford, PA), and 250 μg of this chromatin input was incubated with 10 μl of each of the antibodies to acetylated H3 and H4 (Upstate Biotechnologies, Lake Placid, NY), or in the absence of antibody, in a total volume of 500 μl. Protein A-Sepharose (Amersham Pharmacia Biosciences, Piscataway, NJ) precipitates were generated and washed, and DNA was purified from the pellets (bound) as described previously (23). DNA samples from the input and bound fractions were amplified by PCR using various primer sets listed in Table 2. Parallel dilutions of 1/300, 1/600, and 1/1,200 were made from 15% of the input sample and from the entire immunoprecipitated sample pellet. Aliquots containing a final concentration of 0.16%, 0.08%, and 0.04% of each immunoprecipitated sample were analyzed by PCR to confirm that each assay was within the linear range of amplification. Each product was analyzed by electrophoresis on a 1% agarose gel followed by Southern blotting. The blots were hybridized with 1× to 2× 10^6 cpm/ml random primer-labeled probe at 65°C overnight. The membranes were subsequently washed at 60°C in 0.1% SDS and 0.5× SSC. Signals were quantified by PhosphorImager analysis. Each ratio was normalized to the comparable signal detected at the ubiquitously expressed GAPDH locus.

For the histone H3 acetylation and H4 acetylation ChIP assays, two independent
assays were performed from different preparations of nuclear extracts. For the histone H3 K4 dimethylation ChIP assay, a single experiment was performed.

RESULTS

B-cell-specific DNase I HS are established at the CD79b locus. The chromatin structure at the hCD79b/GH locus was analyzed in a series of B-cell lines representing different stages of differentiation. Consistent with their B-cell characteristics, each line expresses CD79b mRNA (Fig. 1B). However, the levels of CD79b mRNA differ significantly among these lines and fail to correlate with the stage of B-cell differentiation. A subset of these lines representing various levels of CD79b expression was chosen for HS mapping. Control studies were carried out on K562 erythroleukemia cells and a human chorionicarcinoma cell line (JEG3).

HS mapping surveyed two adjacent EcoRI restriction fragments spanning the CD79b gene and 31 kb of 5′-flanking sequences (Fig. 2). HS mapping of the 12.5-kb EcoRI fragment containing the human CD79b gene, the contiguous 8.0 kb of 5′-flanking sequences, and 850 bp of 3′-flanking sequences revealed two HS (HSB1 and HSa; Fig. 2A). HSa mapped within CD79b intron 1 and was detected in all cells studied, irrespective of cell type or CD79b expression. HSB1 mapped to the CD79b promoter and formed specifically in B-cell chromatin. DNase I analysis of the adjacent 5′ EcoRI fragment, 23.1 kb in length, identified three HS (Fig. 2B). Two of these HS corresponded to the previously reported HSIII and HSV of the hGH LCR (17). HSIII was detected in all cell types tested, including pituitary, placenta, B, and erythroid cells, whereas HSV was observed in B-cell lines and pituitary, placenta, and JEG3 cells but not in K562 cells (Fig. 2B and data not shown). A novel HSB2 was also identified in this region. This HS was exclusive to B cells and mapped 14 kb 5′ to the CD79b promoter. HSB2 intensity roughly paralleled the levels of CD79b mRNA in the various B-cell lines. In summary, the DNase I mapping identified three novel HS at the human CD79b/GH locus; a constitutive HSa within the CD79b gene, a B-cell-specific HS mapping to the CD79b promoter, and a second B-cell-specific HS located 14 kb 5′ of the CD79b gene (Fig. 2C).

hCD79b expression is under local control. Expression of the hGH cluster in pituitary and placenta tissue is dependent on a remote LCR (Fig. 1A). Identification of HSB2 suggested that CD79b might be under similar remote regulatory control. The full set of HS mapping data (Fig. 2C) was consistent with a model in which the pituitary tissue-specific HS1 and HSII, the B-cell-specific HSB2, and the placenta tissue-specific HSIV might each act in conjunction with the shared HSV and HSIII to selectively activate their respective target genes. To test this model, sequences necessary for hCD79b expression were mapped in vivo using a series of mouse transgenic models (Fig. 3A). For each transgene, five independent mouse lines were established. For each line, the transgene copy number was determined by Southern blotting (Table 1 and data not shown), and the level of hCD79b mRNA expression in the transgenic B cells was quantified in two or more mice relative to endogenous mouse CD79b (mCD79b) mRNA (Fig. 3B). The ratio of hCD79b mRNA to mCD79b mRNA in each line was divided by the respective transgene copy number. In this manner, hCD79b transgene expression was calculated as a percentage of the expression from a single endogenous mCD79b gene (see Materials and Methods for details).

Prior analyses had revealed that the hGH/P1 transgene supports robust hCD79b expression in mouse B cells at levels of 25 to 42% of a single endogenous mCD79b gene (5). To extend this baseline, we established five lines carrying a 123-kb BAC transgene encompassing CD79b with more extensive flanking regions (details will be reported separately) (Fig. 3A). CD79b mRNA was expressed from this CD/hGH BAC transgene at levels similar to that observed in the hGH/P1 lines (10 to 53% of the endogenous mCD79b locus; Fig. 3C). With these two large transgenes as standards, we next assessed expression from a set of transgenes that isolated CD79b from its remote sequences. The first transgene, −8.0CD79b, contained the CD79b gene along with 8 kb of 5′-flanking sequences. This transgene specifically excluded HSV, HSIII, and HSB2 as well as much of the 3′-flanking region (Fig. 3A). hCD79b was expressed in the splenic B cells of all five −8.0CD79b lines. The mean expression was indistinguishable from that of the larger transgenes, although there was slightly more prominent variation in expression values (6% to 49% of the endogenous mouse locus) (Fig. 3D and H). Two additional hCD79b transgenes were generated that shared a 3′ end with −8.0CD79b (Fig. 3A). The −1.3CD79b transgene excluded the pituitary cell-specific HS1 and HSII, and the −0.5CD79b transgene further excluded a B-cell-specific hypomethylated CpG island (Fig. 3A and unpublished data). The −1.3CD79b transgene was robustly expressed in all five lines. Its expression per copy was 10 to 30% of that of the endogenous mCD79b gene (Fig. 3E and H). The −0.5CD79b transgene maintained a similar level of expression, ranging from 16 to 19% of that of the endogenous gene (Fig. 3F and H). Thus, the five transgenes, CD/hGH BAC, hGH/P1, −8.0CD79b, −1.3CD79b, and −0.5CD79b, expressed mean levels of CD79b mRNA that were within a twofold range (17 to 27%). These data indicated that a complement of cis-acting elements necessary for full activation and expression of CD79b is in close juxtaposition to the structural gene.

A final hCD79b transgene was generated that contained only a 200-bp minimal promoter region (Fig. 3A). It should be noted that the CD79b gene utilizes a GC-rich, TATA-less promoter, and for this reason it has a cluster of multiple transcription initiation sites (5, 28). The expression of the −0.2CD79b transgene (Fig. 3G and H) was remarkable in three respects. First, its level of expression, 4.6% relative to that of the endogenous mCD79b gene (Fig. 3G and H) was remarkable in three respects. First, its level of expression, 4.6% relative to that of the endogenous mCD79b gene, was substantially below that of the other five transgenes. Second, despite its weak expression, the −0.2CD79b transgene retained site-of-integration independence and copy number-dependent expression, with four of five lines demonstrating essentially identical levels (4%) of CD79b mRNA per gene copy. Finally, this transgene retained B-cell specificity (Fig. 4). Thus, determinants sufficient to establish an autonomous and B-cell-specific chromatin locus are encompassed within the CD79b gene or in its immediate 200 bp of 5′-flanking sequences. An additional element(s) necessary for enhancement of expression to full levels is located between −200 and −500 of the transcription start site.

Histone modifications at the hCD79b/GH locus in B-cell chromatin are tightly restricted to the CD79b gene. Transgenic
FIG. 2. Identification of a B-cell-specific DNase I HS at the hCD79b/GH locus. A. Two DNase I HS are detected within and immediately 5' of the CD79b gene; HSB1 is B cell specific. Chromatin samples from the B-cell lines were subjected to DNase I HS mapping. For the left panel, chromatin preparations from six human B-cell lines representing various developmental stages (697, REH, 25A, 9068, 1484, and 1621) were analyzed. The relative level of CD79b mRNA in each cell line, as determined from Northern blot analysis (Fig. 1 and data not shown), is indicated above the corresponding lanes (strong, ++; intermediate, +; low, +/-). The first lane contains a DNA size marker (M). The analyzed EcoRI fragment is 12.5 kb (marked by a black dot). Sub-bands generated by graded DNase I digestions (times of 0 s, 10 s, and 1 min) are indicated by the horizontal arrows to the right of the autoradiograph (HSB1 and HSa). The band at 8 kb was present irrespective of DNase I digestion (time 0) and was considered to be a cross-hybridizing band of unknown origin. For the right panel, DNase I HS mapping of chromatin from a human choriocarcinoma cell line (JEG3) and the erythroleukemia cell line (K562) were compared to a pre-B-cell line (Nalm6). HSB1 was only detectable in Nalm6 cells, whereas HSa was present in all three lines. The diagram below the blots summarizes the strategy and results of the HS mapping studies depicted in the blots. The relevant EcoRI restriction enzyme sites, the probe (Prb1; Table 2; filled box below the CD79b gene), fragment sizes, and positions of HSB1 and HSa, as well as pituitary cell-specific HSI and HSII for reference, are shown within the 12.5-kb EcoRI fragment. B. Three DNase I HS are detected in the remote 5'-flanking region of CD79b; HSB2 is B cell specific. Chromatin mapping and labeling of the autoradiograph are as described for panel A. The 23.1-kb EcoRI fragment (marked by a black dot) studied with probe Prb2 is located immediately 5' to the fragments depicted in panel A. The DNase I sub-bands generated from this fragment are HSV, HSIII, and HSB2 (positions indicated at the right of the autoradiograph). The left panel shows DNase I mapping of the human B-cell lines; positions of HSV, HSIII, and HSB2 are indicated at the right. The right panel shows DNase I mapping of two nonlymphoid lines (JEG3 and K562) and a B-cell line (CA46). HSB2 was formed in the B-cell line but not in JEG3 or K562. The band below the 23.1-kb master band in panel B is a nonspecific, cross-hybridizing band. The diagram at the bottom summarizes the mapping experimental scheme and results as described in the legend to panel A. The hybridization probe (Prb2) is indicated by the filled rectangle. C. Localization of B-cell-specific HS in the hCD79b/GH locus. Arrows (open) above the map indicate B-cell-specific HS and the HSa identified in the current report. Arrows (closed) summarize HS identified in prior studies. HSI and HSII are specific to pituitary chromatin, HSIV is specific to placental chromatin, and HSIII and HSV are constitutive.
FIG. 3. hCD79b mRNA levels in splenic lymphocytes of transgenic mice. A. Transgenes are depicted. The map of the CD79b gene and its 5'-flanking region indicates the positions of B-cell-specific HS (black arrows) and the two HS specific to the pituitary gland (HSI and HSIi; white arrows) for reference. A CpG hypomethylated region of DNA specific to B cells is shown (stippled circle). The four CD79b transgenes are indicated on the map along with their respective sizes. The name of each of these transgenes corresponds to the length of the 5'-flanking region in each case.

B. Quantitation of human versus mouse CD79b mRNAs. hCD79b mRNA and endogenous mCD79b mRNA were reverse transcribed (RT) coamplified from B cells purified from the spleens of the indicated transgenic mice. The coamplification was accomplished using a set of conserved sequence primers (mhIgB forward and reverse; Table 2; white horizontal arrows). The PCR product spans exons 5 and 6, permitting size...
studies indicate that the major determinant(s) of CD79b transcription in B cells is located within, or in close proximity to, the structural gene (Fig. 3 and 4). To explore the basis for this control, we mapped histone modifications at the hCD79b/GH locus in B-cell chromatin. Three histone modifications that are consistently associated with active chromatin domains were assessed by ChIP: acetylated histone H3 (H3ac), acetylated histone H4 (H4ac), and histone H3 dimethylated at the lysine 4 position (H3K4me2). ChIP-enriched DNA was surveyed at 11 sites across the hCD79b/GH locus by semiquantitative PCR (Fig. 5A; Table 2). Each value was calculated as the ratio of DNA in the antibody-bound chromatin to that in the input sample. The values were further normalized to levels of enrichment for the corresponding histone modification at the constitutively active glyceraldehyde-3-phosphate dehydrogenase gene (GAPDH) (see Materials and Methods).

Acetylated histones H3 and H4 were mapped in a B-cell line with high CD79b expression (1484), a B-cell line with trace CD79b expression (U266), and an erythroid line lacking CD79b mRNA (K562) (Fig. 1B). Line 1484 chromatin contained a prominent peak of H3 and H4 acetylation centered on the CD79b promoter (amplimer CDP) and its immediate flanking regions (amplimers HSI and P3; Fig. 5B). A slight enrichment for acetylated H3 and H4 was also noted at HSV (Fig. 5B). The U266 B-cell line lacked significant enrichment for H3 acetylation ChIP study, there was no evidence for H3 acetylation in the hepatic sample (Fig. 5F). Thus, within the resolution of the assay, H3/H4 acetylation in CD79b-expressing B cells was limited to the CD79b promoter. The comparison of the two B-cell lines further established a positive correlation between levels of CD79b expression and local acetylation.

Levels of H3K4me2 were determined by ChIP analysis at the hCD79b/GH locus (Fig. 6). A prominent peak of H3K4me2 was observed at the CD79b promoter (probes CDP and P3) in the high-expressing 1484 cell line (Fig. 6B). A low level of modification also appeared to be present between CD79b and HSV in these cells. In contrast, the region 3′ of the gene remained unmodified. In the U266 cells, which contain only trace levels of CD79b mRNA, a modest peak of modification was identified at the CD79b promoter and at HSV (Fig. 6C). K562 cell chromatin lacked modification throughout the surveyed region (Fig. 6D). The primary B cells from an hGH/P1 transgenic mouse had a strong H3K4me2 peak at the CD79b promoter (probes CDP and P3) and minimal enrichment at HSV (Fig. 6E). Hepatocytes from the same line had low levels of modification at the CD79b promoter (probe CDP) and lacked significant enrichment elsewhere. As was the case in the H3 and H4 acetylation ChIP study, there was no evidence for 3′ extension of H3K4me2 modification within the hGH cluster. The peak of H3K4me2 modification at two adjacent sites in the two expressing cell sources (1484 and transgenic B cells) were mutually confirming. Comparisons among the high- and low-expressing B-cell lines and the nonexpressing K562 cells revealed a direct relationship between gene activation and H3K4 methylation at the CD79b promoter. Analysis of primary B cells and hepatocytes from the hGH/P1 mouse extended these observations. The significance of the H4 acetylation and H3K4 methylation at the CD79b promoter in the hepatocytes remains unclear. Overall, these results reveal that histone modifications associated with chromatin activation in B cells are limited to the immediate vicinity of the hCD79b gene and fully exclude the adjacent hGH cluster.
DISCUSSION

Approximately 20,000 to 25,000 genes are present in the human genome (16). These genes, which for the most part are randomly distributed, are differentially regulated by sets of cis-acting determinants, including enhancers, silencers, insulators, and boundary elements. Since these elements are frequently remote from target promoters, there is a clear potential for genes with distinct expression profiles to cross paths and interfere with each other’s functions (11). Remote regulatory elements can initiate and regulate gene expression via targeted alterations in chromatin structure; these modifications often constitute critical steps in complex developmental pathways. Thus, defining interactions of tightly linked genes necessitates combined structural and functional mapping of chromatin structures in developmentally valid model systems.

The hCD79b/GH locus presents an informative model for analysis of chromatin structures and their developmental regulation. This locus contains six closely packed genes with three distinct tissue specificities: pituitary, placenta, and B cell. In addition, the striated muscle-specific SCN4A gene is juxtaposed 5' of these genes, and a testis-specific TCAM gene is juxtaposed 3' of the locus (Fig. 6). HS formation and histone modifications at this locus have been previously mapped in pituitary and placental chromatin (7, 15, 17, 19). In the pituitary tissue, the hGH LCR establishes a 32-kb acetylated chromatin domain that directly links the LCR with the target hGH-N gene promoter (15). In the placenta tissue, the LCR determinants HSIII-HSV and the target placental genes are each individually acetylated and are separated by a 28-kb region of unmodified chromatin (8, 19). Of note, the CD79b gene is located between the hGH cluster and its LCR. As such, it is situated in the “activated” domain in pituitary chromatin and in a region of hypoacetylated histones in placental chromatin. Significantly, CD79b is robustly transcribed in the pituitary tissue and is inactive in the placenta (5). The “bystander” activation of CD79b in the pituitary tissue is independent of B-cell-specific transcription factors, and as such it appears to
represent a mechanistically independent and epigenetically driven pathway of gene activation (5). Thus, the specific configurations of chromatin modifications at the hCD79b/GH locus in the pituitary and placenta tissues predict divergent pathways of LCR-mediated transcriptional activation and have distinct effects on the adjacent CD79b gene.

To further explore transcriptional interactions at the hCD79b/GH locus, we have mapped its transcriptional determinants and chromatin structures in B cells. HS mapping, which has the capability to scan for critical chromatin determinants (10, 12), identified a set of B-cell-restricted sites, HSB1 and HSB2, and a constitutive site, HSa (Fig. 2). The B-cell specificity of HSB1 and HSB2 suggested that they play a role in CD79b expression. The functional importance of these sites was tested in a series of transgenic mouse lines. The initial studies assessed the expression of hCD79b from transgenes that encompass the entire locus on 123-kb or 87-kb genomic fragments. These levels were then compared to a series of smaller transgenes that isolate the CD79b gene with limited flanking regions (Fig. 3). Three major observations resulted from these studies. First, CD79b expression was maintained within a twofold mean range for all but the smallest transgene, −0.2CD79b. These comparisons led us to conclude that the region between −200 bp and −500 bp upstream from the CD79b gene contains one or more determinants that enhance CD79b expression. Second, the copy number dependence and site-of-integration independence of CD79b expression was maintained in all transgenes, including −0.2CD79b. Whereas we did observe as much as an eightfold variation of expression in some of the transgenes, the remarkable consistency of expression for the −0.2CD79b transgene suggested that an element(s) sufficient to establish an autonomous chromatin domain in B cells is located within or in very close juxtaposition to the hCD79b gene itself. Third, these studies effectively eliminated HSB2 as a critical determinant of CD79b expression. Exclusion of this HS from the CD79b transgene had no appreciable impact on expression levels, and the copy number dependence and B-cell specificity was maintained in its absence. This data set leads us to conclude that control over CD79b is local as opposed to long range, and that the two HS retained in the −0.2CD79b transgene, the B-cell-specific HSB1 and the constitutive HSa, may be sufficient to establish an autonomous B-cell chromatin domain.

Although the −0.2CD79b transgene contained elements sufficient to establish an autonomous chromatin domain, its level of expression was significantly below that of the transgenes with more extensive 5′-flanking regions. As noted above, this comparison suggests that one or more determinants residing in the region between the 5′ termini of −0.2CD79b and −0.5CD79b have transcription-enhancing activity. Prior analyses by others using in vitro and cell transfection approaches (28) have identified a putative B-cell-specific activation element, BCS (for “B29 conserved sequence”), in this region. This BCS can bind to the multifunctional transcription factor Ying Yang 1 (YY1) in vitro (25). YY1, a zinc finger, GLI1-Kruppel family member, can function as either a silencer or an enhancer through interaction with a variety of chromatin-modifying proteins (1, 9, 20). However, the role of this factor in

FIG. 6. Histone H3 lysine 4 dimethylation (H3K4me2) profiles at the hCD79b/GH locus. Experimental details are the same as those described in the legend to Fig. 5, except that anti-H3K4me2 was utilized for immunoprecipitation. The histogram represents a single assay in each case.
CD79b gene expression has not been further evaluated. Previous studies using cell transfection models have also suggested that the minimal promoter of the hCD79b gene, extending to −0.2 kb, exhibits B-cell-specific activity. This minimal promoter region contains EBF, Oct1-2, PU.1 (Ets family), Ikaros, and Sp1 transcription factor motifs (28). Ikaros is essential for normal development and proliferation of lymphoid cells and exists as an integral component of chromatin-remodeling complexes (18, 24). PU.1, a member of the Ets family, is critical for B-lymphocyte development and interacts with the chromatin-modifying enzymes CBP and p300 (29). The activity of these factors may correlate with HS1 formation and function and may contribute to the autonomous chromatin domain established by the −0.2CD79b transe. However, these studies in transfected B cells also suggested that the minimal promoter (−193 bp) has full transcriptional activity, and extension of the 5′-flanking region to −469 bp resulted in a prominent loss of activity (28). These data, which led the authors to propose that repressor determinants are located between −193 and −496, are inconsistent with our transgenic studies. Indeed, we detect enhancer activity in this region based on comparison of the −0.2CD79b versus −0.5CD79b transgenes. Thus, the preceding cell transfection studies, while useful in identifying potential cis-trans interactions at the CD79b promoter, may reflect functional activities that are not in operation during in vivo B-cell differentiation in the intact animal.

The analysis of histone modifications at the hCD79b/GH cluster in B cells (Fig. 5 and 6) revealed that histone H3 and H4 acetylation and H3K4 methylation are predominantly limited to the immediate vicinity of the CD79b promoter. This stands in marked contrast to the distribution of histone modifications at this locus in pituitary and placental chromatin. Therefore, three distinct patterns of chromatin modification are established at the hCD79b/GH locus (Fig. 7). These patterns correspond to three specific gene expression profiles. In the pituitary cell, an extensive domain of histone acetylation links the LCR with the target hGH-N promoter. This pituitary domain, which may be established by linear “spreading” of histone acetyltransferase complexes, encompasses the intervening CD79b gene and results in bystander gene activation. Consistent with this model of bystander activation and its dependence on HS1 action, hCD79b is robustly transcribed in hGH/P1 and −8.0CD79b transgenic mouse pituitary glands but not in the −1.3CD79b, −0.2CD79b, and hGH/P1(HS1) transgenic mouse pituitary glands (5). In the placenta, histone acetylation is limited to the LCR and to the placental genes. These modified regions are separated by an extensive segment of unmodified chromatin containing the pituitary cell-specific LCR determinants HS1 and HSIII, the CD79b gene, and the hGH-N gene. Previous studies have demonstrated that hCD79b, which is excluded from the modified region, is not expressed in the placenta of humans and transgenic mice (5). The discontinuous pattern of histone modification in the placenta predicts a long-distance interaction, or “looping,” between the LCR and the four placental genes to activate expression. Finally, the activating modifications in B-cell chromatin are tightly restricted to the CD79b gene; these modifications exclude all adjacent genes in the region. These data, taken together, reveal a remarkable complexity of chromatin structures at a single locus. These structures impact the expression of neighboring genes in an asymmetric and tissuespecific fashion. How common these sorts of interactions are and how well they represent other multispecificity loci can now be further explored.

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