MOLECULAR AND CELLULAR BIOLOGY, Jan. 1999, p. 671–679 Vol. 19, No. 1
0270-7306/99/$04.00+0

Analysis of Mice with Single and Multiple Copies of Transgenes Reveals a Novel Arrangement for the λ5-V_{preB1} Locus Control Region

PIERANGELA SABBATTINI, ANDREW GEORGIOU, CALUM SINCLAIR, AND NIALL DILLON*

Gene Regulation and Chromatin Group, MRC Clinical Sciences Centre, Imperial College School of Medicine, Hammersmith Hospital, London W12 0NN, United Kingdom

Received 16 July 1998/Returned for modification 7 September 1998/Accepted 19 October 1998

The murine λ5-V_{preB1} locus encodes two proteins that form part of the pre-B-cell receptor and play a key role in B-lymphocyte development. We have identified a locus control region (LCR) which is responsible for coordinate activation of both genes in pre-B cells. Analysis of mice with single and multiple copies of transgenes shows a clear difference in the expression behavior of the genes depending on the transgene copy number. While expression of both λ5 and V_{preB1} in single- and two-copy integrations requires the presence of a set of DNase I hypersensitive sites located 3′ of the λ5 gene, small fragments containing the genes have LCR activity when arranged in multiple-copy tandem arrays, indicating that additional components of the LCR are located within or close to the genes. The complete LCR is capable of driving efficient copy-dependent expression of a λ5 gene in pre-B cells even when it is integrated into centomeric γ-satellite DNA. The finding that activation of expression of the locus by positively acting factors is fully dominant over the silencing effect of heterochromatin has implications for models for chromatin-mediated gene silencing during B-cell development.

The generation of multiple cell types in metazoans requires the establishment of diverse patterns of tissue-specific gene expression. It is now clear that the mechanisms by which this is achieved involve the action of transcription factors in conjunction with changes in chromatin structure. Sequence-specific DNA binding factors, such as MyoD, are capable of determining entire differentiation programs, while chromatin structure is thought to play an important role in the maintenance of specific patterns of expression and transmission of these patterns through the cell cycle. Locus control regions (LCRs) are sequences that mediate reorganization of chromatin and activation of transcription by sequence-specific transcription factors. The defining characteristic of an LCR is the ability to drive gene expression in transgenic mice at any site of integration at levels that are equivalent to those of the gene in its natural location (11). LCRs were first described in the human β-globin and CD2 loci (10, 11). They are composed of clustered DNase I hypersensitive sites (HS) containing binding sites for tissue-specific and ubiquitous factors (3, 33, 38). In the multigene β-globin locus, the LCR HS are located outside the gene cluster and are responsible for activation of all of the genes. In the absence of the sites, the genes give low levels of expression in transgenic mice and expression is highly sensitive to the position of integration of the transgene. Naturally occurring deletions of the β-globin LCR result in inactivation of the locus and conversion to a DNase I-insensitive configuration (8). Rather than insulating the gene from position effects, the β-globin and CD2 LCRs activate expression in a dominant-positive manner.

Differentiation of B cells from the hematopoietic stem cell involves a number of stages characterized by the sequential rearrangement and expression of the heavy- and light-chain immunoglobulin (Ig) loci. The λ5 and V_{preB} genes are early markers of B-cell commitment. They are expressed at the pro- and pre-B-cell stages and silenced in immature and mature B cells. V_{preB1} and λ5 are related to the *V* and *C* genes of the λ Ig locus but are expressed in the germ line configuration (19, 20). The two proteins associate to form the surrogate light chain. In pre-B cells, following rearrangement of the heavy-chain locus, the surrogate light chain acts as a chaperone, mediating transport of the newly synthesized heavy chain μ to the cell surface (35) and together with μ forms part of the pre-B-cell receptor (27). This receptor is thought to mediate signalling by an unknown ligand, which leads to proliferation of pre-B cells that have a productive heavy-chain rearrangement (26). Mice that lack a functional λ5 gene show a drastic reduction in the number of B cells (18). Mutations in λ*14.1*, the human homologue of λ5, are associated with a severe immunodeficiency and almost complete absence of B cells (29).

Expression of the λ5 and V_{preB} genes is B-lineage restricted (27) and is also subject to stage-specific regulation during B-cell development. Although a number of transcription factors have been implicated in B-lineage-specific gene regulation, there is no clear correlation between gene expression at different stages and the presence or absence of specific factors. Binding sites for the transcription factors EBF, E47, Pax-5, and Ikaros are present in the λ5 and V_{preB1} promoters (36, 39). Ectopic expression of EBF and E47 has been shown to activate the genes in an early pro-B-cell line, where they are normally silent (36). In a recent study, the Ikaros protein was shown to form a complex which colocalizes with centromeric γ-satellite DNA, the major component of centromeric heterochromatin in mice (2). The λ5-V_{preB1} locus was found to be associated with the Ikaros-centromere complex in a mature B-cell line that does not express the genes but not in an expressing pre-B-cell line, suggesting that Ikaros might act as a repressor of λ5 and V_{preB1} expression during B-cell development.

In this paper, we describe the identification of a multicomponent LCR in the λ5-V_{preB1} locus. Part of the LCR activity is found in a set of HS located 3′ of the locus, while additional

* Corresponding author. Mailing address: Gene Regulation and Chromatin Group, MRC Clinical Sciences Centre, Imperial College School of Medicine, Hammersmith Hospital, Du Cane Rd., London W12 0NN, United Kingdom. Phone: 44 181 383 8233. Fax: 44 181 383 8338. E-mail: ndillon@rpms.ac.uk.

components are present in small fragments containing either the λ5 or the V_{preB1} gene. Analysis of a transgene integration into centromeric γ-satellite DNA provides information on the relationship between positively acting factors and chromatin-mediated silencing.

MATERIALS AND METHODS

DNA fragments used for generation of transgenic mice. Cosmid 2.41, containing a 30-kb region of the λ5-V_{preB1} locus extending from 8 kb 5′ of V_{preB1} to 15 kb 3′ of λ5, was isolated from a mouse 129SV cosmid library. A 36-bp tag (5′ GAGCAAAAGCTGATTTCTGAGGAGGATCTGGAATTC 3′) was inserted immediately downstream of the first ATG of the λ5 gene by the PCR overlap extension method described previously (15). The following fragments were used to generate transgenic mice: L5F1 (4.5-kb *Pst*I fragment), L5F2 (5.5-kb *Bam*HI-*Pst*I fragment), L5F3 (10-kb *Pst*I-*Bam*HI fragment), L5F4 (11-kb *Bam*HI fragment), L5F5 (12-kb *Eco*RI-*Sph*I fragment), L5F6 (19-kb *Eco*RI-*Bam*HI fragment), and Vp1 (8-kb *Eco*RI-*Bam*HI fragment). The λ5–β-globin construct contains 410 bp extending from the *Pst*I site at −298 to the λ5 ATG fused to the 3.4-kb *Nco*I-*Bgl*II fragment of the human β-globin gene. L5F5, L5F6, and Vp1 each contain a 35-bp tag (5′ GTACCCATACGACGTCCCAGACTACGCGA ATTCGG 3′) in the *Pst*I site of V_{preB1} exon 2.

Transgenic mice. DNA fragments were purified and injected into the pronuclei of C57BL6/CBA F1 mouse eggs as previously described (5). Fetuses were dissected at 16.5 days posttransfer and analyzed as described below. Mosaic animals were excluded from the analysis by measuring the transgene copy numbers in three tissues (placenta, head, and liver). For some constructs, transgenic lines were established and individual transgenics were identified by Southern blotting of tail DNA.

Abelson virus transformation of fetal liver cells. Abelson virus transformation of fetal liver cells was carried out by a modification of the procedure described by Waneck and Rosenberg (41). Fetal livers were disaggregated by multiple passages through a 25-gauge needle in 1× RPMI, 20% fetal calf serum (FCS), 50 μM 2-mercaptoethanol, 50 μg of gentamicin/ml. A 1-ml aliquot of fetal liver cells (2×10^6/ml) in 1× RPMI medium was infected with 1 ml of filtered supernatant of a culture of Abelson murine leukemia virus producer line ABO10 grown to saturation in Dulbecco modified Eagle medium–10% FCS–50 μg of gentamicin/ml. The infection was carried out at 37°C and 5% CO_2 for 4 h in the presence of 4 μg of Polybrene/ml. The infected cells were then plated in 1× RPMI–20% FCS–50 μM 2-mercaptoethanol–0.3% agar (Noble-Difco). After 8 to 12 days, colonies were picked from the agar and expanded in liquid culture in 1× RPMI medium.

Pre-B-cell primary culture system. The 16.5-day fetal livers were disaggregated, and the fetal liver cells were resuspended in a solution of 1× RPMI, 20% FCS, 50 μM 2-mercaptoethanol, 50 μg of gentamicin/ml, and 1 ng of interleukin 7 (IL-7)/ml. The cell suspensions were allowed to form their own feeder layers and were cultured for up to 11 days. When cultures were maintained for longer periods (up to 3 weeks), the cells were subcultured onto ST-2 stromal cell feeder layers (32, 34).

DNA analysis. DNA was extracted from one-fourth of each fetal liver as well as from the fetal head and placenta. After phenol extraction and digestion with restriction enzymes, 5 μg of each sample was separated on 0.6% agarose gels. The transgene fragments were distinguished from the endogenous genes by the presence of an *Eco*RI site in the λ5 and V_{preB1} tags. Southern blotting and hybridization with nick-translated probes was carried out by standard procedures. The blots were scanned with a phosphorimager, and the results were used to calculate the transgene copy number. Single-copy integrations were confirmed by end fragment analysis (5). Transgene integrity was verified by a combination of Southern blotting with restriction fragments specific for the transgene and PCR analysis with one primer derived from the oligonucleotide tag and a second primer from the 5′ or 3′ region of the gene.

RNA analysis. RNA was obtained from 16.5-day fetal livers or transgenic pre-B-cell lines by lithium chloride extraction (reference 1; described in detail in reference 5) and subjected to RNase protection analysis. The riboprobes were synthesized from 1 μg of pGEMT-derived DNA template by using the Promega riboprobe system-Sp6 kit. The Promega RNase ONE kit was used for the RNase protection assay, following conditions of hybridization and RNase ONE digestion suggested by the manufacturer. Briefly, the riboprobe was resuspended in 100 μl of hybridization buffer, and the RNA samples were dried under vacuum and resuspended in 25 μl of hybridization buffer–5 μl of riboprobe. The hybridization was carried out overnight at 42°C. Digestion was carried out with 1.5 U of RNase ONE per μg of RNA, in RNase ONE buffer at 33°C for 1 h. The digested RNA samples were ethanol precipitated and separated by electrophoresis on a 4% polyacrylamide–8 M urea sequencing gel. The protected bands were quantified on a phosphorimager, and the transgene expression level was normalized to the expression derived from a single endogenous allele. RNA analysis in tissues that do not express the endogenous λ5 and V_{preB1} genes was carried out with the β-actin transcript as a loading control. For this purpose, a riboprobe complementary to a 305-bp region of β-actin mRNA (obtained by PCR with primers 5′ GGGCGCCCGGTTCTTTTTG 3′ and 5′ ACACCCAGCCGGCCA CAGTCG 3′ and cloned in pGEMT) was labelled at 1/10 of the specific activity of the λ5 probe and was added to the hybridization mixture.

DNase I HS mapping. Nuclei were prepared from a minimum of 2×10^8 cells as described previously (8), using 20 strokes of a B-type Dounce homogenizer. The nuclei were resuspended in 1 ml of 1× reticulocyte standard buffer, and aliquots of 100 μl were digested with increasing volumes of 0.05 μg of DNase I/ml (from 0.5 to 10 μl) for 4 min at 37°C. The reactions were stopped, the products were digested with proteinase K, and the DNA was phenol-chloroform extracted and ethanol precipitated. The DNA was resuspended in 100 μl, and 20 μl was digested with restriction enzymes and separated by electrophoresis on a 0.6% agarose gel in 1× Tris-borate-EDTA and analyzed by Southern blotting (see the legend to Fig. 2 for details of the probe).

PCR analysis of transgene integration in γ-satellite DNA. The strategy used for PCR analysis of transgene integration in γ-satellite DNA was based on the tagged-primer method of Jeffreys et al. (16). The sequences of the primers used were as follows: primer 1, 5′ TCATGCGTCCATGGTCCGGGGACCTGGAA TATGGCGAG 3′; primer 2, 5′ CCGGTTGTGGTTGGGATGC 3′; and primer 3, 5′ TCATGCGTCCATGGTCCGG 3′. Thirty picomoles of primers 2 and 3 and 0.5 pmol of primer 1 were used in a 30-μl PCR to amplify 25 ng of genomic DNA in PCR buffer (10 mM Tris-HCl [pH 8.3], 50 mM KCl, 1.5 mM $MgCl_2$, 0.001% gelatin), 0.2 mM deoxynucleoside triphosphates, and 0.5 U of Amplitaq (Perkin-Elmer). The PCR conditions of amplification were derived from the protocol of Jeffreys et al. (16). The first nine cycles (94°C for 45 s; 55°C for 1 min; 72°C for 2 min 30 s) allowed amplification between the γ-satellite-specific primer 1, containing at the 5′ end a tag of 19 nucleotides (nt), and the λ5-specific primer 2. These were followed by 11 cycles (94°C for 45 s; 66°C for 1 min; 72°C for 2 min 30 s, with an increment of 10 s/cycle). The higher annealing temperature used for the second round of cycles favored amplification from primer 2 and primer 3 (corresponding to the tag of oligonucleotide 1) of the product generated in the first nine cycles.

FISH. For fluorescence in situ hybridization (FISH) cells were hypotonically swollen in 0.056 M KCl and fixed in 3:1 methanol-acetic acid, and slides were made. The slides were pretreated with 100 μg of RNase A/ml in 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) for 1 h at 37°C, washed in 2× SSC, and put through an ethanol dehydration series (70, 90, and 100% ethanol). The chromosomes were denatured at 70°C for 5 min in 70% formamide–2× SSC, plunged into ice-cold 70% ethanol, and dehydrated as before. One hundred nanograms of probe (fragment L5F4 labelled with biotin by nick translation) was precipitated with 1 μg of cot-1 DNA and 5 μg of salmon sperm DNA; resuspended in 50% formamide–2× SSC–1% Tween 20–10% dextran sulfate; denatured at 75°C; preannealed for 15 min at 37°C; and applied to the slides. Hybridization was carried out overnight at 37°C. The slides were washed four times for 3 min each time in 50% formamide–2× SSC at 45°C, four times for 3 min each time in 2× SSC at 45°C, and four times for 3 min each time in 0.1× SSC at 60°C. After being washed for 5 min in 4× SSC–0.1% Tween 20, the slides were blocked for 5 min in 4× SSC–5% low-fat skim milk. The biotin was detected by 30 min of incubation at 37°C with each of the following: avidin-conjugated fluorescein isothiocyanate (FITC) (Vector) followed by biotinylated anti-avidin (Vector) and avidin-conjugated FITC. Between every two incubations, the slides were washed three times for 2 min each time in 4× SSC–0.1% Tween 20. The slides were mounted and counterstained with 1.25 mg of DAPI (4′,6-diamidino-2-phenylindole) in Vectashield (Vector). Images were examined with an oil ×100 objective on a Leica DMRB fluorescence microscope with a Pinkel no. 1 filter set. The images were captured with a Photometrics cooled charge-coupled device camera and Vysis Smartcapture software.

RESULTS

A minimal λ5 transgene expresses efficiently in mice with multiple copies of transgenes but not in mice with single-copy integrations. In order to identify the control elements involved in the transcriptional regulation of the λ5 gene, we carried out a functional analysis in transgenic mice, using fragments containing the gene and various amounts of flanking sequence. A 36-bp sequence tag was inserted immediately downstream of the ATG, allowing a direct comparison of the levels of transgene and endogenous transcripts by RNase protection analysis. Since the fetal liver contains large numbers of pro- and pre-B cells, expression was measured in livers from fetuses dissected at 16.5 days postinjection, when λ5 expression was maximal. In addition, pre-B cells from some transgenic livers were immortalized with Abelson murine leukemia virus to produce clonal transgenic pre-B-cell lines. A third approach involved culturing cells directly from fetal livers in the presence of IL-7. Culture under these conditions results in preferential expansion of pre-B cells (see Materials and Methods). The use of transformed cell lines and primary cultures containing relatively

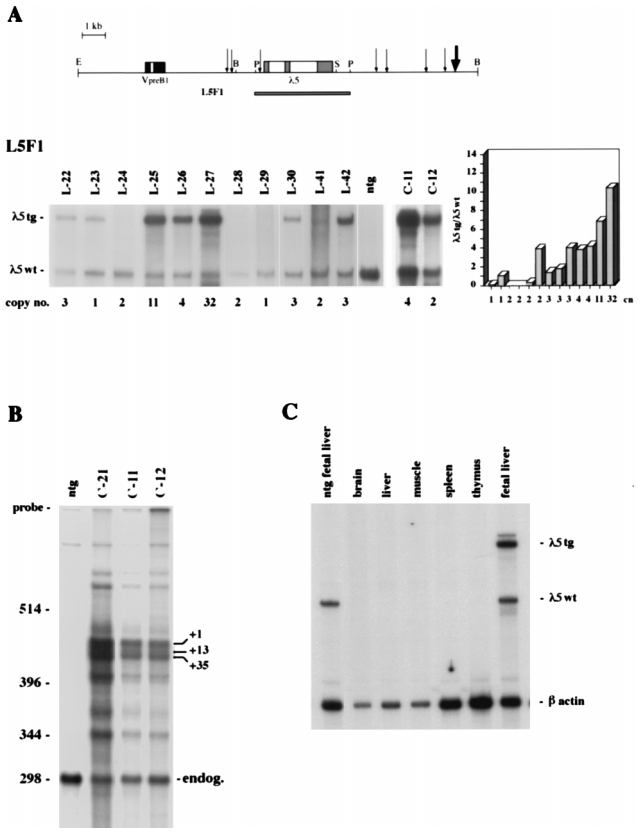

FIG. 1. (A) Expression analysis of λ5 in mice transgenic for fragment L5F1. The map shows the λ5-V_{preB1} locus and the location of fragment L5F1. B, *Bam*HI; E, *Eco*RI; P, *Pst*I; S, *Sph*I. Analysis was carried out on 25 μg of RNA from 16.5-day fetal livers (samples L−) and 3 μg of RNA from Abelson virus-transformed pre-B-cell lines (samples C−). The probe was a 498-bp sequence (probe A) derived from the λ5 cDNA, extending from the *Bgl*II site at +65 (downstream of all mapped transcription initiation sites) to the *Rsa*I site in exon 3 and including a 36-nt tag immediately downstream of the first ATG of the gene (see Materials and Methods). The protected fragments were 498 bp for the λ5 transgene (tg) and 412 bp for the endogenous λ5 (wild type [wt]). The transgene copy number is indicated at the bottom of each lane. Band intensities were quantified with a phosphorimager. The histograms show the expression level (normalized to the expression of one allele of the endogenous λ5) versus the copy number of each transgenic mouse. (B) Mapping of transcription initiation sites of the λ5 transgene in cell lines (C-11 and C-12) transgenic for the L5F1 fragment. The pattern of initiation was compared with that of a cell line (C-21) transgenic for the complete locus (2 kb 5′ of V_{preB1} to 15 kb 3′ of λ5) and with the nontransgenic pre-B-cell line 122-1 (ntg). RNase protection was carried out with 5 μg of RNA for line C-21 (which contains 16 copies of the large locus fragment) and 10 μg of RNA for lines C-11, C-12, and 122-1. The probe (probe B) extends from 281 bp upstream of the first ATG of the gene to the end of the 36-nt tag (see Materials and Methods). The segment of the probe downstream of the ATG is derived from the λ5 cDNA and extends to the *Avr*II site 305 bp from the ATG. This probe includes all previously mapped λ5 transcription initiation sites. The numbers refer to the sizes of the marker bands. As the riboprobe contains the tag, all the transcripts derived from the endogenous λ5 give rise to a protected fragment of 305 bp (endog.). The major transcription starting sites correspond to those mapped for the wild-type λ5 (+1, +13, and +35) (23, 25). Readthrough transcripts would give an additional protected fragment of 622 bp and are not present in significant amounts. (C) RNA was analyzed from brain, liver, spleen, muscle, and thymus tissues obtained from 10-week-old animals from line 42 and was compared with expression in 16.5-day fetal liver (L-42) from the same line. In the RNase protection assay, 25 μg of RNA from fetal liver and 40 μg of RNA from the other tissues were hybridized to probe A. A β-actin-specific riboprobe was added to each reaction mixture as a loading control.

pure populations of λ5- and V_{preB1}-expressing cells gave an increased signal for both the endogenous genes and the transgenes, facilitating quantitation of transgene expression.

Figure 1A shows the analysis of a fragment (L5F1) containing the λ5 gene with 410 bp of sequence upstream of the ATG and 1 kb downstream of the poly(A) site. It can be seen that a proportion of the transgenics (e.g., L-26, L-42, and C-11) express the λ5 gene at levels (per transgene copy) that are comparable to the levels from the endogenous gene. In addition to the expressing transgenics, a number of animals (L-24, L-28, L-29, and L-41) either fail to express the transgene at all or express it only at very low levels. Inspection of the transgene copy numbers (Fig. 2) shows that the expressing animals consistently carry three or more copies of the gene while those that fail to express the transgene or express it at low levels have one or two copies. Multiple-copy transgenes in mice are arranged as head-to-tail tandem repeats, raising the possibility that some of the signal might be the result of readthrough transcription through the array. This possibility was excluded by analyzing the λ5 transcripts with a probe that extends across the promoter (Fig. 1B). The results obtained with fragment L5F1 lead us to conclude that the tandem arrangement of the transgene creates a structure which can drive efficient position-insensitive expression in chromatin and therefore has some of the properties of an LCR. Expression of the λ5 gene in this and other constructs analyzed in this study reached a plateau in animals with high copy numbers (>20 copies). This plateau effect, which has also been observed for the β-globin LCR (37), could be due to limiting levels of transcription or RNA stabilization factors.

To determine whether expression from L5F1 showed correct tissue-specific regulation, lines of transgenic mice carrying the fragment were generated. Figure 1C shows the analysis of

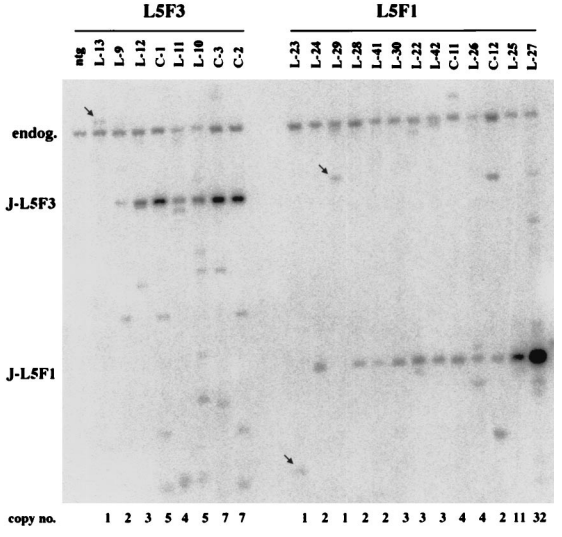

FIG. 2. Transgene copy number analysis for L5F1 and L5F3 transgenic mice. Five micrograms of fetal liver DNA (samples L−) or pre-B-cell line DNA (samples C−) was digested with *Eco*RI, Southern blotted, and hybridized to a 446-bp λ5 probe extending from the *Pst*I site at position −296 to the *Eco*RI site at the 3′ end of the tag. Since the probe is located at the ends of the constructs, it detects end fragments of different sizes, depending on the location of *Eco*RI sites close to the site of integration, and joining fragments resulting from digestion of multiple-copy head-to-tail tandem repeats. The intensities of the bands were measured by phosphorimager. endog., endogenous λ5 band; J-L5F1 and J-L5F3, joining fragments for the two constructs; ntg, nontransgenic cell line. Mice with a single copy of the transgene give one end fragment (indicated by the arrows) and no joining fragment. The integrity of the single- and two-copy integrations was verified as described in Materials and Methods. The presence of more than one end fragment indicates that there have been additional integrations (although some of the smaller fragments observed in mice with multiple copies of the transgene are likely to be degradation products). Since copy numbers of animals with multiple copies have been calculated from the intensities of the joining bands, they refer only to the number of copies in the tandem array.

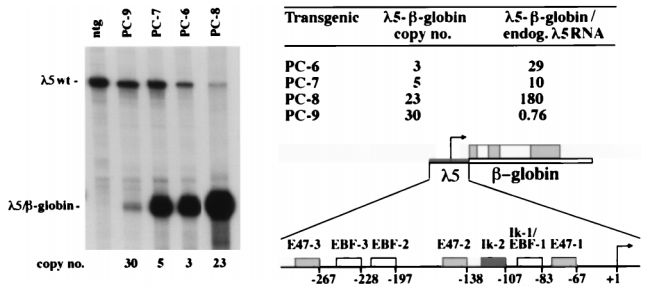

FIG. 3. Expression analysis of the human β-globin gene under the control of the λ5 promoter. The level of human β-globin transcript was compared to the level of the endogenous λ5 by using probes specific for the two genes. RNase protection was carried out by hybridizing 20 μg of RNA extracted from fetal liver cell primary cultures (PC−) grown in the presence of IL-7 to a β-globin-specific probe (probe D) containing the 296-bp *Nco*I-*Bam*HI fragment of the β-globin cDNA. The λ5-specific probe A was also included in the hybridizations. The table shows the level of expression of β-globin normalized to the expression of one endogenous (endog.) λ5 allele. The schematic diagram of the λ5 promoter shows binding sites for EBF, E47, and Ikaros (Ik) together with their positions relative to the major transcription start site. ntg, nontransgenic cell line.

different tissues from one of these lines, which carried three copies of the transgene. Copy-dependent expression of the transgene was observed in fetal liver, while no expression was observed in the spleen, thymus, liver, muscle, and brain. We conclude from this that fragment L5F1 contains sufficient information to give efficient, tissue-specific expression of the λ5 gene. Since immature and mature B cells make up 50% of spleen cells, we can also conclude that transgene expression is silenced at these stages and is therefore subject to stage-specific regulation during B-cell development.

A 410-bp λ5 promoter fragment is capable of giving high-level expression of a reporter gene. A recent study has shown that the ectopic expression of the transcription factors EBF and E47 activates endogenous λ5 and V_{preB1} expression in an early pro-B-cell line (36). Since the λ5 promoter contains binding sites for these factors, we considered the possibility that the promoter directly contributes to the LCR-like activity observed with tandem repeats of L5F1. To test whether this was the case, a 410-bp fragment 5′ of λ5 (from the *Pst*I site to the ATG of the gene) was cloned upstream of the coding region of a human β-globin gene. This region contains approximately 300 bp upstream of the main transcription initiation site for λ5 (Fig. 3). Pre-B cells from livers of 16.5-day fetuses transgenic for this construct were cultured in the presence of IL-7 (see Materials and Methods and the legend to Fig. 3). Fluorescence-activated cell sorter analysis of the cultured cells showed that they were >99% B220 positive. The four transgenics obtained carried the fragment in multiple-copy tandem arrays (between 3 and 30 copies), and all expressed the λ5-β-globin fusion transcript (Fig. 3). Three animals (PC-6, PC-7, and PC-8) gave an expression per copy that was between two- and eightfold higher than that of endogenous λ5. The stronger signal observed for the transgene could be due in part to differences between the hybridization efficiencies of the two probes, but it could also be caused by the greater stability of the β-globin transcript. The fourth transgenic (PC-9), which had the highest copy number (30 copies), expressed the transgene at a level that was much lower than expected. Thus, the promoter alone is able to drive high-level expression of the transgene in pre-B cells of mice with multiple copies of the transgene, although this expression is more sensitive to position effects than that obtained with the 4.5-kb minimal λ5 transgene (L5F1). As with all experiments using reporter gene constructs, we cannot say whether the increased sensitivity to position is due to the absence of sequences in the λ5 gene or the introduction of foreign sequences in the reporter gene that might interfere with interactions in the tandem repeats.

Location of candidate LCR sequences by DNase I HS mapping. The minimal 4.5-kb λ5 gene fragment, while displaying LCR activity in multiple-copy integrations and giving tissue-specific expression of λ5, was unable to promote efficient transcription in transgenic mice carrying one or two copies of the transgene. Since the gene in its normal location is present as a single copy, we set out to determine whether additional flanking sequences could give efficient expression at low copy numbers. Previous reports of a series of DNase I HS 3′ of the λ5 gene (42) suggested to us that this region might contain part of the LCR. To determine the precise location of the HS surrounding the gene, we carried out DNase I sensitivity analysis on the flanking sequences in nuclei from the pre-B-cell line 122-1 (obtained by Abelson virus transformation of fetal liver cells) and the B-cell line WEHI-231. The results are shown in Fig. 4.

In the pre-B-cell line 122-1, the region containing the λ5

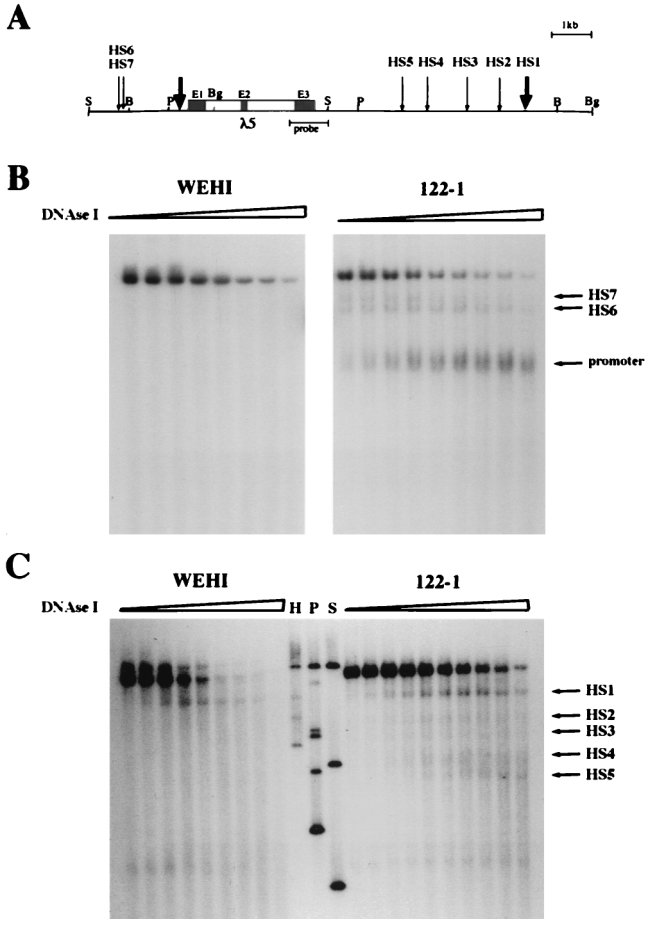

FIG. 4. DNase I HS mapping of the regions flanking the λ5 gene. (A) Map of the λ5 gene and surrounding regions. B, *Bam*HI; Bg, *Bgl*II; P, *Pst*I; S, *Sph*I. (B) DNase I HS 5′ of λ5. Pre-B-cell line 122-1 and B-cell line WEHI-231 nuclei were digested with increasing amounts of DNase I, and genomic DNA was extracted and digested with *Sph*I, followed by electrophoresis and Southern blotting. The blot was hybridized to a probe spanning the third exon of λ5 (0.6-kb *Sac*I-*Sph*I fragment). (C) DNase I HS 3′ of λ5. DNase I-treated DNA was digested with *Bgl*II and probed with the 0.6-kb *Sac*I-*Sph*I fragment. HS1 to -6 were mapped against restriction fragments derived from partial digestion of the *Bgl*II fragment with *Hin*dIII (H), *Pst*I (P), and *Sph*I (S).

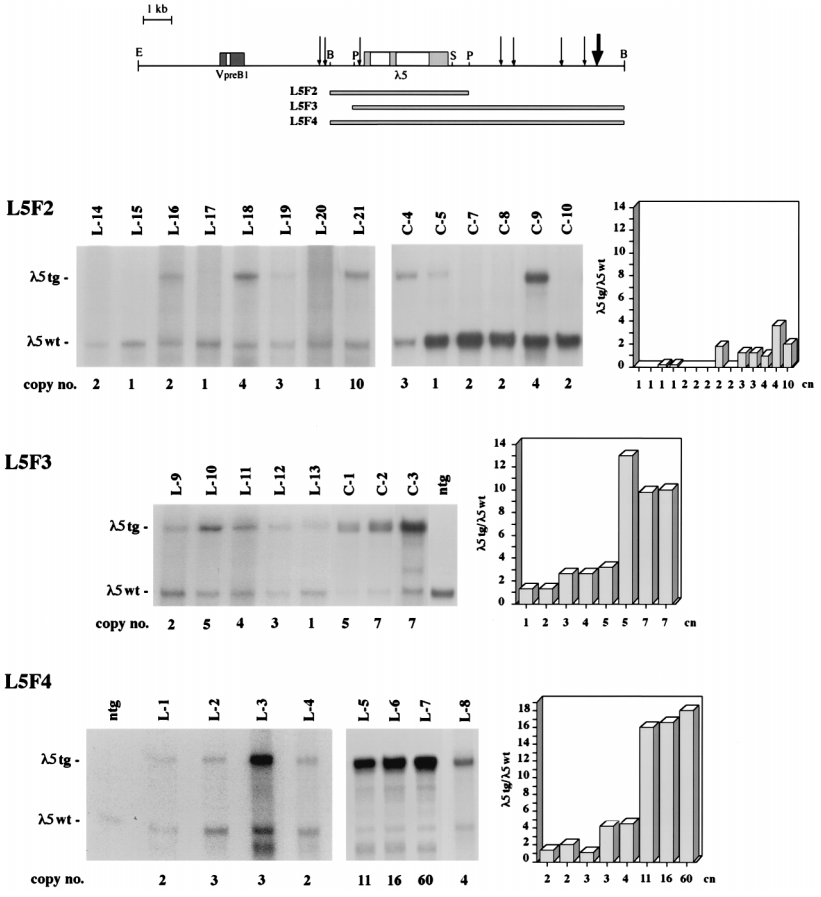

FIG. 5. Expression analysis of λ5 in mice and Abelson virus-transformed pre-B-cell lines transgenic (tg) for fragments L5F2, L5F3, and L5F4. A map of the fragments is shown at the top. The RNase protection assay was carried out as described in Materials and Methods and in the legend to Fig. 1. wt, wild type; cn, copy number.

promoter is strongly hypersensitive and two additional HS (HS6 and HS7) are detected approximately 1.5-kb 5′ of the gene (Fig. 4B). No HS were found in the 5′ region of λ5 in the B-cell lines WEHI-231, J558L, and Bal-17 (Fig. 4B and data not shown). A total of five HS were detected between 2 and 6 kb downstream of the λ5 gene in cell line 122-1 (Fig. 4C). The strongest of these sites, HS1, is also found in IgM-producing WEHI-231 cells, which are derived from a B-cell lymphoma and represent the immature-B-cell stage (21) (Fig. 4C). HS1 was also observed in the Ig λ-expressing myeloma cell line J558L (13) and in the mature-B-cell line Bal-17 (17), which do not express λ5 or V_{preB} (data not shown). The remaining sites (HS2 to -5) are only observed in the pre-B-cell line. A set of HS were previously identified in the same region in the early-pre-B-cell line 70Z/3 (42), while no HS were found 3′ of λ5 in J558L cells in the same study. Our finding that HS-1 is present in WEHI-231, Bal-17, and J558L cells shows that this site is present after the genes have been silenced during B-cell maturation. The discrepancy between our results and those of Yang et al. may be due to the use of different sublines of J558L.

Position-insensitive expression of λ5 in mice with one and two copies of the transgene requires the presence of the 3′ HS. To investigate the sequence requirements for expression of λ5 in single-copy integrations, constructs containing additional 5′ and 3′ flanking sequences (Fig. 5) were analyzed in transgenic fetal livers and Abelson virus-transformed cell lines. The addition of 1 kb of 5′ sequence (L5F2) failed to rescue the impaired expression in animals with one and two copies of the transgene, as was observed in L5F1. In fact, the additional sequences appeared to further reduce the functioning of the transgene, with low expression observed in four of five mice with multiple copies of the transgene (L-19, L-21, C-4, and C-9). The 5′ region does not include HS6 and -7, and no other HS have been mapped within it. The weak expression of L5F2 suggests that the LCR effect of tandem repeats may be quite sensitive to the configuration and/or sequence composition of the repeated fragment.

In contrast, the addition of a 6-kb region containing the HS 3′ to the minimal λ5 gene gave good expression in an animal with a single copy of the transgene and in an animal with two copies (L5F3, L-13, and L-9; see Fig. 2 for copy number determination). Fragment L5F4, which contains both 5′ and 3′ flanking sequences, also expressed the gene efficiently in two animals carrying two transgene copies (L-1 and L-4). Both constructs gave copy-dependent expression in mice with multiple copies of the transgene. Expression of L5F4 in adult animals from line L8 was also found to be tissue and stage specific (data not shown). A strong positive position effect was observed in one of the L5F3 transgenics (C-1). This was not unexpected, since it is known that LCRs do not insulate transgenes from positive position effects (4). We conclude from these results that the presence of the 3′ HS rescues expression of λ5 in single- and two-copy integrations and counteracts the inhibitory effect of the 5′ sequences in mice with multiple copies of the transgene. Our data indicate that the 10-kb frag-

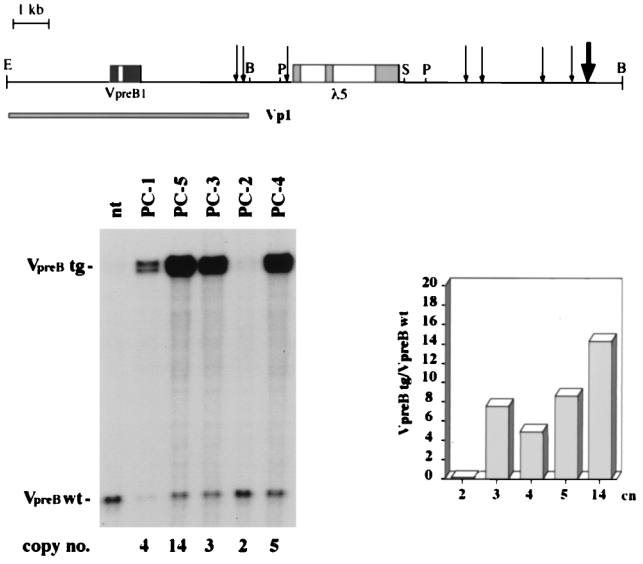

FIG. 6. Expression analysis of V_{preB1} in primary cultures of fetal liver cells transgenic for fragment Vp1. The primary cultures were grown as described in Materials and Methods. Twenty micrograms of each RNA was used in the RNase protection assay. The V_{preB}-specific probe (probe C) spanned the *Kpn*I-*Acc*I region of V_{preB1} exon 2 and contained a 35-bp tag (see Materials and Methods) inserted in the *Pst*I site. The size of the protected fragments is 288 bp for the V_{preB1} transgene (tg) and 146 bp for the endogenous V_{preB1} (wt). Mice have a second V_{preB} gene (V_{preB2}), which differs from V_{preB1} by a single-base substitution in the protected region. We cannot exclude the possibility that some of the endogenous signal arises from V_{preB2} as a result of incomplete RNase digestion at the base mismatch. cn, copy number.

ment containing the 3′ HS together with the λ*5* gene and 300 bp of promoter sequence has the properties of an LCR.

Coordinate regulation of the V_{preB1} and λ*5* genes. The proximity of the λ*5* and V_{preB1} genes to one another and the fact that they are coexpressed raises the possibility that they are coordinately regulated by shared elements. To compare the regulation of the two genes, we generated transgenics by using a fragment containing only the V_{preB1} gene (Fig. 6). Because the smaller probe used to analyze V_{preB1} expression gave a weak signal for RNA extracted from whole fetal liver, V_{preB1} expression was analyzed only in cell lines and primary fetal liver cell cultures. Five transgenics were obtained for the fragment Vp1 and analyzed by culturing pre-B cells from fetal liver in IL-7. Four of the transgenics contained the gene in multiple-copy tandem arrays (3 to 14 copies) and gave efficient copy-dependent expression, apart from one positive position effect (PC-3). A fifth animal (PC-2) contained two copies of the transgene and expressed it at very low levels. Thus, the V_{preB1} transgene appears to behave in a way similar to that of λ*5*, suggesting that it might also depend on the presence of the 3′ HS for efficient expression in single-copy integrations. To directly test whether this is the case, we analyzed the functioning of large constructs containing both genes in the presence and absence of the 3′ HS (L5F6 and L5F5) (Fig. 7). The analysis of λ*5* expression for the two constructs is shown in Fig. 7A. A single-copy transgene containing the 3′ HS gave good expression (Fig. 7A, L5F6, lane C-20), while a single-copy integration lacking the 3′ HS gave expression that was sevenfold lower (Fig. 7A, L5F5, lane C-15). Expression of V_{preB1} was reduced by a similar amount in the single-copy integration that lacked the 3′ HS (Fig. 7B, compare lanes C-20 and C-15). Interestingly, the relative levels of V_{preB1} observed in the mouse with 14 copies of the transgene L5F5, C-13, are 2.5-fold lower than those of λ*5*, suggesting a loss of coordinate regulation of the

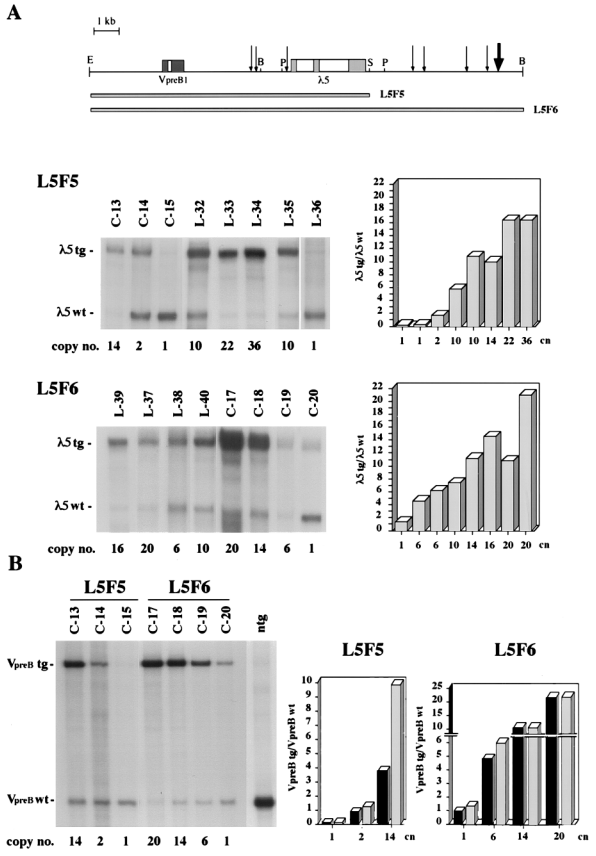

FIG. 7. (A) Analysis of λ*5* expression in mice transgenic for fragments L5F5 or L5F6. The RNase protection assay for the analysis of λ*5* expression was carried out as described in Materials and Methods and in the legend to Fig. 1. (B) RNase protection analysis to measure V_{preB1} expression. Ten micrograms of RNA from transgenic pre-B-cell lines was hybridized to probe C (see the legend to Fig. 6). The histograms compare the expression of V_{preB1} (solid bars) with that of λ*5* (shaded bars) for each cell line. tg, transgene; wt, wild type; ntg, nontransgenic cell line; cn, copy number.

genes in this construct. The finding that V_{preB1} alone and in conjunction with λ*5* fails to express efficiently in transgenics with low copy numbers (Fig. 6, lane PC-2, and Fig. 7B, lane C-15) leads us to conclude that the 3′ HS are involved in activating both genes and form part of an LCR for the entire locus. The importance of the 3′ region is further illustrated by the summarized data for all of the constructs shown in Table 1.

TABLE 1. Transgene expression levels in mice or cell lines transgenic for L5F1-6 and Vp1

3′ HS[a]	Expression level[b]	No. expressing/total mice with:	
		<3 repeats	≥3 repeats
−	None	8/19	0/21
	Low	7/19	7/21[c]
	Good	4/19	14/21
+	None	0/5	0/19
	Low	0/5	2/19
	Good	5/5	17/19

[a] +, present; −, absent.
[b] Low expression is defined as a level of expression per copy which is less than half the level of the endogenous gene.
[c] Four of the seven low expressors in this category were transgenic for fragment L5F2.

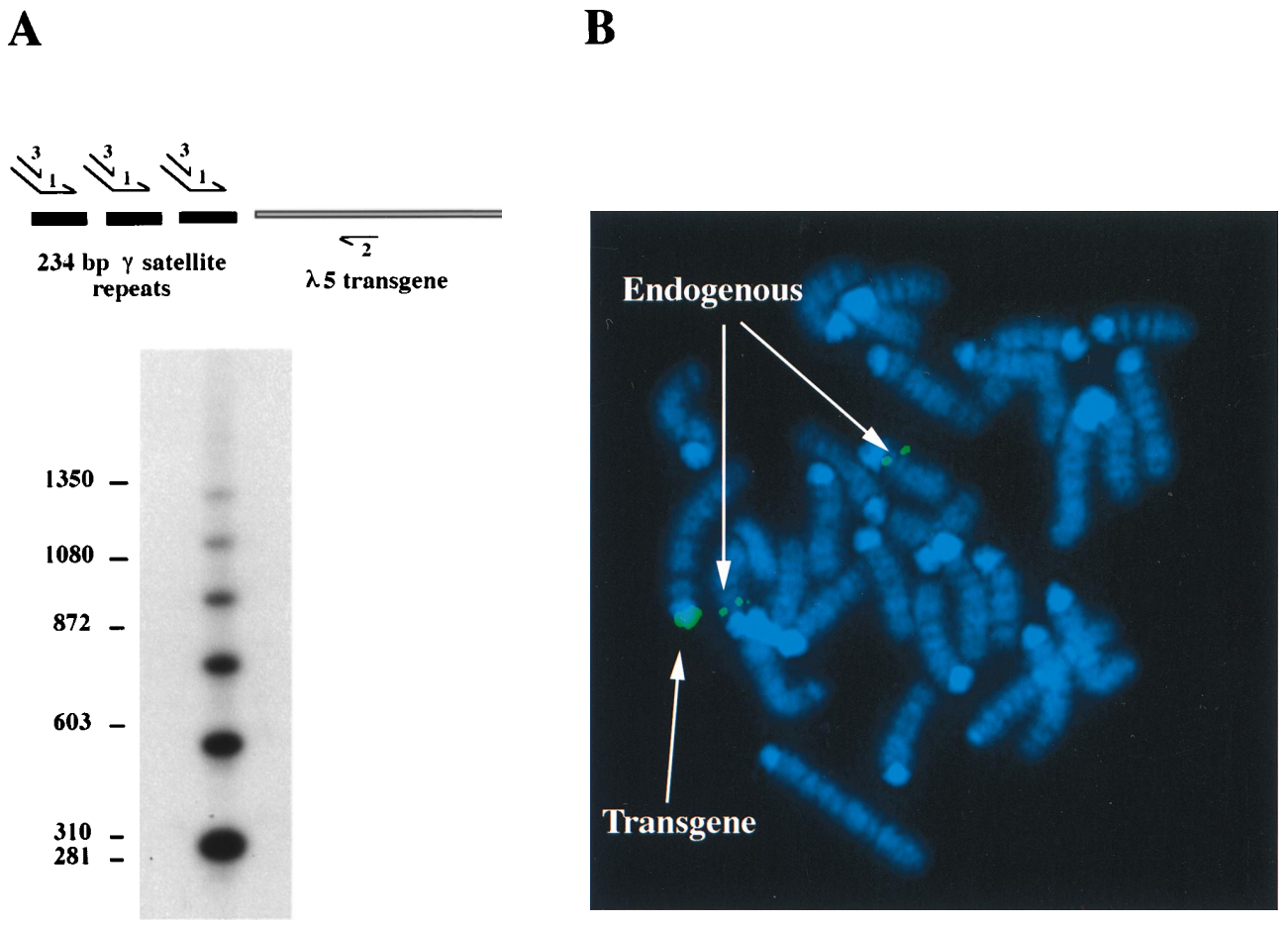

FIG. 8. (A) PCR amplification of the λ5 transgene integrated in γ-satellite DNA in pre-B-cell line C-3. A scheme of the PCR procedure described in Materials and Methods is shown. The PCR product was detected by Southern blotting and probing with a λ5-specific probe. (B) Centromeric localization of the λ5 transgene in pre-B-cell line C-3 by FISH.

Of 19 transgenics with low copy numbers for constructs that lack the 3′ HS, 15 either failed to express the transgenes or expressed them at low levels. In the presence of the HS, five of five transgenics with low copy numbers gave good expression.

The λ5 LCR can activate transcription in centromeric heterochromatin. A defining feature of LCRs is the fact that they are dominant over inhibitory position effects, including those that result from integration close to heterochromatin (7, 28). In order to test whether the λ5 LCR can function efficiently in centromeric heterochromatin, we used a novel PCR-based assay to screen the λ5 transgenics for integrations into γ-satellite DNA, the major satellite component of centromeric heterochromatin in the mouse. The advantage of this approach is that it detects transgenes that are directly integrated into centromeric satellite DNA and provides an extremely stringent test of whether an LCR can function in heterochromatin. Amplification of DNA from cell line C-3 (L5F3) gave a ladder of fragments which was diagnostic for the presence of γ-satellite repeats flanking the transgene (Fig. 8A). Cloning and sequencing of the PCR product showed that λ5 and γ-satellite sequences were present on the same fragment. FISH analysis confirmed that the transgene was indeed located in centromeric DNA (Fig. 8B). Transgenic C-3 contains seven copies of the transgene and expresses it at 10 times the level of each endogenous λ5 allele (Fig. 5). This is within the range of variation for the RNase protection assay and shows that the λ5 LCR gives full copy-dependent expression even when integrated into constitutive heterochromatin.

DISCUSSION

LCRs are dominant activating sequences that are able to activate gene expression at any location in the genome. In this study we describe the identification of an LCR in the pro- and pre-B-cell-specific λ5-V_{preB1} locus. Detailed characterization of the LCR involved a comparison of expression in transgenic mice with low copy numbers (one or two copies) and with multiple copies of the transgene (greater than three copies). This analysis has revealed two quite different types of behavior, depending on the transgene copy number. In transgenic mice with one or two copies, efficient and position-independent expression of both genes requires the presence of a region located 3′ of the locus. This region resembles previously described LCRs, which are composed of clusters of DNase I HS. However, a shorter fragment containing the λ5 gene and lacking the 3′ HS gave full position-independent and copy-dependent expression when present in tandem repeats of three or more copies. Similar behavior was observed with a fragment containing the V_{preB1} gene. We conclude from these results that the shorter fragments contain elements that are capable of functioning as LCRs when they are reiterated in multiple-copy tandem repeats. Since these elements can cooperate to give

rise to an LCR effect, it is reasonable to suppose that they form part of the LCR for the complete locus.

What are the sequences that are responsible for the LCR-like behavior of the minimal gene fragments in tandem repeats? Within the minimal λ*5* gene fragment, the strong HS on the λ*5* promoter is the only HS that has been detected, making it a good candidate for mediating the LCR effect observed with tandem repeats of this fragment. This is supported by the observation that the promoter has enhancer activity in transient expression assays (25, 42) and the observation in this study that a 410-bp λ*5* promoter fragment linked to a β-globin reporter gene gave expression in all of the multiple-copy transgenic mice analyzed. The levels of expression observed were also much higher than is generally observed with minimal promoter fragments. For example, the promoter of the related λ*1* Ig gene gives no expression in mice with multiple copies of the transgene in the absence of additional distal HS (6, 12, 22a). However, the expression from the λ*5* promoter was not completely position insensitive, possibly because of interference by the β-globin sequences within the repeated unit. A similar interference is also observed when a region of 1 kb 5′ of the gene is added to the minimal λ*5* fragment (L5F2), indicating that the configuration of the repeated unit is critical for the LCR effect in tandem repeats.

The organization of the λ*5*-V_{preB1} LCR has a number of important implications for ideas about LCR organization and function in general. The distribution of the LCR components throughout the locus is quite different from that of the human β-globin locus, where the LCR is a discrete unit located some distance from the gene cluster (11). The difference between the λ*5*-V_{preB1} and β-globin LCRs highlights two different aspects of LCRs, namely, their role as dominant-positive activators and the specific organization of LCR HS in individual loci. The dominant activation function is clearly a widespread phenomenon and is arguably the most important and defining feature of LCRs. On the other hand, the arrangement of LCR components appears to be quite flexible and is likely to be as much a product of evolutionary contingency as of selection for specific functions.

A second implication of our results is the need to analyze single- and multiple-copy integrations when analyzing gene regulation in transgenic animals. An analysis that included only integrations of three or more transgene copies would have given a quite different picture of the organization of the regulatory elements in the λ*5*-V_{preB1} locus and would not have detected the role of the 3′ HS. An additional conclusion from our study relates to recent findings that suggest that arrangement in multiple-copy tandem repeats has an intrinsic silencing effect on transgene expression (9, 14). Our results indicate that the opposite effect (repression at single-copy levels and activation at multiple-copy levels) is just as likely to be observed, suggesting that repeats amplify the effects of inhibitory or activating sequences present in the repeated sequence.

The complete unit required for efficient expression of the λ*5* and V_{preB1} genes at single copy extends over a 19-kb region and includes the five HS located 3′ of λ*5*. What are the determinants of tissue and stage specificity within this functional unit? Four of the 3′ sites are present only in pre-B cells, while the strongest site, HS1, is also detected in later stages of the B-cell lineage. The efficient expression that is observed with the tandemly repeated minimal λ*5* transgene (L5F1) is also fully tissue and stage specific, implying that sequences that are important for cell type specificity reside in this fragment. A recent study showed that a 720-bp λ*5* promoter fragment gave tissue- and stage-specific expression when placed upstream of a CD25-β-globin reporter gene in transgenic mice (24), although expression of the transgene was highly position sensitive, with only three of nine transgenic lines with multiple copies giving expression. Taken together, these results indicate that efficient tissue- and stage-specific expression at single-copy levels involves both the promoter and the 3′ HS. Binding sites for EBF, E47, and Ikaros are present in the promoter (22, 36), and ectopic expression of EBF and E47 has been used to demonstrate the importance of these factors for activating λ*5* and V_{preB1} expression (36). Sequence analysis has also revealed the presence of consensus binding sites for EBF, E47, and Ikaros in HS1 (data not shown). Further studies will be required to analyze the dynamics of the interactions between these elements and the roles played by the different regulatory elements in the locus.

Recent studies have shown that the β-globin and CD-2 LCRs can give efficient nonvariegated expression even when integrated in pericentromeric regions (7, 28). In this study, we have taken this type of analysis a step further by using a PCR assay to identify a centromeric integration that is directly flanked by centromeric γ-satellite DNA. The fact that the transgene in this integration gives full copy-dependent expression demonstrates that the λ*5* LCR is able to activate expression in pre-B cells even when directly flanked by heterochromatin-forming sequences. Nuclear-localization studies have shown that the λ*5*-V_{preB1} locus colocalizes with Ikaros-centromeric γ-satellite clusters in nonexpressing mature-B-cell lines but not in expressing pre-B-cell lines (2). This finding raises the possibility that nuclear localization plays a role in silencing and cellular memory. Our results provide important additional information on this phenomenon by showing that localization to the heterochromatin compartment is not in itself sufficient to give silencing when the full spectrum of factors is present. The available data supports a model where silencing is the product of a combination of changes in the factor profile and the position of the gene in the nucleus.

The identification of the λ*5*-V_{preB1} LCR provides the means for obtaining reproducible levels of expression of the λ*5* and V_{preB1} genes. This should facilitate studies that aim to test this type of model. In particular, it will now be possible to carry out mutagenesis studies to determine the precise role of Ikaros and other transcription factors in regulating expression of the locus.

ACKNOWLEDGMENTS

We thank Norman Thompson and Donna Hawkesworth for technical assistance. We are grateful to Niels Gelhardt for providing the 129SV cosmid library. We thank Amanda Fisher, Matthias Merkenschlager, and Mauro Santibanez-Koref for discussions and critical reading of the manuscript.

This work was supported by the Medical Research Council, United Kingdom.

REFERENCES

1. **Auffray, C., and F. Rougeon.** 1980. Purification of mouse immunoglobulin heavy-chain messenger RNAs from total myeloma tumour RNA. Eur. J. Biochem. **107:**303–314.
2. **Brown, K. E., S. S. Guest, S. T. Smale, K. Hahm, M. Merkenschlager, and A. G. Fisher.** 1997. Association of transcriptionally silent genes with Ikaros complexes at centromeric heterochromatin. Cell **91:**845–854.
3. **Caterina, J. J., T. M. Ryan, K. M. Pawlik, R. D. Palmiter, R. L. Brinster, R. R. Behringer, and T. M. Townes.** 1991. Human beta-globin locus control region: analysis of the 5′ DNase I hypersensitive site HS 2 in transgenic mice. Proc. Natl. Acad. Sci. USA **88:**1626–1630.
4. **Dillon, N., and F. Grosveld.** 1993. Transcriptional regulation of multigene loci: multilevel control. Trends Genet. **9:**134–137.
5. **Dillon, N., and F. Grosveld.** 1993. Transcriptional analysis using transgenic animals, p. 153–188. *In* B. D. Hames and S. J. Higgins (ed.), Gene transcription: a practical approach. IRL Press, Oxford, United Kingdom.
6. **Eccles, S., N. Sarner, M. Vidal, A. Cox, and F. Grosveld.** 1990. Enhancer

sequences located 3′ of the mouse immunoglobulin lambda locus specify high-level expression of an immunoglobulin lambda gene in B cells of transgenic mice. New. Biol. **2:**801–811.
7. **Festenstein, R., M. Tolaini, P. Corbella, C. Mamalaki, J. Parrington, M. Fox, A. Miliou, M. Jones, and D. Kioussis.** 1996. Locus control region function and heterochromatin-induced position effect variegation. Science **271:**1123–1125.
8. **Forrester, W. C., E. Epner, M. C. Driscoll, T. Enver, M. Brice, T. Papayannopoulou, and M. Groudine.** 1990. A deletion of the human beta-globin locus activation region causes a major alteration in chromatin structure and replication across the entire beta-globin locus. Genes Dev. **4:**1637–1649.
9. **Garrick, D., S. Fiering, D. I. K. Martin, and E. Whitelaw.** 1998. Repeats-induced gene silencing in mammals. Nat. Genet. **18:**56–59.
10. **Greaves, D. R., F. D. Wilson, G. Lang, and D. Kioussis.** 1989. Human CD2 3′-flanking sequences confer high-level, T cell-specific, position-independent gene expression in transgenic mice. Cell **56:**979–986.
11. **Grosveld, F., G. B. van Assendelft, D. R. Greaves, and G. Kollias.** 1987. Position-independent, high-level expression of the human beta-globin gene in transgenic mice. Cell **51:**975–985.
12. **Hagman, J., D. Lo, L. T. Doglio, J. Hackett, Jr., C. M. Rudin, D. Haasch, R. Brinster, and U. Storb.** 1989. Inhibition of immunoglobulin gene rearrangement by the expression of a lambda 2 transgene. J. Exp. Med. **169:**1911–1929.
13. **Halpren, M. S., and R. Coffman.** 1972. Polymer formation and J-chain synthesis in mouse plasmacytomas. J. Immunol. **109:**674–682.
14. **Henikoff, S.** 1998. Conspiracy of silence among repeated transgenes. Bioessays **20:**532–535.
15. **Ho, S. N., H. D. Hunt, R. M. Horton, J. Pullen, and L. R. Pease.** 1989. Site directed mutation by overlap extension using the polymerase chain reaction. Gene **77:**51–59.
16. **Jeffreys, A. J., A. MacLeod, K. Tamaki, D. L. Neil, and D. G. Monckton.** 1991. Minisatellite repeat coding as a digital approach to DNA typing. Nature **354:**204–209.
17. **Kim, K. J., C. Kanellopoulos-Langevin, R. M. Merwin, D. H. Sachs, and R. Asofsky.** 1979. Establishment and characterisation of BALB/c lymphoma lines with B cell properties. J. Immunol. **122:**549–554.
18. **Kitamura, D., A. Kudo, S. Schaal, W. Muller, F. Melchers, and K. Rajewsky.** 1992. A critical role of lambda 5 protein in B cell development. Cell **69:**823–831.
19. **Kudo, A., and F. Melchers.** 1987. A second gene, VpreB in the lambda5 locus of the mouse, which appears to be selectively expressed in pre-B lymphocytes. EMBO J. **6:**2267–2272.
20. **Kudo, A., N. Sakaguchi, and F. Melchers.** 1987. Organization of the murine Ig-related lambda 5 gene transcribed selectively in pre-B lymphocytes. EMBO J. **6:**103–107.
21. **Lanier, L. L., and N. L. Warner.** 1981. Cell cycle related heterogeneity of Ia antigen expression on a murine lymphoma cell line: analysis by flow cytometry. J. Immunol. **126:**626–631.
22. **Lo, K., N. R. Landau, and S. T. Smale.** 1991. LyF-1, a transcriptional regulator that interacts with a novel class of promoters for lymphocyte-specific genes. Mol. Cell. Biol. **11:**5229–5243.
22a. **MacNeil, A., and N. Dillon.** Unpublished data.
23. **Mai, S., and I. L. Martensson.** 1995. The c-myc protein represses the lambda 5 and TdT initiators. Nucleic Acids Res. **23:**1–9.
24. **Martensson, I., F. Melchers, and T. H. Winkler.** 1997. A transgenic marker for mouse B lymphoid precursors. J. Exp. Med. **185:**653–661.
25. **Martensson, I. L., and F. Melchers.** 1994. Pre-B cell-specific lambda 5 gene expression due to suppression in non-pre-B cells. Int. Immunol. **6:**863–872.
26. **Melchers, F.** 1995. The role of B cell and pre-B cell receptors in development and growth control of the B-lymphocyte cell lineage, p. 33–56. *In* T. Honjo and F. W. Alt (ed.), Immunoglobulin genes. Academic Press, London, United Kingdom.
27. **Melchers, F., H. Karasuyama, D. Haasner, S. Bauer, A. Kudo, N. Sakaguchi, B. Jameson, and A. Rolink.** 1993. The surrogate light chain in B-cell development. Immunol. Today **14:**60–68.
28. **Milot, E., J. Strouboulis, T. Trimborn, M. Wijgerde, E. de Boer, A. Langeveld, K. Tan Un, W. Vergeer, N. Yannoutsos, F. Grosveld, and P. Fraser.** 1996. Heterochromatin effects on the frequency and duration of LCR-mediated gene transcription. Cell **87:**105–114.
29. **Minegishi, Y., E. Coustan-Smith, Y. Wang, M. D. Cooper, D. Campana, and M. Conley.** 1998. Mutations in the human λ5/14.1 gene result in B cell deficiency and agammaglobulinemia. J. Exp. Med. **187:**71–77.
30. **Molnár, Á., and K. Georgopoulos.** 1994. The Ikaros gene encodes a family of functionally diverse zinc finger DNA-binding proteins. Mol. Cell. Biol. **14:**8292–8303.
31. **Murre, C., P. Schonleber-McCaw, H. Vaessin, M. Caudy, L. Y. Jan, Y. N. Jan, C. V. Cabrera, J. N. Buskin, S. D. Hauschka, A. B. Lassar, et al.** 1989. Interactions between heterologous helix-loop-helix proteins generate complexes that bind specifically to a common DNA sequence. Cell **58:**537–544.
32. **Ogawa, M., S. Nishikawa, K. Ikuta, F. Yamamura, M. Naito, K. Takahashi, and S. I. Nishikawa.** 1988. B cell ontogeny in murine embryo studied by a culture system with the monolayer of a stroma cell clone, ST-2: B cell progenitor develops first in the embryonal body rather than in the yolk sac. EMBO J. **7:**1337–1343.
33. **Philipsen, S., D. Talbot, P. Fraser, and F. Grosveld.** 1990. The beta-globin dominant control region: hypersensitive site 2. EMBO J. **9:**2159–2167.
34. **Rolink, A., A. Kudo, H. Karasuyama, Y. Kikuchi, and F. Melchers.** 1991. Long-term proliferating early pre B cell lines and clones with the potential to develop to surface Ig-positive, mitogen reactive B cells in vitro and in vivo. EMBO J. **10:**327–336.
35. **Schaffer, A. L., and M. S. Schlissel.** 1997. A truncated heavy chain protein relieves the requirement for surrogate light chains in early B cell development. J. Immunol. **159:**1265–1275.
36. **Sigvardsson, M., M. O'Riordan, and R. Grosschedl.** 1997. EBF and E47 collaborate to induce expression of the endogenous immunoglobulin surrogate light-chain genes. Immunity **7:**25–36.
37. **Talbot, D., P. Collis, M. Antoniou, M. Vidal, F. Grosveld, and D. R. Greaves.** 1989. A dominant control region from the human beta-globin locus conferring integration site-independent gene expression. Nature **338:**352–355.
38. **Talbot, D., S. Philipsen, P. Fraser, and F. Grosveld.** 1990. Detailed analysis of the site 3 region of the human beta globin dominant control region. EMBO J. **9:**2169–2177.
39. **Tian, J., T. Okabe, T. Miyazaki, S. Takeshita, and A. Kudo.** 1997. Pax-5 is identical to EBB-1/KLP and binds to the VpreB and lambda5 promoters. Eur. J. Immunol. **27:**750–755.
40. **Travis, A., J. Hagman, L. Hwang, and R. Grosschedl.** 1993. Purification of early-B-cell factor and characterization of its DNA-binding specificity. Mol. Cell. Biol. **13:**3392–3400.
41. **Waneck, G. L., and N. Rosenberg.** 1981. Abelson leukemia virus induces lymphoid and erythroid colonies in infected fetal cell cultures. Cell **26:**79–89.
42. **Yang, J., M. A. Glozak, and B. B. Blomberg.** 1995. Identification and localization of a developmental stage-specific promoter activity from the murine lambda 5 gene. J. Immunol. **155:**2498–2514.