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Pre-B cell-specific λ5 gene expression due to suppression in non pre-B cells

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Abstract

The λ5 gene is expressed specifically in pre-B cells; during B lymphocyte differentiation the expression of the λ5 gene is turned on in pre-B cells and turned off again at the mature B cell stage. No other cell type has yet been found to express the λ5 gene. The pre-B cell-specific expression of the λ5 gene is regulated at the level of transcription. We asked whether the region 5′ of the λ5 gene ($5'_{\lambda5}$) could explain the stage- and tissue-specific expression. The λ5 promoter lacks a TATA box and transcription is initiated at multiple sites. In the presence of a heterologous enhancer, $5'_{\lambda5}$ confers pre-B cell-specific expression on the reporter gene chloramphenicol acetyl-transferase. Deletion analysis of $5'_{\lambda5}$ defines two separate regions, referred to as $A_{\lambda5}$ and $B_{\lambda5}$. Region $B_{\lambda5}$ suppresses the expression in non pre-B cells since deletion of $B_{\lambda5}$ allows $A_{\lambda5}$ to promote transcription of the reporter gene in all cell types tested. In addition, $B_{\lambda5}$ acts as an enhancer on the heterologous κ light chain promoter in pre-B cells but not in B cells. Thus region $A_{\lambda5}$ functions as a basal promoter in all cell types tested. Region $B_{\lambda5}$, in concert with a heterologous enhancer, acts as a suppressing region in non-pre-B cells and therefore confers pre-B cell specificity on the expression of the λ5 gene.

Introduction

In the bone marrow, pluripotent stem cells give rise to pre-B cells that can differentiate into mature B cells. Normal pre-B cells in bone marrow lose λ5 expression at the transition from Ig μ heavy chain expressing pre-B cells to immature surface IgM^+ B cells, while transformed pre-B and immature B cells all express the λ5 gene (1–4). Normal and transformed mature B cells, and all other cell types tested, do not express λ5 (1). The pre-B cell-specific expression of λ5 is regulated at the level of transcription (5).

Initiation of transcription of many genes is determined by a TATA element at ~30 bp upstream of the transcription initiation site (6,7); however, the λ5 gene as well as other genes active in pre-B cells such as $V_{pre\text{-}B}$ (8), terminal deoxynucleotidyltransferase (TdT) (9), *mb-1* (10,11) and CD19 (12) do not contain a TATA element. Lack of a TATA element often results in initiation of a transcription at multiple sites, and this has also been observed with the λ5 gene (5,13).

The promoter regions of the TdT (14), *mb-1* (10,11,15) and $V_{pre\text{-}B1}$ (8) genes encode information that ensures their specific expression pattern in cell lines. This could be due to activation in permissive cells, to silencing in non-permissive cells or both. For instance, the turn-off of expression of the λ5 gene in later mature stages of B cell development could involve the loss of a transcriptional activator or the production of a transcriptional repressor. The latter mechanism is favoured by the observation that the expression of λ5 is not detectable in hybrids between fetal liver pre-B cells and a B cell myeloma (3). The data in this paper support the second possibility, i.e. that the λ5 gene expression is regulated by cell-type specific suppression.

Methods

Animals

CBA/J mice were obtained from the Institute für Biologisch-Medizinische Forschung AG (Füllinsdorf, Switzerland).

Tissue culture conditions

Murine spleen cells in single cell suspension were depleted of red cells and thereafter cultured in Iscove's modified Dulbecco's modified Eagles medium supplemented with 5% FCS, antibiotics and 5×10^{-5} M 2-mercaptoethanol. The spleen cell cultures (5×10^6 cells/ml) were incubated in the presence of lipopolysaccharide (LPS, *Escherichia coli* 055:B5; Difco, Detroit, MI) at a final concentration of 50 μg/ml. The murine pre-B cell line 230-238 (16), the murine B cell lymphomas WEHI 279 (17) and K46R (18), and the murine T cell leukaemia EL-4 (19) were

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cultured in medium as above. The murine fibroblast line, 3T3, was cultured in the same medium with the following exceptions: the 2-mercaptoethanol was omitted and the amount of FCS was 10%. All cells described above have been tested for λ5 gene expression as described (1,3) or by us and only the pre-B cell line 230-238 was found positive.

RNA preparation and RNase protection mapping

RNA from non-transfected and transiently transfected cells was prepared as NP40 lysates as described (20). A DNase step was included which turned out not to be necessary.

The RNase protection mapping probe was labelled with [α-^{32}P]UTP according to the manufacturer (Promega, Madison, WI). The pG5′$_{\lambda5}$-globin plasmid was made by us (see DNA constructs). The pG5′$_{\lambda5}$-globin probe was hybridized with 20 μg total RNA at 37°C. The hybridizations were incubated overnight. Next day samples were treated with RNaseA and T1. The protected fragments were separated on a PAGE 8 M urea gel followed by autoradiography. As molecular weight marker, [α-^{32}P]dCTP labelled pBR322 *Msp*I digested DNA (NEB, Beverly, MA) was used.

Transfection and chloramphenicol acetyltransferase (CAT) assay

Cells were transiently transfected using DEAE-dextran as described (21) with the following exceptions: 2.5×10^6 cells per transfection sample were used for all cell lines and the LPS blasts were used at 10^7 cells per sample. The 3T3 cells were plated at 0.7×10^6 cells in a 10 cm Petri dish the day before transfection and transfected as the other cells (in the plates).

Cells were harvested 40 – 46 h after transfection. Cell extracts or RNA were prepared, tested in CAT assay (22) as described (21) or RNase protection mapping. The thin layer chromatography plates (CAT assay) were measured on a phosphor imager (Molecular Dynamics, Sunnyvale, CA). The amount of CAT activity was determined as the amount (%) of acetylated chloramphenicol of the total amount of radioactivity in the respective sample. The variation between duplicates within one experiment was <10% unless stated with the exception of activities <1% in which duplicates sometimes varied by 20%. In case of CAT activities >30%, where the assay is no longer linear, the samples were tested as 2-fold dilutions.

DNA constructs

All DNA constructs were made using conventional DNA techniques (20). The pSV2CAT construct has been described before (22). The transfection construct 5′$_x$-CAT-E$_\mu$ (1.5 kb IgLx promoter and IgH intron, E$_\mu$, enhancer) has been described before as L-CAT-E (21). A$_x$-CAT-E$_\mu$, described elsewhere as x′-CAT-E (23) contains the most 3′ 150 bp of the 5′$_x$ promoter. The λ5 promoters used in the transfection vectors were either made by polymerase chain reaction (PCR) (Gene Amp) or annealed oligonucleotides. All oligonucleotides were made such that they contained a *Hin*dIII site at the 5′ end (with two extra nucleotides to protect the *Hin*dIII site) to make it possible to subclone the fragments in both orientations into the unique 5′ *Hin*dIII site of the pUC-CAT vectors (21), i.e. pUC-CAT and pUC-CAT-E$_\mu$. The CAT reporter gene contains its own ATG start codon. As template in the PCR reactions, the plasmid 7pB12-2 containing the λ5 genomic gene (13) was used. To make the constructs 5′$_{\lambda5}$-CAT, 5′$_{\lambda5}$-CAT-E$_\mu$ and X5′$_{\lambda5}$-CAT-E$_\mu$ (X denotes the 5′$_{\lambda5}$ in the opposite orientation) the 725 bp λ5 promoter PCR product was first cloned in pGEM bluescript (Promega, Madison, WI), then excised from this (pG5′$_{\lambda5}$) plasmid as a *Hin*dIII fragment and subcloned in the respective vectors. For the 5′$_{\lambda5}$-globin-E$_\mu$ the λ5 promoter was excised as a *Hin*dIII fragment, filled in by Kleenow and ligated in the unique *Acc*I site (after Kleenow fill in) of the OVEC-H (24) vector. The 5′$_{\lambda5}$ promoter deletion constructs with the SV40 enhancer, E$_{SV}$, were made by deleting E$_\mu$ from the corresponding construct using *Bam*HI and replacing it with E$_{SV}$. The following PCR 5′ primers were used for the respective deletion contructs:

5′$_{\lambda5}$; TTATATGTCACAGGCTGGCCTTGA,
−272$_{\lambda5}$; CAGGTGTTCAGTTGCTCTCTACGG,
−197$_{\lambda5}$; TGGATATCAGTCAGGCAGAGCTGC,
−81$_{\lambda5}$; AACTCCACAGATGGTGACCATGGGC,
−45$_{\lambda5}$; CCTGCTGGTGGTGGAAACTAGAGA,
−6$_{\lambda5}$; CTACACAGATCCACCTGCACTGGA.

The same 3′ primer was used in all these constructs; TGACCCTCAAGTCCAAAGTCAACT. For the +59$_{\lambda5}$ and the +90$_{\lambda5}$ constructs, annealed oligonucleotides were used. +59$_{\lambda5}$; GGAGATCTACACTGCAAGTGAGGCTAGAGTTGACTTTGGACTTGAGGGTCA and +90$_{\lambda5}$; GACTTTGGACTTGAGGGTCA.

Region B$_{\lambda5}$ of the λ5 promoter was made by PCR using the same 5′ primer as the 5′$_{\lambda5}$ and as 3′ primer; ACAAGCTTCTCTCCCAAAGGACTACCCGGTTG. The PCR product was cloned in both orientations into the unique *Hin*dIII site 5′ of the A$_x$ promoter of the A$_x$-CAT-E$_\mu$ vector to make (X)B$_{\lambda5}$-A$_x$-CAT-E$_\mu$. X denotes B$_{\lambda5}$ in the opposite orientation. (X)B$_{\lambda5}$-A$_x$-CAT was made by excising Eμ from (X)B$_{\lambda5}$-A$_x$-CAT-E$_\mu$. All λ5 promoter constructs were confirmed by sequencing (Sequenase).

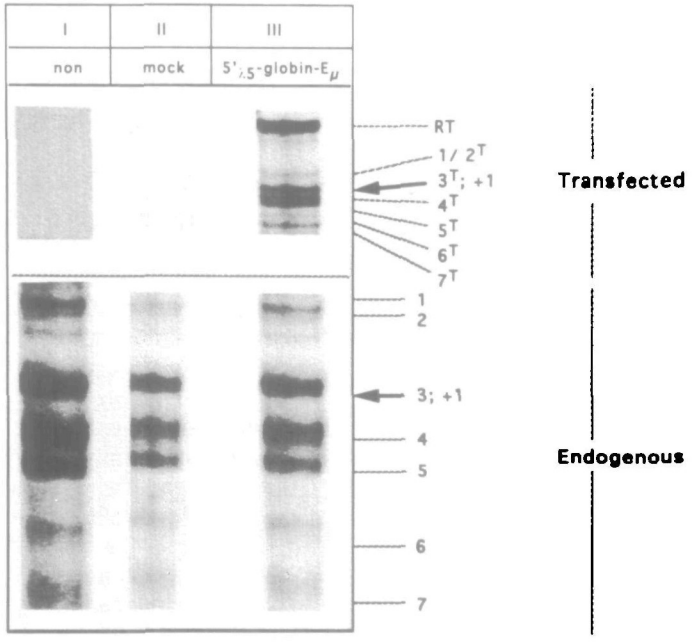
pG5′$_{\lambda5}$-globin was made by excising the 440 bp *Pvu*II – *Bam*HI fragment from 5′$_{\lambda5}$-globin-E$_\mu$ and cloned into pGEM bluescript (Promega). This fragment corresponds to position −140 in 5′$_{\lambda5}$, polylinker and 200 bp of the rabbit β-globin gene.

Results

Multiple initiation sites for transcription of the λ5 gene in pre-B cells

Kudo *et al.* have observed two major transcription start sites in the λ5 gene detectable by primer extension techniques (13) and an additional major start site detectable by S1 nuclease mapping (5). We have confirmed and extended these results with the RNase protection technique and have found several additional minor transcription start sites as shown in Fig. 1(A). In the 230-238 pre-B cell line (Fig. 1A, lane I), transcription of the endogenous λ5 gene was initiated at three major (#3 – #5) and four minor sites (#1, #2, #6 and #7) as detected by the pG5′$_{\lambda5}$-globin probe (Fig. 1B). The same start sites were also found using RNA from another pre-B cell line, NFS-5 (data not shown). Identical results were obtained with a similar probe lacking the globin region (data not shown). The seven transcription initiation sites which we detected are all within a 91 bp region (Fig. 1B). These major and minor transcription start sites have therefore been numbered as #1 – #7. We use the most 5′ major start site, #3, located 109 bp upstream of the translation start codon, as +1.

A) Detection of multiple λ5 transcription initiation sites.



B) The pG5'$_{\lambda5}$-globin probe.

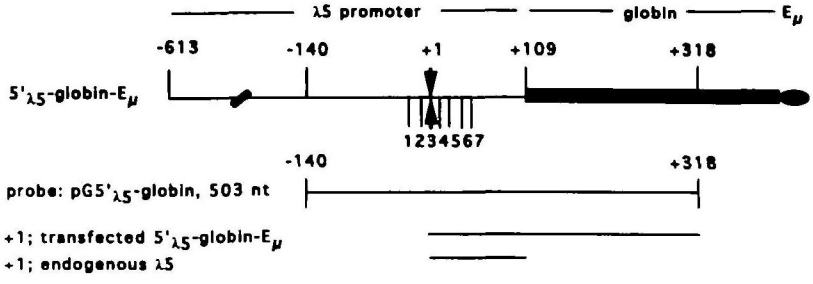


Fig. 1. (A) Detection of λ5 transcripts initiated at multiple sites RNase protection mapping of RNA from 230-238 cells; non-transfected (lane I), mock transfected (lane II) and transfected with 5'$_{\lambda5}$-globin-E$_\mu$ (lane III) The lower part of the gel (Endogenous) shows protected bands corresponding to endogenous λ5 RNA (#1 – #7) and the upper part of the gel (Transfected) shows protected bands (#1^T – #7^T) corresponding to λ5-globin RNA The arrows point to the most 5' major start site #3 (set as +1) protected in endogenous and transfected RNA. RT, read through. (B) The pG5'$_{\lambda5}$-globin probe. Depicted is the 5'$_{\lambda5}$-globin-E$_\mu$ transfection vector including start sites #1 – #7 with the arrows showing start site #3 (+1). Below is shown the 503 nucleotide pG5'$_{\lambda5}$-globin probe corresponding to position −140 in the 5'$_{\lambda5}$ promoter and to position +318 in the globin gene. The probe detects transcription initiation sites used in the endogenous λ5 gene and in the transfected 5'$_{\lambda5}$-globin-E$_\mu$ as shown below for start site #3, i.e. +1.

The 5' region of the λ5 gene promotes expression of reporter genes

To test for regulatory elements upstream of the λ5 coding sequence, a 725 bp fragment, 5'$_{\lambda5}$ (Fig. 2A), was cloned upstream of the CAT reporter gene. A summary of all the constructs used in this paper is given in Fig. 2. One of the constructs, 5'$_{\lambda5}$-CAT, was used to look at the promoter activity of 5'$_{\lambda5}$. Since other promoters, like the Ig promoters, show low activities in the absence of an enhancer (25 – 28) we also constructed 5'$_{\lambda5}$-CAT-E$_\mu$ which contained the IgH intron enhancer (E$_\mu$), 3' of the CAT gene. In a third construct (X5'$_{\lambda5}$-CAT-E$_\mu$) the orientation of the 5'$_{\lambda5}$ fragment was reversed. Expression of the CAT reporter gene was assayed after transfection into the pre-B cell line 230-238. Figure 3(A) shows one representative CAT assay. Below each sample the CAT conversion is shown as percentage (see Methods). 5'$_{\lambda5}$-CAT was poorly expressed (1.0%), while the presence of the E$_\mu$ enhancer (5'$_{\lambda5}$-CAT-E$_\mu$) increased the expression level 10- to 15-fold. With the X5'$_{\lambda5}$-CAT-E$_\mu$ construct only background levels of CAT activity were found (data not shown), showing that there was no promoter activity when 5'$_{\lambda5}$ was positioned in the opposite orientation. Similar results were obtained in the 18-81 pre-B cell line (data not shown). We conclude that in the presence of the E$_\mu$ enhancer the 5'$_{\lambda5}$ fragment, correctly oriented, can promote transcription in pre-B cells.

To determine whether the 5'$_{\lambda5}$ promoter initiated transcription using the same sites as the endogenous λ5 gene, the CAT gene was replaced by a rabbit β-globin gene (24), to make

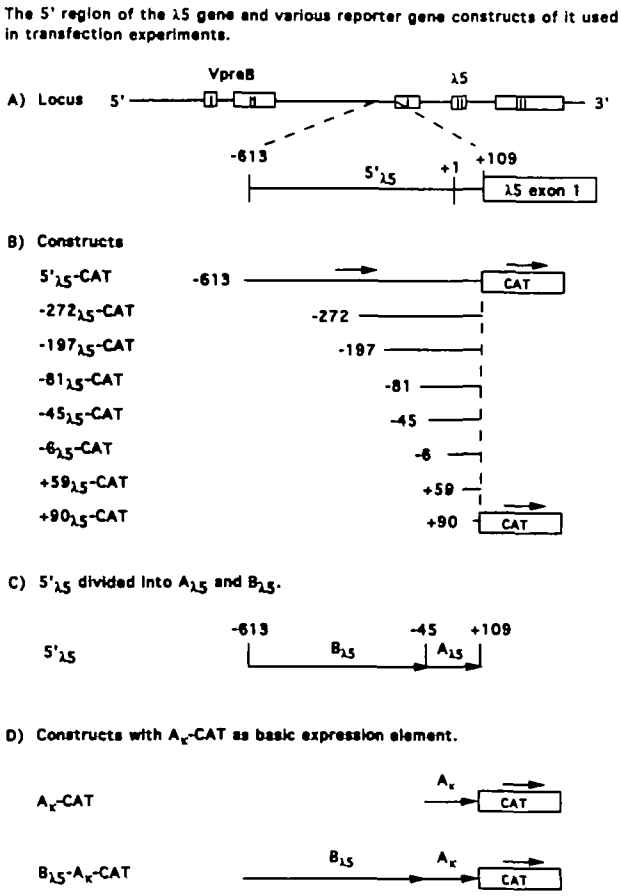


Fig. 2. (A) The 5′ region of the λ5 gene. The murine V_{preB1} (exon I and II) and λ5 (exon I, II and III) locus and below the 5′ region of the λ5 gene used to test for promoter activity, $5'_{\lambda5}$ (position −613 to +109, transcription start site +1) is shown. (B) Various reporter gene constructs of the 5′ region of the λ5 gene. Several constructs were made based on the reporter gene CAT without or with an enhancer 3′ of CAT either the SV40 enhancer, denoted E_{SV}, or the IgH intron enhancer, denoted E_μ. The $5'_{\lambda5}$-CAT construct is shown at the top and below is depicted others with deletions from the 5′ end of $5'_{\lambda5}$ while keeping the 3′ end at +109. The deletion constructs are labelled according to the respective 5′ position, i.e. $-272_{\lambda5}$-CAT starts at position −272 and $+59_{\lambda5}$-CAT starts at position +59. Note that the $+59_{\lambda5}$-CAT vector include the most 3′ minor start site while $+90_{\lambda5}$-CAT contains no transcription initiation site. (C) $5'_{\lambda5}$ divided into two regions. Dividing $5'_{\lambda5}$ into two regions: $A_{\lambda5}$ (position −45 to +109 containing all transcription initiation sites) and $B_{\lambda5}$ (position −613 to −45). (D) Various reporter gene constructs based on A_κ-CAT. All constructs are based on the basal A_κ-CAT construct without or with the E_μ enhancer 3′ of CAT. A_κ, the most 3′ 150 bp of the Sp6 κ promoter including the octamer, TATA box and transcription start site (see Methods); $B_{\lambda5}$, see (C).

$5'_{\lambda5}$-globin-E_μ (Fig. 1B). The 230-238 pre-B cell line was either mock transfected or transfected with $5'_{\lambda5}$-globin-E_μ and RNA was prepared to map transcription start sites by RNase protection (Fig. 1A). Figure 1(B) shows the pG$5'_{\lambda5}$-globin probe and the predicted protected transcripts initiated at +1 in the endogenous λ5 gene and in the $5'_{\lambda5}$-globin-E_μ transfectant. Seven bands (#1 − #7) corresponding to endogenous λ5 transcripts were detected in mock as well as in $5'_{\lambda5}$-globin-E_μ transfected cells. In addition, seven new bands (#1^T − #7^T) were detected in $5'_{\lambda5}$-globin-E_μ transfected cells. The new transcripts were all 209 nucleotides larger than the respective endogenous transcripts as expected for transcripts initiated in the $5'_{\lambda5}$-globin-E_μ construct at the same positions as in the endogenous gene. The intensity of these new bands correlated with the intensity of the respective bands from the endogenous λ5 RNA. We conclude that the $5'_{\lambda5}$ region contains the information needed to initiate transcription similarly to the endogenous gene.

The $5'_{\lambda5}$ region is developmentally regulated

We next asked whether the $5'_{\lambda5}$ region contained sequence information sufficient to explain the developmentally regulated expression pattern of λ5. The IgL κ promoter, $5'_\kappa$ (1.5 kb, see Methods), allows expression of a $5'_\kappa$-CAT-E_μ construct in both pre-B and mature B cells. Thus, the ratios of $5'_{\lambda5}$-CAT-E_μ and $5'_\kappa$-CAT-E_μ expression in these cells should show whether $5'_{\lambda5}$ confer stage-specific expression. Table 1 shows that none of the B cell lymphomas (WEHI 279 and K46R) nor normal LPS stimulated B cells expressed any appreciable level of CAT activity when driven by the $5'_{\lambda5}$ region, while the control $5'_\kappa$-CAT-E_μ was expressed (although at different levels depending on which cells were used). In the 230-238 pre-B cells, $5'_{\lambda5}$-CAT-E_μ and $5'_\kappa$-CAT-E_μ were expressed at similar levels, while in the B cells the ratio varied between 0.1 and 0.003. Thus, the $5'_{\lambda5}$ activity as promoter was poor in all B cells tested. We conclude that the $5'_{\lambda5}$ region functions as a developmentally regulated promoter in pre-B cells but not in mature B cells.

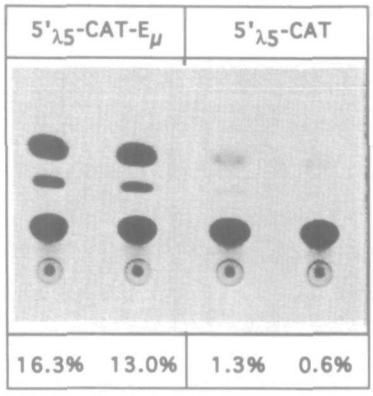
Two regions regulating expression of the $5'_{\lambda5}$-CAT-E_μ construct in pre-B cells

To determine regions important for $5'_{\lambda5}$ promoter activity, constructs with successive deletions in the 5′ end of the promoter were made (Fig. 2B). Since the $5'_{\lambda5}$ promoter alone conferred very low activity unless the E_μ enhancer was provided in the construct (see results in Fig. 3A) such an enhancer was included in all promoter-deletion constructs. Figure 3(B) shows one representative experiment in 230-238 pre-B cells. Actual CAT conversion levels were similar to earlier experiments, see Fig. 2 and Table 1. Deletions in the 5′ end from −613 to −197 showed no major change in CAT activity. At position −81 the activity dropped 2-fold and slightly increased at position −45. Further 5′ deletions showed minor changes. When the last transcription initiation site was deleted (construct $+90_{\lambda5}$-CAT-E_μ) only background levels of activity were noted. The difference in expression level between $5'_{\lambda5}$-CAT-E_μ and $+90_{\lambda5}$-CAT-E_μ was 30-fold. We conclude that in the presence of the E_μ enhancer, the $5'_{\lambda5}$ segment appears to contain several regions which have different effects on the expression of the reporter gene in pre-B cells. In order to simplify the interpretation and because of the results from the following experiments we divided the $5'_{\lambda5}$ segment into two regions, region $A_{\lambda5}$ (position −45 to +109), which included all transcription initiation sites, and region $B_{\lambda5}$ (position −613 to −45).

Region $B_{\lambda5}$ shows stage- and tissue-specific suppression of the basal $A_{\lambda5}$ promoter activity

The activities of the $5'_{\lambda5}$ deletion constructs (Fig. 2B) were determined in several non pre-B cell-types by transfections and CAT assays. Figure 4 shows representative experiments of the relative CAT activities detected in the K46R B cell line (Fig. 4A), in the EL-4 T cell line (Fig. 4B) and in 3T3 fibroblasts (Fig. 4C).

A) CAT activity with constructs 5'$_{\lambda 5}$-CAT and 5'$_{\lambda 5}$-CATE$_{\mu}$ in preB cells.



B) CAT activity with the 5'$_{\lambda 5}$ deletion constructs in 230-238 preB cells.

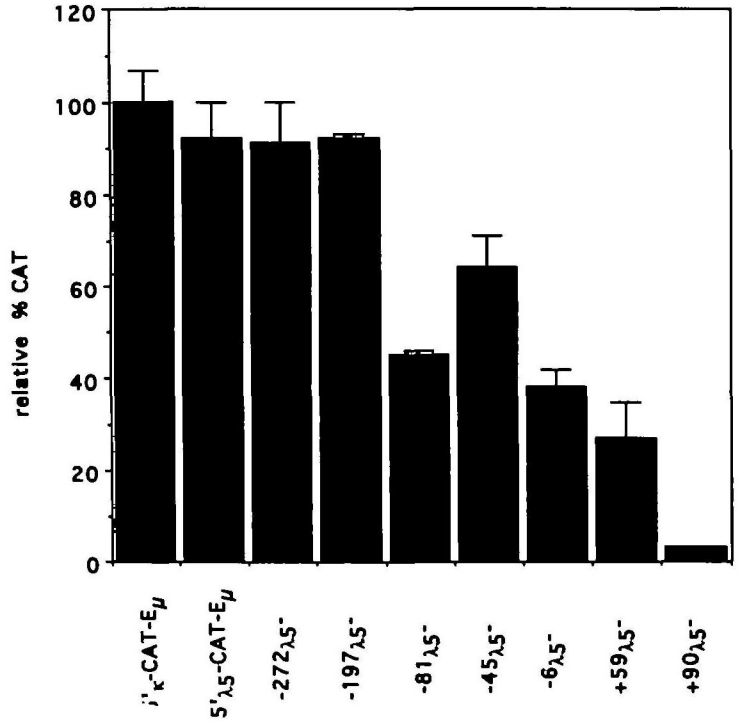


Fig. 3. (A) Transfection of 230-238 pre-B cells with 5'$_{\lambda 5}$-CAT and 5'$_{\lambda 5}$-CAT-E$_{\mu}$. CAT assay after transient transfection of the pre-B cell line 230-238 with the 5'$_{\lambda 5}$-CAT construct described in Fig. 2(B). The construct named 5'$_{\lambda 5}$-CAT-E$_{\mu}$ includes the E$_{\mu}$ enhancer 3' of the CAT gene. The activity is shown below each sample (duplicates) as percentage CAT conversion (see Methods) (B) CAT activity of 5'$_{\lambda 5}$ deletion constructs in 230-238 pre-B cells. The 5'$_{\lambda 5}$ deletion constructs described in Fig. 2(B), all containing the E$_{\mu}$ enhancer, were transiently transfected into the pre-B cell line 230-238, followed by CAT assay. The CAT activities were determined and thereafter calculated as relative percentage of 5'$_{\kappa}$-CAT-E$_{\mu}$ which was set as 100%. One representative experiment is shown

The constructs contained either the E$_{\mu}$ enhancer (Fig. 4A and B) or the E$_{SV}$ enhancer (Fig. 4C).

In the B cell line K46R (Fig. 4A) the relative activity detected with 5'$_{\lambda 5}$-CAT-E$_{\mu}$ was low, as described before (see Table 1). Deletion from the 5' end of 5'$_{\lambda 5}$ showed a stepwise increase in activity up to position −45, with the 45$_{\lambda 5}$-CAT-E$_{\mu}$ construct giving the highest expression level. The relative CAT activity (50%) with this construct was similar to that found in the pre-B cell line (see Fig. 3B). Further 5' deletions showed minor changes until a major drop in expression level with the +90$_{\lambda 5}$-CAT-E vector, where only background CAT activity was detected. Similar results were also obtained in the B cell myeloma X63/O (data not shown).

In the T cell line EL-4 the relative activity detected with 5'$_{\lambda 5}$-CAT-E$_{\mu}$ was very low. Deletion to position −6 gave the highest relative CAT activity (15%). The +59$_{\lambda 5}$-CAT-E$_{\mu}$ construct was active while the +90$_{\lambda 5}$-CAT-E$_{\mu}$ vector was inactive. While the pattern of expression was similar in EL-4 T cells and K46R

Table 1. Relative promoter activity of the 5′ regions of the λ5 and the IgL, $\varkappa$ genes in pre-B and B cells

Cells	Actual % CAT conversion in transient transfection experiments with[a]		Relative promoter activity (ratio I/II)
	$5'_{\lambda 5}$-CAT-E_μ (I)	$5'_\varkappa$-CAT-E_μ (II)	
Pre-B cells:			
230-238 (Exp. 1)	16.5 ± 1.2	17.9 ± 1.2	0.9
230-238 (Exp. 2)	14.8 ± 0.6	19.5 ± 0.5	0.8
B cells:			
WEHI 279 (Exp. 1)	0.4	5.2 ± 0.4	0.08
K46R (Exp. 1)	0.8	9.3	0.09
K46R (Exp. 2)	0.1 ± 0	10.0 ± 1.0	0.01
Normal B cells:			
spleen, LPS (Exp. 1)	0.2 ± 0.1	28.7	0.007
spleen, LPS (Exp. 2)	0.1	31.9	0.003

[a]Details are given in Methods.

B cells, the relative level of expression in B cells was higher than that in T cells.

To test the 5′ deletion vectors in 3T3 cells, the E_μ enhancer was replaced by the E_{SV} enhancer, since E_μ does not function in 3T3 cells. We compared expression with the $5'_{\lambda 5}$-CAT-E_{SV} deletion constructs in K46R B cells and 3T3 fibroblasts. In the B cell line the expression pattern was very similar to that of the corresponding constructs with the E_μ enhancer (Fig. 4A). In 3T3 cells the $5'_{\lambda 5}$-CAT-E_{SV} construct was not expressed (CAT conversion <1%). The most prominent increase in activity, 10-fold, was detected with both the $81_{\lambda 5}$-CAT-E_{SV} and $+59_{\lambda 5}$-CAT-E_{SV} constructs. The $+90_{\lambda 5}$-CAT-E_{SV} vector showed no CAT activity. The relative level of CAT activity was higher in the B cell as compared with that in fibroblasts, however, the pattern of expression was the same.

The data presented above show that in the presence of a heterologous enhancer (E_μ or E_{SV}) the $5'_{\lambda 5}$ segment appears to contain two regions which affect the expression of the reporter gene in B cells, T cells and fibroblasts. We conclude that, in the context of a heterologous enhancer, region $A_{\lambda 5}$ allows expression of the reporter gene in all cell types tested (pre-B, B, T and fibroblasts), suggesting that it acts as a basal promoter. The $B_{\lambda 5}$ region suppresses $A_{\lambda 5}$ driven expression in B cells, T cells and in fibroblasts but not in pre-B cells. Thus $B_{\lambda 5}$ and E_μ/E_{SV} together confer pre-B cell-specific expression of the reporter gene CAT.

$B_{\lambda 5}$ confers pre-B cell stage-specific expression to an IgL $\varkappa$ promoter

The experiments described above showed that $B_{\lambda 5}$ and E_μ together imposed stage- and tissue-specific expression on constructs driven off the $A_{\lambda 5}$ basal promoter. We wanted to ask whether these elements could also regulate another promoter active in both pre-B and B cells. We used a short, 150 bp, IgL $\varkappa$ promoter (here named $A_\varkappa$, see Methods) to give an $A_\varkappa$-CAT construct (Fig. 2D). Experiments summarized in Fig. 5 show that the CAT gene under the control of $A_\varkappa$ was expressed, provided the E_μ enhancer was included ($A_\varkappa$-CAT-E_μ, in both pre-B and B cells. Inserting $B_{\lambda 5}$ upstream of $A_\varkappa$($B_{\lambda 5}$-$A_\varkappa$-CAT-E_μ) increased the activity in B cells 1.5- to 2-fold. However, in the pre-B cells the activity increased 5-fold above $A_\varkappa$-CAT-E_μ. Somewhat surprisingly, when E_μ was removed $B_{\lambda 5}$-$A_\varkappa$-CAT was still expressed (25% CAT conversion) in pre-B cells but not in B cells. Even upon reversing the orientation of $B_{\lambda 5}$ ($XB_{\lambda 5}$-$A_\varkappa$-CAT) this new construct was active (7% CAT conversion) in the pre-B cell line. This was also observed in another pre-B cell line, 18-81 (data not shown). These experiments show that $B_{\lambda 5}$ can act as a stage-specific enhancer on the heterologous $A_\varkappa$ promoter. It also shows that $B_{\lambda 5}$ and E_μ together can convey pre-B cell specific expression to the basal $A_{\lambda 5}$ promoter but not to the $A_\varkappa$ promoter.

Discussion

This study shows that the 5′ regulatory region of the pre-B cell-specific λ5 gene ($5'_{\lambda 5}$) contains two control regions, $A_{\lambda 5}$ and $B_{\lambda 5}$, with strikingly different properties (summarized in Fig. 6). The $A_{\lambda 5}$ region appears to function as a basal promoter in several cell types (pre-B, B, T cells and fibroblasts). The $B_{\lambda 5}$ region, in contrast, can act in a tissue-specific fashion to suppress transcription, but can also act in a stage-specific manner to enhance transcription.

The basal $A_{\lambda 5}$ promoter behaves like other TATA-less promoters in that it initiates transcription at multiple sites. However, expression depends on an appropriate lineage-related enhancer (E_μ for lymphocytes, E_{SV} for fibroblasts).

Region $B_{\lambda 5}$ acts dominantly together with a heterologous enhancer to suppress reporter gene expression driven off its own promoter in cell types other than pre-B cells. One possibility is that suppressor factors bind to $B_{\lambda 5}$ in non-permissive cells. This would be consistent with experiments where pre-B cells were fused with myeloma cells, yielding hybrids in which λ5 gene expression was no longer detectable (3). When region $B_{\lambda 5}$ was introduced upstream of the IgL $\varkappa$ promoter, $A_\varkappa$, in the presence of E_μ, expression in B cells was not suppressed. Surprisingly, in the absence of E_μ, $B_{\lambda 5}$ could act as an enhancer on the $A_\varkappa$ promoter in pre-B cells, but not in B cells, i.e. a pre-B cell stage-specific enhancer. This suggests that factors present in pre-B but not B cells bind to $B_{\lambda 5}$ and activate transcription from $A_\varkappa$.

The different activities obtained with $B_{\lambda 5}$ using the two different promoters could be explained by the fact that $A_{\lambda 5}$ is a TATA-less promoter while $A_\varkappa$ is a TATA-containing promoter. It has been shown that such promoters use different components of the basal machinery to initiate transcription (29,30).

We are interested in identifying the factors which cause the apparent differences in expression patterns imposed by $B_{\lambda 5}$.

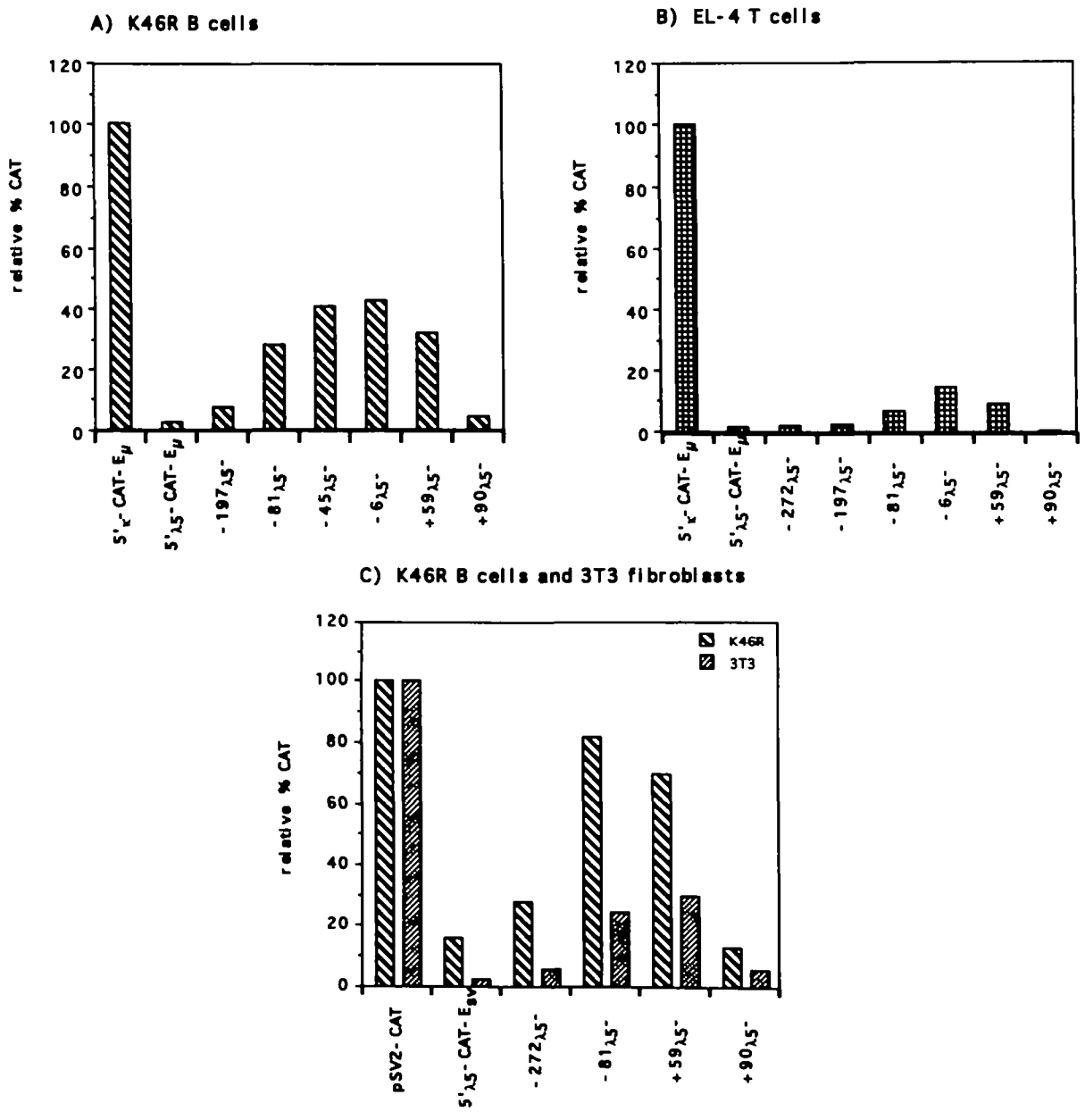


Fig. 4. CAT activity of $5'_{\lambda 5}$ deletion constructs in B cells, T cells and fibroblasts. The cells were transfected with the $5'_{\lambda 5}$ deletion constructs in Fig. 2(B) containing the E_μ (A, B) or the E_{SV} enhancer (C) and the relative CAT activities, compared with $5'_\kappa$-CAT-E_μ (100%) (A, B) or pSV2-CAT (100%) (C) were determined. One representative experiment is shown for each cell type. The variation between duplicates within one experiment was ≤ 10% (A) K46R B cells, (B) EL-4 T cells, (C) 3T3 fibroblasts and K46R B cells as comparison.

Therefore we looked within $B_{\lambda 5}$ for sequence recognition motifs of DNA binding proteins. One DNA binding protein, EBB-1, present in pre-B and B cells, binds to a motif in the murine $V_{pre\text{-}B1}$ promoter and also to a motif in $B_{\lambda 5}$ (31). The EBB-1 protein may be related to Pax-5, which acts on the CD19 promoter (12,32). A protein complex, Lyf-1, detected in lymphoid cells, binds to the TdT promoter and within $B_{\lambda 5}$ (14,33) The consensus sequence recognized by Lyf-1 is the same as that of the μB element present in the E_μ enhancer (34,35) and that of a site in the IgL κ 3′ enhancer (36,37). Several motifs corresponding to μE2, μE4 and the E-box consensus sequence (38) are present in $B_{\lambda 5}$. None of the motifs described above bind proteins expressed only in pre-B cells or only in non-pre-B cells. This might indicate that none of these motifs are involved in the

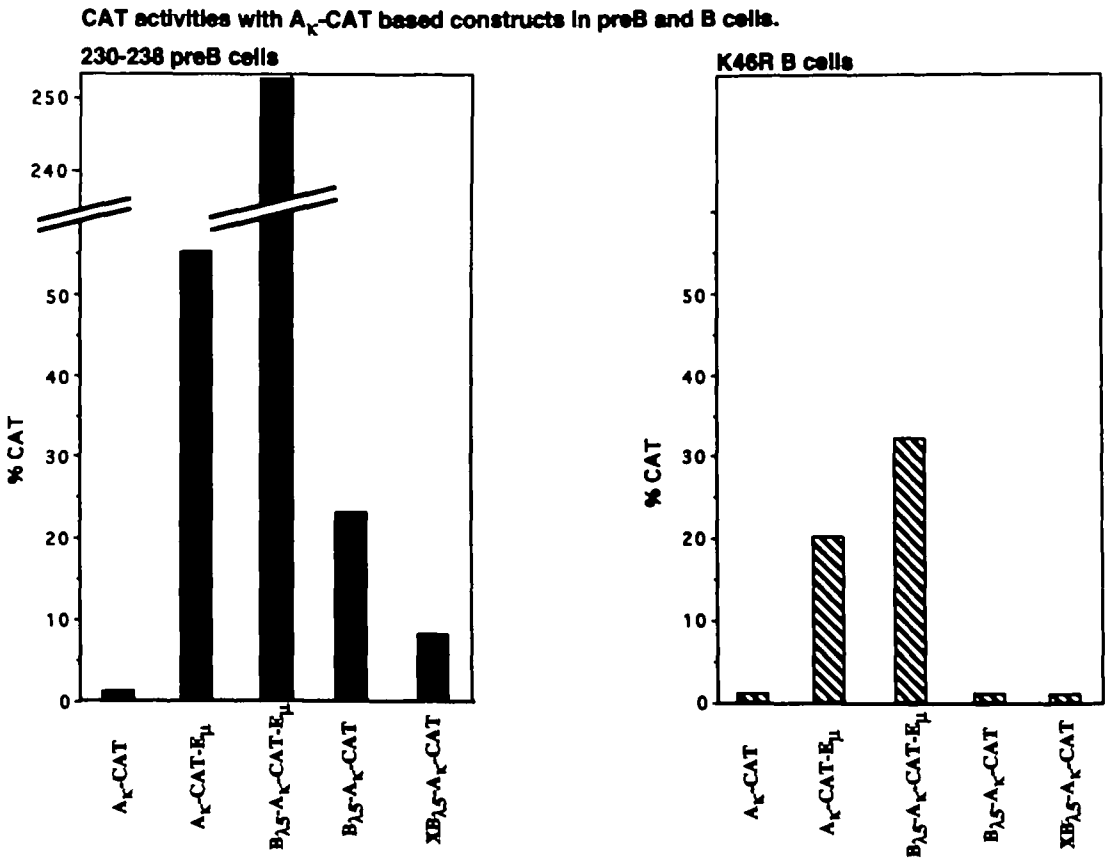


Fig. 5. Relative CAT activities of A_x-CAT based constructs in pre-B and B cells. Transfection of the A_x-CAT based constructs described in Fig. 2(D) into 230-238 pre-B cells (left) and K46R B cells (right). One representative experiment is shown for each cell type. Constructs which include the E_μ enhancer are labelled accordingly. $B_{\lambda 5}$ is described in Fig. 2(C). The activity with A_x-CAT was in some experiments 1% and others <1%, and in the experiments shown normalized to 1%. Note that the activity with $B_{\lambda 5}$-A_x-CAT-E_μ in the pre-B cell line is out of range (>200%). The activity with $XB_{\lambda 5}$-A_x-CAT-E_μ in the pre-B cells was 77% and in the B cells it was 20% (not shown). The variation between duplicates within one experiment was ≤10%.

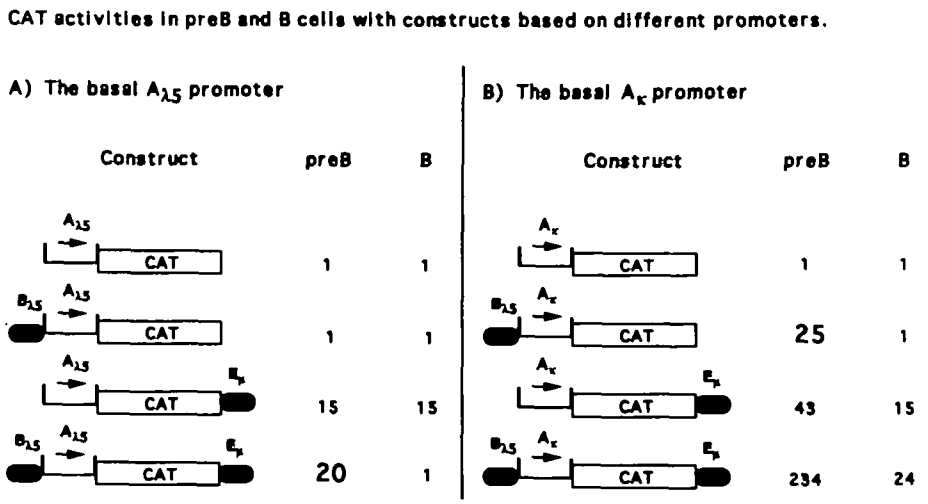


Fig. 6. (A) Constructs based on the basal $A_{\lambda 5}$ promoter and corresponding CAT activities in pre-B and B cells. The transfection vectors contain the basal $A_{\lambda 5}$ promoter upstream of the CAT reporter gene. The E_μ enhancer was cloned 3′ of the CAT gene. $B_{\lambda 5}$ (described in Fig. 2C) was cloned 5′ of the $A_{\lambda 5}$ promoter. A summary of several independent transfection experiments is shown for 230-238 pre-B cells and K46R B cells. Due to variations in transfection efficiency between experiments the actual CAT activity with $A_{\lambda 5}$-CAT and A_x-CAT varied between 0.3 and 1.0%. The data have been normalized to 1% for the basal promoter activity obtained. Standard variations within each experiment are <10% For constructs showing pre-B cell specific expression the values are written as enlarged numbers. (B) Constructs based on the A_x promoter and corresponding CAT activties in pre-B and B cells. As in (A) except that the $A_{\lambda 5}$ promoter was replaced by the 150 bp IgL x, A_x, promoter.

cell type and differentiation stage-specific regulation of λ5 gene expression. Alternatively, the small differences in reporter gene expression observed with the $5'_{\lambda5}$ deletion constructs indicate that more than one factor could be involved in the pre-B cell-specific regulation of gene expression, perhaps binding to more than one motif within $B_{\lambda5}$.

Among the genes differentially expressed during B cell development TdT, $V_{pre\text{-}B}$ and λ5 show a strikingly similar pattern of expression. We would therefore expect that future more detailed analysis of the regulatory elements of these genes would detect the greatest similarities in TdT and surrogate light chain genes. In the TdT gene, a basal promoter element active in all cell types has been described, the so called Inr (9). Furthermore, elements activating expression in appropriate cell types have been found in the 5′ regions of several genes expressed during early B cell development, including TdT (14), $V_{pre\text{-}B}$ (8), mb-1 (10,11,15) and CD19 (12). So far, there is no element described that suppresses expression of these genes in non-permissive cell types. However, other genes like the Ig genes have been described that are regulated by suppression (39).

To detect $5'_{\lambda5}$ driven reporter gene expression, a heterologous enhancer (like E_μ) had to be provided in our constructs. Similar observations have been made with other genes (27,28,37); this suggests that in addition to the $5'_{\lambda5}$ segment described here another element with an activity similar to E_μ should exist. We are currently searching for such E_μ-like, λ5-associated, regulatory elements in the neighbourhood of the λ5 gene

So far, we have only used two B-lymphocyte lineage-related promoters ($A_{\lambda5}$, A_x) in our studies of the regulating activities of $B_{\lambda5}$ on reporter gene expression. It will be interesting to test the capacity of other promoters in combination with $B_{\lambda5}$ (with or without E_μ) to confer pre-B cell-specific expression of a reporter gene.

Future experiments will ascertain the pre-B cell-specific expression pattern conferred by $5'_{\lambda5}$ during normal B cell development. Constructs containing suitable reporter genes will be used to generate transgenic mice and stably transfected normal pre-B-I cells (2). The lineage specific $5'_{\lambda5}$ promoter may allow us to express a series of genes with functions in B cell development, differentiation and deregulation at the cellular stage of the pre-B cell in a transient or permanent way. This should help us to characterize further the genes and molecules which influence these lineage specific processes.

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Abbreviations

CAT	chloramphenicol acetyltransferase
E_μ	IgH intron enhancer
E_{SV}	SV40 enhancer
LPS	lipopolysaccharide
PCR	polymerase chain reaction
SL	surrogate light chain
TdT	terminal deoxynucleotidyl transferase

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