The EMBO Journal vol.6 no.8 pp.2267–2272, 1987

A second gene, V_{preB} in the λ_5 locus of the mouse, which appears to be selectively expressed in pre-B lymphocytes

Akira Kudo and Fritz Melchers

Basel Institute for Immunology, Postfach, CH-4058 Grenzacherstrasse 487, Basel, Switzerland

Communicated by F.Melchers

The murine gene λ_5 is selectively expressed in pre-B lymphocytes. Of the three exons encoding λ_5, exons II and III show strong homologies to immunoglobulin λ light (L) chain gene segments, i.e. to J_λ intron and exon, and C_λ exon sequences respectively. We have now found, 4.6 kb upstream of λ_5, another gene composed of two exons which is selectively expressed in pre-B cell lines as a 0.85 kb mRNA potentially coding for a protein of 142 amino acids including a 19 amino acid-long signal peptide. The 5′ sequences of this gene show homologies to sequences encoding the variable regions of κ and λ L chains and of heavy (H) chains. The deduced amino acid sequence contains the consensus cysteine residues as well as other consensus amino acids at positions which characterize immunoglobulin (Ig) domains. We call the second gene V_{preB}. The 3′ end of V_{preB} encoding the 26 carboxyl terminal amino acids shows no homology to any known nucleotide sequence. The putative protein encoded by V_{preB} is a potential candidate for association with the putative protein encoded by λ_5, and thereby a candidate for association with H chains in pre-B cells. Southern blot analysis of DNA from liver (germ line) and 70Z/3 pre-B cell lines reveals two genes which hybridize to the V_{preB} gene. We call $V_{preB}1$ the gene which is found 5′ of λ_5. The other gene, called $V_{preB}2$, which has not yet been located within the genome, shows 97% nucleotide sequence homology to $V_{preB}1$ in an area of 1 kb which covers the coding region of the gene. Both $V_{preB}1$ and $V_{preB}2$ appear not to be rearranged during B cell development.

Key words: gene organization/Ig super gene family/λ5/pre-B cell

Introduction

The development of B lymphocytes from pluripotent stem cells is one of the best studied lineages of eukaryotic cell differentiation (Tonegawa, 1983; Melchers *et al.*, 1977). Different stages of this development are defined by rearrangements of gene segments encoding immunoglobulin (Ig) H and L chains, by the expression of cell surface markers, and by reactions of cells to stimuli and growth factors. Transformed cell lines and tumors exist which represent these different stages. They have facilitated the identification of a gene, called λ_5, (Sakaguchi and Melchers, 1986) which is selectively expressed in pre-B cells, but not in mature B cells or Ig-secreting plasma cells, nor in any other cell lineage tested thus far (Sakaguchi *et al.*, 1986). The λ_5 gene is expressed as a 1.2 kb mRNA coding for a 209 amino acid-long protein which includes a potential 30 amino acid-long signal peptide (Kudo *et al.*, 1987). Of the three exons encoding λ_5, exons II and III show strong homologies to λ light (L) chain gene segment, i.e. to J_λ intron and exon, and C_λ exon sequences, respectively. Exon I shows no strong homology to any known gene. The putative protein encoded by the λ_5 gene, although not yet identified, is a potential candidate for heterodimer formation with IgH chains and thereby, may influence the function which H chains play in pre-B cell development. We describe here, 4.6 kb upstream of λ_5 another gene named $V_{preB}1$ composed of two exons which is selectively expressed in pre-B cell lines as 0.85 kb mRNA. The putative protein encoded by this gene is a potential candidate for association with the λ_5 protein and may, therefore, also influence the role of H chains in pre-B cell development.

Results

Isolation of the $V_{preB}1$ cDNA clone pZ121

In a search for possible exons upstream of λ_5 with functions in the regulation of expression of this gene, we probed total RNA preparations of 70Z/3 pre-B cell lines (λ_5^+) and of EL4 thymic lymphoma cell lines (λ_5^-) by Northern blot analysis with different radiolabelled segments of the 7pB12 genomic clone (Kudo *et al.*, 1987) containing the λ_5 gene plus 10 kb upstream of it (Figure 3). The *Bam*HI–*Xba*I fragment of the 5′ region of 7pB12 was found to hybridize to 70Z/3, but not to EL4 total RNA

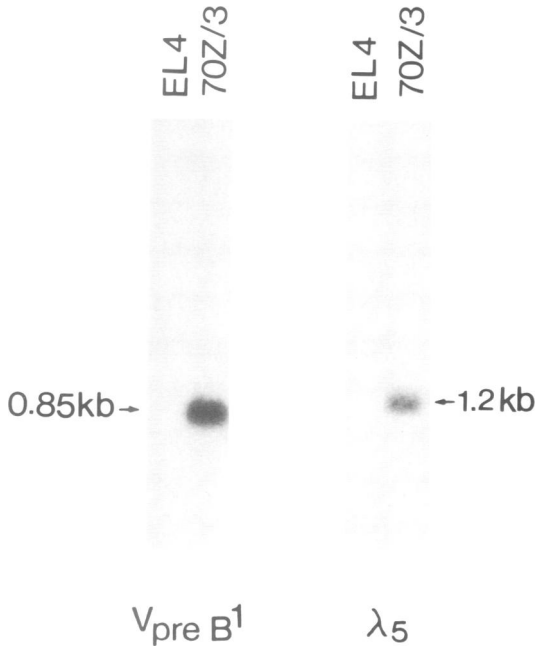

Fig. 1. Northern analysis of 10 μg total RNA from 70Z/3 pre-B lymphoma and from EL4 thymic lymphoma cells probed with the *Bam*HI–*Xba*I fragment of 7pB12 (see Figure 3, subclone a) and with the λ5 *Kpn*I–*Pst*I fragment (see Figure 3, subclone b). The same filter was hybridized first with the oligo-labelled pZ121, washed with 0.1 × SSC, 0.1% SDS at 50°C, exposed to X-ray film (Kodak XS-1) then the radiolabelled probe was washed off at 0.1 × SSC, 0.1% SDS at 95°C, and thereafter exposed to the second oligolabelled probe, pZ183-1 (λ_5) (Sakaguchi and Melchers, 1976) again washed in 0.1 × SSC, 0.1% SDS at 50°C, and again exposed to X-ray film.

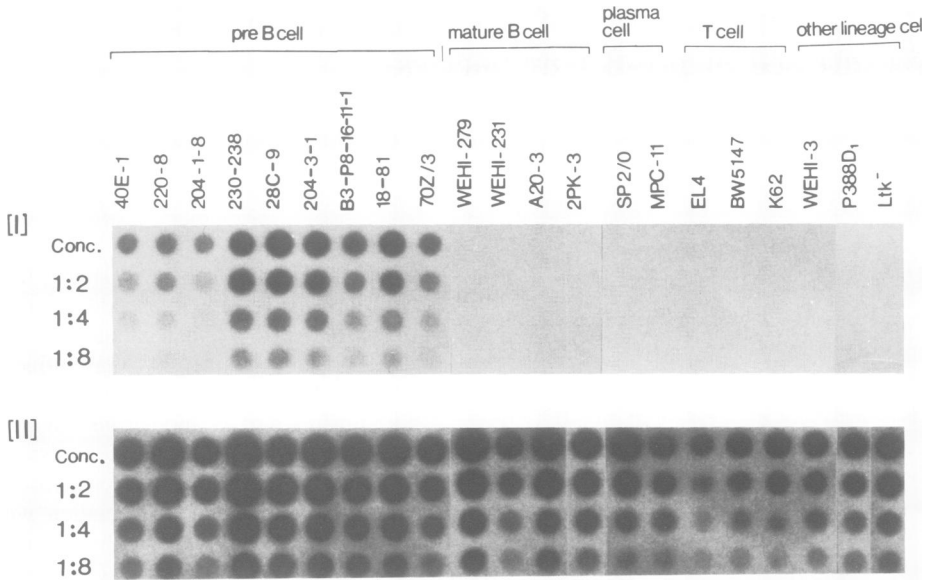

Fig. 2. Quantitative Northern dot blot analysis of pZ121 ($V_{preB}1$) transcripts in cell lines. Serial 2-fold dilutions were blotted on nitrocellulose filters for hybridization with the oligo-labelled *Eco*RI−*Acc*I fragment of pZ121. Filters were first probed with the radiolabelled pZ121 cDNA (I) then washed and thereafter probed with radiolabelled β-actin probe (Alonso *et al.*, 1986), (II) 'Conc.' indicates RNA amounts between 1 and 5 μg per dot.

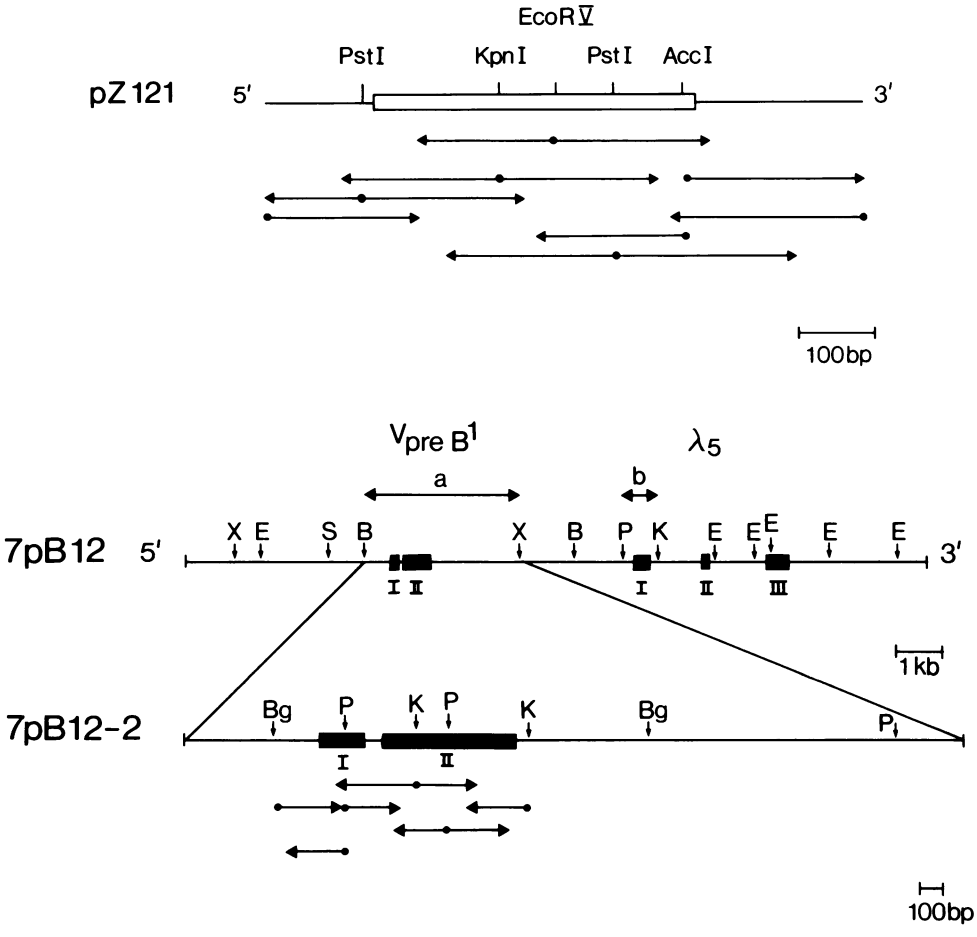

Fig. 3. Restriction map and sequence strategies of the pZ121 cDNA clone and the genomic subclone 7pB12-2, both containing $V_{preB}1$. The 3.5 kb *Bam*HI−*Xba*I fragment 7pB12-2 was subcloned into PUC-18 and a more detailed restriction map was determined. Restriction sites are B, *Bam*HI; Bg, *Bgl*II; P, *Pst*I; E, *Eco*RI; K, *Kpn*I; S, *Sal*I; X, *Xba*I. The positions of the two exons in the $V_{preB}1$ gene are indicated with I and II. The arrows indicate the direction of sequencing lengths of the sequences as determined after subcloning fragments into M13 vectors. **a** and **b** show the 3.5 kb 7pB12 *Bam*HI−*Xba*I fragment and the 0.7 kb 7pB12 *Pst*I−*Kpn*I fragment which were used as $V_{preB}1$ and λ_5 specific probes in Northern blot analyses.

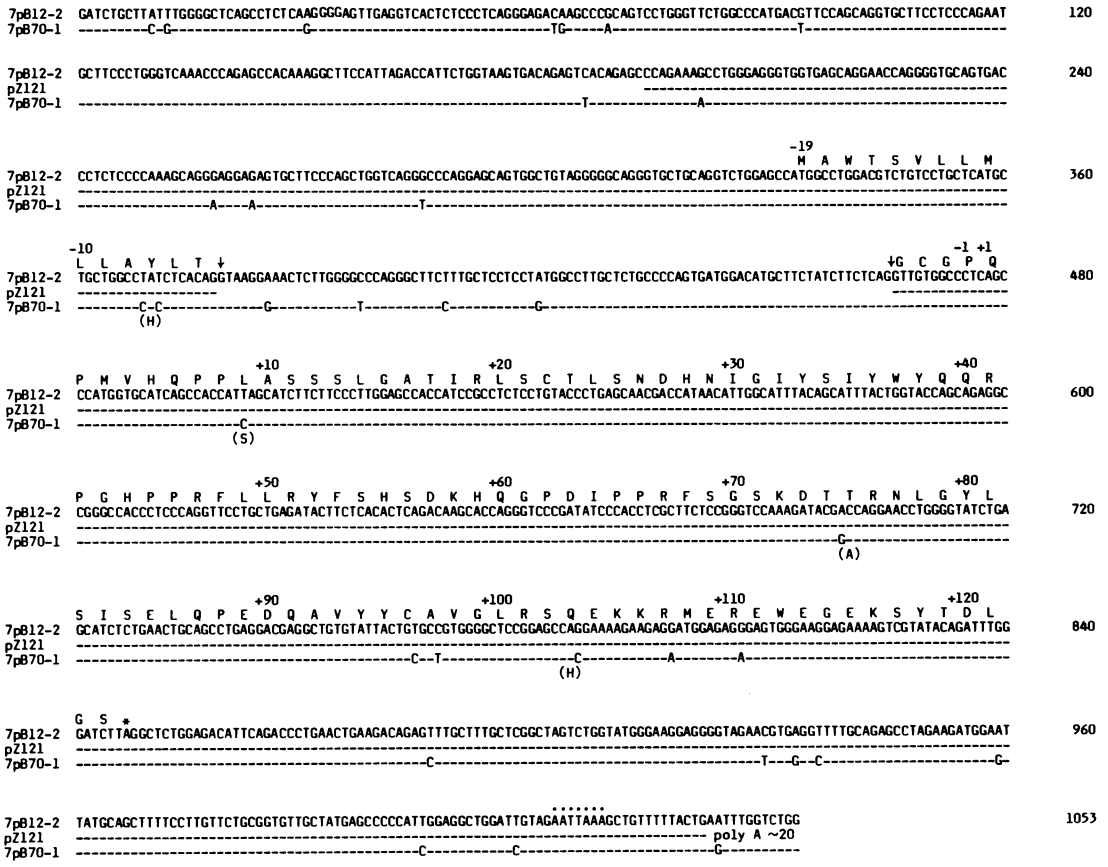

Fig. 4. Nucleotide sequence and deduced amino acid sequence of the $V_{preB}1$ and $V_{preB}2$ gene. For $V_{preB}1$ nucleotide sequences of both the genomic form (7pB12-2) as well as of the cDNA (pZ121) are given. The cDNA sequences are identical with the genomic sequences and are, therefore, only indicated by dashes (-) and follow the genomic sequence in numbering. Numbering of amino acid residues starts with −19 as the first position of the leader and proceeds to +1 as the first position of the putative mature protein. The sequence overlined by closed circles (●●●●●●●) shows the poly(A) addition signal sequence (Proudfoot and Brownlee, 1976). Arrows (↓) indicate potential splice sites. The asterisk (*) points to the termination codon TAG. The $V_{preB}2$ nucleotide sequence of the genomic form (7pB70-1) is given. (For a restriction map of 7pB70-1, see Figure 7). $V_{preB}2$ sequences identical to $V_{preB}1$ sequences are indicated by dashes (−). Wherever the deduced amino acid sequence of $V_{preB}2$ differs from that of $V_{preB}1$ the changed amino acid is given in brackets ().

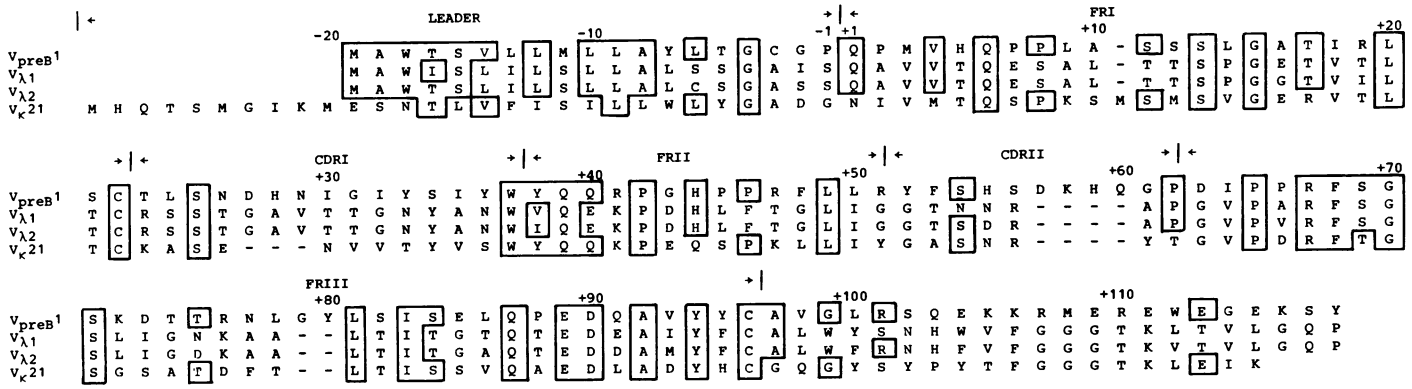

Fig. 5. Alignment of the deduced amino acid sequence of $V_{preB}1$ with sequences of $V_{\lambda 1}$, $V_{\lambda 2}$ (Tonegawa *et al.*, 1978) and $V_{\kappa 21}$ (Fong *et al.*, 1979; Hamlin *et al.*, 1981). Those residues homologous to $V_{preB}1$ and any of the other sequences are boxed. The numbering of positions follows that given for $V_{preB}1$ in Figure 4. Leader, CDRI and II and FRI, II and III indicate the locations of the leader, the complementarity-determining and the framework residues found in variable regions of L chain (Kabat *et al.*, 1983).

(Figure 1). The size of the hybridizing RNAs, however, was 0.85 kb rather than 1.2 kb of λ_5 mRNA.

This result prompted a search for a cDNA clone which would hybridize with this *Bam*HI−*Xba*I fragment of the genomic 7pB12 clone. In a library of 10^6 once amplified cDNA clones constructed from 70Z/3 pre-B cell lines poly(A^+) RNA (Sakaguchi *et al.*, 1987; Kudo *et al.*, 1987), we found around 100 positives of which we selected one, which we call pZ121.

Expression of the $V_{preB}1$ cDNA clone pZ121 in different cells

The expression of pZ121 was probed with RNA from a variety of cell lines by Northern dot blot analysis (Figure 2). It was found

to be expressed in Abelson virus-transformed cell lines of day 13−14 (40E-1), day 17−18 of gestation in fetal liver (28C-9, B3-P8-16-11-1), and of adult bone marrow (220-8, 204-1-8, 230-238, 204-3-1, 18-81), and in 70Z/3 pre-B cell lines from where the gene has been isolated. It is not expressed in mature Ig positive B cells (WEHI-279, WEHI-231, A20-3, 2pK-3), in plasma cells (Sp2/0, MPC-11), in T cells (EL4, BW5147, K62), and in macrophages (WEHI-3, P388D_1) and Ltk^- cells. The pattern of expression is, thereby, so far indistinguishable from that of the λ_5 gene (Sakaguchi and Melchers, 1986) and indicates selective expression in pre-B cell lines.

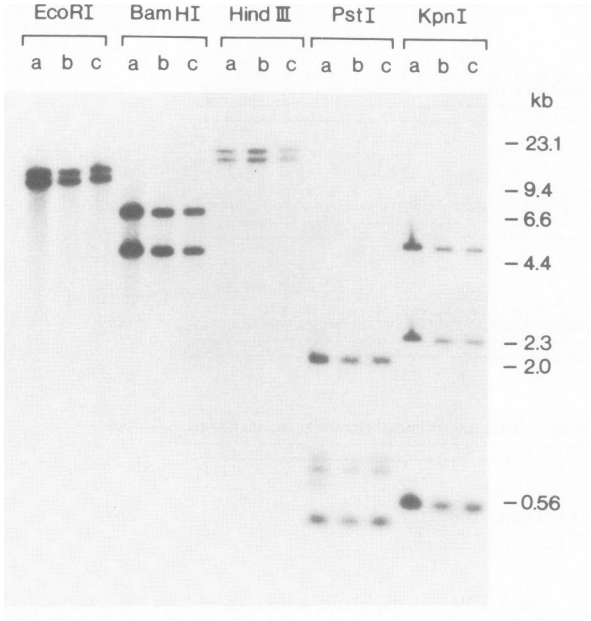

Fig. 6. Southern blot analysis of DNA from DBA/2 liver (**a**), C57BL/6 liver (**b**) and 70Z/3 pre-B cells (**c**). DNA was digested with the indicated enzymes. The filter was hybridized with ^{32}P-labelled insert DNA of the cDNA clone pZ121.

The structure of the $V_{preB}1$ gene and its comparison to other known genes

The nucleotide sequence of pZ121 was determined by the strategy outlined in Figure 3 and is shown in Figure 4. We then searched a DNA sequence data bank (GenBank, Genetic Sequence Data Bank, Release 1986) for possible evolutionary relationships of the pZ121 sequence to known DNA sequences. For positions 477 to 785 homologies were found with sequences encoding murine variable regions V_κ and V_λ of L chains (Figure 4). This nucleotide sequence shows 48% homology to the corresponding sequence of $V_{\lambda 1}$, and 46% to $V_{\kappa 21}$ (43% to framework [FR] II of $V_{\lambda 1}$, 57% to FR II of $V_{\kappa 21}$, 56% to FR III of $V_{\lambda 1}$, and 53% to FR III of $V_{\kappa 21}$. The longest open reading frame of the pZ121 cDNA codes for a protein of 142 amino acids including a 19 amino acid-long leader sequence at its amino terminus (Figure 4). Comparison of the deduced amino acid sequence of pZ121 with the published sequences of $V_{\kappa 21}$ (Fong *et al.*, 1979; Hamlin *et al.*, 1981) $V_{\lambda 1}$ and $V_{\lambda 2}$ (Tonegawa *et al.*, 1978) indicates that consensus cysteine residues as well as other consensus amino acids are conserved in the protein encoded by pZ121 which characterize Ig domains (Amzel and Poljak, 1979) (Figure 5). The homologies of pZ121 to $V_{\kappa 21}$, $V_{\lambda 1}$ and $V_{\lambda 2}$ are in the same ranges as those between V_κ and V_λ. The amino acid sequence of $V_{preB}1$ between position +1 and +103 shows 31% homology to the corresponding sequence of $V_{\lambda 1}$ and 34% to $V_{\kappa 21}$, in an area where $V_{\lambda 1}$ and $V_{\kappa 21}$ are 39% homologous. In addition to V_κ, V_λ and V_H, pZ121 detects a gene which constitutes a fourth locus of V-related sequences, and therefore we call it $V_{preB}1$.

The evolutionary relationship of $V_{preB}1$ to Ig V-gene segments is borne out further by its genomic organization. For the determination of this genomic organization the *Bam*HI−*Xba*I fragment of the 7pB12 genomic clone was subcloned and sequenced as outlined in Figure 3. The sequence is given in Figure 4. It

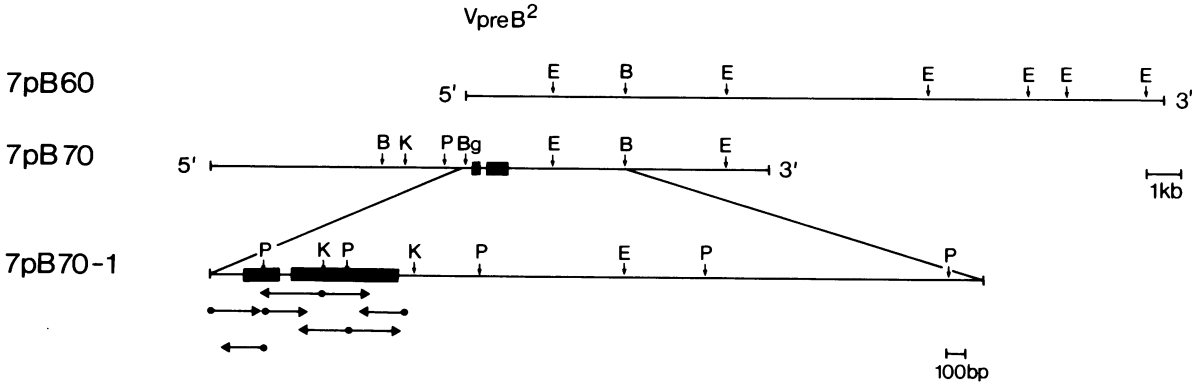

Fig. 7. Restriction map and sequencing strategy of $V_{preB}2$ genomic clones. The restriction maps of two overlapping $V_{preB}2$ genomic clones, 7pB60 and 7pB70 are shown. The 4.4 kb *Bgl*II−*Bam*HI fragment (7pB70-1) of 7pB70 was subcloned into pUC-18 and a more detailed restriction map was determined. Restriction sites are B, *Bam*HI; Bg, *Bgl*II; P, *Pst*I; E, *Eco*RI; K, *Kpn*I. The sequence is shown in Figure 4.

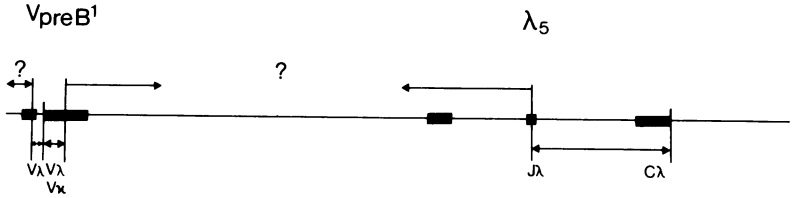

Fig. 8. Homologies of $V_{preB}1$ and λ_5 genomic sequences with genomic λL-chain sequences. The question mark indicates no homology with λL-chain sequences, and no homology to any known murine nucleotide sequence.

shows that $V_{preB}1$ is composed of two exons. Exon I codes for most of the leader peptide and is separated from exon II by 87 bp.

Sequences 5′ of the first exon of $V_{preB}1$ do not contain a TATA box as many Ig-V-gene segments do, nor an octamer consensus sequence (Parslow *et al.*, 1984) which is commonly found at 5′ ends of V_L and V_H genes of mouse and man. This is reminiscent of a similar lack of such sequences 5′ of the λ_5 gene and may indicate that different transcription regulating DNA sequences may be operative for pre-B cell-specific expression of $V_{preB}1$ and λ_5. In fact, no homologies were detectable to any known nucleotide sequences.

The nucleotide sequences encoding the leader peptide show homology to those encoding λ_1, and λ_2L chain leader nucleotide sequences but not to those encoding the $\varkappa$L chain leader peptide in $V_{\varkappa 21}$ (data not shown; Figure 4). However, sequences of $V_{preB}1$ corresponding to the V segment of L chain genes show almost equal homologies to those sequences encoding the V gene segment part of λ as well as $\varkappa$ V regions of L chains. The V-like sequences in the $V_{preB}1$ gene are followed by sequences from position 768 to the end at position 1053 (Figure 4) where no homology to any known nucleotide sequence could be detected.

No recombination heptamer and nonamer signal sequences (Sakano *et al.*, 1979) can be found at the 3′ end of $V_{preB}1$ gene. This makes it further unlikely that $V_{preB}1$ can ever be rearranged as a V gene segment in the way that the Ig and T cell receptor gene loci do. We also have no experimental evidence that $V_{preB}1$, in fact, is ever rearranged since it exists in the same genomic context in all cell lines analyzed, and in particular in those of the B lineage.

Southern blot analysis detects two V_{preB} genes

Southern blot analysis was carried out with liver DNA from DBA/2 and C57BL/6 mice, and with DNA from the mouse pre-B cell line 70Z/3, generated in C57BL/6 × DBA/2 F_1 mice. High mol. wt DNAs were digested with five restriction enzymes, *Eco*RI, *Bam*HI, *Hin*dIII, *Pst*I or *Kpn*I, the fragments separated by gel electrophoresis and probed with the $V_{preB}1$ cDNA clone pZ121. The hybridization pattern shown in Figure 6 indicates that two bands are detectable in *Eco*RI, *Bam*HI and *Hin*dIII digested DNAs from the three sources, while three and four bands were seen with *Kpn*I and *Pst*I digested DNAs. Multiple hybridizing bands with all five enzymes indicated that there were multiple V_{preB} genes in the mouse genome and prompted a structural analysis of the difference of the two genomic DNA fragments.

When DNA of the genomic clone 7pB12 containing the λ_5 associated $V_{preB}1$ gene was digested with either *Kpn*I or *Pst*I and probed in Southern blots with pZ121, it was found to contain the first and the last of the three respectively four bands seen in Figure 6. We conclude that the middle band of *Kpn*I, and the middle two bands of *Pst*I digestions seen in Figure 6 must contain the other V_{preB} gene, which we call $V_{preB}2$. It should be noted that the band with the smallest mol. wt of both *Kpn*I as well as *Pst*I digestions seen in Figure 6, in fact, contains sequences of both $V_{preB}1$ and $V_{preB}2$ genes, as analyses with the appropriate genomic clones (see below) have shown (data not shown). No differences in the lengths of restriction fragments of DNA from liver and from 70Z/3 cell lines have been detected for $V_{preB}1$ as well as $V_{preB}2$. This indicates that $V_{preB}1$ and $V_{preB}2$ genes are not rearranged during pre-B cell development.

Isolation and characterization of the $V_{preB}2$ gene

A genomic library was constructed from DNA of the pre-B cell line 70Z/3 using the EMBL-3 vector (Frischauf *et al.*, 1983). This library was screened with pZ121 as a probe. DNAs of possible clones were then digested with *Pst*I and *Kpn*I and probed in Southern blots with pZ121. Two clones, 7pB60 and 7pB70, contained the appropriate bands, i.e. the $V_{preB}2$ gene.

The *Bgl*II−*Bam*HI fragment of 7pB70 was subcloned into *Bam*HI site of pUC18 and was analyzed by restriction enzyme mapping (Figure 7) and DNA sequencing (Figure 4). Comparison to $V_{preB}1$ indicates that $V_{preB}2$ has a 97% nucleotide homology to $V_{preB}1$ in an area of 1 kb which covers the cDNA coding region of $V_{preB}1$. Although the 3′ region of $V_{preB}2$ was probed for possible homologies to λ_5, such homologies have so far not been detectable within the 7pB60 and 7pB70 genomic clones (see Figure 7).

Discussion

In our search for DNA sequences with possible regulatory functions in the expression of the λ_5 gene we have, surprisingly, come upon another gene closely linked to λ_5 and which is apparently just as selectively expressed in pre-B lymphocytes. The structure of this gene is most closely related to V region gene segments, at least for most of its 5′ portions. We have, therefore, named it $V_{preB}1$. At an unknown distance from these two genes a third gene has been found, with strong homologies to $V_{preB}1$. All three genes are located on chromosome 16 of the mouse (A.Kudo, E.Pravtcheva, N.Sakaguchi, F.Ruddle and F.Melchers, submitted for publication) which also harbors the λL chain genes (D'Eustachio *et al.*, 1981). The third gene, called $V_{preB}2$, differs in the nucleotide sequence of the coding exons from $V_{preB}1$ in nine bases, five of which constitute replacement mutations within the presumed amino acid sequences of the two V_{preB} genes. The two V_{preB} genes are apparently preserved as two copies in many laboratory strains of mice, in wild mice and in other closely related rodents such as hamsters, rats and guinea pigs (S.Bauer, A.Kudo and F.Melchers, in preparation). $V_{preB}2$ has not yet been linked at the molecular level to $V_{preB}1$ and λ_5, nor have any of them been linked to any other known genes such as those encoding the λL chains.

In a little more than the 10 kb of DNA on chromosome 16 in which $V_{preB}1$ and λ_5 are located, five areas of DNA sequences can be discerned which appear to have evolved in quite separate ways (Figure 8). $V_{preB}1$ and λ_5 are flanked at the 5′ and 3′ end by sequences with no homologies to known DNA sequences. The exon encoding the leader sequence of $V_{preB}1$ as well as the intron between this leader and the second exon of $V_{preB}1$ has strong homology to corresponding gene segments of V gene segments of λL chain genes (Tonegawa *et al.*, 1978). $V_{preB}1$ (and $V_{preB}2$) has a much lower degree of homology to V_λ, a homology which is equally high to $V_\varkappa$ segments. This is followed by a third area encoding the 3′ end of $V_{preB}1$, the intervening non-coding sequences between $V_{preB}1$ and λ_5 as well as the 5′ located first exon of the λ_5 gene which shows no homology to any known sequence. This area of sequence may well have evolved by an insertion into a pre-existing V ($V_{preB}1$) and C (λ_5). The fourth area of DNA, exon II, exon III and the intervening intron of the λ_5 gene, shows again strong homology to λL chain genes, as do the leader sequence of $V_{preB}1$. The difference in homologies within the V segments of $V_{preB}1$ (and $V_{preB}2$) indicates that this area of the two genes may have been selected for functions which differ from those of λL chain V regions.

At the present time we do not know whether $V_{preB}1$, $V_{preB}2$, or both genes are selectively expressed in pre-B lymphocytes. However, neither λ_5 nor $V_{preB}1$ (nor $V_{preB}2$) have yet been iden-

tified as proteins. We therefore cannot rule out that both $V_{preB}1$ 0.85 kb poly(A^+) RNA and λ_5 1.2 kb poly(A^+) RNA are sterile transcripts of pre-B cells. If, indeed, they are translated then $V_{preB}1$ protein could associate with itself, or with V_H domains expressed in pre-B cells, or even with λ_5 proteins. In the latter speculation $V_{preB}1$ and λ_5 proteins would form a complete V domain via noncovalent bonds which normally hold V gene segment-encoded amino acids in the three dimensional structure of a V domain together with J-gene segment encoded amino acids (Amzel and Poljak, 1979). The λ_5-encoded protein is a potential candidate for heterodimer formation with H chains in pre-B cells (Sakaguchi and Melchers, 1986), therefore, the $V_{preB}1$ (and $V_{preB}2$) encoded proteins could well associate itself with H chains through the noncovalent association with λ_5. The amino terminal 62 amino acids of λ_5 and the carboxy terminal 26 amino acids of $V_{preB}1$ would be located as a large protrusion at the complementarity determining region (CDR) 3 site. Together with the unusual extra four amino acids found in CDR 2 and two extra ones within framework region (FR) 3 (Figure 5), $V_{preB}1$, in association with λ_5, could well constitute an Ig domain with unusual, constant binding properties, which may be of importance in the regulation of pre-B cell development.

Materials and methods

Cell lines and culture conditions

The pre-B cell Abelson lines, 40E-1, 220-8, 204-1-8, 230-238, 28C-9, 204-3-1, 18-81 were obtained from Dr N.Rosenberg, Tufts University Medical School, Boston, MA. 70Z/3 cells and WEHI-231 were obtained from Dr Christopher Paige from our Institute. The Abelson virus transformed pre-B cell line B3-P8-16-11-1 was given to us by Dr M.Reth (Institute for Genetics, University of Cologne, Cologne, FRG). The B cell lymphoma lines, WEHI-279 (Sibley *et al.*, 1980), 2PK-3 and the thymoma EL4 were given to us by Dr J.McKearn, Dupont Glenolden Laboratories, Glenolden, PA. The B cell tumor line A20-3 was brought to our Institute by Dr J.Kim, National Institutes of Health, Bethesda, MD. The macrophage lines $P388D_1$ and WEHI-3 were given to us by Dr N.Iscove (EMBL, Heidelberg, FRG). The thymic lymphoma BW5147 ($HPRT^-$, Oua^r) was given to us by Dr Hyman, Salk Institute for Biological Studies, La Jolla, CA. The other cell lines were SP2/0 (given to us by Dr G.Köhler, Max Planck Institute for Immunobiology, Freiburg i. Br., FRG), MPC-11 (given to us by Dr M.Scharff, Albert Einstein College of Medicine, Bronx, NY), and K62 T cell hybridoma line (given to us by Dr Antonius Rolink, Basel Institute for Immunology). All cell lines were grown in Dulbecco's medium containing 2 mM glutamine, 5% heat inactivated fetal calf serum (Gibco), β-mercaptoethanol (5×10^{-5} M), streptomycin (100 μg/ml) and penicillin (100 units/ml) in a 10% CO_2 atmosphere.

Northern blot analysis and RNA dot blot

Total RNA for Northern blot analysis (Rave *et al.*, 1979) was prepared by phenol extraction in the presence of 1% sodium dodecylsulphate (SDS). 10 μg of total RNA was electrophoresed by agarose/formaldehyde method and transferred to the nitrocellulose filter. Pre-hybridization and hybridization were performed with 50% formamide at 39°C with oligolabelled probes (Feinberg and Vogelstein, 1984). The filter was finally washed with 0.1 × SSC, 0.1% SDS at 50°C. Preparations of cytoplasmic RNA for RNA dot blot analysis and transfers to nitrocellulose filters were done as described (White and Bancroft, 1982). Prehybridization and hybridization were performed with 1.5 × SSPE, 1.0% SDS, 0.5% non-fat powdered milk, 0.5 mg/ml carrier salmon sperm DNA. Filters were washed finally with 0.1 × SSC, 0.1% SDS at 60°C.

Isolation of cDNA clone pZ121

The cDNA library was constructed from 70Z/3 pre-B lymphoma poly(A^+) RNA by using λgt11 vectors (Young and Davis, 1983). Seven clones were identified as strongly hybridizing clones from around 100 positive clones using the 3.5 kb long 7pB12-2 *Bam*HI−*Xba*I fragment as a probe. After restriction map analyses, pZ121 was chosen because it appeared to have the longest insert with 780 bp including 20 As at the 3′ end. DNA sequencing was carried out using the dideoxy chain termination method (Sanger *et al.*, 1977) by subcloning of fragments into M13mp18 and M13mp19 vectors, using a 17-mer universal M13 primer (Amersham).

Isolation of genomic clones 7pB60 and 7pB70

DNA from the 70Z/3 pre-B lymphoma, partially digested with *Mbo*I to an average size of 20 kb, was used to construct a genomic phage library in the vector EMBL-3 (Frischauf *et al.*, 1983). The genomic library was screened with a ^{32}P-labelled pZ121 probe. Pre-hybridization and hybridization were carried out with 50% formamide at 42°C (Maniatis *et al.*, 1982). Filters were finally washed with 0.2 × SSC, 0.1% SDS at 65°C. After screening of one million recombinant amplified clones, six independent clones were found and further examined by restriction map analysis. Two clones, 7pB60 and 7pB70 were selected. The 4.4 kb *Bam*HI−*Bgl*II fragment of 7pB70 was subcloned into the *Bam*HI site of pUC18 and was further analyzed by restriction enzyme mapping and DNA sequencing.

Southern blot analysis

High mol. wt DNAs were extracted from DBA/2 liver, C57BL/6 liver, and from the 70Z/3 (DBA/2 × C57BL/6) pre-B lymphoma cell lines (Paige *et al.*, 1978). After restriction enzyme digestion, 10 μg of DNA fragments were separated by electrophoresis on agarose gels and transferred to the Zeta probe membrane (BioRad) using 0.4 N NaOH as transfer buffer (Reed and Mann, 1985). After transfer, filters were rinsed twice in 2 × SSC and dried at 80°C under vacuum. Pre-hybridization and hybridization were carried out with 1.5 × SSPE, 1.0% SDS, 0.5% non-fat powdered milk, 0.5 mg/ml carrier salmon sperm DNA and 10% dextran sulfate at 65°C by using ^{32}P-oligo-labelled insert DNA of cDNA clone pZ121 as a probe. Filters were washed finally with 0.1 × SSC, 0.1% SDS at 65°C.

Acknowledgements

The able technical assistance of Ms Heidi Bächtold, Mr Wyn Davies and Ms Denise Richterich is gratefully acknowledged. We thank Drs Steven Bauer, Christopher Coleclough and Jim Kaufman for critical reading of our manuscript. The Basel Institute for Immunology was founded and is supported by F.Hoffmann-La Roche and Co. Ltd., Basel, Switzerland.

References

Alonso,S., Minty,A., Bourlet,Y. and Buckingham,M. (1986) *J. Mol. Evol.*, **23**, 11−22.
Amzel,M.L. and Poljak,R.J. (1979) *Annu. Rev. Biochem.*, **48**, 961−997.
D'Eustachio,P., Bothwell,A.L.M., Takaro,T.K., Baltimore,D. and Ruddle,F.H. (1981) *J. Exp. Med.*, **153**, 793−800.
Feinberg,A.P. and Vogelstein,B. (1984) *Anal. Biochem.*, **137**, 266−267.
Fong,K., Lei,A. and Salser,W. (1979) *Biochem. Biophys. Res. Commun.*, **90**, 832−841.
Frischauf,A.M., Lehrach,H., Pouska,A. and Murray,N. (1983) *J. Mol. Biol.*, **170**, 827−842.
Hamlin,P.H., Gait,M.J. and Milstein,C. (1981) *Nucleic Acids Res.*, **9**, 4485−4494.
Kabat,E.A., Wu,T.T., Bilofsky,H., Reid-Miller,M. and Perry,H. (1983) *Sequences of Proteins of Immunological Interest.* US Department of Health and Human Services, Washington, USA.
Kudo,A., Sakaguchi,N. and Melchers,F. (1987) *EMBO J.*, **6**, 103−107.
Maniatis,T., Fritsch,E.F. and Sambrook,J. (1982) *Molecular Cloning, A Laboratory Manual.* Cold Spring Harbor Laboratory Press, New York.
Melchers,F., Andersson,J. and Phillips,R.A. (1977) *Cold Spring Harbor Symp. Quant. Biol.*, **41**, 147−158.
Parslow,T.G., Blair,D.L., Murphy,W.J. and Granner,D.K. (1984) *Proc. Natl. Acad. Sci. USA*, **81**, 2650−2654.
Paige,C.J., Kincade,P.W. and Ralph,P. (1978) *J. Immunol.*, **121**, 641−647.
Proudfoot,N.J. and Brownlee,G.G. (1976) *Nature*, **263**, 211−214.
Rave,N., Crkvenjakov,R. and Baetker,H. (1979) *Nucleic Acids Res.*, **6**, 3559−3567.
Reed,K.C. and Mann,D.A. (1985) *Nucleic Acids Res.*, **13**, 7201−7221.
Sakaguchi,N. and Melchers,F. (1986) *Nature*, **324**, 579−582.
Sakaguchi,N., Berger,C.N. and Melchers,F. (1986) *EMBO J.*, **5**, 2139−2147.
Sakano,H., Huppi,K., Heinrich,G. and Tonegawa,S. (1979) *Nature*, **280**, 288−294.
Sanger,F., Nicklen,S. and Coulson,A. (1977) *Proc. Natl. Acad. Sci. USA*, **74**, 5462−5487.
Sibley,C.H., Ewald,S.J., Kehry,M.R., Douglas,R.H., Raschke,W.C. and Hood,L.E. (1980) *J. Immunol.*, **125**, 2097−3003.
Tonegawa,S. (1983) *Nature*, **302**, 575−581.
Tonegawa,S., Maxam,A.M., Tizard,R., Bernard,O. and Gilbert,W. (1978) *Proc. Natl. Acad. Sci. USA*, **75**, 1485−1489.
White,B.A. and Bancroft,F.C. (1982) *J. Biol. Chem.*,, **257**, 8569−8572.
Young,R.A. and Davis,R.W. (1983) *Proc. Natl. Acad. Sci. USA*, **84**, 1194−1198.

Received on April 30, 1987; revised on May 25, 1987