
Cloning and sequencing of a rearranged V_{λ} gene from a Burkitt's lymphoma cell line expressing kappa light chains

Lee-Hwei K.Sun, Carlo M.Croce and Louise C.Showe*

The Wistar Institute of Anatomy and Biology, 36th St. at Spruce, Philadelphia, PA 19104, USA

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ABSTRACT

We have cloned and sequenced a rearranged V_{λ} gene from a Burkitt's lymphoma cell line PA682(PB). This cell line has two rearranged κ loci and has been shown to be expressing κ light chains (1). This V_{λ} gene has been identified as a member of the V_{λ} subgroup III gene family based on the homology of the predicted amino acid sequence of PAV_{λ} with the reported sequences of the V_{λ} protein DEL of subgroup III. Nine cross-hybridizing bands have been detected on Southern blots and the chromosomal orientation of the V_{λ} subgroup III gene family has been determined in relation to the V_{λ} subgroup I gene family. Although the PAV_{λ} rearrangement has occurred via a legitimate V-J joining and a normal size transcript is detected on Northern blots, the nucleotide sequence reveals a high level of mutations resulting in multiple termination signals within the V gene coding sequence and only a truncated V_{λ} protein can be translated. This confirms previous observations that although multiple light chain genes may be transcribed, only one functional light chain protein can be synthesized.

INTRODUCTION

Magrath *et al.* (1) have described three PA682 cell lines derived simultaneously from peripheral blood (PB), pleural effusion (PE), and bone marrow (BM) of a patient with Burkitt's lymphoma. The clonal origin of these three lines was established cytologically by the presence of an (8;22) chromosomal translocation in all three cases and by Southern analyses of the heavy chain and light chain loci for immunoglobulins. Identical rearrangements of the μ , κ , and λ loci were found with several restriction enzymes (1). The rearrangements of both μ and κ loci could be directly related to the secretion of μ and κ immunoglobulin proteins in all three cell lines by an Elisa assay (1). In light of the proposed hierarchy for the light chain rearrangement (2), the rearrangement of the λ locus in a cell expressing κ light chains would be a rare event. Therefore, it was suggested that the rearrangement of the C_{λ} locus in the PA682 cell lines might be due to the (8;22) translocation described in these cell lines (1).

We report here the molecular cloning and characterization of the

rearranged C_λ restriction fragment described by Magrath et al. (1). This rearrangement is not associated with the (8;22) chromosomal translocation, but results from a rare λ rearrangement in cells expressing κ light chains (3,4). The rearrangement results from a V-J recombination on the normal chromosome 22. This V_λ gene (PAV_λ) has been sequenced and the predicted amino acid sequence has been found to be related to a human λ protein of subgroup III. Using Southern blotting at low stringency conditions, the PAV_λ probe detected nine cross-hybridizing bands for this V_λ family.

MATERIALS and METHODS

Genomic Library Construction

Genomic library was constructed in lambda phage vector EMBL3A. High molecular weight DNA was partially digested with restriction endonuclease Sau3A and size-fractionated on a 10-40% sucrose gradient. DNA fragments of 18-23 kb were ligated with EMBL3A lambda phage arms and packaged according to Hohn and Murray (5). Packaging efficiency was 4×10^5 recombinant phages per μg of insert DNA. The genomic library was screened at a density of 15,000 recombinant plaques per 150 mm diameter petri dish. Plaque hybridizations were carried out in 5X SSC at 65°C for 18 h. Final washes were in 1.0X or 0.5X SSC at 65°C.

DNA Analysis

Genomic DNA was digested with restriction endonucleases, fractionated by electrophoresis through 0.5% or 0.7% agarose gels, and blotted to nitrocellulose (6). Hybridizations were in 4X SSC and 50% formamide at 37°C for 48 h unless otherwise noted. Final washes were 0.5-0.2X SSC at 65°C.

RNA Isolation and Analysis

Cytoplasmic RNA was extracted from actively dividing cell cultures according to Scott and Frankel (7). RNA samples were denatured at 60°C with 50% formamide and 2.2 M formaldehyde and electrophoresed on 1% agarose gels containing 2.2 M formaldehyde. RNA was transferred to nitrocellulose and hybridized essentially as described by Thomas (8). Removal of hybridized probe was accomplished by washing the blots in 1 mM EDTA at 65°C for 0.5-1 h. The blots were then prehybridized and hybridized with another probe.

Probes

The C_λ probe is an 8 kb EcoR I human genomic DNA fragment which includes C_λ genes #2 (Ke^-0z^-) and #3 (Ke^-0z^+) (9). The C_λ coding probe is a 900 bp BglII/EcoR I fragment which contains the coding sequences for the C_λ

gene #3. ^{32}P -labeled probes (specific activity, 5×10^8 cpm/ μg) were prepared by using calf thymus primers (10). Free nucleotides were removed by centrifugation through a Sephadex G-75 mini-column.

DNA Sequences

DNA sequences were obtained using the dideoxy chain termination method (11) on fragments subcloned into M13 bacteriophage vectors mp18 and mp19.

S1 Nuclease Analysis

S1 nuclease analysis was carried out according to Sharp et al. (12) by using 5'- and 3'-labeled probes derived from clone pPAL 4.2E. The ^{32}P -labeled probes were heat-denatured, hybridized in 80% formamide with 20 μg of cytoplasmic RNA at 57°C overnight. Each reaction was digested with 3000U of S1 nuclease (Boehringer Mannheim), and the products analyzed by electrophoresis on a 6% polyacrylamide gel containing 7M urea.

RESULTS

Cloning of the Rearranged C_λ Fragment

A genomic DNA library was prepared from the PA682 cell line derived from PB [PA682(PB)]. Approximately 3×10^5 lambda phage recombinants were screened directly with the 8 kb C_λ genomic probe which contains coding and flanking regions for C_λ genes 2 and 3 (9). Twelve positive clones were isolated. Figure 1 shows the hybridization pattern with the C_λ probe on a Southern blot which compares the germline pattern in the T cell line Molt 4 and the rearranged pattern of the PA682(PB) cell lines. The 4.2 kb EcoR I and the 13 kb Hind III fragments are equivalent to the rearranged C_λ restriction fragments at 4.8 kb and 16 kb described by Magrath et al. (1). Four of the 12 recombinants were found to contain the rearranged 4.2 kb EcoR I fragment. Three of these four clones extended sufficiently far 5' to contain the intact 13 kb Hind III fragment which includes the 4.2 kb rearranged fragment. Figure 2 shows the restriction maps of these overlapping clones which span 30 kb of the λ light chain locus. Within the 15 kb extending 3' of the 4.2 kb EcoR I fragment (pPAL4.2E), there are three additional restriction fragments which hybridize to the C_λ coding probe. These fragments correspond to the C_λ genes #4, #5, and #6 as described by Hieter et al. (9). However, the sequences 5' to pPAL4.2E diverge from the published restriction map for the λ locus (9). The comparison of restriction maps for pPAL4.2E and the germline C_λ locus indicates that a rearrangement has occurred near the BamHI site within pPAL4.2E as indicated by the arrowhead in Figure 2A. In order to determine whether

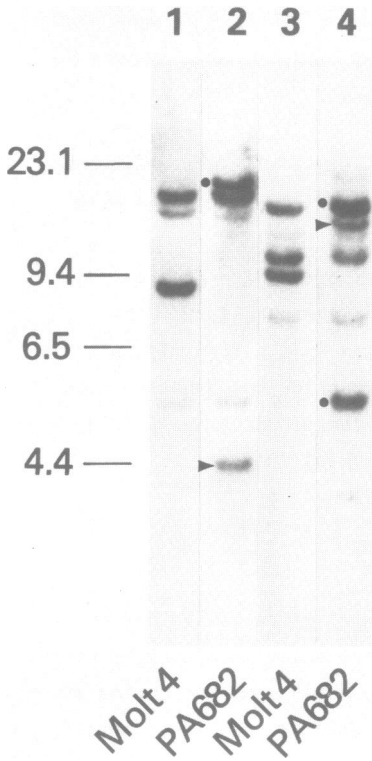


Fig. 1: Rearranged C_λ genes in PA682 cells. Ten micrograms of genomic DNA were digested with *EcoR* I (lanes 1,2) or *Hind* III (lanes 3,4), fractionated on a 0.5% agarose, and transferred to a nitrocellulose filter. The filter was then hybridized with a 900 bp *EcoR* I/*Bgl*III probe containing the coding region of the C_λ gene #3. Lanes 1 and 3, Molt4 which serves as a germline control; lanes 2 and 4, PA682(PB). Arrowheads indicate the 4.2 kb *EcoR* I and the 13 kb *Hind* III rearranged fragments that we have cloned. Other rearranged C_λ fragments are marked with solid circles (●).

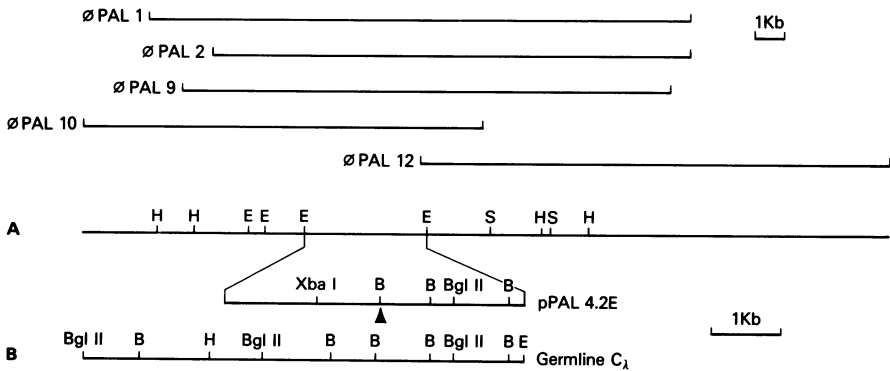


Fig. 2: Overlapping clones of the λ locus in PA682 cells. (A) Restriction map of the rearranged λ locus in PA682(PB). (B) Restriction map of the relevant germline C_λ locus. Arrowhead indicates where these two maps diverge. Abbreviations: E = *EcoR* I; H = *Hind* III; B = *Bam*HI; X = *Xba*I.

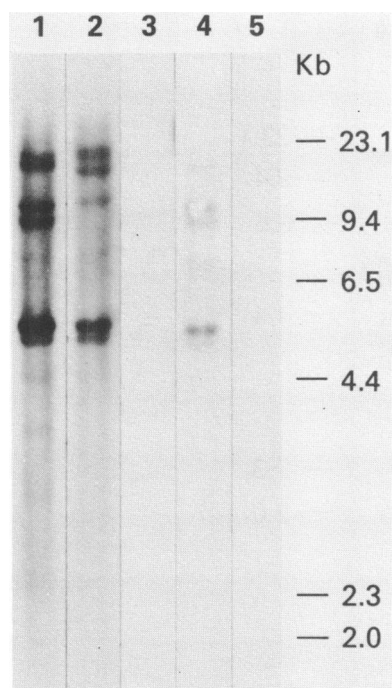


Fig. 3: Southern blot analysis of somatic cell hybrids with pPAL4.2E as a probe. Ten micrograms of genomic DNA were digested with *Hind* III, fractionated on a 0.7% agarose gel, and transferred to a nitrocellulose filter. The filter was hybridized with pPAL4.2E. Lane 1, PAF (SV40-transformed human fibroblast cell line); lane 2, PA682(PB) (κ -producing Burkitt's lymphoma); lane 5, NP3 (mouse plasmacytoma cell line). Lanes 3 and 4 are DNA samples of somatic cell hybrids derived by using NP3 as a fusion partner (23). Hybrids 706CL17 (lane 3) and DSK1B2A5C12 (lane 4), contain human chromosome 8, and 22, respectively.

these rearrangements resulted from the t(8;22) translocation, the 4.2 kb *Eco*R I fragment was isolated and used to analyze somatic cell hybrids which segregated the relevant chromosomes.

In Southern analysis of DNAs from somatic cell hybrids which segregated chromosomes 8 and 22, the subclone pPAL4.2E which contains the rearranged fragment hybridized only to DNA derived from the somatic cell hybrid containing chromosome 22 and not to hybrids containing chromosome 8 (Fig. 3). Since pPAL4.2E contains sequences derived only from chromosome 22, this rearrangement could not result from the t(8;22) translocation. In addition, DNAs from somatic cell hybrids of PA682(PB) which contain the 8q⁺ and 22q⁻ chromosomes, but not the normal chromosome 22, do not contain this rearranged 4.2 kb C_λ band on the Southern blots (J. Erikson and C. M. C., unpublished results). Therefore, this rearranged *Eco*R I fragment must be on the normal chromosome 22, and is not in any way associated with the chromosomal translocation. Furthermore, the probe pPAL4.2E detects multiple cross-hybridizing bands in PA682(PB) DNA in addition to the C_λ bands (Fig. 3, lane 2). This suggests that pPAL4.2E contains sequences which may be detecting a V_λ gene family and that the DNA rearrangement

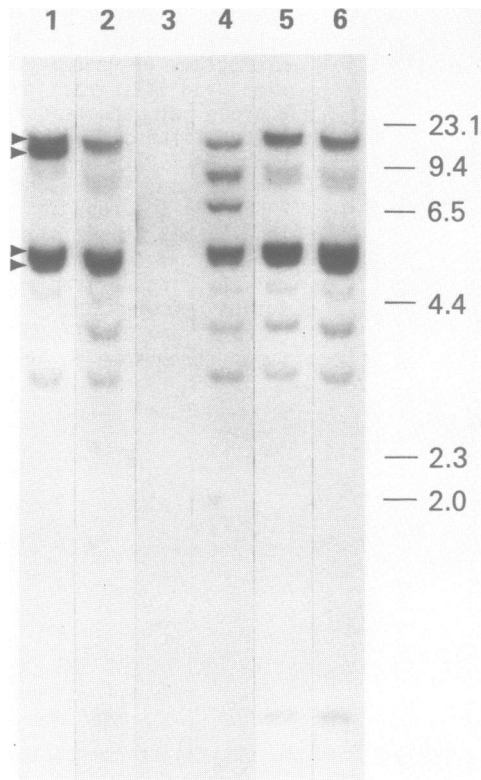


Fig. 4: Southern blot analysis of different cell lines with probe EB derived from pPAL4.2E (see restriction map in Fig. 5). Ten micrograms of DNA were digested with Hind III, fractionated on 0.7% agarose gel, and transferred to a nitrocellulose filter. The filter was hybridized with probe EB in 6 X SSC and 50% formamide at 37°C. The filter was washed in 2 X SSC at 65°C. Lane 1, PA682(PB) (κ -producing Burkitt's lymphoma); lane 2, LY67 (λ -producing Burkitt's lymphoma); lane 3, BL2 (λ -producing Burkitt's lymphoma); lane 4, GM1056 (λ -producing human lymphoblastoid cell line); lane 5, JD38 (κ -producing Burkitt's lymphoma); lane 6, PAF (human fibroblast cell line). Arrowheads indicate the bands detected under conditions of higher stringency: hybridizing in 4 X SSC and 50% formamide at 37°C and washing in 0.2 X SSC at 65°C.

represents a V-J joining within the C_λ locus.

Characterization of pPAL4.2E

In order to determine whether the C_λ rearrangement had occurred as a result of a V-J joining event, pPAL4.2E was further subcloned to separate the sequences which are located 5' of the rearranged BamH1 site from the non-rearranged C_λ sequences at the 3' end of pPAL4.2E (Fig. 5). The EcoR

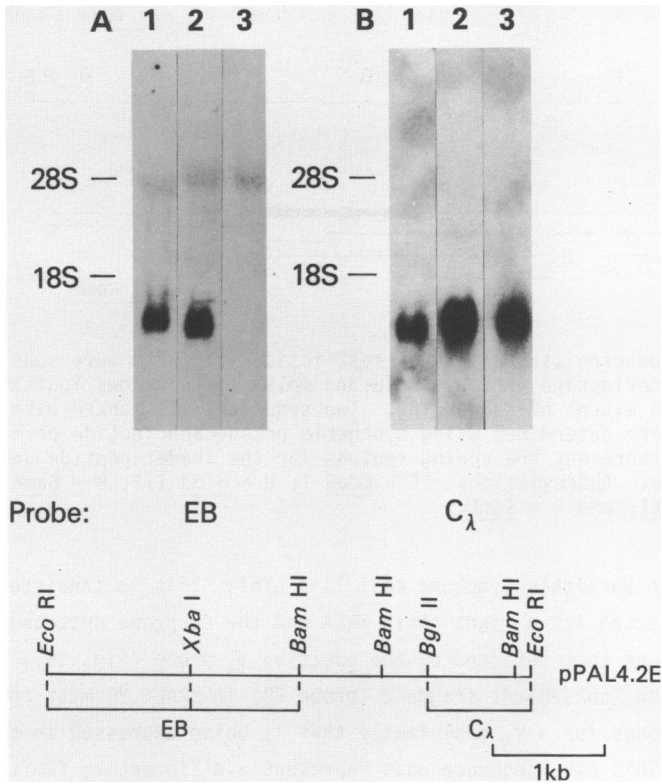


Fig. 5: RNA blot analysis. Twenty micrograms of total cytoplasmic RNA were fractionated on 1% formaldehyde agarose gels, transferred to nitrocellulose and hybridized with probe EB(A) or a C_λ probe (B) as indicated. Lane 1, PA682(PB); lane 2, LY67; lane 3, BL2.

I/BamHI (EB) fragment derived from the 5' half of pPAL4.2E does not cross-hybridize with either the C_λ #2 or #3 coding regions or the flanking sequences included within the 8 kb C_λ probe. On Southern blots, probe EB detects three to four bands under conditions of high stringency, as indicated with arrowheads in Figure 4. Under conditions of lower stringency, 9 to 10 cross-hybridizing bands can be detected. This hybridization pattern is consistent with the hypothesis that the EB restriction fragment codes for a V gene segment.

Since no cross-hybridization is observed between the EB fragment and the C_λ sequences, this EB probe was subsequently hybridized to a Northern blot of RNAs derived from a variety of cell lines which express λ light chains. As shown in Figure 5A, probe EB detect a high level of a 1.2-kb

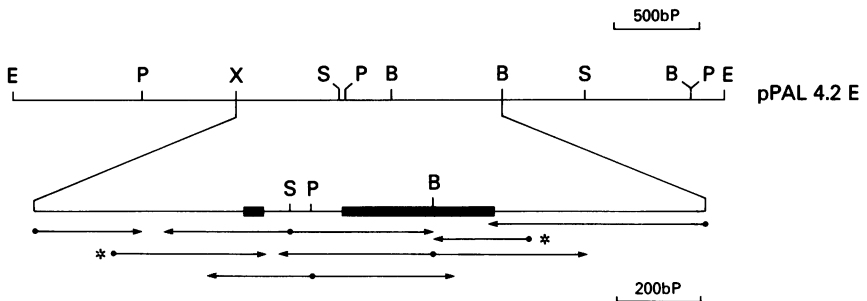


Fig. 6: Sequencing strategy. DNA restriction fragments were subcloned into M13 bacteriophage vectors (mp18 and mp19). The arrows indicate the direction and extent of sequencing. Two sequences, as marked with asterisks, were determined using synthetic octadecanucleotide primers. Solid boxes represent the coding regions for the leader peptide and the variable gene. Abbreviations: E = EcoR I; H = Hind III; B = BamHI; X = XbaI; P = PstI; and S = SstI.

transcript in Burkitt's lymphoma cell line LY67. This is consistent with the size expected for a light chain mRNA and the C_λ probe detected the same size message as that detected by the putative V_λ probe (Fig. 5B). Therefore, the EcoRI/BamHI fragment (probe EB) in pPAL4.2E must contain coding sequences for a V_λ gene family that is being expressed in the LY67 cell line. This PAV_λ sequence must represent a different V_λ family than the V_λ I sequence expressed in the BL2 cell line (13), as probe EB does not hybridize to RNA from BL2 cells (Fig. 5A). Furthermore, Southern blot analysis indicates that the sequences included in the PAV_λ probe are completely deleted in the BL2 cell line (Fig. 4). This observation suggests that the PAV_λ gene family is more proximal to the C_λ gene cluster than the V_λ I gene family, and has been deleted from both chromosomes in the BL2 cell line. Both the V_λ I probe and the PAV_λ probe cross-hybridize with 9 to 10 separate bands on Southern blots but do not cross-hybridize with each other even at low stringencies. If each of the six V_λ subgroups which have been defined by protein sequences (14) are equally large, this would be equivalent to a germline repertoire of at least 60 V_λ genes. The recently cloned V_λ VII gene (15), which is not represented in the six known protein subgroups, would further increase this estimate. The PA682 cell line is expressing a low level of C_λ transcripts in addition to the C_κ transcripts which was previously described (1). The positive hybridization of the λ transcripts with the EB probe suggests the transcription originates from the cloned λ gene.

The genomic clones described in Figure 2 extend 10 kb beyond the 5' end of the PAV λ gene that we have identified. No other sequences which are homologous to either the V λ I (13) or the PAV λ gene have been detected within this region. This would indicate a spacing of greater than 10 kb between V λ genes. We cannot eliminate the possibility that all other members of the PAV λ family have been deleted from this chromosome, however, the intensity of the bands seen on Southern blots comparing the PA682 cell line and a T cell control appear equivalent for most bands.

Characterization of PAV λ

To study the fine structure of the PAV λ gene, we determined the nucleotide sequence of the 1.6 kb XbaI/BamHI restriction fragment. The sequencing strategy is outlined in Figure 6 and the nucleotide sequence is summarized in Figure 7.

Leader Peptide Starting at the translation initiation codon at nucleotide 506 we have identified an exon which codes for the first 16 amino acids of a leader peptide which are indicated as negative numbers. The splice junctions, GT/AG (vertical arrows in Fig. 7), bracket an intron of 187 nucleotides which interrupts the leader peptide within position -4 which codes for a highly conserved glycine residue. The reading frame that resumes after the AG splice encodes three additional amino acids of the leader peptide. Twelve of these 19 residues are homologous to the reported sequence for the leader peptide of a human V λ I gene (13) shown in Figure 7B. The arrowhead between nucleotide position 748 and 749 indicates a presumptive single nucleotide deletion at this point within the leader peptide. If the reading frame is continued from amino acid residue -3 without assuming this deletion, a peptide of 52 amino acid residues is translated with a termination codon at nucleotide position 992-994. This peptide sequence has no homology to any of the published light chain protein sequences. The amino acid sequence which is translated assuming this deletion between nucleotide 748 and 749 (Fig. 7A) is homologous to the human V λ subgroup III protein DEL (16). The sequence of DEL begins at amino acid residue 1 in Figure 7B.

Variable Gene Region The overall homology between the predicted amino acid sequences of PAV λ and the sequence of a subgroup III lambda protein, DEL, is 67%, with 76% homology within the framework regions, and 41% homology within the complementarity-determining regions. Six of the 37 highly conserved amino acid residues, which are indicated with asterisks in Figure 7, have been altered by single base changes. In addition to these changes,

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0   TCTAGAGAAGCAGAAAGTAAACACCCTCCCTCCTGAGCCAGGATGGAATGGAGGAGGGGACTGTGGACCCAGATAA
80   TTCCCCATCACCACCTGTGACTCTGGTAACCTCTTGAACAGGGCCAAACCACTATCCCATAGGAAGGACTTTATATCCCT
160  AGAAAATACAGAGAAACTCAGCTCTGAGTTTTTCCATGACCAACTCAGCCCAGGGCAAAGTGGCACAATCTGGGTAAG
240  ATGTGAACCTAGACCATGGGACCAGTGGGTGGAGAGAATCCCATGGGCTGAGGGGTGGGCAACCAGTGGTCAACCCTT
320  TTCTCTCTCTCTGTGTCCCTGGGATGAGCCCTCTCTGGAACCCAAAGCTCCTCCAGCAGCAACCCCTGACTCTG
400  CTGTTTGCATCATGGGCTGTTCTCTCCAGCAAGGGGATAAGAGAGGCTGGGAGGAACCTGCCTAGCCTGGGCCTCAGG
480  AAGCAGCATCAGCAGTGCCTCAGCCATGGCCTGGACCCCTCTCTCCTCAGCCTCCTCGCTACTGCACAGGTGCTCTGC
(A) MetAlaTrpThrProLeuLeuLeuSerLeuLeuAlaHisCysThrGly
(B) ---ThrCysSer-----Thr-----Ile-----
      -19                      -10                      -4
560  CCAGGGTATCACCACCTGCCATCCCCAGGGCTCTGGGTCCAGTGTGGCCATGACTATGAGCTCAGGAGGGCCCTGCTG
640  TGGTGGGCAGGATGTCATGACCCCTGCAGGGTGAGGGACTGGCGGATGAAGTCCCTCAAATATCCTCTGCTTTTGT
720  TTTATTTTAAATTTTGCAGGCTCTCGACTCGTATGCTCTGACTCAGCCACACTCAGTGTGCTGAGCCACGGCAGAGATG
(A) GlySerArg??SerTyrValLeuThrGlnProHisSerValSerValAlaThrAlaGlnMet
(B) -----TrpAla-----Ser-----* 10 * *---Thr
      -4      -1 1 * 10 * *
800  GCCTGGATCACCTGTGGGAGAAGCAACATTGGAAGTTAAGCTGTGCATGGTCCCAGCAACCCGAGCCAGGACCCTGT
(A) AlaTrpIleThrCysGlyArgSerAsnIleGlySerxxxAlaValHisTrpSerGlnGlnThrAlaGlyGlnAspProVa
(B) ---Arg-----GlyAspGly-----GlyLysSer-----Tyr-----LysPro-----* 40 Ala-----
      20                      30                      * 40
880  GATGGTCATCTATAGCGAGAGTAGCCGGCCCTCAGGGATCCCTGAGCGATTCTCTGGCCCCAACCCAGGGAACACCGCCA
(A) lMetValIleTyrSerArgIleGluAlaGlyAspSerGlyIleProGluArgPheSerGlyProAsnProGlyAsnThrAlaT
(B) -Leu---ValHisGluAspAsnAsp-----Ala-----Ser---Ser-----A
      50                      60                      * 70
960  CCCTAACCATCAGCAGGATCGAGGCTGGGGATGAGGCTGACTTTCTGTGAGGTGGGACAGTACTAGTGATCGCTGG
(A) hrLeuThrIleSerArgIleGluAlaGlyAspGluAlaAspPheSerCysGlnValTrpAspSerThrSerAspArgTrp
(B) la-----Val-----TyrTyr-----AspArgThrAlaHisVal
      80 * 90
1040 GTGTTGCGGGAGGGACCAAGCTGACCCCTCCTAGGTGAGTCTCTTCTCCCTCTCCTTCCCGCTCTTGGGACAATTTCT
(A) ValPheGlyGlyGlyThrLysLeuThrValLeuGly
(B) -----
      100                      109
1120 GTTGTTTTGTGTTTCTGTATCATGTCTCAATTTGTGGTCAGCCTTTCTCCCTGCATCCCAGGCTGAGCAAGGACCT
1200 CTGCCCTCCCTGTTCAAGCCCTTGCTTGCCCTCAGCAGGTCAATTACAACCACCTTCACTCTGACCGCAGGGGCAGGGGACT
1280 AGATAGAATGACGTATTGAGCCTCGTCTGTCTGTCTGTCTGTCTCTCTCTCTGTTTGTCTCTGTCTGTCTGA
1360 CAGGCGCAGGCTGGGTCTCTAAGGCTTGTCTGTTCTGGCCCTCCTCAGTCTGGGTCTTGTGCGAACAGCTTTGCCCTTG
1440 GGTTACCTGGGTCCATCTCTGGGAATTGGGAACAAGGGGTCTGAGGGAGGCACCTCTGGGAGACTTTAGAAGGACC
1520 CAGTGCCTCGGGGCTGATTCTCGGGAATCACAGAGCTGGGACTCAGAGCCAGGATCC

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Fig. 7: The nucleotide sequence of the PAV_λ gene. (A) represents the reading frame which corresponds to a light chain protein and its leader peptide. Negative numbers refer to the amino acids of the leader peptide. The in-phase termination codon at position 30 is marked with xxx. (B) is the amino acid sequence of a human leader peptide (13) and a human λ light chain protein subgroup III, DEL (16). Only residues which differ from those in (A) are shown. Asterisks mark the positions where highly conserved residues have been replaced due to single base substitutions. The octanucleotide element in the 5' control region is underlined. The intron defined by the splice junctions, GT/AG, is marked with vertical arrows. The presumed single base deletion is indicated with an arrowhead.

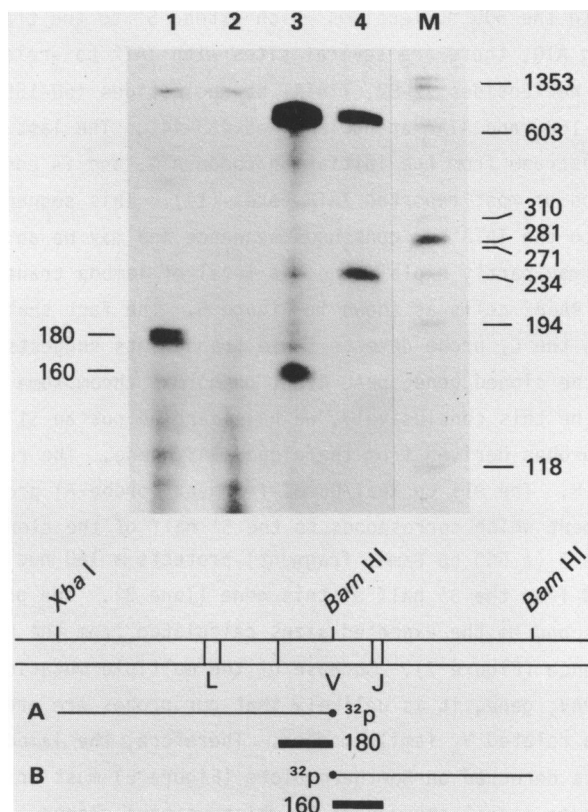


Fig. 8: S1 nuclease protection of V_{λ} transcripts in PA682 cells. Probe A, a double stranded 915 bp XbaI/BamHI fragment, was ^{32}P -labeled at the 5' end and used in lanes 1 and 2. Probe B, a 660 bp BamHI fragment, was 3' ^{32}P -labeled and used in lanes 3 and 4. Probes were hybridized to 20 μg of PA682RNA (lanes 1,3) or 20 μg of t-RNA (lanes 2,4) at 57°C overnight. Each reaction was treated with 3000U of S1 nuclease and samples were analyzed on a 6% polyacrylamide gel containing 7M urea. The protected fragments of 180 nt and 160 nt are shown in the schematic diagram. Size markers are end-labeled $\phi\text{X174-HaeIII}$ fragments.

there is a termination codon in this reading frame located at amino acid position 30 due to a single base mutation. The J segment, which is coded for by amino acids 99 to 109, is 100% homologous to that for the DEL protein. Therefore, a legitimate V-J joining has occurred, and this rearrangement cannot be considered aberrant at this level. However, the changes in invariant residues, the deletion in the leader sequences, and the early termination codons eliminate the possibility of producing a functional lambda light chain.

5' Region Within the 505 nucleotides which extend 5' to the translation initiation codon ATG, there are several sites with TATA box-related sequences: ATAATT at nucleotides 77-82, TTATAT at nucleotides 150-155, AAAATA at nucleotides 163-168, and ATAA at nucleotides 438-441. The last sequence is located 64 bp upstream from the initiation codon ATG, and is consistent with the position of most reported TATA boxes (17). This sequence is only weakly related to the TATA box consensus sequence and may be an inefficient promoter. This may partly explain the low level of lambda transcripts observed in the PA682 cells as shown in Figure 5. The fact that the PAV_λ probe as well as the C_λ probe detects these transcripts suggests that they originate from the cloned gene, pPAL 4.2E, on normal chromosome 22. In order to determine this conclusively, we have carried out an S1 protection analysis using probes derived from the cloned PAV_λ gene. The results are shown in Figure 8. The 915 bp XbaI/BamHI fragment (probe A) protects a 180 nucleotide fragment which corresponds to the 5' half of the cloned PAV_λ gene (lane 1). Probe B (a 660 bp BamHI fragment) protects a 160 nucleotide fragment derived from the 3' half of this gene (lane 3). The protected fragments correspond to the expected sizes calculated from the PAV_λ nucleotide sequence (Figure 7). Because of the multiple mutations detected throughout the PAV_λ gene, it is unlikely that our probes are protecting transcripts of a related V_λ family member. Therefore, the lambda light chain transcripts detected on Northern blots (Figure 5) must originate from the lambda locus on normal chromosome 22 which we have cloned.

Variable genes for both the heavy chain and light chain immunoglobulins have been shown to have a conserved sequence in the 5' flanking region which may be important in the transcriptional control of V gene promoters (18). In the case of the light chain V genes, an octanucleotide with consensus sequence ATTTGCAT is found 90-110 bp upstream from the ATG initiation codon. This sequence is highly conserved evolutionarily, while the flanking sequences have diverged considerably (19). At the corresponding position in the PAV_λ gene, there is an octanucleotide element GTTTGCAT (nucleotide 404-411) with one base substitution as compared to the consensus sequence. This octanucleotide element was also observed upstream of a human V_λVII gene (15). A second conserved element, which is a pentadecanucleotide with consensus sequence, TGCA^GCTGTGNCAG, has been reported to occur at 90-160 bp upstream of the ATG^C codon (19). There are no sequences related to this pentadecanucleotide at the appropriate position upstream of the PAV_λ gene. Anderson *et al.* (15) have reported a sequence which loosely

fits the pentadecanucleotide element in the $V_{\lambda}VII$ gene. Deletion experiments which removed the pentadecanucleotide element did not reduce κ gene transcription (20). This implies that this less well conserved element is not required for efficient κ light chain transcription. Recently, Bergman *et al.* (20) have suggested that both the intron enhancer and the sequence containing the octanucleotide element (69-104 bp upstream of the ATG codon) are necessary for efficient expression of κ light chain genes. The low level of λ transcripts in PA682(PB) may be due to poor homology to a TATA consensus sequence within the promoter region on the single base substitution within the octanucleotide element.

DISCUSSION

PA682 is a Burkitt's lymphoma cell line in which both κ and at least one of the λ loci have been rearranged (1). The rearrangement of λ genes in cells producing κ light chains is a rare event and this is accounted for by the proposed hierarchy of light chain activation which strongly favors κ rearrangements (2-4). Unlike the strong allelic exclusion described for heavy chain transcription, multiple light chain genes have been shown to be transcribed in both mouse myelomas and human B-cell lymphomas (21,22). However, in all cases described, only one functional protein is made, although truncated polypeptides that derive from aberrant rearrangements have been detected (21,22). Previous observations of the synthesis of multiple light chain transcripts have involved the expression of aberrantly rearranged κ genes in cell lines which are producing λ light chains. We observe here the transcription of a rearranged λ gene in a cell line which expresses κ light chains. In addition, the rearrangement of the λ locus in PA682(PB) results from a legitimate V-J joining and a normal size transcript is detected. However, the presence of multiple termination signals in the nucleotide sequence prevents translation of the λ message, thus fulfilling the requirement that only one functional light chain protein can be synthesized. The multiple mutations in the PAV_{λ} gene have presumably arisen as a result of the somatic mutational mechanisms involved in the generation of antibody diversity which are activated as a result of immunoglobulin rearrangement. If the postulated hierarchy of light chain rearrangement holds true, the PAV_{λ} rearrangement would have occurred subsequent to a functional κ rearrangement. This, in turn, would mean that at some point two light chains were expressed with the eventual inactivation of the λ locus by a mutational event. Alternatively, the PAV_{λ} gene might

not be functional in the germ line configuration, or the λ rearrangement preceded at least the functional κ rearrangement with the PAV_λ gene being subsequently inactivated by a mutational event.

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*To whom correspondence should be addressed

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