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Chromosome 8 breakpoint far 3′ of the c-*myc* oncogene in a Burkitt's lymphoma 2;8 variant translocation is equivalent to the murine *pvt*-1 locus

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The 2;8 variant translocation of human Burkitt's lymphomas is closely related cytogenetically to the t(6;15) of murine plasmacytomas; both involve a reciprocal exchange between the Igκ locus and a band region indistinguishable from that bearing the c-*myc* oncogene. To define their molecular relationship, we have compared cloned chromosome 8 DNA from the t(2;8) breakpoint in the human Burkitt's lymphoma JBL2 with cloned DNA from the murine *pvt*-1 locus, the major chromosome 15 breakpoint region in murine t(6;15). DNA sequencing and Southern blot analysis shows that these two regions are homologous. Thus the t(2;8) in JBL2 is the molecular equivalent of many murine t(6;15). The murine *pvt*-1 locus lies an unknown distance 3′ of c-*myc*; analysis of DNA from several tumours with c-*myc* amplification reveals that *pvt*-1 is co-amplified in at least one case, placing *pvt*-1 ~100–500 kb 3′ of c-*myc*. The significance of these results with respect to the role of *pvt*-1 in tumorigenesis is discussed.

Key words: B lymphocyte neoplasia/c-*myc* oncogene expression/gene activation at a distance/murine 6;15 translocation breakpoints/human 2;8 translocation breakpoints

Introduction

Murine plasmacytomas and human Burkitt's lymphomas bear characteristic sets of chromosome translocations that are strongly implicated in their genesis (for reviews see Leder *et al.*, 1983; Klein, 1983; Klein and Klein, 1985; Cory, 1986). The majority (~80%) of such tumours involve a well-characterized reciprocal translocation that fuses the c-*myc* oncogene (on murine chromosome 15, human chromosome 8) to the IgH locus (on murine chromosome 12, human chromosome 14). The murine t(12;15) and human t(8;14) are closely analogous, generally involving an exchange between an IgH switch recombination region near a C_H gene, and a point within, or just 5′ of, the c-*myc* gene. The altered regulation of c-*myc* expression that results from these translocations is believed to be a major predisposing factor for transformation. Compelling support for this view comes from evidence that such activated c-*myc* genes are tumorigenic in transgenic mice (Adams *et al.*, 1985).

About 15% of these B lymphoid tumours bear variant translocations, involving the c-*myc*-bearing chromosome and immunoglobin light-chain loci. The nature of the variant translocations is less well understood. Although levels of c-*myc* mRNA are comparable in tumours bearing the variant and predominant translocations (Cory *et al.*, 1985), the chromosome breakpoints generally do not occur near c-*myc* but instead fall a substantial, usually unknown, distance 3′ of the gene (Croce *et al.*, 1983; Erikson *et al.*, 1983; Davis *et al.*, 1984; Rappold *et al.*, 1984; Banerjee *et al.*, 1985; Sun *et al.*, 1986). In murine plasmacytomas, many of these variant t(6;15) involve a reciprocal exchange between C_κ on chromosome 6, and a chromosome 15 region cytogenetically indistinguishable from c-*myc* that we have termed the *pvt*-1 locus. This region was originally isolated as an aberrantly rearranged C_κ gene in the murine plasmacytoma ABPC4 (Webb *et al.*, 1984) and subsequently shown to be the site of chromosome 15 breakpoints in five other plasmacytomas (Cory *et al.*, 1985). *pvt*-1 was further implicated in tumorigenesis when it was found to be a common site of proviral integration in retrovirally induced murine T lymphomas (Graham *et al.*, 1985). Indeed it has recently been shown to be equivalent to the *Mis*-1 locus (Villeneuve *et al.*, 1986), a common proviral integration site in rat T lymphomas (Lemay and Jolicoeur, 1984). Thus *pvt*-1 has been associated with both B- and T-cell neoplasia.

Cytogenetically, the 2;8 variant translocation in Burkitt's lymphomas is closely analogous to the murine t(6;15), since it involves an exchange between the κ-bearing band on chromosome 2 and the c-*myc*-bearing band on chromosome 8 (Erikson *et al.*, 1983; Davis *et al.*, 1984; Rappold *et al.*, 1984; Manolov *et al.*, 1986). Taub *et al.* (1984) have cloned a t(2;8) breakpoint from the Burkitt's lymphoma JBL2, and shown that this rearrangement involves the C_κ locus and an unidentified chromosome 8 region that lies at least 20 kb 3′ of c-*myc*. We have investigated whether the human t(2;8) and murine t(6;15) are equivalent at the molecular level by comparing cloned human chromosome 8 DNA from JBL2 to cloned DNA from the murine *pvt*-1 locus. We show here that the two regions are equivalent, but that the degree of homology is limited. We also report the localization of another murine breakpoint in *pvt*-1 which also maps to this region.

The murine *pvt*-1 locus lies 3′ of c-*myc* (Banerjee *et al.*, 1985). This result coupled with our previous molecular data (Cory *et al.*, 1985) indicates that some *pvt*-1 breakpoints occur at least 94 kb from the c-*myc* promoters. In order to define further the distance *pvt*-1 lies from c-*myc*, we have analysed DNA from several tumours in which c-*myc* is amplified. To explore the possibility that *pvt*-1 encodes an independent oncogene, we have also searched for regions of *pvt*-1 that are transcribed, that are related to known oncogenes, or that have extensive homology with human DNA.

Results

Translocation breakpoints and proviral insertions cluster within the pvt-1 locus

A number of chromosome 15 breakpoints associated with murine t(6;15) and proviral integrations in retrovirally induced T lymphomas have been mapped within the *pvt*-1 locus, as shown in Figure 1A. A cluster of five translocation breakpoints and four proviral inserts map within an 8.5-kb region (Cory *et al.*, 1985; Graham *et al.*, 1985). Another chromosome 15 breakpoint maps ~13 kb leftwards, fairly close to four proviral inserts mapped within the rat *Mis*-1 locus (Villeneuve *et al.*, 1986). Three additional proviral inserts map further away (see Figure 5).

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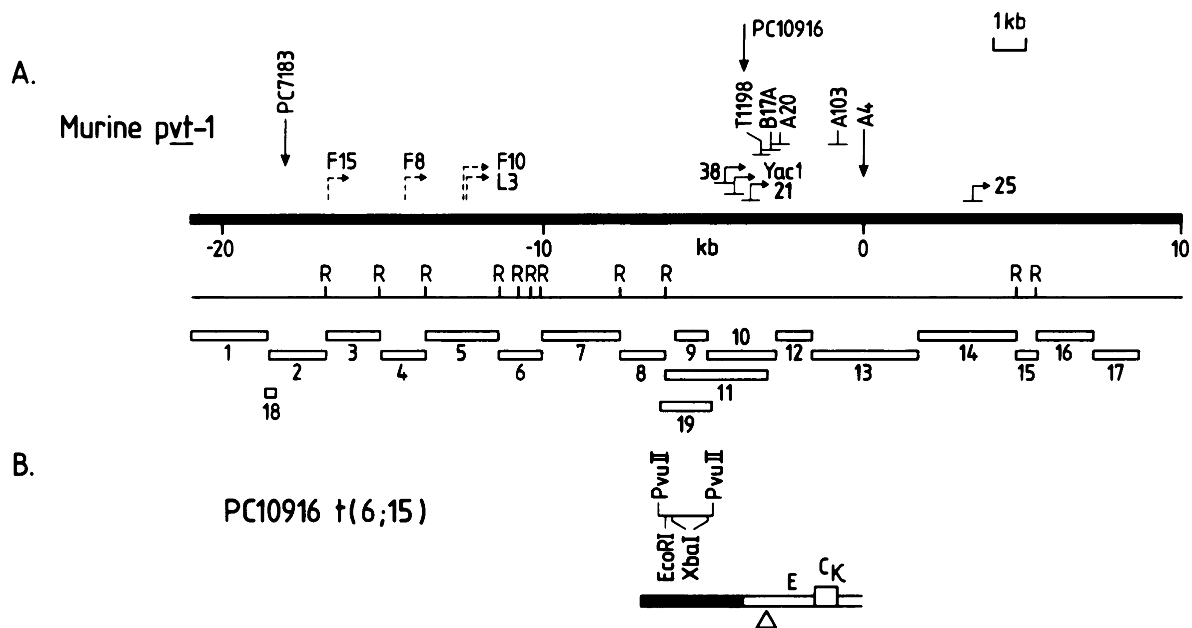

Fig. 1. Chromosome translocations and proviral integrations within the murine *pvt*-1 locus. (**A**) The position of six chromosome 15 breakpoints in murine plasmacytomas (top), four proviral inserts in murine T lymphomas (solid arrows), and the inferred position of four proviral inserts in rat T lymphomas (broken arrows) are shown; arrowheads represent their transcriptional orientation. The position of the chromosome 15 breakpoint in PC10916 is also shown. A partial restriction map, showing *Eco*RI sites (R) is shown below this. The open boxes represent various probes used in Northern and Southern blot analysis (see text). Probes were 1, a *Bam*HI–*Eco*RV fragment; 2, *Eco*RV–*Eco*RI; 3–8, *Eco*RI fragments; 9 and 10, *Xba*I fragments; 11, *Eco*RI–*Sal*I; 12, *Xba*I–*Bam*HI; 13, *Bam*HI–*Bgl*II; 14, *Bgl*II–*Eco*RI; 15, *Eco*RI; 16, *Eco*RI–*Kpn*I; 17, *Kpn*I; 18, *Sma*I–*Fnu*DII and 19, a *Pvu*II fragment. The isolation and characterization of these regions, and more detailed restriction maps, are described in Van Ness *et al.* (1983), Webb *et al.* (1984), Lemay and Jolicoeur (1984), Cory *et al.* (1985), Graham *et al.* (1985), Kelley *et al.* (1985) and Villeneuve *et al.* (1986) (**B**) The bar represents the clone isolated by Kelley *et al.* (1985), shown here to be the t(6;15) junction in the NZB plasmacytoma PC10916. Sequences derived from the C_κ region are shown as an open bar while *pvt*-1-derived sequences are shown filled. The C_κ enhancer is denoted by E, and the triangle represents a deletion that removes J_κ from this clone (Kelley *et al.*, 1985). A partial restriction map is shown above the bar, denoting the *Pvu*II fragment used as a probe, and published restriction sites (Webb *et al.*, 1984; Kelley *et al.*, 1985) used to align the two regions.

An aberrantly rearranged C_κ locus was cloned from the NZB murine plasmacytoma PC10916 by Kelley *et al.* (1985), who suggested that this might reflect a translocation. To establish whether this rearrangement represents a t(6;15) breakpoint involving *pvt*-1, we hybridized the *Pvu*II fragment indicated in Figure 1B, kindly provided by Dr R.Perry, to cloned DNA from the *pvt*-1 locus. This unique-sequence probe cross-hybridized with DNA from *pvt*-1 at high stringency. Furthermore, this probe and probes derived from *pvt*-1 recognize the same rearranged *Eco*RI restriction fragments in genomic Southern blots of DNA from five tumours carrying 6;15 translocations: ABPC4, ABPC103, ABPC20, Baltnlm17A and CAK TEPC1198 (data not shown). These results firmly establish that the aberrantly rearranged C_κ gene in PC10916 represents a t(6;15) involving *pvt*-1 and C_κ. Alignment of the relevant regions based on published maps (Webb *et al.*, 1984; Kelley *et al.*, 1985) indicates that the chromosome 15 breakpoint in PC10916 falls very near five of the breakpoints we mapped previously (Figure 1A).

The chromosome 8 breakpoint region in the t(2;8)-bearing lymphoma JBL2 is homologous to murine pvt-1

The t(2;8) junction cloned by Taub *et al.* (1984) is depicted at the top of Figure 2. In order to establish whether this region corresponds to the murine *pvt*-1 locus, we hybridized the indicated *Eco*RI–*Bam*HI fragment derived from the incoming chromosome 8 DNA (kindly provided by Dr P.Leder) to various restriction digests of cosmids spanning the entire 108-kb cloned region of *pvt*-1 (Cory *et al.*, 1985). No homology was apparent at moderate stringency (2 × SSC, 65°C), but a single *Eco*RI–*Sal*I fragment, lying between −6.4 kb and −3 kb on the *pvt*-1 map (Figure 2), hybridized at low stringency (0.2 × SSC, 37°C). Southern blots of various other digests (not shown) established that the hybridizing regions were confined to a 0.7-kb *Eco*RI–*Sca*I fragment of murine *pvt*-1, and a 0.7-kb *Eco*RI–*Hpa*I fragment of human DNA (Figure 2). Sequence analysis across these regions, coupled with a computer-assisted alignment, revealed a fairly short region that is reasonably well conserved between mouse and human (Figure 3). A 240-bp region of murine DNA (between positions 80 and 320 in Figure 3C) exhibits ~65% homology with human DNA. Maximal alignment within this region requires four single nucleotide gaps, one gap of four nucleotides, and a 21-nucleotide gap that corresponds to an imperfect direct repeat in the murine sequence that is absent in the human.

It might be argued that the low level of homology observed simply represents fortuitous cross-reaction between repetitive elements; however, computer searches of available data banks did not reveal homology with any known human or murine repetitive elements (or indeed any known sequence). Furthermore genomic Southern blot analysis indicates that the homologous regions behave essentially as single-copy elements at low stringency, and recognize the expected fragments in mouse and human DNA. Thus, as shown in Figure 4A, at low stringency (0.2 × SSC, 47°C) the murine *Mbo*II fragment (indicated in Figure 2) recognizes a 9.5-kb *Eco*RI fragment in murine genomic DNA, and the expected 8.5-kb *Eco*RI fragment in human genomic DNA. Conversely, the human *EcoRI*–*Xho*II fragment (indicated in Figure 2) hybridized to the 9.5-kb *Eco*RI fragment

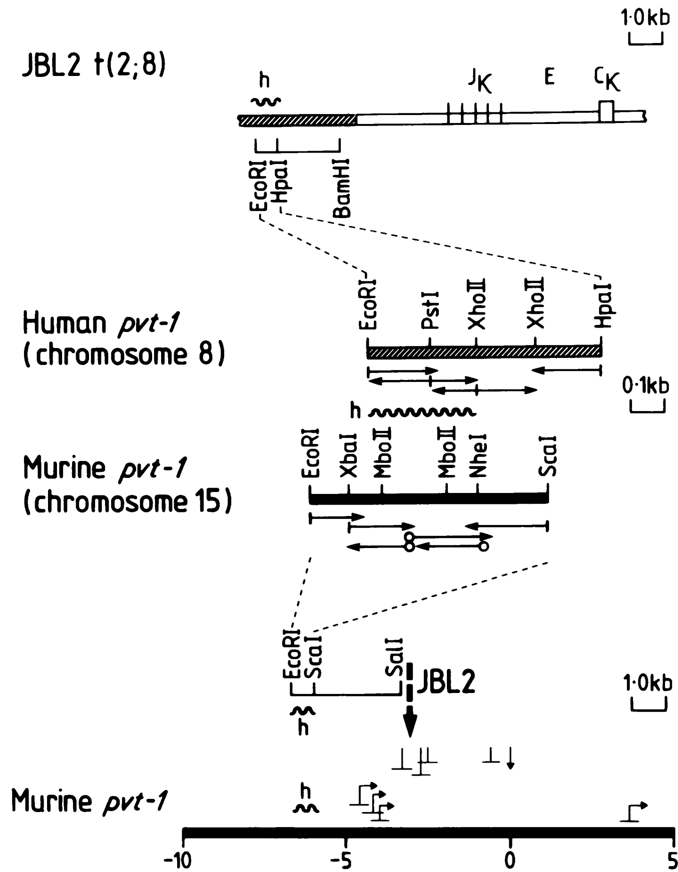

Fig. 2. The relationship between the chromosome 8 breakpoint in JBL2, and the murine *pvt*-1 locus. The top bar represents the t(2;8) junction in the Burkitt's lymphoma JBL2 cloned by Taub *et al.* (1984); the open regions represent C_κ sequences, while the stippled areas represent human chromosome 8 sequences. A partial restriction map is shown below this, delineating the *Eco*RI−*Bam*HI fragment used as a probe. The bottom filled bar represents the murine *pvt*-1 locus, with translocation breakpoints and proviral integrations indicated as in Figure 1. A partial map denoting the hybridizing *Eco*RI−*Sal*I fragment is shown above the bar. Expanded maps of the homologous sequences are also shown, and the sequencing strategy is indicated by arrows; vertical bars represent restriction sites, and circles synthetic oligonucleotides used for sequence determination (see Materials and methods). The expanded maps are aligned to indicate the homology determined by computer-assisted alignment, as shown in Figure 3. The wavy line 'h' denotes the homologous regions. The inferred position of the JBL2 breakpoint relative to the murine *pvt*-1 locus is denoted by the thick broken arrow.

in murine DNA (Figure 4A). Furthermore, Figure 4A shows that this fragment is amplified in the murine tumour ANN-1 that carries an amplified *pvt*-1 locus (see below), indicating that this human probe is recognizing the murine *pvt*-1 locus.

Taken together, all this data suggests that the human chromosome 8 breakpoint region in JBL2 is equivalent to the murine *pvt*-1 locus. An alignment of the cloned murine and human regions based upon this homology places the JBL2 chromosome 8 breakpoint very near the majority of the murine chromosome 15 breakpoints within *pvt*-1, as shown in Figure 2.

The functional significance of this homology is unclear. It appears very unlikely that these regions are part of a coding sequence, since there are no long conserved open-reading frames or conserved initiation codons and maximal alignment of these regions requires multiple insertions/deletions that would change any reading frame. Furthermore there appear to be no conserved splice-donor or splice-acceptor sequences, making it unlikely that these regions are part of an exon. The extent of homology is more reminiscent of that seen within introns of certain genes, such as c-*myc*.

The c-myc gene and the pvt-1 locus are co-amplified in a murine tumour

The distance between the c-*myc* and *pvt*-1 loci is unknown, but in the mouse it must be at least 94 kb (see Introduction). The c-*myc* gene is known to be amplified in some murine and human tumours, and Kinzler *et al.* (1986) have estimated that the size of the c-*myc* amplification unit in five human tumours ranges between 90 and 350 kb. In order to clarify further the relationship between c-*myc* and *pvt*-1, we have examined DNA from several tumours that carry amplified c-*myc* genes, to ascertain whether *pvt*-1 is also amplified.

Genomic Southern blot analysis of two human tumours, HL60 and N82E, clearly shows that the c-*myc* gene is amplified in both cases, but the human *pvt*-1 locus is not (Figure 4B). Kinzler *et al.* (1986) estimated the size of the c-*myc* amplification units in HL60 and N82E as 90 and 120 kb respectively. Unless c-*myc* happens to fall near the 3′ end of both of these amplification units, this data suggests that the human *pvt*-1 locus probably lies at least 60 kb 3′ of c-*myc*.

The murine tumour ANN-1 carries a c-*myc* gene that is amplified ~13-fold (Nepveu *et al.*, 1985). Hybridization with a murine *pvt*-1 probe shows that the *pvt*-1 locus is amplified to a similar extent in this tumour (Figure 4B). It seems highly likely that c-*myc* and *pvt*-1 lie on the same amplification unit. The size of the ANN-1 amplification unit is not known, but since most amplification units are of the order of 100−1000 kb, this result suggests that murine *pvt*-1 lies ~100−500 kb 3′ of c-*myc*.

Search for pvt-1 regions that are transcriptionally active, evolutionarily well-conserved, or related to known oncogenes

The occurrence of chromosome translocations and proviral integrations within *pvt*-1 are consistent with the hypothesis that *pvt*-1 encodes an oncogene, the transcription of which is altered in some way by these lesions (see Discussion). We have searched for such transcripts using Northern blot analysis of total cellular poly(A)+ RNA isolated from a variety of tumour lines, including plasmacytomas with and without translocations involving *pvt*-1. They included the variant t(6;15)-bearing murine plasmacytomas ABPC4, CAK TEPC1198, Baltnlm17A and ABPC103 and t(12;15)-bearing plasmacytomas NBSL and MPC11. Several T lymphomas with and without proviral inserts in *pvt*-1 were also examined. The probes used in this analysis were a series of contiguous fragments spanning the *pvt*-1 locus from −23 kb to +15 kb, most of which are shown in Figure 1A. None of the probes revealed any discrete transcript in any of the lines. The expected c-*myc* transcripts were easily detected in all the lines, indicating that any transcripts from the *pvt*-1 region examined must be of very low abundance, at least 30-fold lower than c-*myc*. In case a poly(A)− transcript might be involved, blots of total cellular RNA from these lines were also screened with probes covering much of the region, but no transcripts were found. Kelly *et al.* (1985) detected traces of discrete poly(A)+ nuclear transcripts in the murine plasmacytomas PC10916 and PC7183 with probes from *pvt*-1. Using the same probes (probes 18 and 19 in Figure 1A, kindly provided by Dr R.Perry), we have been unable to detect any mRNA in total cellular poly(A)+ RNA from the four variant plasmacytomas listed above, even after prolonged autoradiographic exposures (data not shown). Thus the RNA species found by Kelley *et al.* (1985) may be present only in nuclear RNA, or confined to the lines they examined.

A Human (chromosome 8)

```
EcoRI      10         20         30         40         50         60         70         80         90        100
GAATTCAGGA CACTGACTTG GGGCTGGAAA GGCAAAGTGA GGAAGAAAGT ATTCCCCTTT CAGAAGCTCC CCAGTCTGCC TTAAGAAACA AAGGCAGCCC

       110        120        130        140        150        160        170        180  PstI           200
TGTCATCTGC CCAGGACTTT ACAGTTTATA AAGTGATTTC TCATCCTTTC TCCTTTCTTG TATTGCACAA ATATCGAACG AGAACCTGCA GTGTGCCACT

       210        220        230        240        250        260        270        280        290        300
GTGCAGGGTG CCTAGAATAA AGGAGAAAAG CCACTGTGCT TTCTCTGAAG GAATCACAGG GAGTGAAGCT AGCTTCATGG ATGCCAGAAT TTCACAGATG

       310        320        330  XhoII
AAGGGACTGG AGGCTTGGAC AATTGCATTG CTTGAGATCC
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B Murine (chromosome 15)

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XbaI       10         20         30         40         50         60         70         80         90        100
TCTAGATATT CTCTCACTAA CCTGAACCTT TGGCATCAAC ACAGGCTTAA AGGACATACT TAGGGTCTCT AGTGTCAATT GAATGGCAGC ATCCTGACTT

  MboII           120        130        140        150        160        170        180        190        200
TGGTCTTCAA AGCAAAGATG ACACTGAAGT CTGCCCCTTC CAAACAAGGG CTACCCTGCC TGCTTCCAGA AGCAAAGCAC GCCTTACCAT CTGCTTAGGA

       210        220        230        240        250        260        270        280        290        300
CTTCACAGTT CATAAAGTTC TTTCCATCCC GTCTGCTTTC TTTTTATTGC ACAAGTGTTT ACTTTTTATT GCTCAGTATT TACTGAGATA CCGCAGGATG

       310        320        330 MboII 340        350        360        370        380        390        400
CCACTGTGCA GGGCGCCTGC GGTCCTTGAG GAAGAGCTGT TGTTCCCATG CCTAGGCAAT TCAGAAGGCC ATGGCTGGAA TCTGGGGGCA ATTGCATAGC

       410   NheI
CTGAAATCAG GCTGCTAGC
```

C Alignment

```
              10         20         30         40         50         60         70         80 EcoRI  90        100
Human  .......... .......... .......... .......... .......... .......... .......... .......... GAATT.CAGG ACACTGACTT
                                                                                               ****  ***  *  *******
Murine TCTAGATATT CTCTCACTAA CCTGAACCTT TGGCATCAAC ACAGGCTTAA AGGACATACT TAGGGTCTCT AGTGTCAATT GAATGGCAGC ATCCTGACTT
        XbaI
             110        120        130        140        150        160        170        180        190        200
Human  GGGGCTGGAA AGGCAAAGTG AGGAAGAAAG TATTCCCCTT TCAGAA.... GCTCCCCAGT CTGCCTTAAG AAACAAAGGC AGCCCTGTCA TCTGCCCAGG
        ** **  ** **  **  ** *    *** * * * ****** ** *       *** *** *  **** *  ** *****    *** *  ** *****  ***
Murine TGGTCTTCAA AGCAAAGATG ACACTGAA.G TCTGCCCCTT CCAAACAAGG GCTACCCTGC CTGCTTCCAG AAGCAAAGCA CGCCTTACCA TCTGCTTAGG
          MboII
             210        220        230        240        250        260        270        280        290 PstI 300
Human  ACTTTACAGT TTATAAAGTG ATTTCTCATC CTTTCTCCTT TCTTGT.ATT GCACAAAT.. .......... .........A TCGAACGAGA ACCTGCAGTG
       **** ***** * *******   **** **** *  *** *** **** * *** ****** *                    * *  *  ****   * ****
Murine ACTTCACAGT TCATAAAGTT CTTTC.CATC CCGTCTGCTT TCTTTTTATT GCACAAGTGT TTACTTTTTA TTGCTCAGTA TTTACTGAGA TACCGCAGGA

             310        320        330        340        350        360        370        380        390        400
Human  TGCCACTGTG CAGGGTGCCT AGAATAAAGG AGAAAAGCCA CTGTGCTTTC TCTGAAGGAA TCACAGGGAG TGAAGCTAGC TTCATGGATG CCAGAATTTC
       ********** ***** ****     *    * ** **   *   *** ** *   **                           ****              * * ***
Murine TGCCACTGTG CAGGGCGCCT GCGGTCCTTG AGGAAGAGC. .TGTTGTTCC CATGC..... .......... .....CTAG. .......... ...GCAATTC
                                         MboII
             410        420        430        440 XhoII  450
Human  ACAGATGAAG GGACTGGAGG CTTGGACAAT TGCATTGCTT GAGATCC... .......
       * *     *  **   * *     ** **** ***** ** * ** ***
Murine AGAAGGCCAT GGCTGGAATC TGGGGGCAAT TGCATAGCCT GAAATCAGGC TGCTAGC
                                                          NheI
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Fig. 3. Sequence analysis of homologous regions of JBL2 t(2;8) breakpoint and murine *pvt*-1. Human (**A**) and murine (**B**) sequences are shown. The indicated restriction sites correspond to sites shown on the physical maps in Figure 2. (**C**) A computer-assisted alignment of these sequences. The asterisks indicate identical nucleotides, and the dots indicate gaps in the sequence, introduced to maximize homology.

Oncogenes as a group are highly conserved across evolution, reflecting their crucial roles in such processes as cellular proliferation and differentiation. Such homology should be readily detected using genomic Southern blot analysis. We have shown that the murine *pvt*-1 locus is extensively conserved between the mouse and rat (Villeneuve *et al.*, 1986), but we have failed to detect any significant homology with human DNA using genomic Southern blot analysis at moderate stringency (2 × SSC, 65°C) with contiguous probes spanning the *pvt*-1 locus from −23 kb to +15 kb. The limited homology observed around the JBL2 breakpoint (Figure 4A) is not detectable at this stringency, but any well conserved gene would be. We have also examined extensive regions of *pvt*-1 for homology to many known oncogenes, but have failed to detect any (Cory *et al.*, 1985; Villeneuve *et al.*, 1986). In addition to the 23 oncogenes previously examined, we have not detected any low stringency (0.2 × SSC, 37°C) homology to *lsk* (Marth *et al.*, 1985), using a cDNA probe kindly provided by Dr R.Perlmutter. Furthermore, oligonucleotide probes derived from the murine IL4 sequence (Noma *et al.*, 1986) or human *rel* (Brownell *et al.*, 1985) fail to recognize *pvt*-1.

Discussion

Previous studies have strongly implicated the *pvt*-1 locus in lymphomagenesis, both as a site of chromosome 15 breakpoints in murine B lymphoid tumours with 6;15 translocations (Cory *et al.*, 1985), and as a common proviral integration site in retrovirally induced T lymphomas in the mouse and rat (Graham *et al.*,

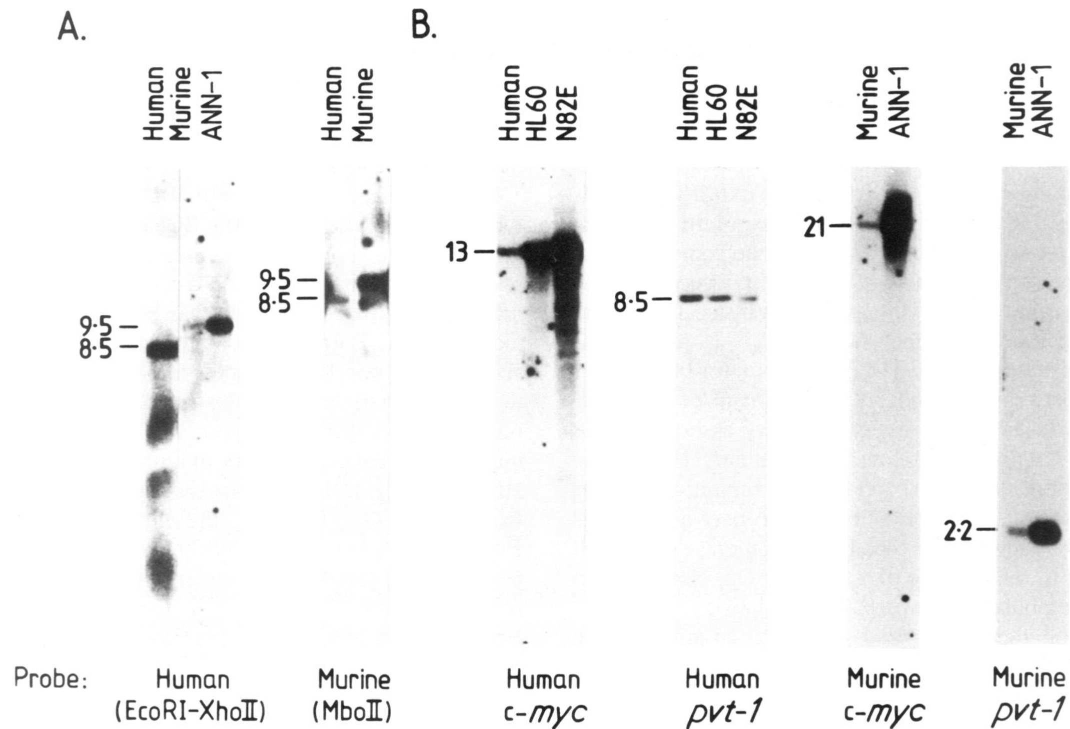

Fig. 4. (**A**) Low-stringency genomic Southern blot analysis showing conservation between murine *pvt*-1 and the JBL2 chromosome 8 breakpoint region. Normal human and murine DNA and ANN-1 murine tumour DNA were restricted with *Eco*RI, run on agarose gels, transferred to nitrocellulose, hybridized at low stringency with the indicated probes and washed extensively in 0.2 × SSC, 0.1% SDS at 47°C as described in Materials and methods. The probes used are shown at the bottom; the human probe was an *Eco*RI–*Xho*II fragment, and the murine probe an *Mbo*II fragment denoted in Figures 2 and 3. The sizes of hybridizing fragments are shown in kilobase pairs. (**B**) Genomic Southern blot analysis showing amplification of the *pvt*-1 locus. Normal human and murine genomic DNA, and DNA from the human tumours HL60 and N82E and the murine tumour ANN-1, were restricted with *Eco*RI, transferred to nitrocellulose and hybridized at high stringency with the indicated probes, as described in Materials and methods. The human c-*myc* probe was a *Sac*I fragment spanning exon 2 (Bernard *et al.*, 1983), and the murine c-*myc* probe was a murine cDNA (Watt *et al.*, 1983). The human *pvt*-1 probe was the *Eco*RI–*Bam*HI fragment shown in Figure 2, and the murine *pvt*-1 probe was an *Eco*RI fragment, probe 5 in Figure 1A.

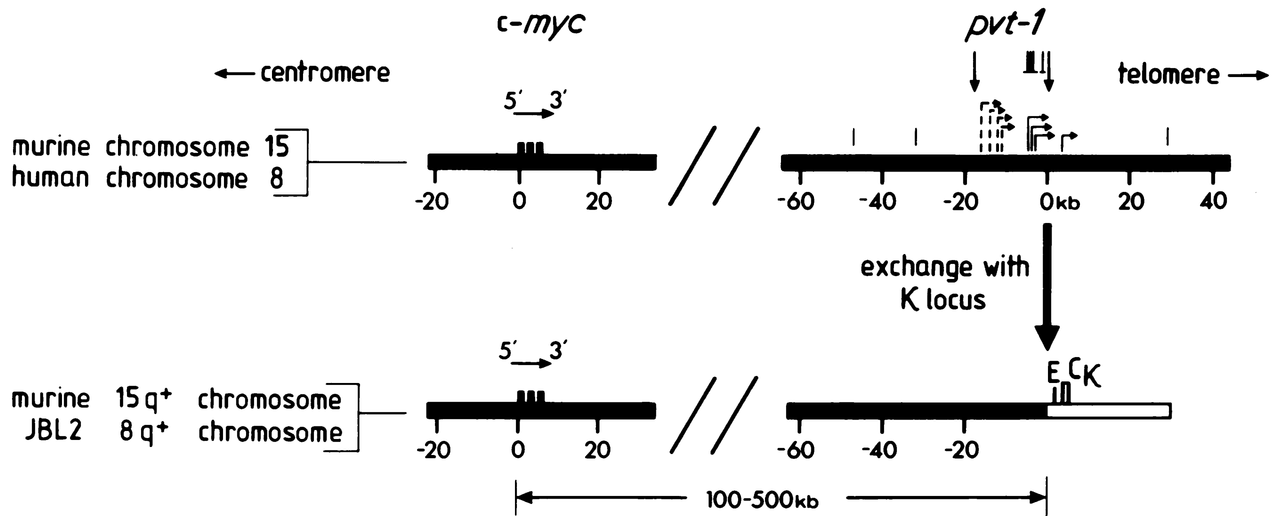

Fig. 5. The relationship between the c-*myc* and *pvt*-1 loci, and the consequence of an exchange with the κ locus. The filled bars represent cloned DNA from the murine c-*myc* and *pvt*-1 loci (Cory *et al.*, 1985), with translocation breakpoints and proviral integrations indicated as in Figure 1. The centromere lies to the left, and the telomere to the right on this map (Banerjee *et al.*, 1985; Manolov *et al.*, 1986). The direction of c-*myc* transcription is shown. Murine t(6;15) bring the C_κ gene and its enhancer onto the $15q^+$ chromosome as indicated. The t(2;8) in JBL2 is identical in this regard. The chromosome 15 breakpoints in murine t(6;15) lie in the order of 100–500 kb 3′ of c-*myc* as indicated, and the chromosome 8 breakpoint in JBL2 lies at least 60 kb 3′ of c-*myc* (see text).

1985; Villeneuve *et al.*, 1986). The present results link these observations to human B lymphomas by showing that the t(2;8) in the JBL2 Burkitt's line is equivalent to most murine t(6;15). The JBL2 translocation, like all the murine t(6;15) characterized molecularly, including that now defined for PC10916 (Kelley *et al.*, 1985) involves a breakpoint upstream of C_κ, and further-

more the chromosome 8 breakpoint in JBL2 maps remarkably near many of the analogous chromosome 15 breakpoints in the mouse. Therefore, in all the best-studied murine t(6;15) and the t(2;8) of JBL2, the C_κ gene and its lymphoid-specific enhancer is linked to the *pvt*-1 locus well downstream of c-*myc*, as indicated in Figure 5.

The mechanism generating the t(6;15) and t(2;8) remains unclear. The fact that neither the JBL2 breakpoint nor that in PC10916 falls close to a J_κ element supports the contention that these rearrangements do not involve the $V_\kappa-J_\kappa$ recombination machinery (Cory *et al.*, 1985). Perhaps these translocations occur at a stage of B-cell development when the $V_\kappa-J_\kappa$ recombination machinery is not operative. The prediliction for breaks near C_κ, which fall between 1.5 and 7.3 kb upstream of the gene, may reflect in part the DNase hypersensitivity associated with an activated C_κ enhancer (Parslow and Granner, 1983). The recently described nuclear matrix attachment region, which lies just upstream of the C_κ enhancer, might also play a role; it is reported to be a binding site for type II topoisomerase (Cockerill and Garrard, 1986), an enzyme of a type that can catalyse non-homologous recombination *in vitro* (Ikeda, 1986).

The observation that c-*myc* and *pvt*-1 are co-amplified in ANN-1 suggests that *pvt*-1 lies some 100−500 kb 3′ of c-*myc*. Nevertheless, alterations within *pvt*-1 appear to be associated with a transcriptionally active c-*myc* gene, since both murine plasmacytomas with t(6;15) and murine T lymphomas with proviral integrations in *pvt*-1 express substantial amounts of c-*myc* mRNA (Cory *et al.*, 1985; Graham *et al.*, 1985).

The ample precedents of oncogene activation by chromosome translocation and proviral integration (Varmus, 1984) suggest that *pvt*-1 might encode an oncogene, the altered expression of which might directly or indirectly affect c-*myc* expression. To search for such a putative oncogene, we have examined extensive regions of murine *pvt*-1 for transcripts or substantial mouse−human homology. Our negative results to date provide no support for the existence of a *pvt*-1 oncogene. However such a gene might not be detected if it were very large, as the widespread distribution of alterations within *pvt*-1 might suggest — only mini-exons, or intron sequences, might then be present within the regions we have examined. Alternatively, *pvt*-1-encoded transcripts might be present at extremely low levels, below our level of detection.

A very different model is that alterations within *pvt*-1 activate c-*myc* expression directly, in *cis*, presumably by some alteration in higher-order chromatin structure. The DNase hypersensitivity studies of Fahrlander *et al.* (1985) on the status of chromatin surrounding c-*myc* in a number of murine lines are consistent with that possibility. Three murine plasmacytomas with t(6;15) known to involve *pvt*-1 gave patterns suggesting that only one c-*myc* allele was active. The cell fusion studies of Erikson *et al.* (1983) indicated that the t(2;8) in the Burkitt's lymphoma JI activated c-*myc* expression: analysis of hybrid segregants showed that the c-*myc* allele on the rearranged $8q^+$ chromosome was active, while that on the unrearranged chromosome was silent. Sun *et al.* (1986) have recently shown that the chromosome 8 breakpoint in JI falls 25−32 kb 3′ of c-*myc*, indicating that this translocation must activate c-*myc* expression in *cis*. Furthermore, Erikson *et al.* (1986) have recently shown that a t(8;14) in a human T-cell leukemia links the T-cell receptor C_α locus to a region cytogenetically indistinguishable from c-*myc*, but at least 38 kb 3′ of it. Again, analysis of hybrid segregants showed that only the c-*myc* allele on the rearranged chromosome 8 was active, which may indicate that translocations a considerable distance 3′ of c-*myc* can activate its expression in *cis*. It is difficult, however, to understand how a provirus integrated in *pvt*-1 could activate c-*myc* expression directly, since integrated retroviruses are thought to promote tumorigenesis via localized effects on transcription (Varmus, 1984). Given the distances between c-*myc* and *pvt*-1, the molecular basis of any such interaction would be remarkable.

It is likely that, in addition to JBL2, many other chromosome 8 breakpoints in t(2;8)-bearing Burkitt's lymphomas will involve the *pvt*-1 locus. However, the breakpoint in JI (Sun *et al.*, 1986) must lie considerably closer to c-*myc* than that in JBL2. Furthermore, probes from another human t(2;8) breakpoint (kindly provided by Dr G.Bornkamm) do not cross-hybridize with the cloned JBL2 region, nor do they appear to bear any significant homology to murine *pvt*-1, although this breakpoint might occur within a region of *pvt*-1 that is very poorly conserved between mouse and man. This breakpoint occurs in a region that is not amplified in HL60 or N82E DNA (data not shown), indicating that it is distinct from that in JI. Therefore, the chromosome 8 breakpoints in Burkitt's lymphomas bearing the variant 2;8 translocation may be somewhat variable. Moreover, we failed previously to detect rearrangements within *pvt*-1 in three t(6;15)-bearing plasmacytomas (Cory *et al.*, 1985) and although the significance of these results remains uncertain, there may be a similar variation in the positions of murine chromosome 15 breakpoints.

The different types of translocation in the plasmacytomas and Burkitt's lymphomas may influence c-*myc* expression by different mechanisms. The human t(8;14) and murine t(12;15) typically involve chromosome breaks quite near c-*myc*, and appear to activate c-*myc* expression directly (Cory, 1986). Translocations involving *pvt*-1 in human t(2;8) and murine t(6;15) must de-regulate c-*myc* expression by some more complex mechanism. Furthermore some Burkitt's lymphomas bear a third type of translocation, involving the C_λ locus. Surprisingly, many of the chromosome 8 breakpoints in such tumours are cytogenetically distinguishable from c-*myc* and *pvt*-1 (Manolov *et al.*, 1986), yet these tumours seem to express elevated levels of c-*myc* mRNA (Sun *et al.*, 1986), implying yet another mechanism of c-*myc* de-regulation. Clearly c-*myc* expression can be altered by diverse mechanisms, some involving regions quite different from the c-*myc* gene itself.

Materials and methods

Southern and Northern blot analysis

DNA was digested, electrophoretically separated and transferred to nitrocellulose as previously described (Cory *et al.*, 1983). Moderate and high stringency hybridizations were performed in 2 × SSC, 5 × Denhardt's, 50 μg/ml denatured salmon sperm DNA, 0.1% SDS, at 65°C, then washed three times in either 2 × SSC, 0.1% SDS (moderate stringency) or 0.2 × SSC, 0.1% SDS (high stringency), before autoradiography. For low stringency, Southern blots were hybridized in 20% (v/v) formamide, 5 × SSC, 5 × Denhardt's, 50 μg/ml denatured salmon sperm DNA, at 42°C, and then washed extensively in 0.2 × SSC, 0.1% SDS at either 37°C or 47°C. The higher temperature was used for the genomic Southerns shown in Figure 4A since the mouse−human hybrids melt between 47°C and 52°C under the conditions used. Northern blot analysis was as previously described (Cory *et al.*, 1985).

Sequence analysis

Fragments of murine and human *pvt*-1 DNA indicated in Figure 2 were subcloned into M13 phage vectors, and sequences derived by the dideoxynucleotide method (Sanger *et al.*, 1980). Specific oligonucleotide primers were used in some cases where convenient restriction sites were unavailable. The strategy is outlined in Figure 2.

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