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# Pvt-1 transcripts are found in normal tissues and are altered by reciprocal(6;15) translocations in mouse plasmacytomas

(protooncogene/B-cell tumors/aberrant transcripts)

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ABSTRACT **The mouse *Pvt-1* (for plasmacytoma variant translocation) region maps to a chromosome 15 breakpoint region that is frequently interrupted by "variant" reciprocal chromosome translocations, rcpt(6;15), in plasmacytomas. This region lies several hundred kilobases (kb) 3′ of the mouse c-*myc* gene (*Myc*) which is deregulated in both rcpt(6;15) and rcpt(12;15) plasmacytomas. rcpt(12;15) translocations apparently activate c-*myc* directly by interrupting the gene itself, but the mechanism causing c-*myc* deregulation in tumors bearing rcpt(6;15) translocations remains unknown. The indirect activation of c-*myc* by *Pvt-1* interruption has remained an appealing possibility, but heretofore it has not been possible to establish such a connection. Furthermore, no genes from the *Pvt-1* locus have been shown to be transcribed in normal tissues or in tumors with rcpt(6;15) translocations. We report the isolation of a cDNA clone, Pvt-1-1, from mouse spleen mRNA that is specific to the *Pvt-1* region. This cDNA probe detects low levels of large (*ca.* 14 kb) RNA transcripts in normal mouse tissues. In plasmacytomas with rcpt(6;15) translocations, the Pvt-1 transcripts are elevated in abundance and truncated in size. Both changes are apparently induced by the chromosomal translocation. Expression of 14-kb Pvt-1 RNA is elevated in B-cell tumor lines that express immunoglobulin light chain genes; thus, we postulate that these translocations are facilitated by the increased DNA accessibility of immunoglobulin κ light chain and *Pvt-1* genes when they are simultaneously expressed at certain times during B-cell ontogeny.**

A common chromosomal disorder found in lymphomas or myelomas (avian, feline, mouse, rat, or human) involves translocation or retroviral integration in the region of the cellular c-*myc* oncogene (1, 2). These chromosomal aberrations result in constitutive expression of c-*myc* from the mutant chromosome, in contrast to low-level expression of c-*myc* from the nonmutated chromosome. Such c-*myc* expression, deregulated by chromosome translocation, is believed to be one of the major contributors to tumorigenesis in mouse plasmacytomas and human Burkitt lymphomas. In 10–20% of these tumors c-*myc* expression is deregulated, but the c-*myc* locus is not involved in the translocation. Instead, a "variant" translocation occurs in a cluster of chromosomal breakpoints called *Pvt-1*,[§] located ≈100–300 kilobases (kb) downstream of c-*myc* (3–6). This chromosomal translocation, rcpt(6;15) in mouse plasmacytomas or t(2;8) in Burkitt lymphomas, juxtaposes *Pvt-1* to immunoglobulin κ light chain *J* segment or enhancer regions. Deregulation of c-*myc* could be achieved by feedback from a putative *Pvt-1* gene product or, alternatively, by long-range effects of chromosomal aberration. Coamplification of *Pvt-1* with c-*myc* in several tumors such as COLO 320 or ANN-1 (7–9) suggests that this entire region may indeed function as a single unit or replicon.

Although Pvt-1 transcripts have not been detected in normal mouse tissues or in plasmacytomas (5, 6), there is evidence in other systems that *Pvt-1* may be a transcriptionally active area. Common sites for retroviral integration in mouse and rat lymphomas, *Mlvi-1* (or *Mis-1*) and *Mlvi-4*, are found in the *Pvt-1* area (10–14), and virus integration is known to favor transcriptionally active loci. RNA transcripts from this area have been detected in four rat tumors, three of which have proviruses integrated into the c-*myc*–*Pvt-1* region (14). Furthermore, Pvt-1 cDNAs have been isolated from human placenta (15), from human tumors with c-*myc*/*Pvt-1* amplifications (8, 9), and in Burkitt lymphomas with t(2;8) translocations (8). Even so, the nature of Pvt-1 transcripts in normal human, rat, or mouse cells remains unclear, and the effect of "variant" translocations on *Pvt-1* expression in the mouse has not been demonstrated. For these reasons we set out to isolate a mouse Pvt-1 cDNA and to characterize its expression in normal tissues and B lymphomas with and without translocations involving the *Pvt-1* locus. We report here the identification and sequence of a Pvt-1 cDNA[¶] from spleen that supports our hypothesis that *Pvt-1* is a transcriptionally active region and thereby susceptible to DNA recombination.

## MATERIALS AND METHODS

**DNA and RNA Hybridization Conditions.** High molecular weight DNA was prepared as described (16) from mouse liver (BALB/cAnPt) or BALB/c plasmacytomas (ABPC4, ABPC20, ABPC47, and TEPC1198) and NZB plasmacytomas (PC7183 and PC10916). Poly(A)$^+$ RNA was prepared from tissue (BALB/c thymus, spleen, and liver), cell lines [HAFTL1, NFS-112, NFS-1437, BALB 1427, NFS-467, NFS-2, and AJ9 (17)] or tumor lines [ABPC52, XRPC24, ABPC33, TEPC1194, MOPC 104E, TEPC1198 (18), ABPC20, and ABPC4] as described (19). Electrophoresis, transfer, and hybridization conditions were as described (16). Final wash conditions, unless specified otherwise, were 0.2 × SSC (0.03 M sodium chloride/0.003 M sodium acetate)/0.1% sodium dodecyl sulfate (SDS)/5 mM EDTA at 65°C.

**DNA Probes and cDNA Library.** The DNA probes for immunoglobulin κ-chain constant (C) region, pECk (20); immunoglobulin λ-chain C region, pCλ (21); glyceraldehyde-3-phosphate dehydrogenase, pGAPDH (22); and *Pvt-1* region, Pvt-1(a–e) (5), were as published.

The cDNA library was generated by oligo(dT)-primed synthesis of cDNA from poly(A)$^+$ RNA from the spleens of BXSB mice. The cDNA inserts containing *Eco*RI linkers were inserted into the λgt10 vector. The library was screened

Abbreviations: rcpt, reciprocal translocation; ORF, open reading frame; C, constant.
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§*Pvt-1* is mouse gene nomenclature; the corresponding gene in human and rat is *PVT1*. c-*myc* in the mouse is *Myc* and in human and rat is *MYC*. For simplicity we use *Pvt-1* and c-*myc* throughout.
¶The sequence reported in this paper has been deposited in the GenBank data base (accession no. M32688).

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with the 600-base-pair (bp) insert from Pvt-1(a), and the clone Pvt-1-1 was isolated and purified. The 1.4-kb Pvt-1-1 cDNA insert was excised and subcloned into the *Eco*RI site of pGEM3Z.

**RNase Protection.** $^{32}$P-labeled single-stranded riboprobes of Pvt-1-1 were synthesized from both the phage T7 or SP6 promoters following linearization of pGEM Pvt-1-1 with *Bam*HI or *Bst*NI, respectively. For RNase protection, 1–20 $\mu$g of poly(A)$^+$ mRNA from ABPC20, ABPC4, TEPC1198, AJ9, and mouse thymus, brain, testes, and liver were hybridized to riboprobes, and, after RNase digestion, samples were electrophoresed on 4% polyacrylamide/urea gels, dried, and exposed to x-ray film.

**DNA Sequencing.** All DNA sequencing reactions were performed by using the Sequenase kit (United States Biochemical) on *Eco*RI, *Hin*dIII, *Sau*3A, and *Xba* I phage M13 subclones of Pvt-1-1. Sequence analysis was performed primarily on the Macintosh program DNA STRYDER.

## RESULTS

To identify a transcript specific to the region of *Pvt-1*, we screened a BXSB mouse splenic cDNA library with a series of genomic DNA probes from chromosome 15 encompassing the rcpt(6;15) breakpoints (5). Among 500,000 cDNA recombinants, a single phage was isolated that hybridized to the DNA probe Pvt-1(a) (Fig. 1). This cDNA clone, Pvt-1-1, contains a 1.4-kb insert that we used as a probe in Southern and Northern blot hybridization experiments to determine the location, complexity, and expression of the gene. Both Pvt-1-1 and Pvt-1(a) probes hybridized to similar patterns in *Bam*HI-digested genomic DNA from rcpt(12;15) or rcpt(6;15) mouse plasmacytomas (Fig. 1 *Upper*). An 18-kb germ line or

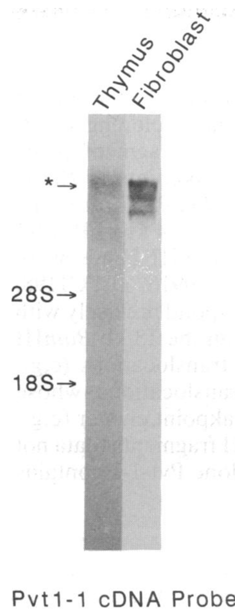


FIG. 2. Pvt-1 RNA 14-kb transcripts are detectable in mouse cells. Poly(A)$^+$ RNA from BALB/cJ thymus (20 $\mu$g) and fibroblast growth factor-stimulated NIH 3T3 cells (5 $\mu$g) were electrophoresed in formaldehyde agarose gels, transferred to Hybond-N membrane filter, and hybridized to a random-primed Pvt-1-1 DNA probe. Positions of 18S and 28S rRNA are indicated. Multiple estimates of the size of the 14-kb Pvt-1 RNA transcripts (asterisk) were made in comparison to RNA size standards (BRL, 0.24- to 95-kb ladder) and calculated on a BRL NA2 analyzer. The autoradiogram was exposed to x-ray film for 96 hr.

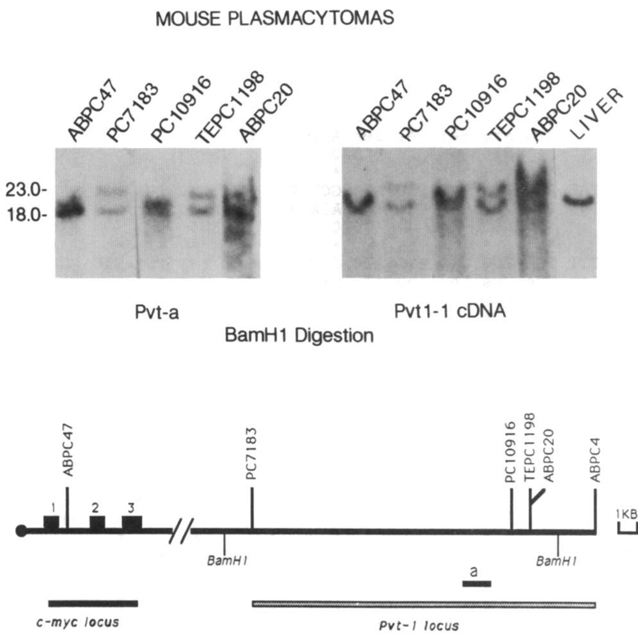


FIG. 1. Pvt-1 DNA rearrangements in mouse plasmacytomas. (*Upper*) *Bam*HI-digested genomic DNAs were size-fractionated by agarose gel electrophoresis, transferred to nylon filters, and hybridized to Pvt-1(a) (*Left*) (4) or Pvt-1-1 (*Right*) as described (16). A nonrearranged 18-kb *Bam*HI fragment is found in germ-line (liver) and rcpt(12;15) plasmacytomas (e.g., line ABPC47). In addition to the 18-kb germ-line fragment, the rcpt(6;15) plasmacytomas PC7183 (NZB), PC10916 (NZB), TEPC1198 (BALB/c × AKR), and ABPC20 (BALB/c) all display a rearranged *Bam*HI fragment containing sequences from both *Pvt-1* and the *C* region of the immunoglobulin $\kappa$-chain gene (refs. 4 and 5; K.H. and J.F.M., unpublished data). Sizes of hybridizing bands were determined by comparison to *Hin*dIII fragments of bacteriophage $\lambda$. (*Lower*) Schematic of chromosome 15 translocation breakpoints in mouse plasmacytomas. The c-*myc* (solid bar) locus is an unknown distance (>72 kb) centromeric to *Pvt-1* (stippled bar). The translocation breakpoints have been previously determined: ABPC47 (K.H., unpublished data), PC7183, PC10916, TEPC1198, ABPC20, and ABPC4 (4, 5). *Bam*HI restriction sites and the location of the DNA probe Pvt-1(a) are also denoted.

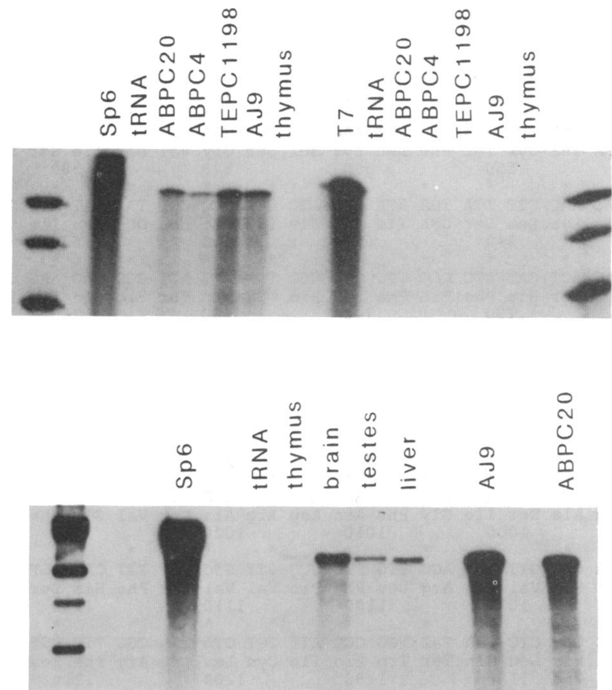


FIG. 3. Pvt-1-1 RNase assay. (*Upper*) Three-day exposure of SP6-directed (*Left*) and T7-directed (*Right*) synthesis of Pvt-1-1 riboprobes. Lanes marked SP6 and T7 represent untreated radioactively labeled probes. These were hybridized with 20 $\mu$g of tRNA (unprotected control) or 5 $\mu$g of poly(A)$^+$ RNA from ABPC20, ABPC4, TEPC1198, AJ9, or BALB/c thymus as indicated. The $\phi$X174 *Hae* III size standard borders the gel (shown are 1353, 1078, and 872 bases). The size differences between untreated transcripts and the protected bands are due to vector sequences included in the labeled RNA. (*Lower*) Six-day exposure of SP6-directed synthesis of Pvt-1-1 riboprobe. The radiolabeled riboprobe (SP6) was hybridized with 20 $\mu$g each of poly(A)$^+$ RNA from mouse thymus, brain, testes, or liver or 1 $\mu$g of poly(A)$^+$ RNA from AJ9 or ABPC20. The size standards on the left side are 1945, 1353, 1078, and 872 bases.

nonmutated *Bam*HI fragment of *Pvt-1* was observed in DNA from all tumors examined and in mouse liver. The *Pvt-1* gene did not appear to be amplified in any of these tumors. Comparison with the original restriction map of the mouse *Pvt-1* locus generated by cosmid or phage cloning (4, 5) permitted identification of the 18-kb *Bam*HI fragment as the region including the major cluster of breakpoints in *Pvt-1*. Further restriction enzyme surveys with *Eco*RI, *Kpn* I, and *Eco*RV assisted us in localizing much of the 1.4 kb of Pvt-1-1 to the region of Pvt-1(a). Rearranged *Bam*HI bands were found in the rcpt(6;15) tumors PC7183, PC10916, TEPC1198, and ABPC20 (Fig. 1 *Lower*), which correspond precisely with breakpoints previously established within the 18-kb *Bam*HI fragment (5). Tumors with rcpt(12;15) translocations (e.g., ABPC47) or those with rcpt(6;15) translocations whose breakpoints lie outside of the major breakpoint cluster (e.g., ABPC4) did not show rearranged *Bam*HI fragments (data not shown). We conclude that the cDNA clone Pvt-1-1 contains sequences derived from a single-copy gene that is transcribed from the *Pvt-1* locus of mouse chromosome 15.

To learn more about the expression of *Pvt-1*, we surveyed RNA from various mouse tissues by Northern blot analysis. Fig. 2 shows that low levels of an unusually large (14 kb), characteristically diffuse RNA transcript are detected by Pvt-1-1 hybridization to BALB/cJ thymus RNA. Similar Pvt-1 RNA transcripts are also detectable in mouse spleen, liver, brain, and testes samples (data not shown). Included for comparison is Pvt-1-1 hybridization to RNA from mitotically stimulated NIH 3T3 fibroblasts, which shows two lower bands in addition to the 14-kb band (Fig. 2). Since the 14-kb band may be diffuse as a result of alternative initiation, processing, or difficulties inherent in preparation of large mRNA, we performed RNase protection assays (*i*) to verify the existence of the distinct Pvt-1 RNA transcripts and (*ii*) to identify the sense strand of Pvt-1-1 for sequence comparisons. The 1.4-kb Pvt-1-1 insert was resubcloned into the

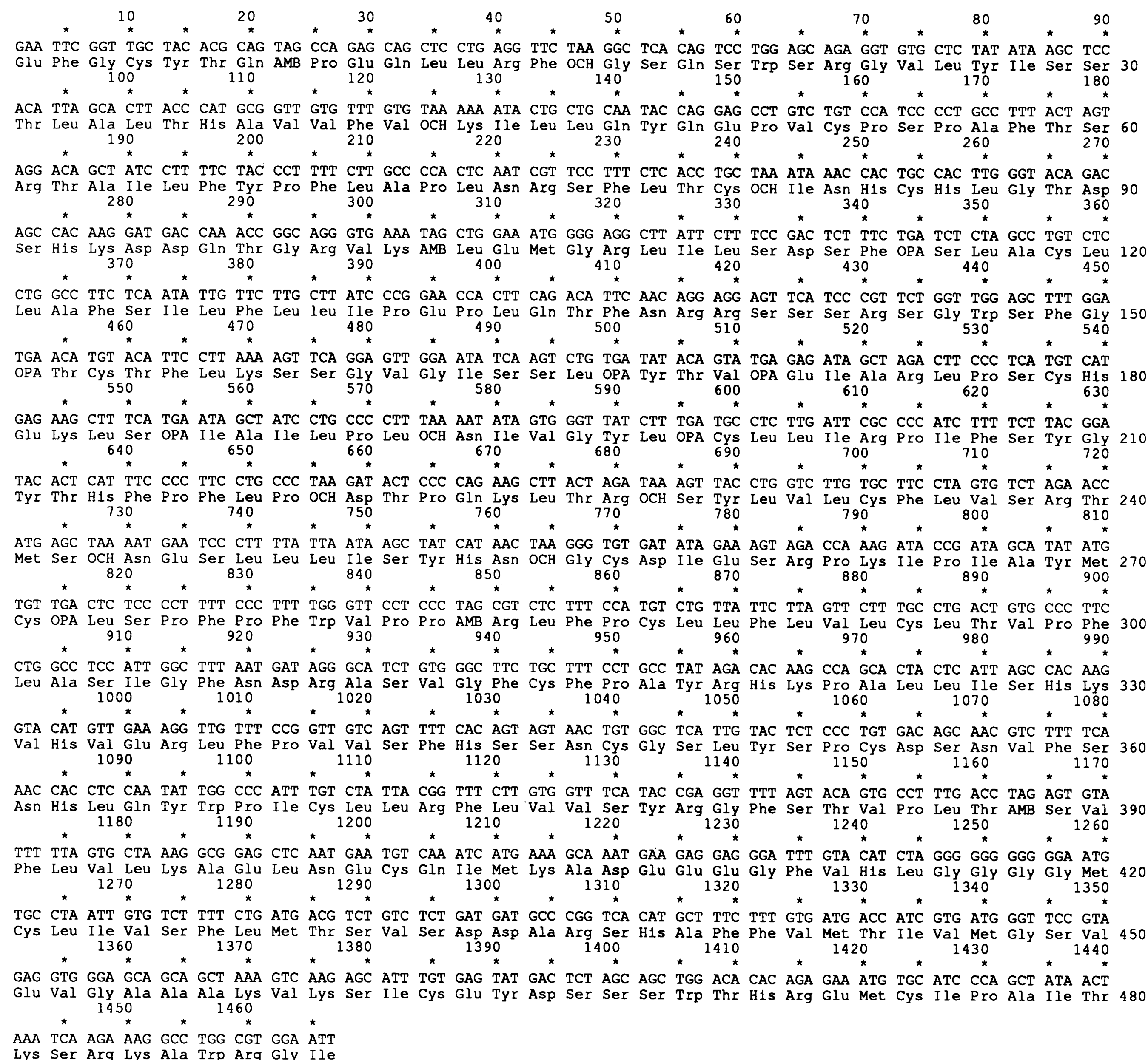


FIG. 4. Pvt-1-1 cDNA sequence. The complete 1467-bp sequence of Pvt-1-1 oriented in the sense direction and translated in the most appropriate reading frame. Numbers in the right hand margin refer to amino acid residue and numbers above to nucleotide position. Terminator codons are indicated by OPA, AMB, and OCH. The T7 promoter precedes this sequence in the pGEM 3Z subclone, and the SP6 promoter follows it.

plasmid vector pGEM3Z, which contains the phage SP6 and T7 promoters on opposite sides of the multiple cloning site. The full-length transcript from the SP6 promoter, but not from the T7 promoter, is protected by RNA from tumors ABPC20, ABPC4, TEPC1198, and AJ9 (Fig. 3 *Upper*). In another RNase protection experiment (Fig. 3 *Lower*), higher levels of SP6-generated signal are protected by 1 μg of poly(A)$^+$ RNA of mouse B-cell tumor lines AJ9 and ABPC20 than are protected by 20 μg of poly(A)$^+$ RNA from normal thymus, brain, testes, or liver. The presence of a single protected fragment indicates that this region is present and uninterrupted in the Pvt-1 mRNA in all of these examples. It does not eliminate the possibility, however, that 5′ or 3′ heterogeneity or alternative splicing may exist in normal and tumor transcripts, which could result in the diffuse broad Pvt-1 band found in thymus or the multiple Pvt-1 mRNAs in NIH 3T3 cells and in some plasmacytomas (see below).

**Pvt-1-1 cDNA Sequence.** The sequence of the entire 1467-bp insert of Pvt-1-1 is shown in Fig. 4. The coding orientation of Pvt-1-1 was established by the results of the RNase protection assay. Two significant open reading frames (ORFs) of 104 and 101 amino acid residues are present at positions 284–387 and 389–489, respectively. In view of the large size of the 14-kb Pvt-1 transcripts, one might expect it to encode a protein that is much larger than that predicted by the 104- or 101-amino acid ORFs. There may be a longer ORF in another part of the Pvt-1 mRNA, since this insert represents only a small percentage of the total transcript. Since the 3′ 101-amino acid ORF contains five AUG codons and no stop codons, this region might represent part of a longer ORF extending beyond our cDNA clone. Sequence comparisons reveal no significant homology with any known sequences, including human PVT (8, 9, 15).

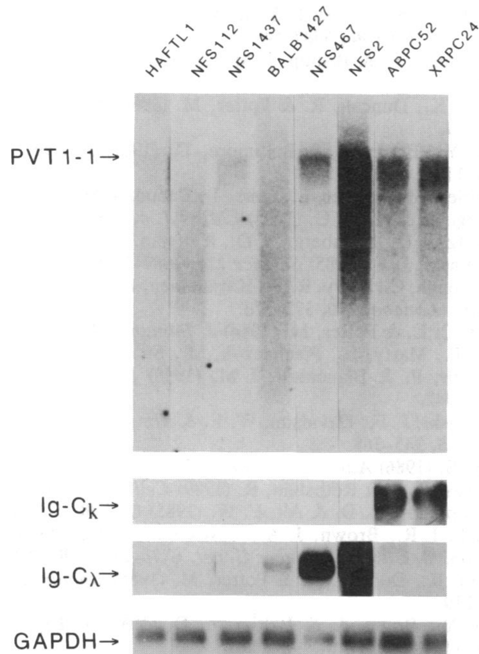


FIG. 5. B-cell spectrum of Pvt-1-1 Northern analysis. Pvt-1 RNA transcripts are more abundant in B lymphocytes expressing IgL. Poly(A)$^+$ RNA (5 μg) from pro-B-cell (HAFTL 1), pre-B-cell (NFS-112 and NFS-1437), small-B-lymphocyte (BALB 1427 and NFS-467) and follicular B-cell lines (NFS-2, a derivative of NFS-1) or plasma cell tumors (ABPC52 and XRPC24) were electrophoresed in a single formaldehyde agarose gel, transferred to Hybond-N membrane, and hybridized to Pvt-1-1. An arrow indicates the 14-kb Pvt-1 RNA transcript. RNA sizes were determined by comparison to size standards of a 0.24- to 9.5-kb ladder (BRL). Autoradiographs were exposed to Kodak XAR-5 film at −70°C for 72 hr with a DuPont Lightning*Plus* intensifying screen. Filters were stripped and rehybridized to probes for the constant region of immunoglobulin κ and λ chain genes (Ig-$C_\kappa$ and Ig-$C_\lambda$) for comparison of IgL expression. The same blot was hybridized to the housekeeping gene encoding glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (22) to normalize RNA levels.

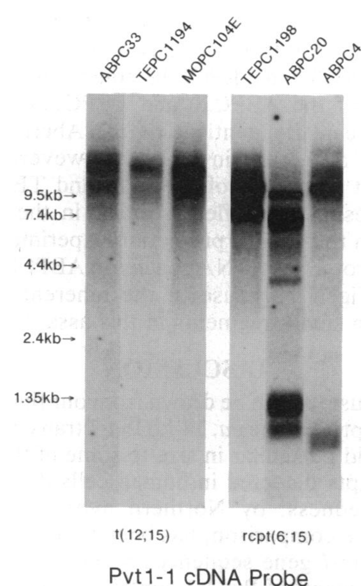


FIG. 6. Northern analysis of mouse plasmacytomas. Altered Pvt-1 RNA transcripts are found in rcpt(6;15) plasmacytomas. A blot containing 5 μg of poly(A)$^+$ RNA from rcpt(12;15) plasmacytomas (ABPC33, TEPC1194, and MOPC 104E) or rcpt(6;15) plasmacytomas (TEPC1198, ABPC20, and ABPC4) was hybridized to Pvt-1-1 as in Fig. 2. Sizes of RNA transcripts were determined by comparisons with multiple RNA size standards (0.24–9.5 kb) on a BRL NA2 analyzer. Autoradiography was at −70°C with intensifying screen for 72 hr for all samples except TEPC1198 (24 hr).

**Expression of Pvt-1 in Lymphoid Tissues.** We investigated whether *Pvt-1* expression might be enhanced at particular stages of lymphoid development—e.g., coinciding with the time of IgL rearrangement and/or expression. By examining mRNA from a spectrum of B-lymphocytic tumors frozen by transformation at different stages of B-cell differentiation, Pvt-1-1 hybridization reveals higher levels of the *ca.* 14-kb transcripts in cells known to have rearranged and expressed IgL genes (23) (Fig. 5). Specifically, low levels of Pvt-1 RNA are found in pro-B-cell (HAFTL 1) or pre-B-cell (NFS-112, NFS-1437, BALB 1427) lines, which do not express abundant IgL. On the other hand, higher levels of Pvt-1 RNA are found in small B lymphocytes (NFS-467 and NFS-2) and plasmacytomas (XRPC24 and ABPC52), all of which contain IgL rearrangements and express abundant immunoglobulin κ-chain or λ-chain constant region (23). All of these B-cell lines are transformed; therefore, *Pvt-1* expression could be associated with the state of transformation as well as with the degree of B-lymphocytic maturation.

**Pvt-1 Transcripts Are Altered in Tumors with rcpt(6;15) Translocations.** To determine whether Pvt-1 transcripts are enhanced or altered following rcpt(6;15) or rcpt(12;15) translocations, we hybridized Pvt-1-1 to Northern blots (Fig. 6) containing RNA from plasmacytomas that contain either rcpt(6;15) or rcpt(12;15) translocations (24). The diffuse *ca.* 14-kb RNA is found in many rcpt(12;15) plasmacytomas with translocation breakpoints 5′ of c-*myc* exon 1 (e.g., ABPC33 and TEPC1194) or within c-*myc* intron 1 (e.g., MOPC 104E). In contrast, rcpt(6;15) plasmacytomas (e.g., TEPC1198, ABPC20, and ABPC4) displayed aberrant or truncated Pvt-1 RNA transcripts. Specifically, TEPC1198 contains two large transcripts of ≈12.1 kb and 8.8 kb. ABPC20 contains two slightly shorter RNA transcripts of 10.5 kb and 7.6 kb in

addition to two small transcripts of 1.2 kb and 0.9 kb. The similarity of the patterns of the two large Pvt-1 RNA transcripts in ABPC20 and TEPC1198 could reflect the fact that the locations of the ABPC20 and TEPC1198 translocation breakpoints are nearly identical (Fig. 1). Aberrant Pvt-1 RNA transcripts are also found in ABPC4; however, the pattern is quite different from that of ABPC20 and TEPC1198. This difference presumably reflects a shift in the translocation breakpoint. In the RNase protection experiment we did not find smaller protected mRNA species in ABPC20, ABPC4, or TEPC1198 (Fig. 3) because of the inherent difficulties in resolving such small fragments in this assay.

## DISCUSSION

Several conclusions can be drawn from our finding of normal Pvt-1 transcripts. (*i*) The *ca.* 14-kb Pvt-1 transcripts identified in mouse could be similar in size to some of the larger Pvt-1 RNA transcripts detected in human cells (9). However, no obvious relatedness, by Northern hybridization or direct DNA sequence comparison, has been found between mouse and human *Pvt-1* gene sequences published so far. (*ii*) The 1.4-kb cDNA insert of Pvt-1-1 contains only a portion of the sequences in the 14-kb Pvt-1 RNA transcripts seen in normal tissues as well as in some tumors in mice. ORFs of 101 and 104 amino acids have been identified in Pvt-1-1, but longer ORFs may be present elsewhere in the 14-kb Pvt-1 transcript. Other portions of the mouse Pvt-1 transcript may also prove to be more similar to sequences in human Pvt-1 cDNAs. (*iii*) Aberrant RNA transcripts of *Pvt-1* are found exclusively in rcpt(6;15) translocations, suggesting that interruption of the *Pvt-1* gene leads to aberrant initiation of transcription, post-transcriptional processing, and/or chimeric transcripts from both chromosomes.[‖] (*iv*) Normal *Pvt-1* expression is highest at stages of B-cell development when IgL genes are rearranging or actively being transcribed. At these stages in B-cell maturation, *Pvt-1* would be in an "open chromatin" configuration, which may render the *Pvt-1* gene more susceptible to DNA breaks and erroneous recombinations with rearranging light chain genes. Increased recombination rates are, indeed, associated with active transcription in yeast (25). Similarly, the transcriptional accessibility model (26, 27) predicts that regions of chromatin that are being transcribed by RNA polymerase are also accessible to chromosomal breakage or viral integration. Since Pvt-1 RNA transcription appears to be stage-specific during B-cell development, rcpt(6;15) plasmacytomas may have been transformed by the chromosomal translocation event that occurred at one stage of B-cell ontogeny—i.e., during immunoglobulin light-chain *V-J* assembly. By analogy rcpt(12;15) plasmacytomas may be immortalized by interchromosomal recombination at a later stage (i.e., during immunoglobulin heavy chain switching).

Although we show a direct correlation between aberrant Pvt-1 RNA transcripts and rcpt(6;15) translocations, it remains to be determined whether alterations in *Pvt-1* transcription are causally related to deregulation of c-*myc* and/or malignant transformation. How *Pvt-1* and its neighboring gene, c-*myc*, interact will require more detailed study of *Pvt-1* transcription and translation. Some rcpt(6;15) plasmacytomas have repeatedly failed to show *Pvt-1* rearrangements with genomic DNA probes (ref. 5; K.H. and J.F.M., unpublished data). We are attempting to generate larger Pvt-1 cDNA probes with the expectation that more extensive *Pvt-1* sequences will be able to define the extent of the genetic locus and to detect DNA rearrangements and aberrant RNA in these tumors.

‖Preliminary experiments have identified a Pvt-1/immunoglobulin κ-chain chimeric cDNA from ABPC20.

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