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Assessing the Pathogenic Potential of the V(D)J Recombinase by Interlocus Immunoglobulin Light-Chain Gene Rearrangement

STEPHANIE N. BAILEY[1] and NAOMI ROSENBERG[1,2]*

Department of Pathology and Graduate Program in Immunology[1] and Department of Molecular Biology and Microbiology,[2] Tufts University School of Medicine, Boston, Massachusetts 02111

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Chromosomal translocations involving antigen receptor genes and oncogenes have been observed in several forms of lymphoid malignancy. Observations of their lymphocyte-restricted occurrence and a molecular analysis of some translocation breakpoints have suggested that some of these rearrangements are generated by V(D)J recombinase activity. However, a direct correlation between this activity and the generation of such rearrangements has never been established. In addition, because these aberrant rearrangements are usually detected only after a tumor has been formed, the frequency with which the recombinase machinery generates translocations has never been assessed directly. To approach these issues, immunoglobulin light-chain gene rearrangements were induced in pre-B cells transformed by temperature-sensitive mutants of Abelson murine leukemia virus and PCR was used to identify interlocus recombinants. VλJκ and VκJλ rearrangements as well as signal joints resulting from the recombination of Vλ and Jκ coding elements were recovered and were found to be similar in structure to conventional intrachromosomal joints. Because these products were detected only when the cells were undergoing active intralocus rearrangement, they provide direct evidence that translocations can be generated by the V(D)J recombinase machinery. Dilution analyses revealed that interlocus rearrangements occur about 1,000 times less frequently than conventional intralocus rearrangements. Considering the large numbers of lymphocytes generated throughout life, aberrant rearrangements generated by the V(D)J recombinase may be relatively common.

V(D)J rearrangement is the site-specific recombination by which antigen receptor genes are assembled during lymphocyte development (20). Recombination signal sequences (RSS), *cis*-acting elements each of which consists of a highly conserved heptamer and nonamer separated by a spacer of either 12 or 23 bases, direct the process (1, 13, 14). *trans*-acting factors, often referred to as the recombinase machinery, mediate recognition and cleavage of the RSS and modification and ligation of the recombining pairs. The RAG-1 and RAG-2 proteins recognize the RSS and cleave at the RSS-coding sequence boundary, generating hairpin-sealed coding ends and blunt, 5′-phosphorylated signal ends (31, 33, 35, 36, 38, 45, 48). These products are then resolved into coding and signal joints, respectively, in part by proteins involved in DNA repair (16, 21, 46).

Normally, joining involves elements located on the same chromosome. However, interlocus recombinants between different T-cell receptor (TCR) loci and rearrangements involving the immunoglobulin (Ig) heavy-chain genes and the BCL-2 gene have been found in normal cells (2, 23, 24, 40–42). These rearrangements are of particular interest because translocations involving antigen receptor genes and oncogenes are a common pathognomonic feature of several human lymphoid malignancies (4, 5, 8, 15, 47). The V(D)J recombinase machinery is thought to play a role in this process (3, 6, 19, 32, 43) and may act on both recombining partners. Alternatively, end donation, a process in which a randomly broken sequence is joined to an end generated by the recombinase, may be involved. Analyses of breakpoint sequences often do not allow distinction between these possibilities. In addition, although extensive studies with recombination substrates have delineated the RSS requirements for efficient recombination (14, 20), very little is known about other constraints that may influence V(D)J recombinase fidelity when recombination of the genomic sequence occurs. Furthermore, the frequency with which aberrant products are generated is difficult to measure in normal cells because only those products that do not adversely influence cell survival are likely to be recovered.

To explore the frequency with which the V(D)J recombinase can generate *trans* rearrangements, we took advantage of pre-B cells transformed by temperature-sensitive (*ts*) Abelson murine leukemia virus (Ab-MuLV) transformation mutants (12). When maintained at the permissive temperature, these cells are blocked at the pre-B-cell stage of differentiation. When the cells are shifted to the nonpermissive temperature and the v-Abl protein tyrosine kinase is inactivated, increased expression of RAG-1 and RAG-2 occurs and a high percentage of the cells rearrange their Ig light-chain genes (10). Analyses of the products generated when V(D)J recombination is activated in these cells revealed that VκJλ and VλJκ joints were formed at a frequency about 1,000-fold less than that for conventional VκJκ rearrangements. The *trans* rearrangements displayed typical hallmarks that characterize V(D)J recombination and were recovered only when the recombinase machinery was active. These data directly demonstrate that the V(D)J recombinase machinery can generate *trans* rearrangements and suggest that such rearrangements, including those which contribute to the development of lymphoid malignancies, are a relatively common by-product of V(D)J recombination.

* Corresponding author. Mailing address: SC-315, Tufts Medical School, 136 Harrison Ave., Boston, MA 02111. Phone: (617) 636-6906. Fax: (617) 636-0337. E-mail: nrosenbe@opal.tufts.edu.

MATERIALS AND METHODS

Cells and DNA preparation. The *ts* Ab-MuLV-transformed pre-B cell lines 103/1, 103/4, and DE/1 were generated with the Ab-MuLV P120/G+H strain (10,

TABLE 1. PCR primers

Primer	Description	Reference
Vκ1,2	5′-GGCTGCAGSTTCAGTGGCAGTGGGTC-3′; S = G or C; external Vκ primer	37
VκI	5′-CAGCAGCATGGAGGCTGALGA-3′; L = A or G; internal Vκ primer	
JκE	5′-TCCTAACATGAAAACCTGTGTC-3′; external Jκ primer	
Jκ5	5′-CCAAGCTTGTACTTACGTTTCAGCT-3′; internal Jκ primer	37
VλE	5′-CTGATTTGCTACTGATGACTGG-3′; external Vλ primer	
Vλ1,2	5′-AGAAGCTTGTGACTCAGGAATCTGCA-3′; internal Vλ primer	49
Jλ1	5′-CAGGATCCTAGGACAGTCAGTTTGGT-3′	49
Jλ2,3	5′-CAGGATCCTAGGACAGTGACCTTG-3′	49
VλRSSE	5′-TTCTGCCAAGTTGATAAACAAAGCTTG-3′; external Vλ RSS primer	
VλRSSI	5′-TTTGTTGCTCGAGACAACAGCTTTT-3′; internal Vλ RSS primer	
JκRSSE	5′-TCTACCTAGGGAGGGTTTTGTGGAG-3′; external Jκ RSS primer	
JκRSSI	5′-GCTCAACTGCTTGTGAAGTTTTGGT-3′; internal Jκ RSS primer	

12). These clones express a human BCL-2 gene which prevents the apoptotic response characteristic of pre-B cells transformed with *ts* Ab-MuLV when incubated at the nonpermissive temperature (9, 10). The cells were grown in RPMI 1640 medium supplemented to contain 10% fetal calf serum (Sigma) and 50 μM 2-mercaptoethanol at the permissive temperature, 34°C. For all experiments, cells were shifted to the nonpermissive temperature, 39.5°C, for 4 days. In some instances, cells were shifted to the nonpermissive temperature for 4 days and then returned to the permissive temperature for 12 to 14 h. NIH 3T3 cells were grown in Dulbecco's modified Eagle's medium supplemented to contain 10% fetal calf serum at 37°C. Genomic DNA to be used as PCR template was prepared by cesium chloride centrifugation as described previously (11).

PCR and Southern blotting. Nested PCR analysis was used for the detection of interlocus light-chain gene rearrangements. Primers used in these studies are listed in Table 1. Each first-round 50-μl reaction mixture contained each primer at a concentration of 2 μM; 150 μM $MgCl_2$; 10 mM Tris-HCl (pH 8.3 at 25°C); 50 mM KCl; 0.001% gelatin; 200 μM (each) dTTP, dGTP, dATP, and dCTP (Pharmacia); 1 U of *Taq* polymerase (Perkin-Elmer Cetus); and 0.2 to 0.5 μg of DNA template. The second-round amplification conditions were exactly the same as the first-round conditions except that 4 μl of the first-round PCR product was used as the template. First- and second-round amplification parameters for VλJκ PCR were 30 cycles of 1 min at 94°C, 1.5 min at 55°C, and 1.5 min at 72°C. Following the last cycle, samples were incubated for 10 min at 72°C and cooled to 4°C. The same conditions were used for the VκJλ PCR except that the first-round annealing temperature was 50°C. The VλJκ reciprocal product of PCR was incubated for 5 min at 95°C prior to adding the reaction mixture to the DNA templates. The reaction cocktail was added to the DNA templates at 80°C, and the reaction mixtures were amplified for 40 cycles of 1 min at 94°C, 1.5 min at 55°C (round 1) or 60°C (round 2), and 1.5 min at 72°C; a 10-min 72°C incubation was done following the final cycle. For dilution PCR analyses, test DNA was serially diluted into DNA prepared from NIH 3T3 cells. Aliquots of the diluted DNAs were taken and used for κ or VλJκ PCR in parallel. Samples were subjected to 30 cycles of 1 min at 94°C, 1.5 min at 50°C, and 1.5 min at 72°C. After the last cycle, the reaction mixtures were incubated for 10 min at 72°C and cooled to 4°C.

All PCR products were fractionated through 1.5% agarose gels, transferred to nylon membranes, UV cross-linked, and hybridized to an appropriate ^{32}P-labeled probe. The probes used include Vκ4, a 178-bp *Bam*HI-*Eco*RI fragment containing Vκ4 and 21 bp of Jκ5 sequences; Jκ5, a 0.6-kb *Acc*I-*Pst*I fragment from pJκ which contains Jκ4 and Jκ5 sequences (10); Vλ1, a 0.4-kb *Eco*RI fragment containing Vλ1 and Cλ1 sequences; Jλ, a probe consisting of pooled TTTTCGGCA/GGTGGAACCAA and TGTTCGGTGGAGGAACC oligonucleotides; and Jκ4Jκ5 RSS, an oligonucleotide probe of the following sequence: GCCTATCTAACTGGATCAGC.

Cloning. To examine the fine structure of amplified interlocus rearrangements, second-round PCR products were cloned with a TA cloning kit (Invitrogen). PCR products were gel purified according to size by either DEAE paper purification or Quiaex gel extraction and cloned. Alternatively, PCR products were cloned directly, transformed colonies were lifted onto nitrocellulose membranes and lysed, and the DNA was bound by UV cross-linking. The membranes were washed in a solution containing 5× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate), 0.5% sodium dodecyl sulfate, and 1 mM EDTA (pH 8.0) at 50°C for 30 min, transferred to prehybridization solution (6× SSC, 5× Denhardt's solution, 0.1% sodium dodecyl sulfate, 0.1 mg of yeast tRNA per ml) for at least 1 h, and hybridized overnight with either ^{32}P-labeled Jλ or Jκ4Jκ5 RSS oligonucleotide probes. VκJλ colony lifts were washed in 6× SSC at 56°C. VλJκ signal joint colony lifts were washed in 6× SSC at 54°C. Rearrangements were sequenced by the dideoxy chain termination method (Sequenase) with either the M13 forward and reverse primers (New England Biolabs) or the appropriate PCR primers. Sequences obtained were compared to those sequences in the GENEMBL rodent databases.

RESULTS

Interlocus κ and λ rearrangements occur when light-chain rearrangement is activated. High levels of V(D)J recombinase activity are induced in the *ts* transformants at the nonpermissive temperature (10). To determine if interlocus recombination between the κ and λ loci occurs under these conditions, a nested PCR assay was designed to identify VλJκ rearrangements (Fig. 1A). When DNA samples prepared from *ts* Ab-MuLV transformants maintained at the permissive temperature were analyzed, very low levels of conventional κ rearrangements were detected (data not shown) and no interlocus rearrangements were recovered (Fig. 1B). In contrast, analysis of DNA samples from *ts* transformants maintained at the nonpermissive temperature for 4 days revealed the presence of fragments that hybridized with κ- and λ-specific probes (Fig. 1B). These fragments were of the appropriate size to represent VλJκ4 and VλJκ5 rearrangements and were detected in both the first- and second-round amplification reaction mixtures. Analyses of DNA samples prepared from cells maintained at the nonpermissive temperature for 1 or 2 days occasionally yielded similar products, but detection was inconsistent.

To demonstrate that interlocus recombination is a general feature of rearranging elements in these cells, a PCR assay was designed to amplify VκJλ rearrangements. Similar to what was revealed by the data obtained by VλJκ PCR, fragments that hybridized with probes specific for the κ and λ loci were recovered from cells incubated at the nonpermissive temperature (Fig. 2). In addition to products of the size expected for a VκJλ rearrangement, fragments of several other sizes that hybridized to the κ locus probe were visualized. In some experiments, additional bands were visualized with the Jλ-specific probe as well (data not shown). At least some of these products reflect the presence of abnormal rearrangements involving κ and λ sequences. One example involved a recombination between a Jλ2 coding sequence and an intronic Vκ sequence, a joining event that may have arisen via cryptic RSS recognition (see below). Other products may result from the degenerate nature of the Vκ primers used and the relatively low-stringency conditions used for the PCR. Similar to the results obtained by VλJκ PCR, no reaction products were recovered from cells incubated at the permissive temperature, indicating that activation of the V(D)J recombinase machinery is required to form the interlocus recombinants.

Interlocus coding joints resemble normal Ig coding joints. Rearrangements mediated by the V(D)J recombinase have a characteristic structure that results from the specificity in-

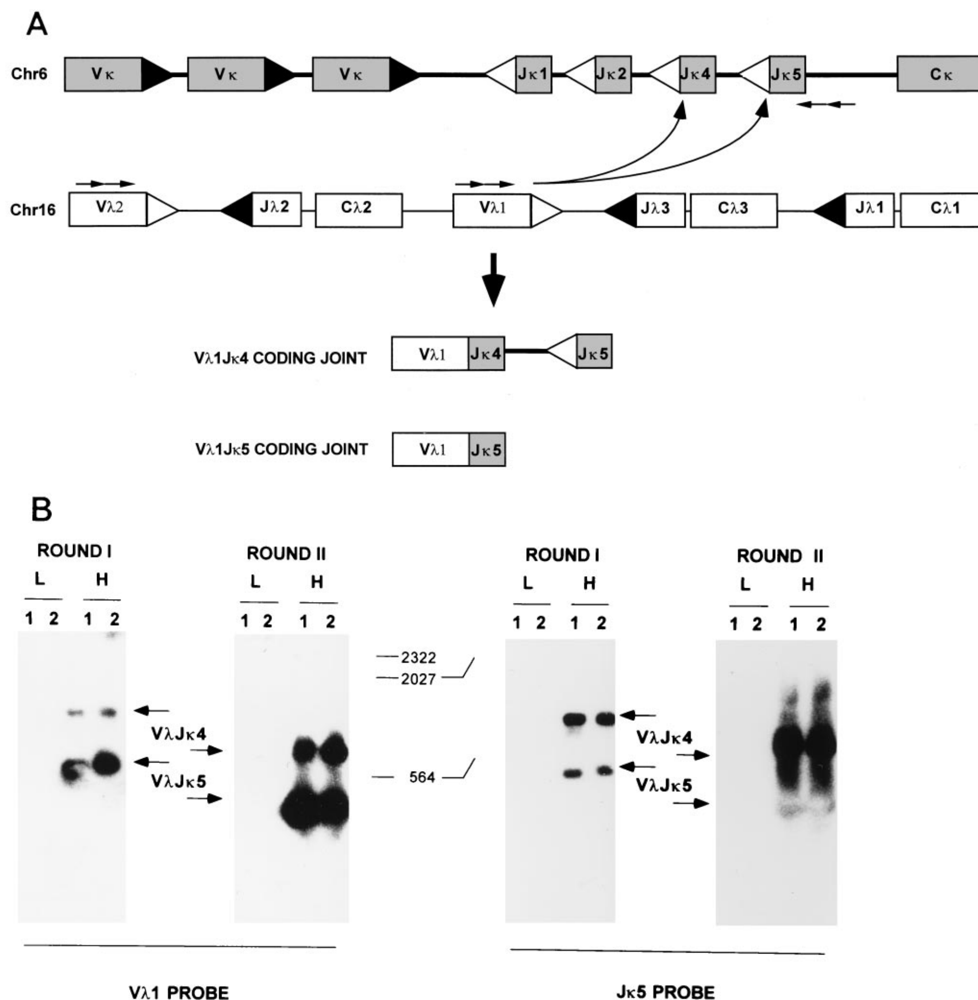

FIG. 1. Detection of VλJκ rearrangements by nested PCR. (A) Diagrammatic representation of the PCR assay used to detect interlocus VλJκ rearrangements. Primers are indicated by the straight arrows. The curved arrows indicate the recombination events occurring during interlocus rearrangement. Coding sequences at the κ locus are indicated by the shaded boxes; coding sequences at the λ locus are indicated by the open boxes; RSS sequences are indicated by the filled (12-bp spacer) and open (23-bp spacer) triangles. The loci are not drawn to scale. (B) PCR analysis of DNA samples from cells maintained at the permissive temperature (L) and at the nonpermissive temperature (H) for 4 days. Two rounds of PCR were performed as described in Materials and Methods. Each reaction was done in duplicate (lanes 1 and 2). The products were fractionated on agarose gels, transferred to nylon membranes, and hybridized first with the Vλ1 probe. The blots were stripped and hybridized to the Jκ5 probe. Arrows indicate the interlocus rearrangement products. In round I, a VλJκ5 product would be expected to be approximately 260 bp and a VλJκ4 product would be expected to be approximately 600 bp; following a second round of amplification, these products would be expected to be about 100 bp smaller. The numbers in the center indicate the positions of molecular weight standards.

volved in cleaving the recombining gene fragments (20). To determine if the sequences detected in the Southern blotting experiments resemble conventional κ and λ rearrangements, the PCR products were cloned and sequenced. Seven independently derived VλJκ coding joints and 16 VκJλ joints isolated from two different *ts* pre-B cell lines were analyzed (Tables 2 and 3). Rearrangements involving both Vλ1 and Vλ2 as well as Jκ4 and Jκ5 gene segments were found among the VλJκ rearrangements; the VκJλ rearrangements contained Vκ sequences from several families and Jλ1, Jλ2, and Jλ3 sequences.

Most features of the rearrangements were similar to those that characterize normal light-chain gene rearrangements, with loss of nucleotides at the coding junction border being the most prominent. The average number of nucleotides lost in these rearrangements (9 bases) is slightly higher than the average of 5 lost in a set of 28 VκJκ coding joints recovered from these cells (7). However, the range in deletion size (0 to 19 nucleotides lost) was similar to that observed in our study. This may reflect differences in the length of time the cells were maintained at the nonpermissive temperature. P nucleotides were not a common feature of either the conventional or the *trans* rearrangements (7) (data not shown). The presence of N nucleotides, a feature that is not normally found in light-chain rearrangements, is an exception to this rule. However, the *ts* Ab-MuLV transformants express high levels of terminal deoxynucleotidyl transferase (TdT), the enzyme that catalyzes N additions, at the nonpermissive temperature (39a) and both VκJκ and VλJλ joints recovered from these cells have N additions (7, 29a).

Some clones contained complex rearrangements. For example, clone H1 (Table 2) contains Vλ, Vκ, and Jκ sequences. This configuration is consistent with a *trans* V gene replacement rearrangement mechanism. Analysis of several Vκ sequences present in the GENEMBL combined database revealed the presence of a 6-for-7 heptamer sequence in the correct orientation just upstream of the breakpoint in the Vκ

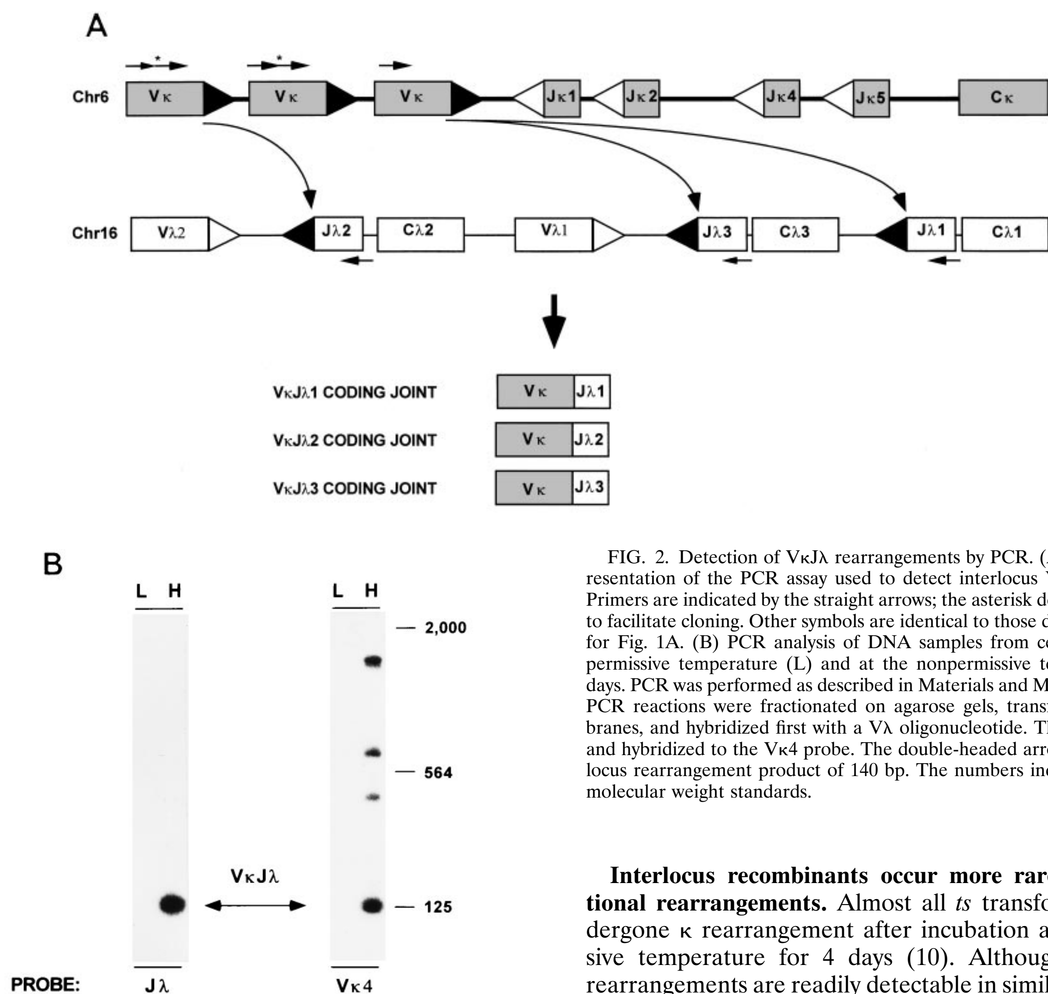

FIG. 2. Detection of VκJλ rearrangements by PCR. (A) Diagrammatic representation of the PCR assay used to detect interlocus VλJκ rearrangements. Primers are indicated by the straight arrows; the asterisk designates primers used to facilitate cloning. Other symbols are identical to those described in the legend for Fig. 1A. (B) PCR analysis of DNA samples from cells maintained at the permissive temperature (L) and at the nonpermissive temperature (H) for 4 days. PCR was performed as described in Materials and Methods. Products from PCR reactions were fractionated on agarose gels, transferred to nylon membranes, and hybridized first with a Vλ oligonucleotide. The blots were stripped and hybridized to the Vκ4 probe. The double-headed arrow indicates the interlocus rearrangement product of 140 bp. The numbers indicate the positions of molecular weight standards.

sequence found in this clone. Although V gene replacement has not been extensively analyzed at the Ig light-chain loci, others have suggested that this mechanism is used at the Ig heavy-chain locus to expand the available antibody repertoire and salvage cells that contain nonproductive rearrangements on both alleles (17, 18, 34, 44).

TABLE 2. Structure of VλJκ coding joints[a]

Clone	Cell line	Vλ usage	Jκ usage	V del	N region	J del
A1	DE/1	Vλ1	Jκ5	−2		−12
B1	DE/1	Vλ2	Jκ5	−3	CGG	0/+1
C1	DE/1	Vλ2	Jκ4	−1	AGG	−3
D1	DE/1	Vλ2	Jκ4	−5	CTCTT	−4
E1	103/4	Vλ1	Jκ4	−5	C	−9
F1	103/4	Vλ1	Jκ5	0	C	−9
G1	103/4	Vλ1	Jκ5	−1		−15
H1	103/4	Vλ2	Jκ5	−7	0/Vκ/A	−1

[a] Molecular clones derived from independent PCR were sequenced, and Vλ and Jκ usages were assigned on the basis of comparison with germ line sequences. The numbers of bases deleted from the Vλ and Jκ germ line sequences are indicated in the V del and J del columns. Nontemplated nucleotides found at the junctions are listed in the N region column. The structure of the rearrangement in clone H1 is discussed in detail in the text.

Interlocus recombinants occur more rarely than conventional rearrangements. Almost all *ts* transformants have undergone κ rearrangement after incubation at the nonpermissive temperature for 4 days (10). Although the interlocus rearrangements are readily detectable in similar DNA samples, the nested PCR assays used to detect these products make it difficult to assess the frequencies with which they occur. To examine this issue more closely, we used single-amplification reactions and limiting-dilution analyses to compare the frequency of VλJκ joint formation to that of VκJκ rearrangement. DNA templates prepared from cells maintained at the nonpermissive temperature were serially diluted into DNA prepared from NIH 3T3 fibroblast cells which do not rearrange Ig genes. A single round of PCR was used to amplify the samples, and the products were analyzed by Southern blotting with appropriate probes (Fig. 3). VκJκ rearrangements could be detected in reaction mixtures containing DNA from 10 to 15 *ts* cells. This frequency of rearrangement is consistent with the frequencies obtained in earlier experiments in which subclones of *ts* cells were analyzed following incubation at the nonpermissive temperature (10). In contrast, comparisons derived from multiple experiments revealed that about 1,000-fold fewer VλJκ products were found. Direct comparison of the frequencies of VκJλ and VλJλ rearrangements was not possible because the VκJλ rearrangements were difficult to detect reproducibly with a single round of amplification. These results could indicate that VκJλ rearrangements occur less frequently than VλJκ rearrangements. Conventional rearrangements involving the λ locus are less frequent than those involving the κ locus in the *ts* cells (10, 29).

Detection of interlocus signal joints. Normal V(D)J rearrangement results in the formation of coding and signal joints. If the interlocus recombinants characterized here arise via

TABLE 3. Structure of VκJλ coding joints[a]

Clone	Cell line	Vκ usage	Jλ usage	V del +/−1	N region	J del
A1	103/4	Vκ1	Jλ3	−9	T	−5
B1	103/4	Vκ24B	Jλ2	−4	AGG	−4
C1	103/4	Vκ1	Jλ1	−9		0
Li-1	103/4	Vκ1	Jλ3	−2	TC	−2
Li-2	103/4	Vκ-OX1	Jλ3	−9	GGAC	−5
D1	DE/1	Vκ1	Jλ2	−2	TC	−7
E1	DE/1	Vκ	Jλ2	−12	AGGGGGA	0
Li-3	DE/1	Vκ-OK1	Jλ2	−4		−1
Li-4	DE/1	Vκ1	Jλ2	−5	GCCC	0
Li-5	DE/1	Vκ4.3	Jλ2	−2	C	−2
Li-6	DE/1	Vκ24	Jλ2	−7		−7
Li-7	DE/1	Vκ-OK1	Jλ2	−2	TTCGC	0
Li-8	DE/1	Vκ	Jλ2	−20	AAGGG	−4
Li-9	DE/1	Vκ	Jλ2	−5	GTCC	0
Li-10	DE/1	Vκ25-39	Jλ2	−5	T	−10
Li-11	DE/1	Vκ	Jλ2	−14		−5

[a] Clones designated with a single letter were derived from individual PCR and cloned following gel purification of the amplified material; clones designated Li were derived by cloning the material from PCR and screening by colony hybridization as described in Materials and Methods. Jλ and Vκ usages were assigned on the basis of a comparison with the germ line sequences. Vκ sequences that could not be assigned to a particular family are designated Vκ. The numbers of bases deleted from the Vκ and Jλ germ line sequences are indicated in the V del +/−1 and J del columns. In cases where Vκ sequences could not be assigned to a particular Vκ gene, estimates of the numbers of bases lost based on a compiled Vκ germ line sequence are given. Nontemplated nucleotides found at the junctions are listed in the N region column.

conventional V(D)J recombination, then interlocus signal joints should also be generated. To detect such rearrangements, a nested PCR assay to amplify VλJκ reciprocal products was designed (Fig. 4A). Because other investigators have shown that conventional κ and λ signal joints can be recovered only after *ts* Ab-MuLV-transformed cells are returned to the nonpermissive temperature (33), we examined DNA prepared from cells that had been maintained at the nonpermissive temperature for 4 days and then shifted back to the permissive temperature for 12 to 14 h. Analyses of these DNA samples revealed that VλJκ signal joints could be detected (Fig. 4B). Occasionally, several fragments could be seen in the second round of amplification (e.g., Fig. 4B, round II, lane H2). Products corresponding in size to these less-intense fragments were not recovered among the cloned products, and the nature of these products is not known. None of these products were detected in DNAs from cells that had remained at the permissive temperature throughout the experiment. In some experiments, signal joints were detected in DNAs from cells maintained at the nonpermissive temperature for 4 days only. However, these products could not be detected on a consistent basis.

Conventional signal joints formed during V(D)J recombination are usually precise joints of heptamer-nonamer sequences (20). However, a sequence analysis of cloned VλJκ signal joints revealed that nucleotides had been added and/or lost at the junction (Table 4). The extent of these processing events may account for the diffuse PCR signal observed by Southern blot analysis of PCR-amplified interlocus signal joints (Fig. 4B). Although the imprecision of interlocus signal joints is unexpected on the basis of conventional signal joint structures, the formation of these reciprocal products substantiates the role of the recombinase in mediating these translocations.

DISCUSSION

Our experiments show that activation of endogenous V(D)J recombinase activity correlates with the appearance of translocations involving Ig light-chain genes. These data demonstrate that the V(D)J recombinase can bring together DNA elements located on different chromosomes in an RSS-directed manner when normal V(D)J rearrangement is activated. Similar events likely give rise to the interlocus TCR rearrangements detected in healthy individuals and to some of the translocations in which antigen receptor sequences and other cellular sequences are joined in normal cells and in lymphoid tumors (4–6, 8, 15, 19, 23, 24, 32, 41–43). It has been suggested that these rearrangements reflect inherent defects in V(D)J recombination (27, 28). However, our ability to detect interlocus recombinants in cells undergoing conventional joining sug-

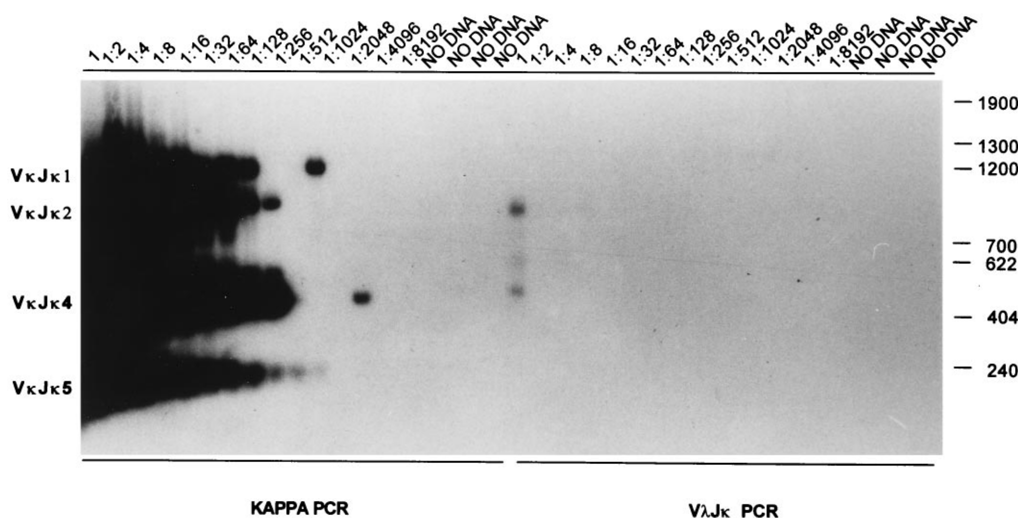

FIG. 3. Comparison of the frequencies of intra- and interlocus rearrangements involving Jκ. DNAs prepared from *ts* cells maintained at the nonpermissive temperature were serially diluted into carrier DNA, and parallel reaction mixtures were amplified with Vκ and Jκ primers or Vλ and Jκ primers. All reaction mixtures contained a total of 500 ng of DNA. The products were fractionated on an agarose gel and transferred to a nylon membrane. The membrane was hybridized with the Jκ5 probe. The autoradiograph shown is representative of results obtained in several experiments. The dilution ratios are indicated at the top. The numbers at the right indicate the positions of molecular weight standards.

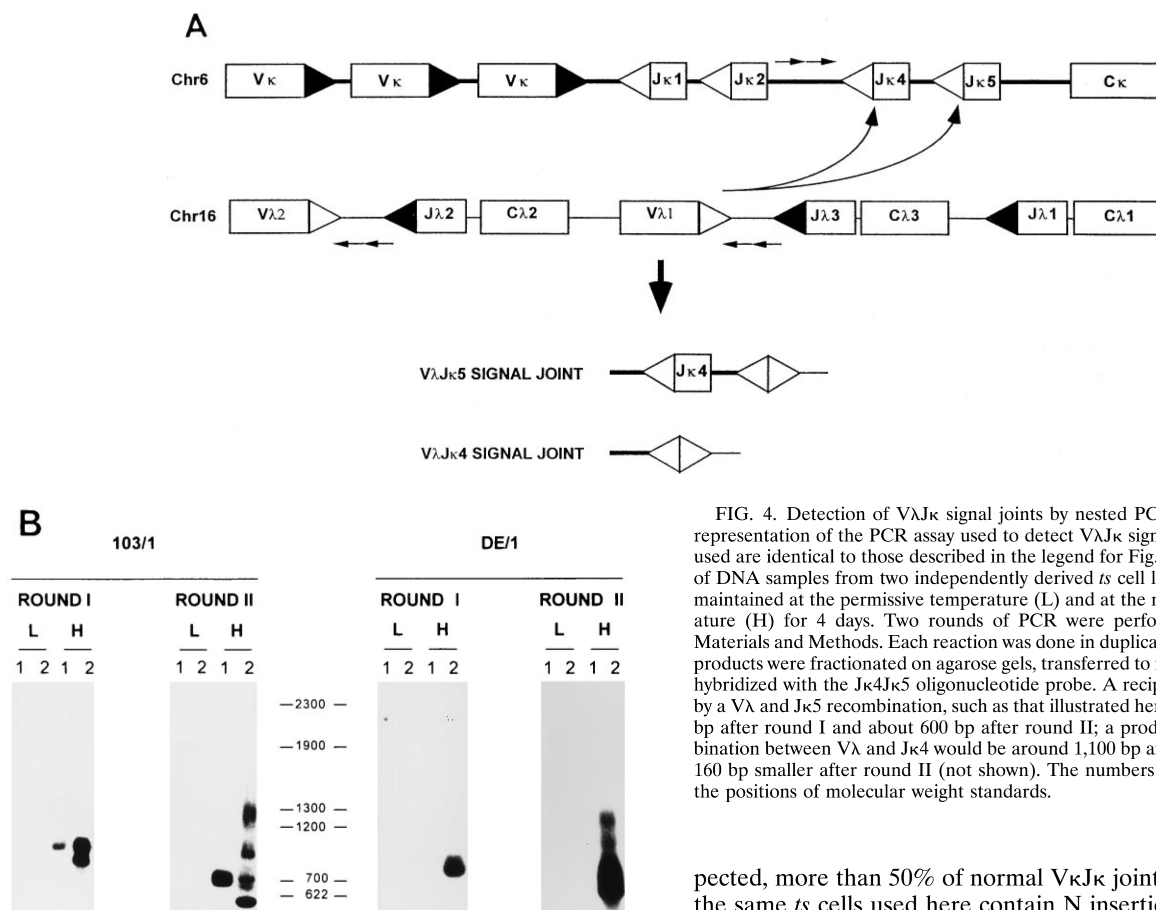

FIG. 4. Detection of VλJκ signal joints by nested PCR. (A) Diagrammatic representation of the PCR assay used to detect VλJκ signal joints. The symbols used are identical to those described in the legend for Fig. 1A. (B) PCR analysis of DNA samples from two independently derived *ts* cell lines (103/4 and DE/1) maintained at the permissive temperature (L) and at the nonpermissive temperature (H) for 4 days. Two rounds of PCR were performed as described in Materials and Methods. Each reaction was done in duplicate (lanes 1 and 2). The products were fractionated on agarose gels, transferred to nylon membranes, and hybridized with the Jκ4Jκ5 oligonucleotide probe. A reciprocal product formed by a Vλ and Jκ5 recombination, such as that illustrated here, would be about 760 bp after round I and about 600 bp after round II; a product formed by recombination between Vλ and Jκ4 would be around 1,100 bp after round I and about 160 bp smaller after round II (not shown). The numbers in the center indicate the positions of molecular weight standards.

gests that they may be a normal, albeit low-frequency, byproduct of V(D)J recombination. Although the recombinase machinery almost certainly does not mediate cleavage of all nonantigen receptor DNA sequences involved in antigen receptor gene translocations, the frequency with which this occurs can be assessed directly with the *ts* pre-B cells.

The structures of the coding and signal joints formed as a consequence of interlocus rearrangement are consistent with V(D)J recombinase-mediated rearrangement. For example, these structures are consistent with cleavage at the RSS-coding sequence border; most clones have lost very few nucleotides at the cleavage site. While the presence of N insertions in rearrangements involving the light-chain loci may seem unexpected, more than 50% of normal VκJκ joints recovered from the same *ts* cells used here contain N insertions (7). The high frequency of such insertions correlates with the large increases in TdT RNA observed following the shift of the *ts* cells to the nonpermissive temperature (39a), a phenomenon that correlates with the loss of an active v-Abl protein. Although the mechanism controlling this phenomenon requires further study, the presence of N insertions in *trans* rearrangements is similar to the situation observed in conventional rearrangements recovered from these cells.

TABLE 4. Structure of interlocus signal joints[a]

Clone	Cell line	Vλ del	N region	Jκ del	Jκ RSS
A	103/4 (H)	−4	CC	−4	Jκ4
B	103/4 (H)	0	ACGCG	−1	Jκ5
S21	103/1 (HL)	0		−11	Jκ4
S22	103/1 (HL)	0		−5	Jκ4
S3A1	103/1 (HL)	0		−17	Jκ4
S3A5	103/1 (HL)	0		−4	Jκ4

[a] Rearrangements derived from cells maintained only at the nonpermissive temperature are designated H; those derived from cells maintained at the nonpermissive temperature and then shifted back to the permissive temperature are designated HL. Molecular clones derived from independent PCR were sequenced, and the Vλ and Jκ usages were assigned on the basis of comparison with the germ line sequences. The numbers of bases deleted from the Vλ and Jκ germ line sequences are indicated in the Vλ del and Jκ del columns. Nontemplated nucleotides found at the junctions are listed in the N region column.

Although most clones have lost only small numbers of nucleotides at the coding border, three clones displayed deletions of >12 nucleotides. Indeed, the average number of nucleotides lost in all the joints was slightly higher than that lost in conventional κ coding junctions cloned from these cells (7). The *trans* rearrangements were recovered from cells that were maintained at the nonpermissive temperature for 48 h longer than the cells from which the VκJκ rearrangements were recovered. Perhaps this time allows for more extensive endonucleolytic processing because nucleases are up regulated under these conditions. Alternatively, the structure of the complex containing the cleaved intermediates may be altered. Normally these complexes are formed by pairing between RSS containing 12- and 23-bp spacers (20); the *trans* rearrangements arise following cleavage of RSS with the same spacer length. Complexes formed as a consequence of this mispairing may be more susceptible to normal nucleolytic activities.

Another important characteristic of rearrangements formed by the V(D)J recombinase is the generation of signal joints. Consistent with the role of the V(D)J recombinase in generating interlocus recombinants, signal joints were recovered only from cells that had been incubated at the nonpermissive temperature. While some signal products could be isolated

from cells after 4 days at the nonpermissive temperature, these products could be detected more readily if the cells were incubated at the nonpermissive temperature for 4 days and then returned to the permissive temperature for a short time. Again, this feature parallels the resolution of normal signal ends; others have shown that these products can be detected only when *ts* cells that have been shifted to the high temperature are returned to the permissive temperature (33).

The interlocus signal joints recovered from the *ts* cells are distinct from most conventional signal joints in two ways. While signal joints are typically precise unions of the 12- and 23-bp spacer RSS, the interlocus signal products show evidence of processing. First, all of the interlocus signal joints contain N insertions. Given the high levels of TdT expressed by the *ts* cells, this modification may not be completely unexpected; N additions have been observed in extrachromosomal substrate signal joints that have formed in the presence of TdT (22). Indeed, the frequency of such insertions correlates with the level of TdT expression. In addition, some endogenous signal joints cloned from γ/δ T cells from adult mouse thymus contain N nucleotides (40).

A second difference, more difficult to interpret, is the presence of deletions. There are only a few examples of nucleotide deletions in conventional signal joints formed in normal cells (22, 40). Extensive nucleotide deletions have been observed in signal joints derived from severe combined immune deficiency cells (22, 30, 39). These must reflect deficiencies in the DNA-dependent protein kinase responsible for the severe combined immune deficiency phenotype. Deletions of DNA sequences at the breakpoints of reciprocal translocations involving antigen receptor genes have also been found. Whether these indicate that the joining events occurred in an aberrant fashion or whether these structures reflect the initial rearrangement is difficult to assess.

Analysis of the frequency with which *trans* light-chain gene rearrangements form suggests that they occur about 1,000-fold less frequently than *cis* rearrangements. Because virtually all cells in the population contain κ rearrangements, this value suggests that about 0.1% of the cells have undergone *trans* joining events. This estimate assumes that the *cis* and *trans* rearrangements are amplified at about the same efficiency. Reconstruction experiments in which the abilities to detect conventional κ and λ rearrangements were compared revealed that both assays were equally sensitive (29). Others have estimated that *trans* rearrangements involving TCR loci are recovered at a frequency of about 1 rearrangement per 10^4 or 10^5 thymocytes in vivo (2, 41). These estimates reflect the levels with which such structures have accumulated in the sampled tissues. Because the half-lives of cells with these rearrangements and the rates at which they form are not known, it is difficult to compare the frequencies observed in the two systems.

Detection of interlocus rearrangements in the *ts* cells may be facilitated because they arrest in G_1 at the nonpermissive temperature (9). The recombinase machinery is active during this phase of the cell cycle (25, 26). In addition, the absence of cell division may allow detection of the rearrangements because those which are detrimental to cell growth will not be lost and those that are advantageous will not be highlighted. This feature lifts the selective constraints which are inherent in analysis of *trans* rearrangements from peripheral blood or thymus, possibly affecting the frequency estimates of such events. Furthermore, unlike tumor cells which carry translocations involving antigen receptor genes, analysis of translocations in the *ts* cells occurs immediately, prior to the accumulation of other rearrangement events, which undoubtably occur in the case of cellular transformation and which can mask the mechanisms by which the initial products were formed.

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