BCL2 Oncogene Translocation Is Mediated by a χ-like Consensus

By Richard T. Wyatt,*† Richard A. Rudders,* Andrew Zelenetz,§ Ronald A. Delellis,‖ and Theodore G. Krontiris*†

From the *Department of Medicine (Hematology/Oncology), New England Medical Center Hospitals, Boston, Massachusetts 02111; the †Graduate Immunology Program, Tufts University School of Medicine, Boston, Massachusetts 02111; the §Department of Medicine, Stanford University Medical Center, Palo Alto, California 94305; and the ‖Department of Pathology, Tufts University School of Medicine, Boston, Massachusetts 02111

Summary

Examination of 64 translocations involving the major breakpoint region (mbr) of the BCL2 oncogene and the immunoglobulin heavy chain locus identified three short (14, 16, and 18 bp) segments within the mbr at which translocations occurred with very high frequency. Each of these clusters was associated with a 15-bp region of sequence homology, the principal one containing an octamer related to χ, the prokaryotic activator of recombination. The presence of short deletions and N nucleotide additions at the breakpoints, as well as involvement of Jα and Dα coding regions, suggested that these sequences served as signals capable of interacting with the VDJ recombinase complex, even though no homology with the traditional heptamer/spacer/nonamer (IgRSS) existed. Furthermore, the BCL2 signal sequences were employed in a bidirectional fashion and could mediate recombination of one mbr region with another. Segments homologous to the BCL2 signal sequences flanked individual members of the XP family of diversity gene segments, which were themselves highly overrepresented in the reciprocal products (18q−) of BCL2 translocation. We propose that the χ-like signal sequences of BCL2 represent a distinct class of recognition sites for the recombinase complex, responsible for initiating interactions between regions of DNA separated by great distances, and that BCL2 translocation begins by a recombination event between mbr and Dαχ signals. Since recombinant joints containing χ, not IgRSS, occur in brain cells expressing RAG-1 (Matsuoka, M., F. Nagawa, K. Okazaki, L. Kingsbury, K. Yoshida, U. Muller, D. T. Larue, J. A. Winer, and H. Sakano. 1991. Science [Wash. DC]. 254:81; reference 1), we further suggest that the product of this gene could mediate both BCL2 translocation and the first step of normal DJ assembly through the creation of χ joints, rather than signal or coding joints.

The activation of some oncogenes occurs as a consequence of the genetic rearrangement accompanying chromosome translocation (2, 3). The mechanisms responsible for such translocations have not, in most cases, been elucidated. In some lymphocyte malignancies, heptamer/nonamer signal sequences identical to those found in Ig and TCR genes occur on the oncogene-bearing chromosome. These signals direct recombination events which, while taking place at aberrant sites within the oncogene locus, are otherwise normal rearrangements mediated by the lymphocyte VDJ recombinase. Thus, the two breakpoints representing reciprocal products of such translocations have the form of a signal joint, in which the participating heptamer/nonamer signals from each chromosome are precisely apposed, and a coding joint, in which several extra nucleotides (N) connect the recombined DNA from the two parental chromosomes (4, 5). As often occurs in physiological rearrangements leading to Ig gene coding joints, short deletions of each parental DNA may be found at the site of the translocation/rearrangement (6).

The BCL2 oncogene was discovered by a detailed molecular analysis of the t(14;18) translocation which occurs in 90% of human follicular lymphoma (7). Although this oncogene spans more than 200 kb, nearly 70% of the translocations are located within a 150-bp segment at the untranslated end of exon 3, designated the major breakpoint region, or mbr (8–10). The translocation breakpoints consist of the 5′ end

1 Abbreviations used in this paper: AFP, α-fetoprotein; ALL, acute lymphocytic leukemia; DPDL, diffuse poorly differentiated lymphocytic lymphomas; IgRSS, Ig gene recombination signal sequences; mbr, major breakpoint region; MDSU, Molecular Diagnostics Laboratory at Stanford University; MGH, Massachusetts General Hospital; NEMC, New England Medical Center.
of mbr (5' mbr) appended to a Jg segment through a coding joint (see Fig. 1). The canonical structure and remarkable specificity of site selection for BCL2 translocation have been attributed to the presence of two sets of heptamer/nonamer sequences, loosely related to the Ig gene recombination signal sequences (IgRSS), located at nearby positions in the mbr (see Fig. 1). Since these signals are oriented in opposite directions, a pseudo-D element is created (8). It has been proposed that this pseudo-D element undergoes rare, interchromosomal recombination with Jg segments on chromosome 14 through the action of the VDJ recombinase. However, DNA sequence analysis of the initial group of BCL2 breakpoints (8-11) revealed that these did not cluster within the coding sequence of the pseudo-D element, as would be predicted by the proposed model (Fig. 1). Furthermore, the reciprocal translocations never contained the expected Jg-3'mbr signal joint. Instead, the 3' portion of the mbr was appended through a second coding joint to a Dn segment 40-kb upstream of Jg (Fig. 1; see also below). This arrangement and the deletion of the intervening Dn-Jg interval presaged a more complex, multistage process (6, 12).

Although the siting of breakpoints on chromosome 14 (Jg/Dn coding) strongly implicated the VDJ recombinase in the creation of BCL2 translocations, the unsatisfactory relationship of breakpoint locations on chromosome 18 to putative IgRSS left open the possibility that other determinants within the mbr were responsible for the targeting of translocations. Clearly, functional considerations, such as the deletion of transcriptional regulatory or mRNA processing signals just distal to the mbr, might have played a role. It was also possible, however, that the mbr possessed recombination signals distinct from IgRSS that were critical for rearrangement. We chose to investigate this latter hypothesis after our observation that the translocations of several different oncogenes were frequently associated with an 8-bp consensus sequence, CC[A/T]CC[A/T]GC, that resembled the procaryotic activator of recombination, χ (CCACCCAGC) (13). The sequence was originally derived from our examination of human minisatellite repeat units in an effort to refine a potential consensus signal for recombination (14). After establishing the consensus, we noted its appearance within, or immediately adjacent to, translocation breakpoints for the oncogenes, MYC and BCL2, and the candidate oncogenes, BCL1, TCL1, and TCL2 (14, and references therein). An important feature of this association was that each translocation bearing a χ-like signal was thought to be mediated by the VDJ recombinase. For BCL2, three published translocation breakpoints (8, 9, 11) occurred at the site of tandemly arrayed 8/8 and 7/7 matches of the consensus within the mbr (Fig. 1 C). Given the predominance of the mbr in BCL2 translocations and the apparent significance of the χ site within the mbr, we decided to analyze rearrangements involving this oncogene region in greater detail.

Materials and Methods

Source and Nature of DNA Samples. Lymphoma samples were obtained from the New England Medical Center Lymphocyte Typing Laboratory (NEMC, Boston, MA), the Molecular Diagnostics Laboratory at Stanford University (MDSU, Stanford, CA), and the laboratory of Dr. Alan Aisenberg at Massachusetts General Hospital (MGH, Boston, MA). BCL2 mbr-Jg translocation fragments were amplified by PCR from lymphomas with varying degrees of nodularity. Of NEMC follicular lymphomas characterized as nodular, small cleaved-cell type, 4/4 contained a BCL2 mbr-Jg translocation. Of NEMC lymphomas characterized as predominantly nodular or follicular lymphoma, 14/32 were BCL2 translocation-positive by PCR. An additional two DNAs from this group possessed a BCL2 mbr-Jg rearrangement by Southern analysis (15, and A. Aisenberg, personal communication), 2/4 NEMC mixed nodular/diffuse lymphomas contained a translocation, as well as 2/5 cases of diffuse lymphoma with areas of nodularity. 2/20 NEMC diffuse histiocytic lymphomas and 1/4 diffuse, poorly differentiated, lymphocytic lymphomas (DPDLL) contained translocations. 1/9 NEMC samples classified as atypical reactive hyperplasia was positive for a translocation. BCL2 translocation fragments from 13/13 MDSU lymphomas containing translocation breakpoints known to map to the BCL2 mbr were successfully amplified by PCR. 5/11 MGH lymphomas previously determined to contain BCL2-Jg translocation by Southern blotting (A. Aisenberg, personal communication) were successfully amplified by PCR.

The translocation-positive samples represented 2/4 nodular, poorly differentiated lymphocytic lymphomas, 1/1 small and large cell nodular/diffuse lymphoma, 1/1 DPDLL, 0/3 lymphocytic/histiocytic, nodular lymphomas, and 0/1 large cell, nodular lymphoma.

DNA Preparation. Lymphoma tissue DNA was prepared by digestion of snap-frozen, finely crushed lymphoma tissue with 100 μg/ml proteinase K (Sigma Chemical Co., St. Louis, MO) in 10 mM Tris (pH 8.0), 25 mM EDTA, 0.5% SDS for 1 h at 65°C. The solution was then extracted twice with saturated phenol-chloroform and precipitated with 0.3 M sodium acetate, (pH 5.2), and 2 vol of 95% ethanol. After washes with 70% ethanol, DNA was resuspended in 10 mM Tris and 1 mM EDTA (pH 8.0).

DNA was also isolated from frozen lymphoma tissue embedded and stored in OCT compound at −70°C. Five 10-μ tissue sections were washed twice with 1 ml of PBS to solubilize the OCT, and then digested overnight at 65°C with proteinase K as described above. Insoluble cellular debris was removed by centrifugation, and the DNA solution was extracted and precipitated as described above.

PCR Amplification. Primers used to amplify BCL2 mbr-Jg translocations and Dn-3'mbr reciprocal breakpoints are listed in Table 1. The Jg consensus primer was described by Crescenzi et al. (16). Base positions for Dn primers are from Ichihara et al. (17), and for the mbr primers, from Cleary et al. (18). For PCR amplifications, 1 μg of genomic DNA was added to a 100-μl reaction mixture of 1 μM primers, 1.25 μM dNTPs, 2 U Taq polymerase and Taq polymerase buffer (both of International Biotechnologies, Inc., New Haven, CT) (10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM MgCl2, 0.01% gelatin, 0.01% Tween 20, and 0.01% NP-40). The reaction mixture was overlaid with 100 μl of mineral oil, and amplification was performed for 30 cycles (1 min denaturing at 94°C, 1 min annealing at 54°C, and 2 min extension at 72°C) in a DNA Thermal Cycler (Perkin-Elmer Cetus Corp., Norwalk, CT). Amplified products were analyzed by electrophoresis through 8% acrylamide-TBE (90 mM Tris-borate, 2 mM EDTA, pH 8.0) gels.

Breakpoint Cloning and DNA Sequencing. For directional cloning, PCR fragments were amplified with the appropriate oligomers to create Sall 5' ends and BamHI 3' ends. After Sall-BamHI double digestion, fragments were gel purified and ligated into Sall-BamHI cut pBS M13 + (Stratagene Inc., La Jolla, CA). Clones containing Sall-BamHI inserts were then subjected to double-strand sequencing.
Figure 1. BCL2 translocation. (A) (Top) Normal DJ intrachromosomal assembly (dark double arrow) between one of ~30 human D genes and one of six J genes. All known D genes, except for the J-proximal DQ52, are found in four 9-kb repeats located over 20 kb upstream of J. (32, 33). The VDJ recombinase complex is directed by the heptamer/spacer/nonamer elements flanking D and J genes (open boxes with designated 12- or 23-bp spacers) to combine these genes in the first step of Ig H gene assembly. After double-stranded cleavage adjacent to each heptamer, the heptamer DNA ends are ligated to form a signal joint (top right), deleting up to 60 kb of intervening DNA. The D and J ends are modified by exonucleolytic nibbling and N nucleotide addition, forming the coding joint (top left). (Bottom) One model for interchromosomal recombination between the BCL2 mbr and J genes (light double arrow). Both genes are transcribed left to right toward their respective centromeres. The mbr functions as a pseudo D gene, with signal sequences represented by stippled boxes labeled "7" and "9", and is cross-ligated by the VDJ recombinase to J genes, just as in normal DJ assembly. Two products are predicted from this model: a characteristically modified 5' mbr:J coding joint (bottom left), and a J:3' mbr signal joint (bottom right). (B) Rather than the proposed signal joint (J:3' mbr), the experimentally determined product is a D:3' mbr coding joint. (C) The initial group of mbr breakpoints (cited in introductory section) are shown above a portion of the mbr germline sequence corresponding to bp 41-182 in Fig. 2. (O) Last mbr base of each breakpoint before encountering N nucleotides. (x) Two sets of hypothetical IGLSS (8). Only two of the seven breakpoints are located in the coding region between the two heptamers. The tandem x-like sequence is boxed.

using a T7 DNA polymerase protocol (Pharmacia, Inc., Piscataway, NJ). Direct sequencing of PCR products was performed as a modification of a previously described technique (19). Briefly, gel-purified PCR fragments were asymmetrically amplified using a 100-fold molar excess of one primer, followed by centrifugation over Centricon columns (Amicon, Beverly, MA). DNA was precipitated in 2 M ammonium acetate and 2 vol of isopropanol. Single-strand sequencing was performed using the limiting primer, the entire asymmetric PCR reaction product, and the Pharmacia T7 sequencing kit. Computer analysis of DNA sequences from GenBank was performed with the Pustell Matrix software (International Biotechnologies, Inc.).

Results

Clustering of BCL2 Translocation within the mbr. If the x-like motif functioned in a manner analogous to x in procaroyotes, we expected to find a high frequency of translocations confined to one side of the repeat, and a gradual decrease in these events...
proceeding unidirectionally away from the putative recombination signal. Therefore, we performed sequence analysis as described in Materials and Methods on 43 new BCL2 translocations PCR amplified from lymphoma cases at NEMC, MGH, and MDSU. As expected, most translocation fragments consisted of three distinct regions: a 5' portion of the BCL2 mbr, followed by a variable stretch of N nucleotides, and ending with a Jn gene coding segment (see below for exceptions). 23 of these translocations used the most distal gene segment, Jn6. The remaining translocations contained one Jn3, ten Jn4, one indeterminate segment (either Jn4 or Jn5), and eight Jn5. We verified the distinct identity of each oligomers sampled at least 900 bp of DNA in this region by other investigators, are summarized in Fig. 2.

mbr translocation sites were nonrandomly distributed. 63/64 breakpoints occurred within a 155-bp segment, an outcome which we anticipated from previous studies. However, within this 155-bp segment, we detected a striking specificity. A cluster of 15 translocations was immediately adjacent to the X-like doublet, confirming this site as a translocation hotspot (cluster 1). However, unlike recombination events mediated by χ, we did not find a gradient of decreasing frequency of translocation distal to the tandem repeat at cluster 1. Instead, we found two other hotspots, clusters 2 and 3, evenly spaced 50 and 100 bp downstream of the X-like motif. An abrupt drop-off in translocation events then occurred. These two clusters contained 23 and 15 translocations, respectively. Although the boundaries of each cluster were somewhat arbitrary, 83% of translocations (53/64) could be accounted for in three groupings 16-, 18-, and 14-bp wide, respectively—a result highly unlikely to have occurred by chance alone (p < 10^-9 by Poisson statistics). Given the fact that our PCR oligomers sampled at least 900 bp of DNA in this region of BCL2 (the distance between our first and last breakpoints), this result represented a much higher degree of site preference than previously suspected.

The single translocation at bp 949 was important for two reasons. First, it demonstrated that breakpoints occurring over a 900-bp region, had they existed, would have been detected by our methods. Second, this translocation showed that follicular lymphoma could still emerge with the BCL2 gene retaining 1 kb of sequence downstream of the mbr.

Sequence Homology Adjacent to Translocation Hotspots. We reexamined DNA sequence within and flanking the three clusters and confirmed that no arrangement of heptamer/nonamer-like signals could account for the pattern of translocation sites. We did find, however, homologous segments within, or just upstream, of each cluster. A 15-bp cluster 3 sequence could be aligned with a sequence just 5' of cluster 1 with over 70% identity. If purine/purine or pyrimidine/pyrimidine substitution were allowed, this homology exceeded 85% (Fig. 3 A). Similarly, 15 bp 5' of cluster 2 contained a 10/15 match with the cluster 1 sequences and, again, 85% homology allowing purine/purine or pyrimidine/pyrimidine substitution (Fig. 3 A). The 15-bp segment at cluster 1, CCAGAGCCCTCTCTGC, contained an 8/8 match for the χ-like sequence (underlined). Immediately upstream was an imperfect repeat of the cluster 1 sequence, CAGACCCACC (Fig. 3 A, CLUSTER 1 5'). We also noted that the single translocation downstream of the the mbr translocation sites at bp 949 was associated with a 5'-flanking sequence similar to that of cluster 1. This sequence contained six bases related to χ (CCTCCT), as well as 5' purine/purine homology (Fig. 3 A). Each cluster was also associated with χ-like sequences of varying fidelity. In addition to the tandem χ-like repeat of cluster 1, cluster 2 was flanked by several χ-like sequences; and cluster 3 contained a 5/8 match, the core of the consensus, CCTCC (Fig. 2).

mbr Cluster Usage in a Variant BCL2/BCL2/J Segment Recombination. The existence of the breakpoint cluster phenomenon suggested that particular regions within the mbr possessed an inherent capacity for recombination. This hypothesis was reinforced by the detection of rare, but revealing, translocation events occurring at exactly the same cluster sites. In the first of these, mbr clusters recombined with each other, as well as with a Jn gene segment (see Fig. 4), resulting in two coding joints at a single translocation breakpoint. At each joint, BCL2:BCL2 and BCL2:Jn, the same mbr cluster sites were employed as for the simple translocations described above. The first mbr was appended through its cluster 2 re-

Table 1. Amplification Primers

| Primer | Sequences 5' to 3' | Use |
|--------|--------------------|-----|
| RTW 2 | CTTTAGAGAGTTGCTTTACGTG | 5' BCL2 mbr |
| RTW 3 | TCCATATTTCATACCTTGAGCA | 3' BCL2 mbr |
| LR 7 | CCCGTCGACCTTTAGAGTTGCTTT | 5' BCL2 mbr SalI site |
| RTW 100 | AAAAGATCCATATTCATCACTTGGACA | 3' BCL2 mbr BamHI site |
| RTW 101 | AAAAGATCCACCTGAGGAGCGGTGACC | 3' Jh consensus BamHI site |
| RTW 112 | AAGTCGACGCAGCGGACACTATCCACATAA | 5' Dαp consensus SalI site |
| RTW 113 | AAGTTCGACGAGTCCTCTCAGGACACCCTGGA | 5' Dαp consensus SalI site |
Figure 2. The mbr breakpoint clusters. Translocation breakpoints are shown mapped within BCL2 germline sequence spanning the mbr. (O) Breakpoints published by others (6, 8–11, 16, 20, 34). (X) Breakpoints sequenced by us. The dark bars below the top line of sequence indicate the cluster boundaries we have selected, with cluster 1 first (5'), and cluster 3 last (3'). (Δ) Position of the 5'mbr oligomer, LR7. The "X" over bp 949 represents the most 3' breakpoint we detected. The thin bar above the top line of sequence denotes the tandem repeat of the χ-like octamers (CCTCGCCGCTCCCTCC; underlined) consensus matches; (dashed lines) 6/8 matches (CTTCCTGA, GCTCCACC, CCACGAAG); (>) 7/8 deleted χ-like sequence in opposite orientation (GCAG TGGT); (dotted line) 5/8 match in cluster 3 (CCTCCCCG).
region to the cluster 1 region of the second mbr fragment. The latter was then attached to J₄ at its cluster 2 site. The presence of N nucleotides at each junction excluded the possibility of PCR artifact or a traditional gene duplication event through homologous recombination. In addition, this translocation fragment was sequenced from two independent clones. The germline BCL2 mbr regions of this individual, as analyzed by denaturing gradient gel electrophoresis, were entirely normal (data not shown). Thus, sites within the mbr that we identified as translocation hotspots were also involved in the somatic recombination of the mbr with itself. We could not exclude the possibility that the mbr-to-mbr recombination may have occurred by sister chromatid exchange, since we were unable to detect with suitable primers the presence of the predicted reciprocal product for exchange between homologues (Fig. 4 C). The absence of this reciprocal may also have resulted from degradation of an extra-chromosomal by-product.

mbr Cluster Site Usage in Variant BCL2/D Segment Recombinations. From lymphoma DNA 1909N, a larger-than-usual translocation fragment of ~400 bp was PCR amplified and sequenced. Analysis of this translocation revealed the presence of a Dₓ gene segment interposed between 5' mbr and J₆, as illustrated in Fig. 5. Once again, an mbr cluster site was involved in the translocation event. The upstream segment of mbr cluster site 2 was appended to the coding region of a Dₓ family member, Dₓ₁. As a result of this rearrangement, 5' Dₓ₁ heptamer-nonamer signal sequences were deleted, along with 10 bp of Dₓ₁ coding sequence. No N nucleotide addition between mbr and the D element was observed. The 3' joint between Dₓ₁ and J₆ was unusual because the conventional 3' D gene heptamer-nonamer signal sequences were retained. Rearrangement appeared to have occurred between the J₆ RSS and the consensus heptamer-nonamer signal sequences located 102 bp downstream from Dₓ₁. Characteristically, 8 bp of J₆ germline sequence had been deleted, and N nucleotides had been inserted between D and J segments. Another example of this type of rare rearrangement, containing Dₓ₁ inserted between mbr cluster 1 and J₆, has been previously reported (20 and Fig. 5 C). To obtain more information about this complex rearrangement, we attempted to amplify the reciprocal breakpoint. Although we were initially unsuccessful with a 5' Dₓ consensus primer, we eventually succeeded in amplifying the 1909N reciprocal product using the Dₓ family consensus primer, 113, and the 3' mbr primer, 3. Sequence analysis revealed that this reciprocal product had Dₓ� appended to mbr cluster 2 in a characteristic coding joint. Only two base pairs of mbr germline sequence had been deleted, while over 10 kb of the D region between Dₓ₁ and Dₓ₄ had been deleted in the translocation process (Fig. 5 B).

These two rare translocations emphasized the bidirectional nature of the signals associated with cluster sites. The usual mbr-to-J₆ join was replaced by an mbr recombination upstream of a Dₓ₁ gene element, mimicking Vₓ-Dₓ₁ gene rearrangement. The capacity of the mbr to recombine upstream of both D and J elements, as well as downstream of D elements (Dₓ₁:3'mbr), contrasted sharply with RSS-mediated recombination.

Nonrandom Usage of Dₓ Family Members in Reciprocal mbr Translocations. Members of the Dₓ family gene family have been observed in multiple reciprocal translocations of both t(14;18) (18q⁻) and t(11;14) (6, 12, 20). To confirm the potentially preferential involvement of Dₓ genes, we used a Dₓ family

---

**Figure 3.** mbr cluster-associated homology and mbr-to-D region homology. (A) (vertical lines) Nucleotide matches between the cluster 1 5' sequence and other 5' homology regions. (y) Pyrimidine/pyrimidine substitution. (x) Purine/purine substitution. The 8/8 match for the x-like consensus in the cluster 1 sequence is underlined with the solid bar; the partial repeat of the cluster 1 sequence is underlined with dashes. The number of exact matches is given in parentheses. (B) The same conventions indicate homology between the repeat sequences associated with Dₓ family members and the cluster 1 5' sequence. (C) The x-like sequence is underlined; and the five D region 8/8 matches, detected by the mbr-to-D region matrix analysis, are listed. Numbering of D region bases follows Ichihara et al. (17).
Figure 4. mbrA:mbrB recombination in an mbr:J_A translocation event. (A) Both the DNA sequence (top) and a schematic diagram of the rearrangement involving two distinct mbr regions (bottom) are depicted. The locations of mbrA and mbrB, on homologous chromosomes or sister chromatids, could not be determined. (Solid line) mbr sequences; (open boxes) breakpoint cluster sites of mbrA. (Solid boxes) mbrB cluster sites. N nucleotides are in lower case. (Thick striped line) D_A coding sequence. (Striped bar) J_A coding sequence. EMBL Accession No. X63226. (B) The reciprocal partner of the (14;18) translocation is shown. (Bottom) Schematic diagram of the (top) DNA sequence. (Open boxes or brackets) 5' D RSS. (Striped bar) D coding. The D_J_A gene segment forms a typical coding joint with the cluster 2 site of mbrB, exhibiting excision of 3' RSS, deletion of germline sequence (12 bp of D, and 2 bp of mbr), and N nucleotide addition. EMBL Accession No. X63230. (C) The expected configuration of the mbr:mbr reciprocal product is schematically depicted. This product was not detected by PCR, indicating that it may have occurred between sister chromatids.
Figure 5. mbr:Dx1;Jx6 translocation. (A) The DNA sequence and schematic depiction of the 5'mbr:Dx1;Jx6 variant translocation is given. (Solid boxes) mbr cluster sites. (Striped bar) D coding. (Brackets or labeled boxes) D and J heptamer/nonamer sequences. (Stippled bar) J coding. EMBL Accession No. X63227. (B) The reciprocal partner of the complex rearrangement, which was amplified using the Dn consensus primer and the 3'mbr primer, is shown. EMBL Accession No. X63232. (C) A schematic representation of a similar 5'mbr:Dx1;Jx6 translocation (20) is shown.
consensus primer to amplify 3' mbr reciprocal translocation fragments from 22 BCL2 translocation-positive DNAs. 9 of the 22 DNAs contained a single, specific band after amplification. Six of the nine reciprocals were cloned and sequenced, and all contained Dx~ family members (Fig. 6). One additional reciprocal was amplified, cloned, and sequenced using a Dx family primer. From our data and the published results of others, 14 of 26 informative reciprocal translocations used a Dx family member. Because of the high degree of specificity of Dx~:mbr amplification, the three unsequenced reciprocals described above were included in this summary. The result we obtained, therefore, represented highly preferential usage of the Dx~ family, because there are six known human D gene families (17) randomly used in normal DJ rearrangement (21). Dx~l and Dx~,l were the most frequently observed, and all contained Dx~ family members (Fig. 6).

We inspected the Dx~:3' mbr breakpoints for the presence of heptamer/nonamer homology in mbr sequences flanking the Dx~ coding element. As expected, none was detected. With complete sequence data from both reciprocal partners of our seven translocations, we could determine the nature and extent of mbr deletion, ranging from 2 to 10 bp, in the translocation event. Again, no heptamer/nonamer homology was evident in the deleted mbr bases. The lack of mbr IgRSS homology adjacent to or deleted from Dx~:3' mbr joints argued strongly against the transient formation of Jc~:3' mbr signal joints predicted by the original model (Fig. 1). Even if signal joints were not evident because of secondary rearrangement events between upstream D genes and the remaining Jc RSS contained in the predicted reciprocal (Fig. 1A), the mbr contribution to the signal joint should still have been observed either flanking the coding element or within the deleted mbr bases.

5' Region of mbr Cluster 1 Is Homologous to a Dx~ Family Repeat Sequence. Because translocation events frequently involved mbr cluster sites and Dx~ family members, we performed a computer-assisted homology search of DNA sequences from the mbr and 15 kb of the human D region spanning D~a4 to D~a5; this included D~a9 and D~a9' (17). We restricted the analysis window to 13 bp, requiring a consecutive 6-bp perfect match and 80% overall homology on either DNA strand. The results of this analysis are graphically represented in Fig. 7A. Selected sequence fragments are shown in Fig. 3, B and C. There was extensive homology between the cluster 1 5' flanking sequence and many short segments (33) interspersed throughout the D region. These matches were of two related types: (a) homology centering on the 8/8 χ-like sequence just upstream of cluster 1 (bp 53-60); and (b) homology extending further 5' of cluster 1 to include the CCAAGGC bases and the adjacent repeat, CAGACC-CACCC, described above. Of the 22 segments detected on the plus (+) strand of the D region, five contained 8/8 matches (Fig. 3C) for the mbr χ-like sequence, and four more were 7/8 matches. This was significant overrepresentation of the motif, since the consensus octamer should have occurred by chance alone only once in 16 kb. In the second homology category, there were multiple matches with a repeat segment highly related to the 5' cluster 1 sequence (Fig. 3B). The sequence motif (CCACAGCCTCCCGA and variations thereof) was found interspersed four times in the 400 bp spanning D~a9 and D~a9', and twice in the D~a4 region (Figs. 3B and 7A). These genes were, as described above, the most frequently observed D segments in mbr reciprocal translocations. Also detected by this analysis was a D region minisatellite (labeled VTR; Fig. 7A) located 900 bp downstream of D~a4. The core element of this tandem repeat was homologous to the χ-like consensus of minisatellites, CC[A/T][CC[A/T]GC, the derivation of which originated our work (14). This result provided unexpected validation of our homology search scheme. An additional 11 D region sequences on the minus (−) DNA strand displayed a similar distribution of homology. Included were two 7/8 χ-like matches and seven homologous matches with the cluster 1 5' flanking sequence. No heptamer or nonamer matches were found between the mbr and the D region on either strand.

We then examined the 3.2-kb human Jc region (22, 23). Seven matches were detected overall, including two 8/8 and one 7/8 χ sequences. One segment which contained homology to the D~a9 repeat unit also appeared (Fig. 7B). The two

Figure 6. DNA sequence of D:3' mbr reciprocal translocations. The DNA sequences of seven D:3' mbr reciprocal breakpoints (18q-) are shown. D sequence, including heptamer/nonamer (boxed) and coding segment, appears on the left side of N nucleotides, which are given in lower case. 3' mbr DNA sequence appears to the right. EMBL Accession Nos. X63225, X63228, X63229, X63230, X63231, X63232, and X63233.
8/8 matches, located between Jα1 and Jα2 coding sequences, were oriented on the (-) DNA strand relative to the mbr and to the 8/8 matches in D region. Finally, we performed an identical analysis on 22 kb of DNA sequence from the human α-fetoprotein gene (AFP) (24). In contrast to the D region pattern, mbr-AFP homology appeared infrequently and was randomly distributed (Fig. 7 C). No χ-like matches were detected in the entire 22 kb of the AFP locus, and only 2/20 segments displayed any overlap with cluster 1-associated sequences (Fig. 7 C).

Discussion

The correlation of elevated BCL2 expression with t(14;18) translocation (25, 26) implies that altered regulation of transcription is the important, selectable outcome of translocation. However, purely functional considerations cannot account for the precise siting of breakpoints that we have observed. The most likely functional consequence of mbr clustering, displacement of message stability control sequences from the 3' untranslated region of BCL2 mRNA, does not
Occur. BCL2 mRNA half-life before and after translocation is the same (27). No other known functional alteration of mRNA would result in such closely grouped events. Furthermore, the existence of translocations downstream of the mbr, within the minor cluster region 30 kb away and, as we report here, at a site 800 bp away, argues against precise truncation of BCL2 mRNA or formation of a BCL2:IgH fusion transcript as a selectable determinant. Therefore, the tight clustering of translocations within the mbr implicates a sequence-specific recombination mechanism. As discussed in the introductory paragraph, classical VDJ recombination signals are unlikely candidates to mediate this process. These considerations lead us to the conclusion that recombination signal sequences distinct from classical IgRSS are critical for mbr:IgH rearrangement. The study of BCL2 translocations reported here strongly implicates our candidate motif.

Other Important \( \chi \) Sightings. In addition to our BCL2 observations, we identified \( \chi \) sequences in two other contexts that strongly supported a role for this consensus in recombination. The first of these was described above: the computer-assisted sequence comparison between the mbr and 15 kb of D region, demonstrating striking homology between the cluster 1 5' region (Fig. 3) and a D sequence repeat motif (consensus: CCACAGCCCTCCCCA) interspersed throughout the D<sub>x31-D<sub>y31 region. The repeats flanked these D<sub>y31 genes, as well as D<sub>x31, and represented 11 of 15 and 13 of 15 matches for the cluster 1 upstream sequence (CCACAGCCCTCCCCA; Fig. 3B). Particularly suggestive was the demonstration that these repeats were the site of recombination that resulted in the evolutionary duplication of D<sub>x/ D<sub>y (17). The other result of this sequence analysis was that the \( \chi \) sequence, itself, was greatly overrepresented throughout the 15 kb of D region that we examined. Our conclusion from these observations, incorporated in the model discussed below, was that these regions of homology represented the principal sites of interaction between the BCL2 mbr and the IGH locus, targeting translocations either through homologous or site-directed recombination.

The second setting in which we observed \( \chi \) sequences was a t(9;22) translocation previously reported by van der Feltz et al. (28) which occurred in a Ph<sup>+</sup>-positive acute lymphocytic leukemia (ALL). This particular translocation provided some insight into the events initiating \( \chi \)-mediated recombination, without further modification of the recombinant joint by the exonuclease and TdT activities associated with the VDJ recombinase complex. ALLs often have low levels of TdT activity and lack VDJ recombinase activity. As shown in Fig. 8, the t(9;22) translocation occurred through \( \chi \)-like sequences on both parental chromosomes, and contained no deletion of germline sequence in either reciprocal product. The 8/8 \( \chi \)-like sequence on chromosome 22 had undergone a staggered, double-stranded cleavage across the center six bases of the motif. The staggered ends were then filled in and ligated to their respective partners from the chromosome 9 breakpoint. In this concerted event, the 7/8 \( \chi \)-like sequence on chromosome 9 appeared to have undergone either a blunt-ended, double-stranded break between bases 5 and 6 of the \( \chi \)-like motif or, alternatively, a staggered double-stranded break, followed by exonucleolytic cleavage of two to three bases. This finding suggested that the 8/8 \( \chi \)-like sequence can serve as the site of double-stranded, staggered cleavage, perhaps by a site-specific endonuclease. This contrasts with procarotic \( \chi \), which is the site of single-stranded cleavage four to six bases downstream. Taken together, the observations on mbr-D<sub>y homology and cleavage in the t(9;22) translocation suggested to us that site-specific recombination between mbr and D<sub>y was mediated by double-stranded, staggered breaks at \( \chi \) sequences.

**A Model for mbr Translocations.** We propose that mbr-to-D rearrangement, not mbr-to-J<sub>y</sub> rearrangement, is the first step in t(14;18) translocation. The homologous sequences present at high density between both D<sub>x31</sub>-D<sub>y31</sub> and the mbr initiate translocation, and the initial breakpoint represents a \( \chi \) crossover, not a VDJ recombinase-mediated coding joint (Fig. 9A). Since 5' cluster 1 sequences share the highest degree of homology with the D<sub>y</sub> repeat, we hypothesize that this cluster interaction is primary. But, as we observed, recombination can occur through any of the three cluster sites. In our model, recombination is mediated by a specific protein complex bound to DNA at the \( \chi \)-like sequences. This complex is responsible for DNA cleavage, either by single-stranded breaks analogous to \( \chi \), or staggered, double-stranded breaks as suggested by the t(9;22) translocation discussed above. Recombination then proceeds through homologous strand invasion and heteroduplex formation. The heteroduplex configuration may transiently resolve, generating the 14q<sup>+</sup> and 18q<sup>+</sup> reciprocal partners shown in Fig. 9B. These intermediates recruit the VDJ recombinase complex, which proceeds short range to generate coding joints through nearby IgRSS. The rearrangements are mediated both by the proposed \( \chi \) sequence-associated recombinase activity, as well as by the VDJ recombinase. Our proposal does not rule out
Figure 9. A model for mbr translocation. (Open boxes) Heptamer/nonamer signal sequences. The open ovals in the DXP1-DXP1 region and the stippled ovals in the mbr represent the homologous DNA sequences shown in Fig. 3, A and B. The arrowheads above and below the stippled ovals in A indicate potential sites of double-stranded (χ) cleavage. Similar cleavage may occur at the D, χ sequences, but is not necessary for the model. In the example depicted here, site-specific recombination occurs between cluster 2 and the DXP1 3' motif (closed double arrow). After cleavage at cluster 2, strand invasion and heteroduplex formation (hybrid white/stippled ovals in B) occur between cluster 2 and DXP1, mediated by a specific binding protein. The transient heteroduplexes diagrammed in B form 18q- and 14q+ activated recombination intermediates. With the initial binding proteins still in place, the VDJ recombinase is recruited. Rearrangement continues on both transient substrates through the χ sequences (filled arrowhead) and through heptamer/nonamer sequences (open arrowheads). The 18q- intermediate in B undergoes one further rearrangement to generate the reciprocal partner observed experimentally (top line; C). The heteroduplex region in the 14q+ intermediate (cluster 2 in this example) usually continues to rearrange downstream to a J₅ element, forming the 14q+ 5'mbr-J₅ coding joint observed experimentally (middle line; C). In variant translocations, cluster 2 rearrangement stops upstream of a D element. The downstream RSS of that D element then recombine with a J₅ element by traditional VDJ recombinase-mediated rearrangement to generate the rare translocations we have observed (bottom line; C).

Figure 10. χ consensus at a RAG-1-mediated breakpoint in mouse brain. χ consensus matches in DNA sequence from Matsuoka et al. (1) is highlighted by the boxes. (Shaded box) 8/8 match. (Open box) 7/8 match. Four bases of additional homology 5' of the χ boxes (TCAG) are present. The two parental sequences (VK21c; LS) are above and below the recombinant (A1-1). Short (12 bp RSS) and long (23 bp RSS) spacer IgRSS are 30 bp upstream and 10 bp downstream, respectively, of the DNA sequence depicted.
the possibility that proteins specifically interacting with the χ-like sequences are additional subunits of the VDJ recombination complex (see below). The process of continuing rearrangement, analogous to secondary D-to-J rearrangement, deletes intervening D-to-J DNA, and juxtaposes the observed mbr cluster sequences with D and J coding sequence (Fig. 9, B and C). Germline deletion and N nucleotide addition occurs by VDJ recombination-associated enzymatic activities. To account for alternate cluster sitings, we hypothesize that, once the recombination apparatus is activated by interacting with cluster 1, the other two cluster sites, by virtue of their similar upstream homology and χ-like sequences, serve as secondary hotspots for translocation. This is presumably accomplished by progression of the recombination complex down the mbr until the signal sequence fragments at cluster 2 or 3 are encountered. Incomplete mbr-to-J rearrangements (Fig. 9 C) leave intervening D sequence and result in rare mbr:Dx,:J, translocations, such as the two described above.

We further suggest that the normal function of χ-related sequences is promoting the close association of distant regions of DNA. Such activity is required in D-to-J joining. Transcription across the 225 kb intron of BCL2 might also require similar means of approximating DNA segments. In this regard, it is interesting to note that both Dn and Jn have χ-like sequences and the Dn repeat motif, although in opposite orientations. The activity of χ sequences may extend to Vn genes, as well. Tutter and Ribblett (29) have shown that the members of the VnIII gene family contain a χ sequence in the 5' region of framework 1 which they suggested played a role in Vn recombination. This sequence and flanking DNA is an 11/15 match with the mbr cluster 1 5' sequence. It is also one of four VnIII sequences conserved at the nucleotide level from mouse to human (29). Finally, Kenter and Birshstein (30) have noted the overrepresentation of χ sequences in mouse Ig genes.

RAG-1 and χ Recombination Events. Our model predicts that a χ joint is the initial interaction of Dn:mbr. It is natural to speculate that such a recombination intermediate may occur during normal DJ rearrangement, as well. We obtained suggestive, but indirect, evidence in favor of this mechanism by examining recently published DNA sequences that were the result of somatic rearrangements in brain cells of transgenic animals bearing a recombination reporter construct (1). Recombination of the construct in these experiments occurred by inversion and was monitored by PCR amplification and DNA sequencing across recombinant joints. In the lymphocytes of transgenic animals, where RAG-1 and RAG-2 were coexpressed, inversion led to the production of normal signal joints. In brain cells, where only RAG-1 was expressed (31), the IgRSS of the reporter were largely ignored. Instead, recombination took place within χ-consensus sequences in all three instances in which DNA sequence was presented. One of the recombinants reported by Matsuoka et al. (1) is shown in Fig. 10. It demonstrated the predicted structure of a χ joint. These results suggested to us the hypothesis that the RAG-1 product might interact with χ signals to cut and splice DNA. (For brevity of presentation, we are assuming genes such as RAG-1 and RAG-2 encode, rather than induce, the actual components of the recombinase complex.) Since not all brain cells expressing RAG-1 rearranged the substrate, other factors, perhaps including substrate chromosome position and additional gene products, may have influenced the recombination process. In any event, it seems plausible that this interaction could occur during DJ rearrangement, producing intermediates related to those depicted in Fig. 9 B, from which other components of the recombinase, such as RAG-2, generate coding and signal joints.

Since the χ consensus was originally derived from human minisatellite sequences, and since these tandem repeats can, in principle, be generated by the end-to-end ligation of fragments arising from a site-specific cleavage mechanism, another implication of our observations is that RAG-1, or a related gene, may be responsible for the generation of vertebrate minisatellite arrays.

We are grateful to Naomi Rosenberg for comments on the manuscript and to Alan Aisenberg for generously providing samples.

This work was supported by grants from the National Institutes of Health CA-51985 and CA-40725. T. G. Krontiris is the recipient of a Faculty Research Award (FRA310) from the American Cancer Society.

Address correspondence to Dr. T. G. Krontiris, Box 245, NEMCH, 750 Washington Street, Boston, MA 02111. R. A. Rudders is presently at Division of Hematology/Oncology, VA Medical Center, Boston, MA 02130. A. Zelenetz is presently at Lymphoma Service, Memorial-Sloan Kettering Institute, New York, NY 10021.

Received for publication 12 December 1991.

References

1. Matsuoka, M., F. Nagawa, K. Okazaki, I. Kingsbury, K. Yoshida, U. Muller, D.T. Larue, J.A. Winer, and H. Sakano. 1991. Detection of somatic DNA recombination in the transgenic mouse brain. Science (Wash. DC). 254:81.

2. Showe, L.C., and C.M. Croce. 1987. The role of chromosomal translocations in B-cell and T-cell neoplasia. Annu. Rev. Im-
munol. 5:253.

3. Boehm, T., and T.H. Rabbits. 1989. A chromosomal basis of lymphoid malignancy in man. Eur. J. Biochem. 185:1.

4. McGuire, E.A., R.D. Hockett, K.M. Pollock, M.F. Bartholdi, S.J. O'Brien, and S.J. Korsmeyer. 1989. The t(11;14)(p13;q11) in a T-cell acute lymphoblastic leukemia cell line activates multiple transcripts, including Ttg-1, a gene encoding a potential zinc finger protein. Mol. Cell. Biol. 9:2124.

5. Cheng, J.-T., C.Y. Yang, J. Hernandez, J. Embrey, and R. Baer. 1990. The chromosome translocation t(11;14)(p13;q11) associated with T cell acute leukemia. J. Exp. Med. 171:489.

6. Tsujimoto, Y., E. Louie, M.M. Bashir, and C.M. Croce. 1988. The reciprocal partners of both the t(14;18) and the t(11;14) translocations involved in B-cell neoplasms are rearranged by the same mechanism. Oncogene. 2:347.

7. Tsujimoto, Y., J. Cossman, E. Jaffe, and C.M. Croce. 1985b. Involvement of the BCL2 gene in human follicular lymphoma. Science (Wash. DC). 228:1440.

8. Tsujimoto, Y., J. Gorham, J. Cossman, E. Jaffe, and C.M. Croce. 1985a. The t(14;18) chromosomal translocations involved in B-cell neoplasms result from mistakes in VDJ joining. Science (Wash. DC). 229:1390.

9. Cleary, M.L., and J. Sklar. 1985. Nucleotide sequence of a t(14;18) chromosomal breakpoint in follicular lymphoma and demonstration of a breakpoint-cluster region near a transcriptionally active locus on chromosome 18. Proc. Natl. Acad. Sci. USA. 82:7439.

10. Bakhshi, A., J.P. Jensen, P. Goldman, J.J. Wright, O.W. McBride, A.L. Epstein, and S.J. Korsmeyer. 1985. Cloning the chromosomal breakpoint of t(14;18) human lymphomas: clustering around J in chromosome 14 and near a transcription unit on 18. Cell. 41:899.

11. Gauerwky, C.E., F.G. Haluska, Y. Tsujimoto, P.C. Nowell, and C.M. Croce. 1988. Evolution of B-cell malignancy: pre-B-cell leukemia resulting from MYC activation in a B-cell neoplasm with a rearranged BCL2 gene. Proc. Natl. Acad. Sci. USA. 85:8548.

12. Bakhshi, A., J.J. Wright, W. Graninger, M. Seto, J. Owens, J. Cossman, J.P. Jensen, P. Goldman, and S.J. Korsmeyer. 1987. Mechanism of the t(14;18) chromosomal translocation: structural analysis of both derivative 14 and 18 reciprocal partners. Proc. Natl. Acad. Sci. USA. 84:2396.

13. Smith, G.R. 1983. Chi hotspots of generalized recombination. Cell. 34:709.

14. Krowczynska, A.M., R.A. Rudders, and T.G. Krantirios. 1990. The human minisatellite consensus at breakpoints of oncogene translocations. Nucleic Acids Res. 18:1121.

15. Aisenberg, A.C., B.M. Wilkes, and J.O. Jacobson. 1988. The BCL2 gene is rearranged in many diffuse B-cell lymphomas. Blood. 71:969.

16. Crescenzi, M., M. Seto, G.P. Herzig, P.D. Weiss, R.C. Griffith, and S.J. Korsmeyer. 1988. Thermostable DNA polymerase chain amplification of t(14;18) chromosome breakpoints and detection of minimal residual disease. Proc. Natl. Acad. Sci. USA. 85:4869.

17. Ichihara, Y., H. Matsuoka, and Y. Kurosawa. 1988. Organization of the human immunoglobulin heavy chain diversity gene loci. EMBO (Eur. Mol. Biol. Organ.) J. 7:4141.

18. Cleary, M.L., S.D. Smith, and J. Sklar. 1986. Cloning and structural analysis of cDNAs for BCL2 and a hybrid BCL2/immunoglobulin transcript resulting from the t(14;18) translocation. Cell. 47:19.

19. Gyhlensten, U. 1989. Direct sequencing of in vitro amplified DNA. In PCR Technology. H.A. Erlich, editor. Stockton Press, New York. 45-60.

20. Cotter, F., C. Price, E. Zucca, and B.D. Young. 1990. Direct sequence analysis of the 14q+ and 18q- chromosome junctions in follicular lymphoma. Blood. 76:131.

21. Yamada, M., R. Wasserman, B.A. Reichard, S. Shane, A.J. Caton, and G. Rovera. 1991. Preferential utilization of specific immunoglobulin heavy chain diversity and joining segments in adult human peripheral blood B lymphocytes. J. Exp. Med. 173:395.

22. Ravetch, J.V., U. Siebenlist, S. Korsmeyer, T. Waldman, and P. Leder. 1981. Structure of the human immunoglobulin μ locus: characterization of embryonic and rearranged J and D genes. Cell. 27:583.

23. Flanagan, J.G., and T.H. Rabbits. 1982. The sequence of a human epsilon heavy chain constant region gene, and evidence for three non-allelic genes. EMBO (Eur. Mol. Biol. Organ.) J. 1:655.

24. Gibbs, P.E.M., R. Zielinski, C. Boyd, and A. Dugaiczky. 1987. Structure, polymorphism, and novel repeated DNA elements revealed by a complete sequence of the human α-fetoprotein gene. Biochemistry. 26:1332.

25. Chen-Levy, Z., J. Nourse, and M.L. Cleary. 1989. The BCL2 candidate proto-oncogene product is a 24-kilodalton integral-membrane protein highly expressed in lymphoid cell lines and lymphomas carrying the t(14;18) translocation. Mol. Cell. Biol. 9:701.

26. Cleary, M.L., B.Y. Ngan, Z. Chen-Levy, and J. Nourse. 1989. The BCL2 proto-oncogene protein associated with t(14;18) translocations: biochemical properties and expression in non-Hodgkin’s lymphoma. Cancer Cells (Cold Spring Harbor). 7:41.

27. Seto, M., U. Jaeger, R.D. Hockett, W. Graninger, S. Bennett, P. Goldman, and S.J. Korsmeyer. 1988. Alternative promoter and exons, somatic mutation and deregulation of the BCL2-Ig fusion gene in lymphoma. EMBO (Eur. Mol. Biol. Organ.) J. 7:123.

28. van der Feltz, M.J.M., J.W. Gow, M.K.K. Shivi, J.G. Morgan, P.B. Allen, A. Hermans, G. Grosvedl, and L.M. Wiedemart. 1989. Molecular analyses of Philadelphia-positive chronic and acute leukemias and the potential applications to diagnosis and detection of residual disease. Cancer Cells (Cold Spring Harbor). 7:27.

29. Tutter, A., and R. Riblet. 1989. Conservation of an immunoglobulin variable-region gene family indicates a specific, non-coding function. Proc. Natl. Acad. Sci. USA. 86:7460.

30. Kenter, A.L., and B.K. Birshtein. 1981. Chi, a promoter of generalized recombination in lambda phage, is present in immunoglobulin genes. Nature (Lond.). 293:402.

31. Schatz, D.G., M.A. Oettinger, and D. Baltimore. 1989. The V(D)J recombination activating gene (RAG-1). Cell. 59:1035.

32. Siebenlist, U., J.V. Ravetch, S. Korsmeyer, T. Waldman, and P. Leder. 1981. Human immunoglobulin D segments encoded in tandem multigenic families. Nature (Lond.). 294:631.

33. Buluwela, L., D.G. Albertson, P. Sherrington, P.H. Rabbits, N. Spurr, and T.H. Rabbits. 1988. The use of chromosomal translocations to study human immunoglobulin gene organization: mapping Ds segments within 35 kb of the C gene and identification of a new Ds locus. EMBO (Eur. Mol. Biol. Organ.) J. 7:2003.

34. Seto, M., H. Osada, R. Ueda, C. Ito, O. Iwate, A. Oyama, T. Suchi, and T. Takahashi. 1991. BCL2 translation in Japanese B cell lymphoma: novel BCL2 translation with immunoglobulin heavy chain diversity segment. Jpn. J. Cancer Res. 82:65.