BCL2 Oncogene Translocation Is Mediated by a χ-like Consensus

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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 procaryotic activator of recombination. The presence of short deletions and N nucleotide additions at the breakpoints, as well as involvement of J_H and D_H 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 D_{XP} χ 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^1 (8–10). The translocation breakpoints consist of the 5' end

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[1] Abbreviations used in this paper: AFP, α-fetoprotein; ALL, acute lymphocytic leukemia; DPDLL, 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 J$_H$ 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 J$_H$ 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 J$_H$-3'*mbr* signal joint. Instead, the 3' portion of the *mbr* was appended through a second coding joint to a D$_H$ segment 40-kb upstream of J$_H$ (Fig. 1; see also below). This arrangement and the deletion of the intervening D$_H$-J$_H$ interval presaged a more complex, multistage process (6, 12).

Although the siting of breakpoints on chromosome 14 (J$_H$/D$_H$ 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 (CCACCAGC) (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 7/8 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*:J$_H$ 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*:J$_H$ 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*:J$_H$ 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:J$_H$ 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*:J$_H$ translocations and D$_H$:3'*mbr* reciprocal breakpoints are listed in Table 1. The J$_H$ consensus primer was described by Crescenzi et al. (16). Base positions for D$_H$ 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 MgCl$_2$, 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 44°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 SalI 5' ends and BamHI 3' ends. After SalI-BamHI double digestion, fragments were gel purified and ligated into SalI-BamHI cut pBS M13$^+$ (Stratagene Inc., La Jolla, CA). Clones containing SalI-BamHI inserts were then subjected to double-strand sequencing

A

DJ Coding Joint   **DJ Signal Joint**

CHROM 14 —[9]¹²[7]■■N■■■— +

23 [7][7]¹²[9]
[9]
~2-60 kb deletion

D_H gene →INTRACHROMOSOMAL RECOMBINATION← J_H gene

CHROM 14 —[9]¹²[7]■■[7]¹²[9]— << —[9]²³[7]■■

BCL2 coding —— CHROM 18 « ———▦¹²▦▨▦¹²▦————

BCL2 mbr

INTERCHROMOSOMAL TRANSLOCATION

BCL2 coding

5' BCL2 mbr:J_H Coding Joint   J_H:3' BCL2 mbr Signal Joint

« ———▦¹²▦▨N■■■—   + ———[9]²³[7]▦¹²▦———

14q+ BCL2 translocation   **18q- reciprocal (model)**

B

D_XP :3' BCL2 mbr Coding Joint

—[9]¹²[7]■■N———

18q- reciprocal (experimental)

C

CCCTCCCAGAAG[CCTCCTGC][CCTCCTTG]COCOGGGGCTTTCTCATGGCTGTCCTTCAGGGTCTTCCTGAAATGCAGTGGTGCTTACOCTCCACCAAGAAAGCAGGAAACCTGTGGTATGAAGCCAGACCTCCCCGGCGGGCCT

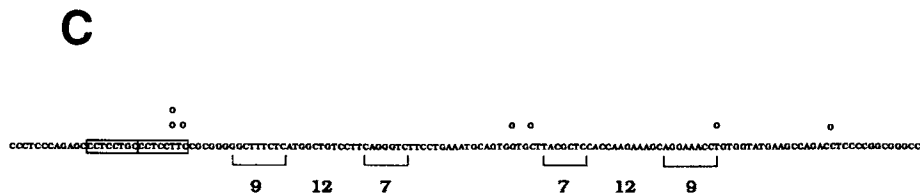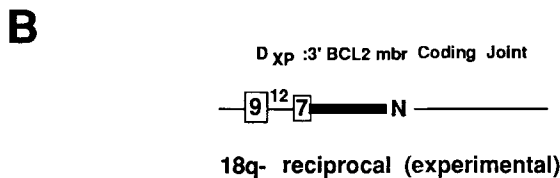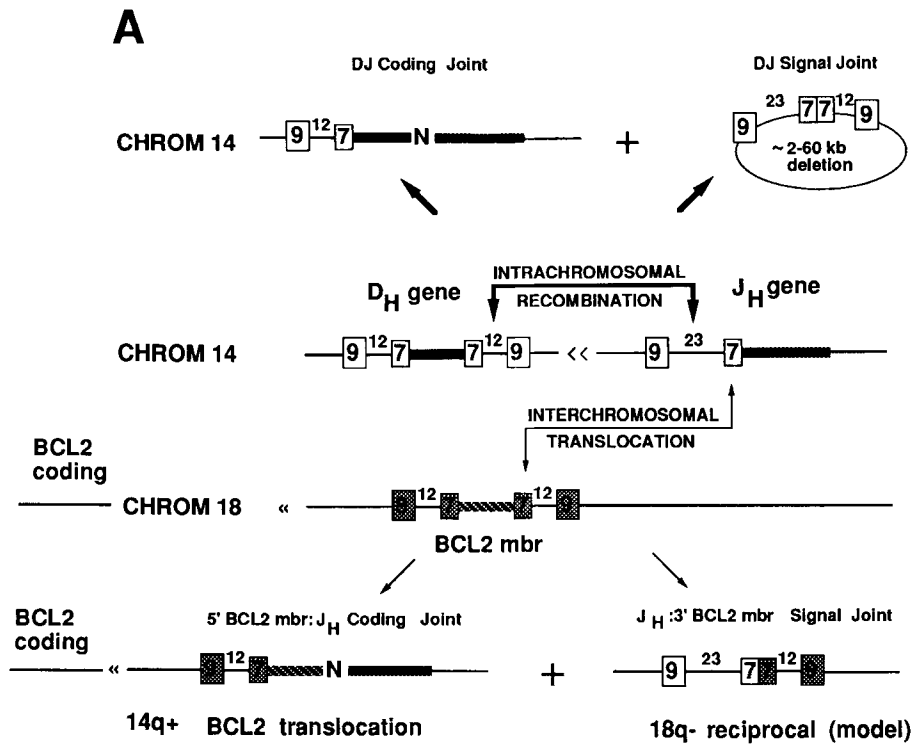9   12   7   7   12   9

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_H 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_H (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_H (*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_H genes, just as in normal DJ assembly. Two products are predicted from this model: a characteristically modified 5'*mbr*:J_H coding joint (*bottom left*), and a J_H: 3'*mbr* signal joint (*bottom right*). (*B*) Rather than the proposed signal joint (J_H:3'*mbr*), the experimentally determined product is a D_H: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. (⌴) Two sets of hypothetical IgRSS (8). Only two of the seven breakpoints are located in the coding region between the two heptamers. The tandem χ-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 Gen-

Bank was performed with the Pushtell Matrix software (International Biotechnologies, Inc.).

Results

Clustering of BCL2 Translocation within the mbr. If the χ-like motif functioned in a manner analogous to χ in procaryotes, we expected to find a high frequency of translocations confined to one side of the repeat, and a gradual decrease in these events

Table 1. *Amplification Primers*

Primer	Sequences 5' to 3'	Use
RTW 2	CTTTAGAGAGTTGCTTTACGTG	5' BCL2 *mbr*
RTW 3	TCCATATTCATCACTTTGACAA	3' BCL2 *mbr*
LR 7	CCCGTCGACCTTTAGAGAGTTGCTTT	5' BCL2 *mbr* SalI site
RTW 100	AAAGGATCCATATTCATCACTTTGACA	3' BCL2 *mbr* BamHI site
RTW 101	AAAGGATCCACCTGAGGAGACGGTGACC	3' J$_H$ consensus BamHI site
RTW 112	AAAGTCGACGCAGCGGACACTATCCACATAA	5' D$_{XP}$ consensus SalI site
RTW 113	AAAGTCGACGAGTCCTCTCCAAGACACCCTGA	5' D$_{LR}$ consensus SalI site

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 J$_H$ gene coding segment (see below for exceptions). 23 of these translocations used the most distal gene segment, J$_H$6. The remaining translocations contained one J$_H$3, ten J$_H$4, one indeterminate segment (either J$_H$4 or J$_H$5), and eight J$_H$5. We verified the distinct identity of each translocation through its *mbr* base position and unique N nucleotide additions. There were two cases, obtained from different institutions and sequenced at different times, that demonstrated identical N nucleotide insertions at identical *mbr* breakpoints. Only one of these was included in this study (1145N). The breakpoints of the translocations we sequenced, together with 20 other breakpoints within the *mbr* published 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 χ-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 χ-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, CCAGAGCCCTCCTGC, contained an 8/8 match for the χ-like sequence (underlined). Immediately upstream was an imperfect repeat of the cluster 1 sequence, CAGACCCACCC (Fig. 3 *A*, CLUSTER 1 5'). We also noted that the single translocation downstream of the the *mbr* cluster 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 J$_H$ gene segment (see Fig. 4), resulting in two coding joints at a single translocation breakpoint. At each joint, BCL2:BCL2 and BCL2:J$_H$, the same *mbr* cluster sites were employed as for the simple translocations described above. The first *mbr* was appended through its cluster 2 re-

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′). (→) 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 (CCTCCTGCCTCCTTC; (*underlined*) consensus matches; (*dashed lines*) 6/8 matches (CTTCCTGA, GCTCCACC, CCACCAAG); (⌃) 7/8 deleted χ-like sequence in opposite orientation (GCAG TGGT); (*dotted line*) 5/8 match in cluster 3 (CCTCCCCG).

A

```
CCAGACCTCCCCGGC   (11/15)   CLUSTER 3
|||||  |y|y|| ||
CCAGAGCCCTCCTGC             CLUSTER 1
y|||u|y|y|||||
TCAGGGTCTTCCTGA   (10/15)   CLUSTER 2
|||| || y|| |
ACAGACCCACCCAGA   (9/15)    CLUSTER 1 5'
|yuuuu ||||||
CTGAGAACCTCCTCG   (7/15)    Breakpoint 949
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B

```
CCACAGCCCTCCTGT   (13/15)   XP4
||| |||||||||y
CCAGAGCCCTCCTGC             CLUSTER 1
||| ||||||||y
CCACAGCCCTCCCTG   (11/15)   XP'1
||| ||||y y|| |
CCACAGCCTCCCCTC   (10/15)   XP1 5'#1
y||||||||| ||||
TCAGAGCCCTGCTGC   (13/15)   XP1 5'#2
||| ||||||||y
CCACAGCCCTCCCCA   (11/15)   XP1 3'
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C

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CCAGAGCCCTCCTGCCCTCCTTC      CLUSTER 1

               D_H Location (bp)

CCCTCCTGCCCTC       1800

CCCTCCTGCtCT        3486

CCCTCCTGCCCT        5780

CCCTCCTGCCCaC       6960

AGgGCCCTCCTGC       11540
```

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. (u) Purine/purine substitution. The 8/8 match for the χ-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 D_xp family members and the cluster 1 5' sequence. (C) The χ-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).

gion to the cluster 1 region of the second *mbr* fragment. The latter was then attached to J_H4 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 ana-
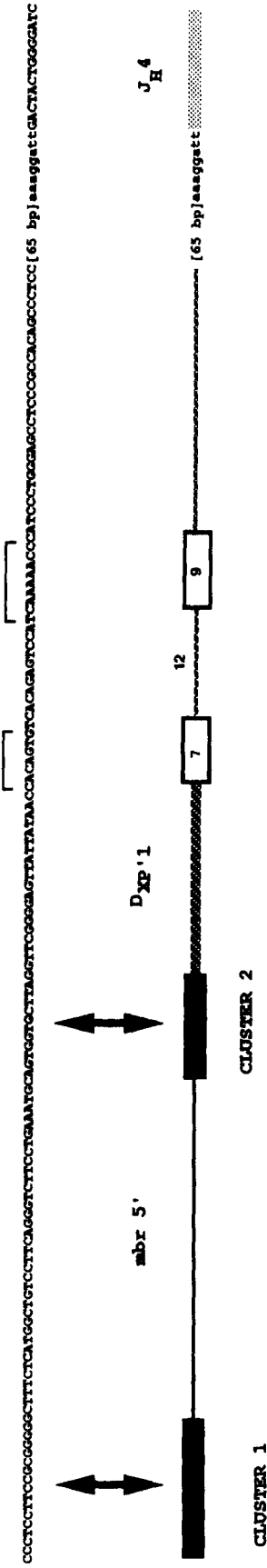
lyzed by denaturing gradient gel electrophoresis, were entirely normal (data not shown). Thus, sites within the *mbr* that we identified as translocation hotspots were also involved in the somatic recombination of the *mbr* with itself. We could not exclude the possibility that the *mbr*-to-*mbr* recombination 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 D_H gene segment interposed between 5'*mbr* and J_H, 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 D_xp family member, D_xp'1. As a result of this rearrangement, 5' D_xp'1 heptamer-nonamer signal sequences were deleted, along with 10 bp of D_xp'1 coding sequence. No N nucleotide addition between *mbr* and the D element was observed. The 3' joint between D_xp'1 and J_H4 was unusual because the conventional 3' D gene heptamer-nonamer signal sequences were retained. Rearrangement appeared to have occurred between the J_H4 RSS and near-consensus heptamer-nonamer signal sequences located 102 bp downstream from D_xp'1. Characteristically, 8 bp of J_H 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 D_xp'1 inserted between *mbr* cluster 1 and J_H6, has been previously reported (20 and Fig. 5 *C*). To obtain more information about this complex rearrangement, we attempted to amplify the reciprocal 18q⁻ breakpoint. Although we were initially unsuccessful with a 5' D_xp consensus amplimer, we eventually succeeded in amplifying the 1909N reciprocal product using the D_LR family consensus primer, 113, and the 3' *mbr* primer, 3. Sequence analysis revealed that this reciprocal product had D_LR4 appended to *mbr* cluster 2 in a characteristic coding joint. Only two bases of *mbr* germline were deleted, while over 10 kb of the D region between D_xp'1 and D_LR4 had been deleted in the translocation process (Fig. 5 *B*).
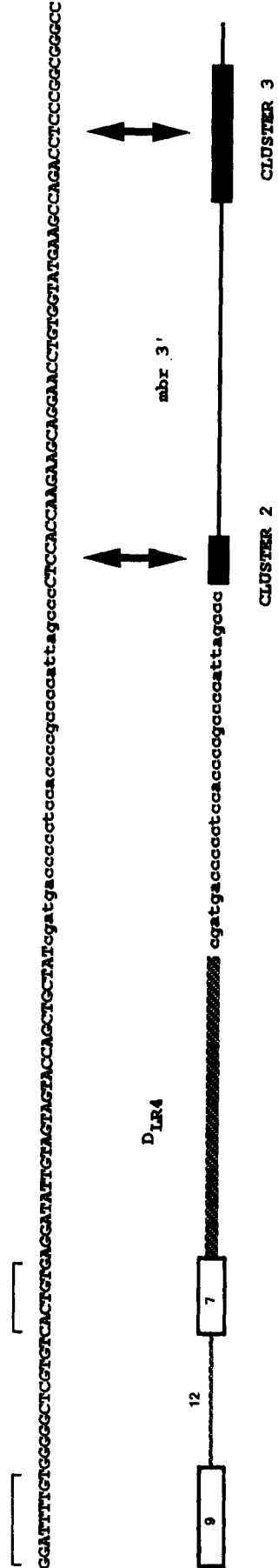
These two rare translocations emphasized the bidirectional nature of the signals associated with cluster sites. The usual *mbr*-to-J_H join was replaced by an *mbr* recombination upstream of a D_xp gene element, mimicking V_H-D_H gene rearrangement. The capacity of the *mbr* to recombine upstream of both D and J elements, as well as downstream of D elements (D_xp:3'*mbr*), contrasted sharply with RSS-mediated recombination.

Nonrandom Usage of D_xp Family Members in Reciprocal mbr Translocations. Members of the D_xp 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 D_xp genes, we used a D_xp family
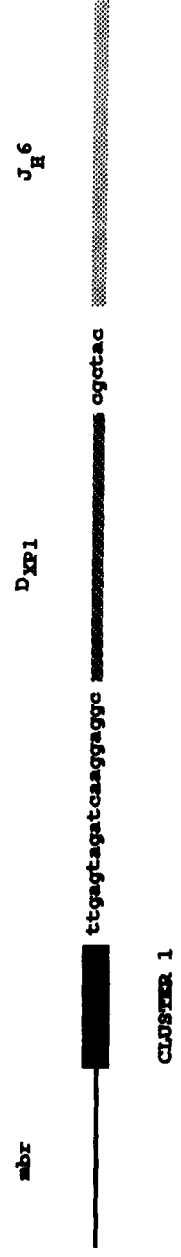
A

CCCTCCTCCCGCGGGGCTTTCTCAGGGTCTCCTGAAATGCAGGGCTCCTGAAATGCAGTGCAGTGCTACCacaggcggtgctttgaagcccGGgcttcgccggggGCTTTCTCAGGGTCTCCTGAAATGCAGTGCAGTGCTAC TGGGCGGAGAACCGTGTCACCGTCTCCTCT

mbr A 5'   mbr B 5'   J_H 4

CLUSTER 1   CLUSTER 2 CLUSTER 1   CLUSTER 2 CLUSTER 1   CLUSTER 2

gctt   gctt   caaggcggtgctttgaagcccc

B

GGTTTGAAGTGAGGTCTGTTGTCACTGTGTGGTATTATGATTGATTACGTTTGGGGGAGTccacCGCTCCACCAAGAAAGCAGAAACCTGTGTATGAAGCCAGACCTCCCCGGCGGGG

                        9   12   7   D_XP2   ccacc   mbr B 3'

                                            CLUSTER 2   CLUSTER 3

C

mbr B   mbr A   nnn

            CLUSTER 1 CLUSTER 2   CLUSTER 3

Figure 4. *mbrA:mbrB* recombination in an *mbr*:J_H4 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_H coding sequence. (*Stippled bar*) J_H4 coding sequence. EMBL Accession No. X63226. (*B*) The reciprocal partner of the t(14;18) translocation is shown. (*Bottom*) Schematic diagram of the (*top*) DNA sequence. (*Open boxes or brackets*) 5' D RSS. (*Striped bar*) D_XP2 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.

A

CCCTCCTTCCCGGGGGCTTTCTCCATGCCTCGTGCCTCCTTCAGGCTCTGAAATCGCAGGATCAGATATAACACAGTGTCAGAGTCCATGCCCTCCGAGCGCTCCCGCGCAGCCTCC[65 bp]aaaggatcGACTACTGGGGATC

mbr 5'

D~XP~'1

J~H~4

CLUSTER 1
CLUSTER 2

$\boxed{9}$ 12 $\boxed{7}$ $\boxed{9}$

[65 bp]aaaggatt

B

GGATTTTGTGGGGGCCTCGTGGCACTGTGAGGATATTGTAGTAGTACCACCTCGTATcgatgaccccctcacccgcccattagcccCTCCACGGAGAGCAGGAGACCTGTCGTATGGTATGGAGCCAGACCTCCCGGCGCGGCCC

cgatgaccccctcacccgcccaccattagccc

D~LR4~

CLUSTER 2

CLUSTER 3

mbr 3'

$\boxed{9}$ 12 $\boxed{7}$

D~LR4~

C

ttgagtagatcaaggaggc .... cgctac

mbr

D~XP1~

J~H~6

CLUSTER 1

Figure 5. *mbr*:D~XP~:J~H~ translocation. (*A*) The DNA sequence and schematic depiction of the 5'*mbr*:D~XP~'1:J~H~4 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 D~LR~ consensus primer and the 3'*mbr* primer, is shown. EMBL Accession No. X63232. (*C*) A schematic representation of a similar 5'*mbr*:D~XP~'1:J~H~6 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 D_{XP} family members (Fig. 6). One additional reciprocal was amplified, cloned, and sequenced using a D_{LR} family primer. From our data and the published results of others, 14 of 26 informative reciprocal translocations used a D_{XP} family member. Because of the high degree of specificity of D_{XP}:*mbr* amplification, the three unsequenced reciprocals described above were included in this summary. The result we obtained, therefore, represented highly preferential usage of the D_{XP} family, because there are six known human D gene families (17) randomly used in normal DJ rearrangement (21). D_{XP1} and $D_{XP'1}$ were the most frequently observed members (four times each in 11 sequenced reciprocal translocations involving D_{XP} genes).

We inspected the D_{XP}:3'*mbr* breakpoints for the presence of heptamer/nonamer homology in *mbr* sequences flanking the D_{XP} 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 D_{XP}:3'*mbr* joints argued strongly against the transient formation of J_H: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 J_H RSS contained in the predicted reciprocal (Fig. 1 A), the *mbr* contribution to the signal joint should still have been observed either flanking the D coding element or within the deleted *mbr* bases.

5' Region of mbr Cluster 1 Is Homologous to a D_{XP} Family Repeat Sequence. Because translocation events frequently involved *mbr* cluster sites and D_{XP} 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_{XP4} to D_{M2}; this included D_{XP1} and $D_{XP'1}$ (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. 7 A. 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 CCAGAGC 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. 3 C) 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. 3 B). The sequence motif (CCACAGCCCTCCCCA and variations thereof) was found interspersed four times in the 400 bp spanning D_{XP1} and $D_{XP'1}$, and twice in the D_{XP4} region (Figs. 3 B and 7 A). 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. 7 A) located 900 bp downstream of D_{XP4}. 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 J_H 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_{XP1} repeat unit also appeared (Fig. 7 B). The two
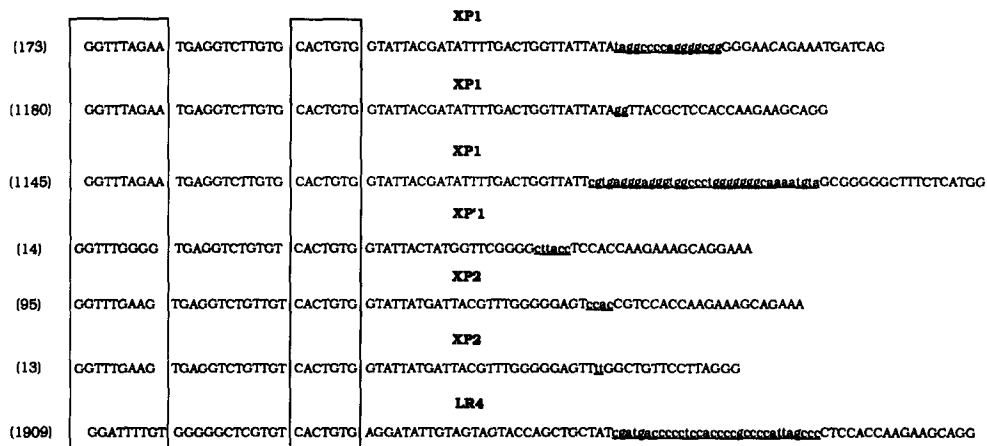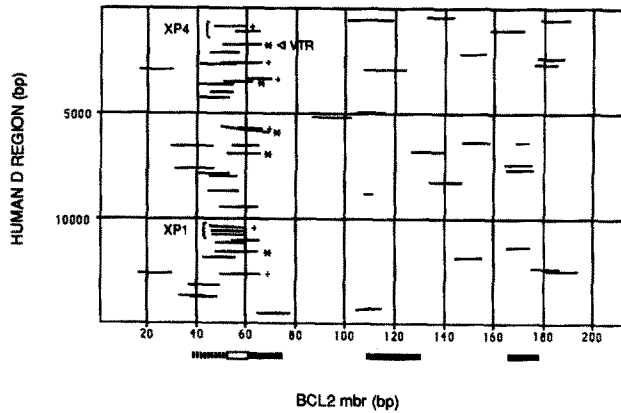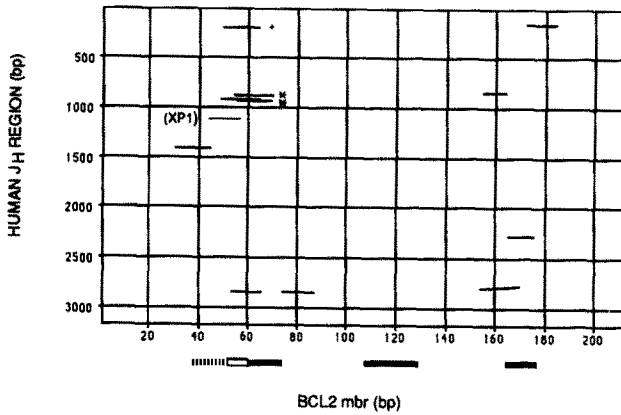

Figure 6. DNA sequence of D:3'*mbr* reciprocal translocations. The DNA sequences of seven D:3'*mbr* reciprocal breakpoints (18q⁻) are shown. D sequence, including heptamer/nonamer (*boxed*) and coding segment, appears on the left side of N nucleotides, which are given in lower case. 3'*mbr* DNA sequence appears to the right. EMBL Accession Nos. X63225, X63228, X63229, X63230, X63231, X63232, and X63233.
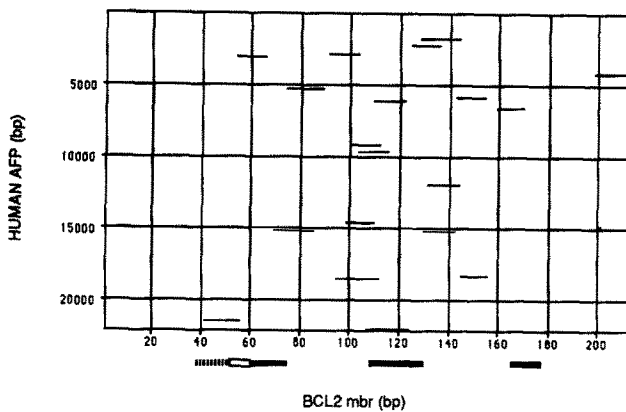
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A

HUMAN D REGION (bp)

XP4

◁ VTR

5000

10000

XP1

20 40 60 80 100 120 140 160 180 200

BCL2 mbr (bp)

B

HUMAN J H REGION (bp)

500

1000

(XP1)

1500

2000

2500

3000

20 40 60 80 100 120 140 160 180 200

BCL2 mbr (bp)

C

HUMAN AFP (bp)

5000

10000

15000

20000

20 40 60 80 100 120 140 160 180 200

BCL2 mbr (bp)

Figure 7. *mbr*-D, *mbr*-J, and *mbr*-AFP homology matrices. (*A*) Graphic representation of the DNA matrix analysis between the *mbr* and the human D region, (*B*) the human J$_H$ region, and (*C*) the human α-fetoprotein (AFP) gene. Numbers on vertical axes indicate bp 5' to 3' (*top* to *bottom*); the horizontal axes depict *mbr* bases 1–215 (*left* to *right*), as assigned in Fig. 2. *mbr* cluster groups 1, 2, and 3 are indicated by the dark bars underneath the horizontal axes. (*Open box*) 8/8 χ-like consensus. (*Dashed line*) Remainder of the 5' cluster 1 homology sequences. Homology detected in either orientation is indicated by the horizontal lines on each graph, drawn to scale. 8/8 (*) and 7/8 (+) matches with the χ-like consensus are labeled, as is homology with the D$_{xp}$ repeat. See also Fig. 3 *B*. The position of a minisatellite detected by our homology search is indicated, as well (*VTR*).

8/8 matches, located between J$_H$1 and J$_H$2 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 χ Sightings. In addition to our BCL2 observations, we identified χ 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_{xp1}-$D_{xp'1}$ region. The repeats flanked these D_{xp} genes, as well as D_{xp4}, and represented 11 of 15 and 13 of 15 matches for the cluster 1 upstream sequence (CCAGAGCCCTCCTGC; Fig. 3 B). Particularly suggestive was the demonstration that these repeats were the site of recombination that resulted in the evolutionary duplication of D_{xp1}/ $D_{xp'1}$ (17). The other result of this sequence analysis was that the χ 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

CHROM 9   CTCCG⎡CCTCCTGG⎤TTCACG
CHROM 22   CCTCACAGT⎡GCTGGAG G⎤CAGGAAGTC

↓

CHROM 9q+   CTCCG⎡CCTCC⎤CTGGAG G⎤CAGGAAGTC
CHROM 22q- CCTCACAGT⎡GCTGGAG⎤TGG⎤TTCACG

Figure 8. A t(9;22) translocation occurring at χ-like sequences. A t(9;22) translocation across two χ-like sequences is illustrated. We have reformatted the DNA sequence data of van der Feltz et al. (28) to highlight the χ sequences we found through which the interchromosomal recombination took place. (*Open box*) 7/8 χ-like sequence on chromosome 9. (*Stippled box*) 8/8 χ-like sequence in opposite orientation on chromosome 22. (*Dotted line*) A 7/8 χ-like sequence that overlaps the 8/8 match by one base, reminiscent of the tandem repeat in the *mbr*. (∇) Chromosomal breakpoints. The white on black sequence highlights the chromosome 22 bases conserved in both reciprocal partners, consistent with a double-stranded, staggered break across these six bases.

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 χ sequences was a t(9;22) translocation previously reported by van der Feltz et al. (28) which occurred in a Ph[1]-positive acute lymphocytic leukemia (ALL). This particular translocation provided some insight into the events initiating χ-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 χ-like sequences on both parental chromosomes, and contained no deletion of germline sequence in either reciprocal product. The 8/8 χ-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 χ-like sequence on chromosome 9 appeared to have undergone either a blunt-ended, double-stranded break between bases 5 and 6 of the χ-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 χ-like sequence can serve as the site of double-stranded, staggered cleavage, perhaps by a site-specific endonuclease. This contrasts with procaryotic χ, which is the site of single-stranded cleavage four to six bases downstream. Taken together, the observations on *mbr*-D_H homology and cleavage in the t(9;22) translocation suggested to us that site-specific recombination between *mbr* and D_H was mediated by double-stranded, staggered breaks at χ sequences.

A Model for mbr Translocations. We propose that *mbr*-to-D rearrangement, not *mbr*-toJ_H rearrangement, is the first step in t(14;18) translocation. The homologous sequences present at high density at both D_{xp1}-$D_{xp'1}$ and the *mbr* initiate translocation, and the initial breakpoint represents a χ crossover, not a VDJ recombinase-mediated coding joint (Fig. 9 A). Since 5' cluster 1 sequences share the highest degree of homology with the D_{xp} 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 χ-like sequences. This complex is responsible for DNA cleavage, either by single-stranded breaks analogous to χ, 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[+] and 18q[−] reciprocal partners shown in Fig. 9 B. 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 χ sequence-associated recombinase activity, as well as by the VDJ recombinase. Our proposal does not rule out
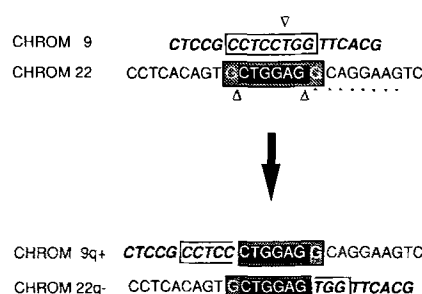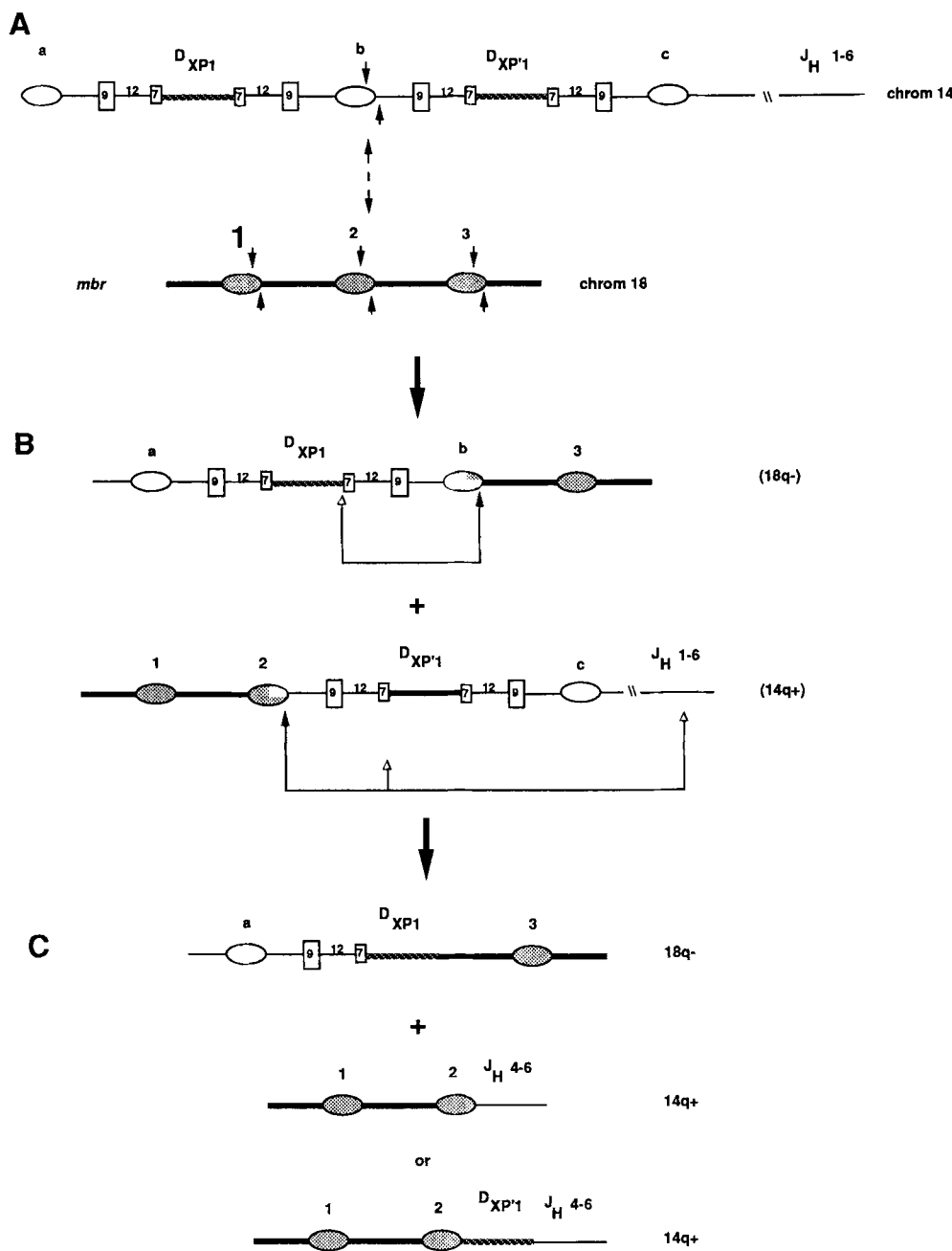
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A

chrom 14

chrom 18

B

(18q-)

+

(14q+)

C

18q-

+

1   2 ^{J}H 4-6

14q+

or

1   2 $D_{XP'1}$ ^{J}H 4-6

14q+

Figure 9. A model for *mbr* translocation. (*Open boxes*) Heptamer/nonamer signal sequences. The open ovals in the D_{XP1}-$D_{XP'1}$ 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_{XP} sequences, but is not necessary for the model. In the example depicted here, site-specific recombination occurs between cluster 2 and the D_{XP1} 3' motif (*b*) (*dashed double arrow*). After cleavage at cluster 2, strand invasion and heteroduplex formation (*hybrid white/stippled ovals* in *B*) occur between cluster 2 and D_{XP} b, 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 J_H element, forming the 14q⁺ 5'*mbr*:J_H 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 J_H element by traditional VDJ recombinase-mediated rearrangement to generate the rare translocations we have observed (*bottom line*; *C*).

12bp RSS--30bp--*TAGGTTGCAACATCATCAG*CCTCCACAGGATTAATGGTGA V_{K21C}

12bp RSS--30bp--*TAGGTTGCAACATCA*TCAGCCTCCAGCTTTGGTCCCAGCAC--10bp--23bp RSS   A1-1

AAGTGGTACTTACGTTTCAGCCTCCAGCTTTGGTCCCAGCAC--10bp--23bp RSS $J_{K}5$

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 (V_{K21c}; $J_{K}5$) 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 recombinase (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 recombinase-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 procession of the recombinase complex down the *mbr* until the signal sequence fragments at cluster 2 or 3 are encountered. Incomplete *mbr*-to-J_H rearrangements (Fig. 9 *C*) leave intervening D sequence and result in rare *mbr*:D_{XP}:J_H 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 D_H and J_H have χ-like sequences and the D_{XP} repeat motif, although in opposite orientations. The activity of χ sequences may extend to V_H genes, as well. Tutter and Riblett (29) have shown that the members of the V_HIII gene family contain a χ sequence in the 5' region of framework 1 which they suggested played a role in V_H recombination. This sequence and flanking DNA is an 11/15 match with the *mbr* cluster 1 5' sequence. It is also one of four V_HIII sequences conserved at the nucleotide level from mouse to human (29). Finally, Kenter and Birshtein (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 D_H:*mbr*. It is nat-ural 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.

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