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Identification of the gene associated with the recurring chromosomal translocations t(3;14)(q27;q32) and t(3;22)(q27;q11) in B-cell lymphomas

(fluorescence *in situ* hybridization/nucleotide sequencing/*IGH* locus)

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ABSTRACT **Chromosomal translocations involving chromosome 3, band q27, are among the most common rearrangements in B-cell non-Hodgkin lymphoma. From a bacteriophage λ library prepared from a lymphoma characterized by a t(3;14)(q27;q32), genomic clones were isolated using a probe from the immunoglobulin heavy chain locus (*IGH*) joining region. In addition to clones containing an apparently normal *IGH* rearrangement, others were found to contain one of the translocation breakpoint junctions. Normal chromosome 3 sequences and the reciprocal breakpoint junction were subsequently isolated. DNA probes on each side of the chromosome 3 breakpoint hybridized at high stringency to the DNA of various mammalian species, demonstrating evolutionary conservation. One such probe from the presumptive der(3) chromosome detected an 11-kilobase transcript when hybridized to RNA of B- and T-cell lines. A probe made from partial cDNA clones isolated from a T-cell line hybridized with genomic DNA from both sides of the chromosome 3 breakpoint, indicating that the t(3;14) is associated with a break within the gene on chromosome 3. *In situ* chromosomal hybridization revealed that the same gene is involved in the t(3;22)(q27;q11). Preliminary nucleotide sequencing shows no identity of the cDNA to gene sequences in available data banks. We propose the name *BCL6* (*B*-cell *l*ymphoma *6*) for this gene, since it is likely to play a role in the pathogenesis of certain B-cell lymphomas.**

It is well known that nonrandom chromosomal rearrangements accompany certain malignant neoplasms (1, 2). Molecular analysis of chromosomal breakpoints associated with some rearrangements has resulted in the identification of a number of genes that have been found to be important for the normal growth and differentiation of lymphocytes; however, when involved in a translocation, these genes may play a role in the development of lymphoid malignancies (e.g., refs. 3–11). In many of the recurring chromosomal translocations, a gene important in the control of normal cell proliferation becomes deregulated, with perturbed synthesis of its usual protein product, because of its transposition next to regulatory sequences from another gene that is actively expressed. An example of an actively expressed gene that is frequently affected in chromosomal translocations in B-cell leukemias and lymphomas is the immunoglobulin heavy chain (*IGH*) locus, located on chromosome 14, band q32 (12–18).

A number of authors (19–26) have noted that chromosome 3, band q27-q29, is frequently involved in various translocations in non-Hodgkin lymphomas. Among these, the t(3;14)(q27;q32) and t(3;22)(q27-29;q11), presumably involving the *IGH* and the λ light chain genes, respectively, are the most common (20, 22). This study concerns a diffuse B large-cell lymphoma with a complex karyotype. Molecular analysis resulted in the cloning of the breakpoint junctions of a t(3;14) and the identification of the involved gene on chromosome 3, band q27. The translocation involves a break within the gene, which has a large (11 kb) transcript. By *in situ* chromosomal hybridization, we show that the same gene is involved in the t(3;22)(q27;q11).

Apparently this gene has not been identified previously. Its recurring association with the t(3;14), t(3;22), and other less commonly described translocations (22) in certain large-cell B lymphomas suggests that when deregulated, it has a role in the pathogenesis of these neoplasms.§

MATERIALS AND METHODS

Cytogenetic Examination. Cytogenetic analysis was performed with a trypsingiemsa banding technique on cells from a spleen biopsy obtained at the time of diagnosis of non-Hodgkin lymphoma. Metaphase cells were examined from short-term (24 and 48 hr) unstimulated cultures. Chromosomal abnormalities are described according to the International System for Human Cytogenetic Nomenclature (1991).

Southern Blot Analysis. Probes for Southern blot hybridization (27) of the patient and control samples included (*i*) for the *IGH* region (Fig. 2*A*, top panel), a 3.9-kb *Bgl* II probe containing the joining (J_H) region, a 0.8-kb *Bgl* II–*Hin*dIII probe 3′ of the J_H region (3′J_H), the μ- and α-chain constant (C) regions, and the γ-switch (S_γ) region (28) and (*ii*) probes from chromosome 3 as described subsequently. Additional Southern blots were made (*i*) from a panel of 38 human–rodent somatic cell hybrids, each digested with *Eco*RI, and (*ii*) from the DNA of rhesus monkey, dog, calf, rat, rabbit, and mouse, digested with *Eco*RI, *Bam*HI, *Bgl* II, and *Hin*dIII; these were hybridized with probes from chromosome 3 as described below.

Molecular Cloning. Genomic DNA from the malignant lymphoma and from the peripheral blood cells of a patient with chronic myelogenous leukemia without abnormalities of chromosome 3 or 14 was partially digested with *Sau*3A. Libraries were made with size-selected DNA ligated to the *Bam*HI site of the λDash II vector (Stratagene). These libraries were screened with a 0.96-kb *Eco*RI fragment of chromosome 3 and/or a 1.4-kb *Eco*RI–*Bgl* II fragment of chromosome 3, as indicated in the text.

***In Situ* Chromosomal Hybridization.** Human metaphase cells were prepared from phytohemagglutinin-stimulated peripheral blood lymphocytes. Fluorescence *in situ* hybridization (FISH) was performed as described (29). Biotin-labeled

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Abbreviations: FISH, fluorescence *in situ* hybridization; J, joining; C, constant.

§The sequences reported in this paper have been deposited in the GenBank data base (accession nos. L11921, L11922, and L11923).

λ phage probes from chromosome 3 were prepared by nick-translation using Bio-11-dUTP (Enzo Diagnostics, New York). Hybridization was detected with fluorescein-conjugated avidin (Vector Laboratories), and chromosomes were identified by staining with 4′,6-diamidino-2-phenylindole. In addition, *in situ* hybridization was also performed using ^{3}H-labeled probes (specific activity, 10^8 dpm/μg) (30).

Nucleotide Sequencing. Genomic DNA fragments encompassing the breakpoint junctions of the t(3;14) from the presumptive rearranged chromosome 3 and 14 homologs [der(3) and der(14) chromosomes], as well as the corresponding region of the normal chromosome 3, were digested with *Nco* I and *Pvu* II [der(3) chromosome] or *Bss*HII [der(14) chromosome and normal chromosome 3] and then subcloned into M13 for sequencing. Nucleotide sequences were determined by the dideoxy DNA sequencing technique using single-stranded templates labeled with ^{35}S (31).

Northern Blot Analysis. SUP-T13 cells (a T-cell acute lymphoblastic leukemia line) (32) were cultured under conditions of 5% O_2/5% CO_2/90% N_2. Poly(A) RNA was extracted from these cells as well as from RS4;11 cells (a pre-B acute lymphocytic leukemia cell line) and RCH-ADD cells (a B-cell line) using a standard protocol (Fast Track; Invitrogen, San Diego). Northern blot analysis was performed as described (9).

Cloning of the cDNA. Poly(A) RNA from SUP-T13 cells was used to prepare double-stranded cDNA with the use of protocols suggested by the manufacturer (BRL). Size-selected DNA was ligated to *Eco*RI sites of λ Zap II bacteriophage. Phage clones of the cDNA library were screened (9) by plaque hybridization (Colony/Plaque Screen; DuPont) with the use of the 1.4-kb *Eco*RI–*Bgl* II probe from chromosome 3 obtained from the genomic library. Hybridization was performed as described (9). The cDNA inserts were subcloned into pBluescript SK− with the use of R408 helper phage (Stratagene), and cDNA probes were made from 1.5-kb fragments obtained after digestion with *Eco*RI. Nucleotide sequencing was performed following denaturation.

RESULTS

Cytogenetic Examination. Cytogenetic analysis of spleen cells revealed the presence of a normal cell line as well as hypertriploid cells characterized by multiple numerical and structural abnormalities. A composite karyotype including the abnormalities observed in all of the cells examined is 77,XXY,add(1)(p11),dup(2)(p13p23),+dup(2),del(2)(q13q23),add(2)(q11),der(3)t(3;14)(q27;q32),der(3)t(3;14)(q27;q32)t(3;7)(q23;q36),add(4)(q11),dup(5)(q31q35),der(5)t(5;17)(q11;q21),+6,add(6)(q13),idic(7)(q31),der(7)t(3;7)(q23;q36),−10,+11,add(11)(q23),der(11)t(X;11)(q11;p11),add(14)(p13),der(14)t(3;14)(q27;q32)x2,−15,add(16)(p13),+18,add(18)(q23),+19,add(19)(q13)x2,+20,+21,idic(21)(p12),−22,+der(?)t(?;17)(?;q11),+mar1,+mar2,+mar3[cp22]/46,XY[2]. Of note was the presence of two rearranged chromosome 3 and 14 homologs resulting from the t(3;14)(q27;q32). Initially, these rearranged chromosomes were interpreted to be due to a del(3)(q27) and the translocation of unknown material to 14q32 [add(14)(q32)]; however, additional cytogenetic analysis, combined with data from molecular cloning and chromosomal mapping studies, resulted in the reinterpretation and identification of the reciprocal translocation.

Genomic Southern Blot Hybridization. The J_H and $3'J_H$ probes detected two rearranged bands in the *IGH* locus of the malignant lymphoma cells (Fig. 1 *a* and *b*). An unusual finding was that one of the rearranged bands showed strong hybridization, whereas hybridization with the other was weaker. Additional hybridization revealed deletion, downstream on *IGH*, of both μ regions as well as the γ3 and γ1 gene segments.

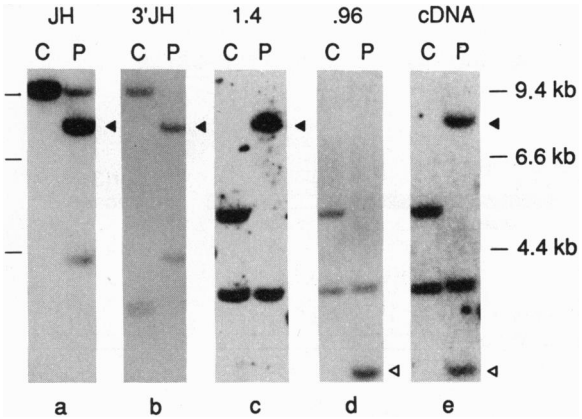


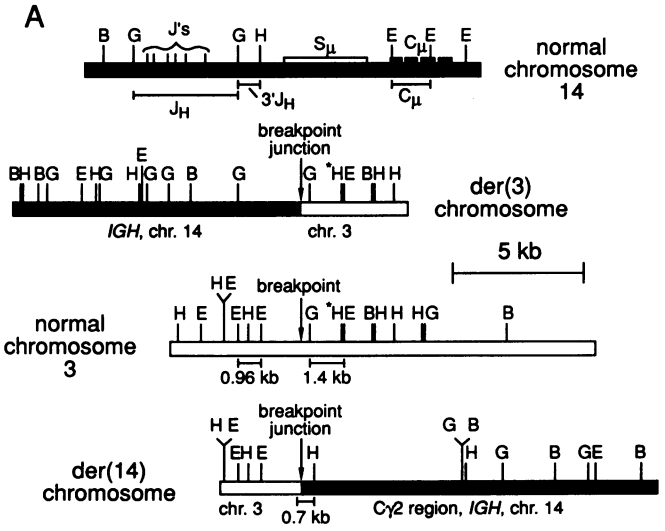
FIG. 1. Southern blot hybridization of DNA digested with *Hin*dIII: Autoradiograms of a blot containing genomic DNA from control placental DNA (C) and the patient's malignant lymphoma (P). The probes used were J_H and $3'J_H$ for *IGH* (*a* and *b*), 1.4-kb *Eco*RI–*Bgl* II and 0.96-kb EcoRI fragments, on opposite sides of the chromosome 3 breakpoint (*c* and *d*), and 1.5-kb cDNA fragments (*e*). The solid and open arrowheads indicate rearranged bands from the presumed der(3) and der(14) chromosomes, respectively. The control lanes in the last 3 panels demonstrate polymorphic *Hin*dIII fragments (see Fig. 2); only the lower one is present in the patient's malignant cells. The 0.96-kb probe also detected a germ-line 1-kb *Hin*dIII fragment (not shown). Size markers are from λ phage digested with *Hin*dIII.

Molecular Cloning and Mapping [der(3) Chromosome]. The λ bacteriophage library constructed from the malignant lymphoma was used to clone and map the DNA corresponding to the two rearranged bands detected by the J_H probe. The fainter band appeared to correspond to a normal (variable–diversity–joining) rearrangement. The clones corresponding to the more strongly hybridizing band yielded a map (Fig. 2*A*, second panel) that did not correspond to any of the mapped DNA region of *IGH*.

Fig. 1*c* demonstrates one of the rearrangements detected when the 1.4-kb *Eco*RI–*Bgl* II probe was hybridized with the genomic DNA of the lymphoma. As expected, the rearranged band detected with this probe is identical in position to that detected with the J_H and $3'J_H$ probes.

Chromosomal Localization. When the 1.4-kb *Eco*RI–*Bgl* II fragment (Fig. 2) from one of the clones was used as a probe in hybridization to Southern blots of a panel of somatic cell hybrids, the pattern was most consistent with localization to chromosome 3. *In situ* hybridization of this radiolabeled probe to mitotic chromosomes confirmed the chromosome 3 localization and further localized the probe to bands q27-q28 (results not shown). The cytogenetic appearance of the der(3) and der(14) chromosomes was compatible with their derivation from a t(3;14)(q27;q32). The orientation of *IGH* in the clones suggested that they were derived from the der(3) chromosome. No additional material from the lymphoma was available for *in situ* hybridization analysis to confirm this interpretation.

The 1.4-kb *Eco*RI–*Bgl* II probe then was hybridized to a second bacteriophage library from the peripheral blood lymphocytes of a patient with no abnormalities of chromosome 3 or 14, and three clones were mapped. As expected, a portion of the normal map of chromosome 3 was identical to that of the presumptive der(3) clones (Fig. 2*A*). The three clones were localized to normal prometaphase and metaphase cells by FISH; the results confirmed those of previous studies using radiolabeled probes and sublocalized the clones to 3q27 (Fig. 3). Of 25 cells analyzed following hybridization to one of the chromosome 3 phage clones (the map of which is illustrated in Fig. 2*A*), specific labeling of 3q27-q28 was observed on two (6



B

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CGAGGAGAGAGACTGATGGCGGGACAGGGCTTCTTGGGGTGGGTGGGAAA  der(14)
CGAGGAGAGAGACTGATGGCGGGACAGGGCTTCTTGGGGTGGGTGGGAAA  normal 3
GCCTGGGCAGAGTCAGCCAGAGCCTGTGGTGCAGGTAAGGGGAAGGGGTG  der(3)

GGGGTGCGCACTGCACCTGCGATGCCTTTCACTGTGGTGACTGAGACTCC  der(14)
GGGGTGCGCACTGCACCTGCGATGCCTTTCAGTGTGCGGGGGGACTCAGG  normal 3
GTGAGCAGGGCCCTGGGGCTGAGCAGAGGGGATGGCCTGGCTGACTCAGG  der(3)

AGAAGAAATGCAGGACACTGGGAGGCAGGGGGCATCCAGGCACTCAGGGC  der(14)
GAACCTCGACAGGACAGCCTGGGAGAACGAGAAAGGTGGCGGGATTTCTG  normal 3
GAACCTCGACAGGACAGCCTGGGAGAACGAGAAAGGTGGCGGGATTTCTG  der(3)
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FIG. 2. Map and nucleotide sequence of genomic DNA. (*A*) First panel: Partial map of the germ-line *IGH* locus illustrating the location of the J_H, $3'J_H$, and the μ-chain probes. Second panel: Restriction map of clones isolated using probes for the *IGH* J region. This map corresponded to the more intensely hybridizing rearranged band on Southern blots. B, *Bam*HI; E, *Eco*RI; H, *Hin*dIII; G, *Bgl* II. Portions of the clones derived from chromosomes 3 and 14 are indicated. The asterisk indicates a polymorphic *Hin*dIII site. Third panel: Map of a representative clone containing normal chromosome 3 sequences. The 0.96- and 1.4-kb fragments were used as probes, as indicated in the text. The entire bacteriophage clone was used for FISH analysis. Fourth panel: Restriction map of a representative clone that hybridized with the 0.96-kb probe but not the 1.4-kb probe from chromosome 3. The 0.7-kb fragment was used as a probe as indicated in the text. (*B*) Nucleotide sequences of der(14), der(3), and the corresponding region of normal chromosome 3 adjacent to the breakpoint. The solid arrowhead and second open arrowhead delineate a 6-bp deletion of chromosome 3; adjacent to the first open arrowhead is a point mutation in the der(14) (an alternative interpretation is an 11-bp deletion of chromosome 3 between the first open arrowhead and the solid arrowhead).

cells), three (10 cells), or all four (9 cells) chromatids of the chromosome 3 homologs. Of 78 signals observed, 59 (75%) were located on 3q27, 13 (17%) were located at the junction of 3q27-q28, and 6 (8%) were located on 3q28.

To determine whether the same locus is involved in another recurring translocation involving 3q27, the same chromosome 3 probe was used for FISH to metaphase cells prepared from the malignant tissue of three patients with non-Hodgkin lymphoma characterized by the t(3;22)(q27;q11). In each case, the probe hybridized to the normal chromosome 3 homolog as well as to the rearranged chromosome 3 and 22 homologs at 3q27 and 22q11 (Fig. 3). These results suggest that the breakpoints in the t(3;22)(q27;q11) and the t(3;14)(q27;q32) involve the same locus.

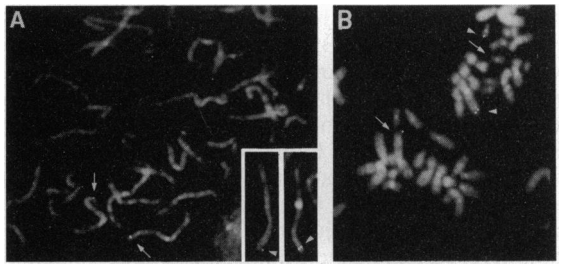

FIG. 3. Chromosomal localization by *in situ* hybridization. (*A*) *In situ* hybridization of a biotin-labeled chromosome 3 phage probe (Fig. 2*A*) to human metaphase cells from phytohemagglutinin-stimulated peripheral blood lymphocytes. The chromosome 3 homologs are identified by arrows; specific labeling was observed at 3q27. (*Inset*) Partial karyotypes of two chromosome 3 homologs illustrating specific labeling at 3q27 (arrowhead). (*B*) *In situ* hybridization of the chromosome 3 phage probe to a metaphase cell characterized by the t(3;22)(q27;q11). The hybridization signal was detected on the normal chromosome 3 (arrow) and on the rearranged chromosome 3 and 22 homologs (arrowheads). No signal was observed on the normal chromosome 22 homolog (arrow). Images were obtained using a Zeiss Axiophot microscope coupled to a thermoelectrically cooled charge-coupled device camera. Separate images of chromosomes stained with 4',6-diamidino-2-phenylindole and the hybridization signal were merged using image analysis software (IPLab Spectrum and GeneJoin). (×≈2400.)

Molecular Cloning and Mapping of the Presumptive der(14) Chromosome. A probe made from a 0.96-kb *Eco*RI fragment of chromosome 3 (Fig. 2*A*) detected additional rearranged bands when hybridized to a Southern blot made from DNA of the malignant lymphoma cells (Fig. 1*d*). This 0.96-kb probe and the 1.4-kb *Eco*RI–*Bgl* II probe (both from chromosome 3, on either side of the region corresponding to the breakpoint, Fig. 2) then were used for hybridization with an amplified bacteriophage library of DNA from the malignant lymphoma. The plaques that hybridized only with the 0.96-kb fragment were selected for DNA extraction and mapping. The map of one such clone is illustrated (Fig. 2*A*, fourth panel).

To determine the origin of the sequences adjacent to the breakpoint of the presumed der(14) chromosome, a 0.7-kb probe (*Bss*HII–*Hin*dIII fragment) encompassing the breakpoint junction (see Fig. 2*A*, fourth panel) was hybridized to a Southern blot made from the DNA of fresh peripheral blood lymphocytes from two patients without abnormalities of chromosome 14. In each restriction digest, there were multiple hybridizing bands in a pattern characteristic of the γ C region gene segments as well as the expected bands derived from chromosome 3. Restriction mapping was consistent with localization of the breakpoint on chromosome 14 to the $S_{\gamma 2}$ region of *IGH*.

Nucleotide Sequencing. There was complete identity of the chromosome 3 sequences in the chromosome 3 portions of the der(3) and der(14) chromosomes, except for a point mutation and a 6-bp deletion of chromosome 3 (an alternative interpretation is an 11-bp deletion of chromosome 3 without a point mutation, Fig. 2*B*). The *IGH* sequences of the der(3) chromosome showed 87% sequence identity with the σ regions 5' of the $C_{\gamma 3}$ and $C_{\gamma 4}$ genes of human *IGH* (33). Most likely, the breakpoint occurs within one of the other σ regions ($\sigma_{\gamma 1}$, or, more likely, $\sigma_{\gamma 2}$), for which sequences are not available. The σ-like sequences adjacent to the breakpoint are in an inverted orientation in comparison to those found upstream of $C_{\gamma 3}$ and $C_{\gamma 4}$. Thus, it is possible that this region was inverted in association with the translocation. The sequences adjacent to chromosome 3 sequences in the presumptive der(14) chromosome did not match sequences in GenBank; they may be derived from a region of *IGH* not yet sequenced.

Hybridization with "Zoo" Blots. The 1.4-kb *Eco*RI–*Bgl* II fragment and the 0.96-kb *Eco*RI fragment from each side of the chromosome 3 breakpoint hybridized strongly with Southern blots prepared from the DNA of various animal species under stringent conditions, indicating evolutionary conservation (Fig. 4). Since exons are usually more highly evolutionarily conserved than introns and intergenic regions, it seemed likely that these DNA fragments might contain gene exons.

Northern Blot Analysis. When the 1.4-kb *Eco*RI–*Bgl* II probe from chromosome 3 was hybridized to Northern blots made from B- and T-cell lines, a prominent 11-kb transcript was detected in RNA extracted from SUP-T13 cells (Fig. 5). A much weaker 11-kb band was also detected in RNA from RS4;11 cells. Clear hybridization to a band at this position was not seen in 10 other hematopoietic cell lines or in 3 nonhematopoietic tissues tested. It is not clear whether the weak hybridization at the approximate position of the 28S RNA is specific.

Isolation of cDNA and Additional Southern Blot Analysis. A number of cDNA libraries from various B- and T-cell lines were screened with the 1.4-kb *Eco*RI–*Bgl* II DNA probe made from the genomic DNA of chromosome 3; four cDNA clones were successfully isolated only from a library of the SUP-T13 cell line. Since the transcript is large (11 kb), multiple rounds of cloning will be needed to isolate the entire cDNA. The two largest clones were analyzed further. *Eco*RI digestion released an ≈1.5-kb cDNA fragment from each clone. These 1.5-kb fragments were combined for use as a probe. When hybridized to the original Southern blots made from the genomic DNA of the lymphoma, the rearrangements detected indicated that the probe had hybridized to DNA on each side of the chromosome 3 breakpoint (Fig. 1*e*).

DISCUSSION

We have cloned the breakpoint junctions of the t(3;14) in a non-Hodgkin lymphoma. The strong hybridization of chromosome 3 fragments proximal and distal to the translocation breakpoint with the DNA of several different animal species demonstrates evolutionary conservation. The hybridization of the partial cDNA with chromosome 3 on each side of the breakpoint supports the hypothesis that a break within a gene on chromosome 3 has occurred. The initial sequences from the cDNA are not identical to any in GenBank, indicating that this gene apparently has not been identified previously (data not shown).

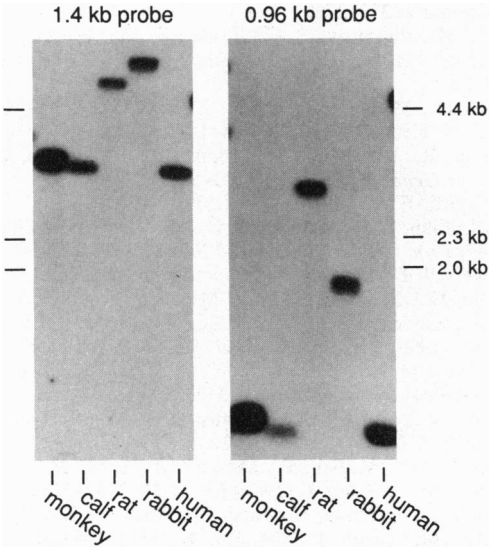

FIG. 4. Southern blot analysis. Lanes left to right: DNA from rhesus monkey, calf, rat, rabbit, and human placenta, each digested with *Eco*RI. (*Left*) The 1.4-kb probe (*Bgl* II–*Eco*RI fragment of chromosome 3). (*Right*) The 0.96-kb probe (*Eco*RI fragment of chromosome 3). Hybridization washes were at high stringency: 0.1× SSC (1× SSC = 0.15 M NaCl/15 mM sodium citrate)/1% SDS, 65°C (1.4-kb probe); 1× SSC/1% SDS, 65°C (0.96-kb probe). Size markers are from λ phage digested with *Hin*dIII.

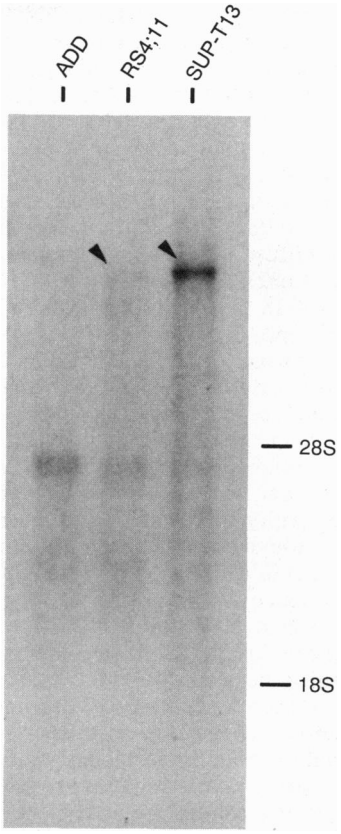

FIG. 5. Northern blot analysis. Shown is poly(A)$^+$ RNA isolated from RCH-ADD (a B-cell line, left), RS4;11 (a pre-B-cell acute lymphoblastic leukemia line, middle), and SUP-T13 (a T-cell acute lymphoblastic leukemia line, right) hybridized with the 1.4-kb *Bgl* II–*Eco*RI fragment from chromosome 3. Size markers are 28S and 18S rRNAs. Arrowheads indicate bands representing specific hybridization in the SUP-T13 and RS4;11 (weak) cell lines. It is not clear whether the hybridization at the approximate position of the 28S RNA is specific.

The transcript detected here is unusually large. It may encode a correspondingly large protein; alternatively, it is possible that only a portion of the 11-kb transcript codes for a protein, and that the remainder includes large 5′ or 3′ untranslated regions. This is the case in the *REL* locus, which is associated with a 12-kb mRNA but only 1.8 kb of coding sequences (34). Our analysis to date suggests that the 11-kb transcriptional activity is not ubiquitous; of the samples analyzed so far, expression of the gene is strongest in a malignant T-cell line. Since there is evidence of transcriptional activity in B- and T-cell lines, this gene may have a role in the control of lymphocyte proliferation.

This hypothesis is supported by the fact that t(3;14) has been reported in a number of diffuse, noncleaved large-cell lymphomas (19–21). The 3;14 translocation is difficult to identify by cytogenetic analysis, since the break occurs close to the end of chromosome 3 and, therefore, may have been overlooked in other studies; in fact, initial cytogenetic analysis in our case failed to identify the translocation. Bastard *et al.* (20, 21) present 15 cases of the t(3;14)(q27;q32) and find this to be the third most common recurring translocation

(4.7%) in their series of non-Hodgkin lymphomas. As in the patient we describe, most of their patients had a complex karyotype, but in two of their patients, one with t(3;14)(q27;q32) and one with t(2;3)(p12;q27), the translocation involving 3q27 was the only cytogenetic abnormality. Additionally, a number of other cases of lymphoma have been reported with a t(3;22)(q27-29;q11) (22–26); we have shown that the same gene is involved in this rearrangement. Offit *et al.* (22) consider the t(3;22) the third most common recurring translocation in diffuse non-Hodgkin lymphomas.

The t(8;14)(q24;q32), t(14;18)(q32;q21), t(10;14)(q24;q32), and t(7;9)(q34;q34.3), involving the genes *MYC* (13), *BCL2* (16, 35), *NFKB2* (previously *LYT10*) (11), and *TAN1* (36), respectively, are examples of translocations also associated with a break within the affected protooncogene. *MYC* and *BCL2* may be involved in translocations with *IGH* as well as with the λ light chain locus on chromosome 22 and the κ light chain locus on chromosome 2. The gene we have isolated is similar in this respect. In a majority of cases of the t(14;18), the break occurs within 3′ untranslated sequences in the last exon of *BCL2* without truncation of the protein. In those cases of the t(8;14) in which a break occurs within *MYC*, 5′ untranslated sequences are removed, and the major protein product is not truncated. In contrast, however, the t(10;14) results in a truncated protein, which functionally resembles a processed form of the normal product. In the case of *TAN1*, multiple major transcripts correspond to truncated versions of the gene, and it is speculated that the translocation may result in removal of the extracellular domain of the normal protein (36). Clearly, much additional work is needed to elucidate how translocations involving the gene on 3q27 contribute to malignancy; however, we speculate that since a break in the gene has occurred in the case we present, and since translocation of the same gene to various loci on other chromosomes may result in a similar type of lymphoid neoplasm, it is possible that truncation of the protein product is a consequence of these translocations.

The gene under investigation here apparently has not been identified previously, and we propose the name *BCL6* (*B-cell lymphoma 6*). Just as other protooncogenes have been found to be significant in the regulation of cell proliferation and to contribute to oncogenesis when aberrantly expressed, it is likely that *BCL6* will be found to have an important role in the pathogenesis of certain B-cell lymphomas.

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1. Rowley, J. D. (1990) *Cancer Res.* **50**, 3816–3825.
2. Solomon, E., Borrow, J. & Goddard, A. D. (1991) *Science* **254**, 1153–1160.
3. Tsujimoto, Y., Yunis, J., Onorato-Showe, L., Erikson, J., Nowell, P. C. & Croce, C. M. (1984) *Science* **224**, 1403–1406.
4. Bakhshi, A., Jensen, J. P., Goldman, P., Wright, J. J., McBride, O. W., Epstein, A. L. & Korsmeyer, S. J. (1985) *Cell* **41**, 899–906.
5. Mellentin, J. D., Murre, C., Donlon, T. A., McCaw, P. S., Smith, S. D., Carroll, A. J., McDonald, M. E., Baltimore, D. & Cleary, M. L. (1989) *Science* **246**, 379–382.
6. McGuire, E., Hockett, R. D., Pollock, K. M., Bartholdi, M. F., O'Brien, S. J. & Korsmeyer, S. J. (1989) *Mol. Cell. Biol.* **9**, 2124–2132.
7. Nourse, J., Mellentin, J. D., Galili, N., Wilkinson, J., Stanbridge, E., Smith, S. D. & Cleary, M. L. (1990) *Cell* **60**, 535–545.
8. Kamps, M. P., Murre, C., Sun, X. H. & Baltimore, D. (1990) *Cell* **60**, 547–555.
9. Ohno, H., Takimoto, G. & McKeithan, T. W. (1990) *Cell* **60**, 991–997.
10. Zutter, M., Hockett, R. D., Roberts, C. W. M., McGuire, E. A., Bloomstone, J., Morton, C. C., Deaven, L. L., Crist, W. M., Carrol, A. J. & Korsmeyer, S. J. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 3161–3165.
11. Neri, A., Chang, C.-C., Lombardi, L., Salina, M., Corradini, P., Maiolo, A. T., Chaganti, R. S. K. & Dalla-Favera, R. (1991) *Cell* **67**, 1075–1087.
12. Fukuhara, S., Rowley, J. D., Variakojis, D. & Golomb, H. M. (1979) *Cancer Res.* **39**, 3119–3128.
13. Dalla-Favera, R., Bregni, M., Erikson, J., Patterson, D., Gallo, R. C. & Croce, C. M. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 7824–7827.
14. Taub, R., Kirsch, I., Morton, C., Lenoir, G., Swan, D., Tronick, S., Aaronson, S. & Leder, P. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 7837–7841.
15. Yunis, J. J., Oken, M. M., Kaplan, M. E., Ensrud, K. M., Howe, R. R. & Theologides, A. (1982) *N. Engl. J. Med.* **307**, 1231–1236.
16. Tsujimoto, Y., Finger, L. R., Yunis, J., Nowell, P. C. & Croce, C. M. (1984) *Science* **226**, 1097–1099.
17. Adams, J. M., Harris, A. W., Pinkert, C. A., Corcoran, L. M., Alexander, W. S., Cory, S., Palmiter, R. D. & Brinster, R. L. (1985) *Nature (London)* **318**, 533–538.
18. Nishida, K., Taniwaki, M., Misawa, S. & Abe, T. (1989) *Cancer Res.* **49**, 1275–1281.
19. Yunis, J. J., Oken, M. M., Theologides, A., Howe, R. B. & Kaplan, M. E. (1984) *Cancer Genet. Cytogenet.* **13**, 17–28.
20. Bastard, C., Tilly, H., Lenormand, B., Bigorgne, C., Boulet, D., Kunlin, A., Monconduit, M. & Piguet, H. (1992) *Blood* **79**, 2527–2531.
21. Bastard, C., Deweindt, C., Tilly, H. & Kerckaert, J. P. (1992) *Blood* **80** (Suppl. 1), 255a (Abstr. #1012).
22. Offit, K., Jhanwar, S., Ebrahim, S. A. D., Filippa, D., Clarkson, B. D. & Chaganti, R. S. K. (1989) *Blood* **74**, 1876–1879.
23. Leroux, D., Stul, M., Sotto, J. J., Bastard, C., Couderc, P., Bensa, J. C., Monteil, M., Cassiman, J. J. & Jalbert, P. (1990) *Leukemia* **4**, 373–376.
24. Offit, K., Jhanwar, S. C., Ladanyi, M., Filippa, D. A. & Chaganti, R. S. K. (1991) *Genes Chromosomes Cancer* **3**, 189–201.
25. Offit, K., Wong, G., Filippa, D. A., Tao, Y. & Chaganti, R. S. K. (1991) *Blood* **77**, 1508–1515.
26. Berger, R., Bernheim, A., Valensi, F. & Flandrin, G. (1983) *Cancer Genet. Cytogenet.* **8**, 91–92.
27. Southern, E. M. (1975) *J. Mol. Biol.* **98**, 503–517.
28. McKeithan, T. W., Rowley, J. D., Shows, T. B. & Diaz, M. O. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 9257–9260.
29. Rowley, J. D., Diaz, M. O., Espinosa, R., III, Patel, Y. D., van Melle, E., Ziemin, S., Taillon-Miller, P., Lichter, P., Evans, G. A., Kersey, J. H., Ward, D. C., Domer, P. H. & Le Beau, M. M. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 9358–9362.
30. Le Beau, M. M., Westbrook, C. A., Diaz, M. O. & Rowley, J. D. (1984) *Nature (London)* **312**, 70–71.
31. Sanger, F., Nicklen, S. & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 5463–5467.
32. Smith, S. D., McFall, P., Morgan, R., Link, M., Hecht, F., Cleary, M. & Sklar, J. (1989) *Blood* **73**, 2182–2187.
33. Akahori, Y., Handa, H., Imai, K., Abe, M., Kameyama, K., Hibiya, M., Yasui, H., Okamura, K., Naito, M., Matsuoka, H. & Kurosawa, Y. (1988) *Nucleic Acids Res.* **16**, 9497–9511.
34. Brownell, E., Mittereder, N. & Rice, N. R. (1989) *Oncogene* **4**, 935–942.
35. Cleary, M. L., Smith, S. D. & Sklar, J. (1986) *Cell* **47**, 19–28.
36. Ellisen, L. W., Bird, J., West, D. C., Soreng, A. L., Reynolds, T. C., Smith, S. D. & Sklar, J. (1991) *Cell* **66**, 649–661.