RESEARCH ARTICLE

Molecular Cloning of the Chromosomal Breakpoint of B-Cell Lymphomas and Leukemias with the t(11;14) Chromosome Translocation

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Abstract. *The chromosomal breakpoint of chronic lymphocytic leukemia (CLL) cells of the B-cell type carrying the translocated long arms of chromosomes 11 and 14 [t(11;14) (q13;q32)] was cloned. The breakpoint was found to be within the joining segment of the human heavy chain locus on the translocated long arm of chromosome 14. A probe that is specific for chromosome 11 and that maps immediately 5′ to the breakpoint on the 14q$^+$ chromosome was isolated. The probe detected a rearrangement of the homologous genomic DNA segment in the parental CLL cells and also in DNA from a diffuse large cell lymphoma with the t(11;14) translocation. This rearranged DNA segment was not present in Burkitt lymphoma cells with the t(8;14) translocation or in nonneoplastic human lymphoblastoid cells. The probe can thus be used to identify and characterize a gene located on band q13 of chromosome 11 that appears to be involved in the malignant transformation of human B cells carrying the t(11;14) translocation. This gene, named* bcl-1, *appears to be unrelated to any of the known retrovirus oncogenes described to date.*

In Burkitt lymphoma the distal segment (q24 → qter) segment of the long (q) arm of human chromosome 8 translocates to chromosome 14, 22, or 2 (*1–4*). These chromosomes carry the loci for immunoglobulin heavy (H), the λ, and the κ chains, respectively (*5–7*). The c-*myc* oncogene, which is located on band q24 of chromosome 8, translocates to the H chain locus on chromosome 14 in Burkitt lymphomas with the t(8;14) translocation (*8, 9*). In the variants t(8;22) and t(2;8), the c-*myc* oncogene remains on chromosome 8, while the loci for the constant (C) region of the λ and κ chains, respectively, translocate to the involved chromosome 8 and rearrange with a region distal (3′) to the c-*myc* oncogene (*10, 11*). These three different chromosomal rearrangements result in a deregulation of transcription of c-*myc* so that it is expressed constitutively at high levels, while the normal c-*myc* oncogene on the uninvolved chromosome 8 is transcriptionally silent (*10–13*). Since we find that the translocated c-*myc* gene of Burkitt lymphoma is transcribed in Burkitt lymphoma cells and in murine plasmacytoma cells (*14*), but not in hybrids with EBV-transformed lymphoblastoid cells, we have postulated the existence of enhancer elements, within the three immunoglobulin loci genes, which are capable of activating transcription of the involved c-*myc* oncogene at precise stages of B-cell differentiation (*14*).

Other translocations involving 14q have been observed in human lymphoid tumors (*15–17*). A translocation between chromosomes 14 and 18 with breakpoints at 14q32 and 18q21.3 has been described as particularly characteristic of follicular lymphomas (*17*). Similarly, a translocation between chromosomes 11 and 14 with breakpoints at 14q32.3 and 11q13 has been more common in diffuse small cell lymphocytic and diffuse large cell lymphomas (*17*).

The breakpoint on chromosome 14 in these circumstances occurs in the region carrying the H chain locus (*18*). We have used chronic lymphocytic leukemia (CLL) cells of the B-cell type with the t(11;14) (q13;q32) translocation (*19*) in an attempt to clone the chromosomal breakpoint in order to obtain nucleic acid probes to identify and characterize a putative new human oncogene that could be activated by translocation to the H chain locus. This oncogene appears to be unrelated to any of the known retrovirus oncogenes.

Cloning of the joining of chromosomes 11 and 14. The CLL 271 cells, derived from a 65-year-old male with CLL of the B-cell type, carry a t(11;14) (q13;q32) chromosomal translocation (*19, 20*). The complete karyotype of the CLL 271 cells has been reported previously (*19, 20*), with clarification, from multiple studies, of what originally appeared to be a t(9;11;14) translocation. The breakpoints on chromosomes 11 and 14 in this leukemia are similar to those described in other B-cell neoplasms (*15, 17, 19*) (Fig. 1). As shown in Fig. 2, DNA from the CLL 271 cells and from a diffuse large cell lymphoma (LN87), with the same t(11;14) translocation (Fig. 1), showed two rearranged Bam HI restriction fragments hybridizing with an immunoglobulin genomic C region μ chain probe (pCμ 0.9) (*9, 20*) containing a 0.9-kb Eco RI fragment of the human genomic C_μ gene or an H chain joining region (J_H) probe (pHj) (*9, 18*) containing an Eco RI–Hind III fragment of the human genomic C_μ clone (λH18 Cl10) (*9*). Figure 2C shows that CLL 271 DNA has two rearranged Eco RI restriction fragments hybridizing with the J_H probe. DNA from SV40 transformed human fibroblasts (PAF) showed only a single restriction fragment representing the germ line C_μ gene. By using somatic cell hybrids between NP3 mouse myeloma and human CLL 271 cells we have shown that the Bam HI 14-kb C_μ and J_H bands correspond to the productively rearranged gene whereas the 19.5-kb C_μ and J_H bands correspond to the excluded gene on the 14q$^+$ chromosome (*20*).

A full genomic library was prepared from the DNA of CLL 271 cells. Genomic DNA was partially digested with the restriction enzyme Sau 3A, and DNA fragments 15 to 23 kb in length were purified by sucrose gradient centrifugation. The fragments were then ligated with DNA of the λ phage vector EMBL3A (*21*), which was cut with Bam HI. After packaging in vitro, 375,000

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Table 1. Presence of the relevant human chromosomes and of the germ line and of the rearranged *bcl-1* locus in mouse myeloma × CLL 271 hybrids.

Cells	Human chromosomes				*bcl-1*	
	11	11q⁻	14	14q⁺	Germ line locus	Rearranged locus
CLL 271	+	+	+	+	+	+
NP 3	−	−	−	−	−	−
280-1C3-AD4	+	−	+	−	+	−
280-1C3-AE2	+	−	−	−	+	−
280-1C3-AG8-CE4	−	−	−	+	−	+
280-1C3-AG8-EE9	+	−	−	+	+	+

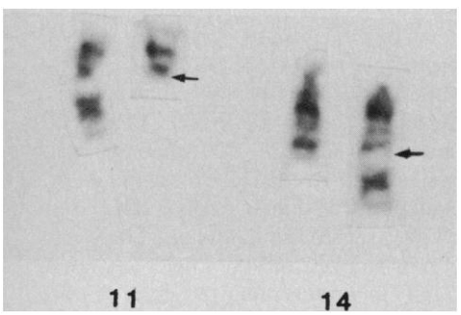
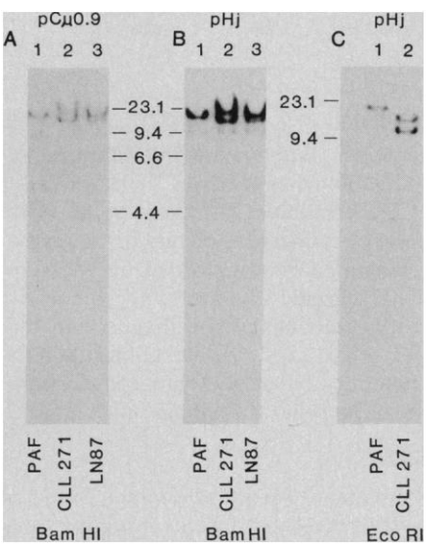


Fig. 1 (left). The reciprocal t(11;14) (q13,q32) translocation in the neoplastic cells of a patient with diffuse large cell lymphoma (LN87). Fig. 2 (right). (A to C) Southern blotting analysis of CLL 271 and LN87 DNA's for rearrangements of the C_μ and J_H DNA segments. PAF cells are SV40 transformed human fibroblasts that carry a germ line C_μ gene. In (A) and (B), the rearranged C_μ and J_H bands have the same size in CLL 271 and LN87 DNA's.



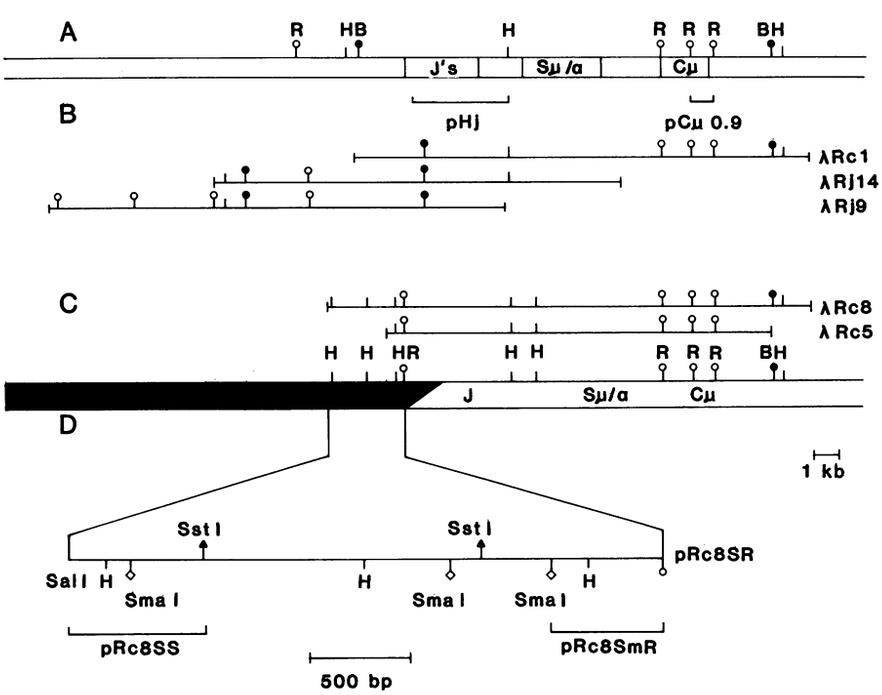


Fig. 3. Restriction maps of the germ line C_μ gene (A), and of the two classes of recombinant clones we have obtained (B and C). The clones shown in (B) represent the productively rearranged C_μ gene while the clones in (C) represent the excluded C_μ allele on the $14q^+$ chromosome. *H*, Hind III; *R*, Eco RI; *B*, Bam HI. The black box represents the chromosome 11–derived sequences, while the white box represents the chromosome 14–derived sequences (D).

independent recombinant phages were screened with the pCμ 0.9 and pHj probes. Ten clones were obtained, and their restriction map analysis allowed us to classify them into two groups that represented the sequences derived from the uninvolved chromosome 14 and from the $14q^+$ chromosome, respectively.

The restriction maps and representative overlapping recombinant clones of each group are shown in Fig. 3, B and C. The recombinant clones shown in Fig. 3B contain the shorter Bam HI fragment hybridizing with the pCμ 0.9 or pHj probes and represent the productively rearranged μ gene on the uninvolved chromosome 14 (*20*). The other group, illustrated in Fig. 3C, represents the excluded μ allele on the $14q^+$ chromosome. As shown in Fig. 3, the restriction maps of the two groups, 5′ of the J_H segments, are completely different from each other and also from that of germ line DNA.

To test whether the recombinant clones of the second group, shown in Fig. 3C, contain the t(11;14) breakpoint, we searched for DNA sequences derived from chromosome 11 within the λRc8 and λRc5 cloned DNA's. As a hybridization probe, we isolated a single-copy DNA sequence without repetitive sequences from the recombinant clones. The longest Sst I–Sst I fragment of the insert of pRc8SR (Fig. 3D) contained repetitive DNA sequences while the other two shorter fragments, the Sal I–Sst I and the Sst I–Eco RI, did not.

The Sal I–Sst I fragment and a Sma I–Eco RI fragment of the Sst I–Eco RI fragment were then subcloned into the plasmid vectors pUC19 (*22*) and pYT13 (*23*), and the subclones were named pRc8SS and pRc8SmR, respectively (Fig. 3D). These two probes were used for hybridization with Southern blots of DNA from somatic cell hybrids between rodent cells and human cells that retained either human chromosome 14 or human chromosome 11. Hybrid J1 is a Chinese hamster × human hybrid cell line containing only human chromosome 11 and no other human chromosome (*24*). Hybrid clone H11 is a mouse × human hybrid containing only human chromosome 11 with the long arm of the human X chromosome (*25*) and no other human chromosome. This hybrid was derived from the fusion of hypoxanthine phosphoribosyltransferase deficient mouse cells with cells derived from an individual with a t(X;11) (q25–q26,q23) translocation (*25*). As shown in Fig. 4, A, B, and C, the DNA of J1, H11, and CLL 271 cells showed the same size

fragment hybridizing with the two probes, while mouse and Chinese hamster DNA did not hybridize with the probes. Furthermore, the pRc8SS probe did not hybridize with DNA from hybrid cells containing human chromosome 14 (Fig. 4A, lane 4). These results prove that the recombinant clones λRc8 and λRc5 contain the joining site between chromosomes 11 and 14 on the $14q^+$ chromosome of CLL 271 cells.

Since an Eco RI digest of CLL 271 DNA showed a single restriction fragment hybridizing with chromosome 11 DNA which is the same as that observed in P3HR-1 Burkitt lymphoma cells with the t(8;14) translocation (Fig. 4A, lane 5), we can infer that the breakpoint on $14q^+$ occurred in a region 3′ to the most 5′ Eco RI site of the λRc8 and λRc5 clones (Fig. 3).

DNA rearrangements associated with the t(11;14) translocation in neoplastic B cells from CLL 271 and LN87. Since the λRc8 clone contains the joining between chromosomes 11 and 14, we might expect that the DNA of CLL 271 cells, cleaved with some restriction enzyme, should show a rearranged fragment after hybridization with a chromosome 11–derived DNA probe mapped close to the breakpoint. The Bam HI digestion of CLL 271 DNA, however, did not show any rearranged fragment after hybridization with the pRc8SS probe (data not shown). This finding can be explained by the comigration of the rearranged and of the germ line fragments. Since the Hind III–Hind III fragment containing J_H sequences of λRc5 and λRc8 DNA also contains chromosome 11–derived sequences (Fig. 3), we should expect to find both germ line and rearranged Hind III fragments hybridizing with the chromosome 11–specific pRc8SmR probe. Since pRc8SmR DNA has the Hind III site within the insert, three hybridizing fragments should be expected in CLL 271. As shown in Fig. 5, all human DNA samples tested, except for the CLL 271 DNA, showed two Hind III fragments (Hind III-2 and -3); the CLL 271 DNA showed one additional rearrangement fragment (Hind III-1). The DNA fragments Hind III-1 and Hind III-3 in CLL 271 DNA are the same as those expected from λRc8 (Fig. 3). Therefore, DNA fragment Hind III-2, of approximately 2.5 kb, apparently represents the normal chromosome 11 sequence counterpart of the rearranged fragment Hind III-1. This indicates that the chromosomal break on $14q^+$ occurs within a region of 2.1 kb 3′ to the most 5′ Eco RI site of λRc8 DNA (Fig. 3D).

In view of these results it became important to determine whether the chromosomal breakpoint involved the same segment of chromosome 11 in the cells of LN87, a diffuse large cell lymphoma with the t(11;14) (q13;q32) translocation (Fig. 1). As shown in Fig. 5 (lane 8) Hind III digestion of LN87 DNA did not show any rearranged fragments. However, digestion with Bcl I showed a rearranged fragment (Bcl I-2) in LN87 cell DNA (Fig. 5, lane 9) as well as in CLL 271 cell DNA (Bcl I-1) (Fig. 5, lane 10). This result indicates that the break-

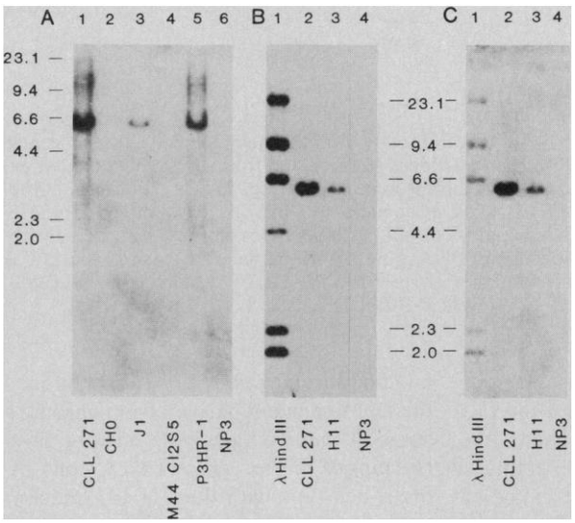


Fig. 4. Hybridization of Eco RI digested human chromosome 11 containing rodent × human hybrid cell DNA's with sequence of pRc8SS and pRc8SmR. Samples of 5 μg of DNA for (A) and 10 μg for (B) and (C) were loaded on the gels. CHO cells are derived from Chinese hamster. J1 cells are human × CHO hybrids containing only human chromosome 11. H11 cells are human × mouse hybrids containing only human chromosomes 11 and Xq. P3HR-1 cells are derived from a Burkitt lymphoma. Hybrid M44 Cl2S5 is a human × mouse cell line containing only the human chromosome $14q^+$ from P3HR-1 Burkitt lymphoma (*9*), and NP3 cells are derived from a mouse myeloma cell line. The DNA samples were digested with Eco RI and Southern blot filters were hybridized with probe pRc8SS (A and B) and with pRc8SmR (C). The final washing of filters was done with 2× SSC (standard saline citrate) at 65°C for (A) and with 0.2× SSC at 65°C for (B) and (C). The molecular size of the Hind III cut λ DNA markers is given in kilobases.

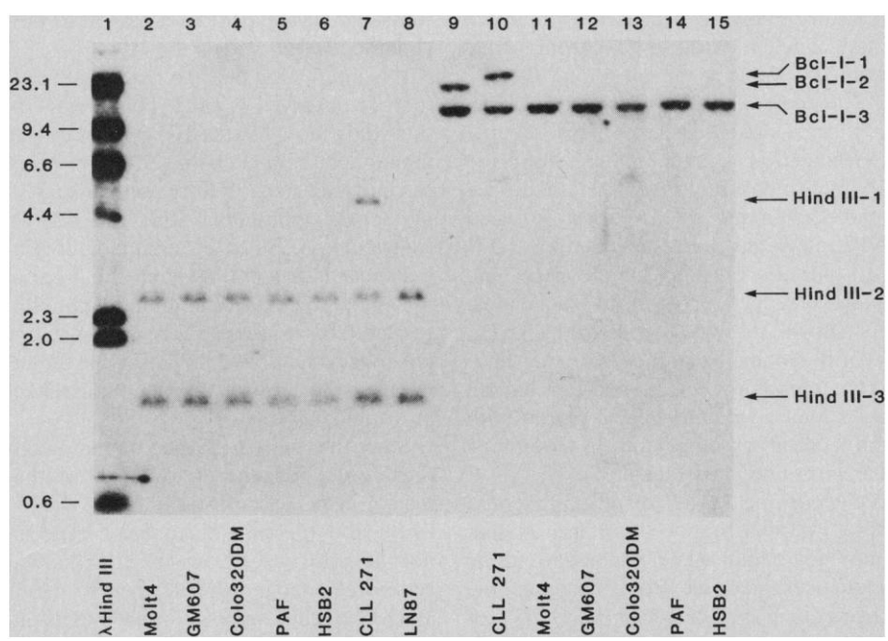
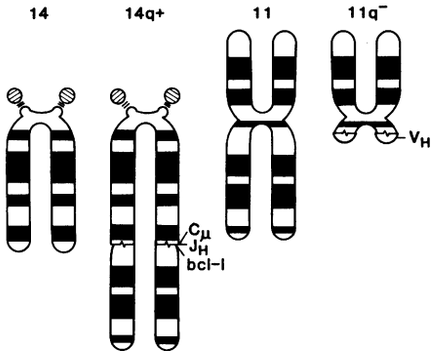


Fig. 5. Southern blot hybridization of CLL 271 and LN87 DNA's with a chromosome 11 specific DNA probe mapping close to the breakpoint of CLL 271 t(11;14). The cellular DNA (5 μg) was digested with Hind III (lanes 2 to 8) and Bcl I (lanes 9 to 15) and fractionated by agarose gel electrophoresis. The Southern blot filter was hybridized with the pRc8SmR probe and finally washed with 0.2× SSC at 65°C. The molecular size of the Hind III cut marker DNA (lane 1) is given in kilobases. Lanes 2 and 11, molt 4 (human T-cell line) DNA; lanes 3 and 12, GM607 (human lymphoblastoid cell line) DNA; lanes 4 and 13, Colo320DM (human carcinoid) DNA; lanes 5 and 14, PAF (SV40 transformed human fibroblast) DNA; lanes 6 and 15, HSB2 (human T-cell line) DNA; lanes 7 and 10, CLL 271 (human B-cell leukemia) DNA; lanes 8 and 9, LN87 (human B-cell lymphoma) DNA.



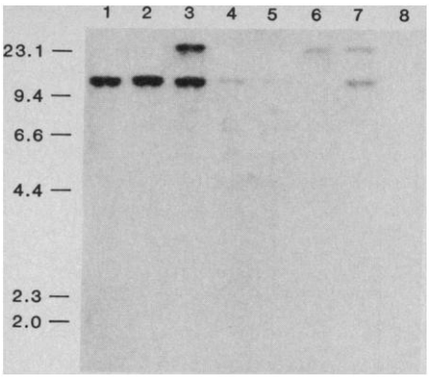


Fig. 6 (left). Diagram of the t(11;14) (q13; q32) translocation observed in B-cell neoplasias. The breakpoint on chromosome 14 involves directly the H chain locus. The V_H genes translocated to the deleted 11 ($11q^-$). The *bcl-1* locus translocated from its normal position on band q13 of chromosome 11 to the involved chromosome 14. Fig. 7 (right). Presence of the germ line and of the rearranged *bcl-1* locus in somatic cell hybrids between NP3 mouse myeloma cells and CLL 271 human leukemic cells (*20*). The Southern blot was hybridized with the pRc8SmR probe. Lane 1, GM607 human DNA; lane 2, PAF human DNA; lane 3, CLL 271 DNA; lane 4, hybrid 280-1C3-AD4 DNA; lane 5, hybrid 280-1C3-AE2 DNA; lane 6, hybrid 280-1C3-AG8-CE4 DNA; lane 7, hybrid 280-1C3-AG8-EE9 DNA; lane 8, NP3 mouse DNA.

point in LN87 occurred in a chromosomal region that is outside the Hind III-2 site (as this is not rearranged) but inside the germ line Bcl I-3 site (as this is rearranged). Therefore, the breakpoint in LN87 must be close to the breakpoint observed in CLL 271 cells. This consistent location of the breakpoints in a case of CLL and a diffuse large cell lymphoma, both with the t(11;14) translocation, suggest that the same gene, which we call *bcl-1* (B-cell lymphoma/leukemia 1), may be involved in a variety of B-cell malignancies with this chromosomal translocation (Fig. 6).

The rearranged bcl-1 *locus is on the* $14q^+$ *chromosome*. To confirm that the rearranged *bcl-1* locus is located on the $14q^+$ chromosome of CLL 271 cells, we studied somatic cell hybrids between NP3 mouse myeloma cells and CLL 271 cells for the presence of the germ line and rearranged *bcl-1* DNA sequences. As shown in Fig. 7 and Table 1, the hybrid clones with the $14q^+$ chromosome contained the rearranged human *bcl-1* locus, whereas hybrid clones with the normal chromosome 11 contained the germ line *bcl-1* locus.

Conclusions. The results presented here indicate that we can use human immunoglobulin gene probes to clone sequences derived from chromosome segments translocated to band q32 of human chromosome 14. Furthermore, the DNA segment derived from chromosome 11 that we have cloned is not only rearranged in the parental CLL cells of the B-cell type with the t(11;14) translocation but is also rearranged in a diffuse large cell lymphoma with the same translocation. These findings are consistent with the involvement of a specific gene, which we call *bcl-1*, that is normally located on band q13 of chromosome 11 and that may become activated by being translocated adjacent to a rearranged H chain locus.

It is interesting that the rearrangement that we observed in the H chain locus in this study involved the J_H segment of the immunoglobulin H chain gene. This type of rearrangement is infrequent in cases of Burkitt lymphoma with the t(8;14) translocation. Since an immunoglobulin enhancer is located between the J_H and the switch region of the immunoglobulin μ gene (*26*), it seems likely that such an enhancer could have an activating effect on the transcription of the juxtaposed *bcl-1* gene (*13*).

Since no known human homolog of retroviral oncogenes is located on the long arm of chromosome 11, it seems likely that the postulated *bcl-1* gene is not homologous to any of the known retroviral oncogenes described to date. While the rearrangement of *bcl-1* locus in B cells carrying the t(11;14) translocation indicates an important role in the neoplastic transformation of these cells, it will be necessary to develop transformation assays in vitro and in vivo to assess conclusively its transforming ability.

A specific chromosomal translocation between chromosomes 14 and 18 is also found in a proportion of human lymphoid tumors. Approaches similar to the one used here may be useful in the isolation and characterization of a gene located on the long arm of chromosome 18 which may be important in the development of these disorders.

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