

Molecular Genetics of Human B Cell Neoplasia

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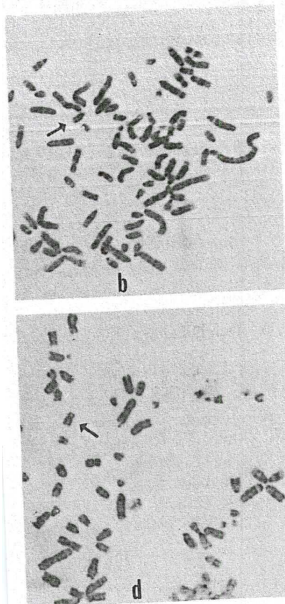
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I. Introduction

Since the discovery of the Philadelphia chromosome in the neoplastic cells of patients with chronic myelogenous leukemia (Nowell and Hungerford, 1960), consistent cytogenetic changes have been detected in numerous malignancies of the hematopoietic system (Rowley, 1973, 1983). Many of these chromosomal alterations consist of reciprocal translocations (Rowley, 1983; Yunis, 1983).

While the consistency of such rearrangements in the various leukemias and lymphomas suggested a possible role for these changes in the pathogenesis of human hematopoietic neoplasms, this could not be demonstrated definitely with available techniques.

Recent developments in the identification and characterization of viral genes capable of inducing tumors in experimental animals, however, have been clearly providing major advances in our understanding of the molecular basis of the neoplastic process and of the cellular genes that may be involved in human cancer. The demonstration that chromosomal segments involved in the specific translocations contain the human homologues of viral oncogenes capable of inducing tumors in experimental animals has opened new avenues in our understanding of the role of specific chromosomal alterations in the development of human neoplastic diseases (Dalla Favera *et al.*, 1982a, 1983; Taub *et al.*, 1982; Erikson *et al.*, 1983a).



(b) t(8;14) (q24;q32) (arrows) and small majority of the cells. (b) Trypsin-Giemsa normal 14 (arrows) and no 14q⁺. (c) 14q⁺ (arrow) and no normal 14. G-11 origin of 14q⁺. (d) Trypsin-Giemsa an 8q⁻ (arrow) and no 14 or 14q⁺.

B Cell Neoplasia

onsistent chromosome marker lymphoma, a B cell neoplasm r (14q⁺) derives from a chromon added at the tip of the long sociates (1976) found that the on of the distal end of the long o the distal end of the long (q) was found in cases of Burkitt e tumor is endemic, and from urope and America. ed to have a role in the patho-

genesis of Burkitt lymphoma, since approximately 98% of cases of African Burkitt lymphoma carry the DNA sequences of this virus that can immortalize B cells *in vitro* (Klein, 1981). The great majority (approximately 90%) of Burkitt lymphomas outside Africa, however, do not contain the Epstein-Barr virus genome, suggesting that the virus per se is not essential for the development of this tumor.

Until a few years ago it was thought that the t(8;14) translocation was the hallmark of Burkitt lymphomas. During the last few years, however, several investigators have described variant chromosome translocations in some cases from different countries (Van den Berghe *et al.*, 1979a; Lenoir *et al.*, 1982). These variant translocations involve the same segment of chromosome 8, but instead of translocating to chromosome 14, this segment is translocated to either the long arm of human chromosome 22 (band q11) or to the short arm of chromosome 2 (band p11.2) (Van den Berghe *et al.*, 1979b; Lenoir *et al.*, 1982; Emanuel *et al.*, 1984). Thus, the common denominator of all cases of Burkitt lymphomas is a translocation of the distal end (band q24-→qter) of the long arm of chromosome 8 to either chromosome 14 (band q32), 22 (band q11), or 2 (band p11.2). Approximately 75% of Burkitt lymphomas carry the t(8;14) translocation, 16% carry the t(8;22) translocation and 9% carry the t(2;8) translocation. Somatic cell genetic studies have shown that the three immunoglobulin loci for heavy chains, λ light chains and κ light chains, are located on human chromosomes 14 (Croce *et al.*, 1979), 22 (Erikson *et al.*, 1981), and 2 (Malcolm *et al.*, 1982; McBride *et al.*, 1982), respectively, strongly suggesting a relationship between chromosome translocations and immunoglobulin genes in Burkitt lymphoma (Erikson *et al.*, 1981). The same reciprocal translocation involving human chromosome 8 and chromosome 14 has also been detected in very aggressive human acute lymphocytic leukemias (L3 according to FAB classification) of the B cell type.

Translocations involving human chromosome 14 and different "donor" chromosomes have been observed in other B cell neoplasms of adults. In a fraction of human chronic lymphocytic leukemias of the B cell type, in diffuse large cell and small cell lymphoma, and in a fraction of cases of multiple myeloma the reciprocal chromosome translocation involves human chromosomes 11 (band q13) and 14 (band q32) (Van den Berghe *et al.*, 1984). In follicular lymphomas, the reciprocal chromosomal translocation often involves human chromosomes 14 (band q32) and 18 (band q21) (Yuns, 1983). Thus, it can be concluded that translocations involving the chromosomal regions carrying the immunoglobulin loci are observed quite often in B cell neoplasms of humans.

Similar chromosomal translocations have also been observed in B cell tumors of other mammals. In mineral oil induced myelomas of mice and rats,

chromosomes carrying immunoglobulin genes are commonly involved in translocations (Klein, 1981). In mineral oil-induced multiple myeloma of the mouse the reciprocal translocations involve either chromosome 12 (that carries the heavy chain locus) and chromosome 15, or chromosome 6 (that carries the κ chain locus) and chromosome 15 (Klein, 1981).

In order to determine whether the human immunoglobulin genes are directly involved in the chromosomal rearrangements observed in Burkitt lymphomas, we have used somatic cell hybridization techniques to segregate the relevant human chromosomes on a mouse myeloma chromosome background (Erikson *et al.*, 1982).

As shown in Fig. 1 the human chromosomes involved in the t(8;14) chromosomal translocations segregated in the various hybrid clones, allowing the determination that while the genes for the variable regions of heavy chains are translocated to the involved chromosome 8 (8q⁻) the genes for the constant regions of heavy chains remain on the involved chromosome 14 (14q⁺) (Erikson *et al.*, 1982). This could be achieved by Southern blotting analysis of hybrid cell DNAs with nucleic acid probes specific for the variable and the constant regions of heavy chains (Erikson *et al.*, 1982). These results indicate that the chromosome breakpoints in Burkitt lymphoma with the t(8;14) chromosome translocation involve directly the heavy chain locus and that the genes for the variable regions of heavy chains are more distal than the genes for the constant regions on band q32 of chromosome 14 (Erikson *et al.*, 1982). Since hybrids containing the normal human chromosome 14 expressed human heavy chains, while hybrids containing the 14q⁺ did not (Erikson *et al.*, 1982) it can also be concluded that the chromosomal translocation involves the excluded heavy chain locus (Erikson *et al.*, 1982, 1983a).

III. Translocation of the *c-myc* Oncogene in Burkitt Lymphomas with the t(8;14) Chromosome Translocation

By taking advantage of nucleic acid probes specific for the human homologs of retroviral (Dalla Favera *et al.*, 1982b) oncogenes and rodent-human somatic cell hybrids, it has been possible to determine the chromosomal location of several human oncogenes (Dalla Favera *et al.*, 1982a, 1983). Of particular importance was the chromosomal localization of the human homolog of the *c-myc* oncogene, since the *myc* containing avian myelocytomatosis virus can induce B cell lymphomas in chicken (Duesberg, 1979; Hayward *et al.*, 1981). Analysis of the DNA of a panel of somatic cell hybrids between rodent and human cells indicated that the *c-myc* oncogene is located on human chromosome 8 (Dalla Favera *et al.*, 1982a). In order to determine whether the oncogene is located in that small segment of chromosome 8 that is

involved in Burkitt lymphomas carrying the 14q⁺ some 8 and of the 8q⁻ *v* oncogene. The results of consistently translocated lymphomas with the t(8;14) (Erikson *et al.*, 1983; Erikson *et al.*, 1982).

Restriction enzyme analysis of *myc* DNA probe indicate: *c-myc* oncogene is structurally shows a germ line pattern (1982). When the *c-myc* head fashion (5' to 5') genes, predominantly C (1982).

Considerable heterogeneity in Burkitt lymphomas with the t(8;14) chromosome translocation. On chromosome 14 the genes (Erikson *et al.*, 1983) and in the switch region (Showe *et al.*, 1985) of the breakpoint may occur at or it may involve either shown in Fig. 3 the *c-myc* exon contains termination represents an untranslated (1983). The first ATC beginning of the second encoding for a protein *c* oncogene has also two cleotides, and the *c-myc* (Fig. 4) (Watt *et al.*, 1982).

When the *c-myc* gene is translocated, new cleotides and the *c-myc* transcribed intron (ar-Rushdi *et al.*, 1982). DNA region in front of codons on all three reading frames the *c-myc* oncogene is Recently, Rabbitts *et al.* of the *c-myc* oncogene. Burkitt lymphomas and are observed in

are commonly involved in multiple myeloma of the chromosome 12 (that carries the heavy chain), or chromosome 6 (that carries the light chain) (Erikson, 1981).

Immunoglobulin genes are rearranged in Burkitt lymphoma. Techniques to segregate chromosomal back-

ground involved in the t(8;14) chromosome translocation in hybrid clones, allowing the identification of the regions of heavy chains (8q⁺) and the genes for the involved chromosome 14 (14q⁺) by Southern blotting using probes specific for the variable regions of the immunoglobulin genes (Erikson *et al.*, 1982). These results in Burkitt lymphoma with the t(8;14) translocation show that the heavy chain locus and the light chain locus are more distal than the chromosome 14 (Erikson *et al.*, 1982). Human chromosome 14 containing the 14q⁺ did not undergo the chromosomal translocation (Erikson *et al.*, 1982).

Lymphomas with the t(8;14)

Comparison of the human homologs of the c-myc gene and rodent-human hybrids to determine the chromosomal location of the human homolog of the c-myc gene. Of the human homologs of the c-myc gene, the avian myelocytomatosis virus (RC-1) (Riesberg, 1979; Hayward *et al.*, 1983) and the human c-myc gene are located on chromosome 8. In order to determine the chromosomal location of chromosome 8 that is

involved in Burkitt lymphoma with the t(8;14) translocation, somatic cell hybrids carrying the 14q⁺ chromosome in the absence of the normal chromosome 8 and of the 8q⁺ were studied for the presence of the human c-myc oncogene. The results of these studies indicated that the c-myc oncogene is consistently translocated to the involved human chromosome 14 in Burkitt lymphomas with the t(8;14) translocation (Fig. 2) (Dalla Favera *et al.*, 1982a, 1983; Erikson *et al.*, 1983a).

Restriction enzyme analysis of DNA of Burkitt lymphoma cells using a c-myc DNA probe indicates that in approximately 50% of the cases studied the c-myc oncogene is structurally rearranged, while, in the remaining 50% it shows a germ line pattern (Dalla Favera *et al.*, 1982a, 1983; Taub *et al.*, 1982). When the c-myc gene is rearranged it is recombined in a head-to-head fashion (5' to 5') with one of the immunoglobulin constant region genes, predominantly C_μ (Fig. 2) (Dalla Favera *et al.*, 1982a, 1983; Taub *et al.*, 1982).

Considerable heterogeneity of breakpoints is observed in Burkitt lymphomas with the t(8;14) chromosome translocation (Croce and Nowell, 1985). On chromosome 14 the breakpoint may occur in the region carrying V_H genes (Erikson *et al.*, 1982), in the joining (J_H) segment (Dalla Favera *et al.*, 1983) and in the switch regions (Taub *et al.*, 1982; Dalla Favera *et al.*, 1983; Showe *et al.*, 1985) of the heavy chain genes (Fig. 3). On chromosome 8 the breakpoint may occur at variable distances from the 5' end of the c-myc gene or it may involve either the first c-myc exon or the first c-myc intron. As shown in Fig. 3 the c-myc gene is formed by three separated exons. The first exon contains termination codons on all three reading frames and therefore represents an untranslated leader sequence (Watt *et al.*, 1983a,b; Leder *et al.*, 1983). The first ATG (methionine) signal for protein synthesis is at the beginning of the second exon and it is followed by an open reading frame encoding for a protein of 439 amino acids (Watt *et al.*, 1983a). The c-myc oncogene has also two promoters separated by approximately 160 nucleotides, and the c-myc transcripts initiate from two different initiation sites (Fig. 4) (Watt *et al.*, 1983b; ar-Rushdi *et al.*, 1983).

When the c-myc gene is decapitated by the chromosomal break leading to the translocation, new cryptic promoters are activated within the c-myc first intron and the c-myc transcripts initiate from different sites within the first intron (ar-Rushdi *et al.*, 1983) (Fig. 4). As shown by Colby *et al.* (1983), the DNA region in front of the c-myc second exon also contains termination codons on all three reading frames and it is noncoding. Therefore, whether the c-myc oncogene is rearranged or not the c-myc protein is the same. Recently, Rabbitts *et al.* (1984) have reported mutations in the coding exons of the c-myc oncogene. Such alterations are not the rule in Burkitt lymphomas and are observed in only a minority of cases, indicating that alterations

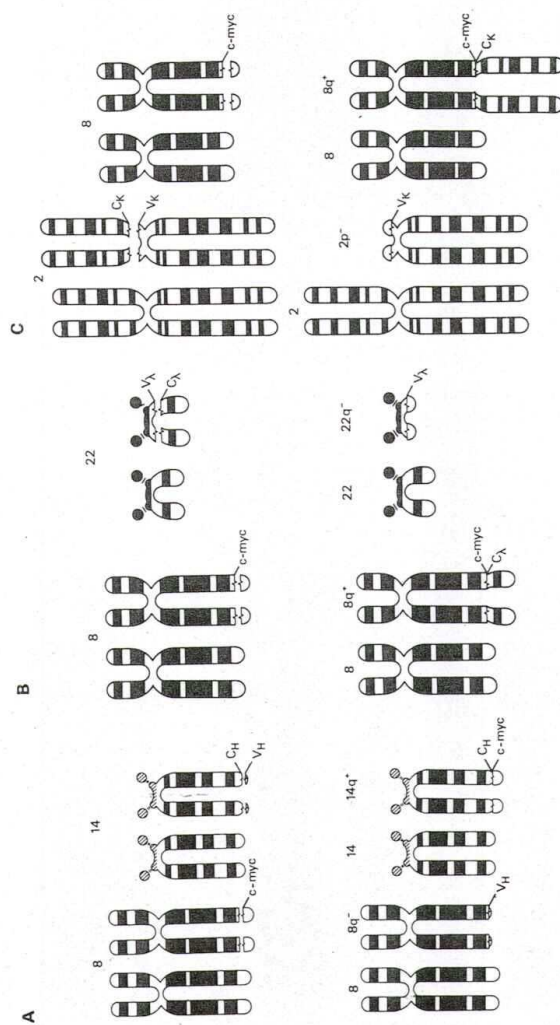


FIG. 2. Diagram of the t(8;14) (A), t(8;22) (B), and t(2;8) (C) chromosome translocations observed in Burkitt's lymphoma.

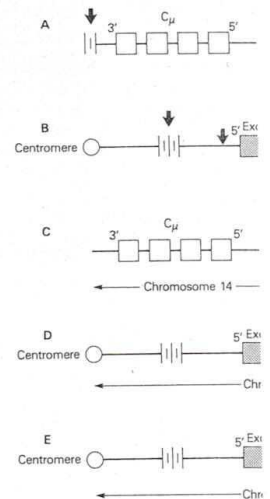


FIG. 3. DNA rearrangement t(8;14) translocation the chromosome region carrying V_H genes, in the J_H , in a switch region, and may. The arrows indicate possible site enhancer (E) is indicated. (B) In mosomal breakpoints on chromosome oncogene (thick arrows). In some break and the first exon of the ge translocate to chromosome 14 (se translocations the breakpoints are Burkitt lymphoma with the t(8;14), the *c-myc* oncogene are involved *c-myc* and of the C_μ genes are in the t(8;22) translocation the *c-m* translocates to a chromosomal re lymphomas with the t(2;8) trans while the κ locus translocates to a

of the *c-myc* coding sequence.

Figures 2 and 3 summarize Burkitt lymphomas with mosomal breakpoint on hu *c-myc* coding exons (exons

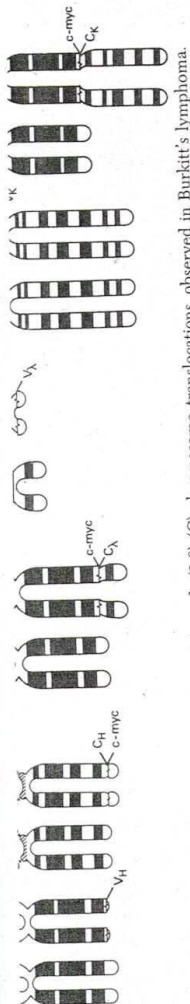


FIG. 2. Diagram of the t(8;14) (A), t(8;22) (B), and t(2;8) (C) chromosome translocations observed in Burkitt's lymphoma.

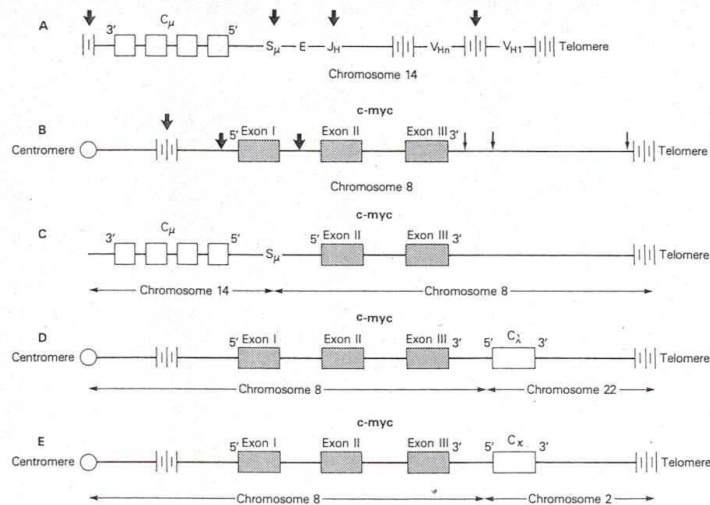


FIG. 3. DNA rearrangements in Burkitt lymphoma. (A) In Burkitt lymphomas with the t(8;14) translocation the chromosomal breakpoints within the heavy chain locus may occur in the region carrying V_H genes, in the region between J_H and V_H , in the heavy chain joining segment (J_H), in a switch region, and may involve a different heavy chain gene such as C_μ , C_γ , and C_α . The arrows indicate possible sites for chromosomal break. The position of the heavy chain enhancer (E) is indicated. (B) In Burkitt lymphomas with the t(8;14) translocation the chromosomal breakpoints on chromosome 8 are 5' of the two coding exons (II and III) of the *c-myc* oncogene (thick arrows). In some cases the *c-myc* oncogene is decapitated by the chromosomal break and the first exon of the gene remains on the 8q- chromosome, while the coding exons translocate to chromosome 14 (see C). In Burkitt lymphomas with the variant t(8;22) and t(2;8) translocations the breakpoints are distal to the *c-myc* oncogene (thin arrows). (C) Example of a Burkitt lymphoma with the t(8;14) translocation and a rearranged *c-myc* gene. The C_μ gene and the *c-myc* oncogene are involved in a head-to-head rearrangement. The transcription of the *c-myc* and of the C_μ genes are in opposite directions (5'→3'). (D) In Burkitt lymphomas with the t(8;22) translocation the *c-myc* oncogene remains on chromosome 8, while the λ locus translocates to a chromosomal region 3' (distal) to the *c-myc* oncogene (see B). (E) In Burkitt lymphomas with the t(2;8) translocation the *c-myc* oncogene also remains on chromosome 8, while the κ locus translocates to a chromosomal region 3' (distal) to the *c-myc* oncogene (see B).

of the *c-myc* coding sequences are not necessary for malignant transformation.

Figures 2 and 3 summarize the findings concerning the genetic changes in Burkitt lymphomas with the t(8;14) translocation. Note that the chromosomal breakpoint on human chromosome 8 is always 5' (proximal) to the *c-myc* coding exons (exons II and III) (Figs. 2 and 3).

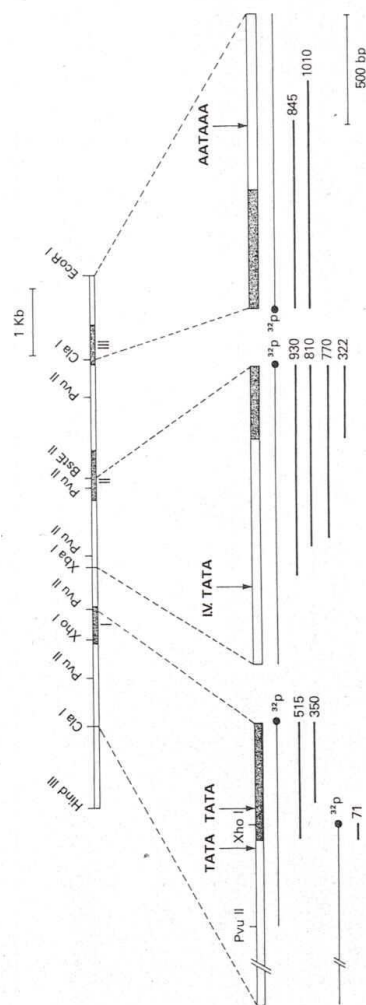


FIG. 4. Schematic representation of DNA probes used for S1 nuclease analysis. The structure of the human *c-myc* genomic DNA is shown schematically according to previous data. A pBR322 subclone, pMyc41-HE carrying the 8.3 kb *HindIII*-*EcoRI* DNA fragment shown in this figure, was used to prepare various S1 probes. A double-stranded 1.3 kb *ClaI*-*XhoI* fragment, 5' 32 P labeled at the *XhoI* site within the first exon, and a 0.8 kb fragment 5' 32 P labeled at the *PvuII* site, were used to analyze the initiation sites. The probe used for S1 mapping analysis to detect the novel initiation sites or cryptic splicing sites within the intervening sequences between the first and second exons was a double-stranded 1.4 kb *XbaI*-*BstEII* fragment, 5' 32 P labeled at the *BstEII* site within second exon. The probe used for S1 mapping analysis of the 3' end of the *c-myc* messages was a 1.4 kb DNA fragment, *ClaI*-*EcoRI*, labeled with 32 P at the 3' end. The location and size of S1 nuclease-resistant DNA products are shown together by the solid bars in the diagram. The approximate location of the authentic TATA boxes found by us and another "TATA box-like" sequence (1.V. TATA) found within the first intron are indicated. The location of the recognition signal sequence (AATAAA) for polyadenylation found by Colby *et al.* (1983) is indicated.



FIG. 5. Detection of the *c-myc* message in human lymphoma cells and human lymphoma cells cleaved with *BclI* and 5' 32 P-enriched 80% formamide to 20 μ g of cytoplasm by electrophoresis in a 7 M urea gel. Lymphoma cells Daudi, CA46, from GM607, GM2669, and G1 leukemia cell line HL-60. Labeled. The size of the probe nucleotides.

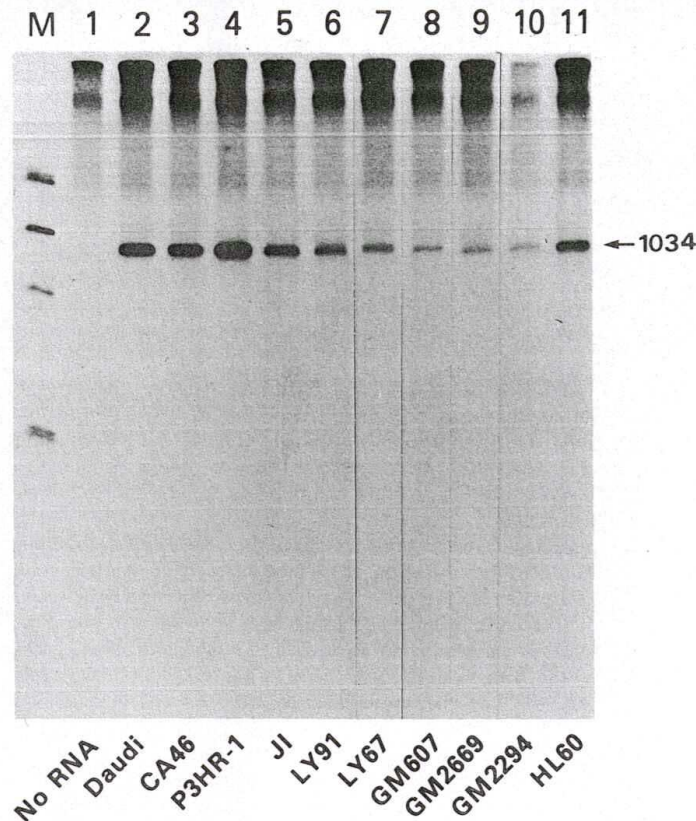


FIG. 5. Detection of the transcripts produced from the *c-myc* gene in various Burkitt lymphoma cells and human lymphoblastoid cell lines by S1 nuclease analysis. The probe, cleaved with *Bcl*I and 5' 32 P-end-labeled pRyc 7.4 plasmid, was heat denatured, hybridized in 80% formamide to 20 μ g of cytoplasmic RNA at 55°C, digested with S1 nuclease, and analyzed by electrophoresis in a 7 M urea 4% polyacrylamide gel. Lanes 2–7, RNAs were from Burkitt lymphoma cells Daudi, CA46, P3HR-1, JI, LY91, and LY67, respectively. Lanes 8–10, RNAs from GM607, GM2669, and GM2294 lymphoblastoid cells. Lane 11, RNA from promyelocytic leukemia cell line HL-60. Lane M, size marker: ϕ X174 digested with *Hae*III and 5' 32 P-end labeled. The size of the protected DNA fragment by using the pRyc 7.4 probe was 1034 nucleotides.

FIG. 4. Schematic representation of DNA probes used for S1 nuclease analysis. The 8.3 kb *Hind*III–*Eco*RI DNA fragment shown schematically according to previous data. A pBR322 subclone, pMyel1, HE carrying the 8.3 kb *Hind*III–*Eco*RI DNA fragment shown in this figure, was used to prepare various S1 probes. A double-stranded 1.3 kb *Clal*–*Xba*I fragment, 5' 32 P labeled at the *Xba*I site within the first exon, and a 0.8 kb fragment 5' 32 P labeled at the *Pvu*II site, were used to analyze the initiation sites. The probe used for S1 mapping analysis to detect the novel initiation sites or cryptic splicing sites within the intervening sequences between the first and second exons was a double-stranded 1.4 kb *Xba*I–*Bst*II fragment, 5' 32 P labeled at the *Bst*II site within second exon. The probe used for S1 nuclease analysis of the 3' end of the *c-myc* messages was a 1.4 kb DNA fragment, *Clal*–*Eco*RI, labeled with 32 P at the 3' end. The location and size of S1 nuclease-resistant DNA products are shown together by the solid bars in the diagram. The approximate location of the authentic TATA boxes found by us and another "TATA box-like" sequence (1.V. TATA) found within the first intron are indicated. The location of the recognition signal sequence (AATAAA) for polyadenylation found by Colby *et al.* (1983) is indicated.

IV. Deregulation of the Translocated *c-myc* Oncogene in Burkitt Lymphoma

If the *myc* gene product is the same in Burkitt lymphoma cells and in human normal B cells, what are the consequences of the t(8;14) chromosome translocation leading to malignant transformation? By examining the steady state levels of human *myc* transcripts in different Burkitt lymphoma cell lines, we found that these levels are quite variable from case to case but are generally elevated (Erikson *et al.*, 1983a; Nishikura *et al.*, 1983). As shown in Fig. 5 the *c-myc* DNA levels in Burkitt lymphomas are similar to those observed in the HL-60 human promyelocytic leukemia cell line where the *c-myc* gene is present in approximately 40 copies per cell because of gene amplification (Dalla Favera *et al.*, 1982a; Nowell *et al.*, 1983) and higher than in three EBV transformed human lymphoblastoid cell lines which were nontumorigenic in nude mice (Nishikura *et al.*, 1983) (Fig. 5). The difficulty with the experiment just described is that we do not know what are the normal counterparts of the Burkitt lymphoma cells that were used in this study. Therefore, it was impossible to compare the *c-myc* RNA levels in malignant and normal cells at the same stage of B cell differentiation.

In order to overcome this problem, we decided to determine whether there is a difference in the levels of transcripts of the normal versus the translocated *c-myc* oncogene in the same cells. Thus, we introduced either the normal *c-myc* gene on chromosome 8 or the translocated *c-myc* oncogene on the 14q⁺ chromosome into the same mouse myeloma cells by using somatic cell hybridization techniques (Nishikura *et al.*, 1983). As shown in Fig. 6 the normal *c-myc* gene is not expressed in a mouse myeloma background, while the translocated *c-myc* gene is expressed at high levels (Nishikura *et al.*, 1983). These results indicate that there is a fundamental difference between the expression of the normal and the translocated *c-myc* gene: the normal *c-myc* gene is transcriptionally silent in the background of terminally differentiated B cells, but the translocated *c-myc* gene is expressed constitutively at elevated levels (Nishikura *et al.*, 1983; Croce *et al.*, 1983).

To determine whether the normal *c-myc* oncogene is capable of responding to normal transcriptional regulation in a terminally differentiated B cell background we have hybridized human lymphoblastoid cells in which the *c-myc* oncogene is in its germ line configuration on a normal chromosome 8 and is expressed at moderately elevated levels with mouse myeloma cells. The introduction of the active *c-myc* oncogene derived from a lymphoblastoid cell into a mouse myeloma cell results in its repression (Nishikura *et al.*, 1983). Thus we conclude that while the normal *c-myc* gene is capable of responding to normal transcriptional control in a differentiated B cell, the translocated *c-myc* gene fails to respond to these normal control mechanisms

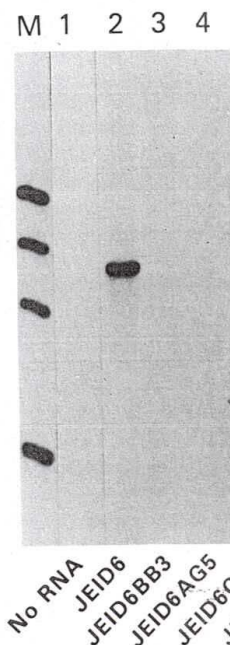


FIG. 6. Expression of the *c-myc* gene in hybrids with mouse myeloma. The expression of the human *c-myc* gene can be protected only by human myeloma parental cells expressing high levels of human *myc* transcripts (lanes 7 and 9). In the case of an NP3 × Burkitt hybrid (JE14q⁺), as shown in lane 2, high levels of human *myc* transcripts were detected. The hybrid JE1D6 was subcloned as JE1D6BB3 and JE1D6AG5 (lanes 3 and 4) but not the normal 8 (JE1D6CC4) hybrid subclones with only chromosome 8 (lanes 5 and 6). In lane 8 is the normal chromosome 8 and low levels of *c-myc* expression in Daudi cells which has retained

ine in Burkitt Lymphoma

it lymphoma cells and in of the t(8;14) chromosome

By examining the steady it Burkitt lymphoma cell from case to case but are *et al.*, 1983). As shown in mas are similar to those kemia cell line where the per cell because of gene *al.*, 1983) and higher than id cell lines which were 83) (Fig. 5). The difficulty o not know what are the lls that were used in this the *c-myc* RNA levels in cell differentiation.

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ene is capable of respond- nally differentiated B cell lastoid cells in which the n a normal chromosome 8 ith mouse myeloma cells. derived from a lympho- ts repression (Nishikura *et* l *c-myc* gene is capable of differentiated B cell, the ormal control mechanisms

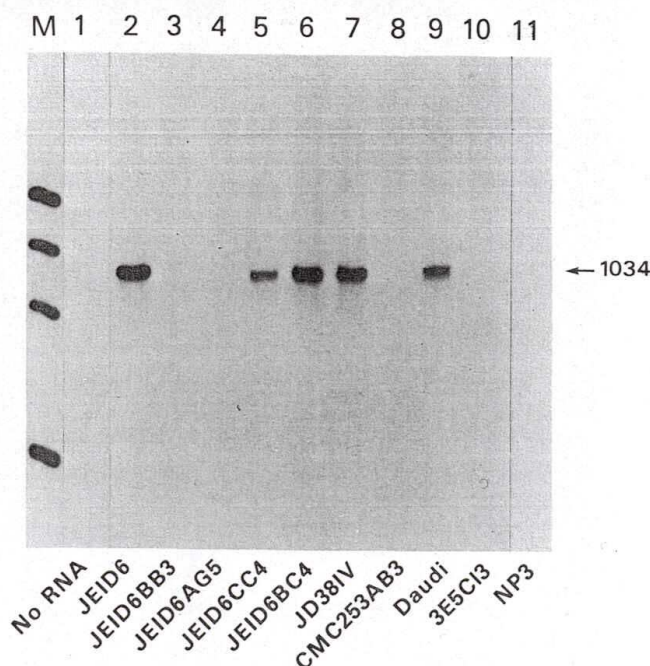


FIG. 6. Expression of the *c-myc* oncogene on the normal chromosome 8 and on the 14q⁺ in hybrids with mouse myeloma. An S1 nuclease protection assay was used to quantitate the expression of the human *c-myc* transcripts in the hybrids. By this method a human *c-myc* cDNA clone can be protected only by human *myc* RNA and not by mouse *myc* RNA. The NP3 mouse myeloma parental cells express high levels of mouse *myc* transcripts (Nishikura *et al.*, 1983). As shown in lane 11, the RNA derived from NP3 does not protect the human *myc* cDNA clone. On the contrary we detect the expression (the protected fragment is 1034 nucleotides in length) of high levels of human *myc* transcripts in two lymphomas, JD38IV and Daudi, carrying the t(8;14) translocation (lanes 7 and 9). In lane 2 is the result of the protection experiment using the RNA of an NP3 × Burkitt hybrid (JE1D6) that contains both the normal chromosome 8 and the 14q⁺. As shown in lane 2 high levels of human *myc* transcripts are expressed in this hybrid. The hybrid JE1D6 was subcloned and hybrid subclones carrying chromosome 8 but not 14q⁺ (JE1D6BB3 and JE1D6AG5) (lanes 3 and 4) and hybrid subclones carrying chromosome 14q⁺ but not the normal 8 (JE1D6CC4 and JE1D6BC4) were analyzed. As shown in Fig. 6, the two hybrid subclones with only chromosome 6 did not express *myc* transcripts (lanes 3–4), while the two hybrid subclones with the 14q⁺ chromosome expressed high levels of human *myc* transcripts (lanes 5 and 6). In lane 8 is a hybrid between JD38IV and NP3 cells which has retained the normal chromosome 8 and lost the 14q⁺ chromosome. In lane 10 is a hybrid between NP3 and Daudi cells which has retained the normal chromosome 8 and lost the 14q⁺ chromosome.

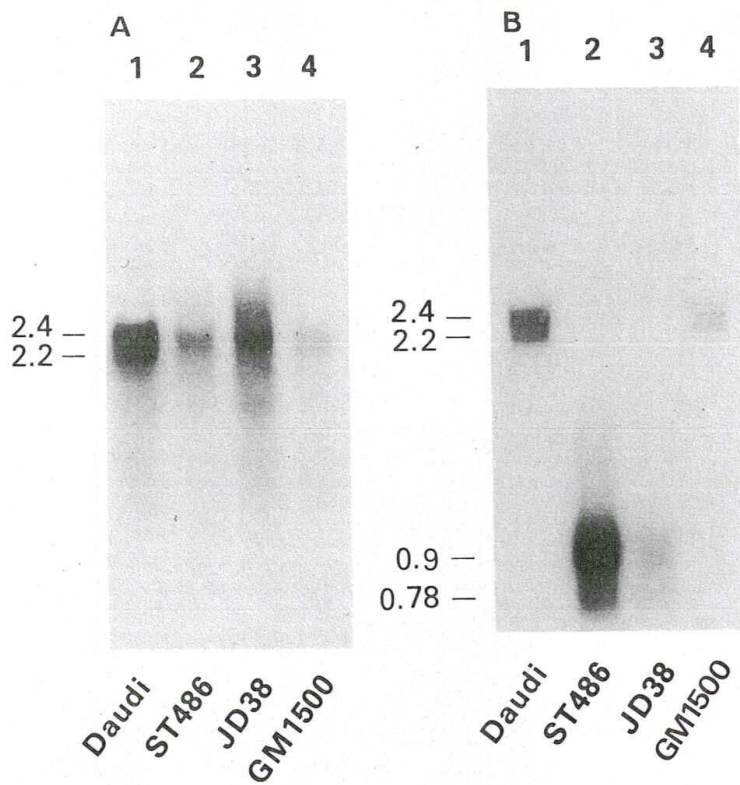


FIG. 7. (A,B) Northern blotting analysis of RNA from three Burkitt's lymphoma cell lines and from an Epstein-Barr virus-transformed lymphoblastoid cell line (GM1500). Polyadenylated RNA was extracted, and 5 μ g of RNA was added to each lane of 1.4% agarose gel. After agarose gel electrophoresis and transfer to nitrocellulose filters, the RNA was hybridized (A) with the Ryc 7.4 probe specific for the two coding exons and (B) with the 5' exon probe. Lanes 1, 2, and 3 show the RNA from the lymphoma cell lines Daudi, ST486 and JD38 IV, respectively. Lane 4 shows the RNA from GM1500 lymphoblastoid cells. All cell lines showed the 2.4 to 2.2 kb *c-myc* transcripts with the Ryc 7.4 probe, whereas the 2.4 to 2.2 kb *c-myc* transcripts were detected only in Daudi and GM1500 cells with the 5' exon probe. We detected 0.9 to 0.7 transcripts hybridizing with the 5' exon probe in the two lymphoma cell lines with a rearranged *c-myc* oncogene (ST486 and JD38 IV).

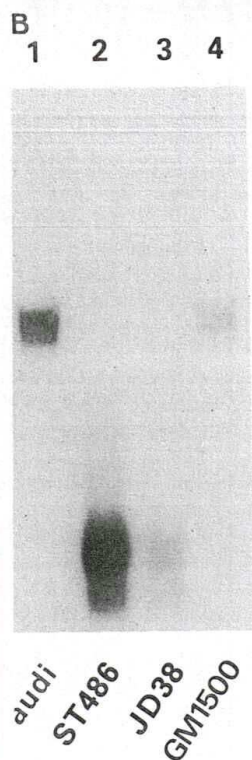
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V. Molecular Genetic

By analyzing somatic
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As shown in Fig. 2, w
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Burkitt's lymphoma cell lines (GM1500). Polyadenylated RNA of 1.4% agarose gel. After electrophoresis, the RNA was hybridized (A) with the 5' exon probe. Lanes 1, ST486 and JD38 IV, respectively. All cell lines showed the 2.4 to 2.2 kb *c-myc* transcripts probe. We detected 0.9 to 0.7% of the total RNA in cell lines with a rearranged

which suppress *c-myc* transcription in the context of a terminally differentiated B cell (Nishikura *et al.*, 1983).

Analysis of Burkitt lymphomas carrying a decapitated *c-myc* oncogene translocated to chromosome 14 also indicates that while the translocated *c-myc* gene is expressed at elevated levels, the normal *c-myc* gene is transcriptionally silent in the neoplastic B cells (ar-Rushdi *et al.*, 1983). As shown in Fig. 7A and B hybridization of RNA derived from two lymphomas carrying the t(8;14) chromosome translocation and a decapitated *myc* with a first exon probe indicates that they do not express normal *myc* transcripts (lanes 2 and 3). On the contrary the first exon probes detect 2.2–2.4 *c-myc* transcripts in Daudi Burkitt lymphoma cells in which the translocated *myc* is not decapitated (Dalla Favera *et al.*, 1982a) (lane 1) and also in human lymphoblastoid cells (lane 4) (ar-Rushdi *et al.*, 1983). Expression of the normal *c-myc* oncogene has been detected in few Burkitt lymphoma cell lines (Rabbitts *et al.*, 1984), but the levels of transcripts of the uninvolved *c-myc* gene are much lower than those of the translocated *c-myc* gene (Croce *et al.*, 1985). Rabbitts *et al.* (1984) have speculated that the *c-myc* product is capable of autoregulating *c-myc* transcription and that the lack of expression of the normal *c-myc* allele is due to such autoregulation. This interpretation, however, is not consistent with the observation that in somatic cell hybrids between Daudi cells and human lymphoblastoid cells, both the translocated and the normal *c-myc* genes are transcribed (Croce *et al.*, 1985).

Interestingly, Adams *et al.* (1983) have also shown the lack of expression of the normal *c-myc* gene in mouse plasmacytomas. It was shown previously that such neoplastic B cells have a translocation between chromosomes 12 and 15 (Klein, 1981) where mouse chromosome 12 carries the heavy chain locus and mouse chromosome 15 carries the *c-myc* oncogene (Taub *et al.*, 1982; Marcu *et al.*, 1983).

V. Molecular Genetics of the Variant Chromosome Translocations in Burkitt Lymphoma

By analyzing somatic cell hybrids between mouse myeloma cells and Burkitt lymphoma cells carrying either that t(8;22) or the t(2;8) chromosome translocation, it has been possible to follow the segregation of the relevant human chromosomes and to understand the genetic rearrangements occurring in Burkitt lymphoma cells with the variant chromosomal translocations. As shown in Fig. 2, we have shown that in Burkitt lymphomas with the t(8;22) translocation the *c-myc* oncogene is not translocated but remains on chromosome 8 in its germ line configuration (Croce *et al.*, 1983). Interestingly, the excluded lambda locus translocates to a region 3' (distal) to the *c-myc* oncogene (Croce *et al.*, 1983). The breakpoint may occur a few hun-

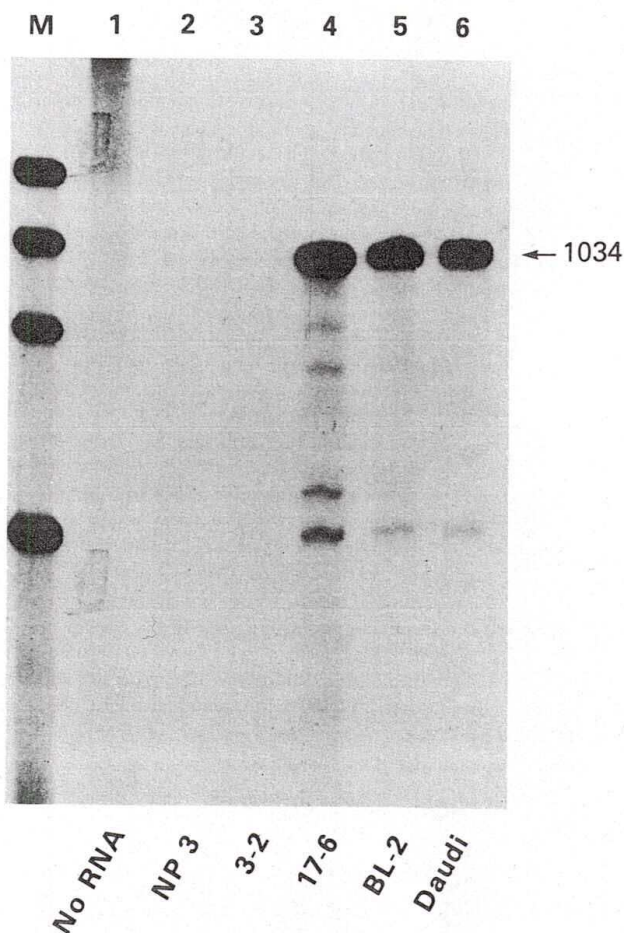


FIG. 8. S1 nuclease protection experiment using a human *c-myc* cDNA probe (Ryc 7.4) specific for the second and third exon of the *c-myc* gene. The human *myc* RNA protects a DNA fragment 1034 nucleotides long. Hybrid BL 17-6, which carries the 8q⁺ chromosome, expresses high levels of *c-myc* transcripts (lane 4). On the other hand hybrid BL 3-2, which contains the normal 8, the normal 22, and the normal 22q⁻ chromosomes, does not express human *myc* transcript (lane 3). The parental BL2 Burkitt Lymphoma cells and Daudi Burkitt lymphoma cells that carry a t(8;14) chromosome translocation express high levels of human *c-myc* transcripts (lanes 5 and 6, respectively). Lane M, marker DNAs.

dred nucleotides or a few Erikson and Croce, 1985). *c-myc* oncogene (Lee and between mouse myeloma c t(8;22) translocation indica chromosome is expressed : oncogene on the normal cl al., 1983) (Fig. 8). Therefore which is involved in the even if it is not translocate is distal to the activated c indicate that the orientatio the heavy chain locus in

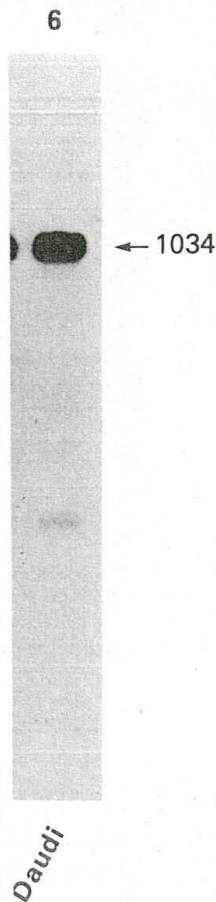
We have also examined mosome translocation by tl this case also the chromo oncogene, and the light c (distal) to the *c-myc* oncog rize the different genetic the t(8;14), t(8;22), and t(2

Translocation of the κ results in the transcription (Erikson *et al.*, 1983b). Al kitt lymphoma cells with t some express elevated leve ing the normal chromoso (Erikson *et al.*, 1983).

In view of these results somatic cell hybrids, it can lymphoma is a transcriptio the reciprocal chromosom pression at elevated levels *et al.*, 1983b). It may be ir silent or expressed at very lymphoma cells, the close noglobulin locus results expression.

VI. I

Why is the *c-myc* gen chromosomal translocatio sibility is that the involv



n *c-myc* cDNA probe (Ryc 7.4) human *myc* RNA protects a DNA rries the 8q⁺ chromosome, ex- ter hand hybrid BL 3-2, which chromosomes, does not express aphoma cells and Daudi Burkitt n express high levels of human er DNAs.

dred nucleotides or a few kilobases as in BL2 cells (Croce *et al.*, 1983; Erikson and Croce, 1985), or more than 30–40 kb from the 3' end of the *c-myc* oncogene (Lee and Croce, 1985). Analysis of somatic cell hybrids between mouse myeloma cells and BL2 Burkitt lymphoma cells carrying the t(8;22) translocation indicates that the involved *c-myc* oncogene on the 8q⁺ chromosome is expressed at elevated levels in the hybrids, while the *c-myc* oncogene on the normal chromosome 8 is transcriptionally inactive (Croce *et al.*, 1983) (Fig. 8). Therefore, these results indicate that the *c-myc* oncogene, which is involved in the reciprocal chromosome exchange, is deregulated even if it is not translocated and even if the involved immunoglobulin locus is distal to the activated oncogene (Croce *et al.*, 1983). These results also indicate that the orientation of the λ locus is opposite to the orientation of the heavy chain locus in man (Croce *et al.*, 1983; Emanuel *et al.*, 1985).

We have also examined Burkitt lymphoma cells carrying the t(2;8) chromosome translocation by the somatic cell genetic approach and found that in this case also the chromosomal breakpoint occurs 3' (distal) to the *c-myc* oncogene, and the light chain locus (κ) translocates to a region that is 3' (distal) to the *c-myc* oncogene (Erikson *et al.*, 1983b). In Fig. 2 we summarize the different genetic exchanges occurring in Burkitt lymphomas with the t(8;14), t(8;22), and t(2;8) chromosome translocations.

Translocation of the κ locus to a region 3' to the *c-myc* oncogene also results in the transcriptional deregulation of the involved *c-myc* oncogene (Erikson *et al.*, 1983b). All hybrids between mouse myeloma cells and Burkitt lymphoma cells with the t(2;8) translocation that carry the 8q⁺ chromosome express elevated levels of *c-myc* transcripts, while the hybrids containing the normal chromosome 8 do not express the human *c-myc* gene (Erikson *et al.*, 1983).

In view of these results concerning the regulation of *c-myc* expression in somatic cell hybrids, it can be concluded that the common feature in Burkitt lymphoma is a transcriptional activation of the *c-myc* oncogene involved in the reciprocal chromosomal translocations, leading to its constitutive expression at elevated levels (Nishikura *et al.*, 1983; Croce *et al.*, 1983; Erikson *et al.*, 1983b). It may be inferred that while the *c-myc* gene should be either silent or expressed at very low levels in the normal counterparts of Burkitt lymphoma cells, the close proximity of the involved *c-myc* gene to an immunoglobulin locus results in its transcriptional activation and constitutive expression.

VI. Mechanisms of *c-myc* Activation

Why is the *c-myc* gene that is involved in one of the three different chromosomal translocations deregulated in Burkitt lymphoma? One possibility is that the involved *c-myc* gene is activated by genetic elements

within the three immunoglobulin loci capable of activating gene transcription *in cis* (Croce *et al.*, 1983, 1984, 1985). A different possibility has been proposed by Leder *et al.* (1983), who suggested that the involved *c-myc* oncogene is deregulated because of decapitation or alterations of its 5' exon or of a region 5' to the *c-myc* oncogene, with resultant failure to bind a repressor and subsequent transcriptional activation.

In order to determine the mechanisms of *c-myc* activation in Burkitt lymphoma, at first we have asked the question of whether such activation by chromosomal translocation is B cell specific. Results mentioned in the preceding section indicate that the involved *c-myc* gene is expressed in hybrids with mouse myeloma cells. Similarly human immunoglobulin genes are expressed in hybrids between mouse and human B cells (Croce *et al.*, 1979; Erikson *et al.*, 1981; Erikson and Croce, 1982). We have also hybridized Burkitt lymphoma cells carrying the t(8;14) chromosome translocation in which the translocated *c-myc* oncogene has been decapitated of its 5' exon with different mouse fibroblasts (Nishikura *et al.*, 1984). The hybrids were then studied for the presence of the translocated *c-myc* oncogene and of the productively rearranged μ heavy chain gene. The hybrids containing both the translocated *c-myc* oncogene and the productively rearranged μ gene failed to express both genes. This result indicates that mouse fibroblasts are incapable of transcribing immunoglobulin genes and a translocated *c-myc* oncogene (Nishikura *et al.*, 1983, 1984). Since the hybrids contained a decapitated *c-myc* oncogene, it seems quite unlikely that the model proposed by Leder *et al.* (1983) concerning the mechanism of *c-myc* deregulation is correct. If decapitation of the first exon resulted in the failure of the gene to respond to a repressor leading to its deregulation, the decapitated *c-myc* gene should have been expressed in fibroblast hybrids and this was not the case (Nishikura *et al.*, 1984).

To determine whether the activation of the involved *c-myc* oncogene depends on the stage of differentiation of the B cells harboring the translocation chromosome carrying the activated *c-myc* oncogene, we have also hybridized Burkitt lymphoma cells with Epstein-Barr virus (EBV) transformed human lymphoblastoid cells (Croce *et al.*, 1984, 1985). Interestingly we found that such hybrids are phenotypically similar to the lymphoblastoid parental cells, indicating that the lymphoblastoid phenotype is dominant in somatic cell hybrids between these cells (Croce *et al.*, 1984, 1985). Having thus introduced a translocated and activated *c-myc* gene into human lymphoblastoid cells, we carried out S1 nuclease analysis of hybrids containing both the germ line and the translocated *c-myc* oncogene. The results indicated that the translocated *c-myc* gene is not expressed in a lymphoblastoid background (Croce *et al.*, 1984). In the two Burkitt lymphoma cell lines (ST486 and CA46) that we have used for hybridization with human lymphoblastoid cells the decapitated and translocated *c-myc* gene was recombined

with the μ switch region (S α) in the c locus. This result suggests that in these cases the enhancer of the heavy chain gene is not in front of the translocation. We observe translocated *c-myc* in Burkitt lymphoma cells and in the translocated *c-myc* gene in the heavy chain locus of plasma cells and Burkitt lymphoma cells (Croce *et al.*, 1984). A corollary of the above results is that the *c-myc* gene must interact with the enhancer elements expressed in plasma cells and blastoid cells (Croce *et al.*, 1984) also because they indicate that the heavy chain locus elements capable of activating gene expression are present in the stage of differentiation.

In ST486 Burkitt lymphoma cells, the translocation occurred within the first *c-myc* exon, which is located between J_H and S_H (distal to the cut to 3'). Interestingly we observed that the *c-myc* exon on the 8q-24 chromosome of hybrids with human lymphoblastoid cells (Croce *et al.*, 1984). This result indicates that the enhancer between J_H and S_H and the first *c-myc* exon in lymphoma cells predict that a *c-myc* oncogene in the heavy chain locus should be expressed in human lymphoblastoid cells and human lymphoblastoid cells where the translocated *c-myc* gene is expressed in regions (and therefore is human lymphoblastoid cells) where the phenotype of the lymphoma is expressed (Croce *et al.*, 1984). In the two Burkitt lymphoma cell lines (ST486 and CA46) that we have used for hybridization with human lymphoblastoid cells the decapitated and translocated *c-myc* gene was recombined

As shown in Fig. 9 the

activating gene transcription. A different possibility has been that the involved *c-myc* alterations of its 5' exon result in a failure to bind a protein.

c-myc activation in Burkitt lymphoma cells, whether such activation by itself or by the mechanisms mentioned in the preceding paragraph, is expressed in hybrids with human immunoglobulin genes are expressed in plasma cells (Croce *et al.*, 1979; Croce *et al.*, 1984). We have also hybridized human chromosome translocation in decapitated of its 5' exon (Croce *et al.*, 1984). The hybrids were *c-myc* oncogene and of the heavy chain locus. The hybrids containing both the rearranged μ gene and the mouse fibroblasts are and a translocated *c-myc* gene. The hybrids contained a decapitated *c-myc* gene that the model proposed for *c-myc* deregulation is the failure of the gene to be transcribed, the decapitated *c-myc* gene in the hybrids and this was not the

involved *c-myc* oncogene. Cells harboring the translocated *c-myc* oncogene, we have also observed that the EBV translocation (Croce *et al.*, 1984, 1985). Interestingly, the phenotype of the lymphoblastoid cells is dominant in the hybrids (Croce *et al.*, 1984, 1985). Having the *c-myc* gene into human lymphoblastoid cells, the results indicated that the *c-myc* gene is expressed in a lymphoblastoid cell line. The results indicated that the *c-myc* gene was recombined

with the μ switch region ($S\mu$) in the case of ST486 cells and with the alpha switch region ($S\alpha$) in the case of CA46 cells (Showe *et al.*, 1985). Therefore, in these cases the enhancer located between $S\mu$ and the joining (J_H) segment of the heavy chain gene was translocated to the 8q- chromosome and was not in front of the translocated *c-myc* gene (see Fig. 2C). Since we did not observe translocated *c-myc* activation in the lymphoblastoid hybrids although the translocated *c-myc* oncogene is expressed in the parental human lymphoma cells and in their hybrids with plasma cells, we concluded that the translocated *c-myc* gene is activated by cis-acting genetic elements within the heavy chain locus that are capable of activating transcription in plasma cells and Burkitt lymphoma cells, but not in lymphoblastoid cells (Croce *et al.*, 1984). A corollary of this is that in order to be expressed, the translocated *c-myc* gene must interact with B cell specific transacting factors that are expressed in plasma cells and Burkitt lymphoma cells but not in lymphoblastoid cells (Croce *et al.*, 1984). These results are of considerable interest also because they indicate that the expression of an activated oncogene may be either dominant or recessive depending on the differentiated stage of the cells harboring that activated oncogene. Our inference from these results is that the heavy chain locus contains additional, not yet identified, genetic elements capable of activating gene transcription *in cis* in cells at the appropriate stage of differentiation.

In ST486 Burkitt lymphoma cells the breakpoint on chromosome 8 occurred within the first *c-myc* intron and the breakpoint on chromosome 14 occurred within the $S\mu$ heavy chain region, thus the enhancer, normally located between J_H and $S\mu$, is translocated to the 8q- chromosome in these cells, 3' (distal) to the cut off *c-myc* 5' exon in a tail-to-tail rearrangement (3' to 3'). Interestingly we observed high levels of transcripts of the cut off first *c-myc* exon on the 8q- chromosome in ST486 cells (Fig. 7B) and in their hybrids with human lymphoblastoid cells (ar-Rushdi *et al.*, 1983; Croce *et al.*, 1984). This result indicates that genetic elements 5' to $S\mu$ (possibly the enhancer between J_H and $S\mu$) are capable of activating the transcription of the first *c-myc* exon in lymphoblastoid cells (Croce *et al.*, 1984). Thus we could predict that a *c-myc* oncogene translocated 5' to the J_H segment of the heavy chain locus should be expressed in hybrids between Burkitt lymphoma cells and human lymphoblastoid cells. Therefore we hybridized Daudi Burkitt lymphoma cells where the *c-myc* gene is translocated into one of the V_H regions (and therefore is 5' to the J_H region) (Erikson *et al.*, 1982) with human lymphoblastoid cells. As shown in Table I, these hybrids retained the phenotype of the lymphoblastoid parental cells. Since the first exon of the translocated *c-myc* oncogene is altered in Daudi cells, we could distinguish between the transcripts of the normal and of the translocated *c-myc* oncogene (Fig. 9) (Croce *et al.*, 1985).

As shown in Fig. 9 the hybrids expressed both the normal and the translo-

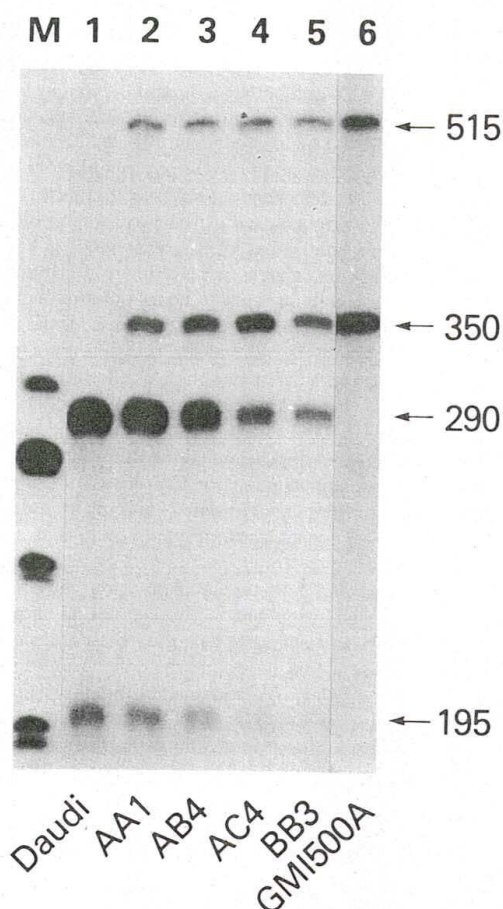


FIG. 9. S1 mapping analysis of *c-myc* mRNAs synthesized in Daudi Burkitt lymphoma and somatic cell hybrids. The RNA was analyzed by the S1 nuclease mapping procedure with modification using a uniformly labeled DNA probe. The *Xma*I-*Pvu*II DNA fragment (610 bp) of the *c-myc* gene containing a portion of the 5' flanking sequences and the first exon was cloned in M13 and the clone was uniformly labeled with 32 P and used as an S1 probe. Cytoplasmic RNAs (20 μ g), prepared from various cells by the cesium chloride method, were used for each assay. As previously described, the 32 P-labeled DNA probe was heat denatured, hybridized in 80% formamide to cytoplasmic RNAs at 57.5°C for 10 hours, digested with 80 units of nuclease S1, and analyzed by electrophoresis on a 7 M urea 4% polyacrylamide gel. M, 5'- 32 P-labeled ϕ X174 *Hae*III digests.

PHENOTYPE OF SOMATIC CELL HYBRIDS

| Cells | Immunoglobulin |
|----------------|----------------|
| Daudi | + |
| GM1500-6TG-OUB | - |
| AA1 | + |
| AC4 | + |
| BB3 | + |
| AB4 | + |

^a Secreted and cytosol immunoprecipitated by using addition of 50 μ l of a 10% (Croce *et al.*, 1985).

^b Daudi cells produce myeloma (κ¹) (Croce, *et al.*, 1985). Or

^c Expression of the anti-immunofluorescence as des

cated *c-myc* oncogenes the J_H segment of heavy chain. This result confirms the proximity between J_H and S μ as a result of transcription in Burkitt lymphoma blastoid cells (Croce *et al.*, 1984) to lymphoblastoid cells. In the other conclusion, the analysis of somatic cell hybridization lymphoma is a transcribed to proximity to different loci capable of activating somal distances (Croce *et al.*, 1984).

These results are of insight into the molecular

TABLE I
PHENOTYPE OF SOMATIC CELL HYBRIDS BETWEEN DAUDI BURKITT LYMPHOMA AND
GM1500-6TG-OUB LYMPHOBLASTOID CELLS

| Cells | Immunoglobulin chains ^a | | | | Secretion ^b | Antigen recognized by the B532 antibodies ^c |
|----------------|------------------------------------|----------|----------|------------|------------------------|--|
| | μ | γ | κ | κ^L | | |
| Daudi | + | - | - | + | - | - |
| GM1500-6TG-OUB | - | + | + | - | + | + |
| AA1 | + | + | + | + | + | + |
| AC4 | + | + | + | + | + | + |
| BB3 | + | + | + | + | + | + |
| AB4 | + | + | + | + | + | + |

^a Secreted and cytosol immunoglobulins were labeled with [³H]leucine (100 μ Ci/ml) and immunoprecipitated by using a rabbit anti-human immunoglobulin antiserum followed by the addition of 50 μ l of a 10% suspension of fixed *Staphylococcus aureus* antibodies as described (Croce *et al.*, 1985).

^b Daudi cells produce membrane bound IgM. Daudi cells express an aberrant κ polypeptide (κ^L) (Croce, *et al.*, 1985). On the contrary GM1500-6TG-OUB secrete IgG (Croce *et al.*, 1985).

^c Expression of the antigen recognized by the B532 antibodies was studied by indirect immunofluorescence as described (Croce *et al.*, 1985).

cated *c-myc* oncogenes, indicating that a *c-myc* oncogene translocated 5' to the J_H segment of heavy chain can be expressed in lymphoblastoid cells. This result confirms that genetic elements 5' to S μ (possibly the enhancer between J_H and S μ) are capable of activating translocated *c-myc* gene transcription in Burkitt lymphoma cells, plasma cells, and human lymphoblastoid cells (Croce *et al.*, 1985). Thus it seems that Burkitt lymphoma is a heterogeneous disease in which different genetic elements are involved in the in cis activation of the translocated *c-myc* oncogene. In one case, where the *c-myc* oncogene is translocated to a switch region, the translocated *c-myc* oncogene can be activated only in terminally or near terminally differentiated B cells. In the other case, where the *c-myc* oncogene is translocated to J_H or a region 5' to J_H, the translocated *c-myc* oncogene can be activated at the different stages of B cell differentiation from pre B cells (Pegoraro *et al.*, 1984) to lymphoblastoid cells, and to plasma cells (Croce *et al.*, 1985). In conclusion, the analysis of *c-myc* activation in Burkitt lymphoma cells using somatic cell hybridization techniques indicates that the hallmark of Burkitt lymphoma is a transcriptional deregulation of the involved *c-myc* gene due to proximity to different genetic elements within the three immunoglobulin loci capable of activating gene transcription in cis over considerable chromosomal distances (Croce *et al.*, 1984, 1985).

These results are of considerable interest not only because they provide insight into the molecular mechanisms of oncogene activation in human

in Daudi Burkitt lymphoma and release mapping procedure with *Pvu*II DNA fragment (610 bp) of the first exon was cloned in an S1 probe. Cytoplasmic RNAs were used for each assay. t denatured, hybridized in 80% and with 80 units of nuclease S1, the gel. M, 5'-³²P-labeled ϕ X174

neoplasia, but also because they indicate the existence of different genetic elements within the three immunoglobulin loci capable of activating gene transcription (Croce *et al.*, 1985). Clearly Burkitt lymphoma seems to be a heterogeneous group of diseases in which different genetic elements play an important role in the transcriptional deregulation of the *c-myc* oncogene. In addition it seems quite clear that Burkitt-like translocations are also involved in the pathogenesis of other B cell neoplastic diseases such as acute lymphocytic leukemias of the B cell type (L3 according to the FAB classification). Interestingly, we have recently discovered a human pre B cell leukemia carrying both a t(8;14) and a t(14;18) chromosome translocation characteristic of Burkitt lymphoma and follicular lymphoma, respectively, in which the translocated *c-myc* oncogene is in its germ line configuration and is located more than 15 kb 5' of the involved J_H segment (Pegoraro *et al.*, 1984). Since expression of the translocated oncogene is elevated in these leukemic cells (ar-Rushdi and Croce, 1985) it can be inferred that activation of the *c-myc* oncogene by chromosomal translocation can occur as early as in pre B cells.

The different rearrangements of the *c-myc* oncogene and of the immunoglobulin loci in malignancies with the t(8;14), t(8;22), and t(2;8) translocations indicate that *c-myc* activation occurs when the *c-myc* oncogene independently of its orientation is located in front (5') of the involved immunoglobulin locus (Dalla Favera *et al.*, 1982a, 1983; Croce *et al.*, 1983; Erikson *et al.*, 1983a). This finding suggests a polarity in the effect of the immunoglobulin loci in *c-myc* activation. It would be of considerable interest, however, to determine whether there are some cases of Burkitt lymphoma with the t(8;14) chromosomal translocation in which the *c-myc* oncogene remains on chromosome 8 and the enhancer located between J_H and S_μ is translocated 3' to the involved *c-myc* oncogene. In this case the *c-myc* oncogene and the heavy chain enhancer would be rearranged in a tail-to-tail fashion.

VII. Molecular Genetics of the t(11;14) Chromosome Translocation

Chromosome 14 is involved in reciprocal chromosomal translocations with chromosome 11 in a fraction of chronic lymphocytic leukemias of the B cell type (Nowell *et al.*, 1981; Erikson *et al.*, 1984) in diffuse small cell and large cell lymphomas (Van den Berghe *et al.*, 1979b) and in a sizable fraction (30–40%) of multiple myelomas. Interestingly, while a 14q⁺ chromosome is frequently observed in human multiple myeloma, Burkitt-like translocations are observed quite rarely in this disease or group of diseases. This suggests that mineral oil-induced mouse plasmacytomas may represent only one of the types of plasmacytomas in the mouse. Possibly plasmacytomas carrying other types of translocations may also occur in the rodents.

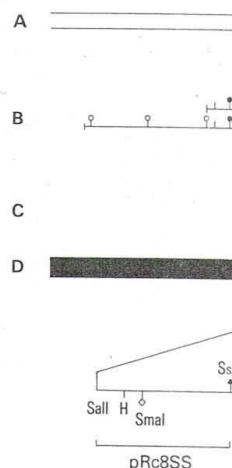


FIG. 10. Restriction maps of DNA clones we have obtained (B the 14q⁺ chromosome. H, *Hin* some 11-derived sequences, w sequences (D).

Since in human B cell chromosomal breakpoints are that the same human loci different B cell neoplastic cell hybrids between mouse cells with the t(11;14) chromosome translocation, it is clear that the chromosomal breakpoint for the heavy chain locus on the chromosome 14 is on the normal chromosome 14. This observation in Burkitt lymphoma (Erikson *et al.*, 1983).

We then produced a DNA in a λ phage vector a specific for the joining (J_H *et al.*, 1984a). As shown in the figure, one class represents

tence of different genetic elements of activating gene lymphoma seems to be a genetic elements play an of the *c-myc* oncogene. In locations are also involved ses such as acute lympho o the FAB classification). nan pre B cell leukemia anslocation characteristic spectively, in which the nfiguration and is located goraro *et al.*, 1984). Since d in these leukemic cells at activation of the *c-myc* as early as in pre B cells. ogene and of the immu-t(8;22), and t(2;8) trans- sen the *c-myc* oncogene 5') of the involved immu- Croce *et al.*, 1983; Erik- n the effect of the immu- of considerable interest, ses of Burkitt lymphoma ich the *c-myc* oncogene d between J_H and $S\mu$ is

In this case the *c-myc* rearranged in a tail-to-tail

ome Translocation

somal translocations with c leukemias of the B cell ffuse small cell and large in a sizable fraction (30– a $14q^+$ chromosome is urkitt-like translocations f diseases. This suggests y represent only one of plasmacytomas carrying rodents.

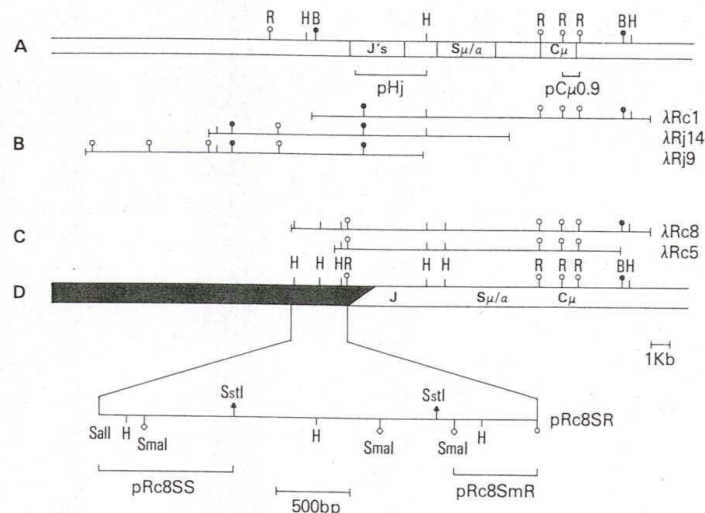


FIG. 10. Restriction maps of the germ line $C\mu$ gene (A) and of the two classes of recombinant clones we have obtained (B and C). Clones shown in B represent the excluded $C\mu$ allele on the $14q^+$ chromosome. H, *HindIII*; R, *EcoRI*; B, *BamHI*. Black box represents the chromosome 11-derived sequences, whereas the white box represents the chromosome 14-derived sequences (D).

Since in human B cell neoplasms with the t(11;14) translocation the chromosomal breakpoints are consistently observed at band q13, we reasoned that the same human locus on chromosome 11 may be involved in the different B cell neoplastic diseases with translocation. By analyzing somatic cell hybrids between mouse cells and chronic lymphocytic leukemia (CLL) cells with the t(11;14) chromosome translocation it was possible to establish that the chromosomal breakpoint on chromosome 14 involves directly the heavy chain locus on the $14q^+$ and that the productively rearranged heavy chain gene is on the normal chromosome 14 (Erikson *et al.*, 1984), similar to the observations in Burkitt lymphoma with the t(8;14) chromosome translocation (Erikson *et al.*, 1982).

We then produced a DNA library of the chronic lymphocytic leukemia DNA in a λ phage vector and screened the recombinant phages with a probe specific for the joining (J_H) segment of the heavy chain locus (Tsujimoto *et al.*, 1984a). As shown in Fig. 10, two classes of recombinant clones were obtained. One class represented the productively rearranged μ heavy chain

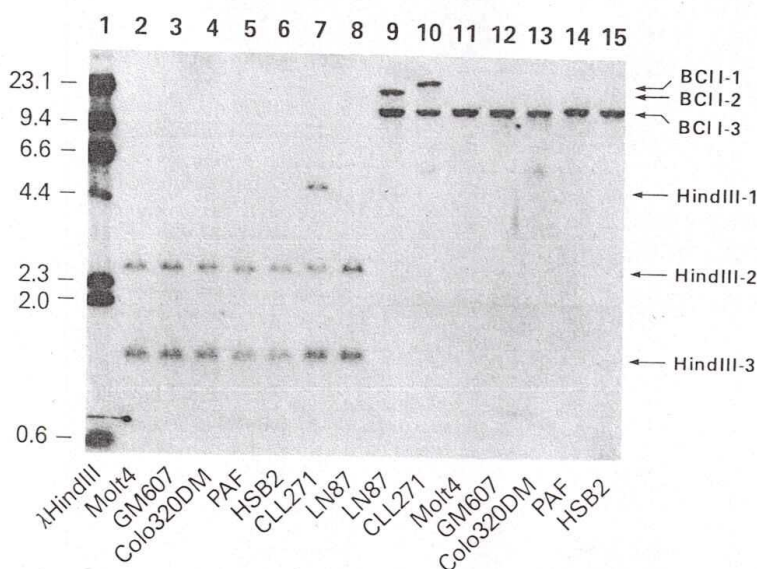


FIG. 11. Southern blot hybridization of CLL 271 and LN87 DNAs with a chromosome 11 specific DNA probe mapping close to the breakpoint of CLL 271 t(11;14). The cellular DNA (5 μ g) was digested with *Hind*III (lanes 2 to 8) and *Bcl*I (lanes 9 to 15) and fractionated by agarose gel electrophoresis. The Southern blot filter was hybridized with the pRc8SmR probe and finally washed with $0.2 \times$ SSCS 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 2 and 12, GM607 (human lymphoblastoid cell line) DNA; lanes 7 and 10, CLL 271 (human B cell leukemia) DNA; lanes 8 and 9, LN87 (human diffuse B-cell lymphoma) DNA.

gene on chromosome 14 (Erikson *et al.*, 1984; Tsujimoto *et al.*, 1984b), while the other represented the unproductively rearranged μ gene on the $14q^+$ chromosome (Tsujimoto *et al.*, 1984b). We then subcloned unique DNA sequences 5' to the rearranged J_H segment of the unproductively rearranged heavy chain locus and found that these DNA probes hybridized to DNA derived from human chromosome 11 but did not hybridize to DNA derived from the other chromosomes, indicating that the second class of recombinant clones contain the joining between chromosomes 11 and 14 on the $14q^+$ chromosome (Tsujimoto *et al.*, 1984a). We then used the chromosome 11 specific probes flanking the breakpoint in the CLL271 cells to detect rearrangements of the homologous DNA sequences in lymphomas and leukemias with the t(11;14) chromosome translocation (Tsujimoto *et al.*, 1984a). As



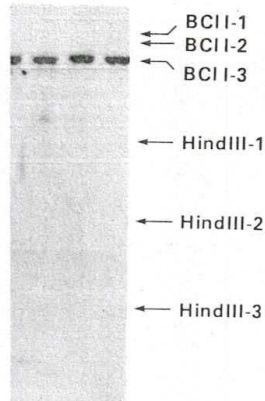
FIG. 12. Diagram of the

shown in Fig. 11 we detected some 11 specific DNA probes of CLL and of diffuse B-cell lymphoma indicate that the breakpoint DNA segment (3.5 kb) is located on chromosome 11 (Tsujimoto *et al.*, 1984a; Tsujimoto *et al.*, 1984b). We have inferred that on the basis of these results we have proposed the t(11;14) translocation is activated by its close proximity to the μ gene (Tsujimoto *et al.*, 1984a) (Fig. 12). Since the long arm of human chromosome 14 is not homologous to chromosome 11, it is interestingly since the t(11;14) translocation is possible to use specific probes to detect the translocation in malignant B-cell lymphomas (Tsujimoto *et al.*, 1984a).

VIII. Molecular Cloning

Chromosome 14 is also involved in some 18 in the great number of human

2 13 14 15



DM PAF HSB2

DNA with a chromosome 11 t(11;14). The cellular DNA (5) and fractionated by agarose with the pReSSmR probe and the HindIII cut marker DNA (cell line) DNA; lanes 2 and 12, LL 271 (human B cell leukemia) DNA.

oto *et al.*, 1984b), while d μ gene on the 14q⁺ abcloned unique DNA reductively rearranged es hybridized to DNA ridize to DNA derived nd class of recombinant l and 14 on the 14q⁺ d the chromosome 11 71 cells to detect rear-ymphomas and leuke-imoto *et al.*, 1984a). As

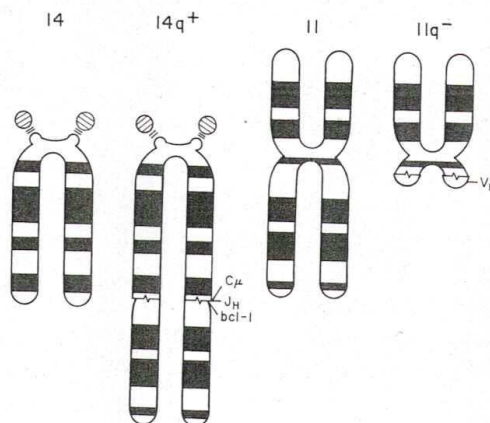


FIG. 12. Diagram of the t(11;14) translocation occurring in human B cell neoplasia.

shown in Fig. 11 we detected rearrangements of the homologous chromosome 11 specific DNA segments in lymphomas and leukemias with the t(11;14) translocation (lanes 9–10) (Tsujimoto *et al.*, 1984a). Additional studies of CLL and of diffuse B cell lymphomas with the same translocation indicate that the breakpoints in different tumors are clustered within a small DNA segment (3.5 kb length) on band q13 of chromosome 11 (Tsujimoto *et al.*, 1984a; Tsujimoto and Croce, 1985). On the basis of these results, we have inferred that on band q13 of chromosome 11 there is a gene, for which we have proposed the name of bcl-1 (B cell lymphoma/leukemia 1) that is activated by its close proximity to the heavy chain locus (Tsujimoto *et al.*, 1984a) (Fig. 12). Since no known homolog of retrovirus oncogenes maps on the long arm of human chromosome 11, we can also infer that the bcl-1 gene is not homologous to any of the presently known retrovirus oncogenes. Interestingly since the chromosome 11 breakpoints in neoplastic B cells with the t(11;14) translocation cluster within a short segment of DNA, it becomes possible to use specific DNA probes to detect the occurrence of a t(11;14) translocation in malignant lymphoid tissues by Southern blotting analysis (Tsujimoto *et al.*, 1984a).

VIII. Molecular Genetics of the t(14;18) Chromosome Translocation

Chromosome 14 is also involved in a reciprocal translocation with chromosome 18 in the great majority of cases of human follicular lymphoma, the

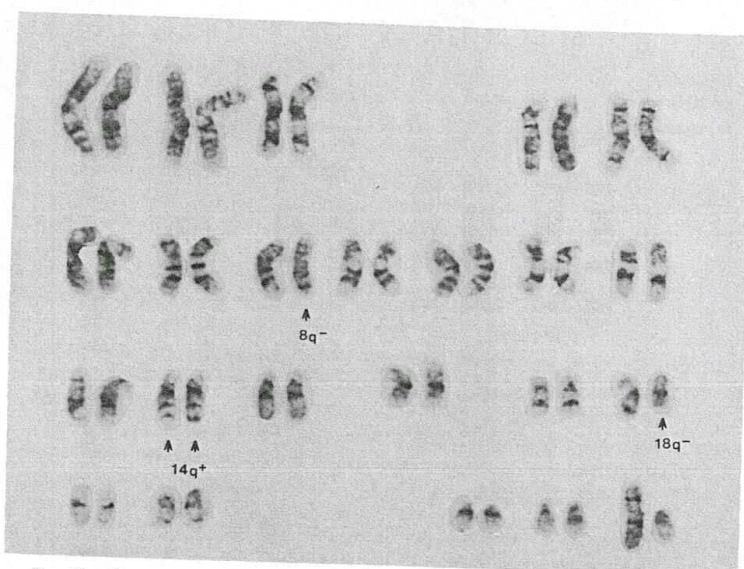


FIG. 13. Karyotype of the 380 cells: 46 XY, t(8;14) (q24;q32). As a result of the two reciprocal chromosomal translocations, the 380 cells have two abnormal chromosomes 14 (14q⁺), one rearranged chromosome 8 (8q⁻), and one abnormal chromosome 18 (18q⁻).

most frequent B cell malignancy in the United States and Western Europe (Yunis, 1983). By taking advantage of an acute B cell leukemia carrying two reciprocal translocations, t(8;14) and t(14;18) (Fig. 13) (Pegoraro *et al.*, 1984), we have been able to clone both chromosomal breakpoints on the two 14q⁺ chromosomes (Fig. 14). As shown in Fig. 14 two classes of recombinant clones were obtained. One class contained the joining between chromosome 8 and 14 on one 14q⁺ chromosome (Fig. 14) (Tsujimoto *et al.*, 1984b). The other class contained the joining between chromosomes 14 and 18 on the other 14q⁺ chromosome (Tsujimoto *et al.*, 1984b). In fact, DNA probes free of repetitive sequences 5' to the J_H regions of the involved heavy chain loci hybridized with either chromosome 8 or chromosome 18, but not with human chromosome 14 (Tsujimoto *et al.*, 1984b).

We then asked whether the same chromosomal region on chromosome 18 is involved in chromosome translocations in follicular lymphomas. By using two DNA probes approximately 15 kb apart we could detect rearrangements in more than 75% of follicular lymphomas. Therefore we inferred that on band q21 of chromosome 18 there is a gene, for which we propose the name

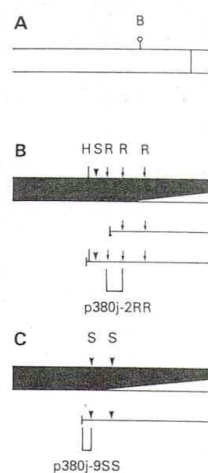


FIG. 14. Restriction mapping of recombinant clones from the 14q⁺ translocations. H, HindIII; R, RsaI; some 18-derived sequences; bar represents the chromosome.

of bcl-2, which is active (Fig. 15). Since the rearrangement occurred within a short segment, probes to detect the oncoprotein in neoplastic tissues.

Of considerable interest in these translocations, a t(8;14) patient with acute lymphocytic leukemia (ALL) carried the 380 leukemic clone. This clone expressed monoclonal antibodies to the heavy chain genes, but not to the light chain genes (Pegoraro *et al.*, 1984). In the leukemia, the joining (J_H) of one of the 14q⁺ chromosomes to the c-myc oncogene on chromosome 8, 5' to the c-myc oncogene in its germ line configuration, was expressed at elevated levels in activated c-myc transcr



32). As a result of the two re-
normal chromosomes 14 (14q⁺),
osome 18 (18q⁻).

tes and Western Europe
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3) (Pegoraro *et al.*, 1984),
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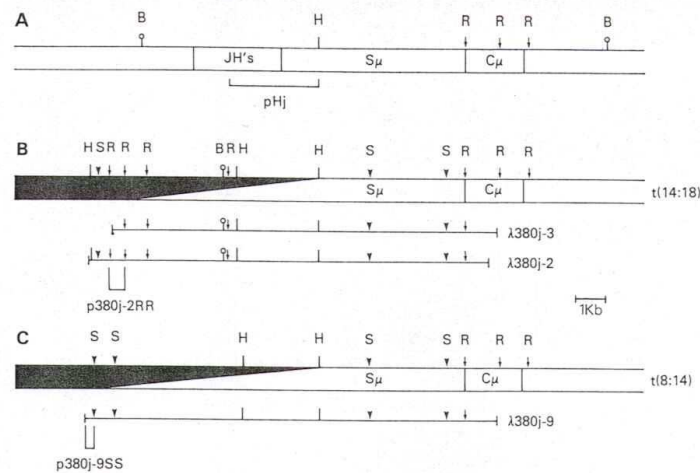


FIG. 14. Restriction maps of the germ line C μ gene (A) and of the two classes of recombinant clones from the 14q⁺ chromosomes resulting from the t(14;18) (B) and the t(8;14) (C) translocations. H, *Hind*III; R, *Eco*RI; B, *Bam*HI; S, *Sst*I. The black bars represent the chromosome 18-derived sequences in (B) and the chromosome 8-derived sequences in (C). The open bar represents the chromosome 14-derived sequences.

of *bcl-2*, which is activated because of its proximity to the heavy chain locus (Fig. 15). Since the rearrangements in different neoplastic samples are clustered within a short segment of DNA, it becomes possible to use nucleic acid probes to detect the occurrence of a t(14;18) chromosomal translocation in neoplastic tissues.

Of considerable interest is the fact that we have observed two reciprocal translocations, a t(8;14) and a t(14;18), in the same neoplastic cells of a patient with acute lymphocytic leukemia of the B cell type. We have examined the 380 leukemic cells and found that they are EBV negative, react with monoclonal antibodies specific for B cells, and contain rearranged heavy and light chain genes, but do not express human immunoglobulins (Pegoraro *et al.*, 1984). In the leukemic cells, both μ heavy chain loci are rearranged within the joining (J_H) DNA segment (Fig. 14). One of the J_H segments on one of the 14q⁺ chromosomes is rearranged with a segment of chromosome 8, 5' to the *c-myc* oncogene (Tsujimoto *et al.*, 1984b). The *c-myc* oncogene is in its germ line configuration more than 15 kb from the breakpoint and is expressed at elevated levels (ar-Rushdi and Croce, 1985). In addition, the activated *c-myc* transcripts of 380 cells are initiated at the two proper sites

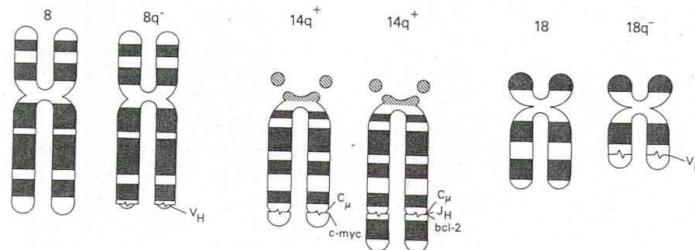


FIG. 15. Diagram of the reciprocal 8;14 and 14;18 translocation carried in 380 cells. Both the *bcl-2* locus on band q21 of chromosome 18 and the *c-myc* locus on band q24 of chromosome 8 are translocated in the involved immunoglobulin heavy chain loci on the two chromosomes 14 in 380 cells.

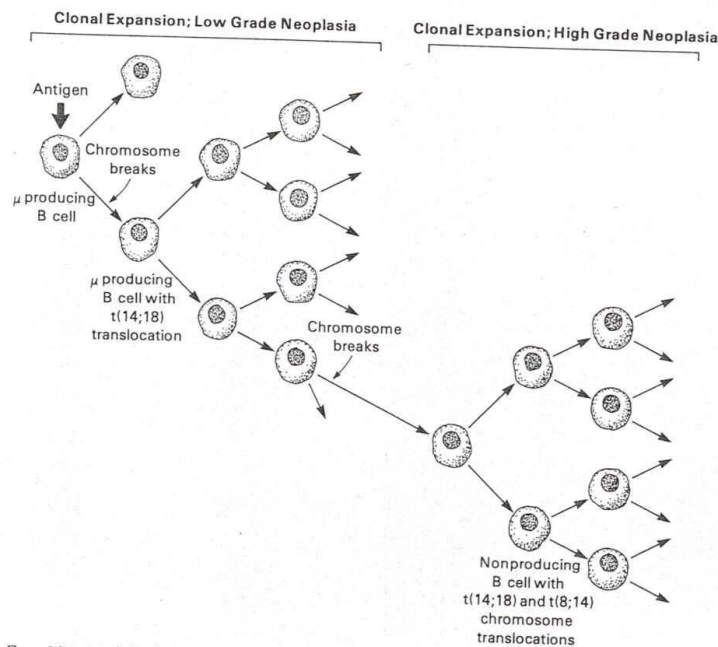
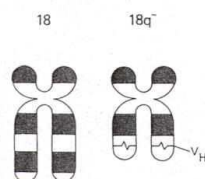


FIG. 16. Model of B cell oncogenesis in B cells carrying two reciprocal translocations involving the two human chromosomes 14 and chromosomes 18 and 8, respectively.

(Nishikura and Croce, rearranged with a segment *bcl-2*, is located (Fig. cell oncogenesis according involving the two heavy chain loci (Fig. 16). The t(14;18) involving the *bcl-2* locus on chromosome 18 in close proximity to the immunoglobulin heavy chain locus, constituting a constitutive expression of the *bcl-2* gene. The consequence of this clone of B cells carrying the t(14;18) translocation is malignancy such as follicular lymphoma. Within the expanded clone, additional random chromosomal changes, which have occurred in one cell, lead to a more aggressive malignancy such as diffuse large cell lymphoma (Fig. 16).

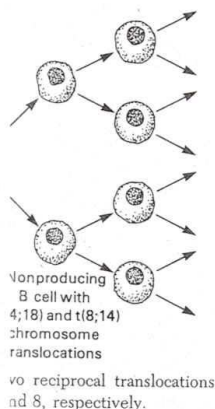
In this postulated model, the first step in the expanding the pool of translocation might be exposure to a strong immunosuppressive agent, such as the Epstein-Barr virus, that the second translocation involves the *c-myc* locus on chromosome 8, leading to a more aggressive malignancy carrying two reciprocal translocations involving the two human chromosomes 14 and chromosomes 18 and 8, respectively.

The studies concerning B cell lymphoma have indicated that the proximity to genetic elements of activating gene translocation fails to respond to the growth inhibitory signals.



on carried in 380 cells. Both on band q24 of chromosome on the two chromosomes 14

translocation; High Grade Neoplasia



(Nishikura and Croce, 1985). The J_H segment of the other chromosome 14 is rearranged with a segment of chromosome 18 where a putative oncogene, bcl-2, is located (Fig. 15). Based on these findings we propose a model of B cell oncogenesis according to which B cell neoplasms carrying translocations involving the two heavy chain loci are the result of a multiple step process (Fig. 16). The t(14;18) translocation may have occurred in an activated B cell, involving the excluded heavy chain locus on chromosome 14 and the bcl-2 locus on chromosome 18 (Fig. 16). This translocation placed the bcl-2 gene in close proximity to the heavy chain locus and its enhancers leading to the activation and constitutive expression of this putative oncogene (Fig. 15). The consequence of bcl-2 deregulation was the expansion of a malignant clone of B cells carrying the t(14;18) translocation and a relatively low-grade malignancy such as follicular lymphoma (Pegoraro *et al.*, 1984) (Fig. 16). Within the expanded clone of neoplastic B cells, the probability for additional random chromosome rearrangements was increased. One of these additional changes, a translocation between chromosomes 8 and 14, might have occurred in one of the neoplastic B cells (Fig. 16).

This additional translocation then resulted in the activation and constitutive expression of the translocated *c-myc* oncogene, leading to a high grade malignancy such as Burkitt lymphoma or Burkitt-type leukemia (Fig. 16).

In this postulated sequence, the t(14;18) translocation is considered to be an early step in the pathogenesis of the 380 leukemia. A similar role in expanding the pool of proliferating B cells susceptible to the effect of *c-myc* translocation might be ascribed, in other circumstances, to continuous exposure to a strong immunogen such as malaria and/or a virus such as Epstein-Barr virus, that is capable of inducing B cell proliferation. The role of the second translocation [t(8;14)], might be analogous to that of sequential karyotypic alterations in the biological progression of other types of neoplasms, to a more aggressive phase. Thus the study of leukemia and lymphomas carrying two reciprocal chromosomal translocations should allow a better understanding of the sequential steps and multiple genes that may be involved in the pathogenesis of many B cell neoplasms in man.

IX. Conclusions

The studies concerning the involvement of the *c-myc* oncogene in Burkitt lymphoma have indicated that a cellular protooncogene, *c-myc*, can be activated through chromosomal translocations. This activation results from close proximity to genetic elements within the three immunoglobulin loci capable of activating gene transcription in cis in B cells. Thus, the involved *c-myc* oncogene fails to respond to the control mechanisms that are able to regulate

the transcription of the uninvolved *c-myc* oncogene and is expressed constitutively at elevated levels. This derangement in *c-myc* regulation is the common feature of all cases of Burkitt lymphomas and of other B cell malignancies carrying Burkitt-like chromosome translocations (Nishikura *et al.*, 1983; ar-Rushdi *et al.*, 1983; Croce *et al.*, 1983; Erikson *et al.*, 1983a,b). Clearly the *c-myc* product must have a role in the control of B cell proliferation. Its constitutive expression at elevated levels in Burkitt lymphoma cells apparently contributes to their continuous proliferation resulting in a high grade neoplasm.

Consistent reciprocal chromosome translocations involving the heavy chain locus are also observed in other B cell neoplasms of man. For example, the t(11;14) (q13;q32) chromosome translocation is observed in CLL, diffuse B cell lymphoma and multiple myeloma, and the t(14;18) (q32;q21) translocation is observed in follicular lymphomas. Since we have shown that in these cases also the chromosomal breakpoints on chromosome 14 directly involve the heavy chain locus (Erikson *et al.*, 1984; Tsujimoto *et al.*, 1984a,b) it was logical to postulate that specific loci on chromosomes 11 and 18 might be involved in the oncogenic process in malignant B cells carrying these translocations. By "walking" on the 14q⁺ chromosome it has been possible to isolate and characterize the chromosomal regions involved in these rearrangements, providing the tools to isolate and characterize two putative oncogenes, *bcl-1* and *bcl-2*, that might be responsible for many of the B cell neoplasms in man.

Thus specific chromosome rearrangements observed in malignant cells provide us with an opportunity to identify "oncogenes" that are involved in the malignant process which would, otherwise, be undetectable. This approach may result not only in a better understanding of the genes involved in the neoplastic process in B cells, and possibly of the role of these genes in B cell differentiation and/or proliferation, but also provides extremely useful probes to detect specific chromosomal changes in human neoplasms. Thus we can suggest that in the near future the diagnosis of some human B cell malignancies may be carried out by the examination of the DNA of the neoplastic cells using DNA probes flanking the chromosomal breakpoints.

ACKNOWLEDGMENTS

This work was supported in part by grants from the National Cancer Institute, CA36521 and CA25875, and grants from the National Foundation-March of Dimes and the American Cancer Society. We thank Kathleen Reinersmann for preparation of this manuscript.

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re and is expressed constitutively. *c-myc* regulation is the kind of other B cell malignancies (Nishikura *et al.*, Erikson *et al.*, 1983a,b). Control of B cell proliferation in Burkitt lymphoma cells resulting in a high

number of cells involving the heavy chain genes of man. For example, observed in CLL, diffuse large cell lymphoma, and follicular lymphoma, we have shown that in chromosome 14 directly involved in these rearrangements (Tsujimoto *et al.*, 1984a,b). Chromosomes 11 and 18 might be involved in these rearrangements. B cells carrying these rearrangements are involved in these rearrangements. We have characterized two putative genes for many of the B cell

genes involved in malignant cells. These genes are "that are involved in the rearrangement of the DNA of the cell." This approach of the genes involved in the rearrangement of the DNA of the cell provides extremely useful information on human neoplasms. Thus, the study of some human B cell genes involved in the rearrangement of the DNA of the cell provides extremely useful information on human neoplasms.

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Somatic cell hybridization. Previously, the procedure usually required the participation of multiple animals, and the reagents were jealously guarded. Imprecise and irreproducible results, with relative ease, lymphoma cell lines against impure antigens, and antibodies may be prepared.