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Cloning and Sequencing of a c-*myc* Oncogene in a Burkitt's Lymphoma Cell Line That Is Translocated to a Germ Line Alpha Switch Region

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We have cloned and sequenced the translocated c-*myc* gene from the Burkitt's lymphoma CA46 cell line that carries a reciprocal translocation between chromosomes 8 and 14. The breakpoint lies within the first intron of c-*myc*, so that the first noncoding exon of the gene remains on the $8q^-$ chromosome. The second and third coding exons are translocated to the $14q^+$ chromosome into the switch region of C-alpha 1. The orientation of the c-*myc* gene with relationship to alpha 1 is 5′ to 5′, with directions of transcription in opposite orientation. DNA sequencing studies predict five changes in the amino acid sequence of the *myc* protein, two of which occur in a region within the second exon which is highly conserved in evolution. Southern blotting data indicate that the first exon of c-*myc* is rearranged 3′ to 3′ with the pseudo-epsilon gene. Because CA46 cells contain two rearranged mu genes, the translocation must have occurred after immunoglobulin rearrangement. The position of the breakpoint in CA46 occurs within a 20-base-pair region of the first intron of c-*myc* to which breakpoints have been mapped for two additional B-cell lymphomas with the t(8;14) translocation, ST486 and the Manca cell line. The region of the heavy chain locus to which c-*myc* has translocated is different in each case. Comparisons have been made of the levels of transcripts of the translocated c-*myc* gene in ST486 and CA46, where the gene is not associated with the heavy chain enhancer, with its expression in the Manca cell, in which it is. The c-*myc* gene is transcribed at similar levels in all three cases.

The chromosomal translocations that characterize the majority of Burkitt's lymphomas and mouse plasmacytomas place the c-*myc* gene in proximity to one of the three immunoglobulin loci (1, 8, 12, 14, 15, 31, 52). These rearrangements result in altered expression of c-*myc* (3, 30, 39, 48) or an altered c-*myc* product (43), and both types of changes have been proposed as determinants of the Burkitt phenotype. However, the fact that several translocated c-*myc* genes are identical in nucleotide sequences to their normal counterparts (5, 43) argues against a primary role for c-*myc* protein alterations in determining malignant transformation. Elevated c-*myc* transcription (28) has been found in many Burkitt's cell lines as well as in mouse plasmacytomas, and the degree of elevation varies over a wide range (19, 31, 39). The c-*myc* gene involved in the translocation is very active, although the c-*myc* gene on the normal chromosome 8 is either depressed or transcriptionally silent (3, 11, 17, 39). Therefore, transcription of the normal and translocation-associated c-*myc* alleles must be differentially regulated.

Translocations into the heavy chain region constitute 75% of those observed, and rearrangements occur that place *myc* into the V_H, J_H, $S\mu$, or C-γ region (14, 18, 43, 45) and join c-*myc* and the immunoglobulin H (IgH) locus in an opposite transcriptional orientation (12, 52). In Burkitt's lymphomas with the t(8;14) chromosomal translocation, the breakpoints on chromosome 8 are always at the 5′ end of c-*myc* and either separate the first noncoding exon from the second and third coding exons or occur within the flanking sequences 5′ of the first exon. The c-*myc* coding sequences are always translocated to chromosome 14, and the positions of the breakpoints in the 5′ flanking region of c-*myc* may vary from 500 base pairs (bp) to more than 12,000 bp upstream of the first exon (19, 43). The heterogeneity of these rearrangements raises the question as to what effect the associated heavy chain loci and the alterations in the 5′ region of the c-*myc* gene have on the regulation of expression of the translocated c-*myc* gene.

We describe here the cloned, rearranged c-*myc* gene from the Burkitt's cell line CA46. This rearrangement belongs to the class in which the noncoding first exon I remains on the $8q^-$ chromosome and the coding sequences are translocated to the $14q^+$ chromosome. The breakpoint on chromosome 8 is almost identical to that described for the ST486 and the Manca cell lines (22, 45); however, the rearrangement into the IgH locus is different in each case. In CA46 cells, c-*myc* is translocated into the switch region of the $C\alpha 1$ gene. This is the predominant site of c-*myc* translocations, which is seen in mouse plasmacytomas (1, 22). In ST486 cells, c-*myc* associates with the switch region of $C\mu$, and in Manca cells it is translocated into the J_H region associated with $C\mu$, placing it in close proximity to a defined IgH enhancer on the $14q^+$ chromosome. By contrast, this enhancer in CA46 and ST486 cells is located on the $8q^-$ chromosome as a result of the reciprocal chromosome transfer. We describe here the characteristics of the c-*myc* translocation in CA46 and examine the effects of the J_H, $S\mu$, and $S\alpha$ heavy chain regions on the transcription of the translocated c-*myc* genes in CA46, ST48, and Manca cell lines in which the breakpoints in the c-*myc* intron differ by only 10 to 20 bp. Sequence changes within the translocation c-*myc* gene of CA46 are discussed in relation to changes seen in other translocated c-*myc* genes.

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MATERIALS AND METHODS

Cell lines. Burkitt's lymphoma cell line CA46 has a reciprocal translocation between band q24 on chromosome 8 and band q32 on chromosome 14 (13). CA46, JD38, ST486, Daudi (6), and Manca (40) cells were all established from nondifferentiated B-cell lymphomas. Molt 4 and HSB2 cells were established from a T-cell lymphoma and a leukemia, respectively (36). GM1500A is a human lymphoblastoid cell line obtained from the Human Genetic Mutant Cell Depository, Camden, N.J.

Construction of genomic libraries. Genomic libraries were constructed in the lambda phage EMBL-3A (21). High-molecular-weight DNA was isolated from suspension cultures of the Burkitt's cell line CA46 (6) and digested to completion with *Bam*HI restriction endonuclease which has been shown to yield a 19-kilobase (kb) rearranged band in addition to the normal 27-kb band (12). The digested DNA was size fractionated on a 10 to 40% sucrose gradient in an SW41 rotor and collected as 0.5-ml fractions. Samples of the fractions were separated on 0.8% agarose gels, blotted to nitrocellulose, and hybridized to a probe for the second and third exons of c-*myc*. Fractions containing the rearranged c-*myc* gene were ligated to EMBL-3A arms and packaged with BHB2688 and BHB2690 lambda packaging strains as described by Hohn (27). The normal *Bam*HI fragment is excluded from packaging due to its large size. Recombinant phage were screened at a density of 10,000 to 15,000 plaques per plate with plaque lifts onto nitrocellulose (25). Approximately 1.5×10^5 recombinant phage were screened, and 12 c-*myc*-positive clones were isolated. Three of these clones were amplified and shown by restriction mapping to be identical.

Probes. The probe for the c-*myc* coding exons is a 1,092-bp fragment of a cDNA clone, pRyc 7.4 (55), which includes 200 bp of the second exon and all of the third exon. The first exon probe is a 449-bp *Xho-Pvu*II fragment derived from the genomic clone λMC41 (3, 13). The Cμ probe is a 1.2-kb *Eco*RI genomic fragment, and the Sμ and J_H probes are *Eco*RI-*Hin*dIII fragments previously described (12). The Cα probe is the 600-bp XP-8 genomic fragment in M13 phage which hybridizes with Cα1 and Cα2 regions (20). The Cε probe is a 2.7-kb *Bam*HI fragment which we subcloned from λCH38 and which also hybridizes with the pseudo-ε gene (32). The probe used for S1 analysis of c-*myc* expression was a 1,400-bp *Xba-Bst*EII genomic fragment, subcloned from λMC41, which encompasses the first intron and part of the second exon of c-*myc* (39).

Hybridization probes were prepared with calf thymus primers (51). Specific activities were routinely 10^9 cpm/μg. Unincorporated [^{32}P]dNTPs were removed by centrifugation through a Sephadex G100 mini-column before use. The S1 probe was 5′ labeled with γ-^{32}P at the *Bst*EII site in the second exon. Specific activities are 10^6 cpm/μg.

S1 analysis. The S1 nuclease protection experiments were carried out as described (7, 47), with modifications previously described (39).

Subcloning. Restriction fragments of lambda genomic clones were ligated into pBR322, pUC18, or pUC19 and used to transform *Escherichia coli* HB101 (37).

Southern hybridization and genomic library screening. High-molecular-weight DNA was digested with restriction endonucleases for 4 to 6 h, and 10-μg samples were fractionated on agarose gels and blotted to nitrocellulose. In the analysis of the digests of cloned DNA, several filters were usually pulled from one agarose gel at 15-min intervals for hybridization with various probes. Hybridizations were carried out in either 5× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate)–Denhardt solution at 65°C for 16 h or in 4× SSC–50% formamide at 37°C for 24 to 48 h. Final washes were generally 0.1× SSC–0.1% sodium dodecyl sulfate at 65°C. The screenings of genomic libraries were carried out at 65°C and 5× SSC, but final washings were 0.5 to 1.0× SSC at 65°C.

DNA sequences. DNA sequences were obtained by using the dideoxy chain termination method (46) on fragments cloned into M13 phage. Vectors mp8, mp9, mp10, mp11, mp18, and mp19 were used (35).

RESULTS

Characterization of the rearranged c-*myc* gene in CA46 cells. Figure 1 shows the genomic organization of the rearranged c-*myc* gene in the clone λ CA-11B derived from the cell line CA46. This clone contains 11.5 kb of the c-*myc* locus and 8 kb of the IgH locus. The breakpoint in c-*myc* occurs within the first intron, thereby removing the noncoding first exon as described for several other Burkitt's translocations (22, 45). The arrow indicates the breakpoint 5′ of an *Sst*I site within the first intron of c-*myc*. Restriction fragments which hybridize to radioactive probes for various regions of the IgH locus are indicated below the map. Only the 1.3-kb *Sst*I fragment immediately downstream from the breakpoint hybridizes strongly with the 5.5-kb probe derived from the Sμ region (Fig. 1B). This probe cross-hybridizes with Sα and Sγ sequences.

The translocation in CA46 cells places c-*myc* into the switch region of a Cα1 gene. The association of c-*myc* with the Cα1 gene was confirmed both by the alignment of the restriction maps of the Cα loci with the restriction map of CA-11B and by cross-hybridization with a Cα probe (XP-8) (20). The restriction fragment which hybridizes with XP-8 is indicated in Fig. 1B. The identification of this locus as Cα1 was accomplished by partially sequencing the *Xho-Sma*I fragment. Comparison of the sequence of the 200 bp 5′ from the *Xho* site with the published sequences from the Cα1 and Cα2 regions (20) confirmed that the translocation is into the Cα1 region (data not shown). The Sα region is in the germ line configuration. No cross-hybridization was detected between the sequences in CA-11B and the J_H probe which contains the associated heavy chain enhancer and sequences flanking Sμ. This rearrangement of c-*myc* to Cα1 in genomic DNA was confirmed by Southern blotting (Fig. 2). *Eco*RI or *Hin*dIII restriction fragments hybridized to both the Cα (XP-8) and the c-*myc* cDNA pRyc 7.4 probes. The 13.5-kb *Hin*dIII fragment included in the CA-11B clone, which also hybridizes with both probes, is the same size as the *Hin*dIII fragment seen on the Southern blot.

Sequence of the c-*myc* breakpoint. The chromosomal breakpoint of CA-11B was mapped (Fig. 1) to a 400-bp *Sst*I restriction fragment by the divergence at the 3′ *Sst*I site from the restriction map for the normal c-*myc* gene (56). Figure 3 shows the nucleotide sequence of this *Sst*I fragment. The vertical arrows indicate the position of the breakpoint in CA46 cells and those of the published breakpoints for ST486 (22) and Manca cells (45). The boxed areas flanking these breakpoints in the normal c-*myc* sequence are homologous to pentameric repeats associated with IgH switch regions. The DNA of CA46 cells contains a single base pair change within this *Sst*I restriction fragment at position 270 which creates the tetranucleotide GAGG. This sequence was found to be associated with several c-*myc* translocations in mouse plasmacytomas by Piccoli et al. (41), who suggested that

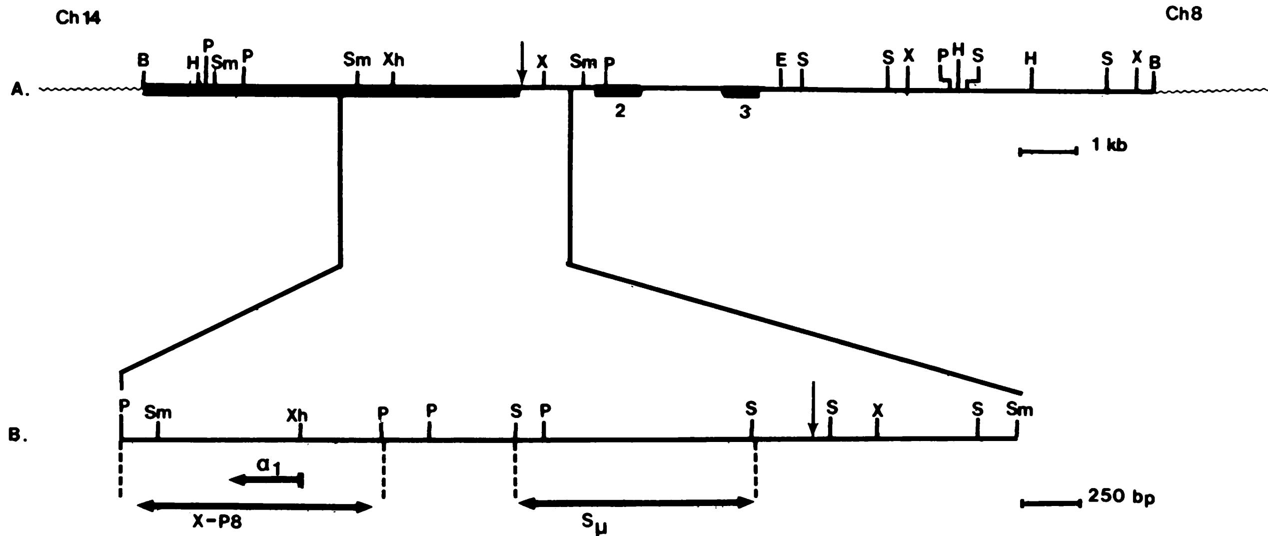

FIG. 1. Restriction map of the c-*myc* clone λCA-11B. (A) Restriction enzyme sites within the cloned *Bam*HI fragment which contains the c-*myc* and Cα1 loci. The vertical arrow indicates where the restriction maps of CA-11B and a normal c-*myc* gene diverge. Solid blocks indicate the positions of the second and third exons. (B) A detailed restriction map of the rearrangement. The arrow labeled α1 indicates the restriction fragment for which the nucleotide sequence was determined and which identifies this region as Cα1. X-P8, Cα probe. The Sμ probe is a 5.5-kb *Eco*RI-*Hin*dIII fragment. Abbreviations: E, *Eco*RI; H, *Hin*dIII; B, *Bam*HI; X, *Xba*I; S, *Sst*I; Sm, *Sma*I; Xh, *Xho*I; and P, *Pst*I.

GAGG may be important in defining the c-*myc* breakpoints. We cannot exclude the possibility that the base pair change at position 270 occurred after the translocation; however, there are two additional GAGG tetranucleotides within the normal sequence of c-*myc* 5′ of the breakpoints. These sequences are underlined in the figure.

Sequence changes in the second and third c-*myc* exons CA46. Figure 4 shows the strategy used to determine the nucleotide sequence of the CA-11B c-*myc* gene. The second and third exons were sequenced completely, as were the borders of the second intron and 200 bp 3′ of the third exon. Table 1 lists the positions of the predicted amino acid changes in the c-*myc* gene. Also listed are some of the changes for the translocated genes from the Raji and Ly67 Burkitt cell lines (43, 44) as well as changes in the c-*myc* gene from the chicken bursal lymphoma LL3 (57). Both CA46 and the Raji c-*myc* genes have mutations at the N-terminus, position 5 in CA46 cells, and positions 6 and 7 in Raji cells. The amino acid change at position 179 in CA46 cells, which converts a leucine to valine, affects the most 3′ *Pst*I site in the second exon. In addition, we find a glutamine-to-histidine change at position 306, the first example of an amino acid change in the third exon. However, the cluster of mutations between positions 54 and 62 is most striking. Each of the altered human c-*myc* sequences has one or more changes in this region. There are three mutations in CA46, one of which is silent at position 54, and changes at positions 57 and 60 which convert two prolines to a serine and histidine, respectively. The Raji clone has an insertion of 3 nucleotides between positions 56 and 57, resulting in the addition of a leucine. Ly67, which carries a variant translocation associated with the λ light chain locus, has a serine-to-proline change at position 62 (43). These changes occur in an area which is highly conserved in the v-*myc* (2) and in the mouse, human (9, 56), and chicken (54) c-*myc* homologs. Westaway et al. (57) have reported that the chicken bursal lymphoma LL-3 also exhibits a sequence change within this region (at the equivalent of the human position 60) which converts a proline to a threonine.

Pathway of c-*myc* translocation. Was c-*myc* directly translocated into the Cα1 region? If translocation occurred in a two-step fashion (as has been suggested in the case of Raji; 26) i.e., *myc* is first translocated to Cμ and then to Cα1 in a class switch-like event, we would expect a deletion of one of the Cμ genes. This is not the case, as CA46 cells have been shown to contain two rearranged Cμ genes (12). In addition, initial translocation of the c-*myc* second and third exons into the switch region of Cμ would place the first exon on the $8q^-$ chromosome adjacent to the J_H sequences. On the other hand, the translocation directly to Cα1 would place the first exon adjacent to sequences derived from the pseudo-ε gene located upstream of Cα1. Figure 5A represents the possible recombinations, and Fig. 5B shows the results from South-

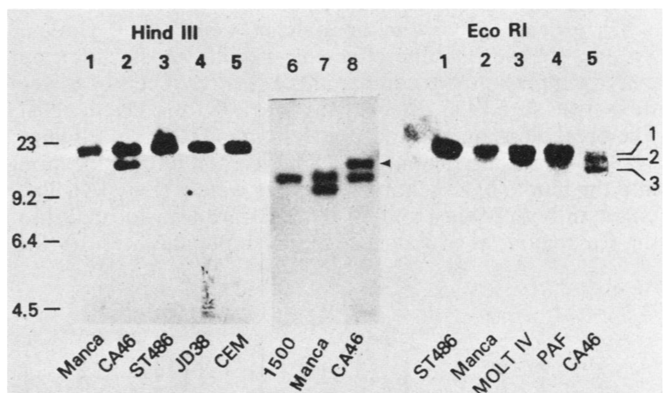

FIG. 2. Rearrangement of Cα genes in the Burkitt lymphoma cell line CA46. JD38, ST486, CA46 and Mance are Burkitt's or Burkitt-like B cell lymphoma cell lines; Molt 4 (MOLT IV) and CEM (T-cell lines) and PAF (a fibroblast line) served as controls for the germ line IgH pattern. The probe was a 2.9-kb *Sma*I fragment from a genomic Cα clone. Bands 1 and 2 are the germ line Cα1 and Cα2 genes, respectively, and band 3 is the rearranged Cα1 gene band. The arrow indicates the band that also hybridized with a probe for the second and third exons of c-*myc*.

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A CTCCCATTCCTGCGCTATTGACACTTTTCTCAGAGTAGTTATGGTAACTGGGGCTGGGGTGGGGGGTAATCCAGAACGGA

A TCGGGGTAAAGTGACTTGTCAAGATGGGAGAGGAGAAGGCAGAGGGAAAACGGGAATGGTTTTTAAGACTACCCTTTGCG
B TCATCTAGCCCAGCCCAGGTCAGCCTAGCCCAGCCGAACCCAGCTCAGCCCGGTCAACCCAATTCAGCTCAGCGTCAGCC

                              ST486        Manca     CA46
A AGATTTCTGCCTTATGAATATATTCACGCTGACTCCCGGCCGGTCGGACATTCCTGCTTTATTGTGTTAATTGCTCTCTG
B CAGGTCAACCCAACCAAGCTCAACGCAGCCCTAGCCCAGTCCAGCTAGCCCAGCTGCTTTATTGTGTTAATTGCTCTCTG

A GGTTTTGGGGGGCTGGGGGTTGCTTTGCGTGGCAGAAAGCCCCTTGCATCCTGAGCTC
B GGTTTTGGGGGGCTGGGGGTTGCTTTGCGAGGCAGAAAGCCCCTTGCATCCTGAGCTC
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FIG. 3. Nucleotide sequence spanning the c-*myc* breakpoint. (A) Sequence of the normal c-*myc* (from Watt et al. [56]). (B) Sequence of the *SstI* fragment to which the breakpoint is mapped (indicated by vertical arrow in Fig. 1B). The arrows indicate the breakpoint for CA46 cells and the published breakpoints for ST486 (22) and Manca (45) cells. The blocked sequences are immunoglobulin switch-like repeats flanking the breakpoint. The tetranucleotides GAGG are underlined. ★, Positions of homology.

ern blot hybridization. In addition to the germ line patterns, the probe for the first exon of c-*myc* hybridizes with a 15-kb *Hin*dIII or a 21-kb *Eco*RI rearranged fragment. Restriction fragments of the same sizes also hybridize with a probe for the ε region. The ε genes are clearly rearranged in CA46 cells whereas germ line patterns are found in the other examples. The fact that this rearrangement is observed with both enzymes makes it unlikely that the restriction fragments containing the first exon sequences are comigrating with the ε seqences. From these data, we can conclude that the reciprocal translocation occurred in a single step, placing the pseudo-ε region downstream of the first exon of c-*myc* and the second and third exons upstream of the Cα1 region. Since Southern (50) analysis reveals two rearranged Cμ genes (12), the c-*myc* translocation most probably occurred after the heavy chain rearrangement.

S1 nuclease protection analysis of c-*myc* transcription. To analyze the possible effect of specific translocation on c-*myc* expression, we compared the relative levels of c-*myc* transcripts in ST486, Manca, CA46, JD38, and Daudi cells. The breakpoint on chromosome 8 in the first three cell lines lies within a 20-bp region, although the point of translocation into the immunoglobulin region varies widely (Fig. 6A). The c-*myc* in both Manca and ST486 cells are translocated into the Cμ region, as are most Burkitt lymphomas with t(8;14) translocation. In the former cell line, c-*myc* is in close proximity to the heavy chain enhancer sequences located between the J_H and Sμ regions (45). In ST486 cells, c-*myc* is translocated into the Sμ region (22), and in CA46 cells, the c-*myc* gene is translocated to the Cα region. In both cases, the heavy chain enhancer has been moved to the $8q^-$ chromosome. In Daudi cells, c-*myc* has been translocated to a V_H region (18), and in JD38 cells, the gene has been translocated to a heavy chain switch region (14).

Comparison of the levels of c-*myc* transcripts in these cell lines (Fig. 6B), with an *XbaI-Bst*EII restriction fragment containing a part of the first intron and the second exon as the probe, reveals a 322-nucleotide, S1-resistant product which is protected by the probe in the lanes for Daudi and for GM1500A, the human lymphoblastoid cells used as a control. This 322-bp fragment is derived from the second exon. In addition to this fragment, as expected, longer fragments are protected in the lanes for CA46, ST486, JD38, and Manca cells. These fragments derive from c-*myc* transcripts initiated from cryptic sites within the first intron (3). The levels of c-*myc* transcription as well as the initiation sites utilized are almost identical among these four cell lines (Fig. 6B, lanes 2 to 5). These results suggest that the previously identified IgH enhancer has little if any effect on the activation of the translocated c-*myc* gene.

FIG. 4. Strategy for DNA sequence studies. Various digests of restriction of the 4.5-kb *Xba-Eco*RI fragments were subcloned into an appropriate M13 phage vector (either mp8, mp9, mp10, mp11, mp18, or mp19) and sequenced. The arrows represent the length and direction of the sequenced fragments. Abbreviations: X, *XbaI*; PV, *PvuII*; S, *SstI*, T, *TaqI*; P, *PstI*; S3, *Sau*3A; C, *ClaI*; R, *Eco*RI. P*, a *PstI* site found in the normal c-*myc* but not present in clone CA-11B.

TABLE 1. Predicted amino acid changes in the protein sequence of the translocated c-*myc* gene[a]

Cell line	Amino acid position												
	5	179	306	54	55	56	56A	57	58	59	60	61	62
Normal	Val / GTT	Leu / CTG	Gln / CAG	Glu / GAG	Leu / CTG	Leu / CTG		Pro / CCC	Thr / ACC	Pro / CCG	Pro / CCC	Leu / CTG	Ser / TCC
CA46	Ile / ATT	Val / GTG	His / CAC	Glu / GAA				Ser / TCC			His / CAC		
RAJI				Glu / GAA			Leu / CTG		Asn / AAT				
Ly67													Pro / CCC
LL3				Glu / GAA							Thr / ACC		

[a] Positions 5 and 179 are in the second exon, and position 306 is in the third exon. The normal sequences are from Watt et al. (55). Amino acid changes in Raji (44) are listed only for positions 54 to 62. Amino acid changes in Ly67 are from Rabbitts et al. (42), and those in LL3 are from Westaway et al. (57).

DISCUSSION

Breakpoints in the first intron of c-*myc*. We have described the rearrangement of a c-*myc* gene in a Burkitt's lymphoma with a breakpoint within the first intron. The position of this breakpoint in c-*myc* for CA46 cells and the previously published breakpoints for ST486 (12) and Manca cells (45) map within a 20-bp stretch of DNA. An additional breakpoint in the non-Burkitt's lymphoma JD38 has been mapped close to this region (unpublished results). Gerondakis et al. (23) reported a similar conservation of breakpoints in the first intron of c-*myc* for mouse plasmacytomas and suggested that those sites define a region in which breakpoints can occur and still result in a functional c-*myc* gene. The positions of the breakpoints within the first intron of the human c-*myc* appear to define a similar region. The switch-

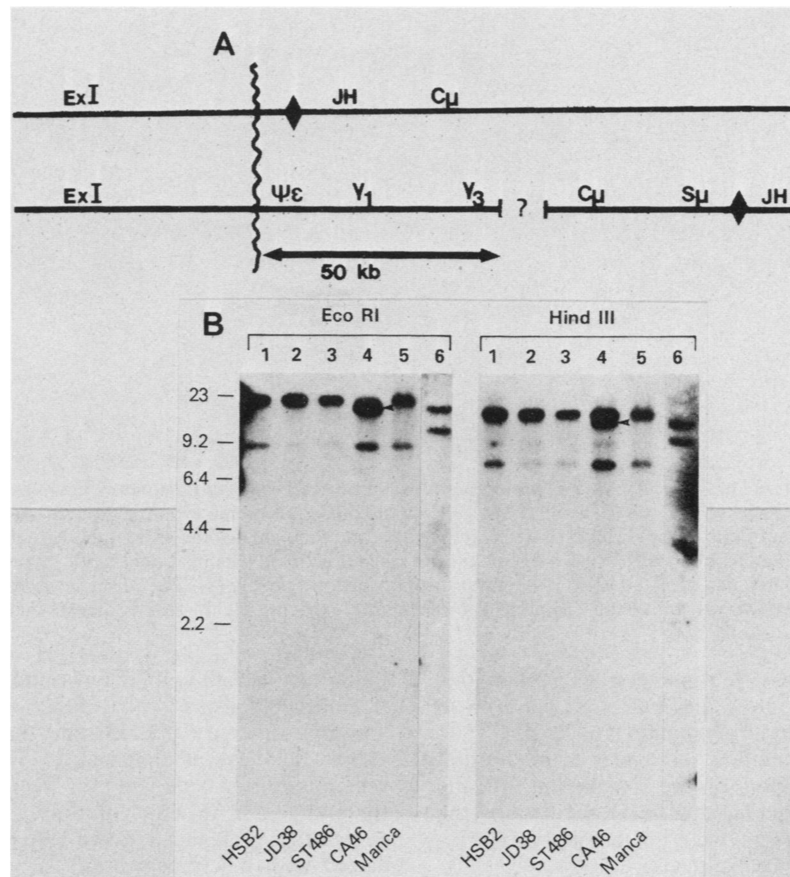

FIG. 5. Rearrangement of the first exon of c-*myc* on the $8q^-$ chromosome. (A) The upper diagram shows the expected arrangement of the c-*myc* first exon, assuming translocation of c-*myc* first to the Cμ region and then to Cα1. The lower diagram shows the arrangement assuming direct translocation of c-*myc* to Cα1. (◆) The position of the IgH enhancer in relation to the first exon in each case. (B) Southern blot demonstrates the comigration of ε sequences and c-*myc* first exon sequences in both *Hin*dIII and *Eco*RI digestions. HSB2 human T-cell leukemia cells give the germ line ε pattern. Lanes 1 to 5 show results of hybridization with a *Bam*HI fragment from the ε locus (32); lane 6 contains the CA46 on the same filter which was washed and then rehybridized with the *Xho*I-*Pvu*II probe for the c-*myc* first exon. The arrows indicate the positions of the bands that hybridized with both probes.

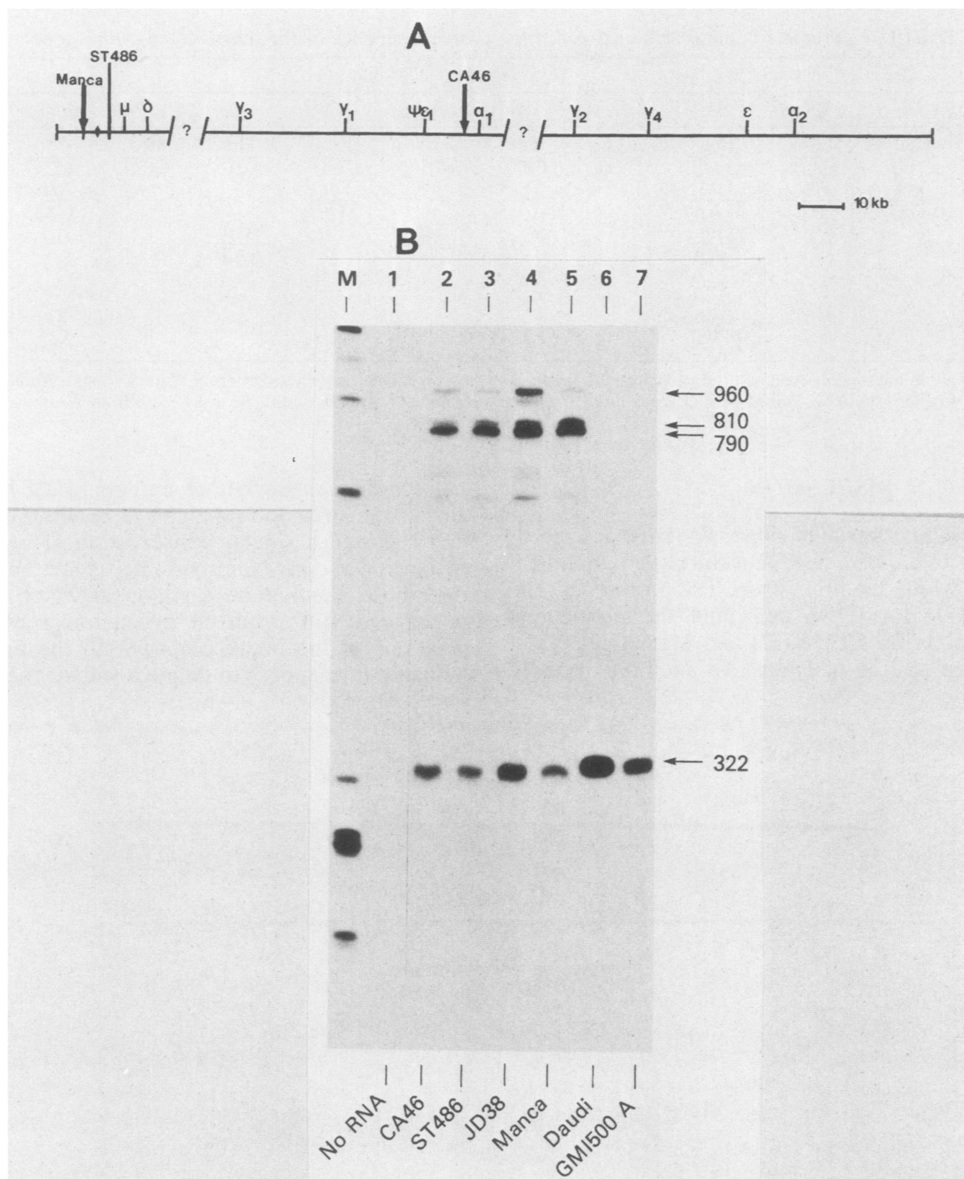

FIG. 6. S1 nuclease protection studies. (A) The position of the translocation of c-*myc* into the heavy chain genes. (◆) The position of the heavy chain enhancers. The translocations in ST486 and CA46 cells would place this element on the $8q^-$ chromosome. The translocation in Manca cells is directly upstream of this enhancer, and that in Daudi cells is an unknown distance 5′ to the V_H region. (B) S1 mapping analysis of c-*myc* RNAs. The S1 probe, an *Xba*I-*Bst*EII DNA fragment 5′ end labeled with ^{32}P was heat denatured, hybridized in 80% formamide to 20 μg of cytoplasmic RNA at 55°C, and digested with S1 nuclease as described previously (3). The S1 nuclease-resistant DNA products were fractionated on a 4% polyacrylamide gel containing 7 M urea. The size markers (M) are pX174-*Hae*III digests that are 5′ end labeled with ^{32}P.

like sequences and the GAGG tetranucleotides flanking this 20-bp region on chromosome 8 may also play a role in defining the position for these breakpoints (16, 23, 41). Taub et al. (52) have noted switch-like sequences 5′ of the first exon of c-*myc* in the region of the breakpoint for the Burkitt's cell line BL22. Piccoli et al. (41) have found the GAGG tetranucleotide present near the breakpoints of c-*myc* in several mouse plasmacytomas.

Changes in amino acid sequences of c-*myc* in Burkitt's lymphomas. The rearranged c-*myc* gene in CA46 cells has six nucleotide changes within the coding sequences which predict five amino acid substitutions relative to the amino acid sequence reported for a normal c-*myc* gene (9, 55). These changes are few compared with the 16 changes reported for Raji cells (44). Rabbitts et al. have suggested that the mutations in Raji cells may be the result of somatic mutational mechanisms which function in the generation of antibody diversity (33, 53) and that translocated c-*myc* is subject to these mechanisms by virtue of its position in a region normally occupied by a V_H gene. Furthermore, since the Sγ1 region to which the c-*myc* gene is translocated in Raji cells is rearranged, a two-step process has been hypothesized with the translocation to Sγ1 occurring as a class-switch event, thereby subjecting the c-*myc* gene to two rounds of mutation (44). The translocation of c-*myc* in CA46 cells has occurred by a single step into a germ line Cα1 region, an event that must then activate this locus for somatic mutation by mimicking a class-switch event. The spatial relation between c-*myc* and Cα1 is more appropriate to distances between J_H or S_H and IgH constant regions than

to distances between V_H and constant regions. Thus, a ruler model for generating somatic diversity (42) does not seem to apply in this case, nor does it apply in the case of Ly67 or Daudi which are more than 14 kb upstream of immunoglobulin constant regions in both cases.

We cannot completely exclude the possibility that some of the amino acid changes are polymorphic, but it is difficult to attribute the cluster of mutations around amino acid 58 to polymorphisms alone. These mutations are in an area that is highly conserved through evolution, suggesting a functional importance for the protein. The possible significance of mutations in these specific regions for protein function is unclear. Westaway et al. (57) have proposed that the mutation in the c-*myc* gene within this hypermutable region in chicken bursal lymphoma cell line LL-3 confers a selective advantage during tumor progression for cells that carry this mutation. Alternatively, it is possible that the function of the protein domain encoded by this hypermutable region is not necessary in maintaining the malignant phenotype; it is, rather, a second function encoded primarily by the stable third exon. Rabbitts et al. (43) have shown that the normal c-*myc* in Raji cells is expressed. We also find low-level expression of the normal c-*myc* gene (<5% of levels for translocated c-*myc*) in CA46 cells (unpublished data) which might well compensate for that altered domain of the translocated c-*myc* product encoded by the second exon.

In contrast to CA46 and Raji cells, normal nucleotide sequences have been reported for the coding exons of the translocated c-*myc* gene from Burkitt cell lines BL22 (5) and Daudi (43). This indicates that amino acid sequence changes are not required for the expression of the Burkitt's phenotype. If a functionally normal c-*myc* protein is necessary to maintain the transformed phenotype in Daudi and BL22 cell lines, this function may be fulfilled by that protein domain which is unaltered in both the Raji and CA46 cell lines. The accumulation of mutations in the latter two cell lines may be a function of the stage of B-cell differentiation they represent, and the level of mutation may be a function of continued mutation during clonal expansion which has been suggested to be the case for immunoglobulin genes (32).

Expression of the translocated c-*myc*. Two hypotheses have been proposed to explain the deregulation of c-*myc* transcription after its translocation: one focuses on the role of a repressor and its binding site(s) within the c-*myc* locus; the other focuses on the influence of the immunoglobulin locus on c-*myc* expression. Siebenlist et al. (49) have recently identified a putative repressor binding site at the 5′ end of the first exon of c-*myc*. Translocations which separate this exon from the rest of the c-*myc* gene as well as those with accumulated mutations within this region would release the c-*myc* gene from the action of this repressor. However, this would not explain the deregulation of the translocation-associated c-*myc* genes in which the first exon is normal. In addition, studies with somatic cell hybrids between 3T3 mouse fibroblasts and Burkitt's lymphoma cells in which the translocated c-*myc* gene is separated from the first exon showed that the hybrids (i) did not transcribe the translocated c-*myc* gene but only the mouse c-*myc* gene and (ii) failed to transcribe the productively rearranged immunoglobulin locus (38). Because the normal c-*myc* gene is expressed in 3T3 cells, those results suggested a coregulation of the translocated c-*myc* gene with the associated immunoglobulin locus. Hybrids between human lymphoblastoid and Burkitt's lymphoma cells in which the lymphoblastoid phenotype is dominant also fail to express the decapitated translocated c-*myc* gene which would lack the putative repressor binding site. However, the hybrids do express the productively rearranged immunoglobulin genes, so that c-*myc* expression must be further regulated at another level (10). In hybrids with mouse plasmacytoma cells in which the plasma cell phenotype is dominant, the decapitated c-*myc* from the Burkitt's lymphoma was expressed at high levels, although the normal c-*myc* on chromosome 8 was not expressed (10). This finding suggests the involvement of transacting factors that are differentiation stage specific and that affect only the c-*myc* gene associated with an immunoglobulin locus.

The differential regulation of a c-*myc* gene that is associated with an immunoglobulin locus versus one that is not raises the question of how that influence is applied and reflected in enhanced c-*myc* expression. Of the CA46, ST486, and Manca cell lines, only Manca cells have a translocation which places c-*myc* near a defined enhancer (45). However, the level of c-*myc* transcription in Manca cells is similar to that for ST486, JD38, CA46, and Daudi cells (Fig. 6), suggesting that the IgH enhancer (4) between J_H and Sμ may not be the primary cause of elevated expression, even in Manca cells. Furthermore, in many t(8;14) translocations, this enhancer element is moved to the $8q^-$ chromosome. In Daudi cells, the translocation of c-*myc* is into the V_H regions, placing the gene more than 14 kb upstream of the heavy chain enhancer (18, 44); this is well beyond the postulated range of influence (~10 kb) of this enhancer (24, 29). Thus, elevated transcription from the translocation-associated c-*myc* genes must be due to other factors.

Based on the dispersed character of the translocations seen in Burkitt's lymphomas, Croce et al. (10) suggested that enhancer-like elements acting over very long distances may be involved in c-*myc* expression. Many of the t(8;14) translocations previously described for Burkitt's lymphomas place the c-*myc* gene into the Cμ region. Since the translocation in CA46 cells places c-*myc* 5′ to Cα1, at least one such enhancing element would be expected 3′ of the Sα1 region. Because the translocations in both Manca and ST486 cells are into the Cμ locus, the range of activity of this enhancing element would have to extend for more than 50 kb to activate these genes. If additional elements reside 5′ of the Cα1 in the heavy chain locus, one would expect enhancement of expression of the first exon of CA46 cells, since those elements would be translocated to chromosome 8 in the reciprocal chromosomal exchange. However, Northern blotting studies show that neither the first exon of CA46 cells, which is associated with the pseudo-ε region, nor that of Manca, which associates with the region 5′ of J, is expressed from the $8q^-$ chromosome (A. ar-Rushdi, unpublished data). By this analysis, any long-range enhancing element would have to be located either within or 3′ of the Cα1 region defined by the CA46 translocation. Attempts to identify enhancer activity within the Cα region in the mouse have not succeeded (34), and our preliminary data also indicate that the alpha region sequences contained in the CA-11B clone do not have enhancer activity in lymphoblastoid or plasmacytoma backgrounds (S. Feo and L. C. Showe, unpublished data). We have now isolated lambda recombinants which extend 3′ of the human Cα1 in an effort to identify any putative additional enhancing elements that may exist. However, certain aspects of the rearrangements of c-*myc* with either the heavy or light chain loci suggest that the influence of these loci on c-*myc* expression does not adhere to the criteria used in defining such enhancing elements. The rearrangements of c-*myc* with either heavy or light chain genes which have been observed always place the

c-*myc* gene upstream of an immunoglobulin constant region gene. Thus, the effect of the immunoglobulin loci on c-*myc* transcription is unidirectional, and that of enhancers, as classically defined, is bidirectional. The "enhancing" effect of the immunoglobulin locus on c-*myc* expression over a distance of more than 50 kb must then be redefined and may well be a general effect of the entire locus on a gene that penetrates this domain.

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LITERATURE CITED

1. **Adams, J. M., S. Gerondakis, E. Webb, L. Corcoran, and S. Cory.** 1983. Cellular *myc* oncogene is altered by chromosome translocation to an immunoglobulin locus in murine plasmacytomas and is rearranged similarly in human Burkitt lymphomas. Proc. Natl. Acad. Sci. U.S.A. **80:**1982–1986.
2. **Alitalo, K., J. M. Bishop, D. H. Smith, E. Y. Chen, W. W. Colby, and A. D. Levinson.** 1983. Nucleotide sequence of the v-myc oncogene of avian retrovirus MC29. Proc. Natl. Acad. Sci. U.S.A. **80:**100–104.
3. **ar-Rushdi, A., K. Nishikura, J. Erikson, R. Watt, G. Rovera, and C. Croce.** 1983. Differential expression of the translocated and the untranslocated c-*myc* oncogene in Burkitt lymphoma. Science **222:**390–393.
4. **Banerji, J., L. Olson, and W. Schaffner.** 1983. A lymphocyte-specific cellular enhancer is located downstream of the joining region in immunoglobulin heavy chain genes. Cell **33:**729–740.
5. **Battey, J., C. Moulding, R. Taub, W. Murphy, T. Stewart, H. Potter, G. Lenoir, and P. Leder.** 1983. The human c-*myc* oncogene: structural consequences of translocation into the IgH locus in Burkitt lymphoma. Cell **34:**779–787.
6. **Benjamin, D., I. Magrath, R. Maguire, C. Janus, H. Todd, and R. Parsons.** 1982. Immunoglobulin secretion by cell lines derived from African and American undifferentiated lymphomas of Burkitt's and non-Burkitt's type. J. Immunol. **129:**1337–1342.
7. **Berk, A., and P. Sharp.** 1977. Sizing and mapping of early adenovirus mRNAs by gel electrophoresis of S1 endonuclease-digested hybrids. Cell **12:**721–732.
8. **Calame, K., S. Kim, P. Lalley, R. Hill, M. Davis, and L. Hood.** 1982. Molecular cloning of translocations involving chromosome 15 and the immunoglobulin C-alpha gene from chromosome 12 in two murine plasmacytomas. Proc. Natl. Acad. Sci. U.S.A. **79:**6994–6998.
9. **Colby, W. W., E. Y. Chen, D. H. Sith, and A. D. Levinson.** 1983. Identification and nucleotide sequence of a human locus homologous to the v-myc oncogene of avian myelocytomatosis virus MC29. Nature (London) **301:**722–725.
10. **Croce, C., J. Erikson, A. ar-Rushdi, D. Aden, and K. Nishikura.** 1984. Translocated c-*myc* oncogene of Burkitt's lymphoma is transcribed in plasma cells and repressed in lymphoblastoid cells. Proc. Natl. Acad. Sci. U.S.A. **81:**3170–3174.
11. **Croce, C., W. Thierfelder, J. Erikson, K. Nishikura, J. Firan, G. Lenoir, and P. C. Nowell.** 1983. Transcriptional activation of an unrearranged and untranslocated c-*myc* oncogene by translocation of a Cλ locus in Burkitt lymphoma cells. Proc. Natl. Acad. Sci. U.S.A. **80:**6922–6926.
12. **Dalla-Favera, R., M. Bregni, J. Erikson, D. Patterson, R. Gallo, and C. M. Croce.** 1982. Human c-*myc* onc-gene is located on the region of chromosome 8 that is translocated in Burkitt lymphoma cells. Proc. Natl. Acad. Sci. U.S.A. **79:**7824–7827.
13. **Dalla-Favera, R., E. P.Gelmann, S. Martinotti, G. Franchini, T. S. Papas, R. C. Gallo, and F. Wong-Staal.** 1982. Cloning and characterization of different human sequences related to the *onc* gene (v-*myc*) of avian myelocytomatosis virus (MC29). Proc. Natl. Acad. Sci. U.S.A. **79:**6497–6501.
14. **Dalla-Favera, R., S. Martinotti, R. C. Gallo, J. Erikson, and C. M. Croce.** 1983. Translocation and rearrangements of the c-*myc* oncogene locus in human undifferentiated B-cell lymphomas. Science **219:**963–967.
15. **Dean, M., R. B. Kent, and G. E. Sonenshein.** 1983. Transcriptional activation of immunoglobulin α heavy-chain genes by translocation of the c-*myc* oncogene. Nature (London) **305:**443–446.
16. **Dunnick, W., B. E. Shell, and C. Dery.** 1983. DNA sequence near the site of reciprocal recombination between a c-*myc* oncogene and an immunoglobulin switch region. Proc. Natl. Acad. Sci. U.S.A. **80:**7269–7273.
17. **Erikson, J., A. ar-Rushdi, H. L. Drwinga, P. C. Nowell, and C. M. Croce.** 1983. Transcriptional activation of the translocated c-*myc* oncogene in Burkitt lymphoma. Proc. Natl. Acad. Sci. U.S.A. **80:**820–824.
18. **Erikson, J., J. Finan, P. Nowell, and C. M. Croce.** 1982. Translocation of V_H genes in Burkitt lymphoma. Proc. Natl. Acad. Sci. U.S.A. **79:**5611–5615.
19. **Erikson, J., K. Nishikura, A. ar-Rushdi, J. Finan, B. Emanuel, G. Lenoir, P. C. Nowell, and C. M. Croce.** 1983. Translocation of an immunoglobulin κ locus to a region 3′ of an unrearranged c-*myc* oncogene enhanced c-*myc* transcription. Proc. Natl. Acad. Sci. U.S.A. **80:**7581–7585.
20. **Flanagan, J. G., and T. H. Rabbitts.** 1982. Arrangement of human immunoglobulin heavy chain constant region genes implies evolutionary duplication of a segment containing γ, ε, and α genes. Nature (London) **300:**709–713.
21. **Frischauf, A.-M., H. Lehrach, A. Poustka, and N. Murray.** 1983. Lambda replacement vectors carrying polylinker sequences. J. Mol. Biol. **170:**827–842.
22. **Gelmann, E. P., M. C. Psallidopoulos, T. S. Papas, and R. Dalla-Favera.** 1983. Identification of reciprocal translocation sites within the c-*myc* oncogene and immunoglobulin μ locus in a Burkitt lymphoma. Nature (London) **306:**799–803.
23. **Gerondakis, S., S. Cory, and J. Adams.** 1984. Translocation of the myc-cellular oncogene to the immunoglobulin heavy chain locus in murine plasmacytomas is an imprecise reciprocal exchange. Cell **36:**973–982.
24. **Gillies, S. D., S. L. Morrison, V. T. Oi, and S. Tonegawa.** 1983. A tissue-specific transcription enhancer element is located in the major intron of a rearranged immunoglobulin heavy chain gene. Cell **33:**717–728.
25. **Grunstein, M., and K. Wallis.** 1980. Colony hybridization. Methods Enzymol. **68:**379–389.
26. **Hamlyn, P., and T. Rabbitts.** 1983. Translocation joins a c-*myc* and immunoglobulin γ1 genes in a Burkitt lymphoma revealing a third exon in the c-*myc* oncogene. Nature (London) **304:**135–139.
27. **Hohn, B., and K. Murray.** 1977. Packaging recombinant DNA molecules into bacteriophage particles in vitro. Proc. Natl. Acad. Sci. U.S.A. **74:**3259–3262.
28. **Kelly, K., B. H. Cochran, C. D. Stiles, and P. Leder.** 1983. Cell-specific regulation of the c-*myc* gene by lymphocyte mitogens and platelet-derived growth factor. Cell **35:**603–610.
29. **Khoury, G., and P. Gruss.** 1983. Enhancer elements. Cell **33:**313–314.
30. **Leder, P., J. Battey, G. Lenoir, C. Moulding, W. Murphy, H. Potter, J. Stewart, and R. Taub.** 1983. Translocations among antibody genes in human cancer. Science **222:**765–770.
31. **Marcu, K. B., L. J. Harris, L. Stanton, J. Erikson, R. Watt, and C. M. Croce.** 1983. Transcriptionally active c-*myc* oncogene is contained within NIARD, a DNA sequence associated with chromosome translocation in B cell neoplasia. Proc. Natl. Acad. Sci. U.S.A. **80:**519–523.
32. **Max, E. E., J. Battey, I. R. Karsch, and P. Leder.** 1982. Duplication and deletion in the human immunoglobulin ε genes. Cell **29:**691–699.

33. **McKean, D., K. Huppi, M. Bell, L. Staudt, W. Gerhard, and M. Weigert.** 1984. Generation of antibody diversity in the immune response of Balb/c mice to influenza virus hemagglutinin. Proc. Natl. Acad. Sci. U.S.A. **81**:3180–3184.
34. **Mercola, M., J. Olsen, X. F. Wang, and K. Calame.** 1983. Transcriptional enhancers for the SV40 early promoter occur in mouse DNA, p. 129–135. *In* Y. Glitzman and T. Shenk (ed.), Enhancers and eukaryotic gene expression. Cold Spring Harbor Laboratories, Cold Spring Harbor, N.Y.
35. **Messing, J.** 1981. M13mp2 and derivatives: a molecular cloning system for DNA sequencing, strand-specific hybridization, and *in vitro* mutagenesis, p. 22–26. *In* A. G. Walton (ed.), Recombinant DNA, Proceedings of the Third Cleveland Symposium on Macromolecules. Elsevier Science Publishers B.V., Amsterdam.
36. **Minowada, J.** 1983. Immunology of leukemic cells, p. 119–139. *In* S. W. Gunz and E. S. Henderson (ed.), Leukemia, 4th ed. Grune and Stratton Publishers, New York, N.Y.
37. **Morrison, D. A.** 1980. Transformation and preservation of competent bacterial cells by freezing. Methods Enzymol. **68**:326–331.
38. **Nishikura, K., A. ar-Rushdi, J. Erikson, E. DeJesus, D. Dugan, and C. M. Croce.** 1984. Repression of rearranged μ gene and translocated c-*myc* in mouse 3T3 cells × Burkitt's lymphoma cell hybrids. Science **224**:399–402.
39. **Nishikura, K., A. ar-Rushdi, J. Erickson, R. Watt, G. Rovera, and C. Croce.** 1983. Differential expression of the normal and of the translocated human c-*myc* oncogenes in B cells. Proc. Natl. Acad. Sci. U.S.A. **80**:4822–4826.
40. **Nishikuri, M., H. Hansen, S. Jhanwar, J. Fried, P. Sorkillo, B. Kosizer, K. Lloyd, and B. Clarkson.** 1984. Establishment of a near-tetraploid B-cell lymphoma line with duplication of the 8;14 translocation. Cancer Genet. Cytogenet. **12**:39–50.
41. **Piccoli, S. P., P. G. Calm, and M. D. Cole.** 1984. A conserved sequence at the c-*myc* oncogene translocation breakpoints in plasmacytomas. Nature (London) **310**:327–330.
42. **Rabbitts, T. H., A. Forster, R. Baer, and P. Hamlyn.** 1983. Transcription enhancer identified near the human C-mu immunoglobulin heavy chain gene is unavailable to the translocated c-*myc* gene in a Burkitt lymphoma. Nature (London) **306**:806–809.
43. **Rabbitts, T. H., A. Forster, D. Hamlyn, and R. Baer.** 1984. Effect of somatic mutation within translocated c-*myc* genes in Burkitt lymphoma. Nature (London) **309**:592–597.
44. **Rabbits, T. H., P. H. Hamlyn, and R. Baer.** 1983. Altered nucleotide sequence of a translocated c-*myc* gene in Burkitt lymphoma. Nature (London) **306**:760–765.
45. **Saito, H., A. C. Hayday, K. Wiman, W. S. Hayward, and S. Tonegara.** 1983. Activation of the c-*myc* gene by translocation: a model for translational control. Proc. Natl. Acad. Sci. U.S.A. **80**:7476–7480.
46. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain terminating inhibitors. Proc. Natl. Acad. Sci. U.S.A. **74**:5463–5467.
47. **Sharp, P. S., A. J. Berk, and S. M. Berget.** 1980. Transcription maps of adenovirus. Methods Enzymol. **65**:750–758.
48. **Shen-Ong, G. L. C., E. J. Keath, S. P. Piccoli, and M. D. Cole.** 1982. Novel *myc* oncogene RNA from abortive immunoglobulin gene recombination in mouse plasmacytomas. Cell **31**:443–452.
49. **Siebenlist, U., L. Hennighausen, J. Battey, and P. Leder.** 1984. Chromatin structure and protein binding in the putative regulatory region of the c-*myc* gene in Burkitt's lymphoma. Cell **37**:381–391.
50. **Southern, E. M.** 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98**:503–517.
51. **Summers, J.** 1975. Physical map of polyoma viral DNA fragments produced by cleavage with a restriction enzyme from *Haemophilus aegyptius*, Endonuclease R · HaeIII. J. Virol. **15**:946–953.
52. **Taub, R., I. Kirsch, C. Morton, G. Lenoir, D. Swan, S. Tronick, S. Aaronson, and P. Leder.** 1982. Translocation of the c-*myc* gene into the immunoglobulin heavy chain locus in human Burkitt lymphoma and mouse plasmacytoma cells. Proc. Natl. Acad. Sci. U.S.A. **79**:7837–7841.
53. **Tonegawa, S.** 1983. Somatic generation of antibody diversity. Nature (London) **302**:575–581.
54. **Watson, D. K., E. Premkumat-Reddy, P. H. Duesberg, and T. S. Papas.** 1983. Nucleotide sequence analysis of the chicken c-*myc* gene reveals homologous and unique coding regions by comparison with the transforming gene of avian myelocytomatosis virus MC29, Δ*gag-myc*. Proc. Natl. Acad. Sci. U.S.A. **80**:2146–2150.
55. **Watt, R., K. Nishikura, J. Sorrentino, A. ar-Rushdi, C. M. Croce, and G. Rovera.** 1983. The structure and nucleotide sequence of the 5′ end of the human c-*myc* oncogene. Proc. Natl. Acad. Sci. U.S.A. **80**:6307–6311.
56. **Watt, R., L. W. Stanton, K. B. Marcu, R. C. Gallo, C. M. Croce, and G. Rovera.** 1983. Nucleotide sequence of cloned cDNA of human c-myc oncogene. Nature (London) **303**:725–728.
57. **Westaway, D., G. Payne, and H. Varmus.** 1984. Proviral deletion and oncogene base-substitution in insertionally mutagenized c-*myc* alleles may contribute to the progression of avian bursal tumors. Proc. Natl. Acad. Sci. U.S.A. **81**:843–847.