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Genetics

Sequence analysis of the *MYC* oncogene involved in the t(8;14)(q24;q11) chromosome translocation in a human leukemia T-cell line indicates that putative regulatory regions are not altered

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ABSTRACT **We have cloned the translocation-associated and homologous normal *MYC* alleles from SKW-3, a leukemia T-cell line with the t(8;14)(q24;q11) translocation, and determined the sequence of the *MYC* oncogene first exon and flanking 5′ putative regulatory regions. S1 nuclease protection experiments utilizing a *MYC* first exon probe demonstrated transcriptional deregulation of the *MYC* gene associated with the T-cell receptor α locus on the 8q+ chromosome of SKW-3 cells. Nucleotide sequence analysis of the translocation-associated (8q+) *MYC* allele identified a single base substitution within the upstream flanking region; the homologous nontranslocated allele contained an additional substitution and a two-base deletion. None of the deletions or substitutions localized to putative 5′ regulatory regions. The *MYC* first exon sequence was germ line in both alleles. These results demonstrate that alterations within the putative 5′ *MYC* regulatory regions are not necessarily involved in *MYC* deregulation in T-cell leukemias, and they show that juxtaposition of the T-cell receptor α locus to a germ-line *MYC* oncogene results in *MYC* deregulation.**

Many human tumors, in particular leukemias and lymphomas, are characterized by nonrandom cytogenetic abnormalities (1–3). Recent experimental evidence indicates that genes located at recurring chromosomal breakpoints are directly involved in tumor pathogenesis (3). In Burkitt lymphoma, three distinct translocations [t(8;14), t(2;8), t(8;22)] are observed that involve juxtaposition of the *MYC* locus at 8q24 with an immunoglobulin gene (4–6). Similarly, human follicular lymphomas often harbor a t(14;18) translocation, in which the *BCL-2* oncogene rearranges with the human heavy chain locus (7). As a consequence of these translocations in B-cell malignancies, expression of the involved oncogene is deregulated (5, 7–9).

Many human T-cell neoplasms also carry specific cytogenetic abnormalities, predominantly translocations and inversions (10–12). These rearrangements frequently involve chromosome 14 at band q11, the locus of the T-cell receptor (TCR) α-chain gene (13, 14). In cases of T-cell acute lymphocytic leukemia with a t(11;14)(p13;q11) translocation, the TCRα locus is split, with variable region genes (V_α) proximal to the breakpoint remaining on chromosome 14q−, whereas the constant region (C_α) translocates to chromosome 11 (14). Similarly, the SKW-3 T-cell leukemia line, derived from an adult patient with T-cell leukemia, contains a t(8;14)(q24;q11) translocation that results in juxtaposition of C_α sequences to a region 3′ of the *MYC* oncogene (15, 16).

A consistent consequence of chromosomal translocations in Burkitt lymphoma is alteration of *MYC* regulation. Analysis of somatic cell hybrids between Burkitt lymphoma cells and other B cells has indicated that the untranslocated *MYC* allele responds to normal transcriptional control signals, whereas the *MYC* oncogene juxtaposed to an immunoglobulin gene fails to respond to these controls and is transcribed constitutively at elevated levels (8). *MYC* activation has been attributed to diverse perturbations in *MYC* regulatory elements: alteration/truncation of negative control regions within exon 1 and flanking regions (6, 17, 18), loss of trans-acting factors (17), changes in posttranscriptional stability (19), transcriptional enhancement (20), blockage of transcript elongation (21), and sequence mutations (17, 22, 23). Alternatively, we have proposed that *de novo MYC* association with cis-acting positive regulatory elements in the activated immunoglobulin locus can lead to transcriptional deregulation (24). In this model, the activated *MYC* allele remains structurally intact, without significant sequence alteration within regulatory regions, as reported for the translocation-associated *MYC* allele of an endemic Burkitt lymphoma, BL-2 (24).

To understand the mechanism(s) of oncogene activation in T cells, we have analyzed the *MYC* oncogene in the SKW-3 leukemia T-cell line. Characterization of somatic cell hybrids derived from mouse leukemia T cells and DeF, a human T-cell leukemia with the same t(8;14)(q24;q11) translocation, has demonstrated elevated levels of human *MYC* expression specific to the translocation-associated *MYC* allele (15). T-cell leukemias with this t(8;14) translocation, which places the TCRα gene 3′ of the *MYC* locus, may represent a mechanism of oncogene activation similar to variant translocations in Burkitt lymphoma (5). Therefore, deregulation of *MYC* due to trans-operative mechanisms or disrupted regulatory signals should be reflected in alterations and mutations within *MYC* coding sequences; conversely, cis-activation is consistent with an intact, germ-line configuration. We have therefore sequenced the *MYC* first exon and flanking regions from the translocation-associated and homologous normal *MYC* alleles of SKW-3 leukemia T cells to elucidate their potential role in *MYC* deregulation.§

MATERIALS AND METHODS

Nucleotide Sequences. Nucleotide sequences were determined by the dideoxy chain-termination method of Sanger *et al.* (25) using the Clontech M13 sequencing primer and synthetic oligonucleotide primers derived from the nucleotide sequence (35). Sequencing templates were constructed by inserting *MYC* 5′ *Hin*dIII/*Xba* I and *Sal* I/*Xba* I fragments (Fig. 2) into phage M13mp18 or M13mp19 (26).

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Abbreviations: TCR, T-cell receptor; V, variable; C, constant; J, joining.

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§This sequence is being deposited in the EMBL/GenBank data base (Intelligenetics, Mountain View, CA, and Eur. Mol. Biol. Lab., Heidelberg) (accession no. J03253).

Genomic DNA Libraries. The genomic DNA libraries used to isolate the *MYC* regions were prepared from a *Sau*3A partial digest of high molecular weight SKW-3 DNA, with appropriate pooled fractions [15–23 kilobases (kb)] purified from sucrose gradients and cloned into bacteriophage λ vector EMBL3A (27). Libraries were screened with the 3′ *MYC* probe pCA1.7S (28), derived from a region 2 kb 3′ of *MYC* (Fig. 2*A*), and pRyc7.4, a cDNA clone that codes for human *MYC* exon 3 and part of exon 2 (29).

S1 Nuclease Analysis. RNA was analyzed by the S1 nuclease mapping procedure (30). Uniformly labeled DNA probe encompassing a part of the first exon and 5′ flanking region (31) was prepared according to Ley *et al.* (32). Twenty micrograms of cytoplasmic RNAs was used for each assay. The ^{32}P-labeled DNA probe was heat-denatured, hybridized in 80% formamide to cytoplasmic RNAs at 56°C for 10 hr, digested with 80 units of S1 nuclease, and analyzed by electrophoresis on a 7 M urea/4% polyacrylamide gel (33).

Chromosome Analysis. Parental and hybrid cell chromosomes were studied by the trypsin/Giemsa-banding method and G-11 technique as described (5).

RESULTS

S1 Nuclease Protection Analysis of *MYC* Exon 1. Transcriptional deregulation specific to the translocation-associated c-*myc* allele has been shown in Burkitt lymphomas carrying t(8;14), t(8;22), or t(2;8) translocations (5, 8, 32) and a T-cell leukemia with a t(8;14)(q24;q11) chromosome translocation (15). In view of the postulated role for *MYC* exon 1 in transcriptional and translational regulation of *MYC* expression (17–19, 21–23), we have analyzed *MYC* transcript levels in the SKW-3 human T-cell leukemia line and in somatic hybrids between SKW-3 and the mouse T-cell leukemia line BW5147. In SKW-3 cells, S1 nuclease protection analysis using a human *MYC* exon 1 probe (33) detected two fragments, 515 and 350 nucleotides in length (Fig. 1*A*, lane 2). This result is consistent with previous S1 nuclease protection experiments using normal RNA derived from various sources, where identical 515- and 350-nucleotide bands were detected, corresponding to *MYC* exon 1 transcripts initiating from promoters P1 and P2, respectively (33). Thus, protection experiments did not detect any qualitative change in the expressed *MYC* gene of SKW-3.

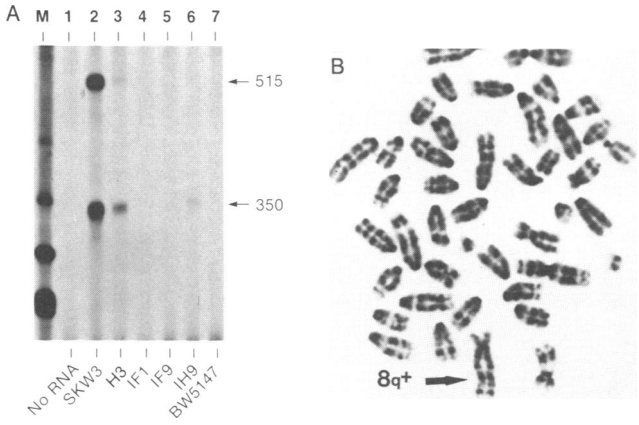

FIG. 1. (*A*) S1 nuclease protection analysis of *MYC* exon 1 RNA expressed in parental and hybrid cells. The 0.8-kb *MYC* exon 1 probe (31) was digested with *Pvu* II, 5′ end-labeled with ^{32}P, and used as a probe as described (30, 33). Two fragments of 515 and 350 base pairs (bp) are protected. *MYC* expression was observed in lane 2 (SKW-3), lane 3 (hybrid H3, which contains an 8q+ chromosome), and lane 6 (hybrid 1H9). Lane 7, BW5147 mouse T-cell leukemia line. Lane 1, no RNA. No *MYC* exon 1 expression was detected in hybrids 1F1 and 1F9 (lanes 4 and 5). Lane M, molecular size markers. (*B*) Portion of a trypsin/Giemsa-banded metaphase from hybrid H3 that retained a human 8q+ chromosome (arrow) but not a normal 8 or the 14q− chromosome.

In mouse–human hybrids, human *MYC* transcripts are readily distinguished from mouse RNA in S1 nuclease analysis (8). As shown in Fig. 1*A*, hybrid 1H9 (lane 6) expressed human *MYC*-encoded RNA, as did hybrid H3 (lane 3), shown cytogenetically to contain an 8q+ chromosome in ≈50% of its cells but not a normal 8 chromosome (Fig. 1*B*). Both hybrids were shown to contain 8q+ chromosomes and to have lost the normal 8 chromosome by Southern blotting analysis (data not shown) using a probe, pSKW1.4X (Fig. 2*A*), immediately 3′ to the c-*myc* oncogene (15, 34). However, hybrid 1H9 contained much less c-*myc* DNA than hybrid 1H3 and, in fact, expressed much lower levels of *MYC* exon 1 transcripts (Fig. 1*A*, lane 6). Hybrids 1F1 and 1F9 have lost the 8q+ chromosome as demonstrated by Southern blotting. We have previously shown that, in hybrids between DeF human leukemia T cells with the t(8;14)(q24;q11) chromosome translocation and mouse BW5147 cells, only the *MYC* gene on the 8q+ chromosome is transcriptionally active, whereas the c-*myc* gene on normal chromosome 8 is transcriptionally silent (15). These results are therefore consistent with the interpretation that the *MYC* gene on the 8q+ chromosome of SKW-3 is transcriptionally deregulated.

Molecular Cloning and Characterization of *MYC* Alleles. The germ-line and translocation-associated *MYC* alleles of SKW-3 were cloned by screening ≈500,000 plaques from a nonamplified SKW-3 genomic DNA library in bacteriophage λ vector EMBL3A (34). By using probes pRyc7.4 (29) and pCA1.7S (28), a 3′ flanking region probe shown previously to detect SKW-3 DNA rearrangements 3′ to the *MYC* third exon (15, 34), a series of overlapping clones covering the *MYC* locus was obtained (34). Physical mapping of recombinant clones allowed their separation into two distinct groups (Fig. 2*A*). Restriction sites on λ SKW13 are in the germ-line configuration, co-linear with the physical map of *MYC* and 3′

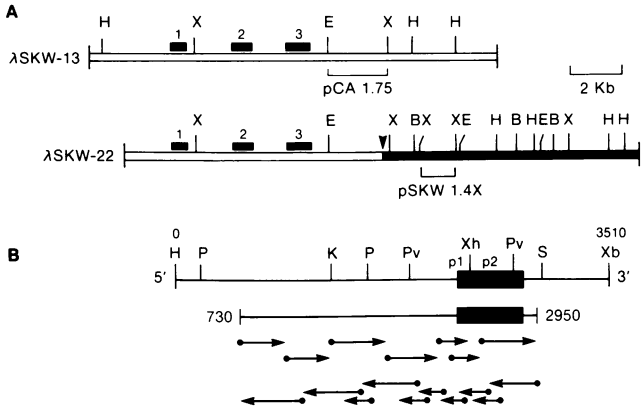

FIG. 2. (*A*) Restriction mapping of SKW-3 clones containing the translocation-associated and germ-line *MYC* alleles. Top, λ SKW13, germ-line *MYC* allele on chromosome 8 (28). Bottom, λ SKW22, with a rearranged *MYC* locus derived from chromosome 8q+ (34). *MYC* exons are indicated by numbered boxes 1, 2, and 3. The chromosomal breakpoint on λ SKW22 is indicated by an arrowhead. Clear bar, chromosome 8 regions; dark bar, region derived from 5′ J_α region on chromosome 14. C_α coding exons are located 27 kb downstream from the *Hin*dIII site at the right end of λ SKW22. E, *Eco*RI; B, *Bam*HI; H, *Hin*dIII; X, *Xba* I. (*B*) Sequencing strategy for *MYC* exon 1 and adjacent flanking regions. *MYC* exon 1 and flanking sequences on the *Hin*dIII–*Xba* I and *Sal* I–*Xba* I restriction fragments of λ SKW13 and λ SKW22, respectively, were subcloned into M13mp18 and M13mp19. H, *Hin*dIII; P, *Pst* I; K, *Kpn* I; Pv, *Pvu* II; Xh, *Xho* I; Xb, *Xba* I; S, *Sau*3A; p1 and p2, promoters. The black bar denotes *MYC* exon 1. Nucleotide sequences were determined by primer extension from synthetic oligonucleotide primers (24) derived from a germ-line 5′ *MYC* sequence (35).

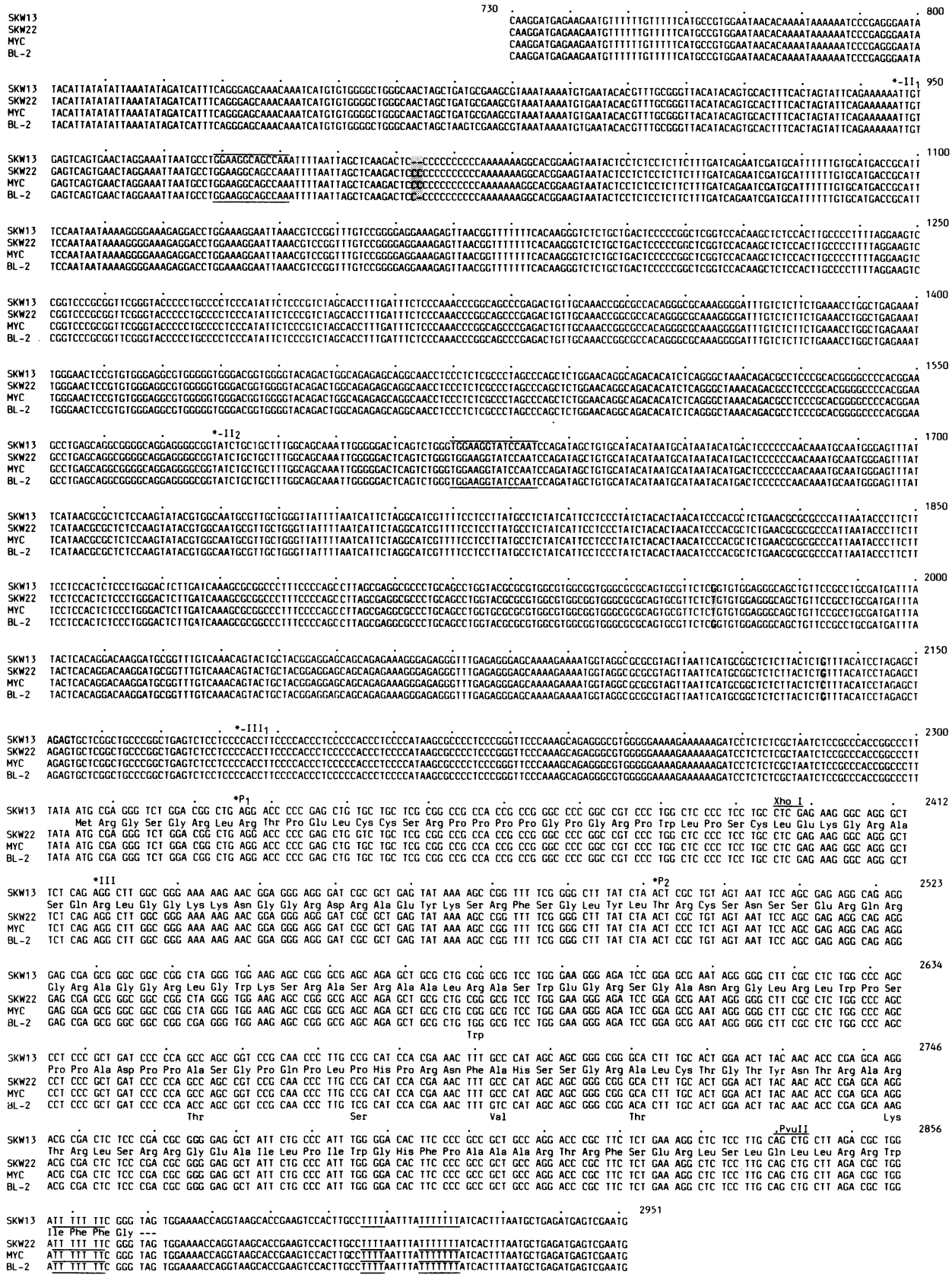

FIG. 3. Nucleotide sequence of *MYC* exon 1 and adjacent flanking regions. Sequences shown are derived from λ SKW13 (unrearranged *MYC* allele of SKW-3), λ SKW22 (translocation-associated *MYC* from SKW-3), normal *MYC* exon 1 (35), and BL-2, a t(8;22) Burkitt lymphoma line (24). Numerical designations from the 5′ end of the *Hin*dIII/*Xba* I fragment and the proposed exon 1 reading frame are from Gazin *et al.* (35). Changes in the proposed amino acid sequence of BL-2 (24) are shown (bottom line). The DNase I-hypersensitive sites (roman numerals), promoters ($*P_1$, $*P_2$), and restriction sites (*Xho* I, *Pvu* II) within the first exon are indicated; thymidine runs at 3′ end are underlined. Shaded areas at nucleotide positions 1965, 2134, and 1014/15 show SKW-3 variation from the germ-line sequence.

flanking regions. λ SKW22 is representative of the second class of *MYC* clones, corresponding to the 8q+ chromosome, and restriction sites diverge from the chromosome 8 germ-line map 3′ to the indicated breakpoint. As reported previously, sequences within the region 3′ of the breakpoint (pSKW1.4X) cross-hybridize in Southern blotting experiments with somatic cell hybrids containing human chromosome 14, and the 3′ physical map of λ SKW22 corresponds to a section of the germ-line joining region (J_α) locus located 36 kb 5′ of the TCRα C region (34).

The Translocation-Associated *MYC* First Exon Is Not Altered in SKW-3. To analyze the potential mechanisms controlling *MYC* expression in T-cell neoplasms, we have determined the nucleotide sequence of the *MYC* first exon and flanking putative regulatory regions for the translocation-associated and germ-line *MYC* alleles. Recombinant phage clones λ SKW13 and λ SKW22 were subcloned into M13 vectors mp18 and mp19 (26) and sequenced according to the strategy outlined in Fig. 2*B*. The nucleotide sequences obtained for the germ-line and translocated *MYC* alleles (λ SKW13 and λ SKW22, respectively) are shown in Fig. 3, along with the sequence of a normal gene described by Gazin *et al.* (35) and of the *MYC* gene involved in a t(8;22) translocation in BL-2, a variant Burkitt lymphoma (24). Within the first exon, the sequences obtained for the SKW-3 *MYC* allele on the 8q+ chromosome and the homologous nonrearranged *MYC* allele are both identical to the normal *MYC* sequence described by Gazin *et al.* (35). This finding indicates that neither SKW-3 *MYC* allele contains the extra nucleotides described by Watt *et al.* (29), which introduce termination codons in all reading frames, and, consequently, the coding capacity for the proposed 188-amino acid peptide (35, 36) remains intact.

Regions flanking *MYC* exon 1 have been implicated in transcriptional activation of this locus. *MYC* alterations clustered around the *Pvu* II site have been correlated with deregulation in endemic Burkitt lymphoma (23), and regions within intron 1 3′ of this *Pvu* II site (in particular, the thymidine runs T7, T4, T7) have been proposed to regulate *MYC* RNA transcription (21). In both SKW-3 *MYC* alleles, however, the DNA sequence of the 3′ flanking region remains germ line. We have detected a single base substitution in both *MYC* alleles 192 nucleotides upstream of the P_1 promoter site 5′ of the *MYC* first exon. Within the nontranslocated *MYC* allele, a second mutation was observed further 5′ at position 1965, and a two-nucleotide cytosine deletion occurred at a position corresponding to 1012 of the germ-line sequence. Interestingly, two of these 5′ alterations, the base substitution at position 2134 in both alleles and the deletion at position 1012 in the nontranslocated *MYC* allele, were also observed in BL-2 and may represent polymorphic changes rather than significant structural abnormalities. These data are consistent with conservation of normal *MYC* coding and regulatory sequences in germ-line and translocation-associated *MYC* alleles of SKW-3 cells, indicating that mutations and alterations within this region, postulated to play a critical role in *MYC* deregulation in Burkitt lymphoma (23, 37–39), are not present in the human leukemia T-cell line investigated here.

DISCUSSION

The picture that has emerged following the molecular characterization of cytogenetic abnormalities in SKW-3 cells is very similar to some cases of Burkitt lymphoma, particularly those involving the variant t(2;8) and t(8;22) translocations (5, 40). DNA rearrangements are detected distal (3′) to *MYC* due to translocation of the TCRα locus to a position 3 kb downstream of *MYC* exon 3 (34). The breakpoint splits the TCRα locus (15), placing the C_α gene ≈36 kb downstream from *MYC* in a "head-to-tail" (5′ → 3′, 5′ → 3′) orientation (34). The translocation involves the J_α region of TCRα and V–J joining signal sequences on chromosomes 8 and 14, implicating errors during recombinase-mediated physiological joining in the etiology of the neoplasm (34). Juxtaposition with C_α results in the deregulation of *MYC* expression specific to the translocation-associated allele (14).

Transcriptional activation of the *MYC* oncogene is generally accepted as a consequence of translocations involving the *MYC* locus on chromosome 8. Recent studies have pinpointed diverse regions adjacent to the *MYC* first exon that may be mechanistically implicated in this specific deregulation. For example, extensive point mutations within this region have been observed in some cases of Burkitt lymphoma (17, 22, 23), and disruption/mutation of negative regulatory elements within *MYC* exon 1 and 5′ flanking sequences (6, 18) or alterations adjacent to the *Pvu* II site (23) have been proposed to account for translocation-specific transcriptional deregulation. Bentley and Groudine (21) have characterized a region at the 3′ end of exon 1 that functions in mRNA elongation and could qualify as such a regulatory sequence. Similarly, mechanisms modulating *MYC* transcript stability as a consequence of gene truncation or mutation have been proposed to account for *MYC* activation (19, 21), and regions within the first exon and 5′ flanking regions that are the target of positive and negative transacting factors have been described in the human (38) and murine (39, 41) systems. However, the sequence obtained suggests that regions playing a role in regulation of human *MYC* expression are normal; observed nucleotide alterations were predominantly in the nontranslocated allele and not associated with areas involved in transcriptional regulation (21) or increased message stability (19). Thus, regions within the *MYC* first exon and 1.6 kb upstream remain structurally intact in SKW-3 cells, arguing against a role for these mechanisms in deregulating *MYC* expression following a t(8;14) translocation in T-cell leukemias.

In a manner directly analogous to immunoglobulin/*MYC* juxtaposition in Burkitt lymphoma, translocation of C_α to the 3′ *MYC* region results in *MYC* transcriptional activation specific to the 8q+ chromosome (15), and the joining event mediating this deregulation has been reported at sites >36 kb upstream of the activated C_α locus (34, 42). We have suggested (24) that the dominant effect on *MYC* transcription may be due to *de novo* association with an activated (immunoglobulin or TCR) locus. Although our data cannot eliminate specific translocation-mediated changes >1.6 kb 5′ of the P_1 promoter site, it appears that structural alteration is not necessary to deregulate *MYC* expression in T-cell leukemias. Thus, our findings that translocation-associated *MYC* exon 1 and putative flanking regulatory sequences were normal in SKW-3, together with the detection of *MYC* transcription specific to the human 8q+ chromosome in hybrids with mouse leukemia T cells, are consistent with the interpretation that juxtaposition of the TCRα locus to a germ-line *MYC* oncogene results in *MYC* deregulation and leads to malignant transformation.

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