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Genetics

The translocated c-*myc* oncogene of Raji Burkitt lymphoma cells is not expressed in human lymphoblastoid cells

(gene regulation/enhancers/first c-*myc* exon/c-*myc* deregulation/B-cell neoplasia)

KAZUKO NISHIKURA, JAN ERIKSON, ABBAS AR-RUSHDI, KAY HUEBNER, AND CARLO M. CROCE

The Wistar Institute of Anatomy and Biology, 36th at Spruce Street, Philadelphia, PA 19104

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ABSTRACT **We hybridized Raji Burkitt lymphoma cells, which carry a t(8;14) chromosome translocation, with human lymphoblastoid cells to study the expression of the translocated cellular *myc* oncogene (c-*myc*) in the hybrid cells. In Raji cells the c-*myc* oncogene is translocated to a switch region of the γ heavy chain locus (S_γ). Because of sequence alterations in the 5′ exon of the translocated c-*myc* oncogene in this cell line, it is possible to distinguish the transcripts of the translocated c-*myc* gene and of the normal c-*myc* gene. S1 nuclease protection experiments with a c-*myc* first exon probe indicate that Raji cells express predominantly the translocated c-*myc* gene, while the level of expression of the normal c-*myc* gene is less than 2% of that of the translocated c-*myc* gene. Somatic cell hybrids between Raji and human lymphoblastoid cells retain the lymphoblastoid phenotype and express only the normal c-*myc* oncogene. This result indicates that the activation of a c-*myc* oncogene translocated to a *S* region depends on the stage of B-cell differentiation of the cells harboring the translocated c-*myc* gene and not on alterations in the structure of the translocated c-*myc* oncogene.**

In Burkitt lymphomas with t(8;14) translocations, the cellular *myc* oncogene, c-*myc*, which is normally on band q24 of chromosome 8 (1–4), translocates to the heavy (H) chain locus (1, 3, 4) on chromosome 14 (5). In Burkitt lymphomas with variant t(8;22) and t(2;8) chromosome translocations, the c-*myc* gene remains in its germ-line configuration on the involved chromosome 8 (8q+), while either the λ or the κ light chain locus translocates to a region distal (3′) to the involved c-*myc* oncogene (6, 7). The consequence of these different types of rearrangements is deregulation of transcription of the involved c-*myc* oncogene, which is expressed constitutively at elevated levels (6–8) in the lymphomas. Since c-*myc* deregulation or, in other terms, constitutive c-*myc* expression is a possible initial cause of these lymphomas, there is great interest in arriving at an understanding of the mechanism(s) of constitutive c-*myc* expression. The first obvious common denominator in the lymphoma is the translocation itself, which juxtaposes a c-*myc* gene and an immunoglobulin locus; the second common denominator is that the involved c-*myc* locus in cases thus far analyzed is always 5′ of the involved immunoglobulin locus; and the third common denominator, aside from the constitutive expression of the involved c-*myc* gene, is the silence or near silence of the untranslocated c-*myc* allele in the lymphomas (8, 9). These common denominators may serve as clues in unraveling the mechanism(s) responsible for constitutive expression of the involved c-*myc* allele, but the heterogeneity of the breakpoints on chromosome 8 and within the immunoglobulin loci have led to differing proposals to explain constitutive c-*myc* expression. Because in many cases of Burkitt lymphoma with the t(8;14) translocation the c-*myc* oncogene translocates to a H-chain switch (*S*) region (predominantly S_μ) on the 14q+ chromosome, with concomitant translocation of the immunoglobulin H-chain gene (*IGH*) enhancer [located between the H-chain joining region (J_H) and the S_μ region] to the 8q− chromosome, it has been speculated that c-*myc* activation in Burkitt lymphoma is not the result of transcriptional enhancement (10, 11). Leder *et al.* (10) have proposed that the first exon of the c-*myc* oncogene and regions 5′ to the first exon mediate negative regulation of c-*myc* transcription by binding to a putative repressor protein. According to this model, truncation of the c-*myc* oncogene at its 5′ end or changes in its 5′ exon would affect the binding of the putative repressor to the c-*myc* gene, resulting in its deregulation (10, 11); thus, the translocated c-*myc* gene would be insensitive to negative regulation (10, 11). Since we observed that a 5′-truncated and translocated c-*myc* oncogene is not expressed (i.e., not deregulated) when introduced into fibroblasts (12), it seemed unlikely that loss of the c-*myc* first exon is responsible *per se* for c-*myc* deregulation. These studies and others to be discussed below demonstrated that the constitutive expression of the translocated c-*myc* allele was observed only in a B-cell background; i.e., activation of the c-*myc* gene involved in the different chromosomal translocations was B-cell specific (8, 12–14).

If constitutive c-*myc* expression from the translocated (or involved) c-*myc* allele were due solely to loss of or change in certain regulatory signals normally contained within or 5′ of the c-*myc* gene so that the involved allele could no longer respond to negative regulation, one would expect the involved c-*myc* allele to remain activated even when placed within a phenotypically different cell type. Thus, we have proposed that the constitutive expression of the involved c-*myc* allele in Burkitt lymphomas must be due to B-cell stage-specific, *cis*-acting, positive-regulatory elements within the immunoglobulin loci (13, 14).

In previous studies (15, 16), two Burkitt lymphoma cell lines, ST486 and CA46, in which the coding portion of the 5′-truncated c-*myc* gene (lacking its 5′ exon) is translocated respectively to the S_μ or the S_α region, were hybridized with human lymphoblastoid cells; the hybrids retained the lymphoblastoid phenotype and expressed the normal but not the translocated c-*myc* gene (13). From these results we concluded that the translocated c-*myc* gene is under the control of thus far unidentified genetic elements within the H-chain locus capable of enhancing c-*myc* transcription in Burkitt lymphoma cells and plasma cells, but incapable of activating gene transcription in lymphoblastoid cells (13). These results also illustrate the point that loss of the 5′ exon and of the DNA region 5′ to the c-*myc* gene does not necessarily result in deregulation of translocated c-*myc* genes. Recently we hybridized lymphoblastoid cells with Daudi cells in which the translocated c-*myc* gene on the 14q+ chromosome is located

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Abbreviations: c, cellular; S, switch; H, heavy; J, joining; C, constant; kb, kilobase(s).

5′ of the J_H segment of the H-chain locus and, therefore, 5′ of the immunoglobulin H-chain gene enhancer located between J_H and S_μ (14). Interestingly, we observed that the hybrid cells expressed both the normal and the translocated c-*myc* genes. These results indicate that genetic elements 5′ of S_μ (possibly the immunoglobulin H-chain gene enhancer between S_μ and J_H) are capable of activating c-*myc* transcription not only in Burkitt lymphoma cells and plasma cells but also in lymphoblastoid cells (14). Thus, it seems likely that Burkitt-like translocations lead to malignant transformation by deregulation of c-*myc* transcription due to proximity to different genetic elements capable of enhancing gene transcription, some of which are more "promiscuous" and can activate gene transcription in B cells at different stages of B-cell differentiation from pre-B-cells (17) to plasma cells (13, 14), while others are able to activate gene transcription only in the more differentiated B cells (13).

In this study we intended to assess the role of changes in the first c-*myc* exon in the deregulation of the c-*myc* gene. Therefore, we hybridized human lymphoblastoid cells with Raji cells which carry a translocated c-*myc* gene that is mutated in its 5′ exon (11). If changes in the c-*myc* 5′ exon or its flanking sequence were responsible for c-*myc* activation in Raji cells, we should observe expression of the translocated c-*myc* gene in the hybrids. On the contrary, if the genetic elements that are responsible for translocated c-*myc* activation in ST486 and CA46 cells are involved in the enhancement of expression of the translocated Raji c-*myc* gene, we should not observe translocated c-*myc* transcription in lymphoblastoid hybrid cells, since the putative enhancer elements that are active in CA46 and ST486 Burkitt lymphoma cells are not active in human lymphoblastoid cells (13).

MATERIALS AND METHODS

Cells. The GM1500-6TG-OUB cells are derived from the hypoxanthine phosphoribosyltransferase-deficient GM1500-6TG human lymphoblastoid cells and are resistant to 100 μM ouabain (13). GM1500A-6TG cells are a late passage of the GM1500-6TG cell line. The difference in levels of c-*myc* transcripts in the GM1500-6TG-OUB and GM1500-6TG has been reported (13). GM1500-6TG-OUB cells carry a germ line c-*myc* gene (13), do not carry constant region μ-chain gene (C_μ) sequences, and express IgG (γ_2 and κ chains) (13). Raji cells are derived from an African Burkitt lymphoma with the t(8;14) translocation and carry a translocated c-*myc* oncogene on the 14q+ chromosome (11). Following fusion with polyethylene glycol, the hybrids were selected in HAT medium (hypoxanthine/aminopterin/thymidine) containing 10 μM ouabain by standard procedures (13). The Burkitt lymphoma cell lines ST486, CA46, Manca, Daudi, and P3HR-1 carrying the t(8;14) chromosome translocation and lines BL2 and JI carrying respectively the t(8;22) or t(2;8) chromosome translocation have been described (1, 3, 6–9, 13–15). The two additional Burkitt lymphomas Ag876 and EW36 with the t(8;14) translocation were a kind gift of Ian McGrath (National Cancer Institute).

Southern Blotting Analysis. Parental and hybrid cell DNAs were digested with restriction enzymes, fractionated by (0.7% or 1%) agarose gel electrophoresis, and blotted to nitrocellulose filters essentially as described by Southern (18). The agarose gel electrophoresis was carried out in 40 mM Tris·HCl/5 mM NaOAc/EDTA, pH 8.0. *Hin*dIII-digested λ phage DNA molecular weight markers (1.0 μg per lane) (Bethesda Research Laboratories) were included on every gel (13). The *Eco*RI 1.2-kilobase (kb) genomic C_μ, the J_H, and the c-*myc* cDNA (Ryc 7.4) probes used in this study have been described (3, 13). The DNA probes were labeled by the nick-translation procedure (19).

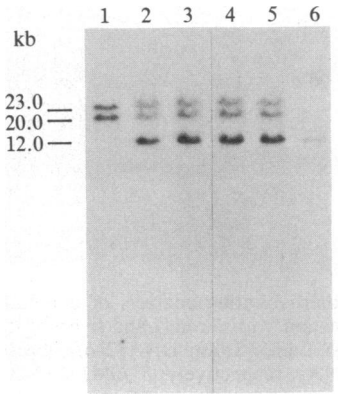

FIG. 1. Southern blotting analysis of parental and hybrid-cell DNAs after digestion with *Bam*HI and hybridization with a probe specific for the J_H region (3). Lanes: 1, Raji DNA; 2–5, hybrid AB2, DB2, CA2, and BD5 cell DNAs, respectively; 6, GM1500-6TG-OUB DNA.

S1 Protection Experiment. Twenty micrograms of cytoplasmic RNAs prepared from various cell lines were subjected to S1 nuclease mapping analysis (20). The M13 clone containing the genomic c-*myc* DNA insert, encompassing the region surrounding the two promotors and the first exon of the c-*myc* gene, was used as an S1 probe. The uniformly ^{32}P-labeled DNA (21) was heat-denatured, hybridized in 80% formamide to the various RNA samples at 65.5°C for 10 hr, digested with S1 nuclease, and analyzed by electrophoresis on a 7 M urea/4% polyacrylamide gel as described (22).

RESULTS

Lymphoblastoid–Raji Hybrids Retain the Lymphoblastoid Phenotype. We hybridized Raji cells and GM1500-6TG-OUB cells at the ratio of 1:1 in the presence of polyethylene glycol (13). The hybrids were selected as described (13). Approximately 95 independent hybrid colonies were obtained by hybridizing 10^7 GM1500-6TG-OUB cells with 10^7 Raji cells. Four independent colonies were picked up and expanded. The four hybrids (AB2, DB2, CA2, and BD5) retained the J_H segments of both GM1500-6TG-OUB and Raji parental cells (Fig. 1 and Table 1). The hybrids also retained the C_μ gene of Raji cells (Fig. 2 and Table 1). Phenotypically, the hybrids resembled the GM1500-6TG-OUB lymphoblastoid parental cells and grew in large clumps. On the contrary, Raji cells grew as single cells. We also tested the hybrid cells

Table 1. Characterization of GM1500-6TG-OUB–Raji somatic cell hybrids

Cells	Fragments present in digestions of parental and hybrid cells: J_H segments,* kb	Fragments present in digestions of parental and hybrid cells: c-*myc* oncogenes,† kb	Surface antigen recognized by the B532 antibody‡
GM1500-6TG-OUB	12	13.5	+
Raji	23, 20	23, 13.5	−
AB2	23, 20, 12	23, 13.5	+
DB2	23, 20, 12	23, 13.5	+
CA2	23, 20, 12	23, 13.5	+
BD5	23, 20, 12	23, 13.5	+

*The cellular DNAs were digested with *Bam*HI.
†The cellular DNAs were digested with *Eco*RI.
‡The expression of the antigen was detected by indirect immunofluorescence as described (13, 23).

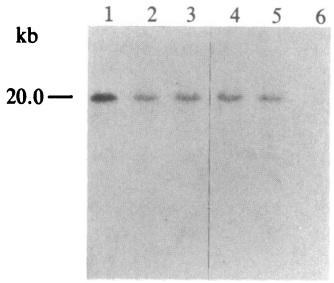

FIG. 2. Southern blotting analysis of parental and hybrid-cell DNAs after digestion with *Bam*HI and hybridization with a probe specific for C_μ (3). Lanes: 1, Raji DNA; 2–5, hybrid AB2, DB2, CA2, and BD5 cell DNAs, respectively; 5, GM1500-6TG-OUB DNA.

for the presence of a surface antigen that is expressed on human lymphoblastoid cells but not in several human Burkitt lymphoma cells (23). This antigen is recognized by a murine monoclonal antibody (B532) (24). The hybrids expressed the marker recognized by the B532 antibody on their surface (Table 1). This surface antigen is not expressed on Raji cells or on other Burkitt lymphoma cells (ST486, CA46, Manca, Daudi, P3HR-1, BL2, and J1) that we have examined (12, 14, 23). The hybrids also were examined for the presence of the normal and of the translocated c-*myc* gene. All four hybrids retained both the normal and the translocated c-*myc* genes (13.5- and 23-kb bands in Fig. 3 and Table 1), indicating that they had retained the 14q+ chromosome derived from the Raji Burkitt lymphoma cells.

Expression of c-*myc* Transcripts in Hybrid Cells. Rabbits *et al.* have shown mutations in the translocated c-*myc* gene of Raji cells (11). These mutations involve the c-*myc* first exon, the first intron, and the second c-*myc* exon (11). Some of these structural alterations can be detected by carrying out S1 nuclease protection experiments using a first-exon probe of the human c-*myc* oncogene (14). By using a first-exon probe, we detected altered c-*myc* transcripts as 440- and 270-nucleotide S1 nuclease-resistant DNA products in Raji cells (Fig. 4*A*, lane 6), whereas the normal c-*myc* transcripts generated 515- and 350-nucleotide products corresponding to the RNAs initiated from the first and second cap site, respectively, in human lymphoblastoid cells (Fig. 4*A*, lane 7). Interestingly we also observed normal c-*myc* transcripts in Raji cells (Figs. 4 and 5). However, the level of normal c-*myc* transcripts in Raji cells was less than 2% of the level of translocated c-*myc* transcripts (Figs. 4 and 5). This result confirms that in Raji, normal c-*myc* transcripts are expressed

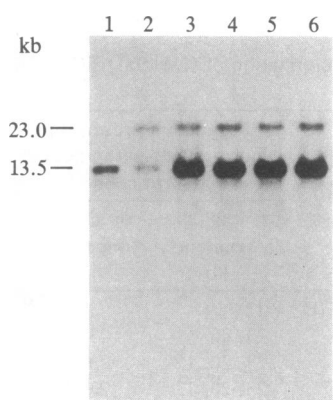

FIG. 3. Southern blotting analysis of parental and hybrid cell DNAs after digestion with *Eco*RI and hybridization with a *myc* DNA probe (Ryc 7.4) specific for the coding exons of the c-*myc* oncogene (3, 6–8). Lanes: 1, GM1500-6TG-OUB DNA; 2, Raji DNA; 3–6, hybrid AB2, DB2, CA2, and BD5 DNAs, respectively.

(11), although at a lower level than found by Rabbitts *et al.* for their Raji line (11).

By S1 nuclease analysis we also detected alteration in the *myc* 5′ exon of other Burkitt lymphoma cells with t(8;14) translocations such as Daudi and P3HR-1 (Fig. 4*A*). We did not detect *myc* 5′ exon changes in Ag876 and EW36 Burkitt lymphomas with t(8;14) translocations (Fig. 4*A*). Leder *et al.* have reported that Burkitt lymphomas utilize predominantly the first promoter of the c-*myc* oncogene and that this might be important in malignant transformation (10). We did not observe a preferential utilization of the first c-*myc* promoter in Ag876 and EW36 Burkitt lymphoma cells (Fig. 4*A*). Therefore, we analyzed several Burkitt lymphomas with the three different Burkitt chromosome translocations. Some cell lines indeed utilized the first promoter more frequently while others did not. Interestingly, the aberrant c-*myc* transcripts derived from the translocated c-*myc* first exon on the 8q− chromosome (9) also utilized the first promoter more extensively (ST486 and JD38; Fig. 4*B*, lanes 2 and 3) Since we could distinguish between the expression of the translocated c-*myc* gene of Raji cells versus the normal c-*myc* gene (Fig. 4*A*, lane 6 versus lane 7), we used the first exon probe to determine whether the hybrids express the Raji translocated c-*myc* gene. The translocated c-*myc* gene of Raji cells is completely silent in the lymphoblastoid hybrids because only the 515- and 350-nucleotide products corresponding to the RNAs initiated at the first and second cap sites were observed in the hybrids (Fig. 5). On the contrary, the normal c-*myc* gene was expressed at levels comparable to the level expressed in GM1500-6TG human lymphoblastoid cells. The levels of normal c-*myc* transcripts in these cells were lower than the level expressed in the GM1500-6TG-OUB double-mutant cells (13), suggesting that complementation with Raji cells results in the reduction of the level of expression of the over-expressed c-*myc* gene of GM1500-6TG-OUB cells. These results indicate that an activated c-*myc* oncogene with a mutated 5′ exon that has been translocated to a *S* region and is expressed at high levels in Burkitt lymphoma cells becomes transcriptionally silent in a lymphoblastoid cell background.

DISCUSSION

In Raji Burkitt lymphoma cells, the translocated c-*myc* gene is mutated in its 5′ exon and is expressed at elevated levels, while the normal c-*myc* gene is expressed at low levels. Since in Raji cells the c-*myc* gene is translocated to a S_γ region (11), the immunoglobulin H-chain gene enhancer normally located between J_H and S_μ is not located in front of the translocated c-*myc* gene on the 14q+ chromosome but is translocated to the 8q− chromosome. Thus, the immunoglobulin H-chain gene enhancer cannot be involved in activation of the translocated c-*myc* gene.

Are the structural alterations in the c-*myc* 5′ exon responsible for translocated c-*myc* activation as suggested by Leder *et al.* (10)? Clearly the results of the experiments described in this paper indicate that structural alterations in the 5′ exon of the c-*myc* oncogene and/or 5′ of the c-*myc* oncogene are not responsible *per se* for c-*myc* activation, since no expression of the translocated c-*myc* oncogene is detectable in human lymphoblastoid cells.

These results are consistent with our previous findings that a 5′-truncated c-*myc* oncogene translocated to a *S* region of the H-chain gene is silent in lymphoblastoid cells (13). Thus, is seems that it does not make much difference whether the translocated c-*myc* gene is 5′-truncated or not. What seems to be important in the differential regulation of the translocated c-*myc* gene is whether the c-*myc* gene is translocated to a *S* region or whether it is translocated to a region 5′ to S_μ (5′ to the immunoglobulin H-chain gene enhancer). When the

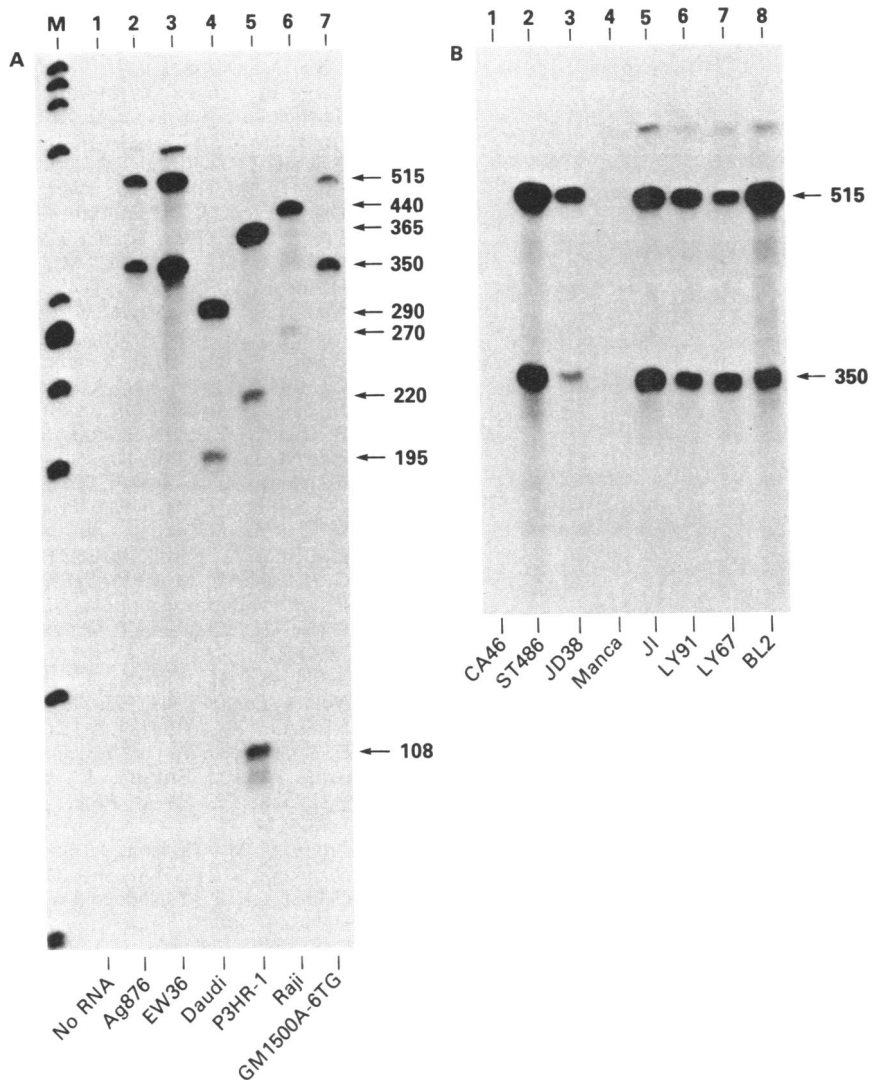

FIG. 4. Detection of specific 5′ ends of the human c-*myc* mRNAs from Burkitt lymphoma cell lines. (*A*) S1 nuclease mapping analysis of the RNAs from Burkitt lymphoma cell lines with a t(8;14) chromosome translocation (lanes 2–6) and human lymphoblastoid cell lines (lanes 7 and 8). Lane M shows size markers: ϕX 174 digested with *Hae* III and 5′-^{32}P-labeled; sizes are shown in kb. (*B*) S1 nuclease mapping analysis of the RNAs from Burkitt lymphoma cell lines with a t(8;14) chromosome translocation (lanes 1–4), from Burkitt lymphoma cell lines with the t(2;8) chromosome translocation (lanes 5–6), and from Burkitt lymphomas with the t(8;22) translocation (lanes 7–9).

c-*myc* gene is translocated to a *S* region (CA46, ST486, and Raji lines) it can be transcribed in Burkitt lymphoma cells and plasma cells, but it cannot be transcribed in lymphoblastoid cells; when c-*myc* is translocated upstream to a region 5′ to J_H (Daudi line), it can be transcribed in a broader range of stages in the B-cell lineage, from pre-B-cells to lymphoblastoid and plasma cells. These findings are consistent with the existence in the H-chain locus of genetic elements capable of activating c-*myc* transcription *in cis* that are active in terminally or near terminally differentiated B cells but are inactive in lymphoblastoid cells. An obvious inference is that, in order to activate gene transcription, these genetic elements must interact with *trans*-acting factors expressed in the more differentiated B cells. A corollary of this interpretation is that Burkitt lymphoma cells carrying a c-*myc* gene translocated to a *S* region are the more differentiated types of B cells. Translocation of the c-*myc* gene to positions upstream of a region 5′ of S_μ should allow deregulation of c-*myc* transcription in B cells at less-differentiated B-cell stages. For example, recently we have observed high levels of c-*myc* transcripts in an acute lymphocytic leukemia of the pre-B-cell type that carried two reciprocal chromosome translocations, a t(14;18) and a t(8;14) (17). In this leukemia the translocated c-*myc* gene was located 5′ of the involved J_H segment on the 14q+ chromosome (17).

Possibly, sequences within the 5′ exon and 5′ flanking sequences of c-*myc* as dissected and discussed by others (10, 11) are involved in negative- and positive-regulatory mechanisms to control expression of the normal c-*myc* allele during performance of its normal function in dividing cells of various cell lineages. However, we have demonstrated in this and previous studies that loss of or changes in the 5′ flanking sequences of the transcriptionally activated c-*myc* allele of Burkitt lymphoma cannot account for the exquisite stage specificity of activated c-*myc* expression. Furthermore, these somatic cell hybrid studies have shown that the lymphomas thus far studied fall into two classes: (*i*) the Daudi class [and possibly Manca (23, 25) and the acute lymphocytic leukemia with the double translocation discussed above (17)] in which the translocated c-*myc* is active, perhaps due to the immunoglobulin H-chain gene enhancer, in early and late B-cell stages; and (*ii*) the S_μ, S_γ, and S_α class, including CA46, ST486, and Raji, in which the more stage-restricted activity of the translocated c-*myc* depends upon element(s) within the immunoglobulin H-chain locus downstream of the *S* region(s). It will be interesting to determine if the variant

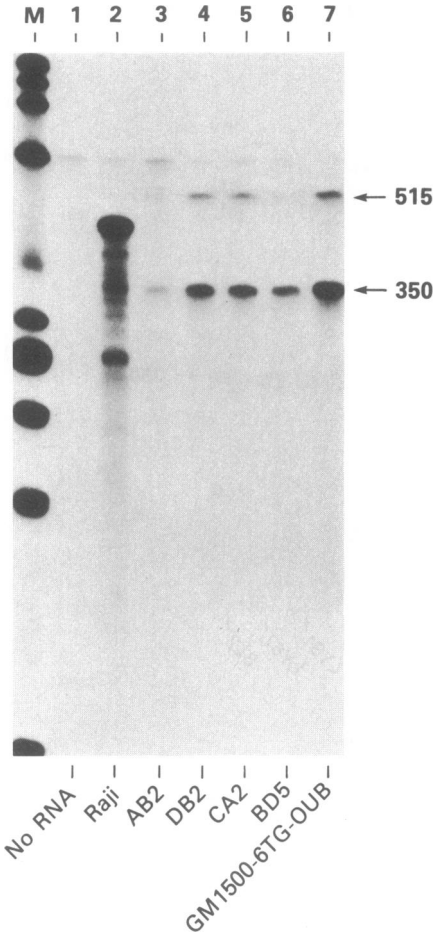

FIG. 5. S1 nuclease mapping analysis of the RNAs from somatic cell hybrids made between Raji and GM1500 cell lines. Conditions for S1 nuclease analysis were described in text.

Burkitt lymphomas in which c-*myc* is activated by proximity to light chain immunoglobulin loci fall into similar classes.

In conclusion, by somatic cell hybridization studies, we have been able to broadly define the B-cell stage specificity of transcriptional c-*myc* activation as a positive-regulatory event involving interaction of *trans*-acting factors with *cis* sequences (*cis* to *myc*) within the H-chain locus. To narrow the search for the putative *cis* sequences will require transfection of large, rearranged c-*myc*: H-chain gene molecular clones into cells of the more differentiated stages of the B-cell lineage.

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