Differential Expression of the Translocated and the Untranslocated c-*myc* Oncogene in Burkitt Lymphoma

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Abstract. *Burkitt lymphoma cells carrying either a rearranged or unrearranged* c-myc *oncogene were examined with the use of probes from the 5′ exon and for the second and third exon of the oncogene. The results indicate that the normal* c-myc *gene on chromosome 8 and the 5′ noncoding and 3′ coding segments of the* c-myc *oncogene separated by the chromosomal translocation are under different transcriptional control in the lymphoma cells. Burkitt lymphoma cells carrying a translocated but unrearranged* c-myc *oncogene express normal* c-myc *transcripts. In contrast, lymphoma cells carrying a* c-myc *gene rearranged head to head with the immunoglobulin constant* μ *region gene express* c-myc *transcripts lacking the normal untranslated leader.*

In approximately 90 percent of Burkitt lymphomas the malignant cells show a reciprocal translocation between chromosomes 8 and 14 [t(8;14)], whereas in the remaining 10 percent the cells show either a t(2;8) or a t(8;22) chromosome translocation, the breakpoint on chromosome 8 being consistently on band q24 (*1*). By examining somatic cell hybrids between mouse cells and Burkitt lymphoma cells we have found that the breakpoint on chromosome 14 in Burkitt lymphoma cells with the t(8;14) translocation is within the immunoglobulin heavy chain locus (*2*) and that genes for the heavy chain variable region (V_H) translocate from chromosome 14, where they normally reside (*3*), to the deleted chromosome 8 in the lymphoma cells (*2, 4*). The human cellular homolog, c-*myc*, of the transforming gene of the avian myelocytomatosis virus, v-*myc*, is normally located on human chromosome 8 (*5–6*). In Burkitt lymphoma cells, however, c-*myc* translocates to the heavy chain locus (*5*). In some cases, the translocated c-*myc* gene is not rearranged within a large DNA restriction (Bam HI) fragment, in others it rearranges 5′ to 5′ (head to head) with the immunoglobulin constant μ region gene (C_μ gene) (*5, 7*).

The cell lines JD38 IV and ST486 contain a rearranged and a normal c-myc *gene.* Recently, we showed that high levels of c-*myc* transcripts are detectable in Burkitt lymphoma cell lines (*4, 8*). We also showed that hybrids between mouse myeloma and Burkitt lymphoma cells carrying the translocated human c-*myc* oncogene express high levels of human c-*myc* transcripts, whereas hybrids carrying the untranslocated c-*myc* oncogene on the normal chromosome 8 do not (*8*). These results indicate that the translocated and untranslocated c-*myc* oncogene are under different control in B cells (*8*). In the present study we investigated the expression of human c-*myc* transcripts in three lymphoma cell lines. One of these cell lines, Daudi, carries an unrearranged c-*myc* oncogene translocated to human chromosome 14 (*5, 7*). The other two, JD38 IV and ST486, carry a c-*myc* oncogene rearranged head to head with an immunoglobulin C_μ gene (*5, 7*). We demonstrated recently that the human c-*myc* oncogene has three exons separated by two introns (*9, 10*), and that the first exon codes for an untranslated leader (*9*) which is normally expressed in lymphoblastoid cell lines and in other human cell lines (*10*).

Using the Southern blotting technique, we examined Bam HI, Hind III, and Xba I digests of the DNA from three independent lymphoma cell lines with a complementary DNA (cDNA) probe (Ryc7.4) specific for the second and third exon of the c-*myc* gene (Fig. 1, probe B) and with a genomic probe specific for the first c-*myc* exon (Fig. 1, probe A). As shown in Fig. 2, while Daudi cells contain both c-*myc* genes unrearranged, the other two lymphoma cell lines (JD38 IV and ST486) contain a rearranged and a normal c-*myc* gene. Hybridization of the DNA from these cell lines with a probe specific for the 5′ exon (Figs. 3 and 4), however, indicated that the first noncoding exon is separated from the two coding exons in JD38 IV and ST486 lymphoma cells that contain one rearranged c-*myc* gene (Figs. 3 and 4). Thus, in JD38 IV and ST486 cells the break occurred between the first and the second exon. The two segments of the rearranged c-*myc* gene are on two different chromosomes: the 5′ noncoding exon remains of the long arm of chromosome 8 ($8q^-$) and the two coding exons translocate to the long arm of chromosome 14 ($14q^+$). Previous studies have shown that the 5′ exon is proximal and the 3′ exon distal on band q24 of chromosome 8 (*5, 7–8*).

Only the translocated myc *gene is transcribed in Burkitt lymphoma.* The c-

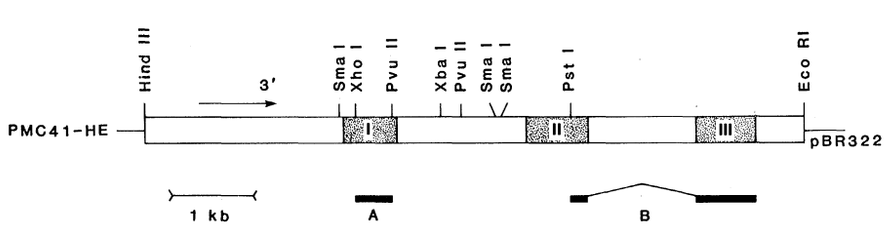

Fig. 1. A simplified map of the human c-*myc* gene in pBR322 (pMyc41 · HE) (*10*) and the restriction sites relevant to the studies in this article. Genomic and cDNA probes used in Southern and Northern hybridizations are: (a) an Xho I–Pvu II leader fragment of 449 base pairs within exon-I (probe A); (b) a 1029-base pair fragment obtained from the Pst I digest of cDNA clone (pRyc7.4) which includes 221 base pairs of the 3′ end of exon-II and all of exon-III (*8*). Shaded areas indicate exons; unshaded areas, intron and flanking regions.

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myc transcripts of the three lymphoma cell lines were studied by the Northern blotting procedure (*8–9, 11*) with DNA probes specific for either the first 5′ noncoding exon or for the two 3′ coding exons of the c-*myc* gene (Fig. 1). As shown in Fig. 5A, 2.4- to 2.2-kilobase (kb) transcripts are evident in the three lymphoma cell lines examined with the Ryc7.4 probe (Fig. 1). When we used a genomic probe specific for the 5′ noncoding exon, however, we detected the 2.4- to 2.2-kb *myc* transcripts only in Daudi Burkitt lymphoma cells that contain one normal c-*myc* and one translocated but unrearranged c-*myc* gene (*5, 7, 9*) (Fig. 5B). We showed previously that the Daudi cell line produces c-*myc* transcripts identical to the normal *myc* transcripts (*10*). We did not detect 2.4- to 2.2-kb *myc* transcripts, by using the 5′ exon probe, in the lymphoma cell lines carrying a rearranged *myc* gene on the $14q^+$ chromosome and a normal *myc* gene on the chromosome 8 (JD38 IV and ST486, Fig. 5B). Therefore, we conclude that only the translocated c-*myc* gene is expressed in Burkitt lymphoma cells. Suppression of transcriptional activity of the normal untranslocated c-*myc* oncogene seems to occur in Burkitt lymphoma as well as in somatic cell hybrids between mouse myeloma and Burkitt lymphoma cells (*8*). We also observed 0.9- to 0.7-kb transcripts hybridizing with the 5′ noncoding *myc* exon probe in JD38 IV and ST486 cells in which the first *myc* exon is separated from the other two (Figs. 3 to 5). This result suggests that while the normal untranslocated c-*myc* gene is silent and the translocated c-*myc* gene is expressed at high levels, the 5′ exon of the *myc* gene that has been decapitated by the chromosomal breakage and remains on the $8q^-$ chromosome is also transcriptionally active in Burkitt lymphoma.

Transcripts of the c-myc *oncogenes in Burkitt lymphoma cells with the rearranged* myc *gene*. We also examined the levels of expression of the 5′ exon of the c-*myc* gene in the same lymphoma cell lines by the S1 nuclease protection method (*8*). The probe, a genomic *myc* Cla I–Xho I fragment or a Pvu II fragment labeled with ^{32}P at the 5′ end (Fig. 6) was hybridized with cytoplasmic RNA from various cell lines, and the S1 nuclease–resistant DNA products were analyzed by electrophoresis on a polyacrylamide gel containing 7*M* urea. The unrearranged c-*myc* gene has two TATA boxes and cap sites at the 5′ end of the gene (*10*). The RNA transcribed from one of the promoters (*10*) protect 71 nucleotides

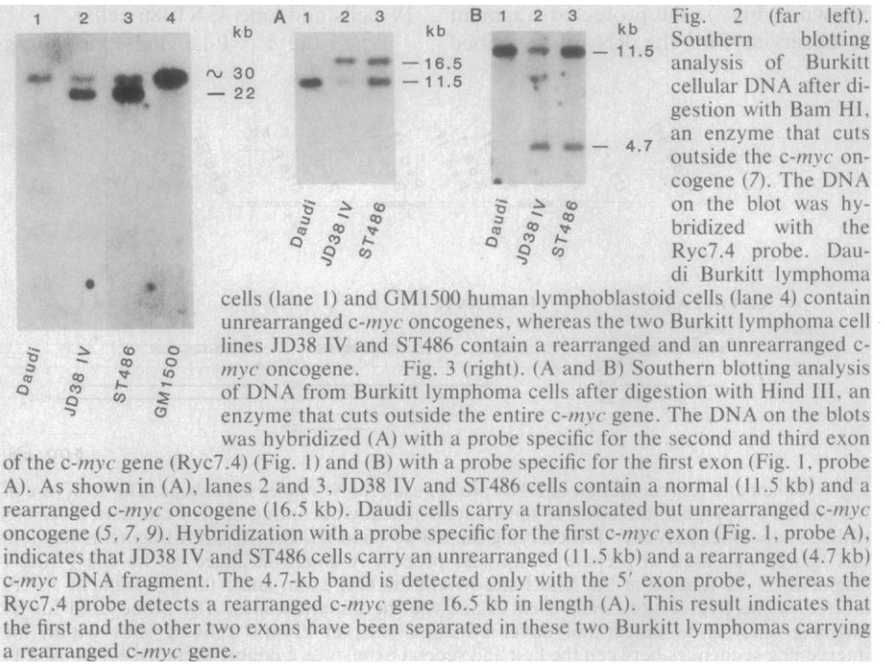


Fig. 2 (far left). Southern blotting analysis of Burkitt cellular DNA after digestion with Bam HI, an enzyme that cuts outside the c-*myc* oncogene (*7*). The DNA on the blot was hybridized with the Ryc7.4 probe. Daudi Burkitt lymphoma cells (lane 1) and GM1500 human lymphoblastoid cells (lane 4) contain unrearranged c-*myc* oncogenes, whereas the two Burkitt lymphoma cell lines JD38 IV and ST486 contain a rearranged and an unrearranged c-*myc* oncogene. Fig. 3 (right). (A and B) Southern blotting analysis of DNA from Burkitt lymphoma cells after digestion with Hind III, an enzyme that cuts outside the entire c-*myc* gene. The DNA on the blots was hybridized (A) with a probe specific for the second and third exon of the c-*myc* gene (Ryc7.4) (Fig. 1) and (B) with a probe specific for the first exon (Fig. 1, probe A). As shown in (A), lanes 2 and 3, JD38 IV and ST486 cells contain a normal (11.5 kb) and a rearranged c-*myc* oncogene (16.5 kb). Daudi cells carry a translocated but unrearranged c-*myc* oncogene (*5, 7, 9*). Hybridization with a probe specific for the first c-*myc* exon (Fig. 1, probe A), indicates that JD38 IV and ST486 cells carry an unrearranged (11.5 kb) and a rearranged (4.7 kb) c-*myc* DNA fragment. The 4.7-kb band is detected only with the 5′ exon probe, whereas the Ryc7.4 probe detects a rearranged c-*myc* gene 16.5 kb in length (A). This result indicates that the first and the other two exons have been separated in these two Burkitt lymphomas carrying a rearranged c-*myc* gene.

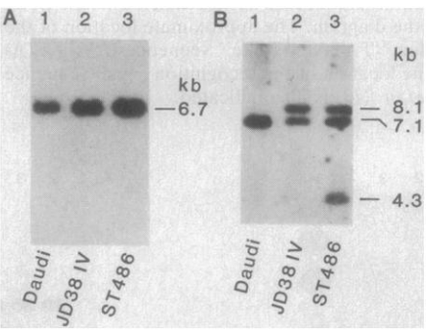


Fig. 4. Southern blotting analysis of DNA from Burkitt lymphoma cells after digestion with Xba I, an enzyme that cuts the c-*myc* oncogene between the first and the second exon (*7*). (A) Hybridization with a probe specific for the second and third exon (Ryc7.4) indicates that these two exons are intact in all of the three Burkitt lymphoma cell lines. (B) On the contrary, c-*myc* rearrangements are detected with the probe specific for the first exon (probe A) of the c-*myc* oncogene in JD38 IV and ST486 cells (lanes 2 and 3) but not in Daudi cells (lane 1).

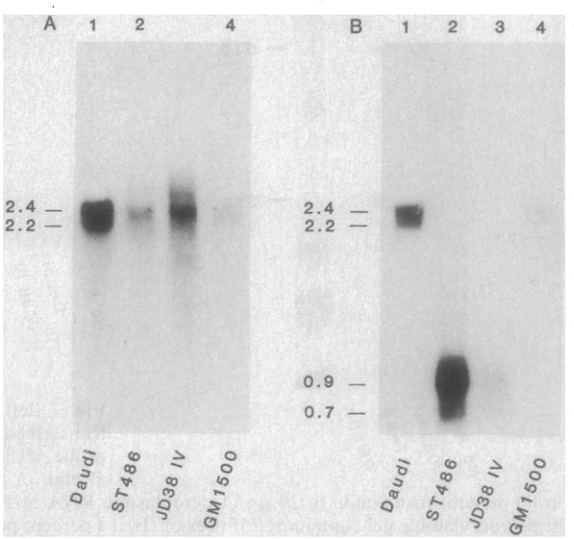


Fig. 5. Northern blotting analysis of RNA from three Burkitt lymphoma cell lines and from an Epstein-Barr virus–transformed lymphoblastoid cell line (GM1500). Polyadenylated RNA was extracted (*4*) and 5 μg of RNA was added to each lane of 1.4 percent agarose gel. After agarose gel electrophoresis and transfer to nitrocellulose filters, the RNA was hybridized (A) with the Ryc7.4 probe (probe B) and (B) with the 5′ exon probe (probe A). Lanes 1, 2, and 3 show the RNA from the lymphoma cell lines Daudi, ST486, and JD38 IV, respectively. Lane 4 shows the RNA from GM1500 cells. All cell lines showed the 2.4- to 2.2-kb c-*myc* transcript with the Ryc7.4 probe, whereas the 2.4- to 2.2-kb c-*myc* transcripts were detected only in Daudi and GM1500 cells with the 5′ exon probe. We detected 0.9- and 0.7-kb transcripts hybridizing with the 5′ exon probe in the two lymphoma cell lines with a rearranged c-*myc* oncogene (ST486 and JD38 1V) .

of the probe corresponding to the distance between the upstream cap site and Xho I site of the c-*myc* gene (Fig. 6). As shown in Fig. 7, the protected fragment is observed with the RNA transcribed from the unrearranged c-*myc* gene (lane 2, Daudi cells) as well as the RNA from the rearranged c-*myc* gene (lane 3, JD38 IV cells and lane 4, ST486 cells).

When the 5′ ^{32}P-labeled Pvu II fragment was used as probe in S1 protection experiments, we found two S1 nuclease–resistant DNA products (515 and 350 nucleotides) corresponding to the RNA initiated from the first (upstream) and the second (downstream) cap sites, respectively (Fig. 6). These two fragments are seen with the RNA derived from the unrearranged c-*myc* gene (lane 4, GM 607) and the rearranged c-*myc* gene (lane 2, JD38 IV; lane 3, ST486) with different intensity. It seems that the c-*myc* RNA detected in Northern blot analysis (Fig. 5), 2.4 and 2.2 kb, may correspond to the S1 nuclease–resistant DNA products of 515 and 350 nucleotides, respectively. Recently, Hamlyn and Rabbitts (*12*) reported the existence of two c-*myc* transcripts. However, it is likely that the DNA products detected in the lanes for JD38 IV and ST486 cells are generated by the aberrant 0.9- to 0.7-long RNA seen on Northern blots (Fig. 5B) as discussed above.

It is of interest that such abnormal transcripts also utilize the authentic c-*myc* gene promoters and cap sites. As shown in Fig. 7A, the first 5′ noncoding exon is transcribed at a much higher level in ST486 cells than in JD38 IV cells. Because of the rearrangement of the c-*myc* gene in ST486 and JD38 IV cells, the

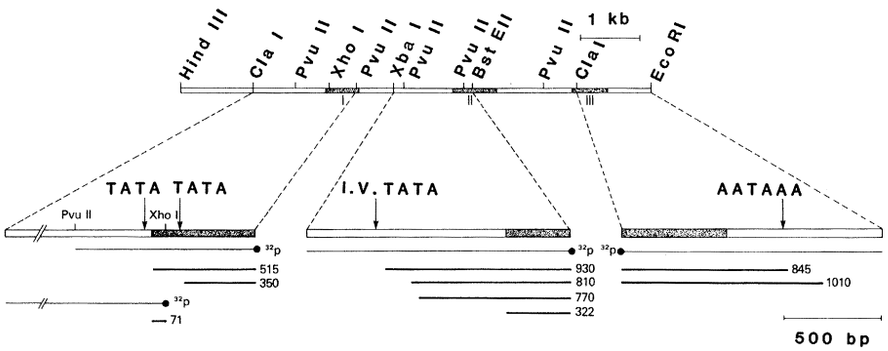
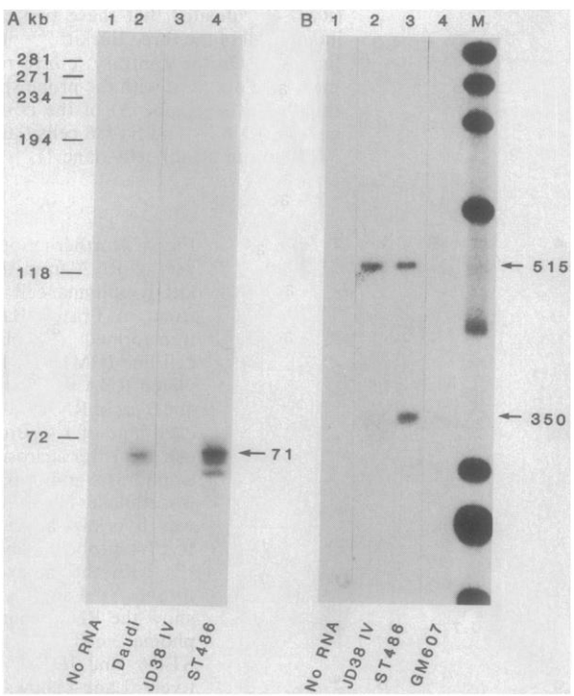


Fig. 6. Schematic representation of DNA probes used for S1 nuclease analysis. The structure of the human c-*myc* genomic DNA is shown schematically according to previous data (*9–11*). A pBR322 subclone, pMyc41 · HE carrying the 8.3-kb Hind III–Eco RI DNA fragment shown in this figure, was used to prepare various S1 probes. A double-stranded 1.3-kb Cla I–Xho I fragment, 5′ ^{32}P-labeled at the Xho I site within the first exon, and a 0.8-kb fragment 5′ ^{32}P-labeled at the Pvu II site, were used to analyze the initiation sites. The probe used for S1 mapping analysis to detect the novel initiation sites or cryptic splicing sites within the intervening sequences between the first and second exons was a double-stranded 1.4-kb Xba I–Bst EII fragment, 5′ ^{32}P-labeled at the Bst EII site within the second exon. The probe used for S1 nuclease analysis of the 3′ end of the c-*myc* messages was a 1.4-kb DNA fragment, Cla I–Eco RI, labeled with ^{32}P at the 3′ end. The location and size of S1 nuclease–resistant DNA products are shown together by the solid bars in the diagram. The approximate location of the authentic TATA box found by us (*10*) and another "TATA box-like" sequence (*I.V. TATA*) found within the first intron (*13*) are indicated. The location of the recognition signal sequence (*AATAAA*) for polyadenylation found by Colby *et al.* (*13*) is also indicated.



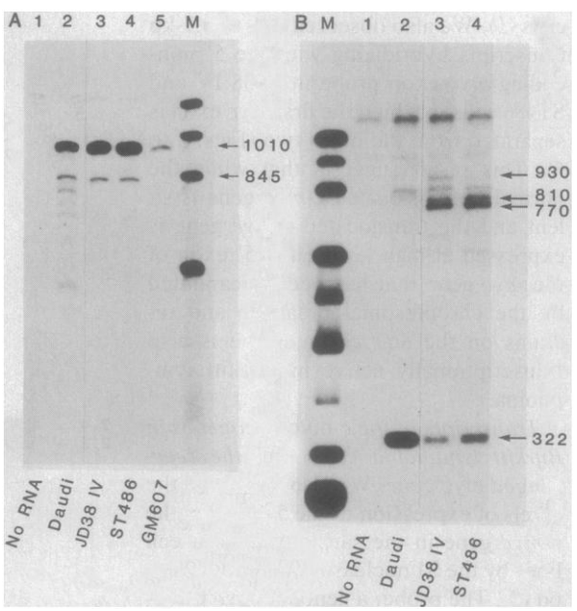


Fig. 7 (left). Mapping of the initiation sites of c-*myc* RNA transcribed from the authentic TATA box region. The S1 probe, a Cla I–Xho I DNA fragment labeled with 5′ ^{32}P at the 5′ end, or a Pvu II DNA fragment ^{32}P-labeled at the 5′ end was heat denatured, hybridized in 80 percent formamide to 20 μg of cytoplasmic RNA at 55°C, digested with S1 nuclease, and analyzed by electrophoresis on (A) an 8 percent polyacrylamide gel containing 7*M* urea or (B) a 4 percent polyacrylamide gel. Lanes 2 to 4, RNA from Daudi, JD38 IV, and ST486 cells, respectively. The location of the size marker (*M*), øX174 digested with Hae III, is indicated in the figure. Fig. 8 (right). (A) Detection of the normal c-*myc* RNA initiating within the first intervening sequences. RNA's were subjected to S1 nuclease analysis with the double-stranded Xba I–Bst EII probe labeled with ^{32}P at the 5′ end. The conditions for hybridization and S1 nuclease digestion were the same as those described in the legend for Fig. 7. The S1 nuclease–resistant DNA products were fractionated on a 4 percent polyacrylamide gel containing 7*M* urea. The size markers (*M*) are øX174–Hae III digests labeled with ^{32}P at the 5′ end. (B) Mapping of the 3′ ends of c-*myc* RNA. The S1 probe used was the Cla I–Eco RI DNA fragment, ^{32}P-labeled at the 3′ end. The S1 nuclease–resistant DNA products were analyzed on a 4 percent polyacrylamide gel containing 7*M* urea.

5′ noncoding exon with the authentic c-*myc* gene promoters have been left behind on chromosome 8q$^-$, whereas the decapitated coding exons have been translocated.

We also tried to determine the initiation sites used for transcription of the 2.4- to 2.2-kb *myc* RNA in ST486 and JD38 IV. A DNA fragment Xba 1–Bst EII, encompassing a part of the first intron and the second exon, ^{32}P-labeled at the 5′ end of the Bst EII site, was used as a probe for S1 mapping analysis (Fig. 6). In addition to a 322-nucleotide band corresponding to the distance between the splice acceptor site of the second exon and the Bst EII site within the second exon (Fig. 6), several S1 nuclease–resistant DNA products longer than 322 nucleotides were protected by messenger RNA (mRNA) from JD38 IV and ST486 cells (Fig. 8, lanes 3 and 4). The lengths of the three major protected fragments are 770, 810, and 930 nucleotides (Fig. 8A). These lengths may measure the distance between initiation sites and the Bst EII site or they may measure the distance between cryptic splicing acceptor sites and the Bst EII site; such cryptic sites are, perhaps, normally silent but can be activated by translocation and rearrangement of the c-*myc* gene.

On the basis of the results of Northern blotting and S1 nuclease mapping analysis we conclude that transcription from the rearranged and decapitated c-*myc* gene without the first exon initiates within the retained part of the 5′ intron. Possibly, the TATA box and sequences similar to cap sites within this region, as described by Colby *et al.* (*13*), are used for transcription of the rearranged c-*myc* gene. Thus, transcription of rearranged c-*myc* genes in Burkitt lymphomas is similar to transcription of rearranged c-*myc* genes in mouse plasmacytomas (*11*).

We also analyzed the 3′ end of the message derived from the unrearranged and rearranged c-*myc* genes by S1 nuclease analysis using the 3′ ^{32}P-labeled Cla 1–Eco RI fragment as a probe (Fig. 6). The signal sequence AATAAA, for polyadenylation, has been found at the position about 300 base pairs downstream from the end of the third exon (*13*) (Fig. 6). The c-*myc* mRNA polyadenylated around this signal sequence would give rise to an S1 nuclease–resistant DNA product about 850 nucleotides long (Fig. 6). Although such DNA products were indeed detected (Fig. 8B), the length of the major protected fragment by the RNA transcribed from the unrearranged (Fig. 8B, lanes 2 and 5) and rearranged (Fig. 8, lanes 2 and 3) c-*myc* genes was 1010 nucleotides, suggesting that a second polyadenylation site exists in the c-*myc* gene and that this site is more dominantly utilized.

Conclusions

Our results indicate that although transcripts of the translocated c-*myc* oncogene are expressed at high levels, the untranslocated c-*myc* oncogene on the normal chromosome 8 is silent in Burkitt lymphoma cells. These results complement our findings that only the translocated c-*myc* oncogene is expressed in hybrids between Burkitt lymphoma and mouse myeloma cells, whereas the untranslocated c-*myc* oncogene on the normal chromosome 8 is not (*9*). These results demonstrate that translocated and untranslocated c-*myc* oncogenes are under different control in Burkitt lymphoma cells. We have also shown that in those cases of Burkitt lymphoma in which a direct rearrangement between c-*myc* and C_μ occurs, the normal 5′ end of one c-*myc* gene is left behind on the 8q$^-$ chromosome. Consequently, transcription from the translocated c-*myc* gene initiates by a new promoter (or promoters) possibly located within the c-*myc* 5′ intron between the first and second exon. As a result, novel c-*myc* transcripts are expressed which contain the same body of the message coded for by the second and third exons but with new 5′ leader sequences from the 5′ intron. Although we have not defined the precise location of the initiation site (or sites) used for rearranged c-*myc* transcription, it is possible that the TATA box-like sequence described by Colby *et al.* (*13*) is used to initiate *myc* mRNA synthesis from the counterpart rearranged c-*myc* gene.

We also observed high levels of transcripts of the 5′ exon of the c-*myc* oncogene remaining on chromosome 8q$^-$ in ST486 lymphoma cells. This finding is of particular interest because it indicates not only that enhancement of c-*myc* transcription can occur as a result of a 5′ C_μ to 5′ c-*myc* (head to head) DNA rearrangements, but also that enhancers of transcription may be located on both sides of the breakpoint on the heavy chain locus on chromosome 14. Since enhancement of transcription of c-*myc* sequences can occur by placing immunoglobulin gene sequences 3′ of the promoter of the c-*myc* oncogene it is possible that in some Burkitt lymphomas the activation of the c-*myc* oncogene may be due to translocation of an immunoglobulin gene to a region 3′ of the c-*myc* gene on chromosome 8 and not to a translocation of the c-*myc* oncogene to one of the three chromosomes carrying immunoglobulin chain genes.

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