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Aberrant c-*myc* RNAs of Burkitt's lymphoma cells have longer half-lives

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BL67 and BL18 are Burkitt's lymphoma cell lines with t(8;14) translocations (the breakpoint is in the first exon and first intron, respectively) in which the μ-heavy chain switch region is fused to the c-*myc* gene in head to head orientation. In both cell lines only aberrant c-*myc* RNAs are found. BL67 cells contain two c-*myc* RNA species of 2.4 and 3.5 kb. The 2.4-kb RNA is initiated at several cryptic promoters in the first intron. The 3.5-kb RNA is transcribed from the immunoglobulin heavy chain anti-sense strand across the breakpoint of the translocation into the first exon of the c-*myc* gene and is then normally spliced using the physiological splice donor and acceptor sites of the c-*myc* gene. BL18 contains c-*myc* RNA of 2.4 kb initiated at cryptic promoters in the first intron and additional RNAs of 0.90 kb and 0.74 kb transcribed from the dual c-*myc* promoters on the reciprocal fragment of the translocation. The cytoplasmic turnover of these RNAs differs significantly from that of the normal c-*myc* message. The 3.5-kb RNA of BL67 cells and the 0.90-kb and 0.74-kb RNAs of BL18 cells, which are both hybrid molecules consisting of c-*myc* and immunoglobulin sequences, have a half-life of several hours in contrast to the normal c-*myc* message with a half-life of 15 min. The aberrant 2.4-kb c-*myc* RNAs of BL67 and BL18 cells are also more stable than the normal c-*myc* message and disappear with a half-life of 50 min. The results are compatible with the model that the secondary structure of the normal c-*myc* RNA is required for the fast, regulated decay of the c-*myc* message in the cytoplasm.

Key words: Burkitt's lymphoma/c-*myc*/aberrant RNA/half-lives

Introduction

Characteristic chromosomal translocations, first observed in Burkitt's lymphoma cells by Manolov and Manolova (1972), always involve band q24 on the long arm of chromosome 8 (Zech *et al.*, 1976; Manolova *et al.*, 1979) and one of the loci of chromosomes 14, 2 or 22 carrying the immunoglobulin heavy or light chain genes (Bernheim *et al.*, 1981). Analogous translocations involving chromosome 15 and the immunoglobulin gene loci on chromosomes 6 and 12 have been described in mouse plasmacytoma (Ohno *et al.*, 1979; for review see Klein 1981, 1983; Perry, 1983). The localization of the c-*myc* proto-oncogene on band q24 of the human chromosome 8 (Neel *et al.*, 1982; Taub *et al.*, 1982) and its move to chromosome 14 in some cases of Burkitt's lymphoma (Dalla-Favera *et al.*, 1982), as well as the demonstration of its fusion to immunoglobulin heavy chain genes (Shen-Ong *et al.*, 1982; Taub *et al.*, 1982), has for the first time provided a link between classical tumour cytogenetics and molecular biology.

In some t(8;14) translocations the chromosomal translocation disrupts the c-*myc* gene and separates the gene from its physiological promoters.

In many other t(8;14) translocations, however, the breakpoints are upstream (Bernard *et al.*,1983; Battey *et al.*, 1983), and in almost all cases of variant translocations they are far downstream of the c-*myc* gene (Erikson *et al.*, 1983; Taub *et al.*, 1984b; Davis *et al.*, 1984; Rappold *et al.*, 1984).

High levels of c-*myc* RNA are observed in many Burkitt's lymphoma and mouse plasmacytoma lines (Maguire *et al.*, 1983; ar-Rushdi *et al.*, 1983; Mushinski *et al.*, 1983; Stanton *et al.*, 1983; Adams *et al.*, 1983). This is, however, not a general feature of Burkitt's lymphoma cells and the amount of c-*myc* mRNA varies considerably among different cell lines (Taub *et al.*, 1984a; Eick *et al.*, unpublished results). In some cases, where the translocation has not directly affected the c-*myc* gene, multiple mutations in the first exon and sometimes in the second exon were described (Taub *et al.*, 1984a, Rabbitts *et al.*, 1983, 1984). The disruption of the c-*myc* gene in most cases with t(8;14) translocations, and structural alterations in the non-coding part of the gene in cases with the breakpoint distant from the c-*myc* gene, were taken as an indication that the c-*myc* gene on the aberrant chromosome becomes transcriptionally deregulated (Leder *et al.*, 1983; Croce *et al.*, 1983; Rabbitts *et al.*, 1984). The concept of transcriptional deregulation was supported by the finding that the c-*myc* RNA in most Burkitt's lymphoma lines is derived from the aberrant chromosome while the c-*myc* gene on the normal chromosome appears to be allelically excluded (Bernard *et al.*, 1983; Nishikura *et al.*, 1983; ar-Rushdi *et al.*, 1983; Taub *et al.*, 1984a).

Evidence for the importance of the post-transcriptional regulation of the c-*myc* gene has been provided by Dani *et al.* (1984) who showed that the c-*myc* RNA has a rapid turnover and a half-life of ~15 min. The disappearance of c-*myc* RNA after treatment of Daudi cells with interferon was found to be post-transcriptionally regulated (Knight *et al.*, 1985; Dani *et al.*, 1985) even though this view is still debated (Einat *et al.*, 1985). Recently Piechaczyk *et al.* (1985) have reported that truncated transcripts found in mouse plasmacytomas, where the c-*myc* gene structure is altered, are significantly more stable than their normal counterparts in control cells.

The rapid turnover of c-*myc* RNA raises the question of the structural targets in the RNA for the rapid decay and of possible alterations in the stability of c-*myc* RNA in Burkitt's lymphoma cells. Here we describe the chromosomal translocation, and c-*myc* RNAs of one Burkitt's lymphoma line (BL67) and, in lesser detail, of a second (BL18), whose structural peculiarities allowed us to address these questions.

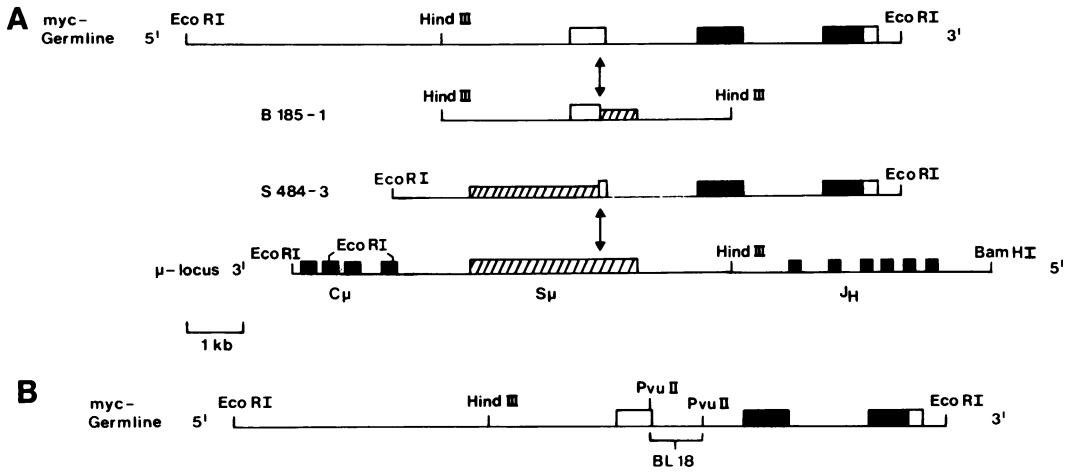

Fig. 1. The breakpoints of the chromosomal translocations in BL67 (**A**) and BL18 cells (**B**). (**A**) The top line illustrates the germ line configuration of the c-*myc* gene in 5′ to 3′ orientation, the bottom line the germ line configuration of the immunoglobulin μ-heavy chain locus in opposite orientation. The untranslated first exon and the 3′-untranslated region of the third exon of the c-*myc* gene are shown by open boxes, translated c-*myc* sequences by filled boxes, μ-switch sequences by a hatched box. Clone B185-1 contains 5′ sequences of the first c-*myc* exon fused to immunoglobulin μ-switch sequences and is derived from chromosome $8q^-$. Clone S484-3 contains the protein-coding sequence of the c-*myc* gene linked to μ-switch and μ-constant sequences and is derived from chromosome $14q^+$. The breakpoint within the c-*myc* gene is designated by an arrow. (**B**) The region where the c-*myc* gene is broken in BL18 cells, as determined by Southern blotting, is shown by a bracket.

Results

Structural characterization of the breakpoints of the chromosomal translocation in BL67 and BL18 cells

BL67 and BL18 are Epstein-Barr virus (EBV)-positive Burkitt's lymphoma lines with reciprocal t(8;14) translocations. Southern blot analysis of *Eco*RI-digested DNA with a human c-*myc* probe containing the third exon revealed fragments of 9.0 kb (BL67) and 18 kb (BL18) in addition to the 12.5-kb germ line fragment. Southern blot analysis with additional enzymes and various c-*myc* probes allowed us to localize the breakpoints in the first exon (BL67) and first intron (BL18), respectively (Figure 1A and B), and to identify a 4.5-kb *Hind*III fragment in BL67 cells carrying the reciprocal recombination (data not shown). The 9.0-kb *Eco*RI and the 4.5-kb *Hind*III fragments of BL67 cells were cloned in phage λgt WES and L47, respectively. The inserts of both clones are schematically described in Figure 1A. The inserts and fragments thereof were subcloned into pUC12 for sequencing of the fragments containing the breakpoints. In clone S484-3, carrying the coding region of the c-*myc* gene, the breakpoint is located within the first exon, 122 bp upstream of the border between first exon and intron (Figure 2). The reciprocal fragment (clone B185-1) contains the sequences of the first exon except for 128 bp at its 3′ side. This implies that five nucleotides of the germ line c-*myc* sequence were deleted at the site of the chromosomal translocation. The break has occurred six nucleotides upstream of a 5′ GAGG 3′ sequence in the c-*myc* gene (underlined in Figure 2), a sequence motif frequently observed near the translocation breakpoints in mouse plasmacytomas (Piccoli *et al.*, 1984). The nucleotide sequence of the disrupted c-*myc* gene was determined 650 bp upstream and 1200 bp downstream of the breakpoint and no difference to the germ line sequence was observed (Gazin *et al.*, 1984) except for the 5-bp deletion already mentioned above. On the immunoglobulin heavy chain locus ~70 nucleotides were determined on both sides of the breakpoint. Similar to other translocation breakpoints analyzed so far, no homology was found between the c-*myc* and μ-switch sequences at or close to the breakpoint. Even though it has not been formally proven, the immunoglobulin heavy chain enhancer

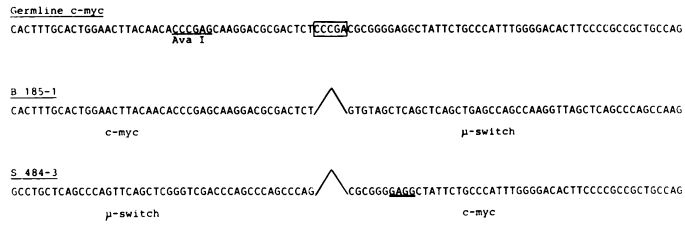

Fig. 2. Comparison of DNA sequences of the normal and translocated c-*myc* gene from BL67 cells. The DNA sequence in the top line represents part of the first exon of the germ line c-*myc* gene (Gazin *et al.*, 1984). The clones B185-1 and S484-3 carrying the breakpoints of the translocation of BL67 cells are described in Figure 1. The five nucleotides deleted at the breakpoint are boxed in the germ line c-*myc* sequence.

should be translocated to chromosome $8q^-$ in this cell line and is thus not available for the c-*myc* gene.

Transcription of the c-myc and IgM gene in BL67 cells

In the Burkitt's lymphoma line BL67 the coding part of the c-*myc* gene is separated from the physiological dual promoters on the aberrant chromosome as a result of the chromosomal translocation. RNAs transcribed from the aberrant c-*myc* allele should, therefore, start at sites which are not physiologically used for the synthesis of c-*myc* mRNA. Some examples have been detailed in various human lymphomas (Bernard *et al.*, 1983; Hayday *et al.*, 1984) and mouse plasmacytomas (Bernard *et al.*, 1983; Stanton *et al.*, 1983; Keath *et al.*, 1984). Northern blot analysis with a probe specific for the second and third exon revealed RNAs of two distinct size classes, one of 2.4 kb and one of 3.5 kb in BL67 RNA (Figure 3, probes 7 and 8). RNA from the cell line IARC 309, an EBV-immortalized lymphoblastoid cell line without chromosomal translocation, which was established from the same patient, showed only the expected 2.4-kb c-*myc* RNA. A probe specific for the first exon (a 862-bp *Pvu*II-*Pvu*II fragment) revealed the same RNA species of 2.4 kb in IARC 309 cells, but hybridized only faintly with the 3.5-kb RNA and failed to hybridize with the 2.4-kb RNA of BL67 cells (Figure 3, probe 5). The 2.4-kb c-*myc* RNA of BL67 must, therefore, be initiated

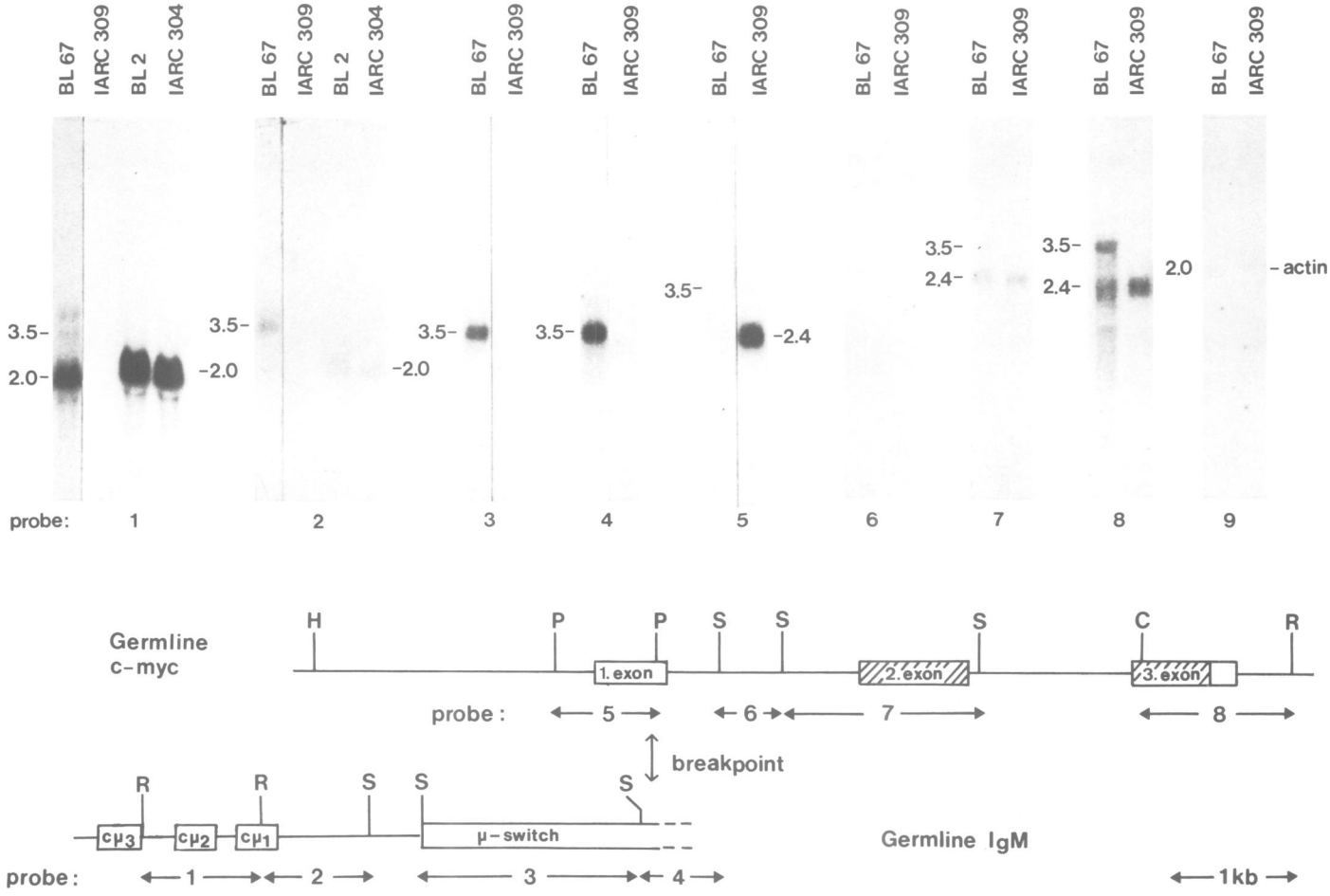

Fig. 3. Northern blot analysis of cytoplasmic c-*myc* and immunoglobulin μ-RNAs of BL67 and IARC 309 cells. The probes used for hybridization are described in the lower part. The following abbreviations were used; C, *Cla*I; H, *Hind*III; P, *Pvu*II, R, *Eco*RI; S, *Sac*I.

at a site other than the normal 2.4-kb c-*myc* RNA seen in IARC 309 cells.

To analyse further the 2.4- and 3.5-kb RNAs of BL67 cells, Northern blots were hybridized with a probe derived from the first intron of the c-*myc* gene and with a probe spanning the breakpoint of the chromosomal translocation. While the intron probe failed to hybridize to any RNA of BL67 and IARC 309 cells (Figure 3, probe 6), the probe spanning the region containing the breakpoint hybridized strongly to RNA of 3.5 kb, which we presumed might be the 3.5-kb c-*myc* RNA. This probe hybridized also weakly to the 2.4-kb c-*myc* RNA of IARC 309 cells (Figure 3, probe 4) due to the presence of sequences from the first exon.

The strong hybridization of the breakpoint probe to the 3.5-kb RNA prompted us to include probes of the μ-locus corresponding to the region beyond the breakpoint into the analysis. All three probes used hybridized with a 3.5-kb RNA of BL67. Since IARC 309 cells do not express μ-specific RNA, the IgM-positive cell lines BL2 and IARC 304 were included as controls. With the immunoglobulin probes, RNA of 3.5 kb was detected only in BL67 cells and not in BL2 and IARC 304 cells (Figure 3, probes 1 and 2).

From these data we presumed that the 3.5-kb c-*myc* RNA of BL67 would probably be initiated in the μ-locus on chromosome 14, while the 2.4-kb RNA would most likely start at cryptic promoters in the first intron, a situation which is reminiscent of that described for the Manca cell line by Hayday *et al.* (1984). These hypotheses were tested by S1 analysis.

No detectable transcripts from the dual c-myc promoters in cytoplasmic RNA of BL67 cells.

The failure to detect c-*myc* RNA of normal size in BL67 cells with the first exon probe indicated that normal c-*myc* transcripts derived from the untranslocated c-*myc* allele are absent. The first exon probe had hybridized, however, to an RNA of 3.5 kb. To exclude the possibility that the RNA visualized by this probe is initiated at the dual c-*myc* promoters, an S1 protection experiment was performed using the 862-bp *Pvu*II-*Pvu*II fragment as a probe (probe A) which carries both promoters. After hybridization to IARC 309 cytoplasmic RNA and S1 digestion, fragments of 513 and 351 bp were protected, as expected, corresponding to the first and second promoter (Figure 4A), (Watt *et al.*, 1983; Saito *et al.*, 1983; Battey *et al.*, 1983). A 1042-bp *Sma*I-*Sac*I probe, comprising the entire first exon (Figure 4B) generated protected fragments of 554 and 392 bp with IARC 309 RNA and confirmed the results obtained with probe A. In contrast, neither of these probes protected fragments of >150 bp with BL67 cytoplasmic RNA. This excludes the possibility that the 3.5-kb RNA is initiated at the dual c-*myc* promoters. It demonstrates that in BL67 cells, as in other Burkitt's lymphoma cases studied in detail (Taub *et al.*, 1984a), neither the promoters

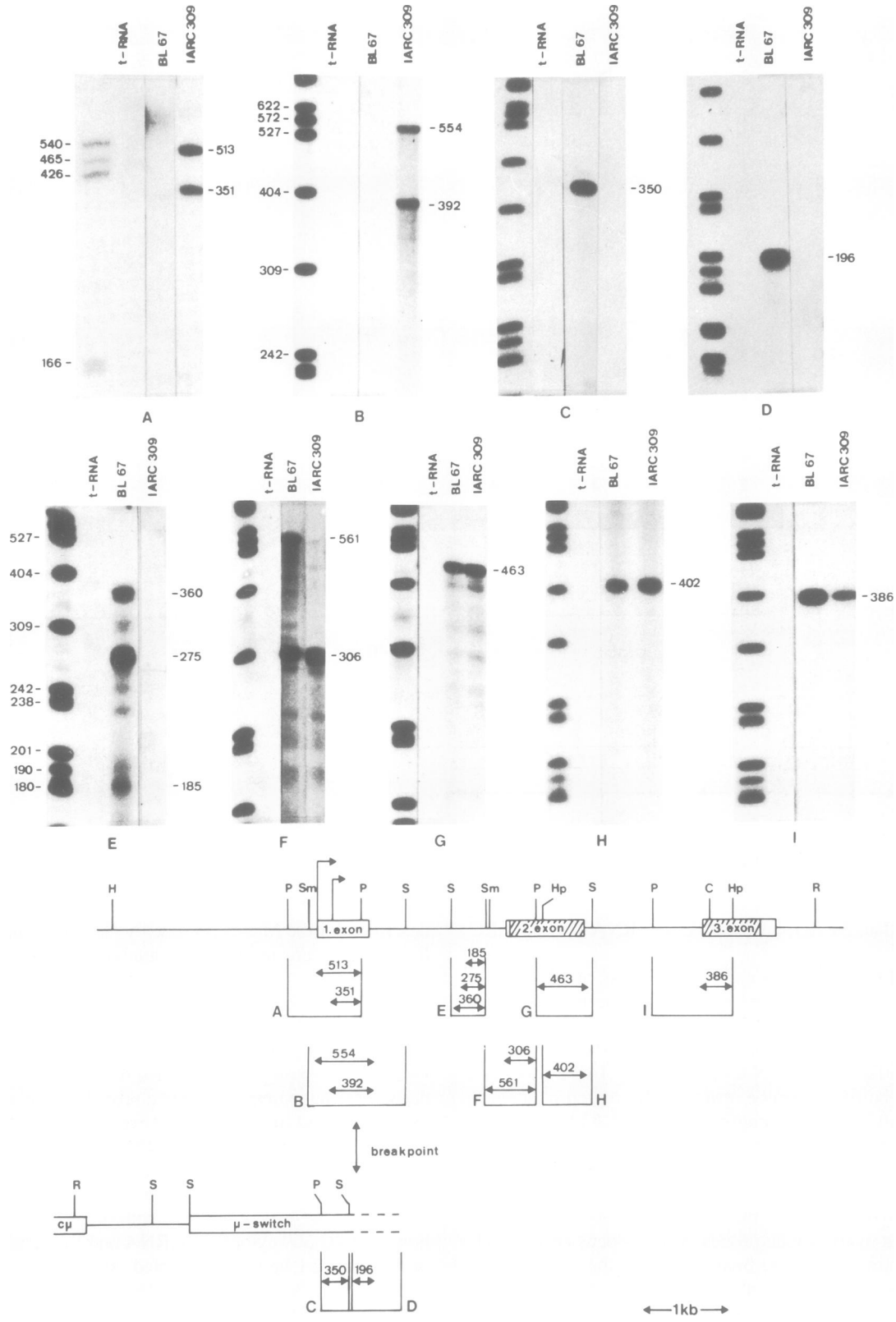

Fig. 4. S1 analysis of cytoplasmic c-*myc* and immunoglobulin μ-RNAs of BL67 and IARC 309 cells. Labelled DNA probes were hybridized to 30 μg cytoplasmic RNA or yeast tRNA (Boehringer, Mannheim). M13 mp10/mp11 clones containing an 862-bp *Pvu*II-*Pvu*II fragment (**A**), a 1042-bp *Sma*I-*Sac*I fragment (**B**), a 561-bp *Sma*I-*Pvu*II fragment (**F**) and a 593-bp *Pvu*II-*Sac*I fragment (**G**) were labelled with ^{32}P by primer extension. The pUC12 clones containing a 583-bp *Sac*I-*Sac*I fragment (containing the breakpoint) (**D**) and a 360-bp *Sac*I-*Sma*I fragment (**E**) were labelled by T4 polymerase. The probes containing a 350-bp *Pvu*II-*Sac*I fragment (**C**), a 532-bp *Hpa*II-*Sac*I fragment (**H**) and a 885-bp *Pvu*II-*Hpa*II fragment (**I**) were labelled by T4 polynucleotide kinase. Probes **C** and **D** were hybridized at 53°C, **A**, **B** and **I** at 56°C and **E**, **G**, **F** and **H** at 60°C. The probes are described in the map shown below the autoradiograms. The upper line represents the map of the human c-*myc* locus in the 5′ and 3′ orientation, the lower line part of the immunoglobulin μ-locus in the opposite orientation. Mol. wt. standards are end-labelled *Hpa*II fragments of the lymphotropic papova virus (LPV) cloned in pBR322. Abbreviations of restriction enzymes: C, *Cla*I; H, *Hind*III; Hp, *Hpa*II; P, *Pvu*II; R, *Eco*RI; S, *Sac*I; Sm, *Sma*I.

on the normal allele nor on the reciprocal fragment (clone B185-1) give rise to detectable amounts of transcripts in the cytoplasm.

The 2.4-kb c-myc RNA is transcribed from cryptic promoters in the first intron in BL67 cells.

Since the 2.4-kb RNA of BL67 cells was shown by Northern blot analysis to be an aberrant transcript, it was tempting to assume that the RNA is initiated at cryptic promoters in the first intron, as described by Hayday *et al.* (1984) for the Burkitt's lymphoma line Manca. This was tested by S1 analysis using a 561-bp *Sma*I-*Pvu*II fragment as a probe (Figure 4F) spanning the border between the first intron and the second exon. This probe generated a protected fragment of 306 bp with cytoplasmic IARC 309 RNA, corresponding to a normal intron-exon boundary. In BL67 RNA this probe protected the same 306-bp fragment and, in addition, a fragment of 561 bp, not seen in IARC 309 RNA. This indicates that one part of the BL67 c-*myc* RNA, presumably the 3.5-kb RNA, is normally spliced at the boundary between first intron and second exon, whereas another part of the RNA, the 2.4-kb RNA, is not spliced.

To map in more detail the transcription initiation sites of the unspliced transcripts in the first intron, a 360-bp *Sac*I-*Sma*I probe from the first intron (Figure 4E) was used for S1 analysis. With BL67 RNA, but not with IARC 309 RNA, fragments of 185, 275 and the complete 360-bp fragment were protected, while with the 606-bp *Sac*I-*Sac*I fragment from the first intron no protection was observed in either BL67 or IARC 309 RNA (data not shown).

We conclude from the S1 analysis that the 2.4-kb *myc* RNA of BL67 cells consists of three distinct species, the largest one starting at the second *Sac*I site in the first intron, and the other two 85 and 175 bp downstream.

To see whether the usage of cryptic transcription initiation sites was correlated with changes in the nucleotide sequence, the first intron was sequenced without revealing, however, any deviation from the germ line c-*myc* gene (Gazin *et al.*, 1984).

The 3.5-kb c-myc RNA is transcribed from the anti-sense strand of the immunoglobulin heavy chain gene in BL67

The Northern and S1 analysis described so far had suggested that the 3.5-kb c-*myc* RNA of BL67 cells is initiated in the immunoglobulin heavy chain locus and transcribed from the heavy chain anti-sense strand towards and across the breakpoint of the translocation into the distal part of the c-*myc* gene. An RNA of 3.5 kb transcribed in this direction was only demonstrated in BL67 cells (Figure 5) and not in the other IgM-producing cell lines. The S1 protection experiment using the probe spanning the breakpoint is shown in Figure 4D. Using the 583-bp *Sac*I-*Sac*I breakpoint probe (probe D) a 196-bp fragment was protected with BL67 RNA. This fragment is composed of 74 bp derived from the μ-switch region on chromosome 14 and of 122 bp derived from the distal part of the first exon of the c-*myc* gene on chromosome 8, thus providing evidence for a contiguous transcript across the breakpoint. The usage of the splice donor site at the 3′ end of the first c-*myc* exon is consistent with the described utilization of the splice acceptor site at the 5′ end of the second exon in BL67 cells (Figure 4F). A 350-bp *Pvu*II-*Sac*I probe from the heavy chain locus adjacent to the breakpoint fragment was fully protected after hybridization with BL67 RNA (Figure 4C), providing additional evidence for the transcription of the heavy chain anti-sense strand towards the breakpoint of the translocation.

The use of additional S1 probes from the immunoglobulin heavy chain locus revealed at least one more splice in the immunoglobulin part of the 3.5-kb RNA (data not shown).

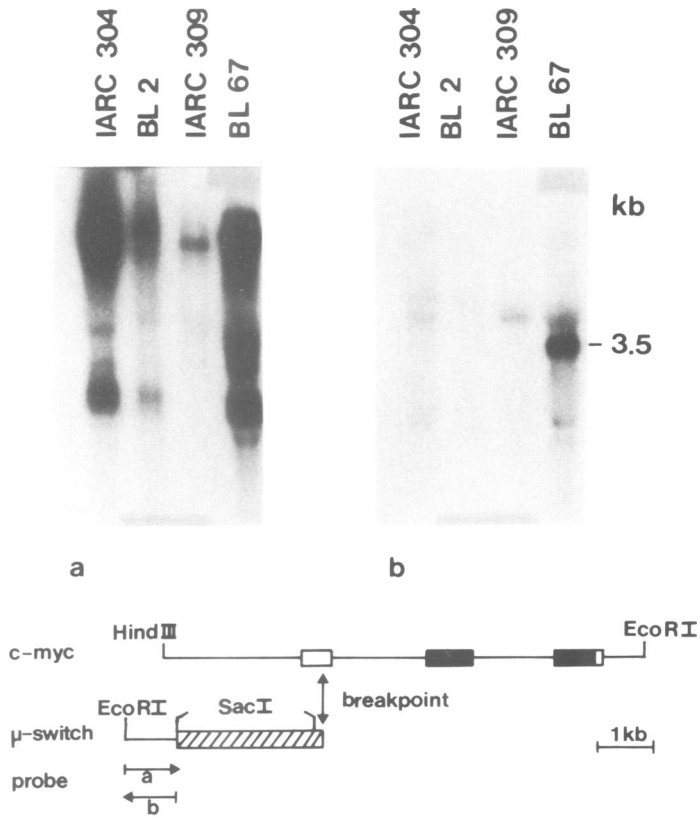

Fig. 5. Two identical Northern blots with total cellular RNA were hybridized with RNAs labelled with [^{32}P]UTP by SP6 polymerase and transcribed from a 0.9-kb *Eco*RI-*Sac*I fragment of the immunoglobulin μ-locus cloned in both orientations into pSP64/65 vectors. Probe a is complementary to immunoglobulin μ-constant RNA and probe b to RNA transcribed in the anti-sense orientation. Probe a was used in a, and probe b in b.

Aberrant c-myc RNAs in BL18 cells

In BL18 cells a similar pattern of c-*myc* transcripts was found as in BL67 cells (Figure 6). The dominant species is a 2.4-kb RNA which is initiated in the first intron. Small amounts of aberrant 3.5-kb c-*myc* RNAs transcribed from the anti-sense strand of the μ-locus were also detected (Figure 6B). Hybridization of the first exon probe to a Northern blot with BL18 RNA demonstrated the absence of normal c-*myc* RNA in these cells (Figure 6A). This probe detected, however, two small RNA species of 0.90 and 0.74 kb in BL18 cells which were absent from BL67 cells. S1 analysis showed that the first exon is completely represented in these RNAs (Figure 6C). The 0.90- and 0.74-kb RNAs are thus transcripts initiated at both c-*myc* promoters in the reciprocal fragment of the translocation and are presumably spliced to sequences in the heavy chain locus, similarly to the small RNA species described in ST486 cells by ar-Rushdi *et al.* (1983).

Stability of the aberrant c-myc RNA of BL67 and BL18 cells

The structural alteration in either the 5′ or the 3′ part of the c-*myc* RNAs of BL18 and BL67 cells raised the question whether the absence of part of the c-*myc* message and/or the acquisition of sequences from the immunoglobulin locus would affect the stability of the c-*myc* RNA. The transcriptional block by actinomycin D was shown to be a reliable tool for measuring c-*myc* RNA turnover in various cellular contexts (Dani *et al.*,1984; Piechac-

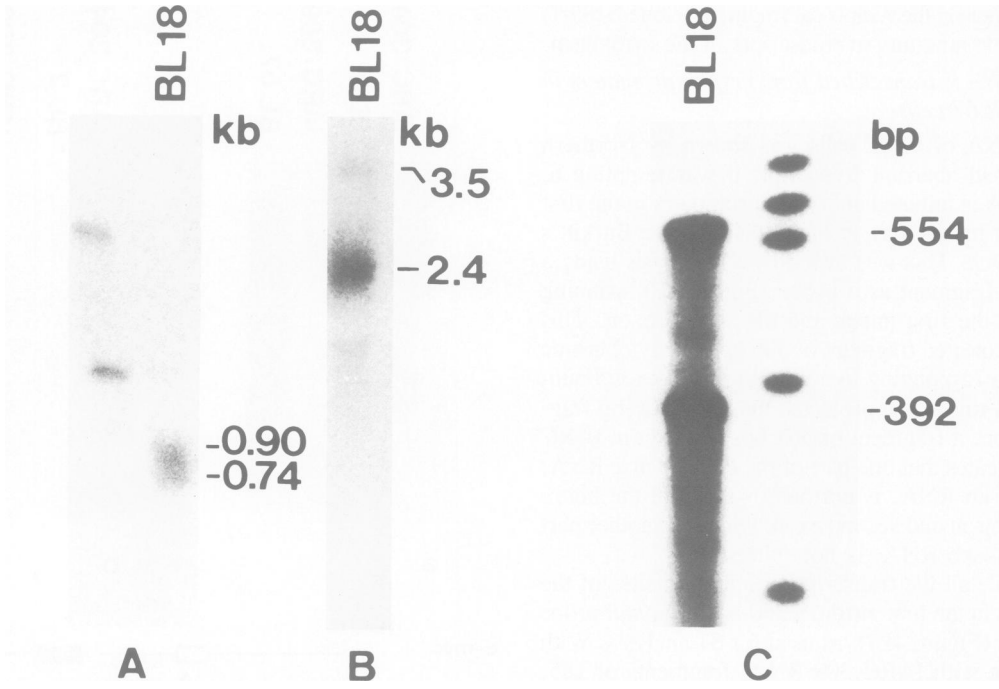

Fig. 6. Northern blot (**A** and **B**) and S1 analysis (**C**) of c-*myc* RNA of BL18 cells. The probes used for the first c-*myc* exon in (**A**) and (**C**) are probe 5 of Figure 3 and probe B of Figure 4, respectively. The third exon probe used in (**B**) is probe 8 of Figure 3. The bands in the left lane of (**A**) are mol. wt. markers.

zyk *et al.*, 1985). Therefore RNA was isolated from BL67, BL18 and IARC 309 cells at different times after the addition of the drug. Equal amounts of each RNA sample were spotted onto nitrocellulose and hybridized with a third exon c-*myc* probe (Figure 7A, lower part). c-*myc* RNA from IARC 309 cells disappeared much more rapidly than that from BL67 cells. A quantitative evaluation of the kinetics of disappearance of total c-*myc* RNA revealed half-lives of ~15 and 110 min in IARC 309 and BL67 cells, respectively (Figure 7B). To analyse the turnover of the individual RNA species in BL67 cells, the RNA preparations were analysed by Northern blotting using the same third exon c-*myc* probe (Figure 7A, upper part). The 3.5-kb RNA species is more stable than the 2.4-kb RNA and contributes largely to the slow turnover of total c-*myc* RNA in BL67 cells. The quantitative evaluation revealed a half-life of 50 min for the 2.4-kb RNA and of several hours (>3 h) for the 3.5-kb RNA.

The increased stability of both c-*myc* RNA species in BL67 cells raised the possibility that the first exon of the c-*myc* gene would carry a target determining the extremely rapid decay of the normal c-*myc* message. The 0.90-kb and 0.74-kb RNAs of BL18 cells span the first c-*myc* exon entirely and thus allow us to address this question. Figure 8 shows a Northern blot of BL18 RNA isolated at different times after actinomycin D treatment. The 2.4-kb c-*myc* RNA disappeared with kinetics similar to the 2.4-kb RNA of BL67 cells and has a half-life of ~1 h. The two small RNA species visualized with the first exon probe are significantly more stable than the 2.4-kb RNA and have half-lives of several hours.

Discussion

We have studied the chromosomal translocations of the Burkitt's lymphoma lines BL67 and BL18 and the consequences of these translocations for the transcription of the c-*myc* gene.

In both lines, the c-*myc* gene is fused to the immunoglobulin heavy chain locus in chromosome 14. On chromosome 8 the breakpoint is located within the first exon (BL67) and the first intron (BL18) of the c-*myc* gene, respectively. The translocation of BL67 cells was analysed at a molecular level and was shown to be reciprocal leading to the loss of 5 bp in the first exon of the c-*myc* gene. Except for this deletion, no change in the nucleotide sequence of this aberrant first exon was observed.

Two classes of c-*myc* RNA of 2.4 and 3.5 kb are found in BL67 cells. The 2.4-kb RNA consists of several species initiated at several sites in the first intron. These cryptic promoters are similar but not identical to those described for Manca cells (Hayday *et al.*, 1984). No changes in the nucleotide sequence of the first intron were observed which could have given rise to the formation of new transcription initiation sites. The structure of the 3.5-kb c-*myc* RNA turned out to be paticularly interesting, because the RNA is initiated within the heavy chain locus on chromosome 14 and is transcribed from the immunogloublin antisense strand towards and across the breakpoint of the chromosomal translocation into the first exon of the c-*myc* gene. This is the first example of a Burkitt's lymphoma in which abnormal c-*myc* transcripts are shown to be initiated in the Ig locus. This has been suggested to occur in some of the Burkitt's lymphoma lines studied by Adams *et al.* (1983) and has probably been underestimated up to now. Since BL67 cells produce IgM, μ-sense and anti-sense RNA are apparently synthesized in the same cell without functional interference with respect to IgM synthesis.

Similarly to most other Burkitt's lymphoma lines, no normal c-*myc* transcripts derived from the untranslocated chromosome are found in BL67 and BL18 cells. In BL67 cells no cytoplasmic RNA initiated at the dual c-*myc* promoters on the reciprocal fragment of the translcoation could be detected, even though the Ig-enhancer is presumably adjacent.

This is different to BL18 cells in which small RNAs transcribed from both c-*myc* promoters on the reciprocal fragment of the translocation are easily detectable. In BL18 cells the total steady-

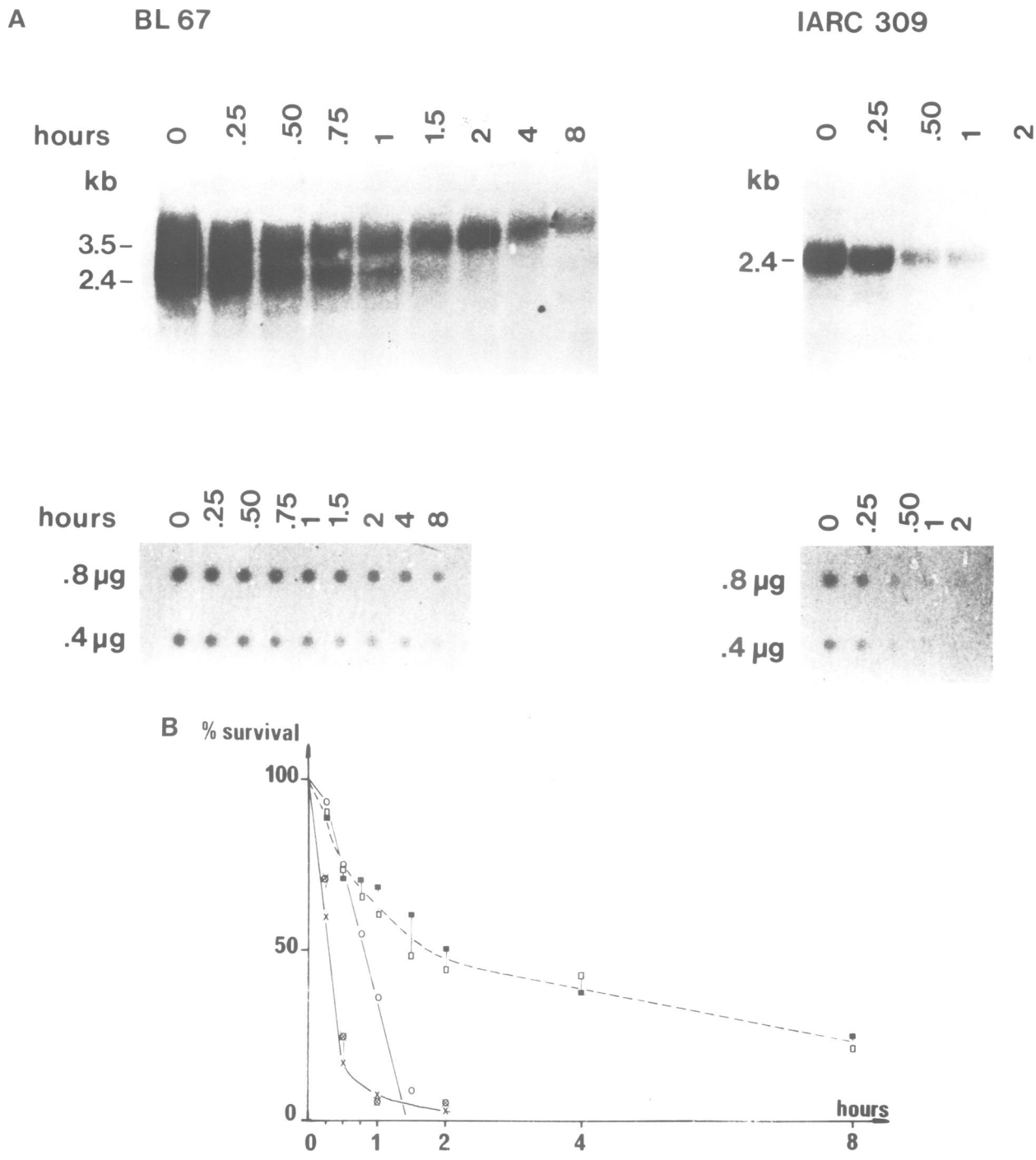

Fig. 7. (**A**) Determination of the half-life of c-*myc* RNAs from BL67 and IARC 309 cells. Cells were incubated in the presence of actinomycin D (5 µg/ml). RNA was extracted from aliquots at various times after addition of the drug. The RNA was analysed on Northern blots hybridized with the third exon c-*myc* probe (Figure 3, probe 8), (**A, upper part**). Aliquots of 0.8 and 0.4 µg of total cellular RNA were spotted onto nitrocellulose filters according to Thomas (1980) and hybridized with the same probe (**A, lower part**). (**B**) The decay of total c-*myc* RNA of BL67 cells (open and filled rectangles), of the 2.4-kb RNA of BL67 cells (open circles), of total RNA of IARC 309 cells (crosses) and of 2.4-kb RNA of IARC 309 cells (open circles with crosses) was quantitated by densitometric scanning of the bands or spots visualized on the autoradiograms shown in (**A**) after shorter exposure. The amount of c-*myc* RNA at the time of actinomycin D treatment is taken as 100%.

state level of c-*myc* RNA is rather low. A 2.4-kb RNA initiated in the first intron is the dominant species. Small amounts of a large RNA initiated in the heavy chain locus are also found similar to the 3.5-kb RNA of BL67 cells. All c-*myc* transcripts from BL67 and BL18 cells are joined at their 5′ or 3′ part to sequences not found in normal cytoplasmic c-*myc* RNA and lack either the head or the body of the normal message.

The occurrence of these aberrant transcripts allowed us to ask the question whether the structural alterations are related to differences in the turnover of these RNAs compared with the normal c-*myc* message. In fact, the half-life of total c-*myc* RNA is ~2 h in BL67 cells and ~1 h in BL18 cells compared with 15 min in the EBV-immortalized normal lymphocytes as determined by actinomycin D treatment.

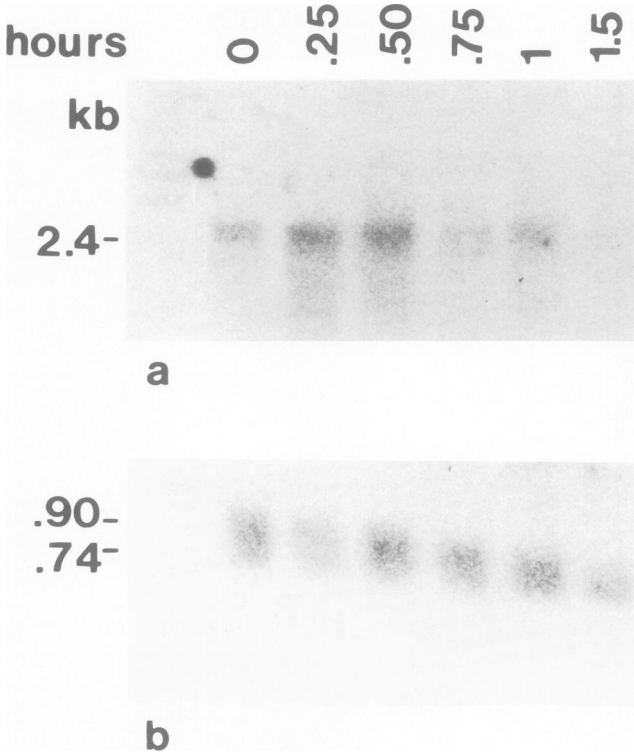

Fig. 8. Decay of c-*myc* in BL18 cells after actinomycin D treatment. RNA isolated at different time points after addition of the drug was subjected to Northern blot analysis and hybridized with a third exon probe (**a**) (Figure 3, probe 8) and a first exon probe (**b**) (Figure 3, probe 5).

The increased RNA stability thus contributes by a factor of 6–8 to the steady-state level of c-*myc* message in BL67 cells and by a factor of 3–4 in BL18 cells. These are probably minimum values, since the nuclear dwell time as well as the time lag necessary for actinomycin D to block transcription [which is ~5 min (Blanchard, unpublished observation; Piechaczyk *et al.*, 1985)], were not subtracted from the apparent half-lives that were measured.

Neither of the cell lines BL67 nor BL18, exhibit particularly high steady-state c-*myc* RNA levels compared with other cell lines. Conversely, a high steady-state level of c-*myc* RNA is found in BL2 cells, a cell line with a t(8;22) translocation leaving the c-*myc* gene intact, in which the half-life of c-*myc* RNA is 15 min (Eick *et al.*, in preparation). This indicates that other mechanisms such as variations in transcriptional rates, RNA processing and transport, also contribute significantly to the steady-state c-*myc* RNA level in the cytoplasm.

The two abnormal c-*myc* transcripts of BL67 contribute differently to the increase in stability. The 2.4-kb RNA of BL67 as well as of BL18 has a half-life of 50 min, which is very similar to that of the structurally truncated c-*myc* RNAs of mouse plasmacytomas MPC11 and J558L (Piechaczyk *et al.*, 1985). If we assume that the stabilization reflects intrinsic properties of these RNA molecules and not an influence of the cellular context, it is impossible to decide whether the stabilization is due to the presence of intronic sequences or to the absence of exon 1. The 3.5-kb RNA of BL67 cells is still much more stable and has a half-life of several hours indicating that long exogenous sequences are able to stabilize dramatically truncated c-*myc* RNA molecules. We do not know, however, whether this is a *cis* effect. Alternatively, one could even speculate that stabilization is exerted in *trans* by the μ-sense RNA which is present in great excess over the μ-anti-sense/c-*myc* hybrid RNA.

The increased stability of c-*myc* RNAs completely or partially lacking the first exon raised the question whether the first exon carries a target structure conferring, solely by its presence, the rapid decay of the c-*myc* message. This was excluded by demonstrating that the small RNAs of BL18 cells which contain the entire first exon are very stable molecules. Again, it is impossible to decide whether the stabilization of the small RNAs is due to the fusion to exogenous sequences or due to the absence of exons 2 and 3. The latter possibility would imply that the secondary structure of the RNA could be an important factor determining the rapid decay of c-*myc* RNA. A long stem structure in the c-*myc* RNA formed between partially homologous sequences in the first and second exon has been proposed by Saito *et al.* (1983). Morevover, a detailed computer analysis revealed the possibility of a high degree of secondary structure in the c-*myc* RNA (Edlind, personal communication). The analysis of chimeric messengers bearing various c-*myc* RNA regions and of c-*myc* RNAs transcribed from c-*myc* deletion constructs will perhaps cast some light on the precise nature of the sequences which are involved. The ability of the RNA to be translated into protein might be another important variable determining the turnover of the RNA. It will, therefore, be interesting to see whether the different aberrant c-*myc* RNAs are found on polysomes and how they contribute to the synthesis of the c-*myc* protein.

Materials and methods

Cell lines

The cell lines have been established at the International Agency for Research on Cancer (Lenoir *et al.*, 1985). BL67 was established from the ascites of a French female with an EBV genome-positive Burkitt's lymphoma with a t(8;14) translocation and μλ phenotype. The lymphoblastoid cell line IARC 309 was obtained by immortalization of normal peripheral lymphocytes of the same patient by the B95-8 EBV strain. BL18 was established from the tumour of an Algerian boy with an EBV-associated tumour carrying a t(8;14) translocation.

RNA extraction

Cytoplasmic RNA was prepared by the Nonidet P-40 method and subsequently extracted with phenol/CIA (Maniatis *et al.*, 1982). Total cellular RNA was extracted by the LiCl-urea method described by Auffray and Rougeon (1980).

Cloning and sequencing

Genomic clones containing the breakpoints of the translocation were obtained by screening recombinant λgtWES and L47 phages containing size-selected *Eco*RI and *Hind*III fragments of BL67 DNA, respectively with probes specific for the third and first exon of the c-*myc* gene. The inserts of the phages were subcloned into pUC12. Fragments were labelled with [α-^{32}P]dNTP by Klenow polymerase and sequenced according to Maxam and Gilbert (1980).

RNA blotting

RNA blot analysis was performed by fractionation of RNA samples on a 1% agarose/formaldehyde gel (Maniatis *et al.*, 1982). Standard procedures were followed for hybridization of blots with nick-translated (Rigby *et al.*, 1977) or SP6-transcribed probes (Green *et al.*, 1983), washing and autoradiography (Maniatis *et al.*, 1982).

S1 mapping

Single-stranded uniformly labelled DNA probes were prepared by primer extension of M13 clones (Ley *et al.*, 1982). 5′-labelled probes were made by dephosphorylation followed by kinase reaction and 3′-labelled probes wiht T4 polymerase (Maniatis *et al.*, 1982). Hybridization of labelled DNA fragments to cytoplasmic or total RNA was carried out in a modification of the method of Berk and Sharp (1977). Hybridization mixtures of 40 μl containing ~100 000 c.p.m. of the probe (sp. act. ~10^8 c.p.m./μg), 20–40 μg RNA in 90% formamide, 400 mM NaCl, 40 mM Pipes pH 6.5, 1 mM EDTA were denatured at 90°C for 5 min and immediately transferred to 53–60°C as indicated for each DNA fragment. After 15 h the hybridization was terminated by addition of 360 μl ice-cold buffer containing 250 mM NaCl, 30 mM Na-acetate pH 4.5, 2 mM Zn-acetate, 5% glycerol, and 4000 U nuclease S1 (Boehringer, Mannheim). The samples were incubated at 37°C for 1 h, extracted with phenol-chloroform-

isoamylalcohol (25:24:1, v/v/v), and precipitated with ethanol. Protected DNA fragments were separated on 5% polyacrylamide gels with 7 M urea.

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