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Post-transcriptional mechanisms of deregulation of *MYC* following conversion of a human B cell line by Epstein – Barr virus

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By utilizing an Epstein – Barr virus (EBV)-negative Burkitt's lymphoma line (BJAB) and several EBV-positive sub-lines derived from it by *in vitro* infection, it has been shown previously that the presence of the EBV genome in this B cell line is reversibly associated with deregulation of *MYC* expression. Specifically, the decline in the level of *MYC* transcripts observed in the EBV-negative BJAB line as cells approach stationary phase of growth is abrogated in the presence of the virus. In the studies described herein, the mechanism of deregulation of *MYC* in EBV-converted BJAB cells was examined. It was shown that the presence of EBV in BJAB cells was not associated with changes in the rate of transcription from exons I, II or III of *MYC* as cells approached stationary phase of growth. In contrast, the stability of *MYC* mRNA was altered in EBV-positive BJAB cells. Specifically, the half-life of *MYC* mRNA increased from <36 to >70 min in EBV-positive BJAB lines as cells progressed from early to late exponential phase of growth. This alteration in stability of *MYC* transcripts was reversibly associated with the presence of the virus, since an EBV-negative revertant BJAB line did not display an increase in stability of *MYC* transcripts in late exponential phase of growth. In addition, progression from early to late exponential phase of growth in two EBV-immortalized lymphoblastoid lines derived from normal human B cells was also associated with prolongation of the half-life of *MYC*. These findings suggest that EBV may directly or indirectly alter regulation of *MYC* expression by post-transcriptional mechanisms in human B cells.

Key words: Burkitt's lymphoma/Epstein – Barr virus/immortalization/*MYC* regulation/oncogene

Introduction

Epstein – Barr virus (EBV) is a human B-lymphotropic herpesvirus that causes infectious mononucleosis and is associated with several human B cell malignancies, including endemic Burkitt's lymphoma (BL) (Miller, 1985). *In vitro* infection of normal human B lymphocytes by EBV results in transformation to 'immortalized' lymphoblastoid cells capable of proliferating indefinitely in culture (Pope, 1979). The mechanisms involved in transformation of human B cells by EBV are not known, and studies to determine the molecular events by which EBV transforms human B cells have been inherently difficult due to the lack of clonal lines of normal non-transformed proliferating human B cells that can be utilized as appropriate EBV-negative controls. In lieu of such lines, we have utilized an EBV-negative human B cell lymphoma line, BJAB (Klein *et al.*, 1974; Menezes *et al.*, 1975), as a control for comparative studies with EBV-positive lines that had been derived from it by *in vitro* infection with the B958 or P3HR1 strains of EBV (Fresen *et al.*, 1977; Fresen and zur Hausen, 1976). Conversion of BJAB to stable EBV-positive lines results in phenotypic changes in growth properties, including reduced serum requirements and increased resistance to saturation conditions, indicating that viral functions mediate altered growth in infected BJAB cells (Steinitz and Klein, 1975, 1976). As in immortalized human B cells, EBV is present in a latent state in BJAB with restricted expression of viral genes. Thus, these isogenic EBV-negative and -positive BJAB lines provide a useful system for examining alterations in molecular processes that are associated with latent infection and transformation by EBV.

We have previously utilized the EBV-positive and -negative BJAB lines to determine whether EBV may alter *MYC* expression in human B cells (Lacy *et al.*, 1987). Transcriptional expression of the *MYC* oncogene is controlled with respect to the cell cycle in a variety of cell types (Kelly and Siebenlist, 1986), including normal human B cells (Lacy *et al.*, 1986), and the protein product of *MYC* is believed to be involved in regulating cell cycling (Kelly *et al.*, 1983; Campisi *et al.*, 1984; Kelly and Siebenlist, 1986; Studzinski *et al.*, 1986). Thus, it is plausible that deregulation of *MYC* expression may be involved in the immortalization of normal human B cells by EBV. Indeed, deregulation of *MYC* has been shown to occur in association with EBV in B cell lymphoma lines derived from endemic BL (Kelly and Siebenlist, 1986; Lenoir, 1986). Although the chromosomal translocation in the region of the *MYC* oncogene is believed to cause deregulation of *MYC* in BL (Kelly and Siebenlist, 1986; Lenoir, 1986), it is possible that EBV may contribute to altered *MYC* expression in these B cell lines. Our previous studies in EBV-negative and -positive BJAB lines support the hypothesis that EBV may cause deregulation of *MYC* in human B cells even in the absence of a chromosomal translocation (Lacy *et al.*, 1987). We observed that the presence of EBV in BJAB cells is reversibly associated with altered transcriptional expression of *MYC*. In the EBV-negative parental BJAB line, the level of *MYC* transcripts declines progressively as cells approach and enter stationary phase of growth. In contrast, there is no decline in the level of *MYC* transcripts in the EBV-positive BJAB lines as the cells approach stationary phase of growth. Thus, in the early exponential phase of growth, the level of *MYC* transcripts in the EBV-positive and -negative BJAB lines are similar. However, in late exponential phase of growth the levels of *MYC* transcripts are 5- to 20-fold less in the EBV-

negative BJAB lines compared with the EBV-positive lines. Deregulation of *MYC* in EBV-positive BJAB cells is reversibly associated with the presence of the virus, since an EBV-negative revertant clone of P3HR1-infected BJAB cells expresses levels of *MYC* transcripts that are similar to those observed in the EBV-negative parental BJAB line. These previous studies indicate that EBV infection of human B cells can lead to altered transcriptional expression of *MYC* and implicate deregulation of *MYC* in the process of immortalization of human B cells by EBV. In addition, these findings suggest that EBV may contribute to the deregulation of *MYC* in EBV-associated BLs.

In this study we have examined the mechanism of deregulation of *MYC* expression in EBV-converted BJAB cells. Studies from other cell systems have shown that regulation of *MYC* expression is complex and can occur by multiple mechanisms. Normal *MYC* transcripts have a short half-life, and alteration in levels of *MYC* mRNA can result from either altered rates of transcription (Endo and Nadal-Ginard, 1986; Greenberg *et al.*, 1986; Yarden and Kimchi, 1986; Polack *et al.*, 1987) or post-transcriptional modifications in *MYC* mRNA stability (Dony *et al.*, 1985; Kindy and Sonenshein, 1986; Knight *et al.*, 1985; Piechaczyk *et al.*, 1985). Recently it has been shown that intragenic pausing of transcription at the 3′ end of the first exon is important in regulating *MYC* transcription in normal as well as malignant cells (Eick and Bornkamm, 1986; Nepveu and Marcu, 1986; Cesarman *et al.*, 1987). In this study we have examined possible mechanisms by which *MYC* deregulation occurs in EBV-converted BJAB cells. It was shown that the reversal of down-regulation of *MYC* expression in association with EBV is due to a post-transcriptional mechanism rather than a transcriptional mechanism. Specifically, the presence of EBV does not cause a shift in promoter utilization of *MYC* or an increase in the overall rate of transcription of exons I, II or III of *MYC*. Furthermore, the presence of EBV does not abrogate the pause in transcription between the first and second exons of *MYC*. However, the presence of EBV does lead to stabilization of *MYC* transcripts in late exponential phase of growth. Thus, the deregulation of *MYC* in association with EBV infection of BJAB appears to be due to post-transcriptional changes in stability of mature *MYC* mRNA.

Results

S1 nuclease analysis of MYC transcripts in EBV-negative and -positive BJAB lines

To determine whether the presence of EBV in BJAB cells is associated with a shift in the utilization of the *MYC* promoters, S1 nuclease analysis of the 5′ end of the *MYC* gene was performed. Since differences in levels of *MYC* transcripts in EBV-positive and -negative BJAB cells were previously shown to be maximum in late exponential phase of growth, the cell lines were examined in both early and late exponential phases of growth. Cells from each line were cultured at low density on day 0 and harvested on day 1 (early exponential phase) and day 3 (late exponential phase) (Figure 1). RNA was extracted at both time points for S1 nuclease analysis. These experiments showed that the majority of *MYC* transcripts were initiated from the second promoter of *MYC* in all four cell lines (Figure 2). There was no difference in promoter utilization in the EBV-negative

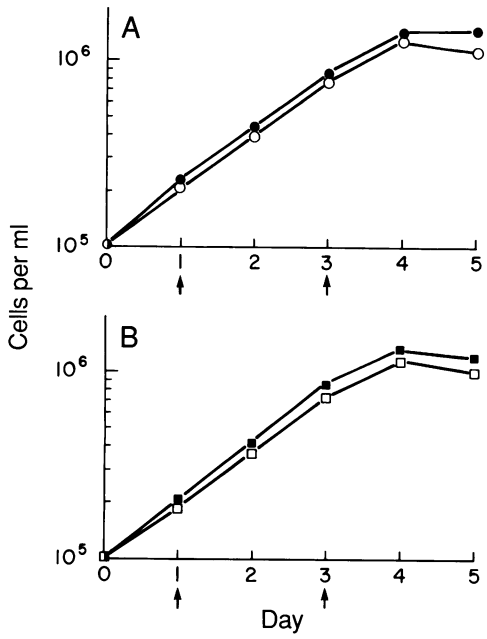

Fig. 1. Growth curves of the EBV-negative and -positive BJAB lines from a representative experiment. (**A**) BJAB cells (○) and BJAB-B958 cells (●); (**B**) BJAB-B1-107 cells (□) and BJAB-B1 cells (■). Cells were harvested on day 1 (early exponential phase) and day 3 (late exponential phase) for the experiments described.

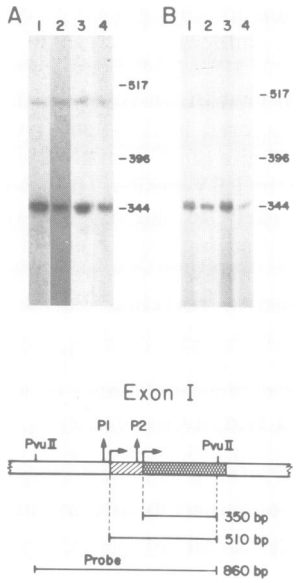

Fig. 2. S1 nuclease analysis of the 5′ end of *MYC* transcripts in EBV-negative (BJAB and BJAB-B1-107) and EBV-positive (BJAB-B958 and BJAB-B1) lines in early and late exponential phases of growth. The probe utilized for hybridization in these experiments is depicted in the scheme of the 5′ region of the *MYC* gene. The size markers shown on the right were derived from pBR322 plasmid DNA digested with *Bam*HI and *Hin*fI. (**A**) Early exponential phase. Total RNA was purified from cells in early exponential phase of growth. **Lane 1**, BJAB-B958; **lane 2**, BJAB; **lane 3**, BJAB-B1; **lane 4**, BJAB-B1-107. Total RNA (5 μg) from each cell line was hybridized to the probe prior to S1 nuclease digestion. (**B**) Late exponential phase. Total RNA was purified from cells in late exponential phase of growth. **Lane 1**, BJAB-B958; **lane 2**, BJAB; **lane 3**, BJAB-B1; **lane 4**, BJAB-B1-107. Total RNA (5 μg) from BJAB-B958 and BJAB-B1 was hybridized to the probe prior to digestion with S1 nuclease; 20 μg of total RNA from BJAB and BJAB-B1-197 was hybridized to the probe.

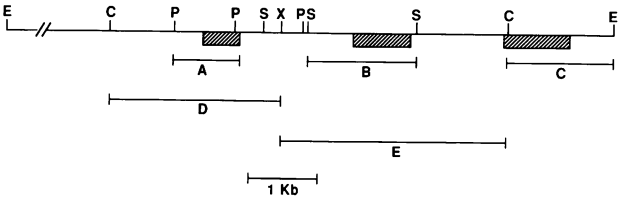

Fig. 3. Partial restriction endonuclease map of the human *MYC* gene and the probes utilized in nuclear runoff experiments. Hatched boxes represent exons I, II and III. The *Eco*RI genomic fragment depicted is 13 kb in length. C, *Cla*I; E, *Eco*RI; P, *Pvu*II; S, *Sac*I; X, *Xba*I.

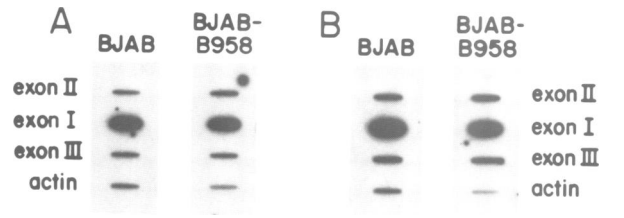

Fig. 4. Nuclear runoff assays of *MYC* transcripts in EBV-negative (BJAB) and EBV-positive (BJAB-B958) lines in early and late exponential phases of growth using exon-specific probes. Probes D, E and C (Figure 3) were utilized for exons I, II and III respectively. (**A**) Nuclei were harvested from cells in early exponential phase of growth (2×10^5 cells/ml). (**B**) Nuclei were harvested 2 days later from cells in late exponential phase of growth (8.0×10^5/ml).

and -positive BJAB lines in either early or late exponential phases of growth. Furthermore, there was no shift in promoter utilization as the cells progressed from early to late exponential phase of growth. Thus, altered utilization of promoters was not involved in deregulation of *MYC* expression in the EBV-positive lines.

Nuclear runoff analysis of rates of MYC transcription

To determine if increased levels of *MYC* transcripts in EBV-positive BJAB cells results from increased transcriptional activity of the gene, the EBV-positive and negative lines were assayed for relative amounts of nascent *MYC* transcripts by nuclear runoff analysis using nuclei isolated from all four lines in both early and late exponential phases of growth. Since it has been shown that transcriptional regulation of *MYC* expression can result from alterations in a block to transcriptional elongation at the 3′ end of the first exon (Eick and Bornkamm, 1986; Nepveu and Marcu, 1986; Cesarman *et al.*, 1987) nuclear runoff experiments were done with exon-specific probes to determine if this block occurred in the EBV-negative BJAB cells and whether it may be involved in the deregulation of *MYC* expression observed in EBV-positive BJAB cells. Both pairs of EBV-positive and EBV-negative BJAB lines were examined in at least two separate experiments using different sets of exon-specific probes (Figure 3) plus the gamma actin probe and a plasmid control. Hybridization to the plasmid control was not detected in these experiments. Equal amounts of radiolabeled RNA from each cell line were used in the hybridizations.

Nuclear runoff analysis of the EBV-negative BJAB line and the EBV-positive BJAB-B958 line in early and late exponential phases of growth showed that the rate of transcription from exons I, II or III was essentially the same in the two lines in both early and late exponential phases of growth (Figure 4). Furthermore, there was a decrease

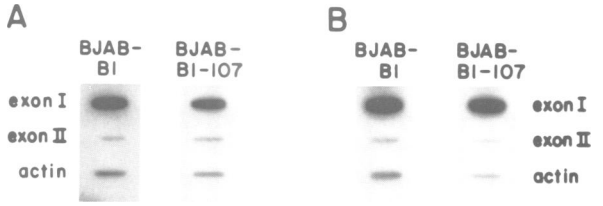

Fig. 5. Nuclear runoff assays of *MYC* transcripts in EBV-negative revertant BJAB-B1-107 and EBV-positive BJAB-B1 lines in early and late exponential phases of growth using exon-specific probes. Probes A and B (Figure 3) were utilized for exons I and II respectively. (**A**) Nuclei were harvested from cells in early exponential phase of growth (2×10^5 cells/ml). (**B**) Nuclei were harvested 2 days later from cells in late exponential phase of growth (8.0×10^5 cells/ml).

in transcription between exons I and II in the EBV-negative BJAB line that was unaffected by the presence of EBV in the EBV-positive line. When the amount of transcripts from each exon of *MYC* was normalized to the amount of transcripts from actin, it was found that the rate of transcription from each exon actually increased modestly (~2-fold) relative to actin in both lines as the cells progressed from early to late exponential phase of growth. Similar results were obtained when different probes were used for exons I and II (probes A and B, Figure 3; data not shown).

Nuclear runoff analysis of the second pair of EBV-positive and -negative BJAB lines (BJAB-B1 and its revertant, BJAB-B1-107) confirmed the findings observed in the BJAB-B958 and BJAB lines (Figure 5). The rate of transcription from exons I and II of *MYC* were similar in both BJAB-B1 and BJAB-B1-107 in early and late exponential phases of growth, and transcription decreased from exon I to exon II in both lines. Relative to actin transcripts, there was a modest increase in the rate of transcription in both lines with progression from early to late exponential phase of growth. These observations were corroborated using probes D, E and C (Figure 3) for exons I, II and III respectively (data not shown).

These nuclear runoff experiments in both pairs of EBV-negative and -positive BJAB lines suggested that altered rates of transcription or abrogation of the block to transcriptional elongation did not appear to be the mechanisms that led to the deregulation of *MYC* expression in the EBV-positive BJAB lines.

Stability of MYC mRNA

The nuclear runoff and S1 nuclease experiments suggested that deregulation of *MYC* expression in EBV-positive BJAB lines did not occur at the transcriptional level, but rather, resulted from post-transcriptional mechanisms. To determine if the presence of EBV in BJAB cells was associated with altered stability of *MYC* transcripts, the half-life of *MYC* mRNA was determined by utilizing actinomycin D as an inhibitor of transcription and measuring the decay of mature *MYC* transcripts by Northern blot analysis. Since differences in steady-state levels of *MYC* transcripts between EBV-positive and -negative BJAB lines are greatest in late exponential phase of growth, the half-life determinations of *MYC* transcripts were performed on cells in both early and late exponential phases of growth. These experiments confirmed our previously published results that steady-state levels of *MYC* transcripts decline in the EBV-negative BJAB

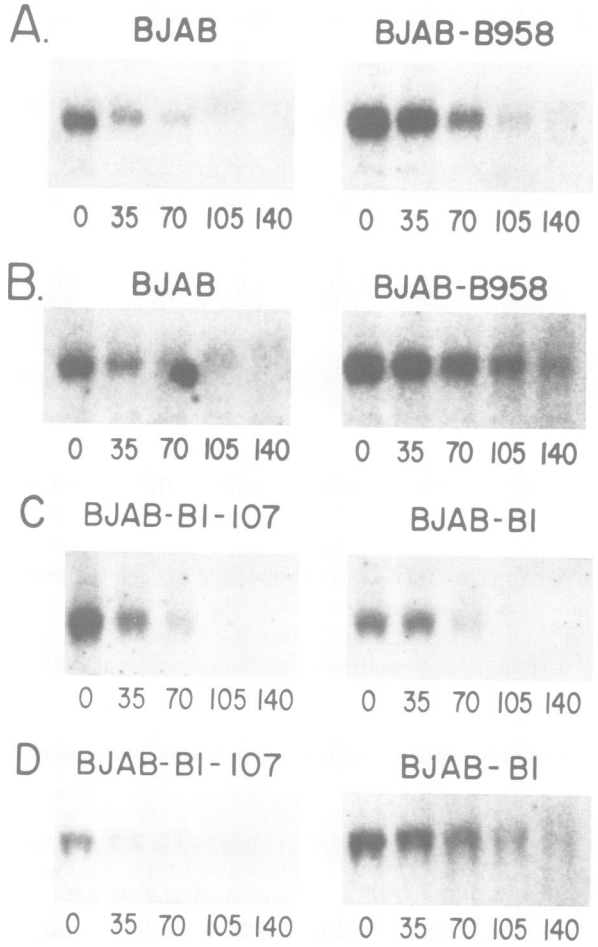

Fig. 6. RNA blot anlaysis of *MYC* transcripts from EBV-negative (BJAB and BJAB-B1-107) and EBV-positive (BJAB-B958 and BJAB-B1) lines in early and late exponential phases of growth during inhibition of transcription with actinomycin D. Cells were incubated in the presence of actinomycin D (10 μg/ml), and aliquots were removed at 35 min intervals for RNA preparation. (**A** and **C**) Cells were examined in early exponential phase of growth (2×10^5 cells/ml). 15 μg samples of total RNA were loaded per lane. (**B** and **D**) Cells were examined 2 days later in late exponential phase of growth (8×10^5 cells/ml). 45 μg samples of total RNA from BJAB and BJAB-B1-107 cells were loaded per lane; 15 μg samples of total RNA from BJAB-B958 and BJAB-B1 cells were loaded per lane.

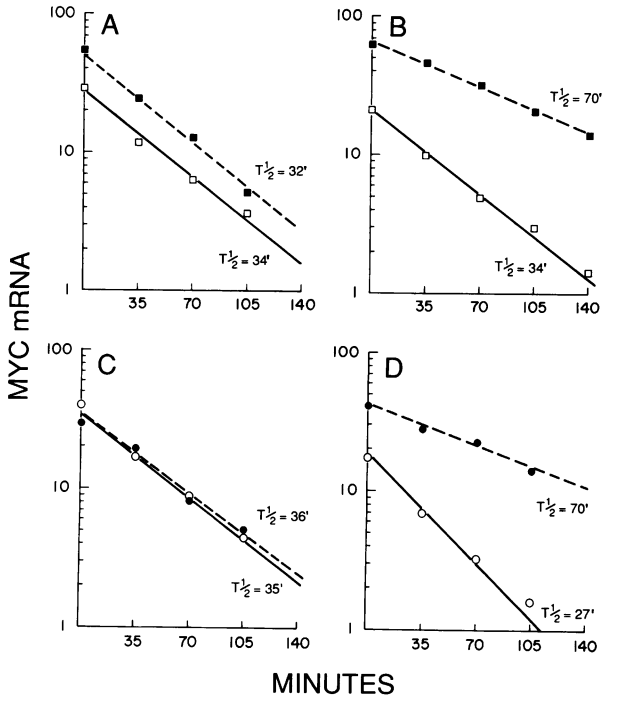

Fig. 7. Half-lives of *MYC* transcripts ($t_{1/2}$) from EBV-negative and -positive BJAB lines in early and late exponential phases of growth from best-fit semi-logarithmic lines of relative amounts of *MYC* mRNA from the Northern blots shown in Figure 6 plotted against time of exposure (min) to actinomycin D. (**A** and **C**) Half-lives of *MYC* mRNA in early exponential phase of growth in the four cell lines: BJAB (□), BJAB-B958 (■), BJAB-B1-107 (○) and BJAB-B1 (●). (**B** and **D**). Half-lives of *MYC* mRNA in late exponential phase of growth in the four cell lines: BJAB (□), BJAB-B958 (■), BJAB-B1-107 (○) and BJAB-B1 (●).

lines, but not in the EBV-positive lines, as the cells approach stationary phase of growth (Lacy *et al.*, 1987). The half-life of *MYC* transcripts in early exponential phase of growth in both the EBV-positive lines (BJAB-B958 and BJAB-B1) and the EBV-negative lines (BJAB and BJAB-B1-107) was 32−36 min (Figures 6A and C; 7A and C). In late exponential phase, however, the half-life of *MYC* transcripts in the two EBV-positive lines was prolonged to ~70 min (Figures 6B and D; 7B and D). In contrast, the half-life of *MYC* transcripts in the EBV-negative lines was not prolonged in late exponential phase and remained <35 min. The half-life of *MYC* in all four lines in early and late exponential phase of growth was confirmed in at least two separate experiments. To determine if the enhanced stability of *MYC* transcripts in EBV-converted BJAB cells in late exponential phase of growth reflected non-specific enhanced stability of labile mRNA, the blots were washed and reprobed with a histone probe. The half-life of the histone 2B transcript was not prolonged in the EBV-converted BJAB cells, measuring ~30 min in all four lines in both early and late exponential phase of growth. Thus, the presence of EBV in BJAB cells was associated with enhanced stability of *MYC* mRNA as the cells approached and entered stationary phase of growth, and this altered stability did not simply reflect non-specific stabilization of labile mRNA species. These observations suggest that deregulation of *MYC* expression in association with the presence of EBV in BJAB cells may result from altered stability of mature *MYC* transcripts, rather than altered rates of transcription.

To determine if the differences in stability of *MYC* transcripts in late exponential phase of growth in the EBV-positive and -negative lines were due to the presence of labile protein species, the effect of the protein synthesis inhibitor, cycloheximide, on levels of *MYC* transcripts in the EBV-positive BJAB-B958 line and the EBV-negative BJAB line was determined (Figure 8). These studies showed that there was no substantial increase in levels of *MYC* transcripts after 2 h of exposure to cycloheximide in either the EBV-positive BJAB-B958 line or the EBV-negative BJAB line.

Stability of MYC mRNA in EBV-immortalized lymphoblastoid lines

In order to determine if the altered stability of *MYC* transcripts with progression from early to late exponential phase occurs in EBV-immortalized cells derived from normal

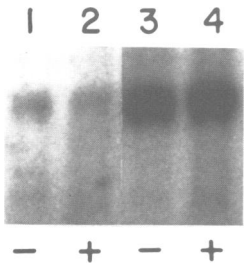

Fig. 8. RNA blot anlaysis of *MYC* transcripts from EBV-negative (BJAB) and EBV-positive (BJAB-B958) lines in late exponential phase of growth in the presence or absence of cycloheximide. **Lanes 1** and **2**, total RNA (15 μg) from BJAB cells before (−) and after (+) exposure to cycloheximide (20 μg/ml) for 2 h; **lanes 3** and **4**, total RNA (15 μg) from BJAB-B958 cells before (−) and after (+) exposure to cycloheximide (20 μg/ml) for 2 h.

human B cells, two EBV-positive lymphoblastoid lines derived from umbilical cord lymphocytes were examined. It had been determined in these lines, as in the EBV-positive BJAB lines, that steady-state levels of *MYC* transcripts remained stable and did not decline as the cells approached and entered stationary phase (Figures 9A and C). Similarly, the half-life of *MYC* transcripts increased significantly as the cells progressed from early exponential phase (~30 min) to late exponential phase (>60 min) in both lines (Figures 9B and 9D). Thus, the altered stability of *MYC* transcripts observed in EBV-positive BJAB cells was not restricted to the BJAB host cell but was also observed in EBV-immortalized lines derived from normal human B cells.

Discussion

We have previously shown that EBV conversion of the EBV-negative BL line, BJAB, is reversibly associated with deregulation of *MYC* expression (Lacy *et al.*, 1987). Specifically, the presence of EBV in BJAB cells is associated with abrogation of down-regulation of *MYC* transcripts as the cells approach and enter stationary phase. This alteration in *MYC* expression is reversibly linked to the presence of the virus, since loss of the EBV genome in an EBV-negative revertant BJAB line is associated with reversion of the pattern of *MYC* expression to that of the parental line. Similar findings were reported by Wennborg *et al.* (1987) utilizing the EBV-negative BJAB line and two EBV-converted sublines derived from it. These previous studies indicate that the presence of EBV in this B cell line leads to altered regulation of *MYC* expression.

In this study we have shown that the deregulation of *MYC* expression in EBV-converted BJAB lines results from post-transcriptional mechanisms rather than transcriptional mechanisms. There was no difference in the rate of transcription from exons I, II or III of the *MYC* gene in the EBV-negative or -positive BJAB lines at any point along the growth curve. A block to transcriptional elongation between exons I and II was observed in the EBV-negative BJAB parental and revertant lines, and this block was unaffected by the presence of EBV. Transcription occurred predominantly from the second promoter of *MYC*, and promoter utilization was unaffected by the presence or absence of EBV in either early or late exponential phase of growth. Although previous studies showed that the EBV-converted BJAB lines

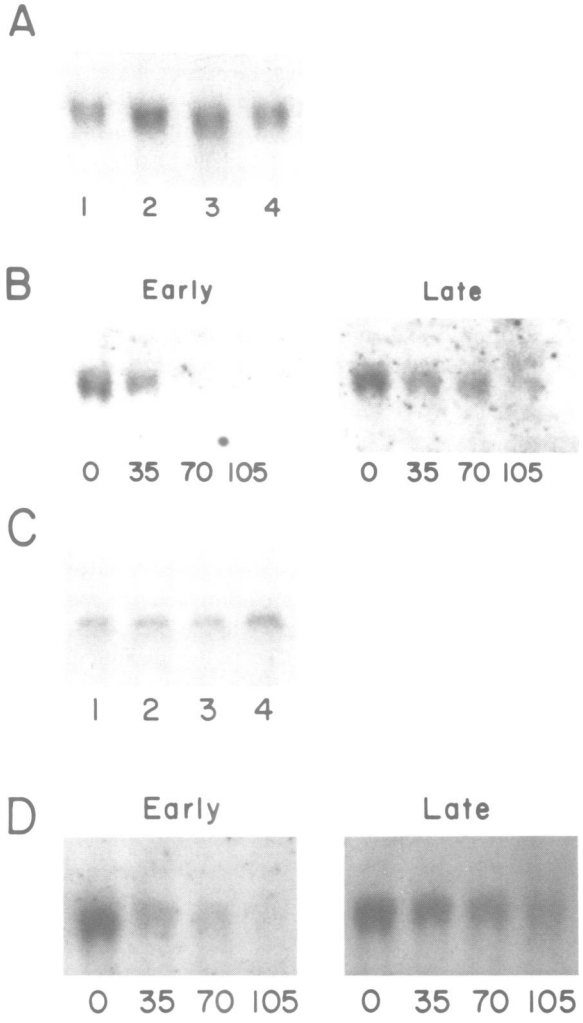

Fig. 9. RNA blot analysis of *MYC* transcripts in the EBV-immortalized lines, LB-11-23 (**A** and **B**) and LB-X50-7 (**C** and **D**). (**A** and **C**) Steady-state levels of *MYC* transcripts as cells progress from early exponential to stationary phase of growth. Aliquots (15 μg) of total RNA were loaded per lane. **Lane 1**, cells harvested on day 1 in early exponential phase (LB-11-23: 3×10^5 cells/ml; LB-X50-7: 3.5×10^5 cells/ml); **lane 2**, cells harvested on day 2 in mid-exponential phase (LB-11-23: 6×10^5 cells/ml; LB-X50-7: 7×10^5 cells/ml); **lane 3**, cells harvested on day 3 in late exponential phase of growth (LB-11-23: 1.2×10^6 cells/ml; LB-X50-7: 1.5×10^6 cells/ml); **lane 4**, cells harvested on day 4 in stationary phase of growth (LB-11-23: 1.5×10^6 cells/ml; LB-X50-7: 2.0×10^6 cells/ml). (**B** and **D**) *MYC* transcripts in early and late exponential phases of growth during inhibition of transcription with actinomycin D. Cells were incubated in the presence of actinomycin D (10 μg/ml), and aliquots were removed at 35 min intervals for RNA preparation. Aliquots (15 μg) of total RNA were loaded per lane.

have a 4- to 8-fold amplification of the *MYC* gene that persists in the EBV-negative revertant line (BJAB-B1-107), the increased copy number of the *MYC* gene in the EBV-positive lines and the EBV-negative revertant line did not lead to an increase in the overall rate of transcription compared with the parent BJAB line in which *MYC* is not amplified. This indicates that the amplified copies of *MYC* may not be active or that there are mechanisms that control the overall rate of transcription. Thus, the amplification of *MYC* did not appear to be involved in the deregulation of *MYC* expression in association with EBV.

The finding that the half-life of *MYC* transcripts was increased in EBV-positive BJAB cells in late exponential phase of growth indicates that the deregulation of *MYC* associated with EBV in this system may result in part from a post-transcriptional increase in stability of mature *MYC* mRNA in the presence of the virus. Interestingly, the half-life of *MYC* transcripts increased in the EBV-positive BJAB lines as the cells progressed from early to late exponential phase of growth, while steady-state levels of *MYC* transcripts remained stable, and the half-life of the *MYC* transcripts in the EBV-negative lines remained stable as steady-state levels of *MYC* transcripts decreased with progression from early to late exponential phase. This suggests that additional regulatory mechanisms may be involved in controlling levels of *MYC* transcripts in both the EBV-positive and -negative lines as the cells approach stationary phase of growth. Since the rates of transcription do not decrease as the cells progress from early to late exponential phase, additional post-transcriptional mechanisms may contribute to the down-regulation of *MYC* transcripts in both EBV-positive and EBV-negative BJAB cells that are abrogated by prolongation of the half-life of *MYC* transcripts in the presence of EBV. For example, alterations in nuclear–cytoplasmic transport or stability of nuclear mRNA can contribute to changes in steady-state levels of mature transcripts (Leys *et al.*, 1984; Fulton and Birnie, 1985).

The increased stability of *MYC* mRNA in EBV-positive BJAB cells in late exponential phase of growth was associated with both the transforming B958 and the non-transforming P3HR1 strains of EBV. The reversal of altered *MYC* stability in the EBV-negative revertant sub-clone of the P3HR1-converted BJAB line strongly supports a causal association between the presence of this strain of EBV and altered stability of *MYC* mRNA in BJAB cells. These results also indicate that the viral functions expressed by the EBNA2-deleted non-transforming P3HR1 strain are sufficient to induce altered *MYC* stability. P3HR1 has been shown previously to be capable of inducing expression of cellular genes that are induced by transformation-competent strains of EBV. For example, *in vitro* conversion of EBV-negative Burkitt lines by P3HR1 induces expression of the *FGR* oncogene (Cheah *et al.*, 1986) and some, but not all, of the lymphoblastoid cell-associated antigens that are induced by B958 (Calendar *et al.*, 1987).

Increased stability of *MYC* mRNA with progression from early to late exponential phase of growth was also observed in two EBV-immortalized lymphoblastoid lines derived from normal human B cells. Athough there are no appropriate EBV-negative normal B cell lines that can be used as comparative controls for these two lines, these findings raise the possibility that enhanced stability of *MYC* mRNA in the late exponential phase of growth may occur in normal B cells as a consequence of infection and immortalization by EBV.

In view of the putative role of the *MYC* protein in cell cycling and DNA synthesis, altered stability of MYC transcripts may be an important mechanism that contributes to the initiation and maintenance of cellular proliferation of B cells induced by infection with EBV. Furthermore, stabilization of *MYC* mRNA may contribute to the deregulation of *MYC* at the post-transcriptional level in EBV-positive Burkitt's lymphomas in which transcriptional mechanisms resulting from the chromosomal translocation are involved as well. The mechanism by which the presence of EBV leads to stabilization of *MYC* mRNA in the BJAB line is not known. It does not appear to result from differences in a labile protein species, since exposing the cells to an inhibitor of protein synthesis did not lead to a differential increase in levels of *MYC* transcripts in late exponential phase in the EBV-negative BJAB lines compared with the EBV-positive lines. It is likely that increased stability of *MYC* mRNA in the presence of EBV in this line results from indirect mechanisms rather than a direct effect of known viral products on *MYC* mRNA. By utilizing the EBV-negative BJAB lines, it should be possible to identify the specific viral genes that mediate the changes in *MYC* mRNA stability.

Table I. Origin and EBV status of cell lines

Cell line	Parental cell line	Superinfecting strain of EBV	EBNA	EBV DNA
BJAB	–	–	Absent	Absent
BJAB-B958	BJAB	B958	Present	Present
BJAB-B1	BJAB	P3HR1	Present	Present
BJAB-B1-107	BJAB-B1	–	Absent	Absent

Presence or absence of intracellular EBV nuclear antigen (EBNA) was determined by anti-complement immunofluorescence (Lacy *et al.*, 1987). Presence or absence of intracellular EBV DNA was determined by Southern blot analysis using the whole EBV genome as a probe (Lacy *et al.*, 1987).

Materials and methods

Cell lines and cell culture

BJAB cells were derived from a human BL that has previously been shown to be negative for the presence of EBV and a chromosomal rearrangement in the region of the *MYC* oncogene (Klein *et al.*, 1974; Menezes *et al.*, 1975; Lacy *et al.*, 1987). BJAB-B958 and BJAB-B1 are EBV-positive sub-lines of BJAB that were derived from BJAB cells by *in vitro* infection with the B958 and P3HR1 strains of EBV respectively (Fresen and zur Hausen, 1976). BJAB-B1 was cloned from P3HR1-infected BJAB cells (Fresen *et al.*, 1977). BJAB-B1-107 was subcloned from BJAB-B1 and is a revertant line in which all cells have lost the EBV genome (Leinbach and Summers, 1979; Lacy *et al.*, 1987). Previous studies have shown that the *MYC* oncogene is amplified 4- to 5-fold in BJAB-B958 and 8- to 10-fold in BJAB-B1 and BJAB-B1-107 relative to normal lymphocytes and the parental BJAB line (Lacy *et al.*, 1987). Cell lines were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum plus penicillin and streptomycin. The origin and characteristics of the BJAB lines are summarized in Table I. The LB-11-23 and LB-X50-7 lines are EBV-positive immortalized lymphoblastoid lines that were derived by infecting umbilical cord lymphocytes with the FF-41 and B958 strains of EBV respectively. LB-11-23 was derived in this laboratory. LB-X50-7 was generously provided by George Miller (Yale University). These lines have been maintained in continuous culture in RPMI-1640 plus 10% fetal calf serum for at least 6 months and are uniformly positive for EBV nuclear antigen (EBNA) by anti-complement immunofluorescence.

S1 nuclease analysis

S1 nuclease analyses of the 5′ end of the *MYC* transcripts were performed essentially as described previously by Chisolm and Summers (1986). Briefly, 5 or 20 μg of total RNA was suspended in 20 μl of hybridization buffer [80% formamide, 0.4 M NaCl, 0.04 M Pipes (pH 6.5) and 1 mM EDTA] with 5×10^5 c.p.m. of radiolabeled probe (probe A in Figure 3) and hybridized for 16 h at 60°C after denaturation at 90°C for 10 min. After the addition of 200 μl of S1 buffer [30 mM Na acetate (pH 4.5), 250 mM NaCl, 3 mM $ZnSO_4$ and 5% glycerol] plus 200 U S1 nuclease (Sigma) the reaction was incubated for 30 min at room temperature. The reaction

was stopped by adding 15 μl of 0.25 M EDTA, extracted with phenol/chloroform (1:1; v/v) and precipitated in ethanol. The pecipitated products of the S1 nuclease digestion were resolved by electrophoresis in a 5% acrylamide denaturing gel in the presence of urea. The dried gel was exposed to Kodak XAR film.

Nuclear runoff transcription assay

Runoff transcription assays were performed using a modification of a method described previously (Linial *et al.*, 1985). Cells were harvested in early (2×10^5 cells/ml) or late (8×10^5 cells/ml) exponential phase of growth. Subsequent steps were performed at 4°C. The cells were washed in phosphate-buffered saline and lysed by trituration in lysis buffer [0.5% NP-40, 10 mM Tris (pH 7.4), 10 mM NaCl and 3 mM $MgCl_2$]. Nuclei were washed three times in lysis buffer, resuspended in 40% glycerol, 50 mM Tris (pH 8.3), 5 mM $MgCl_2$ and 0.1 mM EDTA at 2×10^8 nuclei/ml, and stored at −70°C in 200 μl aliquots. Nuclei (200 μl) were mixed with 60 μl of reaction buffer [25 mM Tris (pH 8), 12.5 mM $MgCl_2$, 750 mM KCl and 1.25 mM each of ATP, CTP and GTP] plus 450 μCi of [α-^{32}P]UTP and incubated for 20 min at 30°C. After incubation nuclei were pelleted for 5 s in a microfuge, resuspended in 100 μl of 10 mM Tris (pH 8), 10 mM NaCl, 6 mM $MgCl_2$ with 200 U of DNase I (RNase-free), and incubated for 30 min at 37°C. After addition of 200 μl containing 1% SDS, 5 mM EDTA, 10 mM Tris (pH 7.4) and proteinase K (1 mg/ml), nuclei were vortexed thoroughly and incubated for 60 min at 37°C. The reaction was extracted twice with phenol/chloroform and the radiolabeled RNA was precipitated twice with isopropanol and ammonium acetate to remove unincorporated nucleotides. Radiolabeled RNA was suspended in 10 mM Tris (pH 8) and 1 mM EDTA and the percentage of [α-^{32}P]UTP incorporated into RNA was determined by precipitation of an aliquot in trichloroacetic acid. An equivalent amount of radiolabeled RNA (1.5×10^7 c.p.m.) from each cell line was hybridized to denatured DNA probes (>2 μg each), immobilized to nitrocellulose filter paper in 3 ml of hybridization buffer [10 mM TES (pH 7.4), 0.2% SDS, 10 mM EDTA, 0.3 M NaCl, 0.02% Ficoll, 0.02% bovine serum albumin, 0.02% polyvinylpyrollidone and 250 μg/ml tRNA] for 48 h at 65°C after preincubation in hybridization buffer for 6 h at 65°C. The filters were washed twice at room temperature in 2 × SSC/0.1% SDS for 15 min and then once in 0.2 × SSC/0.1% SDS at 60°C for 30 min (1 × SSC = 0.15 M NaCl/0.015 M Na citrate). The filters were dried and exposed to Kodak XAR film. Quantification of amounts of RNA hybridized to each probe was determined by densitometry of bands using a Joyce–Loebl automatic recording microdensitometer.

RNA isolation and RNA blot analysis

RNA blot analysis was performed as previously described (Lacy *et al.*, 1987). Briefly, RNA was purified from cells by pelleting cell lysates in guanidium isothiocyanate through a cesium chloride cushion (Glisin *et al.*, 1974). Total RNA (15 or 45 μg/lane) was loaded and separated by electrophoresis through a 6.7% formaldehyde/1% agarose gel. Gels were stained with ethidium bromide to corroborate that equivalent amounts of RNA had been applied in each lane and then transferred to nitrocellulose (Southern, 1975). The filters were hybridized as previously described (Thomas, 1980) for 16 h at 42°C in 50% formamide, 5 × SSC, 50 mM $NaHPO_4$, 1 × Denhardt's solution, 10% dextran sulfate, 100 μg/ml of denatured salmon sperm DNA, and 10^6 c.p.m./ml of denatured purified radiolabeled probe. After hybridization the filters were washed sequentially in 2 × SSC/0.1% SDS for 20 min at room temperature and 0.1 × SSC/0.1% SDS for 30 min at 50°C and exposed to Kodak XAR film for 3–4 days and 7–8 days. The relative amount of hybridization was determined by densitometry of each band using a Joyce–Loebl automatic recording microdensitometer.

Method for determination of mRNA half-lives

The half-life of the RNA ($t_{1/2}$) was defined as $t_{1/2} = 0.693/k$, where k = 2.303 × slope of the best-fit line through the datum points of the $\log_{10}$ of the concentration of the reactant (mRNA) plotted against time (Morris, 1974). The slope of the best-fit line as determined by linear regression analysis was used to calculate the k value in the above equation to determine the half-lives of the mRNAs. The concentration of the mRNA was determined from densitometry of each band and expressed in relative units. Densitometry was performed on at least two different exposures of each autoradiograph to avoid under or over exposure of specific bands. The transcript half-life was calculated using data from both exposures and was found to be independent of the time of exposure. In addition, the blots utilized for half-life determinations of *MYC* were reprobed for a transcript that was stable during the period of exposure to actinomycin D (HLA IB), and the half-life of *MYC* determined after normalization of *MYC* transcripts to HLA transcripts. The calculated half-lives of *MYC* were the same using the normalized concentrations or the absolute concentrations of *MYC* transcripts.

Preparation of probes

All probes were gel-purified from plasmid vectors after restriction endonuclease digestion. The plasmid containing the human *MYC* gene was generously provided by Philip Leder (Harvard University) (Battey *et al.*, 1983); exon III (probe C, Figure 3) was used for RNA blots and exon I (probe A, Figure 3) was used for S1 nuclease analysis. The plasmids containing the human HLA class I (B locus) gene and the human gamma-actin gene were generously provided by Sherman Weissman (Yale University) and Bernard Forget (Yale University) respectively. The histone 2B probe was obtained commercially from Oncor (Gaithersburg, MD, USA). For RNA blot analysis, purified inserts were radiolabeled to a sp. act. of 10^9 c.p.m./μg by random primer extension (Feinberg and Vogelstein, 1983). For S1 nuclease anlaysis, the first exon of *MYC* was radiolabeled at its termini by polynucleotide kinase and [γ-^{32}P]ATP.

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