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Translational Efficiency of cMyc mRNA in Burkitt Lymphoma Cells

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The translational efficiency of cMyc mRNAs was assessed in a variety of cell lines: HeLa cells and Epstein-Barr virus-transformed lymphocytes, both of which contain only the germ line cMyc allele; Daudi, a Burkitt cell line containing a translocated cMyc gene with no apparent alteration; and P3HR-1, a Burkitt line in which the 5′ end of the translocated cMyc gene has been altered by the chromosomal translocation. Translational efficiency was inferred by measuring the number of ribosomes associated with the cMyc mRNA, using a procedure by which individual polysomal fractions were analyzed by blot hybridization. Since polysome size is a function of the length of the translated sequence as well as the rate of initiation of protein synthesis, we also determined the number of ribosomes associated with a control mRNA (α tubulin) which codes for a protein of similar size to cMyc. We found that the cMyc mRNA was associated with a number of ribosomes comparable to that associated with α tubulin mRNA in all the cell lines tested.

Human Burkitt lymphoma cells show characteristic chromosomal translocations involving the cellular oncogene cMyc. The most common of these is a reciprocal exchange between chromosomes 8 and 14 in which the cMyc gene is translocated into the immunoglobulin heavy-chain locus (7, 9, 11, 19, 25). It has been inferred that this event leads to activation of the cMyc gene which may in turn contribute to neoplastic transformation (reviewed in reference 15). No unifying molecular mechanism of activation of cMyc has emerged from the study of several Burkitt cell lines. Available data suggests that activation may occur through several alternative pathways including: (i) enhanced transcription (10, 12, 13), (ii) mutation in the coding sequence for the cMyc protein (22), (iii) loss of normal regulation (4, 17, 26), and (iv) enhanced translation of cMyc mRNA (24).

The human cMyc gene contains three exons, only two of which code for the cMyc protein (4, 6, 8, 11, 28, 29). The first exon encodes an unusually long untranslated leader sequence (4, 28, 29). Transcription of the cMyc gene can initiate at either of two promoter sites, yielding mature mRNAs differing in length by 160 nucleotides (4, 14, 24, 28). The untranslated leader sequence contains four potential hairpin loops, only two of which can form in the shorter transcript (4). Additionally, nucleotide sequence analysis of the entire cMyc gene reveals that a region of exon 1 is highly complementary to a region of exon 2 (24). Saito et al. (24) have shown that the cMyc transcript (from either promoter) can form a very stable stem-loop structure incorporating these regions. In such a stem-loop structure the initiator AUG would be located within the loop and may be unavailable for efficient initiation of translation.

Both this stem-loop structure and the potential secondary structures present within the untranslated leader could be important in cMyc expression. In some Burkitt cells cMyc transcription initiates predominantly from the upstream promoter, whereas in normal cells initiation takes place primarily from the downstream promoter (4, 26). Such a shift in promoter utilization could result in a population of mRNAs with a different secondary structure near the 5′ end. If mRNAs containing this altered secondary structure were translated more efficiently, the net effect of the translocation would be an increase in cMyc protein.

In many Burkitt cell lines, the translocation breakpoint is within the first intervening sequence. Such translocations yield an altered cMyc allele lacking the first exon (2, 24, 26). Any mRNA synthesized from these cMyc genes could not form the stem-loop structure described above (24). Such mRNAs might then be translated more efficiently than wild-type mRNA.

In this report, we have attempted to assess the efficiency of translation of the cMyc mRNA in a variety of cell lines. Translational efficiency was inferred by measuring the number of ribosomes associated with the cMyc mRNA in HeLa cells and Epstein-Barr virus (EBV)-transformed lymphocytes, both of which contain only the wild-type cMyc allele (21, 24, 26), in Daudi, a Burkitt line containing a translocated cMyc gene with no apparent alteration (2, 10), and in P3HR-1, a Burkitt line in which the 5′ end of the translocated cMyc gene has been altered by the 8:14 chromosomal translocation (10).

HeLa S_3 cells were maintained at concentrations between 3×10^5 and 7×10^5 cells per ml in Joklik modified minimal essential medium supplemented with 10% horse serum. Daudi and P3HR-1 cells, obtained from the Cell Culture Laboratory, Children's Hospital of Detroit, Detroit, Mich., were maintained at concentrations between 3.5×10^5 and 1×10^6 cells per ml in RPMI 1640 medium supplemented with 20% fetal bovine serum. EBV-transformed human B lymphocytes, Kwk-B36, obtained from Katherine Klinger of Case Western Reserve Medical School, were maintained as described above for Daudi cells.

To prepare mRNA from various cell lines, we prepared cytoplasmic extracts exactly as described previously (20), except that before preparation of extracts, the cells were treated for 10 min at 37°C with 100 μg of emetine per ml to prevent ribosome runoff during fractionation. Polysomal RNA was prepared by sedimenting cytoplasmic extracts through a 4-ml cushion of 30% (wt/wt) sucrose in 10 mM NaCl–1.5 mM $MgCl_2$–50 μg of dextran sulfate per ml–10 mM Tris-hydrochloride (pH 7.5) (buffer A) for 2 h at 49,000 rpm in a Beckman 50 Ti rotor. Polysomal pellets were suspended in 1 mM EDTA–0.5% sodium dodecyl sulfate–10 mM Tris-hydrochloride (pH 7.5) (buffer B) and made 0.5 M in LiCl.

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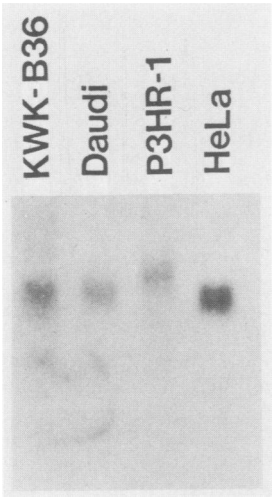

FIG. 1. Northern blot analysis of cMyc mRNAs. Polyadenylated RNA was prepared from the indicated cell lines as described in the text and analyzed (KwK-B36, 5 μg; P3HR-1, Daudi, and HeLa, 2 μg) by blot hybridization with a Myc-specific probe as described previously (18). Size determinations were made relative to 28 and 18S rRNAs electrophoresed in parallel lanes.

Polyadenylated RNA was prepared by oligodeoxythymidylate-cellulose chromatography as described previously (20).

The size of cMyc mRNAs synthesized in HeLa, P3HR-1, and Daudi cells and in the EBV-transformed lymphocyte cell line Kwk-B36 was determined by Northern blot analysis (Fig. 1). The Myc mRNA expressed in HeLa and Daudi cells and in the EBV-transformed lymphocytes had nearly equivalent mobilities, corresponding to a size of ca. 2.3 kilobases (kb). The Myc mRNA expressed in P3HR-1 cells was significantly larger, ca. 2.6 kb (Fig. 1). No. 2.3-kb Myc mRNA could be detected in these cells.

To address the question of translational efficiency of the cMyc mRNA, we adopted an approach that has been used by other investigators to assess translational efficiency of mRNAs during development (23), in cells exposed to heat shock (3), and in virus-infected cells (16). In this approach, the number of ribosomes associated with a specific mRNA is measured. The average size of a polysome translating a given mRNA is proportional to both the size of the translated mRNA sequence and the rate of initiation of protein synthesis and inversely proportional to the rate of chain elongation (16). If it is assumed that elongation rate is similar between different mRNAs, and the size of the translated sequence is known, polysome size can provide a measure of the rate of initiation of protein synthesis on a given mRNA.

To determine the average size of polysomes translating the cMyc mRNA in HeLa cells, cytoplasmic extracts were prepared as described above and fractionated on sucrose gradients (20). After fractionation, polyadenylated RNA was prepared from individual fractions and assayed by Northern blot hybridization with a nick-translated (18) vMyc plasmid (1) (Fig. 2). This analysis showed that the cMyc mRNA was translated on polysomes having an average size of 11 to 12 ribosomes per message. After exposure of this autoradiogram, the same blot was washed and then hybridized with a probe prepared by nick translation of plasmid PT1 containing cloned chicken α tubulin cDNA (5) (Fig. 2). α Tubulin was chosen because its mRNA is abundant in the cell lines analyzed and it codes for a protein (55,000 M_r) only slightly larger than cMyc (27). Thus, the size of its translated sequence is similar to that of the cMyc mRNA even though the total size of the tubulin mRNA (1,800 bases) (5) is 500 bases shorter than the cMyc mRNA. In HeLa cells the α tubulin mRNA was translated on polysomes having the same average size as those translating the cMyc mRNA (Fig. 2).

To rule out the possibility that sedimentation of these mRNAs through polysome gradients was the result of an unusually large ribonucleoprotein complex, we carried out the same analysis on cell extracts pretreated with EDTA (Fig. 2). Under these conditions, ribosomes completely dissociate into subunits, and both the cMyc and α tubulin mRNAs were found to sediment near the top of the gradient (Fig. 2).

We then proceeded to measure the number of ribosomes associated with the cMyc mRNA in Daudi and P3HR-1 cells and in the EBV-transformed lymphocyte cell line Kwk-B36. The polysome size for α tubulin was also measured in these cells (Fig. 3). Again, both the cMyc mRNA and the α tubulin mRNA were found on large polyribosomes of equivalent size. In all three cell lines we determined that the position of the mRNAs in polysome gradients was due to their association with ribosomes by performing EDTA dissociation experiments identical to those described above for HeLa cells.

We estimate a modal polysome size of 11 to 12 ribosomes per message, a value in good agreement with the determinations of other investigators who have measured polysome

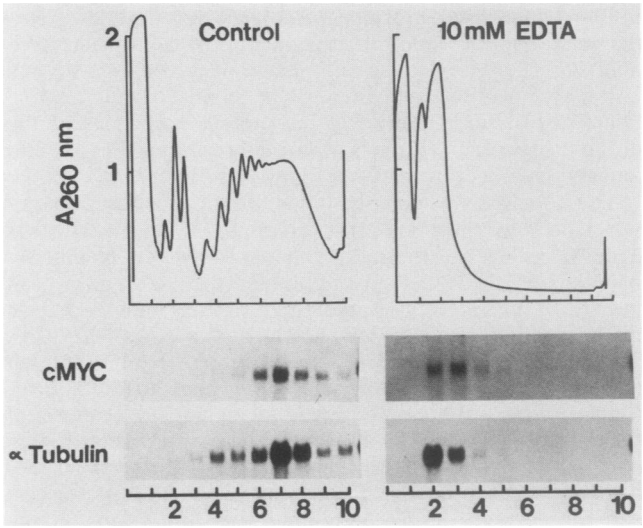

FIG. 2. Translation of cMyc mRNA in HeLa cells. Cytoplasmic extracts were prepared from HeLa cells as described in the text, and 20 units (absorbance at 260 nm) were sedimented through linear sucrose gradients as described previously (20). In the right-hand panel the extract was pretreated for 5 min with 10 mM EDTA before centrifugation. Sedimentation was from left to right. Gradients were fractionated through a flow cell with an Isco density gradient fractionator with continuous monitoring of absorbance at 260 nm. Individual fractions (1.2 ml) were diluted with 4 ml of buffer B, made 0.5 M in LiCl, and passed directly over oligodeoxythymidylate-cellulose columns. Polyadenylated RNA from each fraction was analyzed by blot hybridization as described previously (18). Blots were first probed with a nick-translated vMyc plasmid. After autoradiography the same blots were hybridized with a nick-translated α tubulin plasmid and reautoradiographed. Numbers at bottom are fraction numbers.

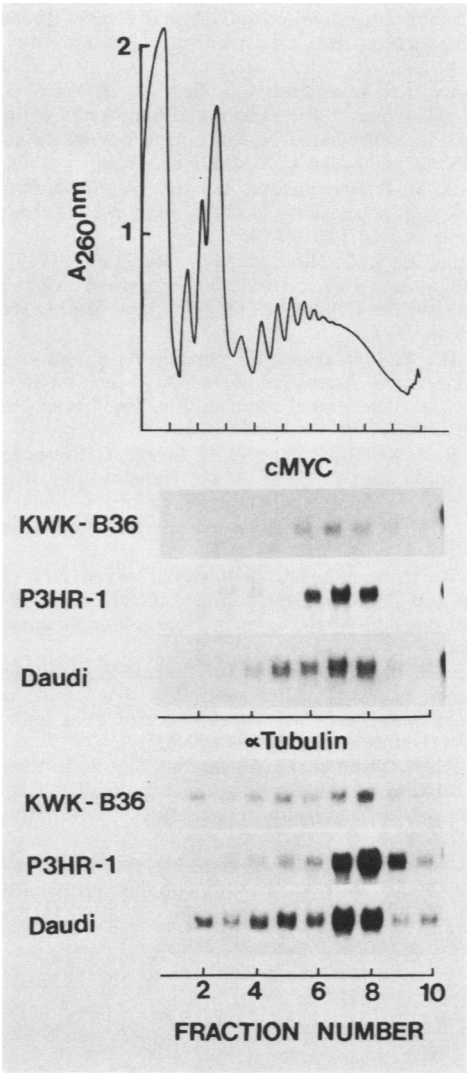

FIG. 3. Translation of cMyc mRNA in Burkitt lymphoma cells and EBV-transformed lymphocytes. Cytoplasmic extracts were prepared from the indicated cell lines as described in the text, and 25 units of extract (absorbance at 260 nm) were sedimented through linear sucrose gradients and fractionated as described in the legend to Fig. 2. Only the absorbance profile from the P3HR-1 gradients is shown. Polyadenylated RNA was isolated from individual fractions as described in the legend to Fig. 2 and analyzed by blot hybridization as described previously (18). Blots were probed first with a Myc-specific probe and autoradiographed and were then probed with an α tubulin probe and reautoradiographed.

size in actively growing cells for mRNAs coding for proteins of similar molecular weight to the cMyc protein (16).

For these experiments, HeLa cells and EBV-transformed lymphocytes served as controls, as they have only the germ line configuration of the cMyc gene (21, 24) and express cMyc mRNAs initiated primarily from the downstream promoter (4, 24, 26). Daudi cells contain an 8:14 chromosomal translocation in which the breakpoint of the translocation is several kb upstream from the 5′ end of the Myc gene and express a cMyc mRNA initated primarily from the upstream promoter (2). The 8:14 chromosomal translocation in P3HR-1 cells alters the 5′ end of the translocated cMyc gene (10), and in agreement with other investigators, we found that only the translocated cMyc allele is expressed in these cells (2, 21; Fig. 1). The size of the transcript (2.6 kb) is not compatible with initiation of transcription from either of the normal promoters, and we assume that initiation of transcription is occurring within the first intron of the altered cMyc gene (2, 24).

We conclude from these experiments that the cMyc mRNA transcribed from its normal promoters is translated efficiently. Furthermore, the number of ribosomes associated with the altered cMyc mRNA expressed in P3HR-1 cells (Fig. 3) is similar to that in the other cell lines. These results indicate either that the potential secondary structure noted by Saito et al. (24) does not form in vivo or that if it forms, this structure does not affect translation.

We did not attempt to quantitate the level of cMyc mRNA in the cell lines we have used nor did we measure the amount of Myc protein present in these cells. Previous reports indicate that cMyc transcription may be elevated in both P3HR-1 and Daudi cells with respect to EBV-transformed lymphocytes (10). Whether this elevation of transcription is relevant to neoplastic transformation remains to be determined.

The results presented here indicate that cMyc mRNAs of different lengths and potential secondary structures are translated with comparable efficiency. Thus, enhanced translation of cMyc mRNA probably does not contribute to the activation of the cMyc gene in these Burkitt lymphoma cells.

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LITERATURE CITED

1. **Alitalo, K., J. M. Bishop, D. H. Smith, E. Y. Chen, W. W. Colby, and A. D. Levinson.** 1983. Nucleotide sequence of the vMyc oncogene of avian retrovirus MC-29. Proc. Natl. Acad. Sci. U.S.A. **80**:100–104.
2. **ar-Rushdi, A., K. Nishakura, J. Erikson, R. Watt, G. Rovera, and C. Croce.** 1983. Differential expression of the translocated and the untranslocated cMyc oncogene in Burkitt lymphoma. Science **222**:390–393.
3. **Ballinger, D. G., and M. L. Pardue.** 1983. The control of protein synthesis during heat shock in Drosophila cells involves altered polypeptide elongation rates. Cell **33**:103–114.
4. **Battey, J., C. Moulding, R. Taub, W. Murphy, T. Stewart, H. Potter, G. Lenoir, and P. Leder.** 1983. The human c-myc oncogene: structural consequences of translocation into the IgH locus in Burkitt lymphoma. Cell **34**:779–787.
5. **Cleveland, D. W., M. A. Lopata, R. W. McDonald, N. J. Cowan, W. J. Rutter, and M. W. Kirschner.** 1980. Number and evolutionary conservation of α and β tubulin and cytoplasmic β and γ actin genes using specific cloned cDNA probes. Cell **20**:95–105.
6. **Colby, W. W., E. Y. Chen, D. H. Smith, and A. D. Levinson.** 1983. Identification and nucleotide sequence of a human locus homologous to the vMyc oncogene of avian myelocytomatosis virus MC29. Nature (London) **301**:722–725.
7. **Dalla-Favera, R., M. Baregni, J. Erikson, D. Patterson, R. C. Gallo, and C. M. Croce.** 1982. Human c-Myc onc gene is located on the region of chromosome 8 that is translocated in Burkitt lymphoma cells. Proc. Natl. Acad. Sci. U.S.A. **79**:7824–7827.
8. **Dalla-Favera, R., E. P. Gelmann, S. Martinotti, G. Franchini, T. S. Papas, R. C. Gallo, and F. Wong-Staal.** 1982. Cloning and characterization of different human sequences related to the onc gene (vMyc) of avian myelocytomatosis virus (MC29). Proc. Natl. Acad. Sci. U.S.A. **79**:6497–6501.
9. **Dalla-Favera, R., S. Martinotti, R. C. Gallo, J. Erikson, and C. M. Croce.** 1983. Translocation and rearrangements of the cMyc oncogene locus in human undifferentiated B-cell lym-

phomas. Science **219**:963–967.
10. **Erikson, J., A. ar-Rushdi, H. L. Drwinga, P. C. Nowell, and C. M. Croce.** 1983. Transcriptional activation of the translocated cMyc oncogene in Burkitt lymphoma. Proc. Natl. Acad. Sci. U.S.A. **80**:820–824.
11. **Hamlyn, P. H., and T. H. Rabbitts.** 1983. Translocation joins c-Myc and immunoglobulin genes in a Burkitt lymphoma revealing a third exon in the c-Myc oncogene. Nature (London) **304**:135–139.
12. **Hayday, A. C., S. D. Gillies, H. Saito, C. Wood, K. Siman, W. S. Hayward, and S. Tonegawa.** 1984. Activation of a translocated human c-Myc gene by an enhancer in the immunoglobulin heavy-chain locus. Nature (London) **307**:334–340.
13. **Hollis, G., K. Mitchell, J. Battey, H. Potter, R. Taub, G. Lenoir, and P. Leder.** 1984. A variant translocation places the immunoglobulin genes 3′ to the c-Myc oncogene in Burkitt's lymphoma. Nature (London) **307**:752–755.
14. **Kelly, K., B. H. Cochran, C. D. Stiles, and P. Leder.** 1983. Cell-specific regulation of the c-Myc gene by lymphocyte mitogens and platelet-derived growth factor. Cell **35**:603–610.
15. **Leder, P., J. Battey, G. Lenoir, C. Moulding, W. Murphy, H. Potter, T. Stewart, and R. Taub.** 1983. Translocations among antibody genes in human cancer. Science **222**:765–771.
16. **Lodish, H. F., and M. Porter.** 1980. Translational control of protein synthesis after infection by vesicular stomatitis virus. J. Virol. **36**:719–733.
17. **Maguire, R. T., T. S. Robins, S. S. Thorgeirsson, and C. A. Heilman.** 1983. Expression of cellular myc and mos genes in undifferentiated B cell lymphomas of Burkitt and non-Burkitt types. Proc. Natl. Acad. Sci. U.S.A. **80**:1947–1950.
18. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Press, Cold Spring Harbor, N.Y.
19. **Marcu, K. B., L. J. Harris, L. W. Stanton, J. Erikson, R. Watt, and C. M. Croce.** 1983. Transcriptionally active c-Myc oncogene is contained within NIARD, a DNA sequence associated with chromosome translocations in B-cell neoplasia. Proc. Natl. Acad. Sci. U.S.A. **80**:519–523.
20. **Nilsen, T. W., P. A. Maroney, and C. Baglioni.** 1982. Synthesis of (2′-5′)oligoadenylate and activation of an endoribonuclease in interferon-treated HeLa cells infected with reovirus. J. Virol. **42**:1039–1045.
21. **Nishikura, K., A. ar-Rushdi, J. Erikson, R. Watt, G. Rovera, and C. M. Croce.** 1983. Differential expression of the normal and of the translocated human c-Myc oncogenes in B cells. Proc. Natl. Acad. Sci. U.S.A. **80**:4822–4826.
22. **Rabbitts, T., P. Hamlyn, and R. Barr.** 1983. Altered nucleotide sequences of a translocated cMyc gene in Burkitt lymphoma. Nature (London) **306**:760–765.
23. **Rosenthal, E. T., T. Hunt, and J. V. Ruderman.** 1980. Selective translation of mRNA controls the pattern of protein synthesis during early development of the surf clam, *Spisula solidissima*. Cell **20**:487–494.
24. **Saito, H., A. C. Hayday, K. Wiman, W. S. Hayward, and S. Tonegawa.** 1983. Activation of the c-Myc gene by translocation: a model for translational control. Proc. Natl. Acad. Sci. U.S.A. **80**:7476–7480.
25. **Taub, R., I. Kirsch, C. Morton, G. Lenoir, D. Swan, S. Tronick, S. Aaronson, and P. Leder.** 1982. Translocation of the c-Myc gene into the immunoglobulin heavy chain locus in human Burkitt lymphoma and murine plasmacytoma cells. Proc. Natl. Acad. Sci. U.S.A. **79**:7837–7841.
26. **Taub, R., C. Moulding, J. Battey, W. Murphy, T. Vasicek, G. Lenoir, and P. Leder.** 1984. Activation and somatic mutation of the translocated c-Myc gene in Burkitt lymphoma cells. Cell **36**:339–348.
27. **Valenzuela, P., M. Quiroga, J. Zaldivar, W. J. Rutter, M. W. Kirschner, and D. W. Cleveland.** 1981. Nucleotide and corresponding amino acid sequences encoded by α and β tubulin mRNAs. Nature (London) **289**:650–655.
28. **Watt, R., K. Nishikura, J. Sorrentino, and A. ar-Rushdi.** 1983. The structure and nucleotide sequence of the 5′ end of the human c-Myc oncogene. Proc. Natl. Acad. Sci. U.S.A. **80**:6307–6311.
29. **Watt, R., L. W. Stanton, K. B. Mareu, R. C. Gallo, C. M. Croce, and G. Rovera.** 1983. Nucleotide sequence of cloned cDNA of human c-Myc oncogene. Nature (London) **303**: 725–728.