Proc. Natl. Acad. Sci. USA
Vol. 82, pp. 2315–2319, April 1985
Biochemistry

Differential efficiencies of *in vitro* translation of mouse c-*myc* transcripts differing in the 5′ untranslated region

(oncogene/translational control)

ANDRE DARVEAU, JERRY PELLETIER, AND NAHUM SONENBERG

Department of Biochemistry and McGill Cancer Center, McGill University, Montreal, PQ, Canada H3G 1Y6

Communicated by Aaron J. Shatkin, December 13, 1984

ABSTRACT **We have studied the *in vitro* translational efficiencies of two murine c-*myc* transcripts synthesized *in vitro* that differ in the lengths of their 5′ noncoding regions (448 and 83 nucleotides) and also in their 3′ noncoding regions. When translated in a reticulocyte translation system, the shorter transcript was translated 10-fold more efficiently. These results are consistent with the hypothesis of Saito *et al.* [Saito, H., Hayday, A. C., Wiman, K., Hayward, W. S. & Tonegawa, S. (1983) *Proc. Natl. Acad. Sci. USA* 80, 7476–7480] that translation of full-length human c-*myc* mRNA is normally repressed, whereas in several Burkitt lymphomas that have deletions of the mRNA 5′ noncoding region (resulting from translocation of the c-*myc* gene), translation of the c-*myc* mRNA is more efficient. Our results suggest that activation of murine c-*myc* expression by production of a more efficient mRNA might in some cases play a role in neoplastic transformation.**

The cellular *myc* gene has been implicated in a variety of hematopoietic malignancies (1). In most mouse plasmacytomas, the distal portion of chromosome 15 containing the c-*myc* gene is translocated into the immunoglobulin heavy chain locus on chromosome 12 (2–7). Similarly, in several human Burkitt lymphoma cell lines, a specific rearrangement results in the translocation of the c-*myc* gene from chromosome 8 into the immunoglobulin heavy chain locus on chromosome 14 (2, 3, 6–8). The murine and human c-*myc* genes contain three exons in which the protein coding region is confined to exons 2 and 3 (9–11). The first exon is thus noncoding and is conserved among different species (≈70% sequence homology between mouse and human; ref. 11), in contrast to the diverse 5′ noncoding nucleotide sequences of most eukaryotic mRNAs among different species (12). This degree of conservation might reflect an important regulatory function of the first exon. Comparative nucleotide sequencing of normal and rearranged c-*myc* genes in mouse plasmacytomas and some Burkitt lymphomas revealed that the translocation event results in breakage of the c-*myc* gene within the first exon or intron (7, 13, 14). Subsequently, initiation of transcription of the rearranged c-*myc* genes in mouse plasmacytomas has been shown to occur from multiple sites in the first intron, thus generating multiple forms of c-*myc* mRNAs in which the 5′ noncoding region consists of sequences derived from the first intron rather than from the first exon of the untranslocated gene (15).

Several hypotheses have been advanced to explain the correlation between the loss of the c-*myc* first exon as a result of translocation and the development of the transformed phenotype. It was suggested that exon 1 serves a negative regulatory function during transcription of the c-*myc* gene and that loss of the first exon as a result of chromosomal translocation leads to increased transcription of the gene or loss of its cell cycle regulated expression (16, 17). However, it is not clear that c-*myc* rearrangement always results in elevated levels of c-*myc* mRNA (5, 7). Alternatively, Saito *et al.* (13) have suggested a model in which the first exon exerts negative translational control of c-*myc* expression. Consequently, when the first exon is lost following translocation, the negative control element is also lost. The mechanism by which the proposed translational control might operate is not known. It is suggested, however, that loss of the first exon following translocation results in increased synthesis of c-*myc* protein, possibly contributing to the development of the transformed phenotype.

In this study, we examined the *in vitro* translational efficiencies of two different murine c-*myc* transcripts synthesized *in vitro*. One of the transcripts contained almost all of exon 1 [426 nucleotides (nt)] whereas the other contained only 46 nt from exon 1. The transcripts were assayed for translatability in a rabbit reticulocyte lysate. The results demonstrate that the transcript containing the long 5′ noncoding region is translated much less efficiently than the transcript with the shorter 5′ noncoding region.

MATERIALS AND METHODS

Molecular Cloning. The plasmic pMc-myc 54 was a generous gift from Elaine Remmers (Stony Brook, NY) and Giovanni Rovera (Wistar Institute, Philadelphia) and contains a 2.2-kilobase cDNA copy of the murine c-*myc* mRNA inserted into the *Pst* I site of pBR322 (see ref. 9). The plasmid pSP64, which is described in detail in ref. 18, was a kind gift from Doug Melton and Michael Green (Harvard, Cambridge). Two subclones of pMc-myc 54 in pSP64 were prepared (see Fig. 1) in which the c-*myc* cDNA was placed under control of the SP6 promoter. For the preparation of pMc-myc/H, pMc-myc 54 was digested with *Hin*dIII, purified from pBR322 sequences by isolating the c-*myc* fragment from an agarose gel by using the method of Vogelstein and Gillespie (19). This fragment was ligated to pSP64 that had been digested previously with *Hin*dIII and treated with calf intestinal alkaline phosphatase (20). For the preparation of pMc-myc/X, the *Xho* I fragment of pMc-myc 54 (see Fig. 1) was inserted into the *Sal* I site of pSP64 as described above. All recombinant and related procedures were carried out as described by Maniatis *et al.* (ref. 20 and references therein).

***In Vitro* Transcription.** *In vitro* transcriptions using *Eco*RI-linearized DNA templates were carried out essentially as described (18) except that 7-methylguanosine triphosphoguanosine (m^7GpppG) was present in the reaction mixture at a concentration of 500 μM and the GTP concentration was lowered to 20 μM. Transcriptions were carried out with 100 μCi of [α-^{32}P]ATP (>400 Ci/mmol; 1 Ci = 37 GBq) or 50 μCi of [5-^{3}H]ATP (21.8 Ci/mmol). Following incubation for 2 hr at 37°C, 2 μg of RNase-free DNase I and 1 μg of carrier calf-

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: nt, nucleotides; bp, base pairs; m^7GpppG, 7-methylguanosine triphosphoguanosine.

liver tRNA were added and incubation was continued for 10 min. The mixture was phenol extracted, passed over a G-50 spun-dialysis column, ethanol precipitated twice with 2 M NH_4OAc and once with 0.15 M KOAc, and finally washed with 70% ethanol. The specific activities of mRNAs obtained were 3×10^6 cpm/μg for [^{32}P]mRNA and 2×10^6 cpm/μg for [^{3}H]mRNA. mRNA size analysis was as described in the legend to Fig. 2.

***In Vitro* Translation.** *In vitro* translations were performed in 12.5 μl of micrococcal nuclease-treated rabbit reticulocyte lysates according to Pelham and Jackson (21) programed with [^{3}H]ATP-labeled capped transcripts. Incorporation of [^{35}S]methionine (>800 Ci/mmol) into acid-precipitable products was determined by spotting 1-μl aliquots on Whatman 3MM filter paper discs and precipitation in 10% trichloroacetic acid. Discs were boiled in 5% trichloroacetic acid for 20 min, washed in ethanol, ethanol/ether (1:1), and finally ether, dried, and assayed for radioactivity in toluene-based scintillation fluid. For gel analysis of translation products, aliquots from translation mixtures were mixed with $NaDodSO_4$/sample buffer (22), boiled for 2 min, and applied on 7.5–15% gradient $NaDodSO_4$/polyacrylamide gels (22). Gels were fixed in 40% methanol/7.5% acetic acid, treated with EN^{3}HANCE (New England Nuclear), and exposed against Fuji RX film at −70°C.

RESULTS

For the preparation of different c-*myc* constructs we used the plasmid pMc-myc 54, which contains a cDNA copy of mouse c-*myc* inserted into the *Pst* I site of pBR322 as described by Stanton and coworkers (9, 23). To prepare a construct that would yield an almost full-length mRNA and thus would resemble the normal c-*myc* mRNA, we cleaved pMc-myc 54 with *Hin*dIII and subcloned the *Hin*dIII fragment into pSP64 as shown in Fig. 1. This fragment contains 441 base pairs (bp) upstream from the ATG initiation codon, which encompasses most of the first exon, except for 140 bp at the 5′ end (23). A short stretch of 15 nt is contributed to the 5′ noncoding region by the second exon. At the 3′ end, this fragment includes 139 bp downstream from the translation termination codon, thus lacking 235 bp as compared to pMc-myc 54 (23). This construct is referred to as pMc-myc/H throughout this paper. *In vitro* transcription from this plasmid after linearization (see below) yields a mRNA containing an extra 7 nt at the 5′ end and an extra 45 nt at the 3′ end originating from the polylinker region of pSP64.

In contrast to pMc-myc/H, a second construct, termed pMc-myc/X, which was constructed by inserting the *Xho* I fragment of pMc-myc 54 into pSP64 (see Fig. 1), contains only a small portion of the mRNA 5′ noncoding region extending 61 bp upstream from the initiator ATG. At the 3′ end this plasmid contains 11 bp downstream from the translation termination codon originating from pMc-myc 54 and therefore is missing 363 bp from the 3′ end of the mRNA sequence as compared to pMc-myc 54 (23). This construct yields a truncated c-*myc* mRNA and, in this respect, resembles the mRNAs produced in some plasmacytomas (11, 15, 24). The mRNA transcribed from *Eco*RI-linearized pMc-myc/X (see below) contains an extra 22 nt at the 5′ end and an extra 30 nt at the 3′ end originating from the polylinker region of pSP64.

In order to synthesize capped transcripts from the two constructs, plasmids were linearized with the restriction enzyme *Eco*RI, which cleaves 30 bp downstream from the c-*myc* insertion site in pMc-myc/X and 45 bp downstream from the c-*myc* insertion site in pMc-myc/H, and were utilized as templates for transcription with SP6 polymerase. The cap analogue m^{7}GpppG was included in the reaction mixture at a concentration of 500 μM, while the concentration of GTP was reduced to 20 μM. We have reported (25) that under these conditions m^{7}GpppG serves as an efficient primer for transcription and consequently the majority of transcripts contained a m^{7}GpppG cap structure. We have analyzed the nature of the 5′ end of transcripts produced in this manner by RNase T2 digestion of [α-^{32}P]ATP-labeled transcripts followed by chromatography on polyethyleneimine plates with 2 M pyridinium formate (pH 3.5) and found that the majority of transcripts (>90%) from both pMc-myc/X and pMc-myc/H contained a 5′ terminal m^{7}GpppG group (data not shown). The expected size transcript from pMc-myc/X is 1455 nt, whereas that from pMc-myc/H is 1963 nt. Size analysis of the *in vitro* synthesized transcripts on denaturing agarose gels shows that the transcripts synthesized were apparently full length and only one species was produced (Fig. 2).

To analyze the relative translational efficiencies of the capped transcripts synthesized *in vitro*, we performed *in vitro* translations in rabbit reticulocyte lysates. Analysis of the translation products on $NaDodSO_4$/polyacrylamide gels from pMc-myc/H and pMc-myc/X mRNAs is shown in Fig.

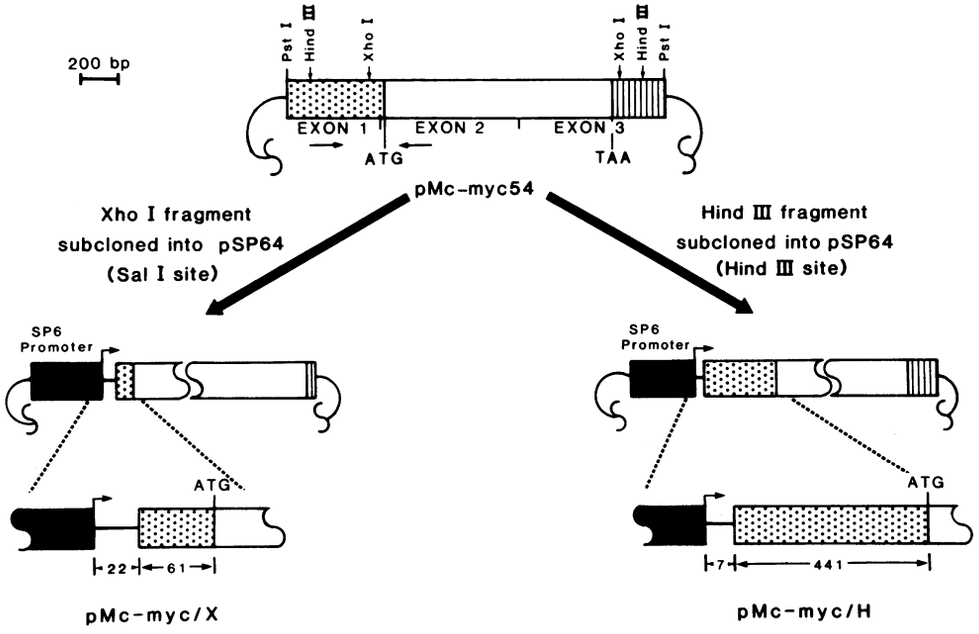

FIG. 1. Structure of pMc-myc 54 and subcloning strategy into pSP64. Subcloning of the *Hin*dIII and *Xho* I fragments generated from pMc-myc 54 was performed. The boundaries of three exons of pMc-myc 54 are denoted by short vertical lines. The 5′ untranslated region is stippled, the coding region is blank, and the 3′ untranslated region is hatched. The distances from the restriction sites to the coding region of the gene are indicated. The number of nucleotides in the 5′ noncoding region derived from pSP64 and from pMc-myc 54 is indicated. The two short horizontal arrows indicate the regions of complementarity between exons 1 and 2.

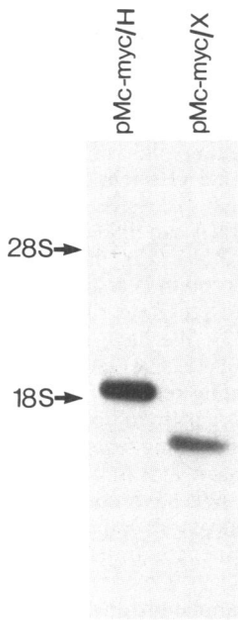

FIG. 2. Size analysis of pMc-myc/X and pMc-myc/H *in vitro* synthesized transcripts. Transcription from pMc-myc constructs was performed with [α-^{32}P]ATP. Samples containing 20,000 cpm of pMc-myc/X transcript and 40,000 cpm of pMc-myc/H transcript were denatured with 5 mM methyl mercuric hydroxide in buffer containing 5 mM sodium borate, 5 mM boric acid, and 10 mM sodium sulfate for 20 min at room temperature and subjected to electrophoresis on a 1.4% agarose gel containing 5 mM methyl mercuric hydroxide as described by Bailey and Davidson (26). Following electrophoresis overnight, the gel was dried and exposed against Fuji RX film for 3 hr at room temperature. HeLa cell rRNA was run in an adjacent lane and visualized by staining with ethidium bromide.

3. Lane 1 shows the translation products in the absence of added mRNA, and lane 2 shows the translation products from pMc-myc/H-derived mRNA. It is evident that translation of this mRNA is very inefficient. [The exposure time in this experiment was intentionally short to discern the different translation products from pMc-myc/X (see below) and therefore the products of pMc-myc/H are not visible at this exposure.] In contrast, the translation of pMc-myc/X-derived mRNA was much more efficient than that of pMc-myc/H-derived mRNA (≈10-fold as determined from the experiment in lanes 7 and 8) and yielded three closely migrating polypeptides with apparent molecular weights between 60,000 and 65,000 (lane 3). To examine the translation products synthesized from pMc-myc/H-derived mRNA we analyzed higher amounts of the translation products by gel electrophoresis and exposed the gel for a longer time period as compared to lane 2. Lane 8 shows that the polypeptides synthesized from pMc-myc/H-derived mRNA migrate with similar mobility to the products made from pMc-myc/X-derived mRNA (compare lane 8 to lane 7). We have confirmed that the polypeptides made in response to pMc-myc/X and pMc-myc/H are authentic c-*myc* polypeptides by immunoprecipitation with rabbit antibodies directed against human c-*myc* polypeptide made in *Escherichia coli* and provided by R. Watt. Lane 4 shows the immunoprecipitated material from the translation mixture directed by pMc-myc/X-derived mRNA and lane 5 represents a control experiment with nonimmune rabbit serum. Identical results were obtained with the translation products of pMc-myc/H-derived mRNA (data not shown). The efficiency of immunoprecipitation in this experiment was ≈25%. However, better yields of immunoprecipitated material were obtained when increased amounts of antibody were used.

The apparent molecular weights of the translation products are similar to those of polypeptides from avian cells that were immunoprecipitated with antibodies prepared against v-*myc* protein or synthetic peptides (27, 28). It is not clear why three polypeptides are made from one mRNA species in our *in vitro* system. Also, the apparent molecular weight of the polypeptides (60,000–65,000), according to their migration on NaDodSO$_4$/polyacrylamide gels is higher than expected from the coding capacity of the mouse *myc* gene that should direct synthesis of a polypeptide with a molecular weight of 48,812 (10). As suggested by Hann *et al.* (27) and Alitalo *et al.* (28) for avian c-*myc* proteins (which also migrate on gels as ≈60,000-dalton polypeptides), this discrepancy might be due to a peculiar conformation or charge property of the c-*myc* protein. It should be noted, however, that Giallongo *et al.* (29) precipitated a 48,000-dalton polypeptide from lysates of mouse cells and also from stimulated human lymphocytes with antiserum prepared against a synthetic peptide of c-*myc*, but the identity of the immunoprecipitated polypeptide has not been investigated. We have learned recently that c-*myc* protein synthesized in bacteria from cloned human cDNA migrates on gels as an ≈64,000-dalton polypeptide (37).

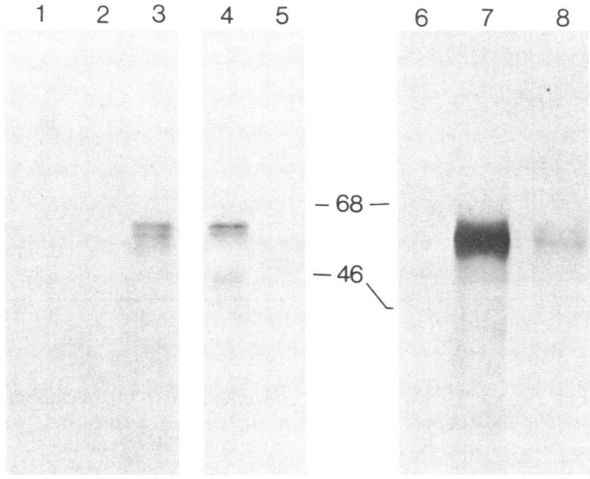

FIG. 3. *In vitro* translation products of pMc-myc/X and pMc-myc/H transcripts. Capped pMc-myc transcripts (0.1 μg of each) were translated for 60 min in 12.5 μl of micrococcal nuclease-treated rabbit reticulocyte lysate. Aliquots (3 μl for lanes 1–3 and 6 μl for lanes 6–8) were mixed with NaDodSO$_4$/sample buffer and analyzed on NaDodSO$_4$/polyacrylamide gels. For the immunoprecipitation experiment, capped mRNA derived from pMc-myc/X was translated in a 12.5-μl rabbit reticulocyte lysate translation system for 60 min followed by the addition of 100 μl of buffer A (phosphate-buffered saline, pH 7.2/1% Triton X-100/0.02% NaN_3/100 mM methionine/1 mM phenylmethylsulfonyl fluoride) and 4 μl of rabbit anti-human c-*myc* serum or normal rabbit serum. Incubations were for 1 hr at 4°C followed by the addition of 40 μl of protein A-Sepharose CL-4B (10% suspension in 0.9% NaCl, Pharmacia) and incubations continued for 1 hr. Immune complexes were collected by 1 min of centrifugation at 15,000 × *g* and washed three times in buffer A and twice in saline (0.9% NaCl). Following addition of electrophoresis sample buffer and boiling for 3 min, the suspension was centrifuged for 5 min at 15,000 × *g* and the supernatant was applied to a NaDodSO$_4$/polyacrylamide gel as above. Gels were treated for autoradiography and exposed against Fuji RX film (24 hr for lanes 1–5 and 3 days for lanes 6–8). Lane 1, no exogenous mRNA added; lane 2, pMc-myc/H; lane 3, pMc-myc/X; lane 4, immunoprecipitation of translation products of pMc-myc/X with rabbit anti-human c-*myc* serum; lane 5, immunoprecipitation of translation products of pMc-myc/X with nonimmune rabbit serum; lane 6, no exogenous mRNA added; lane 7, pMc-myc/X; lane 8, pMc-myc/H. Molecular weights are shown as $M_r \times 10^{-3}$.

To rigorously examine the different translational efficiencies of the two c-*myc* mRNAs, we carried out translations with increasing amounts of mRNAs derived from pMc-myc/X and pMc-myc/H. The results in Fig. 4*A* show that [^{35}S]methionine incorporation is directly proportional to the concentration of mRNA added for both mRNA species up to 8 μg/ml (higher concentrations were not tested), indicating that, in the range examined, mRNA concentration is limiting. In addition, it is seen that under all concentrations of mRNA used, the translation of pMc-myc/X is about 10-fold more efficient than pMc-myc/H mRNA. The translation products were resolved on a NaDodSO$_4$/polyacrylamide gel and the autoradiogram of the dried gel indicated that the expected products were made (data not shown). We have also examined the time course of translation of the two mRNAs.

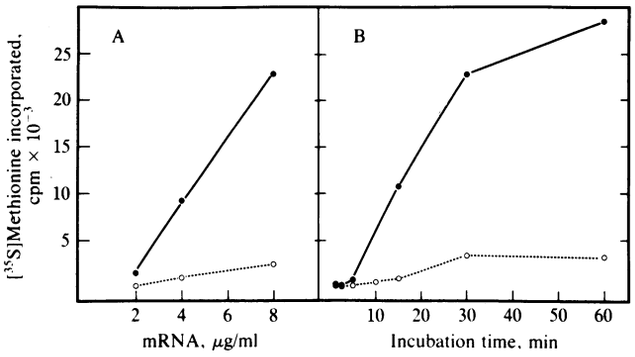

FIG. 4. Concentration curve and time course of translation of pMc-myc transcripts. mRNAs from the pMc-myc constructs were translated in micrococcal nuclease-treated rabbit reticulocyte lysates, and aliquots were withdrawn to determine incorporation of radioactivity into polypeptides or analyzed by $NaDodSO_4$/polyacrylamide gel electrophoresis. (*A*) mRNA concentration curve. pMc-myc/X (●) and pMc-myc/H (○) mRNAs at the indicated concentrations were translated for 60 min and 1-μl aliquots were analyzed for [^{35}S]methionine incorporation into acid-insoluble material. (*B*) Translation time course. pMc-myc/X (●) and pMc-myc/H (○) mRNAs were translated for the indicated times at which aliquots of 1 μl were withdrawn and [^{35}S]methionine incorporation was determined.

The results in Fig. 4*B* show that translation of mRNAs from both constructs is linear for ≈30 min and that translation of pMc-myc/X is 8- to 10-fold more efficient than that of pMc-myc/H. Translation of tobacco mosaic virus RNA and other mRNAs, under similar conditions, was linear for 90–120 min (data not shown), suggesting that the *myc* mRNAs are unstable under our translation conditions. This instability could be attributed to the lack of a 3′ poly(A) tail on both mRNAs (30). Consequently, we compared the stability of the two mRNAs, since faster degradation of the pMc-myc/H mRNA could account for its poor translatability. *A priori*, this possibility would seem unlikely because the pMc-myc/H mRNA contains a longer 3′ noncoding region as compared to pMc-myc/X mRNA (143 nt longer) and therefore its stability should be affected less by 3′ exonucleolytic cleavage. In any event, we analyzed the stability of both mRNAs in rabbit reticulocyte translation systems and found that both mRNAs break down with similar kinetics ($t_{1/2}$ = 20 min; data not shown). Thus, an enhanced degradation rate of pMc-myc/H mRNA is not the reason for the low translational efficiency of this mRNA.

DISCUSSION

We have presented evidence that the translational efficiency of *in vitro* synthesized mouse c-*myc* transcripts is modulated by the 5′ noncoding region of the mRNA. The mRNA containing a 5′ noncoding region of 448 nt, comprising most of the first exon, is translated inefficiently (efficiency lower by a factor of 8–10) as compared to the c-*myc* mRNA containing only a relatively short noncoding region of 83 nt. The two c-*myc* mRNAs differ also with respect to the 3′ noncoding region. The pMc-myc/H-derived mRNA contains 143 more nucleotides than pMc-myc/X construct at the 3′ noncoding region. However, it seems unlikely that these extra nucleotides would have a deleterious effect on translation. For example, experiments by Kronenberg *et al.* (31) in which 3′ noncoding sequences from rabbit β-globin mRNA were deleted indicated no effect in the *in vitro* translation of the mRNA. Furthermore, Soreq *et al.* (32) have reported that removal of the poly(A) tail and large segments of the 3′ noncoding region of human β_1 and β_2 interferon mRNAs did not affect their translational efficiency in *Xenopus* oocytes. However, modulation of translational efficiency mediated by 3′ noncoding sequences might be envisaged, perhaps through base pairing with upstream sequences and subsequent inhibition of translation. On the other hand, there is much evidence that the 5′ noncoding region might play a significant role in determining translational efficiency in eukaryotes (12). It is generally believed that excessive secondary structure at the 5′ region of the mRNA can impede ribosome binding and movement on the mRNA (12). In fact, it has been suggested that a cap binding protein is required to melt 5′ mRNA secondary structure to facilitate ribosome binding during translation initiation (33). We have also recently obtained direct evidence that excessive secondary structure introduced into the 5′ noncoding region of herpes simplex virus I thymidine kinase mRNA acts to inhibit translation. Increase of the length of the 5′ noncoding region without a corresponding increase in secondary structure had no effect on translational efficiency (25). We have concluded that in the latter case the degree of secondary structure at the 5′ noncoding region of the mRNA influences translational efficiency.

A similar interpretation can also be applied to the results presented in this study. Saito *et al.* (13) pointed out the existence in the human c-*myc* gene of a region of 75 bp in exon 1 highly complementary to a region in exon 2 that could theoretically form an extremely stable hairpin loop in the mRNA (ΔG° = −90 kcal/mol; 1 cal = 4.184 J). The 5′ untranslated portion of the c-*myc* mRNA is conserved between mouse and human (11) and analysis of potential mRNA secondary structure of the 5′ noncoding region of mouse c-*myc* mRNA indicates that the secondary structure is also conserved. Using the Zuker program "RNA-2" for predicting RNA secondary structure (34), we found that the major predicted hairpin loop formed between sequences encoded in mouse exon 1 (comprising nt ≈190–240 from the 5′ proximal transcription initiation site) and exon 2 (comprising nt ≈185–235 downstream from the AUG initiation codon) would have a ΔG° value of −70 kcal/mol. Consequently, the transcripts produced from pMc-myc/H have the potential to form the putative hairpin loop structure, whereas those from pMc-myc/X lack the necessary sequences for hairpin formation. Based on these considerations, it is possible that the important determinant for translational efficiency of c-*myc* mRNA is the secondary structure and not the mere length of the 5′ noncoding region. However, direct experiments have to be performed to examine this hypothesis.

In summary, we conclude that the 5′ noncoding region of the normal full-length mouse c-*myc* mRNA interferes somehow with efficient translation. Rearranged c-*myc* mRNAs, generated in some hematopoietic malignancies, in which 5′ noncoding sequences from exon 1 are lost and replaced by intron 1 sequences, might therefore be translated *in vivo* with increased efficiency, thus contributing to neoplastic transformation. It should, however, be emphasized that translational control cannot explain all malignant transformations in which *myc* gene appears to be implicated, since in many Burkitt lymphoma cell lines and some plasmacytomas apparently normal c-*myc* mRNAs are produced.

After submission of this manuscript for publication we learned of a similar study by Persson *et al.* (35), in which the investigators obtained different results from ours. They engineered SP6 constructs of mouse c-*myc* cDNA containing either a long or a short 5′ noncoding sequence. Transcripts prepared from both constructs were capped and subsequently found to be translated in a rabbit reticulocyte lysate with similar efficiencies. The reasons for the apparent differences in results are not immediately clear. We note, however, that Persson *et al.* have used vaccinia guanylyltransferase to cap the SP6-generated transcripts, whereas we used the cap ana-

logue m^7GpppG for cotranscriptional capping of SP6 transcripts. We routinely observed that >90% of the transcripts produced in the latter manner are capped, whereas the extent of capping of SP6 transcripts using guanylyltransferase varies between 20% and 60%. Thus, it is possible that mRNAs containing different primary or secondary structures will be capped to different degrees. In another recent report, Nilsen and Maroney (36) concluded that the translational efficiencies of c-*myc* mRNAs were similar in different human cell lines expressing normal vs. aberrant size mRNAs (the latter generated as a consequence of translocation). However, these conclusions were based on polysome size determinations and might not reflect true rates of translation initiation, because of the unusually long 5′ noncoding region of c-*myc* mRNA that might sequester a substantial number of ribosomes in the absence of polypeptide chain elongation.

We are indebted to Sheelin Howard and Christiane Babin for their skillful technical help. We are grateful to Drs. Giovanni Rovera and Elaine Remmers for providing us with pMc-myc 54 and Rosemary Watt for rabbit anti-human c-*myc* protein. We thank Clifford Stanners, Kevin Lee, and Walter Mushynski for their helpful comments on the manuscript and Dr. Timothy Nilsen for communicating to us his results before publication. This work was supported by a grant from the National Cancer Institute of Canada. N.S. is a recipient of a Terry Fox Cancer Research Scientist Award of the National Cancer Institute of Canada. A.D. was a recipient of a Fonds de Recherche en Santé du Québec Fellowship. J.P. is a recipient of a predoctoral studentship from the Medical Research Council of Canada.

1. Bishop, J. M. (1983) *Annu. Rev. Biochem.* **52,** 301–354.
2. Adams, J. M., Gerondakis, S., Webb, E., Mitchell, J., Bernard, O. & Cory, S. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 6966–6970.
3. Taub, R., Kirsch, I., Morton, C., Lenoir, G., Swan, D., Tronick, S., Aaronson, S. & Leder, P. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 7837–7841.
4. Crews, S., Barth, R., Hood, L., Prehn, J. & Calame, K. (1982) *Science* **218,** 1319–1321.
5. Sheng-Ong, G. L., Keath, E. J., Piccoli, S. P. & Cole, M. D. (1982) *Cell* **31,** 443–452.
6. Marcu, K. B., Harris, L. J., Stanton, L. W., Erikson, J., Watt, R. & Croce, C. M. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 519–524.
7. Adams, J. M., Gerondakis, S., Webb, E., Corcoran, L. M. & Cory, S. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 1982–1986.
8. Dalla-Favera, R., Bregni, M., Erikson, J., Patterson, D., Gallo, R. C. & Croce, C. M. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 7824–7827.
9. Stanton, L. W., Watt, R. & Marcu, K. B. (1983) *Nature (London)* **303,** 401–406.
10. Watt, R., Stanton, L. W., Marcu, K. B., Gallo, R. C., Croce, C. M. & Rovera, G. (1983) *Nature (London)* **303,** 725–728.
11. Bernard, O., Cory, S., Gerondakis, S., Webb, E. & Adams, J. M. (1983) *EMBO J.* **2,** 2375–2383.
12. Kozak, M. (1983) *Microbiol. Rev.* **47,** 1–45.
13. Saito, H., Hayday, A. C., Wiman, K., Hayward, W. S. & Tonegawa, S. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 7476–7480.
14. Perry, R. P. (1983) *Cell* **33,** 647–649.
15. Keath, E. J., Kelekar, A. & Cole, M. D. (1984) *Cell* **37,** 521–528.
16. Leder, P., Battey, J., Lenoir, G., Moulding, G., Murphy, W., Potter, H., Stewart, T. & Taub, R. (1983) *Science* **222,** 765–771.
17. Kelly, K., Cochran, B. H., Stiles, C. D. & Leder, P. (1984) *Cell* **35,** 603–610.
18. Melton, D. A., Krieg, P. A., Rebagliati, M. R., Maniatis, T., Zinn, K. & Green, M. R. (1984) *Nucleic Acids Res.* **12,** 7035–7056.
19. Vogelstein, B. & Gillespie, D. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 615–619.
20. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
21. Pelham, H. P. B. & Jackson, R. J. (1976) *Eur. J. Biochem.* **67,** 247–256.
22. Laemmli, U. K. (1970) *Nature (London)* **227,** 680–685.
23. Stanton, L. W., Fahrlander, P. D., Tesser, P. M. & Marcu, K. B. (1984) *Nature (London)* **310,** 423–425.
24. Stanton, L. W., Yang, J.-Q., Eckhardt, L. A., Harris, L. J., Birshtein, B. K. & Marcu, K. B. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 829–833.
25. Pelletier, J. & Sonenberg, N. (1985) *Cell* **40,** 515–526.
26. Bailey, J. M. & Davidson, N. (1976) *Anal. Biochem.* **70,** 75–85.
27. Hann, S. R., Abrams, H. D., Rohrschneider, L. R. & Eisenman, R. N. (1983) *Cell* **34,** 789–798.
28. Alitalo, K., Ramsay, G., Bishop, J. M., Ohlsson Pfeifer, S., Colby, W. W. & Levinson, A. D. (1983) *Nature (London)* **306,** 274–277.
29. Giallongo, A., Appella, E., Ricciardi, R., Rovera, G. & Croce, C. M. (1983) *Science* **222,** 430–432.
30. Soreq, H., Nudel, U., Salomon, R., Revel, M. & Littauer, U. Z. (1974) *J. Mol. Biol.* **88,** 233–245.
31. Kronenberg, H. M., Roberts, B. E. & Efstratiadis, A. (1979) *Nucleic Acids Res.* **6,** 153–166.
32. Soreq, H., Sagar, A. D. & Sehgal, P. B. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 1741–1745.
33. Sonenberg, N. (1981) *Nucleic Acids Res.* **9,** 1643–1656.
34. Zuker, M. & Stiegler, P. (1981) *Nucleic Acids Res.* **9,** 133–148.
35. Persson, H., Hennighausen, L., Taub, R., DeGrado, W. & Leder, P. (1984) *Science* **225,** 687–693.
36. Nilsen, T. W. & Maroney, P. A. (1984) *Mol. Cell. Biol.* **4,** 2235–2238.
37. Watt, R. A., Shatzman, A. R. & Rosenberg, M. (1985) *Mol. Cell. Biol.*, in press.