MOLECULAR AND CELLULAR BIOLOGY, Dec. 1987, p. 4513–4521
0270-7306/87/124513-09$02.00/0

Vol. 7, No. 12

Rapid Cytoplasmic Turnover of c-*myc* mRNA: Requirement of the 3′ Untranslated Sequences

THOMAS R. JONES AND MICHAEL D. COLE*

Department of Molecular Biology, Lewis Thomas Laboratory, Princeton University, Princeton, New Jersey 08544

Received 8 May 1987/Accepted 9 September 1987

Expression of the c-*myc* gene can be controlled by transcriptional or posttranscriptional mechanisms (or both), depending on the cell type and the growth conditions. An important mechanism of posttranscriptional regulation is modulation of cytoplasmic c-*myc* mRNA stability; normal human and murine c-*myc* mRNAs have cytoplasmic half-lives of 30 min or less. To elucidate the c-*myc* sequences which impart this unusually high rate of cytoplasmic transcript turnover, we have constructed various deletion and hybrid c-*myc* genes and analyzed the cytoplasmic stability of the mRNAs produced from them in stably transfected murine fibroblasts. The results indicate that sequences contained within the 5′ and 3′ ends of the c-*myc* transcript can affect cytoplasmic stability. Specifically, the 3′ untranslated sequences of c-*myc* exon 3 are required for, but do not ensure, a high rate of transcript turnover in the cytoplasm. Exon 2 coding sequences do not seem to be involved, and exon 1 sequences at the 5′ end of the transcript have only a small effect on cytoplasmic transcript stability. The sequences that are primarily responsible for the short c-*myc* RNA half-life were localized to a region of 140 bases in the 3′ untranslated region.

The c-*myc* proto-oncogene is frequently associated with DNA rearrangements and amplification that contribute to cell transformation in naturally occurring and induced cancers (reviewed in reference 8). Expression of the normal c-*myc* gene correlates with the growth state of the cell; that is, c-*myc* mRNA and protein levels are low but constant throughout the cell cycle of growing cells and are significantly reduced in nonproliferating, quiescent (G_0) cells (22, 48). Moreover, c-*myc* gene expression is activated by different mitogens in many cell types (27), and changes in c-*myc* protein levels vary directly with changes in c-*myc* mRNA levels (36). Similarly, growth arrest is associated with reduced c-*myc* mRNA expression, such as when Daudi lymphoblastoid cells are treated with beta interferon or when F9 embryonal carcinoma cells, Friend erythroleukemia cells, or HL-60 promyelocytic leukemia cells are induced to terminally differentiate (5, 24, 29, 40, 50). These changes in c-*myc* mRNA levels are due to both transcriptional and posttranscriptional processes, with the magnitude or contribution of each depending upon the particular system being examined. Changes in c-*myc* gene expression in differentiated Friend cells and HL-60 cells are mainly due to transcriptional regulation (21, 35), and the mechanism of transcriptional down-regulation in HL-60 cells has recently been shown to be due to a block in transcriptional elongation (2, 16). Regulation of c-*myc* in F9 cells, Daudi cells, and Chinese hamster lung fibroblasts is due to posttranscriptional mechanisms (4, 12, 13, 15, 28), whereas a combination of transcriptional and posttranscriptional mechanisms regulates the gene in murine fibroblasts and murine WEHI 231 lymphoma cells (14, 20, 31). In the murine fibroblast system, modulation of the transcriptional rate only partially accounts for the changes in cytoplasmic mRNA levels (14, 20). The posttranscriptional component can be at the level of heterologous nuclear RNA processing, transport from the nucleus, or cytoplasmic stability, and the latter mechanism has been implicated in the Daudi and WEHI 231 cell systems (12, 31). However, the sequences responsible for such modulation have not been identified.

The human c-*myc* gene has a three-exon structure and is highly homologous to the murine c-*myc* gene within the transcribed and 5′-flanking sequences (1, 3, 9). The noncoding first exon is about 550 base pairs in length and contains dual promoters (P1 and P2) separated by 150 base pairs, whereas the third exon contains about 300 base pairs of 3′ untranslated sequences. It has been hypothesized that these regions play an important role in the regulation of c-*myc* expression (30, 47), since the evolutionarily conserved 5′-flanking or first-exon sequences (or both) are often lost or mutated in the translocations observed in plasmacytoma or Burkitt's lymphoma cells (reviewed in reference 10).

In comparison to most eucaryotic mRNAs, c-*myc* mRNA has an unusually high cytoplasmic turnover rate. The half-life of normal c-*myc* mRNA is about 30 min or less in various human and murine cell types (11, 39), whereas the half-life of truncated c-*myc* RNAs (lacking exon 1) in murine plasmacytoma or human Burkitt's lymphoma cells can be up to 10-fold longer (17, 37, 39). Interpretation of the results from these studies has been complicated by the lack of a common cellular context from which the various truncated c-*myc* mRNAs are produced and by the contribution that non-*myc* sequences might have on the stability of aberrant transcripts (17). To address these issues, we have systematically assessed the contribution of individual regions of the human c-*myc* gene upon the stability of cytoplasmic mRNA in a stably transfected murine fibroblast system. Through the use of various deletion and hybrid gene constructs, we have identified the primary regions of the c-*myc* gene that affect cytoplasmic mRNA stability.

MATERIALS AND METHODS

Cell culture and transfection. NIH 3T3 cells were passaged in Dulbecco modified Eagle medium containing 10% fetal calf serum. Transfections were performed by the calcium phosphate method as described previously (51). Plasmid DNA constructs were transfected with selectable marker

* Corresponding author.

plasmid DNA (either pLTR*neo* [see below] or pSV2his [pSV2his was kindly provided by Ihor Lemishka]) at a 10:1 ratio. Stably transfected cells were selected with either 400 μg of G418 (GIBCO Laboratories) per ml or 4.25 mM L-histidinol (Sigma Chemical Co.) beginning 48 h after the addition of the DNA-calcium phosphate precipitate to the cells. Colonies (100 to 500) were pooled and expanded into cell lines for use in experiments. Cells were 60 to 70% confluent in normal growth media for subconfluent cell experiments. For quiescent cell experiments, density-arrested NIH 3T3 cells were maintained in Dulbecco modified Eagle medium containing 0.5% fetal calf serum for 48 h. RNA half-life experiments were initiated by adding actinomycin D (United States Biochemical Corp.; final concentration, 5 μg/ml) to the growth media.

RNase protection assay. Quantitative RNase protection assays were performed as described previously (53). High-specific-activity RNA probes were synthesized by using T7 RNA polymerase (Bethesda Research Laboratories, Inc.) and [α-^{32}P]UTP (ICN Pharmaceuticals) by using the protocol of the supplier. Hybridizations typically contained 5×10^5 cpm of RNA probe complementary to a portion of the exogenous (transfected) gene transcript and 1×10^5 cpm of RNA probe complementary to a portion of the murine β-2-microglobulin (B2M) transcript mixed with 20 μg of total cytoplasmic RNA. RNases A and T_1 (Sigma Chemical Co.) were used at concentrations of 100 and 2 μg/ml, respectively. Protected fragments were electrophoresed in 6% polyacrylamide–8 M urea gels and visualized after exposure to Kodak XAR-5 film with Dupont Cronex intensifying screens. Half-life determinations were made after quantitative densitometric scanning of the autoradiograms.

Plasmid DNA cloning, DNA isolation, and RNA isolation. Plasmid DNA constructs were made and plasmid DNA was isolated by standard techniques (34). Total cytoplasmic RNA from tissue culture cells was isolated as described by Greenberg and Ziff (20).

RNA probes. The DNA fragments used as templates for synthesizing RNA probes were cloned into the polylinker region of pT7-1 or pT7-2 (United States Biochemical Corp.). The cloned DNA fragments were as follows: the 614-base *Sma*I-*Pvu*II fragment spanning the human c-*myc* first exon and its upstream sequences (see genomic map in Fig. 1; pT7Hmex1); the 419-base *Pst*I-*Pst*I fragment contained within the human c-*myc* second exon (Fig. 1; pT7Hmex2-419); the 320-base *Hin*dIII-*Bgl*II fragment spanning the 5′ end of the *neo* transcript (pT7*neo*-320); and an approximately 300-base *Pst*I-*Pst*I fragment spanning the first intron and second exon of the murine B2M gene (pT7B2M; the B2M clone from which pT7B2M was constructed was kindly provided by Gilbert Jay). In addition to sequences within the cloned fragments, sequences from the polylinker region (about 50 bases) of the plasmid are transcribed during probe synthesis, making it possible to differentiate between undigested probe RNA and protected RNA. In an RNase protection assay the following fragments were protected: a probe made from pT7Hmex1 protected fragments of 512 and 342 bases (from P1- and P2-initiated transcripts, respectively) of human c-*myc* RNA (Fig. 1); a probe made from pT7Hmex2-419 protected a 419-base fragment from human c-*myc* transcripts and an approximately 155-base fragment from murine (endogenous) c-*myc* transcripts (due to the high degree of nucleic acid homology between the coding regions of the human and murine c-*myc* genes [3]); a probe made from pT7*neo*-320 protected a 320-base fragment from *neo*-containing transcripts; and a probe made from pT7B2M protected a series of fragments (180 to 220 bases) from murine B2M transcripts.

Plasmid DNA constructs. The approximately 8-kilobase *Hin*dIII-*Eco*RI fragment of the normal human c-*myc* gene was cloned into pBR322 (26). A derivative, pLTRHmyc, was described previously (26) and contains a full-length Moloney leukemia virus (MLV) long terminal repeat (LTR) driving transcription through the human c-*myc* gene second and third exons (construct B of Fig. 1). Another derivative,

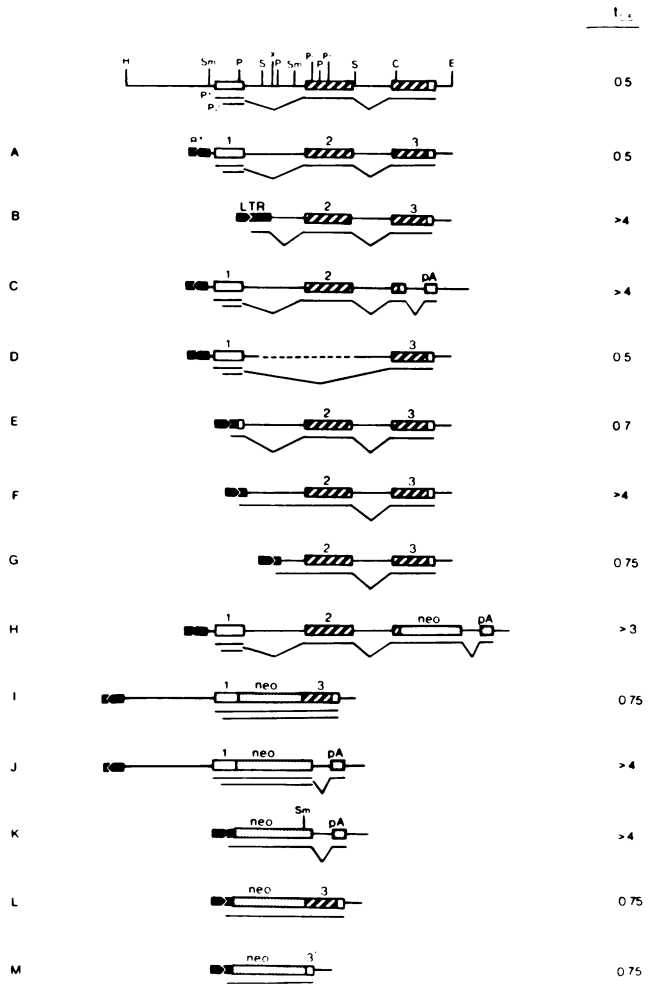

FIG. 1. Summary of the cytoplasmic stability of transcripts from deleted and hybrid c-*myc* genes in proliferating murine fibroblasts. Schematic diagrams and transcription patterns of the endogenous human c-*myc* gene and c-*myc* gene-related constructs (A through M) used in this study are shown. The first diagram shows the restriction pattern of the 8.3-kilobase *Hin*dIII-*Eco*RI fragment containing the human c-*myc* gene. The cytoplasmic half-lives of the respective transcripts are given in hours. Symbols: ——, introns or 5′-3′ flanking sequences; ▭, untranslated c-*myc* exon sequences; ▨, c-*myc* protein-coding sequences; ▩, *neo* gene-coding sequences and the simian virus 40 polyadenylation site sequences, as labeled (*neo* and pA, respectively); ■, full-length MLV LTR (construct B only) or the truncated MLV LTR (all others); the arrow within each indicates the transcriptional orientation of the LTR; ⋁, the exon-intron transcription pattern. Human c-*myc* gene exons are numbered 1 through 3. 3′ indicates the 3′ untranslated sequences of the murine c-*myc* third exon in construct M. Restriction endonuclease site abbreviations: C, *Cla*I; E, *Eco*RI; H, *Hin*dIII; P, *Pvu*II; Ps, *Pst*I; S, *Sac*I; Sm, *Sma*I; X, *Xba*I.

pLTRHm123 (construct A of Fig. 1), was constructed by changing the *Sma*I site at −100 bases (relative to exon 1) to a *Hin*dIII site and then deleting the more upstream c-*myc* sequences. The truncated version of the MLV LTR (see below) was inserted at this new *Hin*dIII site in the opposite transcriptional orientation, so that the transcription unit would be enhanced by the MLV LTR but transcription would still initiate at the normal c-*myc* promoters, P1 and P2 (Fig. 1). The truncated MLV LTR was made from pEVX by changing the *Sma*I site (at +28 bases relative to the transcription initiation site) to a *Hin*dIII site and cloning the resulting 800-base *Hin*dIII fragment (containing the LTR enhancer, promoter, and sequences up to +28 bases) into the *Hin*dIII site of pBR322. pLTR*neo* (construct K of Fig. 1) was made by inserting the truncated MLV LTR into the *Hin*dIII site of A10*neo*. pLTRHm12*neo* (construct H) was constructed from pLTRHm123 by inserting the fragment containing the *Hin*dIII-*Eco*RI *neo* gene from pLTR*neo* at the *Cla*I site near the beginning of the human c-*myc* third exon and thereby replacing the c-*myc* *Cla*I-*Eco*RI fragment containing the third exon-polyadenylation site. A similar construct, pLTRHm12pA (construct C of Fig. 1), was made by replacing the same c-*myc* *Cla*I-*Eco*RI fragment with a DNA fragment containing the simian virus 40 splice and polyadenylation signal. pLTRHm13 (construct D of Fig. 1) was constructed by deleting the second exon-containing *Sac*I fragment from pLTRHm123. Constructs E, F, and G of Fig. 1 (pLTRHm23-5′del1, pLTRHm23-5′del2, and pLTRHm23-5′del3, respectively) were made by progressive *Bal*31 deletion from the *Hin*dIII site just 5′ of the human c-*myc* first exon. A *Hin*dIII site was placed at these new 5′ ends and the *Hin*dIII-*Eco*RI 5′ deleted c-*myc* fragments were placed between the *Hin*dIII-*Eco*RI sites, just downstream of the truncated MLV LTR, in pAFVX (pAFVX was kindly provided by Michael Kriegler). pHmex1-*neo* (construct J) contains the truncated MLV LTR in the opposite transcriptional orientation, about 2.5 kilobases of human c-*myc* 5′-flanking sequences, and most of the human c-*myc* first exon (to the *Pvu*II site in exon 1; Fig. 1) ligated to the full-length *neo* gene. pHmex1-*neo*-ex3 (construct I) derived from pHmex1-*neo* by replacing the *Sma*I-*Eco*RI fragment containing the 3′ *neo* polyadenylation site with the fragment containing the *Cla*I-*Eco*RI human c-*myc* third exon polyadenylation site. pLTR*neo*-Hmex3 (construct L) was constructed from pLTR*neo* in a similar fashion. pLTR*neo*-Mm3′ex3 (construct M) is similar to pLTR*neo*-Hmex3, except that it contains the murine c-*myc* *Xho*I-*Eco*RI fragment (3) with the third-exon 3′ untranslated sequences and polyadenylation site.

Internal deletions in the c-*myc* third exon were made unidirectionally from the *Cla*I site by using *Bal* 31 nuclease. The boundaries of the deletions and the open reading frames were determined by DNA sequencing. With the numbering scheme of Battey et al. (1), in which the *Cla*I site is nucleotide 5008, the deletions extend to nucleotides 5495 (A), 5704 (B), 5846 (C), and 5968 (D). In the same numbering scheme, the recognition sequences (AATAAA) for polyadenylation begin at positions 5839 and 5980. The deletions were cloned into a plasmid containing a retroviral LTR that was 5′ of exon 1 and in an orientation opposite to that of c-*myc*, as in construct A in Fig. 1.

RESULTS

c-*myc* first exon is not sufficient to impart cytoplasmic transcript instability. Our initial experiments were designed to determine the sequences required for the transcriptional and posttranscriptional modulations of c-*myc* gene expression observed in the murine fibroblast system (14, 20, 27). We transfected gene constructs containing approximately 2.5 kilobases of c-*myc* 5′-flanking sequences, either with or without almost the entire c-*myc* first exon ligated to the *neo* gene into BALB/3T3-A31 or NIH 3T3 cells. Transcripts arising from stably integrated, transfected genes were analyzed for appropriate regulation in proliferating subconfluent or density-arrested quiescent cells and in quiescent cells stimulated for 2 h with fresh serum. In time course studies, we had previously determined that 2 h of serum stimulation gave the maximal accumulation of cytoplasmic c-*myc* mRNA. The results indicated that mRNAs arising from the exogenous gene constructs were not regulated in a manner similar to those from the endogenous c-*myc* gene (data not shown). Thus, in this stable assay, the 5′-flanking sequences and first exon are not sufficient to confer regulation by serum on a reporter gene.

Previous studies have demonstrated the rapid cytoplasmic turnover of normal c-*myc* mRNA (11) and suggested that the c-*myc* first exon may be required for cytoplasmic transcript lability (37, 39). Since such turnover might be central to the modulation of c-*myc* gene expression, we examined whether mRNAs from similar *myc* promoter-linked gene constructions were labile in the cytoplasm. In subconfluent proliferating cells (Fig. 2), transcripts arising from the hybrid constructs were very stable ($t_{1/2} > 4$ h), unlike the endogenous c-*myc* transcripts ($t_{1/2}$ = 30 min). The presence or absence of the c-*myc* first exon did not alter the stability of these transcripts. We also tested whether the destabilizing influence of the first exon would be apparent in density-arrested, quiescent cells; however, this was not observed. It was also possible that the murine enzymes responsible for c-*myc* mRNA turnover might not recognize human sequences, but we found that transcripts arising from similar constructs containing the murine flanking and first-exon c-*myc* sequences were also very stable (data not shown). Therefore, the lack of proper turnover of the exogenous transcripts would, in part, explain the apparent unregulated expression of the transfected genes in this system.

Turnover of normal and deleted human c-*myc* transcripts. Since the rapid turnover of c-*myc* mRNA in the cytoplasm seemed to be an important component of gene regulation, we decided to define sequences that are necessary for such turnover by analyzing the stability of transcripts arising from normal and deleted human c-*myc* gene constructs in proliferating murine fibroblasts. A normal human three-exon c-*myc* gene (which is transcriptionally enhanced by a viral LTR but does not contain any viral sequences in the resulting transcript) encodes an mRNA which is very unstable in the cytoplasm ($t_{1/2}$ = 30 min; Fig. 3), similar to the normal endogenous c-*myc* genes of murine and human cells. Using probes which map the transcription initiation sites and splicing patterns, we determined that this normal human gene construct expressed in murine fibroblasts encodes transcripts which initiate at the P2 promoter preferentially over P1 (typical for endogenous c-*myc* genes) and which are properly spliced (data not shown).

To assess the contribution of the first exon to cytoplasmic stability, we analyzed transcripts arising from a human c-*myc* construct in which the first exon was replaced by the MLV LTR. Transcripts from this construct contain 206 bases of viral sequences at their 5′ end and utilize the LTR splice donor site to splice to the normal acceptor site at the beginning of the c-*myc* second exon (data not shown). These

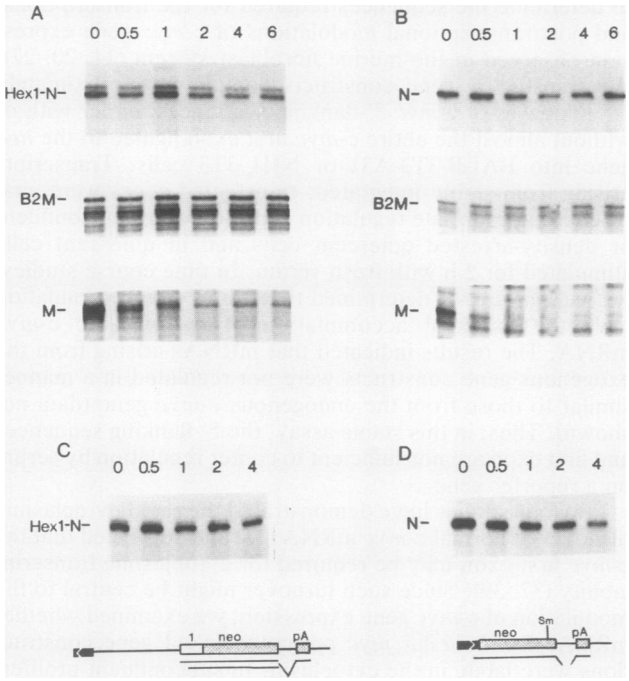

FIG. 2. Cytoplasmic turnover of *neo* transcripts containing the human c-*myc* first exon. The RNAs used were from pools of cells expressing either pHMmex1-*neo* (A and C) or pLTR*neo* (B and D). Turnover of transcripts was analyzed in actinomycin D-RNase protection experiments under subconfluent (proliferating) and density-arrested (quiescent) conditions as described in Materials and Methods. Transcripts arising from pHmex1-*neo* are initiated at the human c-*myc* P1 and P2 promoters and contain most of the c-*myc* first exon (except the 3′ 30 bases) and the entire *neo* gene sequences (construct J in Fig. 1). Transcripts arising from cells transfected with pLTR*neo* (construct K in Fig. 1) serve as a control *neo* transcript lacking c-*myc* sequences. Transcripts from pLTR*neo* initiate in the truncated MLV LTR. Schematic diagrams of these constructs and their transcripts are shown below the panels. The RNA probes were synthesized from pT7*neo*-320, pT7B2M, and pT7Hex2-419 (A and B) or from pT7*neo*-320 (C and D). Bands Hex1-N and N indicate the RNA fragments protected by the *neo* portion of the pHmex1-*neo* and pLTR*neo* genes, respectively. B2M indicates the RNA fragments protected by the pT7B2M probe and is an internal control for RNA content in each lane, since B2M has a very long cytoplasmic half-life. M indicates the pT7Hmex2-419-derived RNA fragment protected by the endogenous murine c-*myc* and is an internal control for the turnover of normal c-*myc* mRNA. The time (in hours) of actinomycin D treatment is shown above each lane. Optimal exposure times are shown for each section, but all panels are from the same gel.

transcripts were very stable ($t_{1/2} > 4$ h; Fig. 4A). In contrast, transcripts arising from a human c-*myc* gene construct in which the second exon was deleted had the same turnover rate as normal three-exon c-*myc* mRNAs (Fig. 4B). This experiment could be interpreted as evidence for a major role of the first exon in cytoplasmic turnover of c-*myc* mRNA, but further experiments described below suggest otherwise.

The influence of the third exon on cytoplasmic turnover rate was determined by replacement of the human c-*myc* third exon (except for the 5′-most 30 bases) by either the full-length *neo* gene or a short segment containing the simian virus 40 small t splice and polyadenylation signal. Although lacking most of the third-exon sequences, mRNAs transcribed from these constructs maintain all of the first- and second-exon sequences, including those which have the potential to form stem-loop structures, which were proposed to be involved in the regulation of c-*myc* expression (43). Transcripts arising from both of these constructs were very stable in the cytoplasm of murine fibroblasts ($t_{1/2} > 3$ to 4 h; Fig. 4C and D).

Stability of c-*myc*/*neo* hybrid transcripts. The results from the studies of the deleted-replaced c-*myc* transcripts suggested that sequences contained within both the first and third exons play a role in imparting cytoplasmic instability to c-*myc* mRNAs. However, the first exon, by itself, did not render the hybrid exon 1–*neo* transcript unstable (Fig. 2). To test whether both the first- and third-exon sequences are required for the rapid turnover rate, we constructed a hybrid gene which encodes transcripts containing almost all of the c-*myc* first- and third-exon sequences flanking the full-length *neo* gene sequences. These transcripts had a cytoplasmic half-life of approximately 45 min (Fig. 5). Using probes for different regions of the transcript, we determined that the entire transcript is degraded at the same rate (Fig. 5A and B), similar to the normal human three-exon transcript (data not shown). To assess the contribution of the third exon by itself, we ligated the human c-*myc* third exon to the 3′ end of the *neo* gene. Transcripts from this construct also turned over rapidly in the cytoplasm ($t_{1/2}$ = 42 min; Fig. 5C), whereas normal *neo* transcripts are stable under the same conditions (Fig. 2B). To localize the third-exon sequences which confer transcript instability, we ligated a DNA fragment containing only the 3′ untranslated sequences (300 bases) from the murine c-*myc* gene to the 3′ end of the *neo* gene. Transcripts from this construct also had a half-life of about 45 min (Fig. 5D).

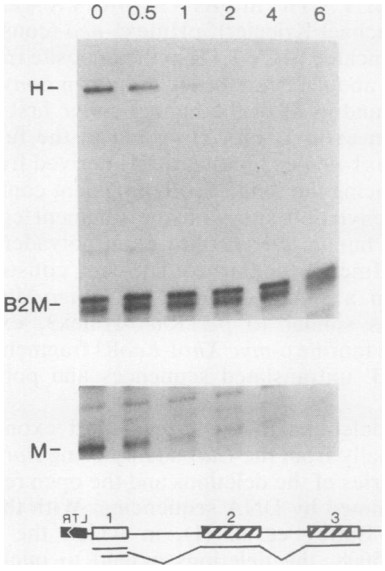

FIG. 3. Turnover of human c-*myc* three-exon transcripts in murine cells. Pooled colonies of proliferating NIH 3T3 cells expressing pLTRHm123 were treated for various times (indicated in hours above each lane) with actinomycin D, and their cytoplasmic RNAs were analyzed as described in the legend to Fig. 2. A schematic diagram of pLTRHm123 and the normal P1- and P2-initiated transcripts arising from it are shown below the panel. The probes (mixed) were from pT7Hmex2-419 and pT7B2M. Protected fragments H and M represent the human (exogenous) and murine (endogenous) gene transcripts, respectively, protected by the pT7Hmex2-419 derived probe. Protected fragments are as described in the legend to Fig. 2.

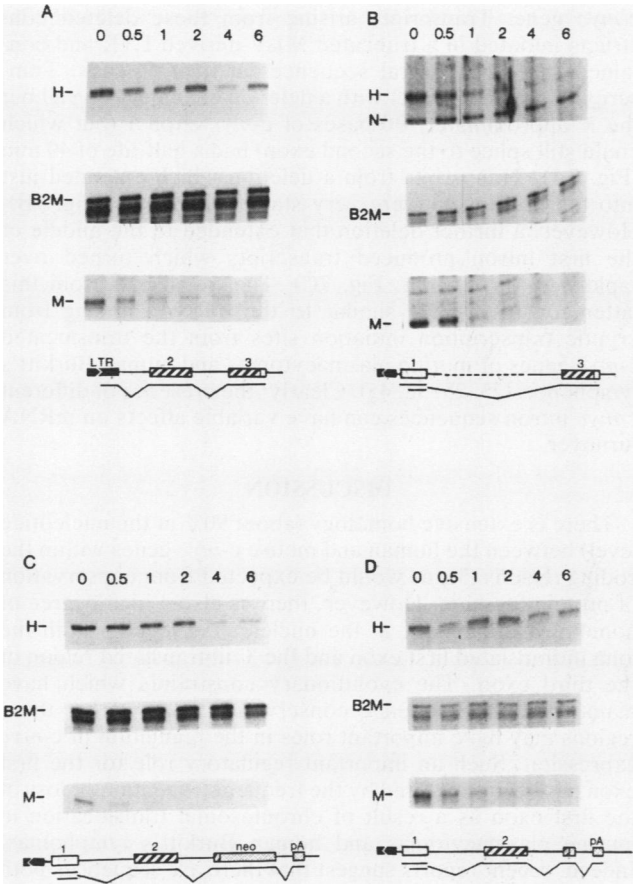

FIG. 4. Turnover of transcripts from human c-*myc* deletion-replacement genes. RNAs from cells were analyzed as described in the legend to Fig. 3. The RNAs used were from pools of cells expressing pLTRHmyc (A), pLTRHm13 (B), pLTRHm12*neo* (C), or pLTRHm12pA (D). Schematics of these constructs and the transcripts that arise from them are shown below the respective panels. The probes were derived from pT7Hmex2-419 and pT7B2M (A, C, and D) or pT7Hmex1, pT7*neo*-320, pT7B2M, and pT7Hmex2-419 (B). The bands labeled H in A, C, and D are the same as those described in the legend to Fig. 3. The band labeled H in panel B is the human P2 initiated transcript fragment protected by a probe derived from pT7Hmex1 (the P1-initiated transcript is not shown because it is too faint). Bands labeled B2M and M are the same as those described in the legend to Fig. 2. The band labeled N (B) is the *neo* transcript fragment protected by the probe derived from pT7*neo*-320. The cells used for B were cotransfected with pLTR*neo* and selected for growth in G418. Hence, the band labeled N, like that labeled B2M, is a control for (stable) RNA content within a lane. Times of actinomycin D treatment are given (in hours) above each lane.

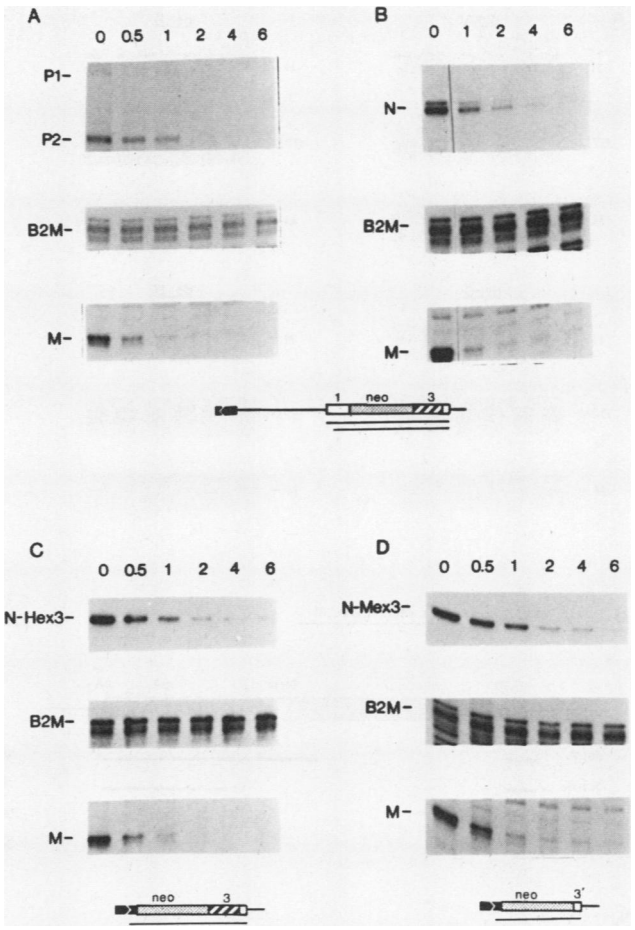

FIG. 5. Turnover of transcripts from human c-*myc*/*neo* hybrid genes. RNAs were analyzed as described in the legend to Fig. 3. The RNAS used were from pools of cells expressing pLTRHmex1-*neo*-ex3 (A and B), pLTR*neo*-Hmex3 (C), or pLTR*neo*-Mm3′ex3 (D). Schematic diagrams of these constructs and the transcripts that arise from them are shown below the respective panels. The probes were derived from pT7Hmex1, pT7B2M, and pT7Hmex2-419 (A) or from pT7*neo*-320, pT7B2M, and pT7Hmex2-419 (B, C, and D). The probe from pT7Hmex1 protects fragments from P1- and P2-initiated transcripts from pLTRHmex1-*neo*-ex3 in A. The probe from pT7*neo*-320 protects a *neo*-specific portion of the human c-*myc*/*neo* hybrid transcripts from pLTRHmex1-*neo*-ex3 (N), pLTR*neo*-Hmex3 (N-Hex3), and pLTR*neo*-Mm3′ex3 (N-Mex3) in B, C, and D, respectively. Bands labeled B2M and M are the same as those described in the legend to Fig. 2. Times of actinomycin D treatment are given (in hours) above each lane.

Localization of sequences conferring a rapid RNA turnover. To better define the sequences in the 3′ untranslated region that are responsible for the short RNA half-life, we made a series of internal deletions within the third exon that preserved the normal polyadenylation site. There are two polyadenylation sites for the c-*myc* gene that are separated by 140 bases (pA1 and pA2 in Fig. 6B). Both sites are utilized, although 80% of the transcripts utilize pA2 (46). Initially, we determined that the half-life was the same for RNAs with either pA1 or pA2 by using cell lines expressing the normal human c-*myc* gene and a probe which distinguished between the two different RNAs (46; data not shown). This indicated that sequences between the two sites are not required for the short half-life. We then analyzed the half-life of derivatives of the human c-*myc* gene in which various amounts of the 3′ untranslated sequences had been deleted. The 5′ boundary of each deletion was the *Cla*I site in the third exon, and the deletions extended through the coding region and into the 3′ untranslated region (Fig. 6B). Each construct was transfected into BALB/3T3 cells, and the half-life was determined as described above (Fig. 6A). Deletion of the third-exon coding region and 5′ portion of the 3′ untranslated region had little affect on the half-life (Fig. 6A, deletions A and B). However, a deletion ending within pA1 had a very long half-life (deletion C), and a deletion extending to pA2 was also quite stable. Thus, the sequence

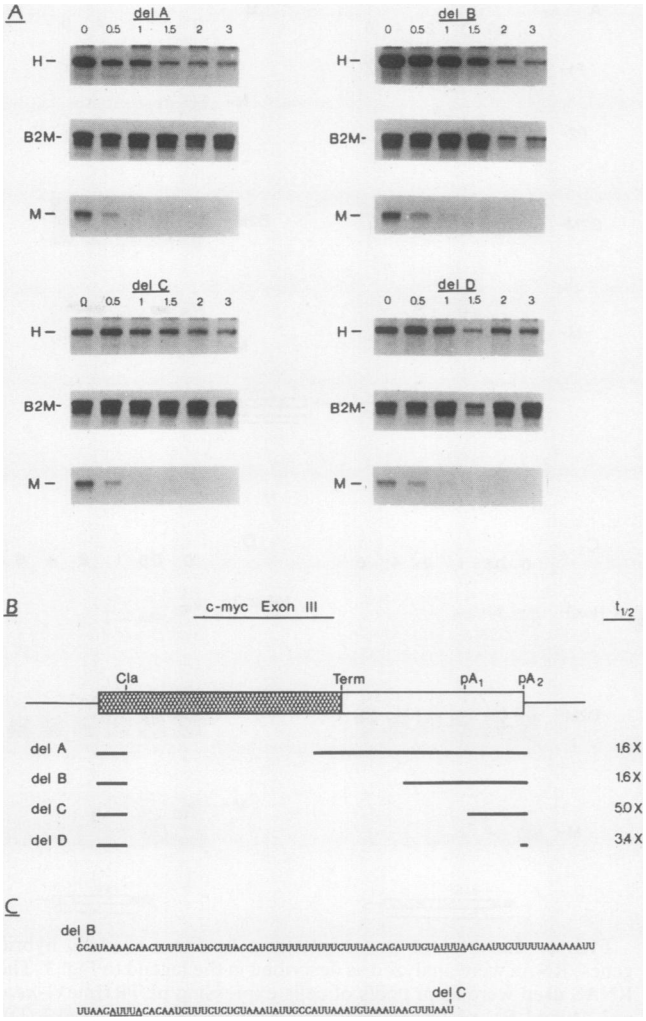

FIG. 6. Localization of sequences in the c-*myc* 3′ untranslated region conferring a short RNA half-life. Internal deletions within the human c-*myc* third exon were prepared and analyzed for half-life as outlined in Materials and Methods. (A) Determination of the RNA half-life for deletions (del) A through C by using three probes simultaneously: human c-*myc* (H), mouse B2M, and mouse c-*myc* (M). Times of actinomycin D treatment (in hours) are given above each lane. (B) Schematic diagram of deletions A through C in relation to the c-*myc* third-exon coding region (▩) and the two polyadenylation signals (pA1 and pA2). The half-life of each transcript was determined from the data in part A and expressed with respect to the half-life of the endogenous mouse c-*myc* RNA in the same experiment (taken as 1X). (C) Sequence of the human c-*myc* 3′ untranslated region (bounded by deletions B and C), which confers a short RNA half-life.

that primarily appeared to be responsible for the short RNA half-life was localized between the boundaries of deletions B and C, which included the 140 bases 5′ of pA1 (Fig. 6C). The most striking feature of this sequence was the high proportion of U (45%), with one stretch of U_{10}.

Effect of various sequences at the 5′ end of the transcript. The results above demonstrate that sequences from the 3′ untranslated region are primarily responsible for rapid cytoplasmic turnover of c-*myc* transcripts. However, to determine whether the first exon or 5′ sequences could influence turnover, we analyzed transcripts arising from a series of progressive 5′ (*Bal* 31-generated) deletions of the human c-*myc* gene. Transcripts arising from these deleted constructs initiated in a truncated MLV-derived LTR and contained 28 bases of viral sequences at their 5′ ends. Transcripts from a construct with a deletion encompassing all but the 3′ approximately 30 bases of c-*myc* exon 1 (but which could still splice to the second exon) had a half-life of 40 min (Fig. 7A). Transcripts from a deletion which extended just into the first intron were very stable ($t_{1/2}$ > 4 h; Fig. 7B). However, a further deletion that extended to the middle of the first intron produced transcripts which turned over rapidly ($t_{1/2}$ = 45 min; Fig. 7C). The transcripts from this latter construct were similar to the mRNAs arising from cryptic transcription initiation sites from the translocated c-*myc* genes of murine plasmacytomas and human Burkitt's lymphomas (23, 25, 38, 45). Clearly, the presence of different c-*myc* intron sequences can have variable affects on mRNA turnover.

DISCUSSION

There is extensive homology (about 90% at the nucleotide level) between the human and mouse c-*myc* genes within the coding regions (3), as would be expected from conservation of protein function. However, there is also a high degree of homology (about 75% at the nucleic acid level) within the long untranslated first exon and the 3′ untranslated region of the third exon. The evolutionary constraints which have maintained this sequence conservation suggest that these regions may have important roles in the regulation of c-*myc* expression. Such an important regulatory role for the first exon has been suggested by the frequent truncation or loss of the first exon as a result of chromosomal translocation in murine plasmacytomas and human Burkitt's lymphomas. Indeed, recent reports suggest that there are sequences both 5′ of the transcription initiation site and within the first exon which mediate both positive and negative regulatory effects on the basal transcription rate from c-*myc* promoter-reporter gene constructs in transient expression assays (7, 33, 41, 52). A posttranscriptional regulatory role has also been suggested for the first exon, since aberrant c-*myc* transcripts lacking exon 1 sequences have been shown to have a 3- to 10-fold longer half-life in tumor cells than normal c-*myc* transcripts (17, 37, 39). These studies suggested that the long untranslated c-*myc* first exon was involved in message stability; specifically, that it was necessary for rapid cytoplasmic turnover of c-*myc* mRNAs. More recently, the 5′ untranslated region of the bacteriophage T4 gene 32 has been shown to affect mRNA stability (18). However, all of the previous c-*myc* mRNA turnover studies suffer from the lack of a common cellular context and the contribution to transcript stability of the varying and diverse non-*myc* sequences present on the aberrant transcripts (17).

In this study we have investigated which sequence(s) in the c-*myc* mRNA contribute to its cytoplasmic instability and therefore may be intimately associated with, and constitute targets for, the posttranscriptional regulation observed in various systems. Our results are summarized in Fig. 1 and 6C, in which all of the data derive from a common cellular context of stably transfected murine fibroblasts expressing nonselected human deletion and hybrid c-*myc* genes. The data indicate that sequences within the 3′ untranslated region of c-*myc* are required for, but do not ensure, the high rate of cytoplasmic turnover. The effects of the incorporation of first-exon and first-intron sequences were also demonstrated.

An LTR-enhanced three-exon human c-*myc* gene expressed in murine fibroblasts produces transcripts which are

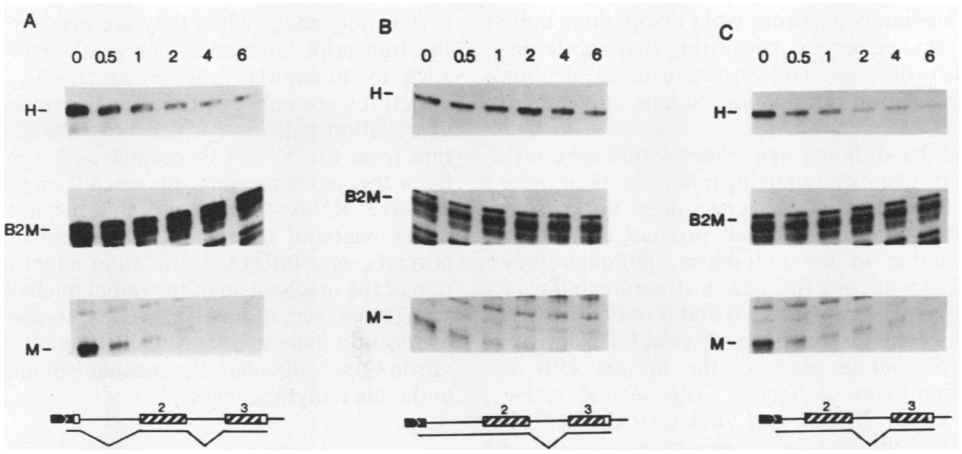

FIG. 7. Turnover of transcripts from 5′ deletions of the human c-*myc* gene. RNAs were analyzed as described in the legend to Fig. 3. RNAs were from pools of cells expressing pLTRHm23-5′del1 (A), pLTRHm23-5′del2 (B), and pLTRHm23-5′del3 (C). Schematic diagrams of these constructs and the transcripts that arise from them are shown below the respective panels. The probes used were derived from pT7Hmex2-419 and pT7B2M. Bands labeled H, B2M, and M are protected fragments as in Fig. 2 and 3. Times of actinomycin D treatment are given (in hours) above each lane.

structurally similar to transcripts arising from the normal endogenous c-*myc* gene. Although the homology between sequences in the human and murine first exon and 3′ untranslated region is less than in the coding region, these changes did not alter the short half-life, since the normal human transcript had the same high turnover rate in mouse cells as the endogenous c-*myc* transcripts of human and mouse cells (construct A of Fig. 1).

Our data with deleted exons demonstrate that the first and second exons of c-*myc* contribute very little, if any, to the cytoplasmic instability of the mRNA. Although transcripts lacking the entire second exon had the same turnover rate as full-length mRNA (construct D), deletion of most or all of the first-exon sequences gave only slightly longer cytoplasmic half-lives (compare half-lives of constructs A, E, and G), but a complete first exon may be required for maximum instability. Our demonstration that the complete first exon only has a slight effect upon increasing transcript c-*myc* turnover contrasts with previous reports (37, 39). These differences are likely due to the various tumor cell lines used in the previous studies to measure the turnover rates of aberrant c-*myc* transcripts.

In contrast, deletion and replacement of most of the third exon results in the production of transcripts that are very stable (constructs C and H of Fig. 1). Our results also show that sequences from the third exon are directly involved in destabilizing the c-*myc* transcript, since ligation of the third exon (or only the 3′ untranslated region) to the 3′ end of the *neo* gene (which otherwise produces transcripts which are stable) produces hybrid transcripts with a high cytoplasmic turnover rate (constructs L and M). These mRNAs are only slightly more stable than normal c-*myc* transcripts and are equivalent to transcripts which lack most or all first exon sequences.

Caput et al. (6) described a group of transiently expressed genes (comprised mainly of interferons, interleukins, and growth factors) which have particularly A+T-rich sequences within their 3′ untranslated regions. A regulatory role for these conserved sequences was suggested, although they did not hypothesize the potential nature of this regulation. By ligating the A+T-rich 3′ untranslated region of the granulocyte-macrophage–colony-stimulating factor (GM-CSF) gene to the human β-globin gene, Shaw and Kamen (44) recently demonstrated that this sequence will destabilize a normally stable globin message. They proposed that the sequence AUUUA might be responsible for the instability, since this sequence is tandemly repeated several times in GM-CSF and several other messages with rapid cytoplasmic turnover. The 3′ untranslated regions of both the murine and human c-*myc* genes are also very A+T rich (77%) and contain this proposed destabilizing motif, although much less frequently and not in tandem as in GM-CSF (1, 3, 44). It is possible that the absolute sequence requirement necessary for the initiation of cytoplasmic degradation is not strict but only requires a particular secondary structure that is recognized by the degradative enzymes. Computer analysis of the potential stem-loop structures that can be formed by the 3′ untranslated regions of the human and murine c-*myc* transcripts shows no striking similarities (data not shown).

Internal deletions within the c-*myc* third exon localize the sequences primarily responsible for the short RNA half-life to a 140-base, U-rich region immediately 5′ of pA1. Shaw and Kamen (44) previously noted a region of c-*myc* which contained the sequence AUUUA, mapping between pA1 and pA2. This particular sequence does not appear to be the primary determinant of c-*myc* RNA turnover, since RNAs terminating at either polyadenylation site have the same half-life (46; data not shown), and RNAs containing this region but lacking the sequences 5′ of pA1 (delC, Fig. 6) are quite stable. Interestingly, the destabilizing region mapped here is quite similar to that noted previously in that both are very U rich and contain the sequence AUUUA (Fig. 6C). There is no obvious correlation between the differential activities of these two sequences in destabilizing c-*myc* RNA and their location with respect to the termination of translation, which could be postulated to be important. Deletion A (Fig. 6B) is in frame, and translation terminates at the normal site. Deletion B removes the normal termination site, and translation continues through a portion of the 3′ untranslated region, terminating 60 bases 5′ of pA1. Thus, in this construct translation continues through much of the U-rich region implicated in RNA turnover, but the RNA is unstable and the same as that in deletion A. In deletion C, translation terminates 40 bases 3′ of pA1 and does not include the second U-rich region in the 3′ untranslated region (the one noted by Shaw and Kamen [44]), yet this RNA is stable.

Thus, it is not immediately apparent what distinctions there are between the two sequences, suggesting that subtle differences in primary or secondary structure or in location with respect to translation termination codons may be important.

Our data suggest that although sequences within the c-*myc* third exon are the primary destabilizing influence, their mere presence at the 3′ end of a transcript does not ensure cytoplasmic instability. Two constructs produce transcripts which are very stable in the cytoplasm, although they maintain a normal second- and third-exon structure and vary only in their 5′ sequences (constructs B and F of Fig. 1). One of these transcripts contains 206 bases of viral LTR-derived sequences at its 5′ end in place of the normal 450- or 550-base c-*myc* first-exon sequences. The second stable transcript contains only 28 bases of viral LTR-derived sequences plus most of the first-intron sequences in place of the first exon. The 28 bases of viral LTR-derived sequences, although common to the 5′ end of both of these transcripts, are not responsible for their observed cytoplasmic stability, since other transcripts with a high rate of turnover have the same LTR sequences (constructs E, G, L, and M). Thus, whereas the first exon by itself does not have a dominant destabilizing effect (construct J), other sequences at the 5′ end of the transcript can negate the potential for rapid cytoplasmic turnover that is imparted by the 3′ untranslated sequences. Clearly, increased transcript target size (length) and degradation rates do not correlate.

The mechanism(s) of mRNA degradation in either eucaryotic or procaryotic systems has not been extensively studied. The best-studied eucaryotic system is the cell cycle-regulated degradation of histone mRNAs. It has been recently reported that properly regulated histone mRNA degradation requires that protein synthesis continue to within 300 bases of the 3′ end of the message (19), which contains a short stem-loop structure which apparently is either the recognition sequence for the degradative enzymes or must be broken by the translational machinery to initiate degradation. Although murine and human c-*myc* mRNAs also contain many potential stem-loop structures, the mechanism for rapid turnover is likely to be different from that of histone mRNAs. However, for both histone and c-*myc*, cytoplasmic mRNA is stabilized in the presence of inhibitors of protein synthesis (11, 27, 32). It has been suggested that cytoplasmic turnover of c-*myc* mRNA is coupled to translation (32), and a similar suggestion has been made recently for turnover of GM-CSF transcripts in macrophages (49). In our c-*myc* deletion construct lacking the entire second exon, the normal translation initiation codon has been deleted, but the resulting transcript maintains the same high cytoplasmic turnover rate as the normal three-exon transcript (constructs D and A, respectively, of Fig. 1). It will be of interest to determine whether this transcript, which lacks exon 2, is found on polysomes and whether c-*myc* mRNA structure influences the contribution of translation to cytoplasmic degradation.

Degradation of normal c-*myc* mRNA and certain c-*myc*/*neo* hybrid transcripts occurs rapidly: the entire transcript is degraded with the same kinetics (data not shown; Fig. 5A). Hence, the initial cleavage event is probably the rate-limiting step. Our data suggest that this first event is activated by sequences present within the 3′ untranslated region of c-*myc*. It is likely that there is an initial endonucleolytic cleavage followed by rapid 3′-to-5′ degradation (by exonuclease activity). This mechanism, however, does not easily account for the modulating effect upon degradation by certain sequences when they are present near the 5′ end of the transcript. A model that would more easily allow for such modulation is one in which the 3′ sequences and structures are only required for the initial activation of the degradation pathway, which is followed by rapid degradation from the 5′ end in normal c-*myc* mRNA. We do not favor this latter mechanism, since it implies the presence of a 5′-to-3′ RNase—an activity that has not been described in either bacterial or eucaryotic systems. The rapid rate of normal c-*myc* mRNA degradation might preclude investigation of the mechanism of the initial nucleolytic cleavage(s) in vivo. However, it may be possible to use cell-free systems, as recently demonstrated for histone mRNA degradation in vitro (42), to elucidate the mechanism and location of these initial nucleolytic events.

ACKNOWLEDGMENTS

We thank Michelle Aman, Ameeta Kelekar, Steven Piccoli, and Mary Ellen Steele for their excellent suggestions or technical assistance. We also thank Beth Ann Rasmussen for her help in the graphic drawing of Fig. 1.

This research was supported by a Public Health Service Postdoctoral Fellowship (T.R.J.) and grants from the National Cancer Institute (M.D.C.) from the National Institutes of Health. M.D.C. is a Pew Scholar in the Biomedical Sciences.

LITERATURE CITED

1. **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.
2. **Bentley, D. L., and M. Groudine.** 1986. A block to elongation is largely responsible for decreased transcription of c-*myc* in differentiated HL60 cells. Nature (London) **321**:702–706.
3. **Bernard, O., S. Cory, S. Gerondakis, E. Webb, and J. M. Adams.** 1983. Sequence of the murine and human cellular *myc* oncogenes and two modes of *myc* transcription resulting from chromosome translocation in B lymphoid tumors. EMBO J. **2**:2375–2383.
4. **Blanchard, J.-M., M. Piechaczyk, C. Dani, J.-C. Chambard, A. Franchi, J. Pouyssegur, and P. Jeanteur.** 1985. C-*myc* gene is transcribed at high rate in G0-arrested fibroblasts and is post-transcriptionally regulated in response to growth factors. Nature (London) **317**:443–445.
5. **Campisi, J., H. E. Gray, A. B. Pardee, M. Dean, and G. E. Sonenshein.** 1984. Cell-cycle control of c-*myc* but not c-*ras* expression is lost following chemical transformation. Cell **36**:241–247.
6. **Caput, D., B. Beutler, K. Hartog, R. Thayer, S. Brown-Shimer, and A. Cerami.** 1986. Identification of a common nucleotide sequence in the 3′-untranslated region of mRNA molecules specifying inflammatory mediators. Proc. Natl. Acad. Sci. USA **83**:1670–1674.
7. **Chung, J., E. Sinn, R. R. Reed, and P. Leder.** 1986. Trans-acting elements modulate expression of the human c-*myc* gene in Burkitt lymphoma cells. Proc. Natl. Acad. Sci. USA **83**:7918–7922.
8. **Cole, M. D.** 1986. The *myc* oncogene: its role in transformation and differentiation. Annu. Rev. Genet. **20**:361–384.
9. **Corcoran, L. M., S. Cory, and J. M. Adams.** 1985. Transposition of the immunoglobulin heavy chain enhancer to the *myc* oncogene in a murine plasmacytoma. Cell **40**:71–79.
10. **Cory, S.** 1986. Activation of cellular oncogenes in hemopoietic cells by chromosome translocation. Adv. Cancer Res. **47**:189–234.
11. **Dani, C., J. M. Blanchard, M. Piechaczyk, S. El Sabouty, L. Marty, and P. Jeanteur.** 1984. Extreme instability of *myc* mRNA in normal and transformed human cells. Proc. Natl. Acad. Sci. USA **81**:7046–7050.
12. **Dani, C., N. Mechti, M. Piechaczyk, B. Lebleu, P. Jeanteur, and**

J. M. Blanchard. 1985. Increased rate of degradation of c-*myc* mRNA in interferon-treated Daudi cells. Proc. Natl. Acad. Sci. USA **82**:4896–4899.

13. **Dean, M., R. A. Levine, and J. Campisi.** 1986. c-*myc* regulation during retinoic acid-induced differentiation of F9 cells is post-transcriptional and associated with growth arrest. Mol. Cell. Biol. **6**:518–524.
14. **Dean, M., R. A. Levine, W. Ran, M. S. Kindy, G. E. Sonenshein, and J. Campisi.** 1986. Regulation of c-*myc* transcription and mRNA abundance by serum growth factors and cell contact. J. Biol. Chem. **261**:9161–9166.
15. **Dony, C., M. Kessel, and P. Gruss.** 1985. Post-transcriptional control of *myc* and p53 expression during differentiation of the embryonal carcinoma cell line F9. Nature (London) **317**:63–639.
16. **Eick, D., and G. Bornkamm.** 1986. Transcriptional arrest within the first exon is a fast control mechanism in c-*myc* gene expression. Nucleic Acids Res. **14**:8331–8346.
17. **Eick, D., M. Piechaczyk, B. Henglein, J.-M. Blanchard, B. Traub, E. Kofler, S. Wiest, G. M. Lenoir, and G. W. Bornkamm.** 1985. Aberrant c-*myc* RNAs of Burkitt's lymphoma cells have longer half-lives. EMBO J. **4**:3717–3725.
18. **Gorski, K., J.-M. Roch, R. Prentki, and H. M. Krisch.** 1985. The stability of bacteriophage T4 gene 32 mRNA: a 5′ leader sequence that can stabilize mRNA transcripts. Cell **43**:461–469.
19. **Graves, R. A., N. B. Pandey, N. Chodchoy, and W. F. Marzluff.** 1987. Translation is required for regulation of histone mRNA degradation. Cell **48**:615–626.
20. **Greenberg, M. E., and E. B. Ziff.** 1984. Stimulation of 3T3 cells induces transcription of the c-*fos* proto-oncogene. Nature (London) **311**:433–438.
21. **Grosso, L. E., and H. C. Pitot.** 1985. Transcriptional regulation of c-*myc* during chemically induced differentiation of HL-60 cultures. Cancer Res. **45**:847–850.
22. **Hann, S. R., C. B. Thompson, and R. N. Eisenman.** 1985. C-*myc* oncogene protein synthesis is independent of the cell cycle in human and avian cells. Nature (London) **314**:366–369.
23. **Hayday, A. C., S. D. Gillies, H. Saito, C. Wood, K. Wiman, 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.
24. **Jonak, G. J., and E. Knight, Jr.** 1984. Selective reduction of c-*myc* mRNA in Daudi cells by human alpha-interferon. Proc. Natl. Acad. Sci. USA **81**:1747–1750.
25. **Keath, E. J., A. Kelekar, and M. D. Cole.** 1984. Transcriptional activation of the translocated c-*myc* oncogene in mouse plasmacytomas: similar RNA levels in tumor and proliferating normal cells. Cell **37**:521–528.
26. **Kelekar, A., and M. D. Cole.** 1986. Tumorigenicity of fibroblast lines expressing the adenovirus E1a, cellular p53, or normal c-*myc* genes. Mol. Cell. Biol. **6**:7–14.
27. **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.
28. **Knight, E., Jr., E. D. Anton, D. Fahey, B. K. Friedland, and G. J. Jonak.** 1985. Interferon regulates c-*myc* gene expression in Daudi cells at the post-transcriptional level. Proc. Natl. Acad. Sci. USA **82**:1151–1154.
29. **Lachman, H. M., and A. I. Skoultchi.** 1984. Expression of c-*myc* changes during differentiation of mouse erythroleukemia cells. Nature (London) **310**:592–594.
30. **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.
31. **Levine, R. A., J. E. McCormack, A. Buckler, and G. E. Sonenshein.** 1986. Transcriptional and posttranscriptional control of c-*myc* gene expression in WEHI 231 cells. Mol. Cell. Biol. **6**:4112–4116.
32. **Linial, M., N. Gunderson, and M. Groudine.** 1985. Enhanced transcription of c-*myc* in bursal lymphoma cells requires continuous protein synthesis. Science **230**:1126–1132.
33. **Lipp, M., R. Schilling, S. Wiest, G. Laux, and G. W. Bornkamm.** 1987. Target sequences for cis-acting regulation within the dual promoter of the human c-*myc* gene. Mol. Cell. Biol. **7**:1393–1400.
34. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
35. **Mechti, N., M. Piechaczyk, J-M. Blanchard, L. Marty, A. Bonnieu, P. Jeanteur, and B. Lebleu.** 1986. Transcription and post-transcriptional regulation of c-*myc* gene expression during the differentiation of murine erythroleukemia Friend cells. Nucleic Acids Res. **14**:9653–9665.
36. **Persson, H., H. E. Gray, and F. Godeau.** 1985. Growth-dependent synthesis of c-*myc*-encoded proteins: early stimulation by serum factors in synchronized mouse 3T3 cells. Mol. Cell. Biol. **5**:2903–2912.
37. **Piechaczyk, M., J.-Q. Yang, J.-M. Blanchard, P. Jeantuer, and K. B. Marcu.** 1985. Posttranscriptional mechanisms are responsible for accumulation of truncated c-*myc* RNAs in murine plasma cell tumors. Cell **42**:589–597.
38. **Prehn, J., M. Mercola, and K. Calame.** 1984. Translocation affects normal promoter usage and activates fifteen cryptic c-*myc* transcription starts in plasmacytoma M603. Nucleic Acids Res. **12**:8987–9007.
39. **Rabbitts, P. H., A. Forster, M. A. Stinson, and T. H. Rabbitts.** 1985. Truncation of exon 1 from the c-*myc* gene results in prolonged c-*myc* mRNA stability. EMBO J. **4**:3727–3733.
40. **Reitsma, P. H., P. G. Rothberg, S. M. Astrin, J. Trial, Z. Bar-Shavit, A. Hall, S. L. Teitelbaum, and A. J. Kahn.** 1983. Regulation of *myc* gene expression in HL-60 leukaemia cells by a vitamin D metabolite. Nature (London) **306**:492–494.
41. **Remmers, E. F., J.-Q. Yang, and K. B. Marcu.** 1986. A negative transcriptional control element located upstream of the murine c-*myc* gene. EMBO J. **5**:899–904.
42. **Ross, J., and G. Kobs.** 1986. H4 histone messenger RNA decay in cell-free extracts initiates at or near the 3′ terminus and proceeds 3′ to 5′. J. Mol. Biol. **188**:579–593.
43. **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. USA **80**:7476–7480.
44. **Shaw, G., and R. Kamen.** 1986. A conserved AU sequence from the 3′ untranslated region of GM-CSF mRNA mediates selective mRNA degradation. Cell **46**:659–667.
45. **Stanton, L. W., R. Watt, and K. B. Marcu.** 1983. Translocation, breakage, and truncated transcripts in c-*myc* oncogene in murine plasmacytomas. Nature (London) **303**:401–406.
46. **Swartwout, S. G., H. Preisler, W. Guan, and A. J. Kinniburgh.** 1987. Relatively stable population of c-*myc* RNA that lacks long poly(A). Mol. Cell. Biol. **7**:2052–2058.
47. **Taub, R., K. Kelly, J. Battey, S. Latt, G. M. Lenoir, U. Tantravahi, Z. Tu, and P. Leder.** 1984. A novel alteration in the structure of an activated c-*myc* gene in a variant t(2;8) Burkitt lymphoma. Cell **37**:511–520.
48. **Thompson, C. B., B. P. Challoner, P. E. Neiman, and M. Groudine.** 1985. Levels of c-*myc* oncogene mRNA are invariant throughout the cell cycle. Nature (London) **314**:363–366.
49. **Thorens, B., J.-J. Mermod, and P. Vassalli.** 1987. Phagocytosis and inflammatory stimuli induce GM-CSF mRNA in macrophages through posttranscriptional regulation. Cell **48**:671–679.
50. **Westin, E. H., F. Wong-Staal, E. P. Gelmann, R. Dalla-Favera, T. S. Papas, J. A. Lautenberger, A. Eva, E. P. Reddy, S. R. Tronick, S. A. Aaronson, and R. C. Gallo.** 1982. Expression of cellular homologues of retroviral onc genes in human hematopoietic cells. Proc. Natl. Acad. Sci. USA **79**:2490–2494.
51. **Wigler, M., A. Pellicer, S. Silverstein, R. Axel, G. Urlaub, and L. Chasin.** 1979. DNA-mediated transfer of the adenine phosphoribosyltransferase locus into mammalian cells. Proc. Natl. Acad. Sci. USA **76**:1373–1376.
52. **Yang, J.-Q., E. F. Remmers, and K. B. Marcu.** 1986. The first exon of the c-*myc* proto-oncogene contains a novel positive control element. EMBO J. **5**:3553–3562.
53. **Zinn, K., D. DiMaio, and T. Maniatis.** 1983. Identification of two distinct regulatory regions adjacent to the human beta-interferon gene. Cell **34**:865–879.