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Relatively Stable Population of c-*myc* RNA That Lacks Long Poly(A)

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We examined the turnover of c-*myc* RNA in the human promyelocytic cell line HL-60. In whole-cell RNA from rapidly growing cells we observed two major size classes of c-*myc* RNA, 2.4 and 2.2 kilobases (kb). When HL-60 cells were treated with actinomycin D for 30 min to inhibit transcription, the 2.4-kb c-*myc* RNA population was rapidly degraded, while the 2.2-kb c-*myc* RNA was degraded much more slowly. S1 nuclease transcript mapping and promoter-specific probes were utilized to show that both the stable 2.2-kb and the labile 2.4-kb c-*myc* RNA populations have 5′ ends at the second promoter site (P2) and 3′ ends at the second poly(A) addition site. To examine further possible structural differences between these two RNA populations, we fractionated RNA on an oligo(dT)-cellulose column to separate RNAs that contained long poly(A) tails from those which did not. We found that the labile 2.4-kb c-*myc* RNA population bound to oligo(dT)-cellulose, while the more stable 2.2-kb c-*myc* RNA population did not. Preliminary estimates of their half-lives ($t_{1/2}$) showed that the poly(A)$^+$ c-*myc* RNA had a $t_{1/2}$ of 12 min, while the c-*myc* RNA that did not bind to oligo(dT)-cellulose had a $t_{1/2}$ of >1 h. Several other cell types contain both poly(A)$^+$ and nonpoly(A)$^+$ c-*myc* RNAs including HeLa cells, normal human bone marrow cells, and normal mouse fetal liver cells. In agreement with the results in HL-60 cell, HeLa cell poly(A)$^+$ c-*myc* RNA was more labile than c-*myc* RNA that lacked poly(A). The stable, nonpoly(A)$^+$ c-*myc* RNA population may be important in the posttranscriptional regulation of c-*myc* expression.

The cellular proto-oncogene c-*myc* is expressed in many cell types. c-*myc* expression is highest in proliferating cells, and it is turned off in differentiating cells (13, 19, 22, 30, 31). The c-*myc* protein is localized to the cell nucleus where it is believed to participate in the regulation of cellular growth (15). c-*myc* is the cellular homolog of several avian retroviruses, including the prototypic MC29 virus. The c-*myc* gene has been implicated in the genesis of several hematopoietic neoplasias. It is translocated to the immunoglobulin heavy-chain locus in Burkitt lymphomas and in mouse plasmacytomas (27–29). In human myeloid and lymphoid leukemias, a subset of patients show highly elevated c-*myc* RNA levels (24).

c-*myc* gene regulation in fibroblasts and tumor cells in culture has been shown to be, in part, posttranscriptional. A human c-*myc* mRNA half-life of 20 to 30 min has been reported for several cell lines in culture, and this half-life can be modulated by interferons (9, 10, 14). The authors of these studies, however, utilized poly(A)$^+$ RNA prepared from whole cells as their source of c-*myc* RNA and not total cellular RNA. We investigated the turnover of c-*myc* mRNA using total cellular RNA from the human promyelocytic leukemia cell line HL-60. We found two classes to c-*myc* RNA, a relatively labile, poly(A)$^+$ population [which binds to oligo(dT)-cellulose] and a more stable c-*myc* RNA population lacking long poly(A) [which does not bind to oligo(dT)-cellulose]. Further structural analysis of these two RNA populations demonstrated that both utilize the c-*myc* downstream promoter site (P2) and both have a predominant 3′ end located at or near the second poly(A) addition site. The labile, poly(A)$^+$ c-*myc* mRNA is approximately 200 nucleotides (nt) larger than the majority of the stable c-*myc* mRNA which does not bind to oligo(dT)-cellulose. Taken together these data suggest that poly(A) is a structural feature of c-*myc* mRNA that is used to regulate its steady-state level.

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MATERIALS AND METHODS

Cell culture, RNA preparation, and Northern blot hybridization. HL-60 cells were grown in RPMI 1640 medium plus 15% fetal calf serum. Cells were split 1:10, and experiments were performed 48 h later. Actinomycin D (Sigma Chemical Co., St. Louis, Mo.) was dissolved in RPMI 1640 plus 15% fetal calf serum at a concentration of 200 μg/ml and added to half of the culture to a final concentration of 10 μg/ml. Cells were harvested from both the treated and untreated cultures, and whole-cell RNA was purified (20), denatured, electrophoresed (21), blotted, and hybridized (7). RNA samples were passed over an oligo(dT)-cellulose column as described by Aviv and Leder (1), and the bound and unbound RNA fractions from the equivalent of 20 or 40 μg of total HL-60 RNA were used for Northern blot analysis or S1 nuclease RNA mapping studies. Cytoplasm and nuclei were prepared as described previously (18).

The c-*myc* probe for total c-*myc* RNA sequences was the 1.5-kilobase (kb) *Cla*I-*Eco*RI exon 3 fragment of human c-*myc*. This DNA restriction fragment was purified by agarose gel electrophoresis and labeled with [α-^{32}P]dATP by the random-primer method (12), and 5×10^6 cpm/ml were used for hybridization. The blots were incubated for 16 to 20 h at 68°C and washed as described by Church and Gilbert (7). The blot was exposed to XAR film (Eastman Kodak Co., Rochester, N.Y.) for various times with an intensifying screen. Blots were simultaneously hybridized with triosephosphate isomerase (TPI) probe (see Figs. 3 and 5) or subsequently rehybridized to an M13, primer-extended TPI cDNA probe (see Fig. 1) (5) as described above.

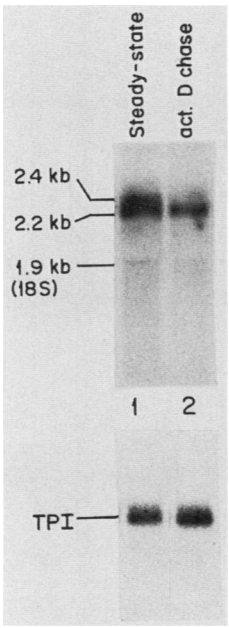

FIG. 1. Northern blot hybridization of c-*myc* RNA in total cellular RNA from HL-60 cells. Lane 1, 20 μg of whole-cell RNA from rapidly growing HL-60 cells (steady state); lane 2, 20 μg of whole-cell RNA from HL-60 cells treated for 30 min with actinomycin D (10 μg/ml) (act. D chase). Probe, *Cla*I-*Eco*RI exon 3 human c-*myc*.

RNA was prepared from mouse tissues by the guanidium isothiocynate method of Chirgwin et al. (6). These RNA samples were a kind gift of Steven Grant of this institute. The P1-specific probe was prepared with a 570-base-pair (bp) *Puv*II-*Xho*I DNA fragment (2). This fragment contains 125 bp of a possible 160 P1-specific base pairs.

S1 nuclease mapping. Samples were hybridized and digested with S1 nuclease by a modification (20) of the Berk and Sharp (4) procedure. Digested hybrids were denatured and run on a 4% acrylamide–7 M urea gel as previously described (20). The 5′-end probe was prepared from a *Pvu*II c-*myc*-containing M13 clone (2) by annealing with universal primer DNA and extending with [α^{32}P]dATP and unlabeled dCTP, dGTP, and TTP with the Klenow fragment of *Escherichia coli* DNA polymerase I. The c-*myc* DNA was excised by using the polylinker sites *Hin*dIII and *Eco*RI, and the 930-nt DNA was purified by denaturing gel electrophoresis. Approximately 20,000 cpm of probe were used in each hybridization reaction. The gel was exposed to XAR film with an intensifying screen. The 3′ c-*myc* end probe was a 3′-end-labeled *Cla*I-*Eco*RI 1.5-kb DNA fragment (2). The DNA fragment was purified by agarose gel electrophoresis and elution. The purified DNA was specifically end labeled at the *Cla*I site with [α-^{32}P]dCTP and Klenow fragment (20).

Deadenylation of HL-60 RNA. RNA digestions were performed 10 μg of total RNA and 10-μg equivalents of oligo(dT)-cellulose-fractionated HL-60 cellular RNA. Total, poly(A)$^+$, and poly(A)$^-$ RNAs were preincubated with 1.5 μg of oligo(dT)$_{15}$ for 30 min at 37°C in a 20-μl reaction volume containing 20 mM HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid)-KOH (pH 8.0), 50 mM KCl, 10 mM $MgCl_2$, and 1 mM dithiothreitol to allow hybridization of the poly(dT) to poly(A). RNase H (2 U) was then added, and incubation was continued for 2 h. The reaction was then stopped by the addition of 0.5 M EDTA to a final concentration of 20 mM. The samples were precipitated with ethanol and suspended in 10 μl of double-distilled H_2O in preparation for Northern blot analysis.

RESULTS

Turnover properties of c-*myc* RNA. The turnover of c-*myc* mRNA was examined in the previously studied HL-60 cell line. We combined the high-resolution glyoxal gel electrophoresis procedure of McMaster and Carmichael (21) with the Church and Gilbert (7) blotting-hybridization technique to analyze total cellular RNA for c-*myc* sequences. Two readily detectable c-*myc* mRNAs (2.4 and 2.2 kb) were present in total RNA that was purified from rapidly growing cells (Fig. 1, lane 1). When total RNA obtained from cells incubated for 30 min with actinomycin D (10 μg/ml) was examined, the smaller, 2.2-kb RNA was the predominant species (Fig. 1, lane 2). This blot was also hybridized to a TPI cDNA probe (5). We observed no turnover of TPI mRNA during the 30-min chase, indicating that the reduction of the 2.4-kb c-*myc* RNA was not due to any secondary effects of actinomycin D (Fig. 1). The data indicate that the majority of the 2.4-kb c-*myc* RNA species is more labile than the 2.2-kb c-*myc* RNA. These results are in contrast to the single, labile c-*myc* RNA species described by others (9, 10).

Transcript mapping of stable and labile c-*myc* RNAs. We next examined the 5′ and 3′ ends of the c-*myc* transcripts for differences that might correlate with their size and turnover properties. The human c-*myc* gene contains two transcriptional promoters (P1 and P2) that are approximately 160 bp apart (2). Preferential promoter usage and turnover could account for the two c-*myc* RNA species observed. To test this idea, we assayed promoter usage in steady-state RNA and in RNA from cells chased with actinomycin D with S1 nuclease protection (4, 20). A uniformly labeled, strand-specific, 860-bp *Pvu*II DNA fragment containing 350 bp of

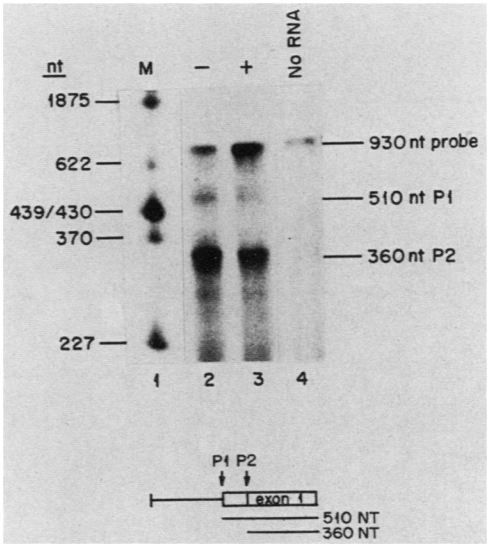

FIG. 2. S1 nuclease mapping of c-*myc* RNA 5′ ends. Lanes: 1, ^{32}P-labeled *Hae*II-digested pBR322 as size markers; 2, 20 μg of whole-cell RNA from rapidly growing HL-60 cells (steady state); 3, 20 μg of whole-cell RNA from actinomycin D-treated HL-60 cells; 4, no RNA, control. The RNAs were annealed to a uniformly ^{32}P-labeled 930-bp *Pvu*II DNA fragment containing exon 1 and 5′-flanking c-*myc* sequences (2). P1 indicates the 5′-most promoter-protected DNA (510 nt), and P2 indicates the second promoter-protected DNA (360 nt).

the 5′-flanking region and 510 bp of c-*myc* exon 1 was utilized in this experiment. The majority of the c-*myc* RNAs protected a 360-nt DNA fragment, consistent with these RNAs being initiated at P2 (Fig. 2). Also, a much lower amount of a 510-nt DNA fragment was protected in this experiment. c-*myc* RNAs initiating at P1 would protect a 510-nt DNA. Further, c-*myc* RNAs transcribed from either promoter site showed no differential instability (Fig. 2, compare lanes 1 and 2). To determine directly whether c-*myc* RNA which is initiated at P1 contributes to the labile 2.4-kb c-*myc* RNA population, we hybridized a P1-specific DNA probe to Northern blots of steady-state RNA (Fig. 3). It is apparent that the P1-initiated c-*myc* RNAs are larger (2.7 kb) and well resolved from the P2-initiated RNAs detectable with an exon 3 probe (Fig. 3, compare lanes 1 and 2). Therefore, three c-*myc* RNAs are observed in HL-60 cells: two which initiate at P2, the 2.4-kb and 2.2-kb species, and one which initiates at P1, the 2.7-kb species. The relatively low level of P1-initiated transcripts can be seen in longer exposures of the exon 3-probed blot (Fig. 3, lane 3). The low level of P1 transcripts is in agreement with the results of others (2, 23).

We also mapped the 3′ ends of c-*myc* mRNA by S1 nuclease protection. The DNA sequence of c-*myc* exon 3 revealed two potential poly(A) addition sites (pA1 and pA2) which are separated by 145 bp and therefore might account for the difference in size and lability of the two P2-initiated c-*myc* RNAs (2). The majority of the c-*myc* steady-state RNA ends at pA2. When compared with RNA from actinomycin D-treated cells, no preferential turnover of any 3′-ended c-*myc* RNA species was observed (Fig. 4A). The S1 nuclease-protected DNA could also indicate the presence of a splice donor site, although there are no reports of c-*myc* cDNAs with sequences 3′ to this site (3).

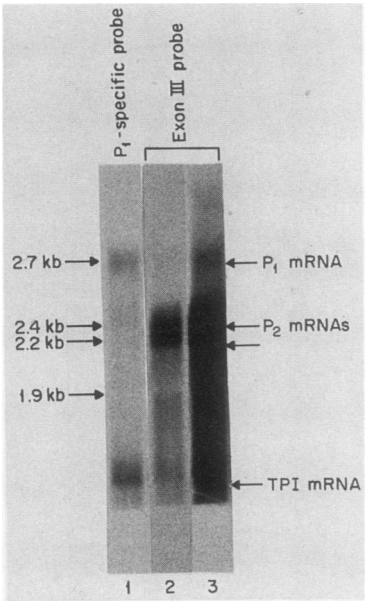

FIG. 3. Northern blot hybridization to detect P1-specific and total c-*myc* RNAs. Three 20-μg samples of HL-60 whole-cell RNA were electrophoresed and blotted as described in the text. Lane 1 was hybridized to a P1-specific *Pvu*II-*Xho*I probe prepared as described in the text and exposed to film for 8 days; lane 2 was hybridized to an exon 3, total c-*myc* probe and exposed to film for 20 h; and lane 3 is the same blot as lane 1 exposed to film for 8 days. All samples were hybridized to a TPI cDNA probe.

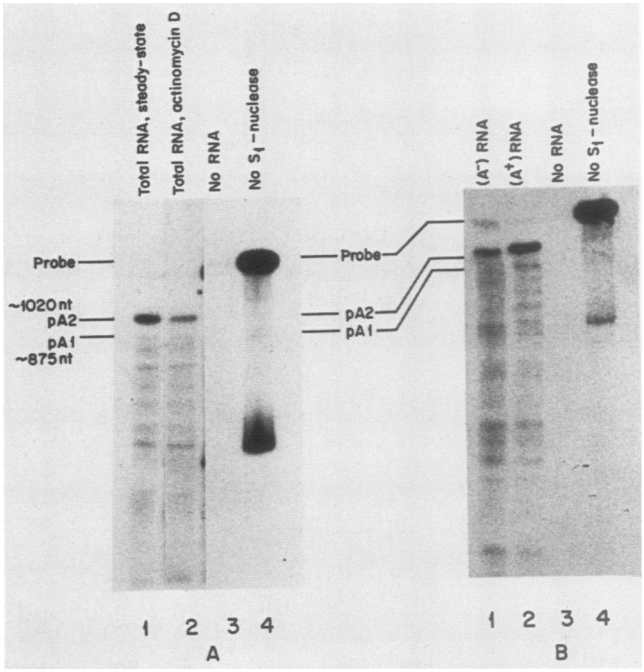

FIG. 4. S1 nuclease mapping of c-*myc* RNA transcribed 3′ ends. (A) Lanes: 1, 40 μg of HL-60 whole-cell RNA; 2, 40 μg of whole-cell RNA from cells treated for 30 min with actinomycin D; 3 and 4, 40 μg of tRNA from *E. coli*. (B) Lanes: 1, 40 μg of non-oligo(dT)-cellulose-bound RNA from HL-60 whole-cell RNA; 2, oligo(dT)-cellulose-bound RNA from 40 μg of whole-cell RNA; 3 and 4, 40 μg of *E. coli* tRNA. The RNAs were hybridized to a 3′-end-labeled *Cla*I-*Eco*RI 1.5-kb fragment of the human c-myc exon 3 and 3′-flanking region (2). The samples were then treated with S1 nuclease (panels A and B, lanes 1 to 3) or incubated without enzyme in S1 nuclease buffer (panels A and B, lane 4). Samples were run on a 4% acrylamide–7 M urea gel (20 by 20 cm) for 16 h at 50 V. Gels were exposed to XAR film with intensifying screens for 48 h.

Polyadenylation and c-*myc* RNA turnover. Since previous studies have shown a single, labile species of poly(A)$^+$ c-*myc* mRNA (9, 10), we next assayed oligo(dT)-cellulose binding of c-*myc* RNA using HL-60 steady-state and actinomycin D-chased RNA. We found that poly(A)$^+$ c-*myc* RNA consists of a broad RNA band on Northern blots. It is approximately 2.4 kb in steady-state RNA. The majority of the non-oligo(dT)-cellulose-bound c-*myc* RNA is 2.2 kb with a much smaller quantity of a 2.4-kb species in the steady-state RNA (Fig. 5). When RNA from actinomycin D-treated HL-60 cells was examined, the poly(A)$^+$ c-*myc* RNA was much more labile than the c-*myc* RNA which does not bind to oligo(dT)-cellulose (Fig. 5A). An estimated 75 and 63% of the combined 2.4- and 2.2-kb nonbound c-*myc* RNAs remained after 30 and 60 min of actinomycin D treatment, respectively. On the other hand, only 16 and 4% of the 2.4-kb poly(A)$^+$ c-*myc* RNA remained after 30 and 60 min of treatment with actinomycin D, respectively (Fig. 5A). A fourfold-longer exposure of the poly(A)$^+$ c-*myc* RNA lanes shows the turnover of this species more clearly (Fig. 5B). This blot was probed simultaneously for TPI mRNA. This mRNA was stable, and the vast majority of it was found in the oligo(dT)-cellulose-bound fraction (Fig. 5A). This internal TPI control demonstrates that the recoveries of HL-60 poly(A)$^+$ RNA in both the untreated and actinomycin D-treated RNA samples were similar. The finding of the vast majority of TPI mRNA in the poly(A)$^+$ RNA samples further demonstrates that the oligo(dT)-cellulose fractionation was

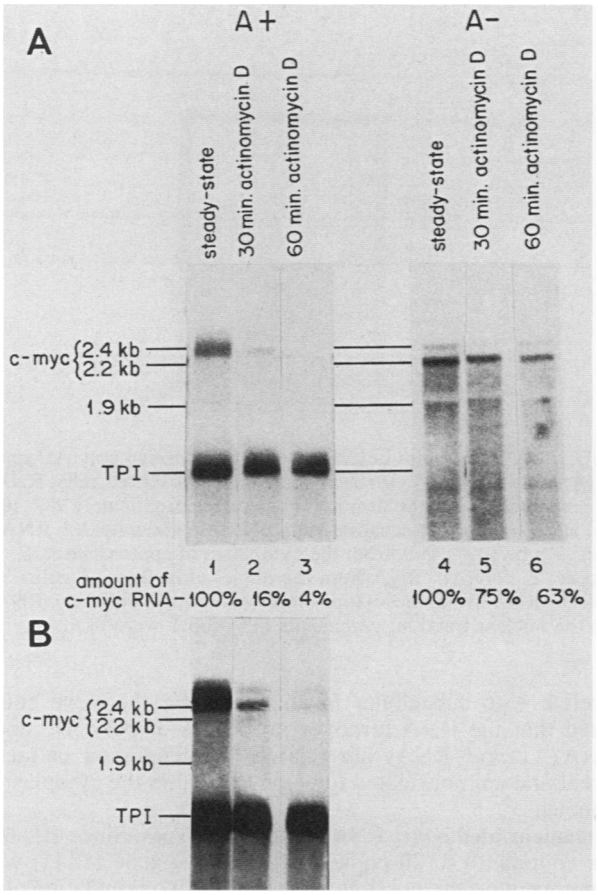

FIG. 5. Northern blot detection of c-*myc* RNA in HL-60 steady-state and actinomycin D-chased RNAs separated on oligo(dT)-cellulose columns. In all samples the amount of poly(A)$^+$ and poly(A)$^-$ RNA that was derived from 20 μg of whole-cell RNA was electrophoresed. (A) Lanes: 1, poly(A)$^+$ steady-state RNA; 2, poly(A)$^+$ RNA from cells treated for 30 min with actinomycin D; 3, poly(A)$^+$ RNA from cells treated with actinomycin D for 60 min; 4, unbound steady-state RNA; 5, unbound RNA from cells treated for 30 min with actinomycin D; 6, unbound RNA from cells treated for 60 min with actinomycin D. The amount of c-*myc* RNA was quantitated by densiometric scanning. Percentages of c-*myc* RNA are relative to 100% of either poly(A)$^-$ or poly(A)$^+$ steady-state RNA. (B) Poly(A)$^+$ lanes from panel A were exposed to X-ray film for four times longer than in panel A (96 h).

efficient. We therefore conclude that a significant fraction of c-*myc* RNA does not bind to oligo(dT)-cellulose and that this unbound c-*myc* RNA is relatively stable when compared with the lability of the poly(A)$^+$ c-*myc* RNA fraction (Fig. 5).

When examining whole-cell RNA we would expect to observe a decrease in the 2.4-kb c-*myc* species relative to the 2.2-kb c-*myc* RNA species after actinomycin D treatment, since the more labile, poly(A)$^+$ c-*myc* RNA is larger than the majority of the c-*myc* RNA that does not bind to oligo(dT)-cellulose, 2.4 versus 2.2 kb. In general, this is what was observed (Fig. 1). However, it should be noted that the amount of poly(A)$^+$ c-*myc* relative to the amount of non-oligo(dT)-cellulose-bound c-*myc* may vary severalfold among experiments. In each experiment a significant amount of c-*myc* RNA that did not bind to oligo(dT)-cellulose was observed, consisting of a minor 2.4-kb species and a major 2.2-kb species.

The data in Fig. 5 were quantitated by densitometry, and the results are presented graphically in Fig. 6. The approximate half-life of the poly(A)$^+$ c-*myc* RNA is 12 min. The half-life of the poly(A)$^-$ c-*myc* cannot be precisely determined from these data but must be greater than 1 h (Fig. 6). We repeated this experiment using time points at 10-min intervals and found very similar results (data not shown). Others have previously described the rapid turnover of poly(A)$^+$ c-*myc* RNA, but these authors did not present experiments on the non-oligo(dT)-cellulose-bound c-*myc* RNA (9, 10). The non-oligo(dT)-cellulose-bound c-*myc* RNAs must either have short poly(A), ≤30 nt, or lack poly(A) entirely. We directly examined poly(A)$^+$ and poly(A)$^-$ c-*myc* RNA 3′ ends. In agreement with our data on actinomycin D-chased RNA samples (Fig. 4A), the poly(A)$^-$ and poly(A)$^+$ c-*myc* RNAs had similarly sized 3′-end fragments, the majority of which mapped at or near the second poly(A) addition site (Fig. 4B). Further, since the *Cla*I-*Eco*RI probe is specific to c-*myc* RNAs that have the same strandedness as c-*myc* mRNA, it may be concluded that HL-60 poly(A)$^+$ and poly(A)$^-$ RNAs have similar levels of mRNA-homologous transcripts.

Further test of structural relatedness of poly(A)$^+$ c-*myc* RNA and poly(A)$^-$ c-*myc* RNA. If differences in the transcribed regions of the poly(A)$^+$ and poly(A)$^-$ c-*myc* RNA exist, then we reasoned that a size comparison of deadenylated poly(A)$^+$ c-*myc* RNA and poly(A)$^-$ c-*myc* RNA should help to reveal these differences. Any poly(A) tracts that would be present in total, poly(A)$^+$, and poly(A)$^-$ HL-60 cell RNAs were removed by annealing with oligo(dT)$_{15}$ and digestion with RNase H. RNase H-digested and undigested samples were analyzed for c-*myc* RNA by Northern blotting

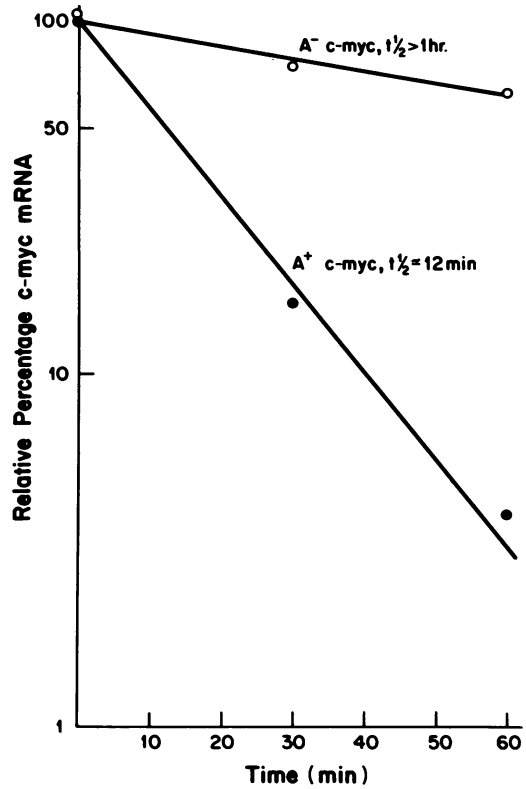

FIG. 6. Semi-log plot of c-*myc* poly(A)$^+$ RNA and poly(A)$^-$ RNA turnover. Densiometric scans of c-*myc* RNAs from Fig. 5 were compiled, and for each c-*myc* RNA species, the steady-state level was taken as 100%.

(Fig. 7). Deadenylation of total HL-60 RNA revealed a major 2.2-kb species and a minor 2.4-kb RNA (Fig. 7, compare lanes 1 and 2).Similarly, deadenylation of the poly(A)$^+$ c-*myc* RNA reduced the diffuse 2.4-kb species to a major 2.2-kb RNA and a minor 2.4-kb RNA (Fig. 7, compare lanes 3 and 4). The c-*myc* RNA species present in the poly(A)$^-$ RNA fraction (whether treated with RNase H or not) appeared identical in size to both the deadenylated total RNA and the deadenylated poly(A)$^+$ RNA. From these results we conclude that the poly(A)$^+$ c-*myc* RNA is very similar, if not identical, in size to the poly(A)$^-$ c-*myc* RNA except for the presence of a poly(A) tract.

Subcellular location of poly(A)$^+$ and poly(A)$^-$ c-*myc* RNA. To assay whether the polyadenylation status of c-*myc* RNA determines or correlates with transport from the nucleus to the cytoplasm, we prepared HL-60 cell nuclear and cytoplasmic RNAs (18). These RNAs were further fractionated into poly(A)$^+$ and poly(A)$^-$ RNA and analyzed by Northern blotting. TPI mRNA was also probed to examine the nuclear and cytoplasmic distribution of a long-lived, poly(A)$^+$ mRNA. The data are consistent with a mainly cytoplasmic distribution of c-*myc* poly(A)$^+$ and poly(A)$^-$ RNA (Fig. 8). Similarly, a majority of the TPI mRNA was found in the cytoplasm. The cytoplasm of HL-60 cells was further fractionated by sucrose density centrifugation. Both the poly(A)$^-$ c-*myc* RNA and the poly(A)$^+$ c-*myc* RNA were present in the polyribosome fractions and were not found in the subribosomal fractions (data not shown). The small yet substantial fraction of nuclear TPI and nuclear c-*myc* RNAs [both poly(A)$^+$ and poly(A)$^-$] is most likely due to cytoplasmic contamination. Supporting this conclusion we observed rRNA in this nuclear RNA preparation (data not shown). It is clear, however, that polyadenylation status does not correlate with subcellular localization. We therefore concluded that the RNA turnover properties of poly(A)$^+$ and poly(A)$^-$ c-*myc* RNAs are related to adenylation or lack thereof and are not related to being located in the cytoplasm or nucleus.

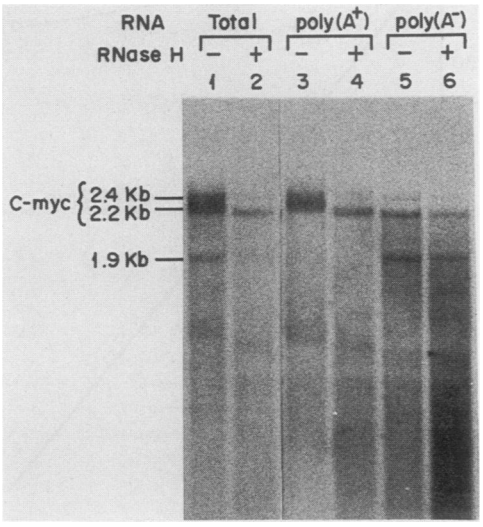

FIG. 7. Sizes of total cellular c-*myc* RNA, poly(A)$^+$ c-*myc* RNA, and poly(A)$^-$ c-*myc* RNA before and after deadenylation. Total cellular, poly(A)$^+$, and poly(A)$^-$ HL-60 RNAs were hybridized to oligo(dT)$_{15}$ and incubated either with or without RNase H. Northern blot analysis was done as described in the text. Lanes: 1, 10 μg of untreated total cellular RNA; 2, 10 μg of total cellular RNA treated with RNase H; 3, 10 μg of untreated poly(A)$^+$ RNA; 4, 10 μg of poly(A)$^+$ RNA treated with RNase H; 5, 10 μg of untreated poly(A)$^-$ RNA; 6, 10 μg of poly(A)$^-$ RNA treated with RNase H. The blot was exposed to film for 5 days. The 1.9-kb RNA is 18S rRNA that shows some nonspecific hybridization to the c-*myc* DNA probe.

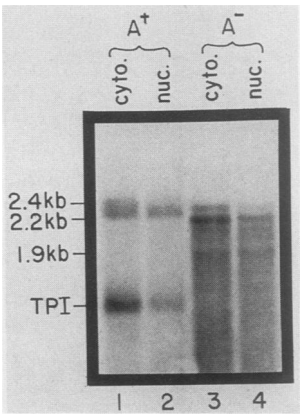

FIG. 8. Northern blot detection of TPI and c-*myc* poly(A)$^+$ and poly(A)$^-$ RNAs in the cytoplasm and nucleus of HL-60 cells. RNA was prepared from cytoplasm and nuclei of approximately 2×10^8 cells. RNA was further separated into poly(A)$^+$ and poly(A)$^-$ RNA. Lanes: 1, poly(A)$^+$ RNA from the cytoplasm of approximately 2×10^6 cells; 2, poly(A)$^+$ RNA from the nuclei ofthe 2×10^6 cells; 3, poly(A)$^-$ RNA from the cytoplasmic fraction; 4, poly(A)$^-$ RNA from the nuclear fraction. c-*myc* and TPI probes were used.

Nonadenylated c-*myc* RNA in other cell types. Since HL-60 cells contain 10 to 20 copies of the c-*myc* gene (8, 11) we cannot exclude the possibility that these two populations of c-*myc* transcripts are derived from different c-*myc* genes. These RNAs might therefore differ in nucleotide sequences other than the length of their poly(A) tracts. These other nucleotide differences might cause differences in polyadenylation and stability. For example, in β^0-thalassemia a single nucleotide deletion produces an extremely labile β-globin mRNA (17, 20). However, Northern blot analysis of RNA from normal and leukemic human bone marrow cells (data not shown) as well as HeLa cells and normal mouse fetal liver (cells without an amplified c-*myc* gene) consistently showed poly(A)$^+$ and poly(A)$^-$ c-*myc* RNA species (Fig. 9). There are nearly equal amounts of 2.4- and 2.2-kb poly(A)$^-$ c-*myc* RNA species in HeLa cells. The structural difference(s) between these two RNAs is at present unknown. Half-life studies have also been performed with HeLa cells in culture. c-*myc* poly(A)$^+$ RNA was more labile than poly(A)$^-$ c-*myc* RNA (data not shown). The half-lives of both the poly(A)$^+$ and non-oligo(dT)-cellulose-bound c-*myc* RNA species in HeLa cells are similar to that observed in HL-60 cells (S. G. Swartwout and A. J. Kinniburgh, unpublished data). Therefore, for two different cultured cell lines the turnover properties and adenylation status of c-*myc* RNA are similar.

From all the above data we conclude that poly(A) length is inversely correlated with c-*myc* RNA stability. Whether it is the direct cause or tightly correlated with a particular nucleotide sequence difference (as might be produced by differential splicing or similar but nonidentical transcribed 3′ ends) is at present unknown. It is also possible that the poly(A) tail must interact with a sequence in the transcribed portion of the c-*myc* RNA to produce the extreme lability of the poly(A)$^+$ c-*myc* RNA. Whether these two species arise

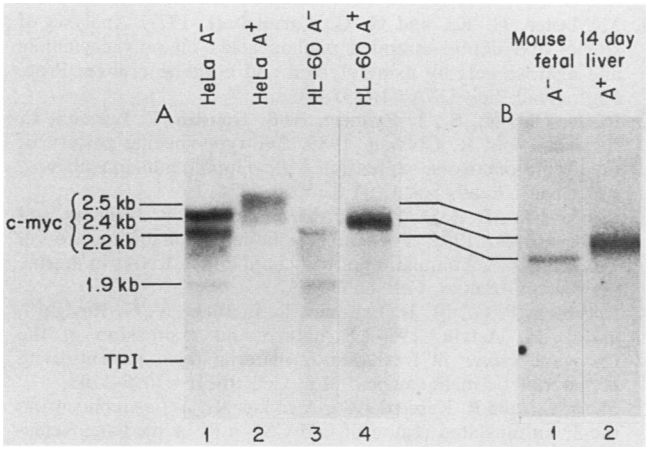

FIG. 9. Northern blot detection of c-*myc* poly(A)+ and poly(A)- RNAs from HeLa cell, HL-60 cell, and mouse fetal liver. (A) The following RNA samples were analyzed: lane 1, the poly(A)- RNA from 20 μg of HeLA whole-cell RNA; lane 2, the poly(A)+ RNA from 20 μg of HeLa whole-cell RNA; lane 3, the poly(A)- RNA from 20 μg of HL-60 whole-cell RNA; and lane 4, the poly(A)+ RNA from 20 μg of HL-60 whole-cell RNA. Lanes 1 and 2 were exposed for 6 days, and lanes 3 and 4 were exposed for 1 day. (B) The following samples were analyzed: lane 1, the poly(A)- RNA from 20 μg of mouse 14-day-old fetal liver RNA; lane 2, the poly(A)+ RNA from 20 μg of mouse 14-day-old fetal liver RNA. The blot in panel B was exposed to film for 7 days.

independently or whether one is a precursor of the other is also not known. However, our data argue against a large-scale interconversion of poly(A)+ and poly(A)- c-*myc* RNA species in rapidly growing cells. For example, if there was substantial conversion of the poly(A)+ c-*myc* RNA to poly(A)- c-*myc* RNA in HL-60 cells, then in our actinomycin D chase experiments we would expect to observe biphasic turnover kinetics for the poly(A)- c-*myc* RNA. The initial poly(A)- turnover would appear to be slower than at a time when little poly(A)+ RNA remains to be converted to poly(A)- c-*myc* RNA. Since we observed stochastic turnover kinetics for the poly(A)- c-*myc* RNA pool, we conclude that in vivo conversion of poly(A)+ c-*myc* RNA to poly(A)- c-*myc* RNA does not occur to a large extent. Our data do not directly address the possible conversion of poly(A)- c-*myc* RNA to poly(A)+ c-*myc* RNA by, for example, cytoplasmic polyadenylation. However, even if all poly(A)- c-*myc* was to become adenylated before turnover, this process could only contribute a small fraction of c-*myc* RNA to the poly(A)+ c-*myc* RNA pool. This is demonstrated by the low level of poly(A)+ c-*myc* RNA present in HL-60 cells in which transcription has been blocked for 60 min, even though substantial amounts of nonadenylated c-*myc* RNA coexist in these cells (Fig. 5 and 6). Further experiments will be needed to examine the question of selective interconversion of c-*myc* RNA species.

DISCUSSION

There are conflicting reports on the role of poly(A) in mRNA turnover. Deadenylated mRNAs have been reported to be both as stable as and more labile than adenylated mRNAs (16, 26). These experiments were performed by injecting in vitro-deadenylated mRNAs into *Xenopus* oocytes and assaying translatability. How these results relate to the present, in vivo situation is unclear, particularly since mRNAs that are normally adenylated and then deadenylated may have different properties than mRNAs that in vivo lack long poly(A). In any case, we are presently performing experiments to determine whether c-*myc* mRNA lacking long poly(A) is translated in HL-60 cells. In addition, the exact sequence of the labile and stable c-*myc* mRNA is being determined by cDNA cloning and sequencing techniques.

Recently Shaw and Kamen (25) have found an AU-rich sequence which is present in granulocyte-macrophage colony-stimulating factor mRNA and several other mRNAs including c-*myc*. When this sequence is inserted into the 3′ untranslated region of a rabbit β-globin gene, the β-globin mRNA transcribed from this gene is extremely labile. Since β-globin mRNA is nearly quantitatively adenylated and c-*myc* has both poly(A)+ and poly(A)- species, we may speculate that poly(A) must interact with a putative AU-rich sequence for rapid c-*myc* RNA turnover to occur. The greater stability of the poly(A)- c-*myc* would result from the lack of this interaction.

In summary, we identified a stable c-*myc* RNA species that lacks long poly(A) in addition to a labile, poly(A)+ c-*myc* RNA, identified by others (9, 10). The 5′ ends of the stable transcripts map to the P2 promoter site, and their 3′ ends map at or near the second poly(A) addition site. There is also a third c-*myc* transcript, initiated at the P1 promoter site. Other workers have utilized poly(A)+ RNA for their c-*myc* RNA stability studies and therefore they could not have observed the stable c-*myc* RNA that lacks long poly(A) (9, 10). The cellular need for both stable and labile populations of c-*myc* RNA is not known. We are currently investigating changes in these c-*myc* RNA species in growth-stimulated cells, differentiating cells, and leukemic cells to determine their role(s), if any, in these processes.

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