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Transcriptional arrest within the first exon is a fast control mechanism in c-myc gene expression

Dirk Eick and Georg W.Bornkamm

Institut für Virologie, Zentrum für Hygiene der Universität Freiburg, Hermann-Herder-Str. 11, 7800 Freiburg, FRG

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ABSTRACT

DMSO (dimethylsulfoxide), a potent inducer of granulocytic differentiation in HL60 cells, causes a rapid decrease of cytoplasmic steady state c-myc RNA. This decrease is regulated mainly at the level of transcript elongation. Elongation is blocked within the untranslated c-myc leader. Twelve hours after transcriptional shut off of c-myc, DNAase I hypersensitive site II was still detectable, indicating that closing of this site upstream of the gene does not correlate with reduction in the steady state level of c-myc RNA.

INTRODUCTION

In many naturally occuring as well as experimentally induced tumours the cellular proto-oncogene c-myc is perturbated in one of several ways including amplification (1-3), retrovirus insertion (4-6) or chromosomal translocation (7-10). In Burkitt's lymphoma and mouse plasmacytoma, the chromosomal translocation has fused the c-myc locus to one of the immunoglobulin constant regions, an event, which is believed to lead to deregulation of the c-myc gene expression (8-10, 11).

In many cases of t(8;14) translocations in man and t(12;15) translocation in mice, the breakpoints are located inside or in the direct vicinity of the c-myc gene suggesting that separation of the gene body from its physiological promoters or upstream regulatory sequences may be an important mechanism leading to c-myc deregulation (10).

However, in a number of cases including the variant translocations the breakpoints are far away upstream or downstream of c-myc (12-20) making a mechanistic interpretation of how the c-myc gene could be deregulated by the chromosomal translocation, difficult.

Attempting to understand, how the chromosomal translocations affect the regulation of the c-myc gene (21), we realized that we need to learn much more about the physiological regulation of the c-myc gene in order to be able to evaluate the role of the chromosomal translocations.

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Therefore we took advantage of cellular systems in which c-myc expression can be easily manipulated, to study in detail at which cellular level c-myc expression is regulated. c-myc RNA is inducible in resting lymphocytes and fibroblasts by the addition of mitogens and growth factors (22, 23). In some rapidly growing hematopoetic or embryonic cell lines, such as Friend erythroleukemia cells (24) the human promyelocytic leukemia line HL60 (25) and the mouse teratocarcinoma line F9 (23) c-myc expression can be efficiently shut off by the addition of drugs which induce differentiation, such as DMSO, sodium butyrate, retinoic acid and others.

We have used the promyelocytic leukemia line HL60 (26) which has an amplified c-myc gene (1, 2) and expresses large amounts of c-myc RNA during proliferation, to study the mechanism of c-myc down regulation upon induction of differentiation by DMSO (25). We demonstrate that a block of transcript elongation is the dominating mechanism leading to transcription arrest of the c-myc gene. When this manuscript was in preparation Bentley and Groudine (27) reported similar results and demonstrated a transcript elongation block of c-myc RNA at the boundary exon1/intron1 in HL60 cells after treatment with retinoic acid.

MATERIALS AND METHODS

Cell lines. HL60 is a promyelotic cell line (26). BL2 is an EBV negative Burkitt lymphoma cell line with a t(8;22) translocation (28).

Extraction and Northern blot analysis of total cellular RNA. RNA was isolated from logarithmically growing cells, cultured in RPMI 1640 medium (Gibco) supplemented with 10% foetal calf serum to a densitiy of 5 x 10^5 cells per ml. RNA was extracted from the cells by the lithium/urea method . 15 µg RNA was separated on a denaturing agarose-formaldehyde gel and transferred to nitrocellulose or Zeta-Probe. Filters were hybridized with cloned DNA fragments labelled with ^{32}P by nick translation (29). Prehybridization, hybridization and washing were as described (30) without using dextran sulfate and with 400 µg/ml heat denatured tRNA as a carrier.

Nuclear run-on transcription. The nuclear extracts were made as described by Greenberg and Ziff (31) with slight modifications. Cells were washed three times in ice-cold phosphate buffered saline. The pellets of 1 x 10^8 cells were resuspended in 10 mM TRIS-HCl, pH7.4, 10 mM NaCl, 3 mM $MgCl_2$, 0.5% (v/v) NP40 and incubated for 5 minutes on ice. After centrifugation at 500 g the supernatant was discarded and the nuclear pellet washed by resuspension in 10 ml of the same buffer followed by a second centrifugation. The nuclei

were then resuspended in storage buffer (50 mM Tris-HCl, pH 8.3; 40% (v/v) glycerol, 5 mM $MgCl_2$, and 0.1 mM EDTA) and frozen in liquid nitrogen in portions of 100 µl corresponding to the nuclei of 10^7 cells. For nuclear run-on RNA synthesis, the nuclei were mixed with 100 µl reaction buffer (10 mM TRIS-HCl, pH8.0, 5 mM $MgCl_2$, 300 mM KCl, 0,5 mM of each ATP, CTP, GTP, and 100 µCi of (α-^{32}P) UTP (800 Ci/mmol, Amersham) and incubated for 25 minutes at 28°C. Subsequently, DNAase I was added to a final concentration of 10 µg/ml and the incubation continued for 5 minutes at 28°C. After addition of 20 µl 10% SDS, 10 µl proteinase K (5 mg/ml, preincubated at 37°C for 1 hour) and 200 µl STE buffer (0.5% SDS, 100 mM Tris-HCl, pH 7.5, and 50 mM EDTA) the samples were incubated for 1 hour at 37°C. Nuclear transcripts were separated from unincorporated nucleotides on a Sephadex G50 column equilibrated with 10 mM TRIS-HCl pH 7.5, 1 mM EDTA, and 1% SDS. The labelled RNA was boiled for 5 minutes, chilled on ice and hybridized to DNA immobilized on nitrocellulose filters (30). After hybridization the filters were washed twice for 30 minutes at 37°C in 2 x SSC, 0.1% SDS, once in 2 x SSC, and twice in 2 x SSC containing 10 µg/ml RNase A (boiled for two minutes before use). The filters were air dried and exposed to a Kodak X-Omat AR film using an Dupont Cronex Lightning Plus Intensifying Screen.

S1 analysis. A single stranded 862 b PvuII-PvuII probe specific for the first exon of the c-myc gene cloned in M13 and labelled with ^{32}P by primer extension (50.000 cpm) (32) was hybridized with 30 µg total cellular RNA. Hybridization was carried out in 80% formamide, 400 mM NaCl, 40 mM PIPES, pH 6.5, 1 mM EDTA at 56°C for 16 hours followed by S1 treatment as described by Berk and Sharp (33) and separation of fragments on 5% denaturing polyacrylamide gels. Fragments of 513 and 351 bases of the probe are protected corresponding to the RNAs initiated at c-myc promoter P1 and P2, respectively.

Purification and DNAaseI treatment of nuclei. Approximately 2 x 10^8 cells were harvested, washed in ice-cold PBS and resuspended in 40 ml ice-cold lysis buffer (40 mM KCl, 10 mM NaCl, 10 mM $MgCl_2$, 10 mM TRIS-HCl, pH 7.5, 0.5% NP40). The cells were lysed for 7 minutes on ice, and the nuclei subsequently spun down at 2000 rpm in a Sorvall SS34 rotor for 10 minutes. The pellet was washed once again in lysis buffer, resuspended in 5 ml of the same buffer and immediately aliquoted into tubes at 500 µl each. Control tube 1 received 50 µl DNAaseI buffer (1 mM $MgCl_2$, 5 mM $CaCl_2$). Tubes 2 to 10 received additionally 0.5, 1, 2, 4, 8, 16, 30, 50, and 100 U DNAaseI (Merck). Nuclei were digested for 4 min at 23°C, and the reaction

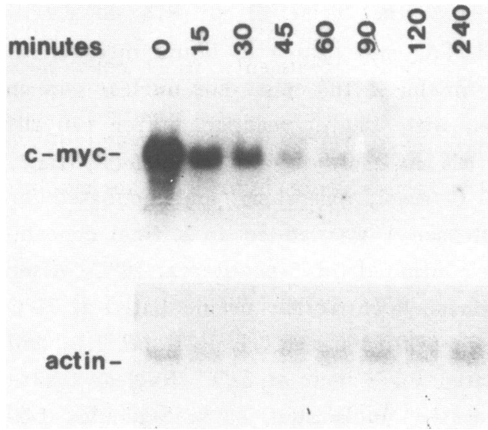


Figure 1. Northern blot analysis of total RNA from HL60 cells after DMSO treatment. HL60 cells were incubated in the presence of 1.25% DMSO. RNA was extracted from aliquots at various times after addition of DMSO and analyzed on Northern blots hybridized with the third exon c-myc probe (Figure 2, probe b) (upper part), or with β-actin probe (lower part).

was stopped by adding 2 ml STE solution (100 mM TRIS-HCl, pH 7.5, 50 mM EDTA, 0.5% SDS) supplemented with 1 mg Proteinase K. After incubation at 37°C for 2 hours DNA was isolated by several phenol/CIA extractions and ethanol precipitations.

RESULTS

Downregulation of c-myc expression is a very early event in HL60 differentiation. The disappearance of c-myc RNA during HL60 differentiation has been amply demonstrated. We were interested to see when during the course of differentiation, c-myc RNA disappears. Figure 1 shows a Northern blot of RNA harvested at different times after addition of 1.25% DMSO which induces granulocytic differentiation within four days in almost 100% of the cells. Apparently, the steady state c-myc RNA starts to decrease immediately after the addition of the inducer and is down to less than one fourth of the initial amount of uninduced cells after already 30 minutes. The analysis of the amount of c-myc RNA in Northern blots gives an estimate of the steady state c-myc RNA levels, but does not allow to decide whether the decrease in c-myc RNA is due to a block of transcription or to increased degradation of c-myc RNA, or eventually to both. Comparison of the c-myc RNA decrease by DMSO and by inhibiting transcription in the presence of actinomycin D revealed similar kinetics (data not shown), suggesting that DMSO might block transcription. To address this question more directly, the c-myc transcription was studied in a nuclear run-on assay.

DMSO induces discontinuous transcription of the c-myc gene. Figure 2 shows an experiment in which elongation of pre-initiated c-myc RNA was

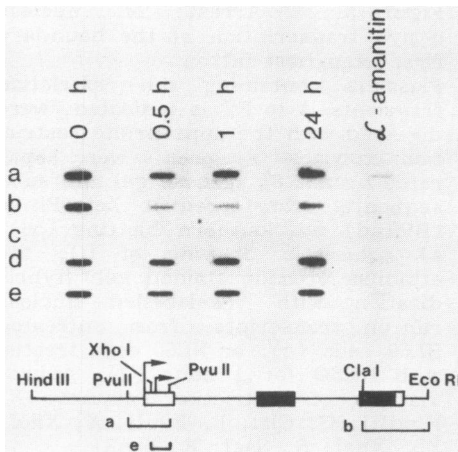


Figure 2. Transcriptional activity of the c-myc gene after DMSO treatment. HL60 cells were incubated for various times with 1.25% DMSO. Subsequently, nuclei were isolated for nuclear run-on experiments. ^{32}P-labelled transcripts were hybridized to immobilized plasmid DNAs, bound to nitrocellulose. Plasmids containing the following DNA fragments were used: a) 862bp PvuII-PvuII, first c-myc exon; b) 1.3 kb ClaI-EcoRI, third c-myc exon; c) vector DNA, pUC12 d) β-actin, and e) 440 bp XhoI-PvuII, internal sequences of the first c-myc exon. In the right lane nuclei of untreated HL60 cells were incubated with α-amanitin (0.25 μM).

studied in nuclear extracts prepared at different times after the addition of DMSO. Hybridization of the labelled RNA to DNA of the third exon revealed that already 30 minutes after addition of DMSO, transcription of the third exon had reached the bottom level of 5 to 10% of that in uninduced cells. Surprisingly, hybridization with a cloned PvuII-PvuII fragment covering almost the complete first exon and sequences upstream thereof, revealed only an about 50% decrease in transcription of the first exon. Transcription of the first as well as the third exon was completely or almost completely abolished in the presence of α-amanitin indicating that both regions are transcribed by RNA polymerase II. The results shown in Figure 2 indicate that the c-myc gene is discontinuously transcribed after addition of DMSO with a much higher RNA polymerase II density on the first compared to the third exon.

The following experiments were designed to identify more closely the site of transcription arrest in the c-myc gene.

RNA elongation is blocked within the first exon in DMSO treated HL60 cells. To look more precisely to the site or sites of discontinuous transcription in the c-myc gene, labelled RNA synthesized in extracts of nuclei prepared without inducer (Fig 3c) or one hour after DMSO treatment (Fig. 3d), was hybridized to a Southern blot containing separated fragments covering the region from the HindIII site upstream of the first exon down to the SacI site in the second intron. Comparison of the hybridization pattern of RNA from untreated and DMSO treated nuclei clearly demonstrated, that all fragments downstream of the PvuII site at the end of the first exon (D, E, and F in Figure 3) lighted up only faintly after induction, whereas the inten-

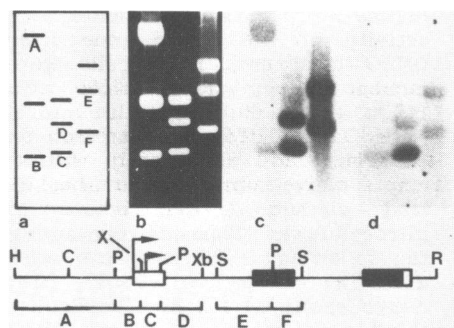


Figure 3. Arrest of nuclear c-myc transcription at the boundary first exon/first intron.
Plasmids containing the restriction fragments A to F, as indicated, were digested with the appropriate restriction enzymes. Fragments were separated on a 1.8% agarose gel and subsequently transferred to Zeta-Probe (Biorad) by Southern blotting (56). a) schematic drawing of b), the ethidium bromide stained gel. Hybridization with ^{32}P-labelled nuclear run-on transcripts, from untreated HL60 cells (c), or HL60 cells treated with DMSO for 1 hour (d). Abbreviations of restriction enzymes: H, HindIII; C, ClaI; P, PvuII; X, XhoI; Xb; XbaI; S, SacI; R, EcoRI.

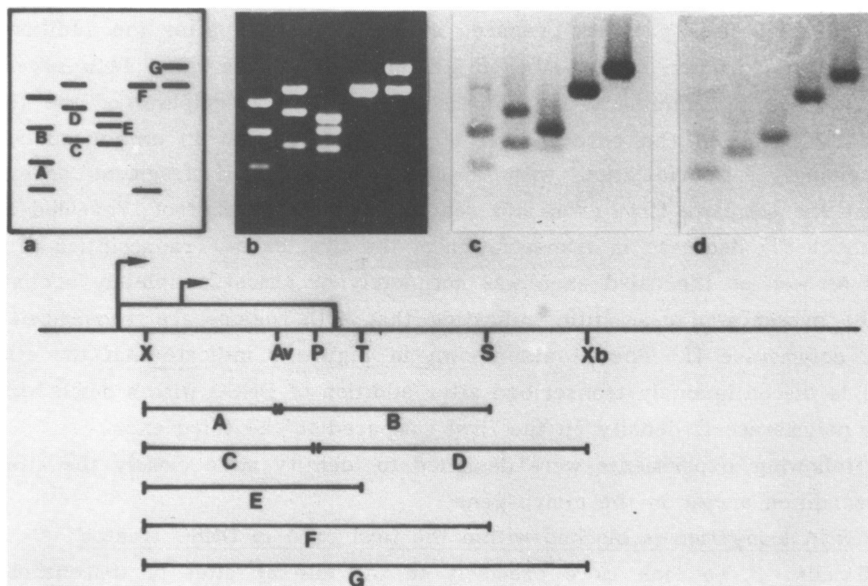


Figure 4. Mapping of the elongation block of c-myc transcription within the first exon.
Plasmid DNA containing the XhoI-XbaI fragment with sequences of the first c-myc exon and intron was cut with restriction enzymes that generate fragments A to G, as schematically shown in (a). Ethidium bromide stained gel after gel electrophoresis (b). Hybridization with ^{32}P-labelled run-on RNA of untreated HL60 cells (c), and cells treated for one hour with DMSO (d). Abbreviations of restriction enzymes: X, XhoI; Av, AvaI; P, PvuII; T, TaqI; S, SacI; Xb, XbaI.

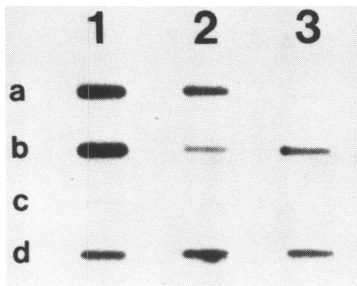


Figure 5. Removal of the elongation block in the first exon by proflavine. For experimental details see Figure 2. Origin of labelled nuclear run-on RNA: HL60 cells without DMSO (1); HL60 cells treated for 1 hour with DMSO (2); HL60 cells treated for 1 hour with DMSO and additionally with 80 μM proflavine for five minutes before harvest (3). Hybridization was carried out to plasmids containing the first c-myc exon (a), third c-myc exon (b), pUC12 vector DNA (c), β-actin (d).

sity of signals of the first exon (B and C) were almost unchanged with and without DMSO treatment. Using the cloned XhoI-XbaI fragment spanning the boundary from first exon to intron, and the restriction enzymes AvaI, PvuII, and TaqI, the site of transcription arrest was further narrowed down.
The signal on fragment E (XhoI-TaqI) (Fig. 4) was diminished substantially after induction, thus locating the block to the left of the TaqI site. The intensity of hybridization to fragment C (XhoI-PvuII) decreased slightly after DMSO treatment, while fragment D disappeared almost completely. Almost no change was observed in the signal of fragment A (XhoI-AvaI), while fragment B again disappeared almost completely after induction. This indicates that the site, where transcription is not allowed to proceed after DMSO treatment, is before or close to the AvaI site.

Proflavine allows readthrough. The intercalating agent proflavine prevents processing of RNA as well as transcription termination, presumably by interfering with the formation of RNA secondary structure. In SV40 infected cells, proflavine can allow readthrough of the RNA polymerase into the SV40 late gene VP1 by releasing a transcription elongation block (34).
To test whether proflavine affects the elongation block within the first exon, HL60 cells were treated with DMSO for one hour and proflavine was added five minutes before preparation of nuclei. The proflavine treatment was restricted to a short period of time to minimize secondary effects of the drug as inhibition of RNA and protein synthesis. The nuclear run-on analysis of this experiment is shown in Figure 5. The addition of proflavine before harvest abolished almost completely the signal obtained with the first exon probe, whereas the signal with the third exon was slightly increased, and β-actin appeared to be unaffected. We interpret this as an indication that the RNA polymerases move on along the gene in the presence of proflavine. This interpretation is compatible with the fact that the polymerase density on the third exon has increased after the proflavine pulse as indicated by the slight

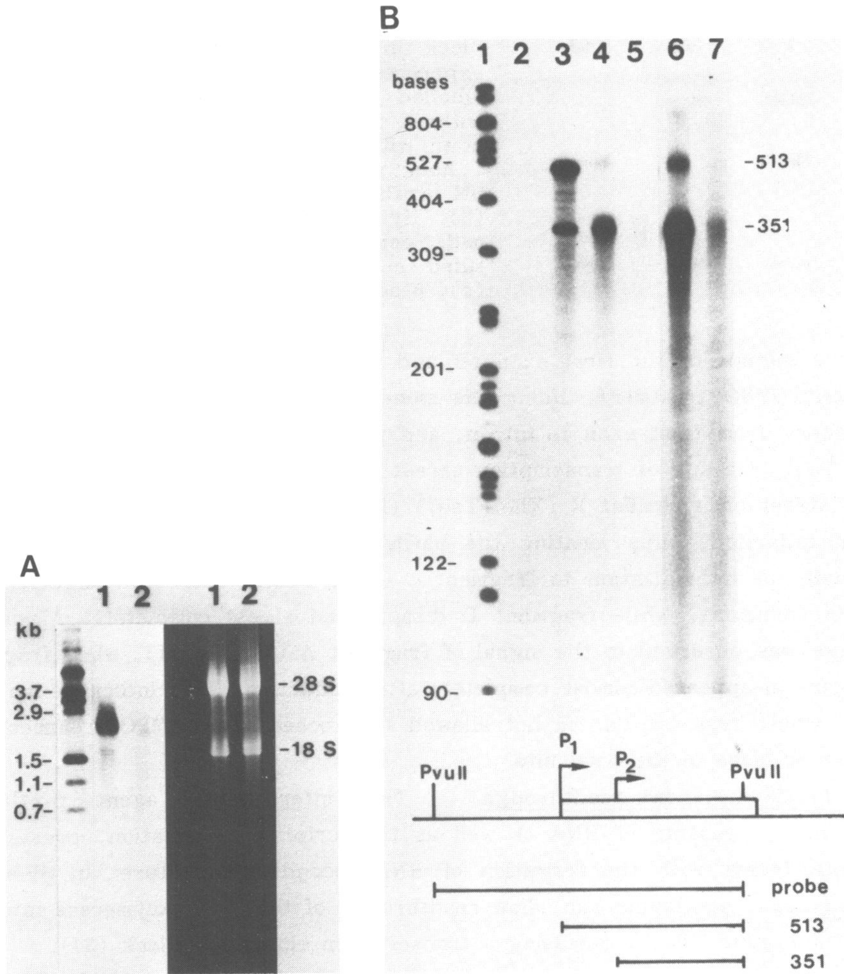


Figure 6. No short RNAs of the first c-myc exon are detectable in total cellular RNA of DMSO treated HL60 cells by Northern (A) and S1 analysis (B).

A. 20 µg of total cellular RNA was separated on a denaturing 1.5% agarose-formaldehyde gel and transferred to Zeta-Probe. HL60 RNA from cells without DMSO (1) and from cells treated for 1 hour with DMSO (2) was hybridized with a nick translated first exon specific, 862 bp PvuII-PvuII probe (see Figure 2, a). The ethidium bromide stained gel before transfer is shown at the right.

B. A 862 bp PvuII-PvuII fragment, cloned in M13 mp 10 and labelled with ^{32}P by primer extension was used as a probe for hybridization to 30 µg cytoplasmic RNA of the variant t(8;22) Burkitt lymphoma line BL2 (lane 3), 50 µg total RNA of HL60 cells treated without (lane 4), and with DMSO for one hour (lane 5). Lane 6 and 7 show a long exposure of lane 4 and 5. Lane 1 contains a molecular weight standard of end-labelled HpaII fragments of the lymphotropic papova virus (LPV). The faint signal (at about 860 bases) in lane 6 is derived from a transcript initiated upstream of P1 and P2 (27).

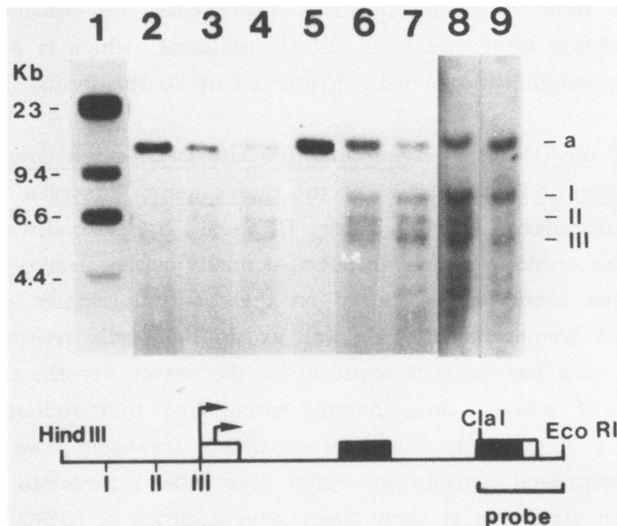


Figure 7. The disappearance of DNAase I hypersensitive site II is a late event in HL60 differentiation.

DNA isolated from nuclei of untreated cells (lane 2 to 4), and from nuclei of cells treated with DMSO for 1 hour (lane 5 to 7), for 12 hours (lane 8) and for 4 days (lane 9), was digested with EcoRI, run on a 0.8% agarose gel, blotted onto nitrocellulose filter and hybridized with a third exon probe (ClaI-EcoRI) labelled with ^{32}P by nick translation. Nuclei of lane 2 and 5 were incubated without DNAase I, in lane 3 and 6, with 1.6 units and those of lane 4 and 7 with 3 units DNAaseI. Lane 8 and 9 contain DNA isolated from mixtures of nuclei incubated with 1.6 and 3 units DNAase I.

increase in the intensity of the signal obtained with the third exon probe.

Small transcripts of c-myc exon1 are not detectable. The block in RNA elongation observed at the end of the first exon could be caused by two different mechanisms, by premature transcription termination or by pausing of the polymerases. Both mechanisms imply that short transcripts of the first exon should be present in the cell (35).

Attempts to detect such small RNAs of the first exon are shown in Figure 6. In Figure 6A, a Northern blot containing RNA of uninduced cells and of cells treated with DMSO for one hour was hybridized with a first exon probe. Apparently, no small RNAs of the first exon could be detected in DMSO treated cells. To search for smaller RNAs, which might have not been detectable in Northern blots, total cellular RNA of uninduced and induced cells was studied by S1 analysis using the 862 bases PvuII-PvuII fragment as a probe (Figure 6B). Again, no distinctly terminated RNA molecules could be identified. At a long exposure (lane 7) only a faint smear starting at around

350 bases was detected. This probably represents the remainder of c-myc RNA still detectable after one hour DMSO treatment, which is initiated at the second c-myc promoter and is fully protected up to the PvuII site at the end of the probe.

Closing of the DNAase I hypersensitive site II does not correlate with transcriptional downregulation of the c-myc gene. Upstream of the dual c-myc promoters, three major DNAaseI hypersensitive sites were described in the chromatin of a transcriptionnally active c-myc gene (36-38). Site II has been found to be closed on the transcriptionally inactive c-myc allele in Burkitt lymphoma cells, as well as in HL60 cells treated with DMSO for four days, and has been thought to be the reason for the transcriptional downregulation of c-myc. Once having established that reduction of c-myc expression is a very early event after DMSO treatment, we have looked whether transcriptional activity of c-myc does indeed correlate with changes in the chromatin structure at early times after addition of DMSO. As shown in Figure 7, the DNAaseI hypersensitive site II continues to be present within 12 hours after DMSO treatment and disappears only after prolonged DMSO treatment. The closing of site II is thus not the cause of transcriptional downregulation of the c-myc gene, but rather a later event.

DISCUSSION

We have studied the mechanism of c-myc downregulation in HL60 cells following induction of differentiation by DMSO. We have shown that the cytoplasmic steady state c-myc RNA starts to decrease immediately after addition of DMSO and is below 5% of the untreated control after 60 minutes. The decrease in c-myc RNA is caused by a block of transcription, which probably starts immediately after addition of DMSO and which is complete after 30 minutes as shown in nuclear run-on experiments. The steady state c-myc RNA decreased with a normal half-life of 10 to 15 minutes (39) after DMSO treatment indicating that DMSO did not speed up the degradation of c-myc RNA, as reported for c-myc RNA in Daudi cells treated with interferon (40).

When using a number of probes to study the transcription rate along the c-myc gene, we made the unexpected observation that the gene is not transcriptionally shut off as a whole, however, that it is transcribed discontinuously after DMSO treatment. The first exon remains transcriptionally active after addition of the drug, at about half the rate of untreated HL60 cells, whereas the transcription rate of the second and third exon is dramatically reduced after induction of differentiation, a finding recently discribed for retinoic acid induced HL60 cells (27). The position where the polymerase

density decreases within the c-myc gene, was mapped to a region around the AvaI site within the first exon. Upstream of the AvaI site, the polymerase density was about the same in untreated and DMSO treated cells and decreased by a factor of about 10 downstream of the PvuII site.

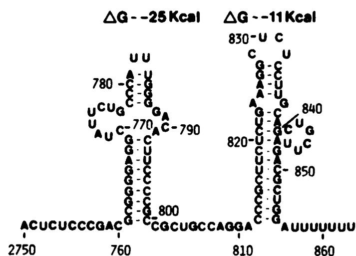
To assess the role of RNA secondary structure for the block in transcript elongation after DMSO treatment we studied the effect of proflavine on c-myc transcription. Proflavine is known to prevent RNA processing (41, 42) as well as transcription termination (43) presumably by interfering with RNA secondary structure. In SV40 infected cells, proflavine was shown to cause readthrough of the RNA polymerase II into the VP1 gene (34). The VP1 gene is negatively regulated at the transcriptional level by a protein (the agnoprotein) which causes premature transcription termination (34, 35) a phenomenon with striking similarity to gene regulation by attenuation in E. coli (44). In SV40 infected cells proflavine allows the polymerase to continue transcription, presumably by removing the secondary structure of the RNA which caused the polymerase to pause or terminate.

In DMSO treated HL60 cells, a short proflavine pulse for 5 minutes before harvest, abolished the signal obtained with the first exon. This is most easily explained by assuming that proflavine removed the secondary structure of the RNA, thus allowing readthrough of the RNA polymerase, while transcription initiation is blocked. This interpretation is supported by the fact that following proflavine treatment the signal obtained with the third exon increased slightly, suggesting that some of the polymerases which were mobilized by proflavine, are still detectable further downstream.

The analogy between the SV40 model and c-myc transcription termination is further strengthened by comparison of possible alternative conformations of the leader regions of the VP1 and c-myc gene. The VP1 leader can form two alternative conformations, a readthrough conformation containing a single stem and loop structure, and an attenuation conformation in which the same sequences can form two stem and loop structures (35). In the second conformation one stem and loop is reported to be a polymerase pausing site, while the second is acting as a transcription termination signal.

Two very similar alternative conformations can be drawn from the regions around the AvaI and PvuII sites within the first c-myc exon: a readthrough conformation with one stem and loop, and alternatively, two stem and loop structures followed by an oligo-U stretch (Figure 8). Just to this region of the c-myc gene the discontinuity of transcription has been assigned to. Once having mapped the site of transcription arrest in the c-myc gene, we

A. Attenuation



B. Readthrough

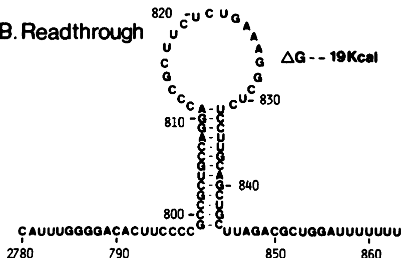


Figure 8. Two alternative c-myc RNA conformations around the PvuII site (position 2850) in the first exon. (A) Attenuation conformation showing a typical pausing signal (left stem and loop) and termination signal (right stem and loop) followed by seven U-residues.
(B) Readthrough confirmation with one stem and loop excluding conformation (A). Both alternative conformations are structurally and energetically very similar to those in the leader of SV40 VP1 m-RNA (35).
The Δ G was calculated as described by Tinoco et al. (57), the sequence and residue numbers are from Gazin et al. (58).

searched for small RNAs derived from the first exon. Such small "attenuator" RNAs have been found in SV40 infected cells and represent the upstream part of the VP1 leader (35). Using Northern blot as well as S1 analysis we were unable to detect such small transcripts derived from the first exon in DMSO treated HL60 cells.

There are two possibilities to explain our failure to detect these small RNAs. Firstly, these RNAs may be immediately degraded when they are generated because they might lack an appropriate 3' end for stabilization. Secondly, the RNA polymerase is pausing upstream of the elongation block and can only move on under artificial conditions in isolated nuclei.

What could be the significance of gene regulation at the level of transcript elongation?

First of all, this type of regulation is extremely fast and reasonably efficient. Secondly, a gene downregulated by transcription arrest should be reactivatable as rapidly as it is shut off. The early events following the addition of the differentiation inducer are in fact reversible for about 24 hours, as demonstrated by the maintainance of the clonogenic potential of the cells (45, 46) and the reappearance of the c-myc RNA upon removal of the drug (47). Interestingly, we found no change in the chromatin structure upstream of the c-myc gene within the first hour after addition of DMSO and observed the closing of DNAaseI hypersensitive site II only after prolonged presence of the drug. It will be interesting to see whether closing of site II

correlates with the irreversible commitment of the cells to differentiation.

In lymphocytes and fibroblasts c-myc RNA is efficiently inducible by mitogens and growth factors and can, on the other hand, be down-regulated either by starvation or by various drugs inducing growth arrest and differentiation, such as DMSO and HL60 cells, which were studied here. It is not clear, whether c-myc regulation is taking place at the same or at different levels in the various systems. Conflicting reports exist in the literature stressing regulation at either the transcriptional (31, 47-49) as well as post-transcriptional level in the various systems (40, 50-52). From the data reported here it is obvious that hybridization to a first exon or a cDNA probe will suggest another interpretation than hybridization to the third c-myc exon. To assess whether regulation of c-myc expression at the level of RNA elongation is a more general phenomenon, it will be important to study the various systems which allow modulation of c-myc RNA, with a set of carefully selected probes.

It is a characteristic feature of Burkitt lymphoma cells that only the c-myc gene on the chromosome affected by the translocation gives rise to c-myc transcripts while the normal allele is silent. Introduction of an active myc gene into a cell leads to shut-off of the cellular c-myc counterpart (53-55) indicating that the c-myc gene product is directly or indirectly involved in its own feedback control (11, 59, 60).In Burkitt lymphoma lines with the breakpoint distant from the c-myc gene including the variant translocations, mutations have been regularly observed in that part of the first exon where the elongation block is located (around the PvuII site) (14, 60, 62-66). It is therefore of interest whether regulation at the level of RNA elongation plays a role for c-myc autoregulation.

We have recently shown that sodium butyrate (47) as well as DMSO (Eick et al., in preparation) are capable of down-regulating c-myc expression in Burkitt lymphoma cells at a transcriptional level, even if the c-myc gene is separated from its physiological promoters. If we infer that both, sodium butyrate and DMSO inhibit RNA elongation, down regulation of c-myc in Burkitt lymphoma cells implies that this type of regulation is not totally abolished by the translocation and that c-myc RNA elongation can still be blocked at other sites of the gene, downstream of the first exon. Nevertheless, by destruction of the regulatory site in the first exon, the fine regulation of c-myc expression in response to physiological growth regulating factors might be severely perturbated in Burkitt lymphoma cells.

Gene regulation by attenuation in the bacterial as well as the SV40 model

implies that, alternatively to the actual product of the gene, a small protein or peptide can be synthesized, whose synthesis regulates transcription arrest versus elongation by interacting with RNA secondary structure. The human c-myc gene has an open reading frame for a putative highly basic protein of 20 K in the first exon (58), whose AUG is located in front of the promoters P1 and P2, thus excluding that its synthesis is promoted by the two major c-myc RNA species initiated at P1 and P2. A c-myc RNA initiated at a promoter located further upstream has recently been demonstrated (27). We have detected this RNA only in marginal amounts in the batch of HL60 cells used in these experiments (Figure 6B), however, in considerable quantity in several Burkitt lymphoma lines (Eick, unpublished results). Therefore the question arises, whether such a protein encoded by the first exon does in fact exist, and if so, whether it is involved in regulation of transcription arrest versus elongation.

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