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Transcriptional control of the endogenous *MYC* protooncogene by antisense RNA

(*MYC* oncogene/cell differentiation)

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ABSTRACT **A plasmid carrying antisense human *MYC* DNA and the gene encoding *Escherichia coli* xanthine/guanine phosphoribosyltransferase (*Ecogpt*) was introduced into human promyelocytic leukemia cell line HL-60 by protoplast fusion. High-level expression of antisense MYC RNA was obtained by selecting cells resistant to progressively higher levels of mycophenolic acid over a period of >6 months. The constitutive production of MYC protein in clones producing high levels of antisense MYC RNA was reduced by 70% compared to parental HL-60 cells. Inhibition of *MYC* expression was observed not only at the translational but also at the transcriptional level, implying that antisense RNA can regulate transcription of the *MYC* gene. The *Pst* I–*Pvu* II fragment (920 base pairs) of the *MYC* leader sequence is the primary transcriptional target of the antisense RNA. The suppression of endogenous *MYC* gene expression by antisense RNA decreases cell proliferation and triggers monocytic differentiation.**

To investigate the biological functions associated with a gene sequence, a valuable genetic approach is to introduce cloned DNA encoding antisense RNA into cells to specifically eliminate the gene product of interest or to block its function (1–4). In the present work we examine the effects of antisense *MYC* transcripts on the constitutive expression of the *MYC* gene and show that a human antisense *MYC* gene stably introduced into the human promyeloleukemia cell line HL-60 can inhibit not only MYC protein synthesis but also transcription of the endogenous *MYC* gene. The decreased transcription of *MYC* appears to commit HL-60 cells to monocytic differentiation without the help of a chemical inducer, suggesting a functional role for *MYC* in differentiation.

MATERIALS AND METHODS

Plasmid Construction. Antisense and sense MYC plasmids pSVgptC5-8 contain the MYC coding sequences cloned in the antisense and sense orientation relative to a simian virus 40 promoter plus the selective marker gene *Ecogpt* (*Escherichia coli* xanthine/guanine phosphoribosyltransferase). Plasmid pC5-8 (ref. 5), which contains a functional MYC cDNA [2.4-kilobase-pair (kbp) *Bam*HI fragment] without exon 1, was the source of the *MYC* sequence. Plasmid structures were confirmed by hybridization with the corresponding single-stranded [α-^{32}P]dCTP-labeled 300-bp *Pst* I–*Cla* I fragment of *MYC* exons 2 and 3 and by DNA sequencing (6). Antisense and sense plasmids pSVH-2K^b and pSVα-globin carry the H-2K^b gene on a 5.0-kbp *Nru* I–*Eco*RI fragment (7) or the 5′-end of the α-globin 68-mer (8), respectively, in place of *MYC*. The strategy of all constructions was to clone *Ecogpt* into pSVMdhfr (9) and to replace the dihydrofolate reductase gene with *MYC* or other coding sequences.

DNA Transformation. Cells were transfected with plasmid DNAs (20 μg) by the protoplast fusion method (10). Two days later, selection for the ability to grow in Dulbecco's modified Eagle's medium (DMEM) containing mycophenolic acid (25 μg/ml), aminopterin, xanthine, hypoxanthine, and thymidine was performed as described by Mulligan and Berg (11). Transformants resistant to a high dose of mycophenolic acid (110 μg/ml) were obtained by culturing cells in DMEM plus mycophenolic acid over a period of 6 months during which the mycophenolic acid concentration was increased at 2-week intervals. Secondary and tertiary transformants were obtained by retransfecting a primary and a secondary transformant, respectively, with plasmid DNA (10 μg/ml) followed by culturing in DMEM containing mycophenolic acid (110 μg/ml). Control antisense and sense clones (pSV2gpt$^+$, pSVgptC5-8, pSVH-2K^b, and pSVα-globin) were obtained by the cotransfection with pSV2gpt$^+$. The same amplification protocol and the repeated transfection were used as described above.

RNA Isolation and Analysis. Total RNA, poly(A)$^+$ mRNA, and cytoplasmic and nuclear RNAs were prepared by the standard protocols (12, 13). RNA gel blot transfer and hybridization were carried out as described by Maniatis *et al.* (13). [α-^{32}P]dCTP-labeled single-stranded probes were prepared from restriction fragments containing β-actin cDNA (14) or exons 2 and 3 of MYC cDNA by exonuclease III digestion, reverse transcriptase treatment (15), and strand separation (13).

RNase Protection. Total RNA (10 μg) was digested with RNases A and T_1. RNase-resistant samples were hybridized with a uniformly radiolabeled [α-^{32}P]UTP-labeled RNA probe for sense MYC RNA prepared by SP6 polymerase (Bethesda Research Laboratories) as described (2, 3). Digestion with RNase-free DNase 1 (Bethesda Research Laboratories) (250 μg/ml) was carried out prior to applying the samples to a 1.2% agarose gel.

Detection of MYC Protein. Transformants were incubated at 37°C for 8 hr with 500 μCi of [^{35}S]methionine (1 Ci = 37 GBq). Preparation of cell lysates, immunoprecipitation by rabbit anti-human MYC antibody, NaDodSO$_4$/polyacrylamide gradient gel electrophoresis (5.4–12%) and fluorography were described (16). MYC antibodies (purchased from Oncor, Gaithersburg, MD, or prepared ourselves) were affinity purified (16). The β-actin antibody was purchased from Bio-Yeda (Rehovot, Israel).

Chloramphenicol Acetyltransferase (CAT) Activity and *in Vitro* Run-On Transcription. CAT derivative pMyc-CAT (10 μg) plus 0.2 μg of pCH110 (17) were used to transfect cells by the DEAE-dextran method (18). After incubation for 48 hr, cell extracts were incubated with 0.8 μCi of [^{14}C]chloramphenicol and acetyl coenzyme A (Pharmacia P-L Biochemi-

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Abbreviation: CAT, chloramphenicol acetyltransferase.

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cals) for 60 min. CAT activity was measured by thin-layer chromatography (19), and β-galactosidase activity was assayed in an *ortho*-nitrophenyl β-D-galactopyranoside (Sigma) as substrate (20). RNA chain elongation in isolated nuclei was performed as described (21).

Other Methods. Cytochemical assays for α-naphthyl acetate esterase and naphthol AS-D chloroacetate esterase were carried out as described (22). Indirect immunofluorescence staining was performed with fluoroscein isothiocyanate-conjugated goat anti-human IgG (Miles) and monoclonal antibodies OKM-1 and OKM-5 (Ortho Diagnostics).

RESULTS

Selection of the Antisense *MYC* Transformants. Stable antisense *MYC* HL-60 transformants were obtained by transfecting with pSVgptC5-8 and selecting for mycophenolic acid resistance.

We used two strategies to try to increase the level of antisense *MYC* transcripts in the transformants. First, the resistance to mycophenolic acid of a primary transformant (AM93) was gradually increased by successive rounds of selection with greater drug concentrations over a 6-month period. Next, secondary (AM93-4) and tertiary (AM93-4-12) transformants were established by successively retransfecting AM93 (6 months) and AM93-4 with antisense *MYC* DNA. To determine whether these procedures resulted in elevated levels of antisense transcripts, RNA was extracted from the transformants and analyzed for antisense MYC RNA by RNA gel blotting. RNA extracted from AM93 after 1 month (Fig. 1*a*, lanes 5 and 9), 2 months (lane 8), 3 months (lane 7), and 6 months culture (lanes 6 and 2) showed gradually increasing amounts of a 2.5-kilobase (kb) transcript; the clone cultured for 6 months contained 5–10 times more antisense MYC RNA than the clone cultured for 1 month. RNA from the secondary (lane 3) and tertiary (lane 4) transformants showed a prominent band at 2.5 kb; these clones contain 10–20 times more antisense MYC RNA than AM93 cultured for 1 month. A quantitative comparison of the amounts of antisense and sense MYC RNA in various clones is given in Table 1. These data clearly show that selection for resistance to high concentrations of mycophenolic acid and repeated transfection with antisense MYC plasmid DNA have resulted in increased levels of antisense MYC RNA. Examination of

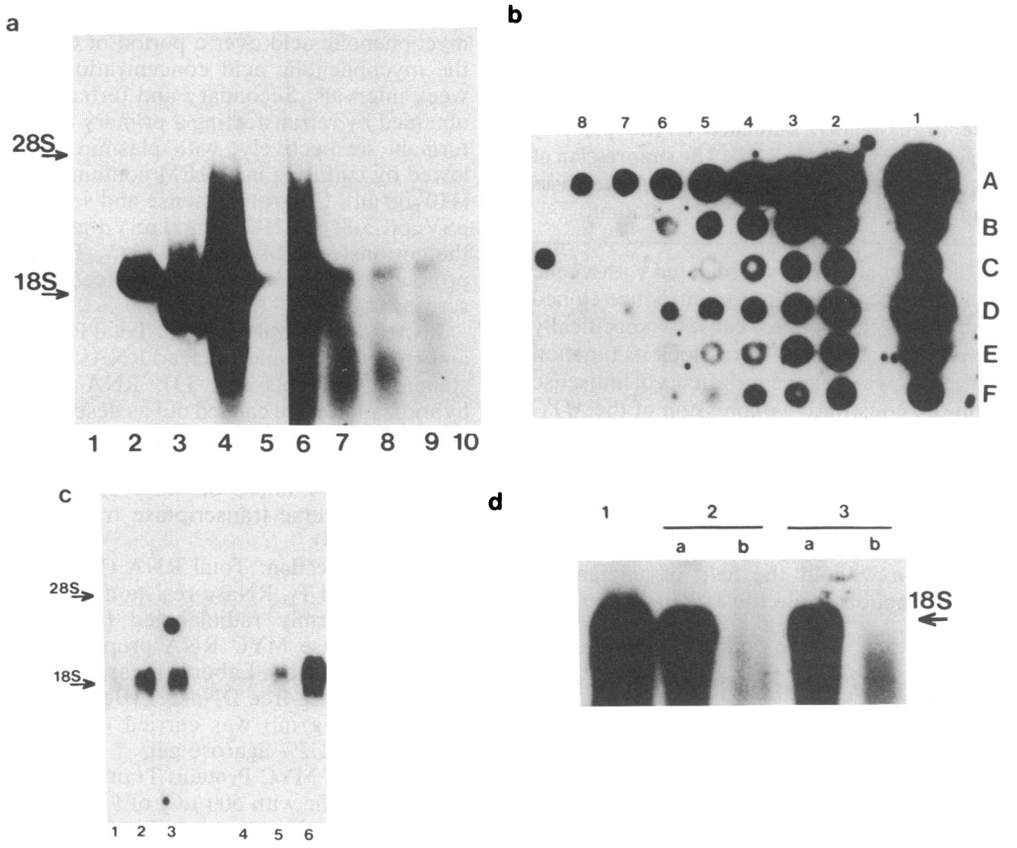

FIG. 1. Hybridization analysis of RNA transcripts. (*a*) Hybridization of RNAs from HL-60 cells and antisense *MYC* transfectants to an antisense MYC probe. Total RNA (10 μg) from the indicated clones was electrophoresed on a 1.2% agarose gel containing 1 M formaldehyde and hybridized to a single-stranded antisense *Pst* I–*Cla* I fragment of MYC exons 2 and 3. Lanes: 1, HL-60; 2 and 6, primary antisense clone AM93 cultured for 6 months (110 μg/ml of mycophenolic acid); 3, secondary antisense clone AM93-4 cultured for 3 months; 4, tertiary antisense clone AM93-4-12; 5 and 9, AM93 cultured for 1 month (35 μg/ml); 7, AM93 cultured for 3 months (95 μg/ml); 8, AM93 cultured for 2 months (65 μg/ml); 10, pSV2gpt$^+$ clone. The arrows on the left indicate 28S and 18S rRNAs. Autoradiography was on Kodak X-Omat S film using Dupont Cronex Quartz intensifying screens at −70°C for 6 hr (lanes 1–5) or 24 hr (lanes 6–10). (*b*) Dot hybridization of mRNAs from antisense *MYC* transformants using a sense MYC probe. Serial dilutions of 50 μg of poly(A)$^+$ mRNA (columns 1–8) were spotted onto nitrocellulose in a microsample filtration manifold (Schleicher & Schuell) and hybridized to a single-stranded ^{32}P-labeled sense MYC DNA probe. Rows: A, HL-60; B, pSV2gpt$^+$ clone; C, AM93-4-12 (tertiary transformant); D, AM93 (1 month); E, AM93 (6 months); F, AM93-4 (secondary transformant). (*c*) RNA·RNA duplex formation analyzed by RNase protection. Lanes: 1 and 4, pSV2gpt$^+$ clone; 2 and 5, AM451-6-30; 3 and 6, AM93-4-12 (the arrows on the left indicate 28S and 18S rRNAs); 4, 5, and 6, RNA was treated with DNase I (250 μg·ml^{-1}) (Bethesda Research Laboratories) prior to the protection analysis. (*d*) Subcellular distribution of antisense RNA. RNase-resistant samples from antisense clones were analyzed by hybridization with uniformly radiolabeled sense MYC RNA as described in Fig. 1*c*. Lanes: 1, 10 μg of AM93-4-12 total cellular RNA; 2a, 10 μg of nuclear RNA from AM93-4-12; 2b, AM93-4-12 cytoplasmic RNA (equivalent to 10 μg of nuclear RNA); 3a, 10 μg of nuclear RNA from AM451-6-30; 3b, AM451-6-30 cytoplasmic RNA derived from a cell number identical to that which yielded 10 μg of nuclear RNA.

Table 1. Summary of sense and antisense transformants

Cells	mRNA, cpm × 10^{-3}			DNA, copy number		p64 protein, cpm × 10^{-3}		Growth rate, hr per cell cycle
	S MYC	Actin	AS MYC	S MYC	AS MYC	MYC	Actin	
HL-60	20.5	18.1	0.1	25	—	2.7	4.2	33
Control pSV2gpt$^+$	17.5	17.2	0.1	22	—	2.5	4.4	36
Sense transformant								
pSVgptC5-8	49.2	16.9	0.4	165	—	3.8	5.3	38
pSVH-2K^b	16.9	16.3	—	25	—	2.5	4.8	36
pSVα-globin 68	18.5	17.0	—	20	—	2.6	4.4	34
Antisense transformant								
AM93 1 month	17.5	17.2	3.1	20	20	2.2	3.9	—
2 months	14.4	—	9.3	—	25	2.0	—	—
3 months	11.3	—	19.4	—	40	1.8	—	—
6 months	6.2	15.4	31.1	20	55	1.6	4.1	—
AM93-4	5.1	16.0	50.6	22	100	1.0	4.0	—
AM93-4-12	3.3	14.9	77.8	20	120	0.6	4.0	135
AM2-3-91	10.3	17.0	27.2	20	170	1.7	3.9	95
AM46-3-2	4.2	15.2	66.9	20	160	0.8	4.0	120
AM451-6-30	4.5	15.6	62.2	22	250	0.8	4.0	125
AM763-11-4	5.1	16.3	46.7	25	200	1.0	4.0	110
AM966-10-64	6.8	15.8	42.8	20	200	1.4	4.1	105
pSVH-2K^b	17.5	17.1	—	20	—	2.6	4.1	36
pSVα-globin 68	18.1	16.0	—	25	—	2.6	4.5	38
pSV2gpt$^+$	18.5	17.2	—	20	—	2.6	4.1	34

Values are means of four experiments. Protein levels and mRNAs of antisense (AS) and sense (S) MYC and actin were measured by counting radioactivity in bands cut out of $NaDodSO_4$ gels (protein) or out of RNA gel blot filters (mRNA). Copy number of the integrated DNA was determined by comparison with known amounts of MYC plasmid DNA (equivalent to 10^{-6} to 10^{-9} molecules).

genomic DNA isolated from the clones demonstrated that amplification of the antisense *MYC* gene has occurred, resulting in 100–250 copies in the tertiary transformants (Table 1).

RNA·RNA Duplex Formation *in Vivo*. To investigate antisense RNA-induced RNA·RNA duplex formation, we examined transformants for the presence of double-stranded RNAs. The gpt$^+$ control clone (Fig. 1*c*, lanes 1 and 4) contained no detectable double-stranded MYC RNA, whereas a distinct 2.2-kb band was detected in the antisense clones (lanes 2 and 3). This protected band was found in the nuclear rather than in the cytoplasmic RNA fraction of the cells (Fig. 1*d*, lanes 2 and 3). The 2.2-kb band appears to be due to the presence of RNA·RNA hybrids in the antisense clones rather than to RNA·DNA heteroduplexes because it is not affected by treatment of the RNA samples with DNase I prior to digestion with RNases A and T_1 (Fig. 1*c*, lanes 5 and 6).

p64 Protein Level in Antisense *MYC* Transformants. Antisense and sense *MYC* transformants were incubated with [^{35}S]methionine for 8 hr to analyze MYC protein synthesis under steady-state conditions. Six representative antisense transformants showed a significant reduction in the amount of *MYC* gene product compared to control clones (Table 1). Assuming that the pool size of methionine is almost the same in all clones, ≈70% less p64 was observed in lysates prepared from the antisense transformants than in the controls. Correcting for cell number, we estimate that the amount of p64 per cell was reduced by >90% in the antisense transformants. This reduction in the steady-state level of p64 is probably not due to increased turnover of the protein in the antisense transformants because the half-life of p64 is about the same in the antisense and control clones (30 min in HL-60 cells and in the pSV2gpt$^+$ transformant; 30–45 min in the antisense clones). The relative amount of p64 in antisense and control clones was not significantly changed by correcting for differences in the half-life of p64 and in the pool sizes of [^{35}S]methionine (data not shown).

Control experiments showed that the reduction in the amount of MYC protein in the antisense transformants apparently depends specifically on the presence of antisense *MYC* sequences. Control antisense clones were constructed and established by transfecting HL-60 cells with plasmids identical to pSVgptC5-8 except that they contained antisense–H-2K^b, antisense–α-globin 68-mer, or antisense–*Ecogpt*$^+$ in place of antisense *MYC* and were screened by the identical protocol as the antisense *MYC* transformants (≈200 copies of the respective plasmid DNAs). These clones exhibited no detectable reduction in p64 synthesis and showed no differentiation markers, indicating that decreased *MYC* expression requires antisense *MYC* sequences and is not merely an artifact of mycophenolic acid selection or of culture conditions. A second control experiment showed that the level of actin protein synthesis is essentially unchanged in all transformants (Table 1).

The HL-60 cell cycle is 30–36 hr under our conditions. In contrast, HL-60 cells transfected with the antisense *MYC* gene grow at a rate of 95–135 hr per cell cycle. This great reduction in growth rate is specific to the antisense *MYC* clones, as the other clones we have constructed grow normally (Table 1). This result suggests that inhibiting synthesis of the *MYC* gene product might cause a substantial alteration in the growth machinery of the cell.

Reduced *MYC* Gene Expression May Commit HL-60 Cells to Differentiate to Monocytes. Immunofluorescence staining and cytochemical studies demonstrated a close correlation between a decrease in the amount of MYC protein in cells and an increase in the number of cells with a monocytic phenotype (Table 2). This correlation suggests that a reduction in *MYC* expression may be the initial committed event in the differentiation of HL-60 cells to adherent monocytes. Although reduced *MYC* expression may not be sufficient to commit HL-60 cells to monocytic differentiation, the presence of antisense *MYC* appears to change the developmental potential of HL-60 cells biasing the cells toward the monocytic pathway in preference to granulocytic development.

Transcriptional Control of the *MYC* Gene. Gene fusion studies were carried out to explore the effect of antisense MYC RNA on constitutive transcription of *MYC*. Two

Table 2. Comparative human MYC protein level and relative cell number of the differentiated phenotypes

Cells	S *MYC* expression,* ratio × 10^{-3}	% reactive cells M†	G‡	OKM-1	OKM-5
HL-60	1.11	0	0	0	0
HL-60 + PMA	—	99	0	95	99
HL-60 + Me_2SO	—	0	98	3	4
Control pSV2gpt$^+$	1.18	12	15	5	6
Transformants					
Sense pSVgptC5-8	1.45	15	18	8	10
Antisense					
AM2-3-91	0.22	27	10	32	29
AM46-3-2	0.10	56	13	61	63
AM93-4-12	0.08	72	12	80	88
AM451-6-30	0.08	58	14	67	74
AM763-11-4	0.22	34	15	49	53
AM966-10-64	0.10	41	17	54	69

Values are means of four experiments. Phorbol 12-myristate 13-acetate (PMA) and dimethyl sulfoxide (Me_2SO) were used as specific inducers of monocyte and granulocyte, respectively. S, sense.
*The ratio of the levels of MYC protein and mRNA per cell were calculated from data such as in Table 1, correcting for differences in cell number and in incorporation rate of methionine.
†Percent of positive cells by staining of α-naphthyl acetate esterase.
‡Percent of positive cells by staining of naphthol AS-D chloroacetate esterase.

fragments of the *MYC* promoter, the *Hin*dIII–*Pvu* II fragment (3 kbp) and the *Pst* I–*Pvu* II fragment (920 bp), were tested in a promoter-probe vector carrying the *E. coli* CAT gene. In parental HL-60 cells and in the pSV2gpt$^+$ clone, weak CAT activity was observed with the larger fragment of the *MYC* promoter (*Hin*dIII–*Pvu* II), while the smaller fragment (*Pst* I–*Pvu* II) produced strong CAT activity (Fig. 2). CAT assay experiments using *MYC* promoter deletion mutants demonstrated that the *Pst* I–*Pvu* II fragment included enhancer-like DNA sequences, since this element stimulated transcription when present upstream or downstream of homologous or heterologous promoters and in either orientation with respect to the direction of transcription (data not shown).

In antisense *MYC* transformants, surprisingly, the enhancer-carrying promoter fragment (*Pst* I–*Pvu* II) gave less CAT activity than the *Hin*dIII–*Pvu* II fragment (less than a factor of 15) (Fig. 2). S1 mapping studies demonstrated that the transcriptional initiation site in antisense transformants was not changed from the regular *P1* and *P2* transcriptional start sites of the *MYC* promoter. Run-on transcription assays using isolated nuclei were carried out to determine whether this inhibition of CAT expression was due to transcriptional control or to post-transcriptional events. As shown in Fig. 3, antisense clones showed decreased transcriptional activities of the *MYC* gene compared to parental HL-60 cells and pSV2gpt$^+$ clones. Thus, our results suggest that antisense MYC RNA may inhibit the expression of an endogenous *MYC* gene at both the translational (Table 1) and the transcriptional (Fig. 2) levels.

DISCUSSION

The results presented here suggest that high levels of cellular antisense MYC RNA can stably reduce the accumulation of sense MYC RNA and the synthesis of protein p64. Plasmids capable of producing *Ecogpt* and antisense MYC RNA were introduced into HL-60 cells by DNA transfer. Resistance to high concentrations of mycophenolic acid usually involves overexpression of *Ecogpt*, and, in the clones isolated in this study, it also resulted in an increase in the amount of antisense MYC RNA: selection for resistance to increasing concentrations of mycophenolic acid followed by repeated transfection

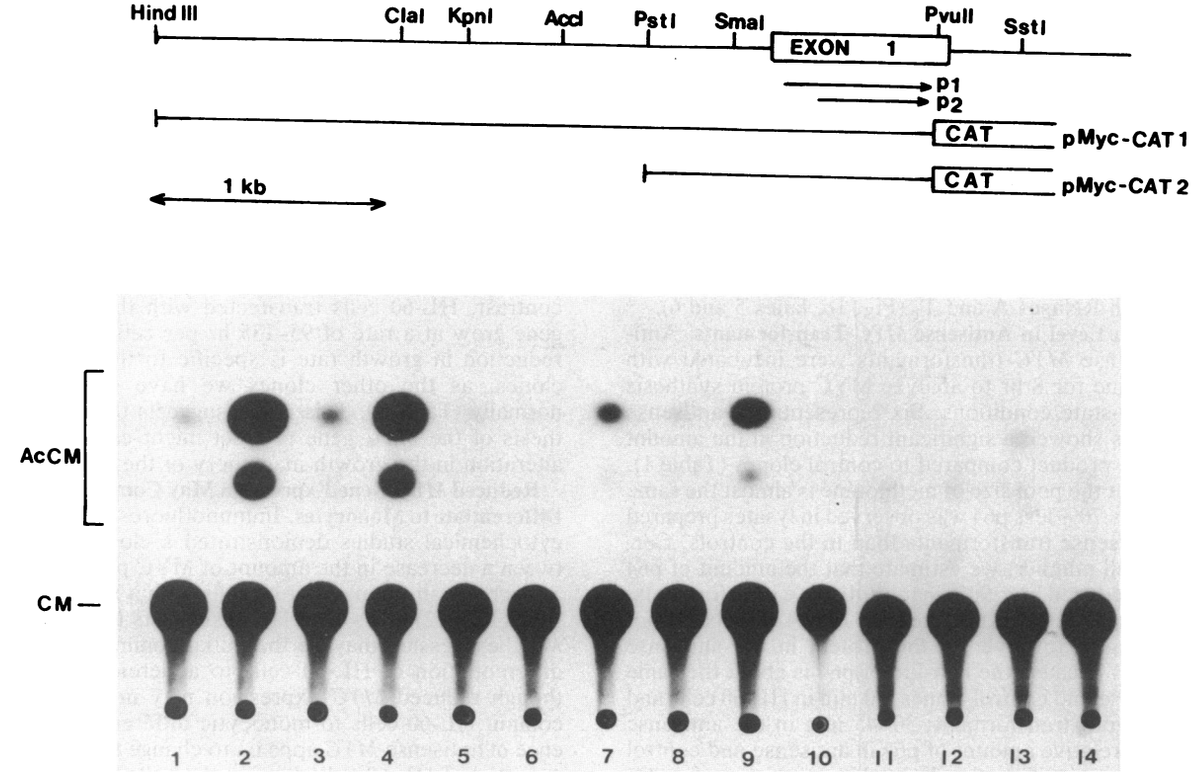

FIG. 2. Expression of the pMyc-CAT construct in HL-60, pSV2gpt$^+$ clone, and antisense *MYC* clones. CAT activity was measured by thin-layer chromatography pMyc-CAT1 (lanes 1, 3, 5, 7, and 9), pMyc-CAT2 (lanes 2, 4, 6, 8, and 10), pSVOCAT (lanes 11, 12, 13, and 14). CM, chloramphenicol; AcCM, acetylated chloramphenicol; *P1* and *P2*, transcriptional start sites of *MYC* gene. Lanes: 1 and 2, HL-60; 3, 4, and 11, pSV2gpt$^+$ clone; 5, 6, and 12, AM46-3-2; 7, 8, and 13, AM451-6-30; 9, 10, and 14, AM93-4-12.

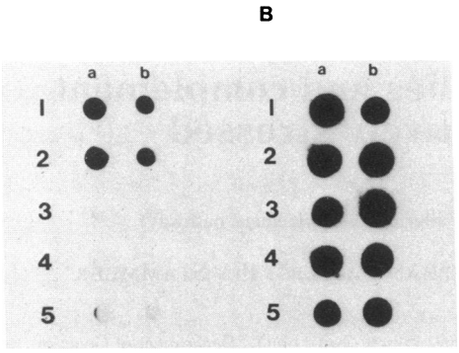

FIG. 3. *In vitro* run-on transcription of antisense *MYC* clones. ^{32}P-labeled transcriptional products (5×10^6 cpm/ml) were hybridized using a manifold dot apparatus (Schleicher & Schuell) onto filters containing 2 μg (columns a) or 1 μg (columns b) of linearized single-stranded probe. *A*, MYC probe (*Pst* I–*Pvu* II fragment); *B*, chicken β-actin probe (0.6-kb *Hin*dIII fragment containing the 3′-untranslated region). Rows: 1, HL-60; 2, pSV2gpt$^+$ clone; 3, AM46-3-2; 4, AM93-4-12; 5, AM451-6-30.

with plasmid DNA caused an increase in antisense RNA resulting from gene amplification (Fig. 1 and Table 1).

Growth inhibition was observed in cells that contain 100–250 copies of the antisense *MYC* DNA sequence. The amount of sense *MYC* DNA in the antisense transformants is 20–25 copies per cell. We find that a minimum ratio of 10:1 antisense to sense DNAs is required for inhibition of *MYC* expression in transfection studies; at this DNA ratio, there is 15- to 25-fold more antisense MYC RNA than sense MYC RNA.

The steady-state level of MYC protein in the antisense MYC clones (tertiary transformants) is reduced by >90% per cell compared to that in HL-60 cells. Control experiments in which cells were transformed with antisense–H-2K^b, antisense–α-globin 68-mer, or antisense–*Ecogpt* plasmids showed no significant inhibition of p64 synthesis. These results suggest that antisense RNA may exert an inhibitory effect on p64 protein synthesis by the formation of a stable antisense–sense RNA hybrid *in vivo*. Our detection of duplex RNA in the antisense *MYC* clones (Fig. 1 *c* and *d*) is consistent with this idea (2).

Reduced expression of MYC transcripts correlates with the triggering of HL-60 cell differentiation. It is well known that MYC mRNA is no longer present in HL-60 cells rendered granulocytic or monocytic differentiation by exposure to chemical inducers (23). Still unknown, however, is whether modulation in oncogene expression is required for cell-cycle progression or terminal differentiation of hematopoietic cell types. The results in Table 2 show a strong correlation between lowered *MYC* transcription and monocytic phenotype, suggesting that antisense MYC RNA directs HL-60 cells into the monocytic pathway in preference to the granulocytic pathway.

As shown in Fig. 1*b*, accumulation of sense MYC mRNA was diminished in antisense *MYC* transformants containing high amounts of antisense RNA. *In vitro* run-on transcription (Fig. 3) and the promoter-specific CAT assay (Fig. 2) demonstrated that the antisense transformants were clearly defective in promoter activity of the endogenous *MYC* gene and in the elongation of MYC RNA in isolated nuclei. These results suggest that MYC RNA transcription in the antisense clones is regulated by the formation of an RNA·RNA duplex in the nucleus. The regulatory sequence that seems to be recognized by antisense RNA was localized to the *Pst* I–*Pvu* II fragment of the promotor region of the *MYC* gene (Fig. 2). One way in which antisense RNA might suppress the transcriptional activities of the endogenous *MYC* gene is by affecting the positive enhancer-like region. Although the molecular mechanism of this repression of the *MYC* gene is not known, the synthesis of trans-acting negative regulatory elements that bind to the enhancer-like *Pst* I–*Pvu* II region might be triggered by RNA·RNA base pairing, by the direct association between antisense RNA and DNA, or by additional regulatory factors induced by the antisense RNA. We have identified 74-kDa and 110-kDa proteins in the nuclei of antisense clones that could be candidates for regulatory proteins of *MYC* gene expression (data not shown). Our run-on analysis revealed that transcriptional initiation from exon 1 can be blocked by antisense RNA.

Studzinski *et al.* (24) reported that the MYC protein is involved in DNA synthesis. Therefore, by interfering with *MYC* gene expression antisense *MYC* transcripts may also interfere with DNA synthesis. If so, it may be that the reduced expression of the *MYC* gene in our antisense clones caused a reduction in cell growth rate by repressing DNA synthesis. However, further experiments are required (*i*) to determine which function, the inhibition of DNA synthesis or the commitment to differentiation, is the direct target of antisense RNA-induced gene regulation and (*ii*) to understand the relationship between the effects of antisense MYC RNA described here and the role of the endogenous antisense *MYC* transcripts detected in HL-60 cells by Bentley *et al.* (25).

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