Inhibition of Messenger RNA Transcriptional Activity in ML-1 Human Myeloblastic Leukemia Cell Nuclei by Antiserum to a c-myb-specific Peptide

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ABSTRACT

Antiserum to a synthetic peptide that defines a hydrophilic region between the putative c-myb translation product was prepared in the rabbit. In lysates from exponentially growing ML-1, human myeloblastic leukemia cells, the antiserum ("anti-myb") reacted with five proteins of M, 58,000, 75,000, 85,000, 90,000 and 105,000. Of these, only p75 and a trace of p85 were detected, by immunoblotting, in extracts derived from ML-1 cell nuclei. The proteins p58, p75 and p90 were present in readily detectable amounts only in the relatively immature myeloid cell lines ML-1 and HL-60, whereas in the more mature myeloid cell line THP-1 and in the lymphoid line BALL-1 only traces of these proteins were found. p85 and p105 were detected in lysates from all cell lines tested, including myeloid and lymphoid leukemia cells and mouse 3T3 cells. In lysates from ML-1 cells induced to differentiate to monocyte/macrophages or to granulocytes, the concentrations of p58 and p75 decreased in parallel with the cell population moving to maturity; in completely mature populations these two proteins were no longer detectable. In ML-1 cells arrested in G, by serum depletion, the amount of p58 and p75 and to a smaller extent that of p90 was decreased, whereas the concentration of p85 and p105 remained unchanged. In nuclei from exponentially growing ML-1 cells, the antisemur or its derived immunoglobulin fraction ("anti-myb IgG") inhibited mRNA transcriptional activity by 30%. DNA synthesis was not affected. In contrast, in nuclei from differentiated ML-1 cells, mRNA transcriptional activity was not significantly inhibited by anti-myb IgG. Similarly, in nuclei from ML-1 cells arrested largely in G, by serum depletion for 2 days, mRNA transcriptional activity was inhibited by only 11%. Upon supplementation with serum, the mRNA transcriptional activity inhibitable by anti-myb IgG increased in parallel with the increasing rate of cell growth. The difference in total mRNA transcriptional activity observed in nuclei from cells of different growth rate was accounted for by the difference in transcriptional activity inhibitable by anti-myb IgG. This correlation between the rate of ML-1 cell growth, the concentration of a specific nuclear protein recognized by anti-myb IgG, and the extent of mRNA transcriptional activity inhibitable by the antibody suggests that a c-myb or c-myb-related product is involved in regulating the expression of some proliferation-related mRNAs.

INTRODUCTION

c-myb (1-10), the cellular homologue of the avian myeloblastosis and avian leukemia virus oncogene v-myb (2, 5, 6, 8, 9, 11-16), is expressed primarily in myeloid cells at early stages of their maturation (3, 15, 17-22) but its expression in other cell types such as CEF has been observed (23). The structure of human c-myb is similar to that found in chickens. It appears as a single locus in both species (6, 24-26), and gives rise to single RNA transcripts of 3.8 kilobases in chicken, and to 4.0-, 4.3-, or 4.5-kilobase transcripts in various human hematopoietic tissues (18, 19, 22, 24, 27). c-myb has been assigned to human chromosome 6 (24, 28, 29) and c-myb gene rearrangements have been observed to occur in various tumor cell lines (30-33). Chicken c-myb encodes a M, 75,000 (14) or a M, 110,000 protein (2), and p45^-v~^v (5, 6, 14, 34, 35) or p48^-v~^v (14) are truncated versions of the cellular protein. These proteins have been observed to be located in the nucleus in close association with the nuclear matrix (36-38).

In ML-1, a human myeloblastic leukemia cell line (25), c-myb expression is amplified (18, 32). This amplification correlates with enhanced levels of c-myb transcription and cell proliferation (18, 32), and c-myb expression declines as the cells are induced to differentiate (18). These observations suggested that, as in other cell types (23, 36, 38) c-myb product(s) may participate in the control of ML-1 cell proliferation. To explore this possibility, we examined the effect of an antiserum, directed against a c-myb-specific peptide, on DNA synthesis and transcription in nuclei from ML-1 cells at diverse stages of their growth and differentiation. We also investigated the localization of the proteins that are the targets of the antibody. The results of these studies are presented in this paper.

MATERIALS AND METHODS

Cells and Cell Culture

ML-1, a line of human myeloblastic leukemia cells; HL-60, a human promyelocytic leukemia cell line (39); THP-1, a human monocytic leukemia cell line (40); and BALL-1, a human lymphoid leukemia cell line (25) were maintained in RPMI 1640 medium supplemented with 10% FBS.4 Mouse 3T3 cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% FBS. Conditioned medium from pokeweed mitogen-stimulated human mononuclear cells was prepared as described in a previous report (41). To induce differentiation, ML-1 cells were harvested from logarithmically growing cultures and were resuspended at a concentration of 3 × 10⁶ cells/ml in the presence of the indicated concentration of conditioned medium. In experiments of longer than 3 days duration, fresh medium was added on day 3 to readjust the cell number to 3 × 10⁶ cells/ml.

Assay of Cell Growth and Differentiation-associated Characteristics

Cell growth was assayed by hemocytometer and viability was estimated by trypan blue dye exclusion. By these criteria, control cultures were found routinely to contain ≥95% viable cells. Differentiation was assessed by morphological change, Fc receptors, and amount of acid phosphatase activity as previously described (42, 43).

Preparation of Antiserum

The peptide YNDEDPEKEKRI ("myb-peptide"), contained in the putative c-myb translation product (6), was selected for synthesis.

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2 Saitama Cancer Center, Ina, Saitama, Japan.

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4 The abbreviations used are: FBS, fetal bovine serum; anti-myb, antiserum to a synthetic peptide present within the putative c-myb translation product; anti-myb IgG, the immunoglobulin fraction derived from the anti-myb serum; BSA, bovine serum albumin; KHL, keyhole limpet hemocyanin; PBS, phosphate buffered saline; HEPS, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; TCA, trichloroacetic acid.

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because it exhibited the highest peak in the \textit{myb} hydropathy profile (44) and this epitope region does not overlap with antigenic determinants in \textit{c-myc}, despite the sequence homology that exists between \textit{myb} and \textit{myc} in three of the flanking residues (45).

The peptide, synthesized by Genetic Design, Watertown, MA, was coupled to BSA by using di-benzalized benzidine (46), lysine residues being blocked by prior treatment with citraconic anhydride (47). The peptide-KLH conjugate was prepared using the glutaraldehyde method (48). KLH (5 mg) and peptide (4 mg) were dissolved in 5 ml of 0.1 M PBS, pH 7.35. An equal volume of 2% glutaraldehyde was added dropwise with stirring at 4°C and, after 1 h, sodium borohydride was added to a final concentration of 10 mg/ml. One hour later, the mixture was dialyzed against PBS.

Rabbits were injected s.c. with 1 mg of conjugate, emulsified in an equal volume of complete Freund's adjuvant. Booster injections were given at 2-week intervals, using conjugate and incomplete Freund's adjuvant. Antisera were drawn 2 weeks after the third booster injection, and the sera were tested for the presence of antibodies to the peptide by the Ouchterlony method and by enzyme immunoassay using protein A-peroxidase reagent. Preimmunization serum served as the control.

**Preparation of IgG from Crude Serum**

To 1.6 ml of antiserum, an equal volume of saturated ammonium sulfate solution was added dropwise with stirring at room temperature. The \(\text{pH}\) was adjusted to 7.8 with 2 N NaOH and the preparation was stirred slowly at room temperature for 2 h. It was then centrifuged for 30 min at 1500 \(\times g\) and the supernatant solution discarded. The precipitate was dissolved in 10 ml saline and was dialyzed against PBS overnight. It was further dialyzed against 0.0175 M phosphate buffer, \(\text{pH}\) 6.3, for 24 h at 4°C, the buffer being changed twice. The dialysate was applied to a 2.5 x 25-cm column of DEAE-cellulose equilibrated with the 0.0175 M phosphate buffer. Elution was carried out with phosphate buffer, IgG eluting almost without delay. Fractions of the first protein peak were selected to a point where the \(A_{280}\) value had fallen to about 50% of maximum. These fractions were combined and stored at a concentration of 20 mg protein/ml. The antibody titer was essentially the same as that of crude serum.

**Immunoblotting**

Protein samples fractionated on 10% sodium dodecyl sulfate-polyacrylamide gel (49) were blotted onto nitrocellulose as described previously (50). All further manipulations were performed at 4°C. The blots were rinsed with rinse buffer (10 mM Tris-HCl, \(\text{pH}\) 7.5, 0.15 M NaCl, 1 mM EDTA, 0.1% Triton X-100) and kept for 7 h in rinse buffer containing 20% fetal bovine serum. The blots were then immersed overnight in anti-\textit{myb} serum (antiserum to a synthetic peptide present within the putative \textit{c-myc} translation product), washed three times with rinse buffer and treated with rinse buffer containing 5% bovine serum albumin and protein A coupled to horseradish peroxidase (Bio-Rad) at a dilution of 1:2000. After washing, the blots were stained with a solution of rinse buffer containing 20 mg of dianisobenzidine and 10 \(\mu\)l of \(H_2O_2\) per 100 ml.

**Isolation of Nuclei.** Nuclei from exponentially growing ML-1 cells were isolated by the method described previously (51). Briefly, ML-1 cells were harvested, washed twice with cold PBS, and suspended at 1 - 2 \(\times 10^7\) cells/ml in hypotonic buffer containing 10 mM Tris-HCl, \(\text{pH}\) 7.9, 24 mM KCl, 10 mM MgCl\(_2\), and 1 mM dithiothreitol. Following centrifugation and resuspension in the same volume of fresh hypotonic buffer for 5 min, NP-40 was added to a concentration of 0.5%, and the cells were homogenized (10-15 strokes) in a dounce homogenizer. Lysis was confirmed by microscope, and the nuclei, collected by centrifugation, were washed once more in hypotonic buffer and resuspended at 2 \(\times 10^7\) nuclei/ml in a buffer containing 40% glycerol, 20 mM HEPES, \(\text{pH}\) 7.6, 2 mM MgCl\(_2\), and 2 mM dithiothreitol.

**Fractionation of Nuclei.** Nuclei were purified by centrifugation through sucrose/buffer containing 2.2 M sucrose, 5 mM Tris-HCl, \(\text{pH}\) 7.0, and 5 mM MgCl\(_2\). The envelope fraction was prepared by successive DNase I digestion of the purified nuclei and the remaining matrix was extracted with Triton X-100 and 2 M NaCl as previously described (52).

**Assay of DNA-replicating Activity in Isolated Nuclei.** This assay was performed by a slight modification of an established method (53). Isolated nuclei (2 \(\times 10^6\) nuclei) were preincubated for 2 h at 4°C in siliconized tubes with anti-\textit{myb} IgG or preimmune IgG. Then, 0.4 ml of a buffer containing 10 mM Tris-HCl, \(\text{pH}\) 7.8, 1 mM EDTA, 4 mM MgCl\(_2\), and 6 mM 2-mercaptoethanol and 0.2 ml of assay mixture containing 300 mM NaCl, 100 mM HEPES, \(\text{pH}\) 7.65, 20 mM MgCl\(_2\), 15 mM ATP, 0.3 mM each of dATP, dTTP, dCTP, and dGTP and 10 \(\mu\)Ci of [\(methyl-^3\text{H}\)]dUTP (15.2 Ci/mmole; New England Nuclear, Boston, MA) were added to each tube. After incubation at 37°C for 1 h, 1 ml of 10% TCA was added to each tube, the tubes were chilled on ice for 15 min, and the acid-insoluble product was collected by vacuum filtration on a GF/C filter prewetted with 10% TCA. The precipitate was washed 3 times with cold 10% TCA followed by cold ethanol. The amount of label incorporated was measured using a liquid scintillation counter.

**Assay of Total RNA Transcriptional Activity in Isolated Nuclei.** The assay was performed by methods previously described (54). Isolated nuclei (2 \(\times 10^6\) nuclei) were preincubated for 2 h at 4°C in siliconized tubes with the specified amount of anti-\textit{myb} IgG or preimmune IgG. The nuclei were then incubated at 25°C in an assay mixture containing 125 mM Tris, 5 mM Mg acetate, 25 mM HEPES, \(\text{pH}\) 7.6, 70 mM KCl, 0.02% 2-mercaptoethanol, 0.4 mM ATP, CTP, and GTP, 0.05 mM [\(5\)-\(^3\text{H}\)]UTP (35 Ci/mmole; ICN Radiochemicals, Irvine, CA), and 10 mM vanadyl ribonucleosides. After incubation for 45 min, the entire sample was spotted onto DE-81 filter paper discs. The disks were washed by immersing them eight times for 5-min periods in 5% Na\(_2\)HPO\(_4\) at room temperature twice for 30 s in H\(_2\)O and twice for 2 min in 95% ethanol, and they were then allowed to dry. Radioactivity was determined in a liquid scintillation counter.

**Assay of mRNA Transcriptional Activity.** An established procedure (Quick-Blot) (55) which depends on the selective immobilization of mRNA on nitrocellulose was used to perform this assay. After incubating the nuclei for 45 min in the same assay mixture as used for determining total RNA transcriptional activity, Pronase solution was added at 1 mg/ml and the suspension incubated at 37°C for 30 min. Brij-35 was added to a concentration of 0.5% and, after mixing, deoxycholate was added to 0.5% and the assay mixture kept on ice for 5 min. An equal volume of supersaturated Na\(_2\)S was added, followed by 1 volume of saturated NaBr and the entire sample was placed onto a nitrocellulose membrane, using a minifold filtration apparatus (Schleicher and Schuell) (55).

After filtration, the filters were soaked for 5 min in three changes of H\(_2\)O, then in three changes of 70% aqueous ethanol, and were air dried at room temperature. To measure the incorporation of [\(5\)-\(^3\text{H}\)]UTP individual dots were excised, placed in liquid scintillation vials, and counted in a liquid scintillation counter.

To examine whether the effects of the antibody are due to changes in polymerase II-catalyzed transcription, \(\alpha\)-amanitin (Sigma Chemical) was added at 1 \(\mu\)g/ml to the reaction mixtures used for assaying total and mRNA transcriptional activity.

**RESULTS**

Specificity of the Antiserum. Initial evaluation showed that the antiserum raised to the KLH-peptide conjugate had a higher titer (>1:10,000) and greater specificity than the serum generated with the BSA-peptide conjugate. Therefore, the KLH-peptide antiserum was used in these studies. When tested by the Ouchterlony method, the antiserum gave rise to a strong precipitation line with KLH-peptide, formed a weak band with the BSA-peptide conjugate, and gave a spur with KLH and the KLH-peptide conjugate. The antiserum did not react with BSA, fetal bovine serum, or human serum albumin. After absorption with KLH, the antiserum generated a precipitation line only with the KLH-peptide conjugate and that activity was completely abolished in the presence of \textit{myb}-peptide (data not shown).

**Target Proteins for the Antiserum in ML-1 Cells.** In lysates...
from exponentially growing ML-1 cells, antiseraum generated with the KLH-(myb)-peptide conjugate reacted with five proteins of M, 58,000, 75,000, 85,000, 90,000, and 105,000 (Fig. 1). The same result was obtained with antiseraum to the BSA-peptide conjugate (data not shown), indicating that the proteins are recognized by antibody to the myb-peptide. Of these proteins, p85 and p105 were found present in all cell lines tested, including the myeloid cell lines ML-1, HL-60, and THP-1, the lymphoid line BALL-1 and the 3T3 fibroblasts obtained from BALB/c mice. The other proteins (p58, p75, and p90) were found present in readily detectable amounts only in ML-1 and HL-60 cells, smaller amounts of p58 and p75 being present in HL-60 than in ML-1 cells. Only trace amounts of these proteins were detected in the more mature myeloid cell line THP-1 and in the lymphoid line BALL-1.

In lysates from ML-1 cells induced to differentiate to monocytes with human leukocyte conditioned medium, to macrophages with tetradecanoylphorbol acetate or to granulocytes with retinoic acid, the amounts of p58 and p75 decreased in parallel with the cell population moving to maturity (Table 1), and in the completely mature population, these two proteins were no longer detectable. A decrease in the amount of these two proteins was also observed in ML-1 cells arrested in G1 by serum depletion (Fig. 1). Under these conditions the amount of p90 decreased only slightly, this decrease being independent of the stage of maturation (Figs. 1 and 2). No decrease in the amounts of p85 and p105 was observed (Figs. 1 and 2).

The nuclear envelopes contained a much smaller amount of p75 (Fig. 3, 1) than did intact nuclei (Fig. 3; 2), indicating that the major fraction of this protein was associated with chromatin. Approximately 10% of total p75 remained with the matrix, even after extraction with Triton X-100 and 2 M NaCl. The amount of p75 present in the lysates was not affected by the procedures used for isolation of the nuclei (Fig. 3; 3 and 4). A trace of p85 was also detectable in these nuclear preparations.

Effect of Anti-myb IgG on DNA Replication and mRNA Formation in ML-1 Cell Nuclei. Under the assay conditions employed, nuclei isolated from ML-1 cells sustained DNA replication for approximately 60 min and transcription for approximately 30 min (Fig. 4) and, because vanadyl complex was present, the plateau reflects the optimal level of RNA synthesis achieved. This activity was unchanged in nuclei preincubated at 4°C for 2 h. As shown in Fig. 5, concentrations of anti-myb IgG, ranging from 0.01–1 μg, did not affect the DNA replicating activity, but interfered in a concentration-dependent manner with total RNA and mRNA transcriptional activity. At a concentration of 1.0 μg anti-myb IgG per 2 × 10⁶ nuclei, mRNA transcriptional activity was inhibited by 30%, whereas total RNA transcriptional activity was decreased by only 16%. In the presence of 0.0125 μg myb-peptide, the inhibition of transcriptional activity by 0.01 μg anti-myb IgG was neutralized by approximately 50%.

Fig. 4 shows that the presence of α-amanitin in the assay mixture decreased total transcriptional activity by 51% in both control and antibody-treated nuclei, indicating that the transcriptional activity inhibited by anti-myb IgG involves primarily polymerase II catalyzed mRNA. This deduction was confirmed by quick-blot analysis, which showed that in the presence of α-amanitin, mRNA transcriptional activity was decreased by an average of 83%.

As shown in Table 2, a correlation is indicated to exist between the rate of cell proliferation and the extent to which mRNA transcriptional activity can be inhibited in the presence of anti-myb IgG. In nuclei from cells arrested in the G1 phase of the cell cycle by serum depletion for 2 days, anti-myb IgG inhibited the mRNA transcriptional activity by only 11%. This residual activity may have derived from the fact that not all the cells of the nonsynchronously growing ML-1 cell population had likely reached the G1 stage. Once proliferation resumed, upon addition of serum, the transcriptional activity inhibitable with anti-myb IgG increased in parallel with the increase in rate of cell proliferation expressed in terms of doubling-time. At day 1 after serum restitution, when the doubling time was 45.6 h, transcription was inhibited by 16%. After 2 days in the presence of serum, the doubling time increased to 38.4 h, and transcription was inhibited by 22%. Once exponential growth was reached, the doubling time of a slow-growing clone reached 33 h, and mRNA transcriptional activity was inhibited by 30%. The doubling time of a fast-growing clone was 25.2 h and the

**Table 1 Decrease of p75 during differentiation of ML-1 cells**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Length of treatment</th>
<th>Morphological differentiation (%)</th>
<th>Amount of p75 (%) of control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td></td>
<td>Blasts</td>
<td>Promonocytes</td>
</tr>
<tr>
<td>Tetradecanoylphorbol acetate</td>
<td>6 h</td>
<td>87</td>
<td>12</td>
</tr>
<tr>
<td>(1.6 × 10⁻⁸ M)</td>
<td>16 h</td>
<td>61</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>24 h</td>
<td>17</td>
<td>54</td>
</tr>
<tr>
<td>Conditioned medium</td>
<td>48 h</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>(10% v/v)</td>
<td>2 day</td>
<td>2</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>3 day</td>
<td>18</td>
<td>73</td>
</tr>
<tr>
<td></td>
<td>4 day</td>
<td>3</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>5-day</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>Retinoic acid</td>
<td>1 day</td>
<td>66</td>
<td>34</td>
</tr>
<tr>
<td>(10⁻⁴ M)</td>
<td>2 day</td>
<td>31</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td>3 day</td>
<td>17</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>4 day</td>
<td>0</td>
<td>25</td>
</tr>
</tbody>
</table>

* Changes observed with p58 were similar to those of p75.
ANTIBODY-INHIBITED TRANSCRIPTION

Fig. 2. Blot of lysates from ML-1 cells induced to differentiate with leukocyte-conditioned medium or retinoic acid, immunostained with anti-myb serum. Lysates from ML-1 cells treated with (1) 10% conditioned medium for 4 days, (2) 10^{-4} M retinoic acid for 2 days, or (3) from untreated ML-1 cells. Arrows localize p75 and p58.

Fig. 3. Localization of p75 in ML-1 cells, determined by immunoblotting with anti-myb serum. Lysates were obtained from (1) nuclear envelope fractions prepared by successive DNAase I digestion of nuclei purified from 10^7 cells, (2) nuclei purified with 2.3 M sucrose from 10^7 cells, (3) nuclei purified as in (2) from 5 x 10^6 cells, (4) nuclei obtained by NP-40 treatment of 5 x 10^6 cells, (5) whole cell lysate of 2.5 x 10^6 cells. Arrow, location of p75.

inhibitable transcriptional activity was 33%. Fig. 6 shows that the correlation that exists between rate of proliferation and inhibitable mRNA transcriptional activity is essentially linear.

Fig. 4. Effect of anti-myb IgG and α-amanitin on total RNA transcriptional activity in ML-1 cell nuclei. Activity in (●) control nuclei, (□) + anti-myb-IgG, (△) + α-amanitin, (○) + anti-myb IgG + α-amanitin.

Fig. 5. Effect of anti-myb IgG on DNA replication and RNA transcriptional activity in nuclei isolated from exponentially growing ML-1 cells. Preimmune IgG served as control. The mean amount of label incorporated into control DNA, total RNA, and mRNA, was 801, 4191, and 1687 cpm, respectively. The values shown are means ± SD derived from three separate experiments.

Table 2 Effect of anti-myb IgG on mRNA-transcriptional activity in nuclei from proliferating, proliferation-arrested, or differentiated ML-1 human myeloblastic leukemia cells and THP-1 human monocytic leukemia cells

<table>
<thead>
<tr>
<th>Nuclei from</th>
<th>mRNA-transcriptional activity in the presence of anti-myb IgG (as % of preimmune IgG control)</th>
<th>Doubling time (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ML-1 cells growth arrested in G_1 by serum depletion</td>
<td>89 ± 4</td>
<td></td>
</tr>
<tr>
<td>Proliferating ML-1 cells</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 day after serum restitution</td>
<td>84 ± 3</td>
<td>45.6</td>
</tr>
<tr>
<td>2 days after serum restitution</td>
<td>78 ± 1</td>
<td>38.4</td>
</tr>
<tr>
<td>Exponentially growing ML-1 cells</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slow-growing clone</td>
<td>70 ± 4</td>
<td>33.0</td>
</tr>
<tr>
<td>Fast-growing clone</td>
<td>67 ± 2</td>
<td>25.2</td>
</tr>
<tr>
<td>Differentiated cells (monocytes)</td>
<td>95 ± 3</td>
<td></td>
</tr>
<tr>
<td>Proliferating THP-1 cells</td>
<td>96 ± 3</td>
<td></td>
</tr>
</tbody>
</table>

After ML-1 cells were induced to differentiate to monocytes by the use of pokeweed mitogen-stimulated human leukocyte-conditioned medium, mRNA transcriptional activity was no longer inhibited (Table 2). Although total mRNA transcriptional activity decreased as cell proliferation decreased, Fig. 7 demonstrates that this decrease was essentially due to the decrease in mRNA transcriptional activity inhibitable by anti-myb IgG.

Anti-myb IgG did not interfere with mRNA transcriptional
activity in the more mature monocytic cell line THP-1 (Table 2). In these cells the expression of c-myb mRNA is not detectable (data not shown).

**DISCUSSION**

Although anti-myb IgG reacts with five proteins present in whole ML-1 cell lysate, p75 and a trace of p85 are the only ones detected in the nuclear fraction. Since anti-myb IgG does not interfere with transcription in nuclei from THP-1 cells, which contain traces of p75 and p85, the observed inhibition of transcriptional activity that occurs in ML-1 cell nuclei is likely due to complexation of p75 with the antibody. Similarly, inhibition of transcriptional activity does not occur in nuclei from ML-1 cells induced to differentiate to a more mature stage at which c-myb is no longer expressed (18) and p75 is not detectable. The relationship p75 bears to the c-myb product in ML-1 cells remains undetermined. The product of chicken c-myb is a protein with a molecular weight of 75,000 (14) and the p75 detected in the nuclear fraction of ML-1 cells may represent the human c-myb product.

The close correlation that exists between the rate of cell proliferation and the extent of transcriptional activity inhibitable by the antibody suggests that p75 may play a role in the regulation of cell growth at a specific stage of maturation, for when the cells mature, p75, antibody-inhibitable transcription, and growth all disappear. In contrast, in the more mature monocytic cell line THP-1 proliferation proceeds even though p75 is present in only trace amounts, and nuclear transcription is insensitive to inhibition by anti-myb IgG.

The relationship the other proteins detected by anti-myb IgG have to c-myb is unknown at present. A series of monoclonal antibodies raised against a bacterially expressed fusion protein, containing a large portion of the p45 myb sequence, also recognized a range of antigens in various avian cell lines, among which p75 was considered to be the likely product of the c-myb gene (56). Similarly, three antisera raised against synthetic peptides representing different regions of avian myeloblastosis virus leukemogenic protein recognized multiple proteins (36). The parallelism observed in the levels of p58 and p75 present in ML-1 cells at various stages of proliferation and differentiation suggests that some relationship exists between the two, but the nature of this relationship remains to be established. Since p58 and p75 were found present in readily detectable amounts only in ML-1 (myeloblastic) and HL-60 (promyelocytic) cells, but not in more mature THP-1 (monocytic) cells that lack a detectable amount of myb RNA (data not shown), these two proteins may have functional significance only in myeloid cells at relatively immature stages of differentiation. The presence of only trace amounts of these two proteins in lymphoid cells (BALL-1) and in fibroblasts (3T3) supports this assumption. The fact that the two proteins are present, albeit in decreased amounts, in ML-1 cells arrested in G1 by serum depletion, but capable of resuming growth after serum supplementation, whereas they are absent in ML-1 cells induced to differentiate to monocyte/macrophages that fail to express myb RNA (52), suggests that their presence signals a continued proliferation potential of the cells, which is lost upon differentiation.

The function of the remaining proteins, p90 detected in ML-1 and HL-60 cells and p85 and p105 found present in all cell lines tested, also remains to be established.

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