c-myc and c-myb Oncoproteins during Induced Maturation of Myeloid and Erythroid Human Leukemic Cell Lines

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ABSTRACT

c-myc and c-myb mRNAs have been found to be tightly regulated during hemopoietic differentiation. We have studied nuclear c-myc and c-myb oncogenes through the cell cycle, during macrophage, granulocyte, erythroid, and megakaryocytic differentiation of KG1, HL60, and HEL cells. p62"myc" and p75"myb" content of propidium iodide-stained nuclei was quantitated by flow cytometry using fluoresceinated antibodies CT14-G4 and MB43, respectively. In uninduced cells p62"myc" content is highest in HL60, followed by HEL, then KG1, while p75"myb" is highest in HEL, followed by HEL and KG1. All lines showed a 2-3 fold increment in both oncoproteins over the cell cycle. Macrophage induction of KG1 and HL60 resulted in early increase in both oncoproteins, followed by a decline to less than starting values by 48 h, concurrent with a reduction of S phase cells and the appearance of adherent α-naphthyl esterase-positive cells. p62"myc" changes were more pronounced in HL60 and p75"myb" changes in KG1. Different patterns of oncoprotein expression were found when different inducing agents were used for granulocyte differentiation of HL60. Under all conditions, however, both oncoproteins declined to basal levels before granulocyte maturity. Hemin-induced erythroid differentiation of HEL to hemoglobin-containing cells resulted in biphasic p62"myc" and p75"myb" kinetics. In contrast, dimethyl sulfoxide-induced megakaryocytic differentiation of HEL was accompanied by an early and steady decline in both oncoproteins. Despite considerable reduction in oncoprotein levels, HEL cells were still actively cycling at 120 h.

It appears that c-myc and c-myb proteins decline with differentiation, well before proliferation ceases in some lineages. The kinetics of the decline differ between the two oncogenes and vary with the lineage induced and the nature of the inducing agent used. The cell cycle distribution of the oncoproteins does not change during maturation. These data suggest disparate roles for c-myc versus c-myb during hemopoietic differentitation and the existence of multiple signal transduction pathways for down-regulation of these genes.

INTRODUCTION

The retroviral oncogenes v-myc and v-myb are the transforming sequences of the avian myelocytomatosis and avian myeloblastosis viruses, respectively, which play a role in the induction of avian, murine, and human hemopoietic neoplasms (1-3). The retroviral oncogenes have a conserved organizational structure and are translated from single open reading frames into related proteins of approximately 45 kDa (4, 5). Both are nucleo-proteins with short half-lives and exhibit DNA binding in vitro (6-9) related to an association of the protein with nuclear matrix I (10) and chromatin (11), suggesting that, in vivo, myc and myb protein may have regulatory roles in DNA replication and RNA processing (12).

Several studies have demonstrated that the expression of both c-myc and c-myb is induced in cells stimulated to proliferate, c-myc in the transition from G0 to G1 (13) and c-myb in the transition from the G1 to the S phase (14) of the cell cycle. The expression of c-myc and c-myb mRNA diminishes when

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Materials and Methods

Cell Lines. HL60, a human myeloblastic leukemia line with promyelocytic differentiation (22), KG1, a human myeloblastic line responsive to growth factors (23), and HEL, a human erythroleukemia line (24), were maintained as continuous cultures in RPMI 1640 with 10% fetal calf serum (Imperial, United Kingdom), 1-glutamine (2 mmol/ml), and streptomycin (100 μg/ml) (complete medium), at 37°C in a 5% CO2 humidified atmosphere. Cell viability, as measured by trypan blue dye exclusion, was consistently greater than 98%. Differentiation was induced with DMSO2 (1.25%), RA (5 x 10−7 M), or TPA (3.3 x 10−9 M). RA was stored at −20°C in absolute ethanol and TPA at −20°C in acetone. Both were diluted in medium immediately prior to use. All inducing agents were purchased from Sigma Chemical Co. (Poole, United Kingdom).

Assessment of Differentiation. Cellular proliferation was assayed by incorporation of [3H]thymidine into DNA, and the results were expressed as a percentage of the value obtained for uninduced cells. The ability to induce differentiation was assessed by a combination of morphological criteria, including the appearance of nuclear staining, and by measurements of the size and density of the cell population. All inducing agents were assayed in triplicate, and the results were expressed as the mean ± SEM. The significance of differences between groups was determined using an unpaired Student's t-test.

Statistical Analysis. The statistical significance of differences between groups was determined using an unpaired Student's t-test.

The abbreviations used are: DMSO, dimethyl sulfoxide; RA, retinoic acid; TPA, 12-O-tetradecanoylphorbol-13-acetate; STC, spermine tetrahydrochloride; MEL, mouse erythroleukemia.

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× 10⁷ methanol-fixed cells harvested at various time points during induction, as described previously (21). Briefly, after a washing in phosphate-buffered saline, cytoplasm was digested by incubation in trypsin (0.03 mg/ml; type IX; Sigma), in 1.5 ml of citrate buffer containing 0.1% (v/v) Nonidet P-40 (Sigma), STC (0.52 mg/ml, Sigma), and Tris (0.06 mg/ml) at pH 7.6 (STC buffer) for 15 min at 20°C. Trypsin inhibitor (0.5 mg/ml; Sigma) and RNase (0.6 mg/ml; Sigma) in 1.5 ml of STC buffer was then added for a further 15 min at 20°C. Twenty ml of STC buffer containing additional spermine HCl (1.682 mg/ml total) (STC* buffer) were then added, and the nuclei were recovered by centrifugation at 1000 × g for 20 min. Nuclei were labeled with anti-p62' monoclonal antibody CT14-G4, diluted to 10 µg/ml in STC* buffer, or anti-p75' rabbit polyclonal antibody MB4.3, diluted 1/40 in STC* buffer, for 60 min on ice (CT14-G4 and MB4.3 were kind gifts of G. Evan, Cambridge, United Kingdom). After washing, nuclei were incubated in fluorescein isothiocyanate-labeled rabbit anti-mouse immunoglobulins [F(ab)2; F313; Dakopatts] or swine anti-rabbit immunoglobulins (Dakopatts; F205) for 30 min on ice. After a further wash, the nuclei were resuspended in STC* buffer containing 0.42 mg/ml propidium iodide. Stained nuclei were analyzed by flow cytometry on a FACS 440 with the laser providing 400 mW at 488 nm. Fluorescent signals were obtained through band pass filters at 535 nm for fluorescein isothiocyanate and 620 nm for propidium iodide and amplified linearly. Data for 10,000 nuclei were recorded on a Hewlett Packard 2000 series computer and analyzed using our own software. Cell cycle distribution was based on the method of Dean (29), fitting G₀-G₁ and G₂-M in normal distributions. S phase cells are those outside 3 SD of the mean for G₀-G₁ and G₂-M.

Fig. 1. p62' in uninduced HL60 cells. Dot display of c-myc protein fluorescence, after subtraction of background (rabbit serum SAR-FITC control), along Y-axis; X-axis, propidium iodide PI fluorescence = DNA content.

Fig. 2. p75' expression in uninduced HEL cells.

Fig. 3. p75' in (A) uninduced HL60 cells and (B) HL60 cells 120 h after induction with DMSO.

Table 1 Comparative p62' and p75' in human leukemic lines

<table>
<thead>
<tr>
<th>Cells</th>
<th>p62'</th>
<th>p75'</th>
</tr>
</thead>
<tbody>
<tr>
<td>HL60</td>
<td>1.0*</td>
<td>1.0</td>
</tr>
<tr>
<td>HEL</td>
<td>0.9</td>
<td>1.1</td>
</tr>
<tr>
<td>KG1</td>
<td>0.68</td>
<td>0.9</td>
</tr>
</tbody>
</table>

* Mean value of 3 experiments.

RESULTS

Antibody Specificity. We (21) and others (30) have previously demonstrated the specificity of CT14-G4 monoclonal antibodies used in this study by synthetic c-myc C'-terminus peptide blocking of nuclear staining, lack of blocking with N-myc peptide, lack of staining of N-myc expressing neuroblastoma line (Kelly), and lack of competitive binding with another isotype-specific monoclonal antibody (data not shown). Approximately 10% of MB4.3 polyclonal antibody staining could be blocked by preincubation in species-specific serum, and this blocked sample was used as the negative (background) control. Ninety % of the remaining staining could be blocked by avian p75' expressed from *Escherichia coli*. MB4.3 did not label NIH 3T3 cells, which are known not to express c-myb mRNA (data not shown).

Cell Cycle Distribution of p62' and p75' As shown in Figs. 1 and 2, the average level of both c-myc and c-myb proteins, in uninduced cells of different leukemic lines, was higher in S than in G₀-G₁ phase and was higher in G₂-M than in S phase. This increment in oncoprotein over the cell cycle was not more than 2-fold, in keeping with the increase in nuclear mass. The cell cycle distribution of neither oncoprotein changed with differentiation, although their absolute levels
declined, as illustrated in Fig. 3 for c-myb protein in (A) uninduced and (B) induced HL60 cells.

Levels of p62^mmy and p75^mb in Human Leukemic Lines. Table 1 demonstrates that, if the mean oncoprotein fluorescence of G0-G1 phase HL60 cells is taken as 1.0, then p62^mmy levels declined, as illustrated in Fig. 3 for c-myb protein in (A) uninduced and (B) induced HL60 cells.

Levels of p62^mmy and p75^mb in Human Leukemic Lines. Table 1 demonstrates that, if the mean oncoprotein fluorescence of G0-G1 phase HL60 cells is taken as 1.0, then p62^mmy levels were highest in HL60, followed by HEL, then KG1. p75^mb levels were similar in all 3 cell lines studied.

Oncoprotein and Cell Cycle Changes during Differentiation Induction of Leukemic Cells. To determine whether oncoprotein kinetics would differ among different cell lines induced to mature along the same pathway, we differentiated HL60 and KG1 to macrophages with TPA. This was accompanied by an overall decline in c-myc and c-myb protein in both cell lines as shown in Table 2 and, in HL60, was accompanied by a reduction in the percentage of S phase cells. We were not able to assess S phase in KG1 cells due to the presence of a hypodiploid subclone (data not shown). Both oncoproteins showed rapid (within 30 min) changes and transient oncoprotein increases in both cell lines before eventually declining; these changes were most prominent for c-myc protein in HL60. Morphological evidence of adherent, macrophage-like cells was present in both cell lines by 48 h; by 72 h, most cells were adherent and showed α-naphthyl acetate esterase positivity.

We next examined whether oncoprotein kinetics of a single cell line, HL60, would vary with the type of chemical inducer used to produce granulocytic differentiation. As shown in Table 3, both oncoproteins declined as HL60 differentiated to granulocytes. However, DMSO induction resulted in a much earlier (30 min) decrease in c-myc protein than did RA induction (120 h). In contrast c-myb protein declined later (24 h) during DMSO induction than during RA induction (30 min). During DMSO induction, the c-myc protein level fell before the percentage of S phase declined; during RA induction, the numbers of S phase cells decreased before the level of c-myc protein declined. These changes contrast with those observed during macrophage differentiation, where numbers of S phase cells fell at approximately the same time as the concentration of c-myc protein. RA induction of HL60, during which c-myb protein declined earlier, resulted in a higher percentage of morphologically mature neutrophils and nitroblue tetrazolium-positive cells, as compared to DMSO induction (Tables 3 and 4).

Having demonstrated that c-myc and c-myb protein kinetics in differentiating HL60 cells were different depending upon the agent used and the type of maturation, we then examined another leukemic line, HEL, induced toward either the erythroid or the megakaryocytic lineage. As shown in Table 5, maturation towards both of these lineages was associated with eventual decrease in c-myc and c-myb protein, but erythroid differentiation induced by hemin resulted in an initial (6 h) increase in p62^mmy. Both oncoproteins declined by 48 h. In contrast, megakaryocytic differentiation induced by DMSO was accompanied by an early (30 min) and sustained decrease in both p62^mmy and p75^mb. Further, the pattern of c-myb protein expression was different in DMSO-induced HEL from that of DMSO induced HL60.

There was no decline in S phase cells during erythroid differentiation and little during megakaryocytic differentiation, despite the more marked oncoprotein reduction in the latter lineage. Forty-two % of hemin-induced HEL cells contained hemoglobin at 120 h, while 99% of DMSO-induced HEL cells developed strong positivity for platelet factor 4. The p75^mb kinetics in DMSO-induced HEL, like those in RA-induced HL60, suggest that more advanced terminal differentiation may be preceded by an earlier decrease in this oncoprotein.

### Table 2 Relative p62^mmy and p75^mb and cell cycle status during TPA induction of leukemic cells to macrophages

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>0</th>
<th>30 min</th>
<th>2 h</th>
<th>6 h</th>
<th>12 h</th>
<th>24 h</th>
<th>48 h</th>
<th>72 h</th>
<th>120 h</th>
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</thead>
<tbody>
<tr>
<td>HL60</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>c-myc</td>
<td>1.0 ± 0.09*</td>
<td>1.5 ± 0.1</td>
<td>2.0 ± 0.15</td>
<td>1.4 ± 0.16</td>
<td>1.5 ± 0.02</td>
<td>0.7 ± 0.2</td>
<td>0.5 ± 0.01*</td>
<td>0.7 ± 0.13</td>
<td>0.7 ± 0.13</td>
</tr>
<tr>
<td>c-myb</td>
<td>1.0 ± 0.07</td>
<td>1.3 ± 0.1</td>
<td>0.95 ± 0.1</td>
<td>1.3 ± 0.13</td>
<td>1.0 ± 0.01</td>
<td>1.0 ± 0.12</td>
<td>0.85 ± 0.02</td>
<td>0.7 ± 0.12</td>
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<tr>
<td>% S</td>
<td>34.7</td>
<td>39.5</td>
<td>34.1</td>
<td>39.8</td>
<td>ND</td>
<td>15.0</td>
<td>15.3</td>
<td>15.9</td>
<td></td>
</tr>
<tr>
<td>KG1</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>c-myc</td>
<td>1.0 ± 0.01</td>
<td>1.0 ± 0.2</td>
<td>1.3 ± 0.06</td>
<td>0.55 ± 0.03</td>
<td>ND</td>
<td>0.35 ± 0.08</td>
<td>0.4 ± 0.02*</td>
<td>ND</td>
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<tr>
<td>c-myb</td>
<td>1.0 ± 0.02</td>
<td>1.1 ± 0.04</td>
<td>1.3 ± 0.04</td>
<td>0.7 ± 0.03</td>
<td>0.9</td>
<td>1.4 ± 0.1</td>
<td>0.4 ± 0.03</td>
<td>ND</td>
<td></td>
</tr>
</tbody>
</table>

* Mean ± SD of 3 experiments.
* 80% nitroblue tetrazolium-positive cells.
° 74% α-naphthyl acetate esterase-positive cells.
° 85% adherent cells.
° ND, not done.

### Table 3 Relative p62^mmy and p75^mb and cell cycle status during induction of HL60 to granulocytes

<table>
<thead>
<tr>
<th>Agent</th>
<th>0</th>
<th>30 min</th>
<th>2 h</th>
<th>6 h</th>
<th>12 h</th>
<th>24 h</th>
<th>48 h</th>
<th>72 h</th>
<th>120 h</th>
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<tbody>
<tr>
<td>DMSO</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>c-myc</td>
<td>1.0 ± 0.04*</td>
<td>0.7 ± 0.08</td>
<td>0.5 ± 0.07</td>
<td>0.7 ± 0.13</td>
<td>0.5 ± 0.09</td>
<td>0.5 ± 0.08</td>
<td>0.5 ± 0.03</td>
<td>ND</td>
<td>0.5 ± 0.1*</td>
</tr>
<tr>
<td>c-myb</td>
<td>1.0 ± 0.06</td>
<td>1.3 ± 0.1</td>
<td>1.1 ± 0.11</td>
<td>1.0 ± 0.16</td>
<td>1.0 ± 0.07</td>
<td>0.8 ± 0.08</td>
<td>0.6 ± 0.05</td>
<td>ND</td>
<td>0.2 ± 0.05</td>
</tr>
<tr>
<td>% S</td>
<td>32.0</td>
<td>34.1</td>
<td>31.2</td>
<td>31.6</td>
<td>29.2</td>
<td>30.1</td>
<td>19.1</td>
<td>ND</td>
<td>7.7*</td>
</tr>
<tr>
<td>Retinoic acid</td>
<td></td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>c-myc</td>
<td>1.0 ± 0.04</td>
<td>1.2 ± 0.07</td>
<td>1.2 ± 0.12</td>
<td>1.4 ± 0.1</td>
<td>1.4 ± 0.08</td>
<td>1.4 ± 0.11</td>
<td>1.2 ± 0.1</td>
<td>1.0</td>
<td>0.7 ± 0.1</td>
</tr>
<tr>
<td>c-myb</td>
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<td>1.2 ± 0.07</td>
<td>0.6 ± 0.09</td>
<td>0.8 ± 0.08</td>
<td>0.7 ± 0.09</td>
<td>0.7 ± 0.07</td>
<td>0.6 ± 0.05</td>
<td>ND</td>
<td>0.3 ± 0.05</td>
</tr>
<tr>
<td>% S</td>
<td>36.3</td>
<td>35.4</td>
<td>40.0</td>
<td>35.8</td>
<td>38.8</td>
<td>29.1</td>
<td>30.9</td>
<td>20.8</td>
<td>10.6</td>
</tr>
</tbody>
</table>

* Mean ± SD of 3 experiments.
* 80% nitroblue tetrazolium-positive cells.
° 92% nitroblue tetrazolium-positive cells.
° ND, not done.

### Table 4 Differential count in uninduced and induced HL60

<table>
<thead>
<tr>
<th>Inducer</th>
<th>Time (h)</th>
<th>Bl*</th>
<th>Prom</th>
<th>My</th>
<th>Meta</th>
<th>BF</th>
<th>SN</th>
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</thead>
<tbody>
<tr>
<td>Control</td>
<td>120</td>
<td>98</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>DMSO</td>
<td>120</td>
<td>7</td>
<td>9</td>
<td>17</td>
<td>22</td>
<td>33</td>
<td>5</td>
</tr>
<tr>
<td>RA</td>
<td>120</td>
<td>2</td>
<td>5</td>
<td>18</td>
<td>14</td>
<td>41</td>
<td>21</td>
</tr>
</tbody>
</table>

* Bl, blasts; Prom, promyelocytes; My, myelocytes; Meta, metamyelocytes; BF, band forms; SN, segmented neutrophils.

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differentiation was markedly different from that occurring during expression of either c-myc and c-myb protein. Therefore, there appeared to be little lineage specificity in the induction known to be present in HL60 (31). In primitive cells, staining.

of species-specific serum to block more than a small amount of lines, despite the c-myc genomic amplification and over-expression of c-myb protein was demonstrated by the near complete blocking of product of the c-myb protooncogene, as reported by Klempner.

nuclear c-myc protein towards quantitation of nuclear c-myb in the nucleus, where, by immunoperoxidase staining, it appears to be associated with chromatin. We have used the flow cytometry technique to quantitate c-myb protein in human hemopoietic cells. The majority of c-myb protein in these hemopoietic cells is located in the nucleus, where, by immunoperoxidase staining, it appears to be associated with chromatin. We have used the flow cytometry technique to quantitate c-myb protein in human hemopoietic cells. The majority of c-myb protein in these hemopoietic cells is located in the nucleus, where, by immunoperoxidase staining, it appears to be associated with chromatin. We have used the flow cytometry technique to quantitate c-myb protein in human hemopoietic cells. The majority of c-myb protein in these hemopoietic cells is located in the nucleus, where, by immunoperoxidase staining, it appears to be associated with chromatin. We have used the flow cytometry technique to quantitate c-myb protein in human hemopoietic cells. The majority of c-myb protein in these hemopoietic cells is located in the nucleus, where, by immunoperoxidase staining, it appears to be associated with chromatin. We have used the flow cytometry technique to quantitate c-myb protein in human hemopoietic cells. The majority of c-myb protein in these hemopoietic cells is located in the nucleus, where, by immunoperoxidase staining, it appears to be associated with chromatin. We have used the flow cytometry technique to quantitate c-myb protein in human hemopoietic cells. The majority of c-myb protein in these hemopoietic cells is located in the nucleus, where, by immunoperoxidase staining, it appears to be associated with chromatin. We have used the flow cytometry technique to quantitate c-myb protein in human hemopoietic cells. The majority of c-myb protein in these hemopoietic cells is located in the nucleus, where, by immunoperoxidase staining, it appears to be associated with chromatin. We have used the flow cytometry technique to quantitate c-myb protein in human hemopoietic cells. The majority of c-myb protein in these hemopoietic cells is located in the nucleus, where, by immunoperoxidase staining, it appears to be associated with chromatin. We have used the flow cytometry technique to quantitate c-myb protein in human hemopoietic cells. The majority of c-myb protein in these hemopoietic cells is located in the nucleus, where, by immunoperoxidase staining, it appears to be associated with chromatin. We have used the flow cytometry technique to quantitate c-myb protein in human hemopoietic cells. The majority of c-myb protein in these hemopoietic cells is located in the nucleus, where, by immunoperoxidase staining, it appears to be associated with chromatin. We have used the flow cytometry technique to quantitate c-myb protein in human hemopoietic cells. The majority of c-myb protein in these hemopoietic cells is located in the nucleus, where, by immunoperoxidase staining, it appears to be associated with chromatin. We have used the flow cytometry technique to quantitate c-myb protein in human hemopoietic cells. The majority of c-myb protein in these hemopoietic cells is located in the nucleus, where, by immunoperoxidase staining, it appears to be associated with chromatin. We have used the flow cytometry technique to quantitate c-myb protein in human hemopoietic cells. The majority of c-myb protein in these hemopoietic cells is located in the nucleus, where, by immunoperoxidase staining, it appears to be associated with chromatin. We have used the flow cytometry technique to quantitate c-myb protein in human hemopoietic cells. The majority of c-myb protein in these hemopoietic cells is located in the nucleus, where, by immunoperoxidase staining, it appears to be associated with chromatin. We have used the flow cytometry technique to quantitate c-myb protein in human hemopoietic cells. The majority of c-myb protein in these hemopoietic cells is located in the nucleus, where, by immunoperoxidase staining, it appears to be associated with chromatin. We have used the flow cytometry technique to quantitate c-myb protein in human hemopoietic cells. The majority of c-myb protein in these hemopoietic cells is located in the nucleus, where, by immunoperoxidase staining, it appears to be associated with chromatin. We have used the flow cytometry technique to quantitate c-myb protein in human hemopoietic cells. The majority of c-myb protein in these hemopoietic cells is located in the nucleus, where, by immunoperoxidase staining, it ap...
sis of a more stable, higher molecular weight isoform, as reported by Wingrove, or in mature cells has a prolonged half-life by virtue of phosphorylation, as described by Persson et al. (41). Oncoprotein decline is not spuriously related to general reduction in nuclear mass with maturation, as demonstrated by the lack of a consistent proportional decrease between the two oncoproteins during the latter stages of differentiation, as illustrated in TPA- and RA-treated HL60 cells.

Throughout differentiation there is no change in the invariant relationship (with regard to nuclear mass) with the cell cycle of both p62-c-myc or p75-c-myb. In particular, we observed no accumulation of either oncoprotein in any phase of the cell cycle as differentiation proceeded, in keeping with data published for c-myc mRNA (41) and protein (42) but in contrast with that of Thompson et al. (14), who noted a 2- to 3-fold increase in c-myc RNA in S phase HL60 cells. The relationship of RNA to protein for these nuclear oncogenes may be abnormal in leukemic cells; failure of both RNA processing and RNA transport to cytoplasm, resulting in sterile transcripts, has been noted in MEL cells (43).

Finally, during RA-induced differentiation of HL60 and DMSO-induced megakaryocytic differentiation of HEL, early decrease in p75-c-myb was accompanied with more complete terminal maturation, suggesting a closer association with hematopoietic differentiation than for p62-c-myc. Lee et al. (44) also noted that terminal differentiation of the human monocytic leukemia line THP-1 was accompanied by down-regulation of c-myc but little change in c-myc expression. Clarke et al. (45) demonstrated that constitutive c-myc expression blocked induced MEL differentiation, even in the face of normally regulated c-myc RNA responses. Ishikura et al. (20) suggested that c-myc protein may have functional significance only in hematopoietic cells at relatively immature stages of differentiation. As yet, no reports exist of c-myc expression in terminally differentiated postmitotic cells, as we and others (46) have described for c-myc protein and RNA. Our data on c-myc protein support the postulate by other authors (45) that permanent reduction in c-myc expression is obligatory for terminal differentiation to occur.

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REFERENCES

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