Defective c-myc and c-myb RNA Turnover in Acute Myeloid Leukemia Cells

By Maria R. Baer, Peter Augustinos, and Alan J. Kinnibrough

Dysregulated expression of the c-myc and c-myb proto-oncogenes has been implicated in the pathogenesis of acute myeloid leukemia (AML). To elucidate mechanisms of c-myc dysregulation in AML cells, we studied c-myc RNA turnover in peripheral blood blasts from eight patients using actinomycin D transcription blockade. Rapid c-myc RNA turnover was seen in cells from six patients, with half-lives of approximately 30 minutes, similar to those reported in normal myeloid cells, in HL-60 cells, and in other cell lines. c-myc RNA turnover was prolonged in cells of the other two patients, with half-lives of greater than 75 minutes. c-fos RNA turnover was rapid in blasts from all eight patients, with half-lives of approximately 15 minutes. Stabilization of GM-CSF transcripts was not observed. In contrast, c-myb RNA half-lives were greater than 75 minutes in cells of the two patients with prolonged c-myc RNA turnover, as compared to 30 minutes in cells of the other six patients. Enhanced stability of both c-myc and c-myb RNA species suggests that a defect exists in a trans-acting factor that destabilizes both of these normally labile RNAs. Incomplete correlation between c-myc RNA levels and half-lives indicates regulation of c-myc expression at the level of transcription or nuclear transport in addition to posttranscriptional regulation.

© 1992 by The American Society of Hematology.

A CUTE MYELOID leukemia is a bone marrow-based malignancy characterized by maturation arrest of the malignant clone, with accumulation of immature myeloid cells and inhibition of normal hematopoesis. The c-myc proto-oncogene encodes a helix-loop-helix DNA-binding protein associated with the control of cell proliferation. This gene is expressed in immature myeloid cells and is downregulated during myeloid maturation. Constitutive c-myc expression inhibits myeloid maturation, and inhibition of c-myc expression by c-myc antisense oligomers induces maturation of leukemia cell lines. These findings support the hypothesis that dysregulation of c-myc expression may play a role in the pathogenesis of AML. The c-myc proto-oncogene is another nuclear proto-oncogene encoding a DNA-binding protein, which regulates myeloid maturation. c-myc downregulation has also been shown to be necessary and sufficient to induce maturation of leukemia cell lines. Therefore, dysregulation of c-myc expression may also be implicated in the pathogenesis of AML.

The c-myc proto-oncogene is expressed in bone marrow and peripheral blood cells of most patients with AML, but with a significant variation in RNA levels. Cells from a subset of patients with AML exhibit markedly elevated c-myc RNA levels as compared with normal marrow cells and other AML patients’ cells. Elevated c-myc RNA levels have been correlated with decreased responsiveness to therapy, with both lower remission rates and shorter remission durations. The presence of persistently elevated c-myc RNA levels 24 hours after initiation of chemotherapy may also correlate with poor therapeutic outcome. Thus, there is substantial evidence that the c-myc proto-oncogene plays an important role in AML.

We sought to determine mechanisms by which c-myc expression may be dysregulated in AML cells. In the studies reported here, we have examined the posttranscriptional regulation of c-myc gene expression by studying c-myc RNA turnover in cells treated with actinomycin D to block transcription. We find significant variation in c-myc RNA turnover, with prolonged half-lives in a subset of patients. We also find impaired turnover of c-myb RNA in AML cells with impaired c-myc RNA turnover.

MATERIALS AND METHODS

Patients. The patient population consisted of eight consecutive patients with AML presenting at diagnosis or at relapse with white blood cell (WBC) counts greater than 20,000/mm³. Clinical data on these eight patients are presented (Table 1). Patients 3 and 4 were treated with standard-dose cytosine arabinoside and daunorubicin, and patients 1 and 2 with high-dose cytosine arabinoside and daunorubicin. Patient 6 did not respond to high-dose cytosine arabinoside and daunorubicin, but then achieved remission after treatment with standard-dose cytosine arabinoside, etoposide, and mitoxantrone. Patients 5 and 7, both with relapsed disease, were treated with etoposide and mitoxantrone. All patients gave informed consent before acquisition of peripheral blood and bone marrow samples for study, and all studies were approved by the Institutional Review Board.

Preparation of cells. Peripheral blood and bone marrow cells were aspirated into syringes containing sodium citrate, which were immediately placed on ice. Mononuclear cell fractions were isolated by centrifugation over 1.077 density Ficoll Hypaque (Pharmacia LKB Biotechnology, Inc, Piscataway, NJ). Differential cell counts were performed on Wright-Giemsa-stained cytospin preparations of the mononuclear cell fractions. For actinomycin D transcription blockade studies, peripheral blood mononuclear cells were cultured in RPMI 1640 medium with 10% fetal calf serum (FCS) and 10 μg/mL actinomycin D at 37°C in 5% CO₂ atmosphere. RNA was prepared from aliquots of 5 × 10⁶ cells harvested at 15, 30, 45, 60, and 75 minutes. To control for effects of serum stimulation, transcription blockade experiments were additionally performed in cells adapted to culture before addition of actinomycin D. In these experiments, actinomycin D was added to cells that had been precultured for 1 hour at a density of 10⁷ cells per milliliter in RPMI 1640 with 10% FCS.

Preparation and analysis of RNA. Cell pellets dissolved in...
guanidium isothiocyanate were layered over 5.8 mol/L cesium chloride and centrifuged at 30,000 rpm for 18 hours. The pelleted RNA was precipitated twice in ethanol. Twenty-five micrograms of whole cell RNA obtained from cells of three patients at serial timepoints after actinomycin D transcription blockade were additionally separated into polyadenylated and nonpolyadenylated fractions by passage over oligo(dT)-cellulose columns, using the method of Aviv and Leder. Gene expression was studied by Northern blot analysis. Ten microgram aliquots of RNA were fractionally separated into polyadenylated and nonadenylated c-myc mRNA, (A) + c-myc mRNA levels were calculated as percentages of TPI mRNA levels and (A)− mRNA levels were calculated as percentages of 18S ribosomal RNA in each lane. 18S rRNA levels were determined by densitometric scanning of Polaroid (Polaroid Corp, Cambridge, MA) photographs of Northern blots taken under short-wave UV light.

RESULTS
There was significant variation in c-myc expression among the eight cases of AML studied (Fig 1). Cells from patients 1, 2, and 4 had marked c-myc overexpression, with c-myc mRNA levels comparable to those observed in growing HL-60 cells. Cells from patient 5 had very low c-myc expression, while cells of patients 3, 6, 7, and 8 had intermediate levels of c-myc expression. c-myc RNA levels were similar in peripheral blood and bone marrow cells from each patient (data not shown).

To test whether differences in c-myc RNA levels could be attributed to differences in the turnover of c-myc RNA, AML cells were cultured with actinomycin D to block transcription. c-myc RNA levels were determined at 15-minute intervals after transcription was blocked. In our initial experiments, c-myc RNA turnover was studied by addition of actinomycin D at the time the cells were placed into culture. Apparent c-myc RNA half-lives were 37 and 39 minutes in two patients, 60 and 68 minutes in two, and greater than 75 minutes in three (Table 2). However, when Northern blots of RNA from transcription blockade experiments performed in this fashion were hybridized with a c-fos cDNA probe, induction of c-fos RNA in the cultured cells was apparent, despite the presence of actinomycin D in the cultures (Fig 2). c-fos expression is highly serum-responsive, with regulation at the level of transcription. We postulated that the induction of c-fos RNA was a rapid effect of serum on c-fos transcription, occurring before establishment of effective transcription blockade by actinomycin D. Transcription rates decline immediately after addition of actinomycin D, but complete transcription blockade requires 10 minutes of actinomycin D exposure. Also, RNA processing events such as poly (A) addition and splicing further delay the appearance of c-fos mRNA as a discrete species at 2.2 kb. c-myc expression also increases rapidly on serum stimulation. Stimulation of c-myc expression by placement of cells into culture (despite the presence of actinomycin D) would result in an apparent prolongation of c-myc RNA turnover in transcription blockade experiments.

<table>
<thead>
<tr>
<th>Patient</th>
<th>WBC Count (/mm3)</th>
<th>Percentage of Blasts in Cell Population Studied</th>
<th>Age/Sex</th>
<th>FAB Type</th>
<th>Cyto genetic Findings</th>
<th>De Novo or Secondary Leukemia</th>
<th>Diagnosis or Relapse</th>
<th>Response to Chemotherapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>159,000</td>
<td>99</td>
<td>73 F</td>
<td>M1</td>
<td>47,XX,+8</td>
<td>Secondary Diagnosis</td>
<td>NR</td>
<td>NT</td>
</tr>
<tr>
<td>2</td>
<td>54,500</td>
<td>93</td>
<td>73 M</td>
<td>M2</td>
<td>46,XY,del(7)(q21q22)</td>
<td>Secondary Diagnosis</td>
<td>D</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>79,100</td>
<td>61</td>
<td>35 M</td>
<td>M4</td>
<td>46,XY,t(6;9)(p23;q34)</td>
<td>De novo Diagnosis</td>
<td>CR</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>301,000</td>
<td>98</td>
<td>59 F</td>
<td>M1</td>
<td>46,XX</td>
<td>De novo Diagnosis</td>
<td>PR</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>32,500</td>
<td>96</td>
<td>78 M</td>
<td>M4</td>
<td>46,XY</td>
<td>Secondary Diagnosis</td>
<td>CR</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>28,400</td>
<td>88</td>
<td>54 M</td>
<td>M2</td>
<td>46,XY</td>
<td>Secondary Diagnosis</td>
<td>Relapse</td>
<td>D</td>
</tr>
<tr>
<td>7</td>
<td>87,300</td>
<td>89</td>
<td>67 F</td>
<td>M1</td>
<td>47,XX,+mar</td>
<td>Secondary Diagnosis</td>
<td>Relapse</td>
<td>NR</td>
</tr>
<tr>
<td>8</td>
<td>113,000</td>
<td>52</td>
<td>75 F</td>
<td>M2</td>
<td>46,XX</td>
<td>Secondary Diagnosis</td>
<td>Relapse</td>
<td>NT</td>
</tr>
</tbody>
</table>

Abbreviations: CR, complete remission; NR, no response; NT, not treated; PR, partial response; D, died during therapy.

For personal use only on September 24, 2017.
To obviate the possible effects of serum stimulation on apparent c-myc RNA turnover, cells from six patients were also studied by addition of actinomycin D to cells precultured for 1 hour in RPMI 1640 medium with 10% FCS. Under these conditions, c-fos RNA is detectable before addition of actinomycin D, and c-fos RNA turnover is rapid following addition of actinomycin D in all cases, with half-lives on the order of 15 minutes (Fig 2 and Table 3). Rapid c-myc RNA turnover is also observed in four of the six patients' cells. c-myc RNA half-lives are 25, 25, 34, and 35 minutes in these four cases (Fig 2 and Table 2). c-myc RNA turnover is significantly prolonged in cells of the other two patients studied in this way, with half-lives of greater than 75 minutes in both (Fig 2 and Table 2).

We sought to determine whether longer c-myc RNA half-lives were specific for c-myc, or whether other normally short-lived mRNA species might be affected. To this end we studied turnover of RNA encoded by the c-fos, GM-CSF, and c-myb genes (Table 3). As noted above, c-fos RNA turnover was rapid in cells from all six patients studied after 1-hour cell culture, including cells of patients 1 and 7, where c-myc turnover was prolonged. GM-CSF mRNA was not detected by Northern blot analysis in cells from any of the eight patients. GM-CSF mRNA accumulation has been shown to occur by mRNA stabilization, and the lack of concordance between GM-CSF and c-myc RNA stabilization has been previously reported in a mouse monocytic tumor. Therefore, two short-lived RNAs, c-fos and GM-CSF, are not stabilized in these patients. A third species examined was c-myb mRNA, detected by Northern blot analysis in all eight cases (Fig 1). Rapid c-myb mRNA turnover was apparent in cells from patients 2, 3, 4, 5, 6, and 8, with half-lives of approximately 30 minutes. In contrast, c-myb mRNA turnover was prolonged in cells of patients 1 and 7, with half-lives of greater than 75 minutes. Thus, there was concordance between c-myb and c-myc RNA stability. We speculate on the reasons for this similarity in c-myc and c-myb RNA turnover below (see Discussion).

There is an incomplete correlation between c-myc RNA levels and half-lives. Notably, cells of patient 1 have very high c-myc expression with a long c-myc RNA half-life, whereas cells of patients 2 and 4 have very high myc expression with rapid c-myc RNA turnover. Northern blots of RNA from two patients are shown, demonstrating rapid and slow c-myc RNA turnover in cells studied after adaptation to culture (Fig 3). The turnover of c-myc RNA in cells of patient 4 is rapid ($t_{1/2} = 34$ minutes) (Fig 3A) and is similar to the turnover of c-myc RNA in cultured leukemia cell lines. In contrast, patient 1’s c-myc RNA is degraded more slowly ($t_{1/2} > 75$ minutes) (Fig 3B). Both patients have elevated c-myc expression, with comparable c-myc RNA levels. Thus, the turnover data indicate different mechanisms of c-myc overexpression in these two patients. Additionally, c-myc RNA levels are not markedly elevated in cells of patient 7, despite the slow turnover of c-myc RNA in this patient’s cells. The level of c-myc RNA in patient 7’s cells was identical to the level in patient 8’s cells, in which c-myc RNA turnover was rapid. This finding also

---

**Table 2. c-myc mRNA Levels and Half-lives**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Relative c-myc mRNA Level</th>
<th>c-myc mRNA Half-life in Cells Studied Without Prior Cell Culture</th>
<th>c-myc mRNA Half-life in Cells Precultured for 1 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.66</td>
<td>&gt; 75</td>
<td>&gt; 75</td>
</tr>
<tr>
<td>2</td>
<td>1.00</td>
<td>60</td>
<td>25</td>
</tr>
<tr>
<td>3</td>
<td>0.39</td>
<td>39</td>
<td>—</td>
</tr>
<tr>
<td>4</td>
<td>0.65</td>
<td>—</td>
<td>34</td>
</tr>
<tr>
<td>5</td>
<td>&lt; 0.01</td>
<td>37</td>
<td>—</td>
</tr>
<tr>
<td>6</td>
<td>0.25</td>
<td>&gt; 75</td>
<td>35</td>
</tr>
<tr>
<td>7</td>
<td>0.12</td>
<td>&gt; 75</td>
<td>&gt; 75</td>
</tr>
<tr>
<td>8</td>
<td>0.13</td>
<td>68</td>
<td>25</td>
</tr>
</tbody>
</table>

Cells treated with actinomycin D to block transcription at the time of initiation of the cell cultures and after 1-hour culture in medium with FCS.
indicates that RNA stability is only one of the factors determining the level of expression of the c-myc gene in AML cells.

Because the c-myc RNA degradation pathway has two steps, deadenylation and degradation of deadenylated c-myc RNA species,\textsuperscript{35,36} we wished to examine which of these steps was slow in cells from our two patients with slow c-myc RNA turnover, or whether both steps were affected. We examined turnover of polyadenylated and of nonadenylated c-myc RNA species in cells from both patients, as well as in cells from a patient with rapid c-myc RNA turnover. Cells of patient 3, with rapid c-myc RNA turnover, show a rapid disappearance of (A)+ c-myc RNA (Fig 4A). The (A)− c-myc RNA shows a lag in disappearance, as expected because the c-myc degradation pathway initiates with deadenylation, followed by degradation of the deadenylated RNA species.\textsuperscript{35,36} After this lag, (A)− c-myc RNA also turns over rapidly in cells of patient 3 (Fig 4A). On the other hand, patient 1’s and patient 7’s cells do not deadenylate c-myc RNA as rapidly as patient 3’s cells, and degradation of deadenylated species also appears to be slower (Fig 4B), consistent with impairment of both steps of the c-myc RNA degradation pathway.

To exclude the possibility that impaired actinomycin D transport into cells was responsible for the apparent lack of c-myc and c-myb RNA turnover, we screened blood and bone marrow cells from all eight patients for expression of the multi-drug resistance 1 (mdr1) gene. mdr1 RNA was detected by Northern blot analysis in cells of patients 4, 5, 6, and 7, but not in cells of patients 1, 2, 3, or 8 (data not shown). There was no correlation between mdr1 gene expression and prolonged c-myc and c-myb mRNA turnover. Effective actinomycin D blockade of transcription could also be inferred from the rapid c-fos turnover seen in cells from all patients when actinomycin D was added to cells already adapted to culture. Therefore, actinomycin D drug resistance as a basis for a lack of c-myc and c-myb RNA turnover in the actinomycin D experiments is unlikely.

Clinical data were compiled on the eight patients (Table 1). There was no apparent relationship between c-myc and c-myb RNA turnover and French-American-British (FAB) type, patient age, or diagnosis versus relapse status. However, prolonged c-myc and c-myb RNA turnover was seen exclusively in cases of secondary leukemia in this series. Cells of all three patients with de novo AML had rapid c-myc and c-myb RNA turnover, whereas cells of two of five patients with secondary AML had slow c-myc and c-myb RNA turnover. Determination of the frequency of c-myc and c-myb RNA turnover defects in AML and their possible
association with subsets of the disease awaits the study of larger numbers of patients.

**DISCUSSION**

To elucidate the mechanisms governing c-myc expression in AML cells, we examined c-myc mRNA turnover in cells from eight patients with AML. Peripheral blood rather than bone marrow cells were used for these studies due to the need for sufficient cells for study of serial timepoints in actinomycin D transcription blockade experiments, with and without prior cell culture. It is likely that our findings with respect to c-myc mRNA turnover in peripheral blood cells also apply to bone marrow cells, because c-myc mRNA levels were similar in our patients’ peripheral blood and bone marrow cells (data not shown), a finding consistent with other reports. Patients were chosen on the basis of availability of sufficient numbers of cells for study and represent a heterogeneous group of AML patients, with both de novo and secondary disease, at diagnosis and at relapse.

Cells from six of the eight patients studied in this series have short c-myc mRNA half-lives, on the order of 30 minutes. These c-myc mRNA half-lives are similar to those previously reported in normal myeloid progenitor cells, normal fibroblasts, uninduced HL-60 cells, and other cell lines. Cells from the two other patients studied exhibit slow c-myc mRNA turnover, with half-lives greater than 75 minutes. This represents a significant decrease in c-myc mRNA turnover in comparison with that observed in cells of other AML patients, and in normal cells and cell lines studied to date. Therefore, c-myc expression appears to be altered posttranscriptionally, at the level of mRNA turnover, in cells from a subset of patients with AML. We also found slow turnover of c-myb RNA in cells of patients with slow c-myc RNA turnover.

This report is, to our knowledge, the first documentation of enhanced stability of c-myc RNA as a mechanism of c-myc dysregulation in acute leukemia cells. The c-myc gene is expressed in cells from most cases of AML, with markedly elevated c-myc RNA levels in approximately a third of patients. Mechanisms of c-myc dysregulation in AML cells have not been previously characterized. c-myc gene amplification is found in the HL-60 cell line, and was present in the AML patient from whose cells this line was derived, but amplification of the c-myc gene has not been observed in other cases of AML studied to date. Moreover, rearrangements of the c-myc gene have not been seen in AML cells. Trisomy of chromosome 8, on which...
the c-myc gene is located, is the most common clonal cytogenetic abnormality in AML, present in 10% of cases, including patient 1 in our series. Altered structure or expression of the c-myc gene has not been seen in AML cells with trisomy 8.41 Thus, AML appears to differ from solid tumors, in which c-myc gene amplification is frequent,42 and from Burkitt’s lymphoma and other B- and T-cell lymphoproliferative disorders, in which the c-myc gene is frequently rearranged.31,43

There are several reports of enhanced stability of 5’ or 3’ truncated c-myc transcripts in malignancies with chromosomal abnormalities involving the locus of the c-myc gene. Murine plasmacytomas with chromosomal translocations resulting in 5’ truncation of c-myc RNA have c-myc RNA half-lives of 60 minutes, compared with 16 minutes and 27 minutes in cell lines without c-myc rearrangements.31 c-myc RNA half-lives of 1 to 2 hours have been demonstrated in Burkitt’s lymphoma cell lines with (8;14) translocations with c-myc gene rearrangements with 5’ RNA truncation, as well as in a human colon carcinoma line, COLO 320, with truncated amplified c-myc genes yielding 5’ truncated c-myc RNA.45,46 Enhanced stability of c-myc RNA has also been demonstrated in a human multiple myeloma and in a T-cell leukemia with c-myc translocations with exon 3 breakpoints, resulting in loss of the 3’ untranslated region of the gene.57,58 Ours is the first report of enhanced c-myc RNA stability in myeloid leukemia, as well as the first report of enhanced c-myb stability in the absence of cytogenetic abnormalities that result in gross structural alterations of the mRNA.

The slower degradation rate of c-myc RNA and c-myb in the same patients in our series may point to a mechanism that works in trans. The trans-acting factor that is defective in these patients might participate in the degradation of a subset of rapidly degraded RNAs. The rapid turnover of c-fos RNA in these patients rules out the possibility of this putative factor regulating all RNA turnover or indeed all turnover of short-lived RNAs. Further supporting this contention is the lack of GM-CSF RNA induction in these patients, because induction of this short-lived mRNA is achieved by RNA stabilization. A labile destabilizer of c-myc mRNA has been demonstrated in cytosol, which accelerates both deadenylation of polyadenylated c-myc RNA and degradation of deadenylated c-myc RNA.49,50 Remarkably, this destabilizing element also destabilizes c-myb RNA, but not γ globin, δ globin, or histone H4 RNA, nor whole cell polyadenylated RNA.50 The destabilizing element appears to be a protein that binds to the AU-rich element in 3’ untranslated c-myc RNA.50 Enhanced stability
of c-myc and c-myb RNA in our two AML patients could result from altered activity of this c-myc- and c-myb-RNA stabilizing element. Further work will be done to test this hypothesis.

Impaired turnover of RNA transcripts of nuclear proto-oncogenes regulating myelopoiesis may contribute to the genesis of AML in the subset of patients whose cells exhibit this defect. The reduced turnover of c-myc and c-myb RNA in patients 1 and 7 is of significant magnitude to increase steady-state c-myc and c-myb levels. The approximately fivefold increase in c-myc and c-myb half-lives should increase c-myc and c-myb RNA levels proportionately. There may also be a disruption of RNA turnover triggered by signals to differentiate. For example, in the myeloblastoid cell line HL-60, signals that cause cellular differentiation also trigger a novel c-myc RNA turnover pathway. This pathway may not be operational in these AML cells. Moreover, enhanced stability of c-myb RNA may play a role in the maturation arrest in these cases of AML. Constitutive c-myb expression, like constitutive c-myc expression, inhibits differentiation of leukemia cell lines.

Our results also demonstrate that mRNA turnover is not the sole determinant of c-myc expression in AML cells. High levels of c-myc expression were observed in cells of two patients even though c-myc mRNA was degraded rapidly, and cells from one patient exhibited only an intermediate level of c-myc expression despite slow c-myc RNA turnover. Therefore, c-myc expression in AML cells appears to be regulated at the level of transcription or nuclear transport, as well as at the level of RNA turnover. We have indeed found greater than 10-fold variation in c-myc transcription rate, determined by nuclear run-on assay, in cells of four AML patients studied to date (unpublished data). The roles of transcriptional and post-transcriptional dysregulation in determining c-myc expression in AML cells are as yet incompletely characterized. Evidence presented here of enhanced c-myc RNA stability in some cases of AML provides the first demonstration of a mechanism of c-myc dysregulation in AML.

ACKNOWLEDGMENT

The authors are grateful to Leon Hall III for expert technical assistance.

REFERENCES

8. Holt JT, Redner RL, Nienhuis AW: An oligomer complemen-
tary to c-myc mRNA inhibits proliferation of HL-60 promyelo-
cytic cells and induces differentiation. Mol Cell Biol 8:963, 1988
11. Anfossi G, Gewirtz AM, Calabretta B: An oligomer complemen-
tary to c-myb-encoded mRNA inhibits proliferation of human myeloid leukemia cells lines. Proc Natl Acad Sci USA 86:3379, 1989
21. Aviv H, Leder P: Purification of biologically active globin-
messenger RNA on oligothymidylic acid-cellulose. Proc Natl Acad Sci USA 69:1408, 1972
22. Feinberg AP, Vogelstein B: A technique for radiolabeling
DNA restriction endonuclease fragments to high specific activity. Anal Biochem 132:6, 1983
26. Ruther U, Wagner EF, Muller R: Analysis of the differentiation-promoting potential of inducible c-fos genes introduced into embryonal carcinoma cells. EMBO J 4:1775, 1985
46. Rabbits PH, Forster A, Stinson MA, Rabbits TH: Truncation of exon 1 from the c-myc gene results in prolonged mRNA stability. EMBO J 4:3727, 1985
Defective c-myc and c-myb RNA turnover in acute myeloid leukemia cells

MR Baer, P Augustinos and AJ Kinniburgh