ABSTRACT

The relative abundance of primary transcript and mature mRNA of the c-myc, calcyclin, S14 ribosomal protein, and rRNA genes was determined densitometrically after reverse transcriptase-polymerase chain reaction and Northern blot analysis in resting and mitogen-stimulated lymphocytes, proliferating and terminally differentiated HL-60 cells, and leukemic blast cells. Transcription and processing of c-myc and rRNA gene transcripts increased proportionally after mitogen stimulation, whereas these processes were independent of cell cycling status in the case of the S14 gene. Normal lymphocytes showed an unexpectedly large amount of primary transcript of the calcyclin gene, whereas the corresponding mRNA was undetectable. The abundance of c-myc, calcyclin, and S14 mRNA in terminally differentiated HL-60 cells decreased sharply, compared to their proliferating counterparts. This decrease reflected post-transcriptional modulation, since the abundance of precursor remained essentially unchanged. After HL-60 differentiation, the 32S rRNA levels remained relatively high, whereas the 45S primary transcript almost disappeared. Leukemic blast cells displayed highly variable precursor/mRNA ratios of c-myc, calcyclin, and S14 genes but consistently high ratios of 32S to 45S RNA, suggesting that the cleavage rate of the 32S rRNA was sharply reduced in these cells, resulting in an accumulation of this molecule. These results suggest the importance of efficient processing of primary transcript to generate adequate functional mRNA, thus regulating gene expression. Furthermore, in terminally differentiated cells and leukemic blast cells a stabilization of the primary transcript without efficient processing can be observed. The role of the stabilization of the primary transcript in terminal differentiation is further supported by the results of run-off transcription, indicating a sharp decrease of c-myc and calcyclin transcription rate in retinoic acid/dimethyl sulfoxide-treated HL-60 cells.

INTRODUCTION

Gene expression is regulated at different levels during the events that occur from the transcription of the template to the formation of an adequate amount of functional protein (1, 2). A critical process in this cascade is the coordination of three essential steps, (a) processing of the rRNA with the formation of mature mRNA (3–5), (b) cleavage of rRNA precursors (6), and (c) assembly of proteins in the functional 40S and 60S ribosomal particles (7). The coordination of such molecular events appears particularly complex since the involved genes are located on many different chromosomes (8–10). The mechanisms through which these genes are regulated in different functional stages remain unclear. However, the mechanisms underlying the changes in the expression of several genes during the transition from G0 to S phase of the cell cycle have been identified. Transcription of many cell cycle-related genes, for instance c-fos, c-myc, c-myb, and H3 histone (11–13), and of the ribosomal genes (14) is activated, whereas other genes, such as ribosomal protein genes, are constitutively expressed (15, 16). The increased abundance of thymidine kinase mRNA, observed before entry into S phase, follows a period of stabilization of the primary transcript (17). Several studies described genes that are regulated at the level of rRNA stability and RNA processing rates (18–21). Under different functional conditions, such as cell differentiation, the mRNA abundance of a large set of genes decreases rapidly to undetectable levels, at least by Northern blot analysis (22–25). In leukemic blast cells, most of which are arrested in the G1 phase of the cycle and are unable to differentiate (26), there is evidence suggesting altered regulation of gene expression at the post-transcriptional level (27, 28). In an effort to delineate the steps involved in the regulation of gene expression in different functional states in normal and leukemic cells, we evaluated the abundance of the precursor RNA molecules and of the processed products of four different genes, namely c-myc, calcyclin, S14 ribosomal protein-encoding gene, and rRNA genes. The rationale for looking at these genes is that all of them are growth related and their mRNAs are undetectable in terminally differentiated cells. Furthermore, their functions are different; c-myc protein is a transcriptional factor (29), calcyclin gene encodes a calcium-binding protein (30), S14 protein is assembled in the 40S ribosomal particle (31), and rRNA genes encode the 18S and 28S ribosomal subunits (6). The calcyclin gene is expressed in a cell typespecific way (30).

MATERIALS AND METHODS

Cells. Blast cell populations were obtained by leukapheresis from four patients with acute myeloid leukemias, before any treatment with antineoplastic drugs. The blast cell populations studied were extremely homogeneous (>95% blast cells in the peripheral blood). Residual granulocytes were lysed with ammonium acetate buffer. The phenotype of each case was defined by morphological, cytochemical, immunological, and molecular criteria. Proliferating and differentiated HL-60 cells were obtained as described (32). Normal quiescent PBL and granulocytes were purified by Ficoll gradient centrifugation. PHA-stimulated lymphocytes were obtained as already described (33).

Molecular Probes and Synthetic Oligodeoxynucleotides. The DNA fragments obtained from the plasmid carrying the gene probes used in this study were a 1.2-kilobase PstI fragment from human c-myc cDNA (34), a 0.6-kilobase BamHI fragment from the human calcyclin gene (30), a 0.56-kilobase PstI fragment from the human S14 cDNA (31), and a 0.55-kilobase PstI fragment of the human β2-microglobulin cDNA (35). The fragments obtained by restriction endonuclease digestion were separated by polyacrylamide gel electrophoresis and recovered by electroelution (36). Nonmodified oligodeoxynucleotides were prepared as already described (37). Table 1 gives the sequences of the synthesized oligomers and their relationship to the organization of c-myc, calcyclin, S14, and ribosomal ITS2 genetic loci (31, 38–41).

Received 8/7/91; accepted 10/16/91.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 Supported by a grant from A.I.R.C. (Associazione Italiana per la Ricerca sul Cancro).

2 To whom requests for reprints should be addressed, at Experimental Hematology Center, Second Medical Clinic, University of Modena, Via Del Pozzo 71, Policlinico, 41100 Modena, Italy.

3 The abbreviations used are: PBL, peripheral blood lymphocytes; PHA, phytohemagglutinin; cDNA, complementary DNA; RA, retinoic acid; PCR, polymerase chain reaction; SSC, standard saline citrate; ITS, internal transcribed spacer; RT, reverse transcriptase; AML, acute myeloid leukemia.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
Radiolabeling of DNA Fragments and Synthetic Oligodeoxynucleotides. purified cDNA fragments were labeled with $[\alpha-\text{32P}]$dCTP, by using the random priming labeling procedure (42), to specific activities ranging from 2 to $3 \times 10^4$ cpm/µg DNA. Synthetic oligomer probes were end-labeled with $[\gamma-\text{32P}]$dATP and polynucleotide kinase, as described (43).

RNA Extraction and Northern Blot Analysis. Total cellular RNA was extracted from cells using the guanidinium isothiocyanate-phenol procedure, with some modification (44). Northern blot analysis was performed as described (45).

RT-PCR Technique and Southern Blot Analysis. These were carried out using a modification of the technique described by Ferrari et al. (37). Briefly, total cellular RNA was extracted from $5 \times 10^6$ cells and 5 µg were reverse-transcribed, using 400 units of murine Moloney leukemia virus reverse transcriptase and 0.5 µg of reverse primer, for 1.5 h at 37°C, in 1× eDNA buffer (10 mM Tris-HCl, pH 8.3, 50 mM KCl, 2 mM MgCl₂, 10 mM dithiothreitol, 100 µM dATP, dCTP, dGTP, and dTPP). The resulting specific cDNA fragments were amplified with 2.5 units of Taq polymerase (Promega), in the presence of 2.5 units of Taq polymerase (Promega), in the presence of 0.5 µg of direct primer and 1× cDNA buffer DNA. Fragments corresponding to precursors of c-myc, calcyclin, and S14 were generated during 10—15 cycles of PCR (denaturation at 94°C for 2 min, annealing at 55°C for 1 min, and extension at 72°C for 4 min each). Each cycle of the PCR reaction mixture (100-µl total reaction volume) were separated on a 4% NuSieve agarose (FMC) gel, denatured in 0.2 M NaOH, 0.4 M NaCl, for 45 min, neutralized in 25 mM phosphate buffer (pH 6.5) for 45 min, and electroblotted (46) onto a GeneScreen membrane (NEN). Blots were hybridized with the labeled oligodeoxynucleotide probes under conditions described by Albretsen et al. (47).

RESULTS

Fig. 1A shows the results obtained in RT-PCR and Northern blotting analysis to determine levels of c-myc precursor and mRNA, respectively. A significant amount of precursor is detectable in quiescent PBL (Fig. 1A; lane 1), and a considerable increase occurs after PHA stimulation (Figs. 1A, lane 2). The mature mRNA is undetectable by Northern blot analysis in quiescent PBL but is readily detected after PHA stimulation. In HL-60 cells, precursor levels are high and very similar in proliferating and in RA-induced terminally differentiated cells (Fig. 1A, lanes 3 and 4, respectively); mature mRNA is abundant in the proliferating cells but undetectable in terminally differentiated cells. In leukemic blast cells precursor levels are variable but consistently detectable, as are c-myc mRNA levels (Fig. 1A, lanes 5—8). No consistent correlation between abundance...
different pattern characterizes the leukemic blast cells, in which both the precursor and the mature mRNA are detectable. However, in cells from two AML patients (Figs. 2A; lanes 6 and 7), very different levels of mRNA correspond to nearly identical levels of precursor.

Fig. 2B shows the relative abundance of calcyclin precursor and mature mRNA, as determined densitometrically.

Both precursor and mature mRNA of S14 ribosomal protein gene are easily detectable in all cell populations studied (Fig. 3A). In HL-60 cells, mRNA levels are reduced in differentiated cells, whereas precursor abundance (Fig. 3A, lane 4) remains substantially nonmodified. In leukemic blast cells a low abundance of precursor does not correspond to proportionally lower mRNA levels.

Fig. 3B shows the relative abundance of S14 ribosomal protein precursor and mature mRNA, as determined densitometrically.

Abundance of 45S and 32S precursors of rRNA subunits was evaluated by studying the hybridization of the ITS2, which is cleaved in the 32S to 28S transition, allowing the simultaneous...
Fig. 3. Levels of S14 primary transcript and mature mRNA. Oligomers 13, 14, and 15 (Table 1) were used in the RT-PCR. No amplification and hybridization were detected without reverse transcription. Panels are as described for Fig. 1. Superimposable results were obtained using oligomers 16, 17, and 18 (Table 1).

Fig. 4B shows the relative abundance of 45S and 32S ribosomal precursors, as determined densitometrically.

The results of the run-off transcription experiments, shown in Fig. 5, indicate that the rate of transcription of c-myc, calcyclin, and rRNA genes decreases sharply in terminally differentiated HL-60 cells. A different behavior is shown by the S14 ribosomal protein-encoding gene, whose rate of transcription decreases more slowly during terminal differentiation. The rates of transcription of these genes were measured in proliferating and terminally differentiated HL-60 cells using nuclear run-off experiments. The results are shown in Fig. 6. The rate of transcription of the c-myc gene decreases sharply in terminally differentiated HL-60 cells, while the rates of transcription of calcyclin and rRNA genes decrease more gradually. The rate of transcription of the S14 ribosomal protein-encoding gene decreases more slowly during terminal differentiation. The rates of transcription of these genes were measured in proliferating and terminally differentiated HL-60 cells using nuclear run-off experiments. The results are shown in Fig. 6. The rate of transcription of the c-myc gene decreases sharply in terminally differentiated HL-60 cells, while the rates of transcription of calcyclin and rRNA genes decrease more gradually. The rate of transcription of the S14 ribosomal protein-encoding gene decreases more slowly during terminal differentiation.

The results of the run-off transcription experiments, shown in Fig. 5, indicate that the rate of transcription of c-myc, calcyclin, and rRNA genes decreases sharply in terminally differentiated HL-60 cells. A different behavior is shown by the S14 ribosomal protein-encoding gene, whose rate of transcription decreases more slowly during terminal differentiation. The rates of transcription of these genes were measured in proliferating and terminally differentiated HL-60 cells using nuclear run-off experiments. The results are shown in Fig. 6. The rate of transcription of the c-myc gene decreases sharply in terminally differentiated HL-60 cells, while the rates of transcription of calcyclin and rRNA genes decrease more gradually. The rate of transcription of the S14 ribosomal protein-encoding gene decreases more slowly during terminal differentiation.

The results of the run-off transcription experiments, shown in Fig. 5, indicate that the rate of transcription of c-myc, calcyclin, and rRNA genes decreases sharply in terminally differentiated HL-60 cells. A different behavior is shown by the S14 ribosomal protein-encoding gene, whose rate of transcription decreases more slowly during terminal differentiation. The rates of transcription of these genes were measured in proliferating and terminally differentiated HL-60 cells using nuclear run-off experiments. The results are shown in Fig. 6. The rate of transcription of the c-myc gene decreases sharply in terminally differentiated HL-60 cells, while the rates of transcription of calcyclin and rRNA genes decrease more gradually. The rate of transcription of the S14 ribosomal protein-encoding gene decreases more slowly during terminal differentiation.
tion is essentially similar in both proliferating and terminally differentiated HL-60 cells.

DISCUSSION

We have analyzed the relative abundance of primary transcripts and mature mRNA of four genes with different well defined roles, ranging from the transactivation ability of c-myc, to the calcium-binding activity of calcycin, to the de novo generation of ribosomal particles for S14 and the RNA genes. The increased abundance of the c-myc precursor in mitogen-stimulated lymphocytes confirms the transcriptional regulation of the expression of this gene in cells induced to proliferate, as demonstrated by transcription run-off experiments (data not shown). An unexpected finding was the close similarity in c-myc precursor level in proliferating and terminally differentiated HL-60 cells, despite the practical disappearance of the mature mRNA in the latter. Since a decrease in the abundance of c-myc mRNA is a necessary prerequisite for cell differentiation (53—55), our observation suggests that this process is not primarily related to a transcriptional process. The stabilization of a precursor molecule seems, therefore, to lead to two different results, depending on the functional state of the cell. In the late G1 period of a proliferating cell the stabilization of the thymidine kinase precursor leads to an increased abundance of the mature mRNA, as mentioned before (17). In the differentiation of the HL-60 cells, the prolonged life of the precursor leads to a reduction in the abundance of mature c-myc mRNA, presumably because of a failure in the activation of the processing mechanisms. This is confirmed by the transcription run-off experiments, indicating that the rate of transcription of c-myc and calcycin are markedly reduced in differentiated cells, in spite of the persistence of a large amount of unprocessed precursor. The detection of calcycin primary precursor in quiescent and proliferating normal lymphocytes and in proliferating and terminally differentiated HL-60 cells was also unexpected, since calcycin expression was not previously detected in these cell types (30, 56). Our finding that both precursor and mature mRNA of the S14 gene are expressed at essentially the same levels in both quiescent and proliferating lymphocytes is in keeping with previous reports that this gene is expressed independently of the cycling status of the cell (27). On the other hand, cell differentiation is accompanied by a decrease in S14 precursor levels, again supporting the conclusion that posttranscriptional mechanisms underlie cell differentiation. The level of ribosomal precursors increases in mitogen-stimulated lymphocytes, but the increase is much smaller than that of the mature rRNA, suggesting that the production of rRNA is regulated through a coordinate increase of both the rate of transcription and the efficiency of processing of the primary transcript. The large amount of the two ribosomal precursors observed in proliferating HL-60 cells is consistent with a rapidly growing cell population. However, in terminally differentiated cells the levels of the primary product decrease much more than those of 32S RNA. Our data thus confirm that both the rate of transcription of the 45S molecule and the rate of cleavage of the 32S molecule are reduced (57). In the leukemic cells, the ratio between the precursor and the mature mRNA appeared to differ for the c-myc, calcycin, and S14 genes. The high levels of c-myc and calcycin mRNAs and the low level of expression of histone H3 (data not shown) are consistent with arrest of these cells in the G1 phase of the cell cycle. Thus, it seems likely that the level of precursor RNA molecules corresponding to these genes is related mainly to an increased half-life of these molecules, rather than to their active transcription. The very low abundance or complete absence of 45S RNA is suggestive of an overall lower transcription rate in these cells. The abundance of 32S RNA, in contrast is remarkably high, suggesting that the cleavage rate of this molecule is much reduced, so that the 32S RNA accumulates in these cells. In conclusion, our results point to the efficient processing of primary transcript as a critical step in the cascade of events regulating gene expression. This is particularly remarkable in terminally differentiated cells and in leukemic blast cells, where a stabilization of the primary transcript or of an intermediate product of cleavage, such as 32S RNA, takes place, instead of efficient processing.

REFERENCES


Abundance of the Primary Transcript and Its Processed Product of Growth-related Genes in Normal and Leukemic Cells during Proliferation and Differentiation

Sergio Ferrari, Enrico Tagliafico, Rossella Manfredini, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/52/1/11

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, use this link
http://cancerres.aacrjournals.org/content/52/1/11.
Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.