Noncoordinated Expression of S6, S11, and S14 Ribosomal Protein Genes in Leukemic Blast Cells

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ABSTRACT

The steady state levels of mRNAs coding for the ribosomal proteins S6, S11, and S14 have been evaluated in quiescent and proliferating human fibroblasts and in resting and proliferating human peripheral blood mononuclear cells. It was found that the amounts of ribosomal protein mRNA are very similar and are not increased by serum or mitogen stimulation. The constitutive expression of these genes appears to be coordinately regulated and it is not modified after protein synthesis inhibition by cycloheximide. The ribosomal protein mRNA was also assayed in 15 different populations of human leukemic blast cells. In these populations the abundance of each ribosomal protein mRNA is remarkably different from the other. The results of our present experiments indicate that the expression of the three ribosomal protein genes undergoes independent noncoordinated changes in the large majority of the leukemic populations studied.

INTRODUCTION

The increased formation of new ribosomes which occurs during G1 progression is a complex process involving the coordinate synthesis and processing of rRNA and the synthesis of the respective ribosomal proteins (1-3). The events by which this process is regulated are still poorly understood. In mouse fibroblasts, the increase in the abundance of the rRNA is due to an increase in the rate of transcription and to the high rate of processing of rRNA precursors (4). The increase in ribosomal proteins in the same cells is due to an increased efficiency of translation of rpmRNA due to a shift in the association with polysomes rather than to an increase in the transcription rate (5). The regulatory mechanisms of the two components of the ribosomes are therefore different, at least in mouse fibroblasts and HeLa cells. However, they must be correlated, since inhibition of ribosomal protein synthesis inhibits processing of the ribosomal precursors (6-8). Since in the blast cells of both myeloid and lymphoid acute leukemia the rate of processing of ribosomal precursors is very low (9) we have assayed the levels of expression of three ribosomal protein genes in several leukemic cell populations. As controls, we have studied the expression of some ribosomal proteins in both resting and growing human fibroblasts and in unstimulated and mitogen-stimulated PBMC. The behavior of the ribosomal protein mRNA was also studied during the in vitro differentiation of HL60 cells.

MATERIALS AND METHODS

Cell Characterization and Cell Cultures

Blast cell populations were obtained by leukapheresis before any treatment with antineoplastic drugs from 13 patients with both myeloid and lymphoid acute leukemia and from two patients with chronic myeloid leukemia in blast crisis. In all cases, the blast cells represented more than 90% of the cells in peripheral blood. The phenotype in each case was defined by morphological, cytochemical, and immunological criteria. Phytohemagglutinin stimulated lymphocytes were cultured according to the method of Torelli et al. (10). The HL60 cells were grown as described by Torelli et al. (11). WI38 human fibroblasts were made quiescent by bringing them to confluence and leaving them in low serum concentration (0.5%) for 15 days. The cells were induced to proliferate by adding 15% fetal calf serum (12). The experiments with cycloheximide were performed in quiescent and mitogen-stimulated PBMC using the experimental conditions of Ritting et al. (13).

Molecular Probes

Synthesis and Purification of Oligomers. The complementary oligodeoxynucleotides were synthesized on an automated solid-phase synthesizer (Applied Biosystems, Inc.; Mod. 381 A) by using standard phosphoramidite chemistry and were purified by several extractions with (NH4)2O, followed by incubation at 56°C for 16 h and concentration by ethanol precipitation or gel electrophoresis. The sequences of the synthesized oligomers and their relationship to the organization of ribosomal protein cDNAs are reported in Table 1.

Purification of Probes. The DNA fragments obtained from the plasmid carrying the gene probes used in this study were a 1.2-kilobase PstI fragment from the human c-myc cDNA as described by Watt et al. (14), a 2.1-kilobase EcoRI genomic fragment from the histone H3 gene (15), a 0.55-kilobase PstI fragment of the human β-2-microglobulin cDNA (16), and a 2.1 BamHI fragment of the human β-actin cDNA obtained from the recombinant plasmid pHFβ-A1 (17).

DNA Labeling

Oligonucleotides were end labeled with polynucleotide kinase (Promega). A solution of oligonucleotides (200–400 ng) was added to 5× kinase buffer (0.25 M Tris-HCl, pH 7.5, 0.1 M MgCl2, 50 mM dithiothreitol, 1 mM spermidine, and 1 mM EDTA) [γ-32P]ATP (100 μCi) and polynucleotide kinase (10 units). The mixture was incubated at 37°C for 30 min followed by enzyme inactivation at 90°C for 2 min. Labeled oligonucleotides were separated from unincorporated ATP by chromatography on Sephadex G-50 fine. All other purified inserts were labeled using the random priming technique described by Feinberg and Vogelstein (18). The specific radioactivities were in the range of 1–3 × 106 cpn/μg DNA.

RNA Extraction and Northern Blot Analysis

Total cellular RNA was extracted from leukemic blast cells and from the other cell populations using a modification of the guanidinium isothiocyanate-phenol procedure as described by Chomczynski and Sacchi (19). The c-myc, histone H3, β-2-microglobulin and β-actin were analyzed by the Northern blot technique described by Ferrari et al. (20). The labeled oligodeoxynucleotides were hybridized as described by Albretsen et al. (21). Densitometer scanning of the filters were performed with the aid of an LKB soft-laser densitometer.

RESULTS

Levels of Expression of the Genes Coding for the Ribosomal Proteins S6, S11, and S14 in Quiescent and Proliferating Fibroblasts and in Resting and Proliferating PBMC. Fig. 1 shows

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3 The abbreviations used are: rpmRNA, ribosomal protein mRNA; PBMC, peripheral blood mononuclear cells; cDNA, complementary DNA.
### Table 1. Sequences of the oligodeoxynucleotides complementary to the S6, S11, and S14 mRNA used in the Northern blot analysis.

<table>
<thead>
<tr>
<th>Genes</th>
<th>Oligomers [5'–3']</th>
<th>Regions</th>
<th>Refs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>S6</td>
<td>GAA GGA GAT GTT CAG CTT TCT TTT GGG GCC CAG GCG GCG AGG</td>
<td>4–21, 23, 24</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>388–411</td>
<td></td>
</tr>
<tr>
<td>S11</td>
<td>GGC ACG CTC AGT CTG AAT CAG GTG TAG AGA CAT GTT CTT GTG</td>
<td>25–42, 331–354</td>
<td>25</td>
</tr>
<tr>
<td>S14</td>
<td>CCC CTT TCG AGG TGC CAT GCC TTT ACC TTC ATC CCA CCA G</td>
<td>34–51, 203–224</td>
<td>26</td>
</tr>
</tbody>
</table>

RNA blots obtained with total cellular RNA extracted from quiescent and proliferating human fibroblasts (Lanes 1 and 2) and from PBMC unstimulated or stimulated for different periods of time with phytohemagglutinin (Lanes 3–7). The RNA was hybridized with synthetic oligonucleotides complementary to the human S6, S11, and S14 cDNAs. The abundances of the rpmRNA are closely similar irrespective of the quiescent or proliferative state of the fibroblasts. No difference in the abundance of the rpmRNAs in PBMC is apparent either in the early hours of stimulation or later when the levels of expression of the histone H3 and c-myc give evidence that the cells have gone...
rpmRNA EXPRESSION IN ACUTE LEUKEMIAS

through the G₁ to the S phase. To monitor the amounts of RNA for each lane, we have hybridized the RNA blots with β-actin probe. Fig. 2A shows the schematic representation of the relationships among the relative abundances of the three rpmRNAs as obtained by densitometric scanning of autoradiographs. Fig. 2A demonstrates that the expression of the three ribosomal protein genes is strictly coordinated, since the abundances of rpmRNA are very similar. Cycloheximide treatment for 4 h at a concentration of 50 μg/ml had no effect on the levels of expression of the three genes (data not shown).

Levels of Expression of the Genes Coding for Ribosomal Proteins S6, S11, and S14 in Proliferating HL60 Cells before and after Induction to Terminal Differentiation. Fig. 3 shows the RNA blots obtained when total cellular RNA was extracted from HL60 cells exponentially growing and after 20, 48, and 110 h of treatment with retinoic acid. In proliferating cells the three ribosomal protein genes were well expressed. The expression of the S6 and S11 genes almost completely ceased after 20 h of treatment with retinoic acid. The behavior of S14 was different, since the expression was decreased but still evident after 110 h. As expected, the mRNAs for c-myc and histone H3 were already undetectable after 20 h.

Levels of Expression of the Genes Coding for S6, S11, and S14 Ribosomal Proteins in Blast Cells of Acute Lymphoid Leukemia, Acute Myeloid Leukemia, and Chronic Myeloid Leukemia Blast Crises. Fig. 4 shows the RNA blots obtained when the synthetic oligodeoxynucleotides complementary to the human S6, S11, and S14 ribosomal protein genes were hybridized to total cellular RNA extracted from blast cell populations. The S6 gene expression was detectable in 13 of 15 cases studied. The expression was not detectable, even after prolonged exposure, in cases 3 and 7. In the other cases the abundances of the mRNA were variable. They were particularly high in cases 1, 8, 9, and 16. S11 gene expression was extremely low in cases 5, 7, and 13. In the other cases, the mRNA was detectable at different levels, and the abundance of the mRNA was particularly high in cases 12, 14, and 16. The S14 gene expression was definitely detectable in all cases studied, but also for this gene the abundance of the mRNA was remarkably variable. Fig. 2B shows the densitometer scanning of the autoradiographs shown in Fig. 4. It is apparent that in the large majority of the leukemic cell populations each single gene was differentially expressed. Whereas in a minority of the cell populations the three genes were expressed approximately at the same level, most leukemic cells either show a preferential expression of one gene or one of the three genes is expressed very scarcely or not at all. It should be noted that six sheets containing the same amount of RNA for each lane were hybridized with the same amount of labeled probe and the exposure time for ribosomal protein genes was 4 days and that for the other genes was 2 days. The β₂-microglobulin probe was hybridized to monitor the amount of RNA for each lane. Each experiment was repeated three times.

DISCUSSION

The results of our experiments in human fibroblasts clearly indicate that the levels of expression of the three ribosomal protein genes studied do not significantly change after serum stimulation. Also in PBMC the abundance of mRNA in the three ribosomal proteins remains unchanged after mitogen stimulation. This indicates that the expression of these genes is not inducible by serum or mitogens, so that the regulation of ribosomal protein synthesis is presumably due to an increased efficiency of translation (5). The control mechanism of ribosomal protein synthesis during cell differentiation is presumably different from that operating during cell growth. In fact our

![Fig. 4. RNA blots of total cellular RNA extracted from leukemic blast cells (Lanes 1-9 and 12-17). The figure also shows the pattern obtained with different preparation of HL60 cells RNA both proliferating (Lanes 10 and 18) and terminally differentiated (Lane 11). Experimental conditions are the same described in the legend to Fig. 1 and in "Materials and Methods."](cancerres.aacrjournals.org)
data show that in HL60 cells induced to terminal differentiation by retinoic acid all rpmRNAs rapidly decrease, some of them becoming undetectable. These events may explain the 70–80% reduction in rRNA synthesis and processing which occurs in terminally differentiated cells (22). This pattern of rpmRNA gene expression has never been observed in leukemic populations. The differential expression of some ribosomal protein genes in leukemic blast cells gives further molecular evidence that their genetic behavior is definitely different from both proliferating and terminally differentiated cells. The results of our experiments indicate that the expression of the three ribosomal proteins genes which we have studied undergo independent, noncoordinated changes in the large majority of the leukemic populations studied. These changes showed no relationship with the phenotype of the cases studied. In these populations the abundance of each rpmRNA is different from the other. This leads us to believe that the coordinated regulation of the ribosomal protein synthesis in these cells is impaired, making difficult all the events depending on a proper assembling of the ribosomes. Since among these events is included the processing of rRNA precursors (8), our results might explain the origin of the inhibition of this processing in leukemic blast cells (9). On the other hand, an alteration in mRNA stability might equally well explain the differential expression that we see at the steady state level in leukemic cell populations and the impaired processing of rRNA precursors in these cells.

REFERENCES

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