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Expression of a gene encoding a tRNA synthetase-like protein is enhanced in tumorigenic human myeloid leukemia cells and is cell cycle stage- and differentiation-dependent

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ABSTRACT **We cloned a tumorigenic phenotype-associated cDNA encoding a tRNA synthetase-like protein from an acute-phase human myeloid leukemia cell line. The cDNA was isolated by reiterative subtraction of cDNAs synthesized from tumor-generating parental leukemia cells versus those from a nontumorigenic variant of the same cells. The selected cDNA encodes a protein that is a close homolog of one subunit of prokaryote and yeast phenylalanyl-tRNA synthetase (PheRS). The expressed protein reacts specificially with polyclonal antibodies raised against mammalian phenylalanyl-tRNA synthetase. Expression of the gene (designated CML33) was directly confirmed by Northern blot hybridization to be substantially enhanced in the tumorigenic cells compared with the nontumorigenic variant. In addition, expression of CML33 in myeloid leukemia cells was sensitive to the stage of the cell cycle and to induction of differentiation. Although the relationship between these observations and the tumorigenic state of the human myeloid leukemia cell line used in these studies is unknown, to our knowledge, this is the first demonstration in mammalian cells of tumor-selective and cell cycle stage- and differentiation-dependent expression of a member of the tRNA synthetase gene family.**

Altered expressions of genes associated with growth and differentiation of cells are considered key genetic events in the malignant transformation process. Cloning and characterization of genes differentially expressed in tumor cells are important steps for understanding the genetic mechanisms underlying malignant transformation. The subtraction hybridization technique has been used to isolate several important genes implicated in tumorigenesis (1, 2). We have used this technique to clone the genes differentially expressed between a tumorigenic human acute-phase chronic myeloid leukemia (CML) cell line and its nontumorigenic variant raised in our laboratory (3). Acute-phase CML cells, when injected into nude mice, rapidly give rise to tumors, whereas the variants have lost the tumorigenic potential. The genetic mechanism(s) responsible for transition of CML, an initially indolent disease, to acute-phase malignancy, is not well understood (4). It was hypothesized that the genes preferentially expressed in the tumorigenic CML cells *in vitro* would also be critical in the evolution of chronic-phase to acute-phase disease *in vivo* (3).

Tumorigenic transformation is known to entail activation of oncogenes that override growth regulatory signals and inactivation of tumor suppressor genes that render cells free of growth restraining mechanisms leading to uncontrolled growth and loss of differentiation (1, 2). These genes have predominantly been found to be involved either in transduction of growth regulatory signals from the cell surface to the cell nucleus (growth factors, growth factors receptors, etc.) or in direct regulation of transcription (transcription factors).

We report in this paper the cloning and characterization of a novel human cDNA that encodes an mRNA preferentially expressed in tumorigenic acute-phase CML cells. To our surprise, this cDNA encoded a protein that has strong homology to one subunit of a class II tRNA synthetase. Subsequent studies showed that this finding was not an artifact of our procedure. In particular, we were able to show directly that the mRNA for this protein was overexpressed in the tumorigenic versus the nontumorigenic variant of the same cell line. These and additional observations demonstrate the sensitivity of an apparent member of the tRNA synthetase family (5, 6) to global regulatory mechanisms in mammalian cells.

MATERIALS AND METHODS

Cell Source and RNA Preparation. Human myeloid leukemia cell lines HL-60 and K562, obtained from the American Type Culture Collection, were maintained in RPMI 1640 medium (GIBCO/BRL) supplemented with 10% fetal bovine serum at 37°C and 5% CO_2 in air. HL-60 cell line was established with peripheral blood leukocytes from a patient with acute promyelocytic leukemia (7) and can be induced to differentiate into monocytic cells in presence of phorbol 12-myristate 13-acetate (PMA). K562 is an acute-phase CML cell line that forms rapidly growing tumors when injected into nude mice. The nontumorigenic variant was identified from among a series of mutants isolated from these cells after treatment with ethyl methanesulfonate. As described (3), among the five mutants tested for their tumorigenic potential in nude mice, one showed complete loss of its ability to form tumors in repeated experiments. Messenger RNA from this tumorigenic variant and the parental tumorigenic cells were used in this study to make the subtractive cDNA library. The poly(A)$^+$ RNA from the cells was isolated by affinity chromatography on oligo(dT) cellulose using the Fast Track mRNA isolation kit (Invitrogen).

Cloning of CML33 cDNA. The cDNA clones were isolated from the subtraction cDNA library by differential screening with the mRNA from the tumorigenic cells and its nontumorigenic variant. The subtraction cDNA library was constructed

Abbreviations: CML, chronic myeloid leukemia; PMA, phorbol 12-myristate 13-acetate; IPTG, isopropyl β-D-thiogalactoside; RACE, rapid amplification of cDNA ends.

Data deposition: The sequence reported in this paper has been deposited in the GenBank database (accession no. U07424).

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by the procedure described by Schweinfest (8). Two independent cDNA libraries with mRNAs from the two cell lines were constructed with the cDNA synthesis kit (Superscript Choice System from GIBCO/BRL) and the predigested λZap II/*Eco*RI/CIAP cloning kit (Stratagene). First-strand cDNA synthesis was primed with a combination of oligo(dT) and random hexamer sequences in the presence of modified Moloney murine leukemia virus reverse transcriptase, Superscript RT (GIBCO/BRL) that lacks RNase H activity. Double-stranded cDNA was ligated with λZap II vector arms (Stratagene). The ligated product was packaged and titered with Gigapack II Gold packaging extract (Stratagene). The libraries yielded $0.7–2 \times 10^6$ plaques per μg of mRNA, with more than 90% being recombinant. Libraries were amplified once to a titer of 5×10^9 plaque-forming units/ml. Single-stranded recombinant phage was rescued from λZap II libraries by *in vivo* excision as pBluescript SK^- ($pBSK^-$) (Stratagene) of helper M13 phage.

To identify the transcripts preferentially expressed in the tumorigenic cell line, single-stranded phage cDNA (2 μg) from these cells was hybridized as "tracer" molecules with 10 μg of biotinylated "driver" single-stranded phage cDNA (labeled Photoprobe biotin from Vector Laboratories) from the non-tumorigenic cells. Hybridized cDNA was subtracted with avidin-agarose (Vector Laboratories). Subtracted tumorigenic cell cDNA was subjected to another round of subtraction hybridization in the presence of 100 ng of biotinylated nontumorigenic cell cDNA. The subtracted single-stranded pBluescript DNA was converted to double-stranded DNA in the presence of a Bluescript sequencing primer and Klenow fragment of DNA polymerase I (Boehringer Mannheim). The DNA was then used to transform *Escherichia coli* DH5α cells and plated on LB agar plates containing ampicillin at 50 μg/ml supplemented with 5-bromo-4-chloro-3-indolyl β-D-galactoside (X-Gal)/isopropyl β-D-thiogalactoside (IPTG). The subtracted cDNA library of about 1,500 cDNA clones was then sequentially screened with mRNA probes from the two cell lines. For this purpose, single-stranded cDNAs complementary to poly$(A)^+$ RNAs were synthesized. Purified labeled probes with about 10^8 cpm were used to screen the subtraction cDNA library by standard protocols (9).

Eight clones showing preferential hybridization with the tumorigenic cell cDNA probe were isolated. Of the eight clones isolated, six were sequenced, one had a short insert of less than 200 bp, and another gave smear signals on Northern and Southern blots, suggesting presence of repeats. The latter two clones were not sequenced. Among the remaining six, one sequence showed significant homology with the human adenine phosphoribosyltransferase gene and another with a human mitochondrial sequence. The rest did not reveal homology with any existing human sequence in the data bank. Of these four clones, one showed strong homology with the gene for yeast phenylalanyl t-RNA synthetase. The insert from this clone was used to screen the cDNA library from the tumorigenic cell line and the full-length clone (designated CML33) was isolated.

5′ Rapid Amplification of cDNA Ends (RACE). To obtain the complete sequence from the 5′ end of the CML33 cDNA, the 5′ RACE technique was used with the mRNA from the tumorigenic cells and the 5′ Amplifier RACE kit (CLONETECH). Positions of the primer P1 for first-strand synthesis of cDNA and of the nested primer P2 for PCR amplification of the cDNA product are shown in Fig. 1. The PCR-amplified product was cloned and sequenced.

DNA Sequencing of cDNA Clones and Computational Analysis. Both strands of the cloned CML33 cDNA were sequenced by the dideoxynucleotide chain-termination method (10) according to the vendors protocol supplied with the Sequenase Version 2.0 DNA sequencing kit (United States Biochemical) and also on an Applied Biosystems model 373A automated DNA sequencer available in a core sequencing facility at the University of Texas M. D. Anderson Cancer Center.

The sequence was examined with the GRAIL-3 software (11) to identify open reading frames in the sequence. An open reading frame with an ATG for the start of translation was located. This open frame extended for 508 codons and ended with TGA. The SwissProt (Release 33.0) and GenPept (Release 96.0) databases were then searched with BLAST using this coding sequence. Searches were also carried out with alternative frames but these showed only a few possible matches, which were rejected by using a low complexity filtering algorithm in the SEG program (12).

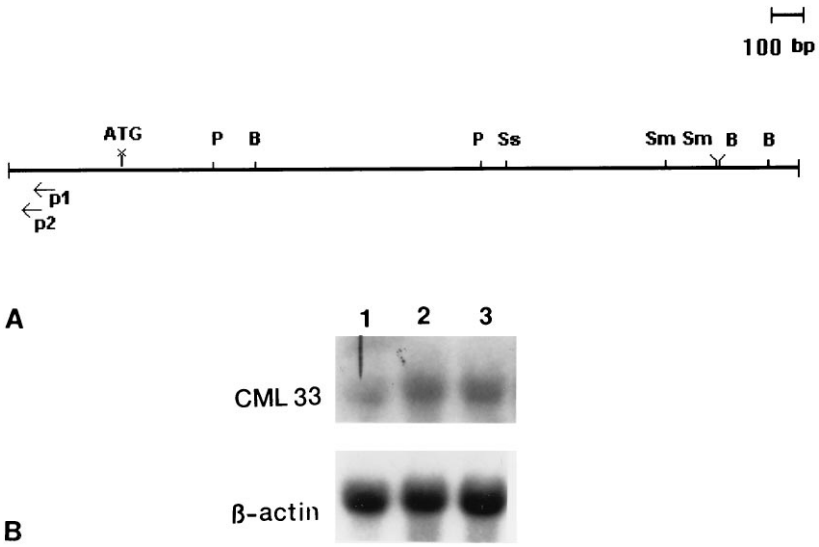

FIG. 1. (*A*) Restriction map of the CML33 cDNA. The full-length cDNA sequence was assembled from three individual overlapping clones isolated. Position of the initiation codon ATG is indicated. P1 and P2 show the positions of the two primers used to identify the 5′ end of the transcript by RACE. Vertical bars represent the restriction enzyme sites (P, *Pst*I; B, *Bgl*I; Ss, *Sst*I; Sm, *Sma*I). (*B*) Expression of CML33 mRNA in peripheral blood lymphocytes (lane 1) and bone marrow mononuclear cells (lanes 2 and 3) of two normal healthy individuals. Lanes 1 and 2 are samples derived from the same individual. Note higher steady-state level of the CML33 transcript in the bone marrow cells. The same blot was hybridized with a β-actin probe to estimate the amount of RNA loaded in each lane.

Those sequences identified as being statistically similar were aligned using the GAP program of the GCG to examine for regions of similarity. As a second approach, the PROSITE database was searched with the coding region of CML33 and the MOTIFS program of the GCG package to identify defined motifs.

Expression of the CML33 cDNA Protein Fused to Glutathione *S*-Transferase. Oligonucleotide directed mutagenesis was used to create a *Bam*HI site upstream from the ATG that was used for cloning the CML33 cDNA into the glutathione-*S*-transferase fusion vector pGEX-3X (Pharmacia). Extracts of *E. coli* containing the CML33 cDNA fused in-frame with glutathione *S*-transferase were prepared without addition of IPTG or after induction of the fusion protein with 0.5 mM IPTG. The fusion protein of the correct molecular weight was detected after electrophoresis of *E. coli* extracts on 8% SDS polyacrylamide gels by using Coomassie blue staining.

Immunoblot Analysis. Proteins from *E. coli* extracts were subjected to PAGE, electrophoretically transferred to Optitran membrane (Schleicher & Schuell), and analyzed for cross-reactivity with antibodies raised in rabbits against sheep liver PheRS, cytoplasmic PheRS from *Saccharomyces cerevisiae,* or mitochondrial PheRS from *S. cerevisiae* (gifts from Marc Mirande, Centre National de la Recherche Scientifique, Cedex, France, and Franco Fasiolo, Centre National de la Recherche Scientifique, Strasbourg, France). Immunoblot analysis was performed with horseradish peroxidase-coupled anti-rabbit secondary antibodies and the ECL Western blotting detection reagents (Amersham).

Chromosomal Localization. For chromosomal localization of the gene encoded by the cloned cDNA, DNA from human–rodent somatic cell hybrids constituting the entire mapping panel (Coriell Cell Repositories, Camden, NJ) was digested with *Eco*RI and analyzed by Southern blotting and hybridization with the CML33 cDNA probe. DNA samples from each of the parental cells (human, mouse, and Chinese hamster) making the cell hybrids were also included in the blot.

Southern and Northern Blotting. Southern and Northern blotting procedures were performed by standard protocols (8).

Cell Synchronization and Cell Cycle Phase-Dependent Expression. Cells synchronized at different phases of the cell cycle were obtained by centrifugal elutriation of an exponentially growing population of human acute myeloid leukemia cells (cell line HL-60) as described (13). Briefly, cells were resuspended in Hanks' balanced salt solution (GIBCO/BRL) containing 3.3% heat-inactivated bovine calf serum and 5 mM 2-naphthol 6,8-disulfonic acid (pH 7.2; Eastman Kodak). The cells were passed through a 23-gauge needle and nylon mesh to remove clumps and loaded into a Beckman J-6M/E centrifugal elutriator (at room temperature) at a rotor speed of 3,000 rpm. Fractions were collected by increasing the flow rate stepwise from 24.9 ml/min to 88.4 ml/min and collecting 50-ml fraction. Cells in each fraction were determined with a FACSCAN flow cytometer (Becton Dickinson) and analyzed with CELLFIT software (Becton Dickinson) after gating out cell doublets and debris. Chicken erythrocytes were used in the flow cytometry measurements for an internal control. Fractions representative of individual cell cycle phases were pooled, and RNA was isolated and assayed for the expression of the CML33 gene by Northern blot hybridization analysis.

Cell Differentiation and Expression. The myeloid leukemia cell line HL-60 was induced to differentiate toward monocyte-like cells by incubation in the presence of PMA as described (14). Cells at a concentration of 1×10^6 cells per ml of culture medium were treated with 5×10^{-8} M PMA for various intervals. At the end of each time point, cells were washed in ice-cold phosphate-buffered saline (0.01 M sodium phosphate/0.15 M sodium chloride, pH 7.2) and processed for isolation of total RNA. An aliquot of each cell sample was verified for monocytic differentiation. Equal amounts of RNA from each treated cell sample was size-fractionated through agarose gel and analyzed by Northern blot hybridization with the CML33 probe.

RESULTS

We cloned and sequenced the full-length (≈1.8 kb) cDNA for a novel gene, CML33, from a myeloid leukemia cell line. The length of the cDNA is in agreement with the mRNA size identified by Northern blot analysis. Restriction maps of the complete cDNA sequence derived from the three partial overlapping cDNA clones are presented in Fig. 1*A*. The clone pK33-4-1 harbored the longest insert, which represented all but about 60 bases at the 3′ end of the full-length transcript. Northern blot hybridization of the pK33 insert DNA with the total RNA from peripheral blood lymphocytes and bone marrow mononuclear cells of a normal healthy individual is shown in Fig. 1*B*. The steady-state level of this 1.8-kb transcript consistently appeared higher in the bone marrow cells compared with peripheral blood lymphocytes from all the five normal individuals screened (data not shown). This result suggested that the expression is higher in proliferating cells of the bone marrow tissue than in differentiated nonproliferative peripheral blood lymphocytes.

Northern blot hybridization of the tumorigenic and nontumorigenic leukemic cell RNAs further confirmed that enhanced expression of this gene also correlated with the tumorigenic phenotype in these cells (Fig. 2). This result also showed that the isolation of CML33 by subtractive hybridization was not an artifact of the procedure used.

Sequencing of CML33 cDNA. The nucleotide corresponding to the most 5′ cDNA end was identified by the RACE technique using the two primers P1 and P2 (shown in Fig. 1*A*). The cDNA sequence revealed that it contains a 3′ untranslated region of 302 bp. A potential polyadenylylation signal AATAAA was at position 1,785.

The search of GenBank using the sequence (with the long polyadenylylate region removed) identified seven sequences with statistically significant homology ($P < 10^{-4}$), including the coding sequences for the β chain of cytoplasmic phenylalanyl-tRNA synthetase from *Bacillus subtillis* (15) and the gene coding for the α subunit of phenylalanyl-tRNA synthetase from *Thermus thermophilus* (16, 17). In addition, four sequences from expressed sequence tags were found. The first and second of these, EST06386 and EST996488 (18), from a human infant brain cDNA library, are essentially identical to

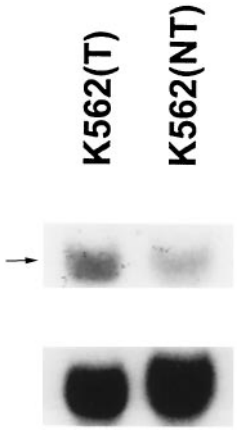

FIG. 2. Northern blot hybridization analyses of total RNA from tumorigenic (K562T) and nontumorigenic (K562NT) human myeloid leukemia cells for expression of the CML33 gene (→). Note marked overexpression of the transcript in tumorigenic cells. Lower row shows β-actin hybridization signal on the same blot to document comparable levels of RNA loading in each lane.

CML33. The first being 94.8% identical to positions 1–232 and the second 97.3% identical to positions 110–365. The second two expressed sequence tags, wEST00002 and wEST02069, from *Caenorhabditis elegans* (19) were located at positions 466–860 (61% identity) and 1,321–1,489 (62% identity), respectively.

The searches of the SwissProt and GenPept data bases identified five peptide sequences with high statistical similarity ($P < 10^{-5}$) including both the *S. cerevisiae* cytoplasmic phenylalanyl-tRNA synthetase β chain and the *T. thermophilus* phenylalanyl-tRNA synthetase α chain.

The results of aligning CML33 with the α subunit of phenylalanyl-tRNA synthetase from *T. thermophilus* are shown in Fig. 3. Only the first 340 of the 508 codons of the CML33 protein are shown. Over the matching region, there is 59.8% similarity and 34.2% identity. There are three motifs characteristic of the type II tRNA synthetases. These are derived from Eriani *et al.* (20) and Cusak *et al.* (21) and constitute motifs I, II, and III. The three motifs are identified in the small α subunit of *T. thermophilus* PheRS. Resolution of the crystal structure of PheRS from *T. thermophilus* has revealed that the three conserved motifs are incorporated into helix–loop–strand, strand–loop–strand, and strand–helix for motifs I, II, and III, respectively (22). Alignments against the *T. thermophilus* enzyme identified all of the three motifs in the sequence encoded by CML33. In addition, the CML33-encoded protein also showed homology with the β chain of *S. cerevisiae* cytoplasmic (66.9% similarity, 52.3% identify), the α chain of *E. coli* (57.9% similarity, 31.3% identity), of *B. subtillis* (52.3% similarity, 29.7% identity), and of *S. cerevisiae* mitochondrial (47.9% similarity and 25% identity) PheRS enzymes.

The CML33 Protein Is Antigenically Related to Cytoplasmic PheRS from Sheep. To determine the relationship between the protein encoded by the CML33 cDNA and PheRS, a fusion protein was created between glutathione *S*-transferase and the CML33 protein. The fusion protein was then tested for its ability to cross-react with antibodies directed against sheep liver PheRS, cytoplasmic PheRS from *S. cerevisiae*, or mitochondrial PheRS from *S. cerevisiae*. Western blot analysis showed that the fusion protein is able to cross-react with only the antibodies directed against sheep liver PheRS (Fig. 4). On the basis of the high sequence identity between the CML33 protein and the β subunit of PheRS, the CML33 protein represents a homolog of the β subunit of the human cytoplasmic or a mitochondrial PheRS.

Chromosomal Localization of CML33. The chromosomal location of the CML33 gene was determined by probing Southern blots of DNA from monochromosomal human/rodent somatic cell hybrids with the pk33–4-1 insert DNA. The hybrid cell line containing the human chromosome 19 revealed the presence of human-specific *Eco*RI restriction fragments (data not shown).

Expression of CML33 Gene Through Cell Division Cycle. An exponentially growing population of HL-60 cells was separated into 15 fractions based on changes in the volume of cells representing different phases of the cell cycle. Fractions containing cells in the same phase of the cell cycle, as indicated by their flow cytometric analyses, were pooled. Relative enrichment of cells in the final eight fractions representing different intervals of G_1, S, and G_2/M phases are shown in the diagram in Fig. 5*A*. Total RNA isolated from cells in the final eight fractions (as described in Fig. 5) were separated on an agarose gel that was hybridized with the CML33 cDNA probe. This analysis showed that the relative abundance of the CML33 transcript was low in G_1 phase and progressively increased as cells traverse through the division cycle. The mRNA level for this gene appeared to peak in the G_2/M phase.

CML33 Expression After Differentiation Induction in Myeloid Leukemia Cells. To study whether CML33 mRNA expression is modulated during differentiation of hematopoi-

```
T thermophilus  .................................MLEEALAAIQNARDLEELKALKARYLG
cml33           MRLPSGKVGFSKAMSNKWIRVDKSAADGPRVFRVVDSMEDEVQ..RRLQLVRGGQAEKLG
                                                      Q  R L       A  LG

T thermophilus  KKGLLT.QEMKGLSALPLEERRKRGQELNAIKAALEAALEAREKALEEAALKEALERERV
cml33           EKERSELRKRKLLAEVTLKTYWVSKG......SAFSTSISKQETELSPEMISSGSWRDRP
                 K        K L    L                          E             R R

T thermophilus  ..DVSLPGASLF..SGGLHPITLMERELVEIFRALGYQAVEGPEV.ESEFFNFDALNIPE
cml33           FKPYNFLAHGVLPDSGHLHPLLKVRSQFRQIFLEMGFTEMPTDNFIESSFWNFDALFQPQ
                              SG LHP          IF   G          ES F NFDAL  P
                                  <<<<<<<<<<Motif I>>>>>>>

T thermophilus  HHPARDMWDTFWLTGEGFRLEGPLG.......................EEVEGRLLLRT
cml33           QHPARDQHDTFFLRDPAEALQLPMDYVQRVKRTHSQGGYGSQGYKYNWKLDEARKNLLRT
                 HPARD  DTF L      L  P                                LLRT


T thermophilus  HTSPMQVR..YMVAHTPPFRIV...VPGRVFRFEQTDATHEAVFHQLEGLVVGEGIAMAH
cml33           HTTSASARALYRLAQKKPFTPVKYFSIDRVFRNETLDATHLAEFHQIEGVVADHGLTLGH
                HT     R  Y  A   PF  V      RVFR E  DATH A FHQ EG V   G    H
                                  <<<<<<<<<<<< Motif II >>>>>>>>>>>>

T thermophilus  LKGAIYELAQALFGPDSKVRFQPVYFPFVEPGAQFAVWWPEGGKWLELGGAGMVHPKVFQ
cml33           LMGVLREFFTKL..GITQLRFKPAYNPYTEPSMEVFSYHQGLKKWVEVGNSGVFRPEMLL
                L G   E    L       RF P Y P  EP            KW E G  G   P

T thermophilus  AVDAYRERLGLPPAYRGVTGFAFGLGVERLAMLRYGIPDIRYFFGGRLKFLEQFKGVL.
cml33           P.......MGLPE...NVSVIAWGLSLERPTMIKYGINNIRELVGHKVNLQMVYDSPLC
                         GLP     V   A GL  ER  M  YGI  IR   G           L
                                  <<<<Motif III>>>>
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FIG. 3. Alignment of the phenylalanyl-tRNA synthetase α chain from *T. thermophilus* with the CML33-encoded enzyme. Only the first 340 codons of the CML33 enzyme are shown. The three characteristic structural motifs identified in the two sequences are indicated with arrows. Invariant residues in the two proteins are shown at the bottom of each row.

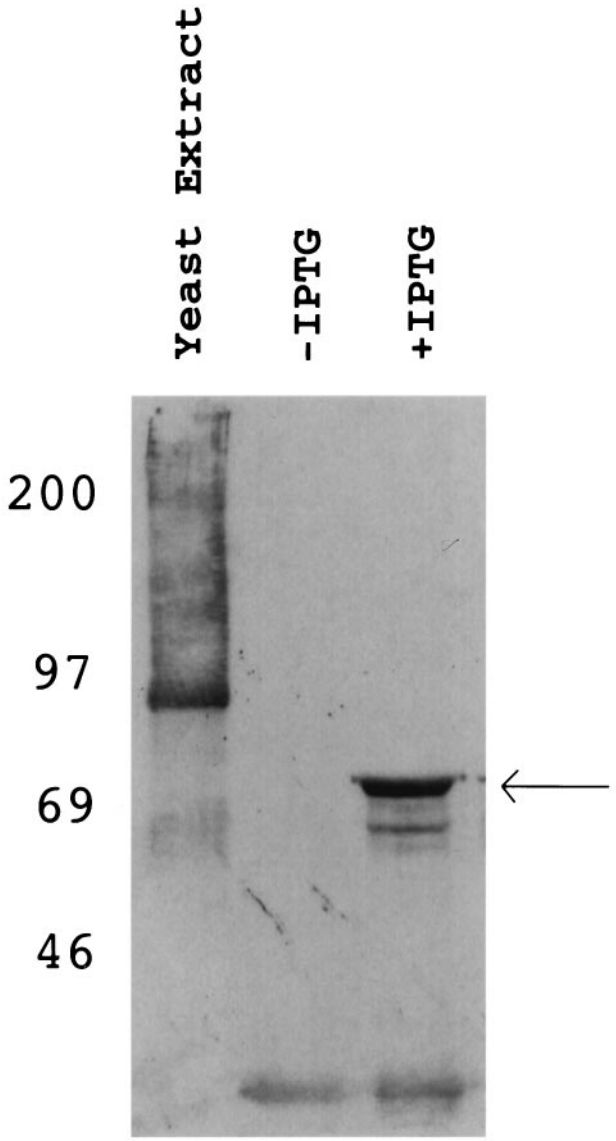

FIG. 4. Protein encoded by the CML33 cDNA cross-reacts with antibodies against sheep PheRS. Total protein from *S. cerevisiae* (lane 1) or total protein from *E. coli* containing a gene encoding the glutathione-*S*-transferase–CML33 fusion protein, uninduced (lane 2) and induced (lane 3) with IPTG, were immunoblotted with antibody against sheep PheRS. The cross-reacting material in the yeast extract lane does not correspond to any subunit of yeast cytoplasmic (approximate molecular weights of 70,000 and 60,000) or mitochondrial (molecular weight of about 57,000) PheRS. Thus, the antibodies are sufficiently specific to distinguish between yeast and mammalian PheRS. Note the fusion protein band in lane 3, marked with an arrow. A molecular weight of 82,000 is predicted for the fusion protein, which is close to what is observed.

etic cells, we analyzed the level of CML33 mRNA in human myeloid leukemia cells induced to differentiate *in vitro*. HL-60 cells were induced to differentiate into monocytic macrophages in the presence of the phorbol ester PMA as observed (14, 23). Northern blot hybridization of RNA isolated from HL-60 cells at various time points after PMA induction is shown in Fig. 6. At the PMA concentration used, about 60% of the cells were induced to differentiate within the first 16 h of treatment. Differentiated cells constituted about 95% of the population after a 96-h incubation with PMA. The CML33 mRNA was found to decrease noticeably within the first 16 h and progressively thereafter up to 96 h of differentiation induction, indicating that the gene is developmentally regulated in cells of myeloid lineage.

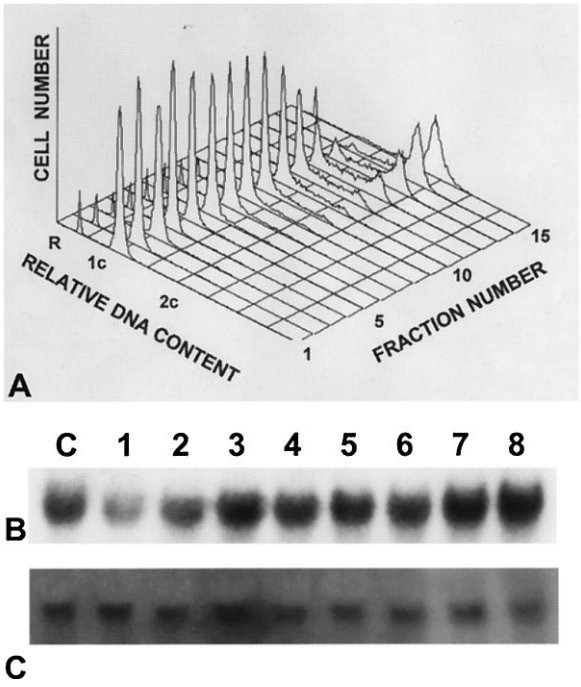

FIG. 5. Cell cycle stage-dependent expression of CML33 gene in human myeloid leukemia cell line HL-60. (*A*) Cells separated by centrifugal elutriation into 15 fractions with relative DNA content increasing from 1C to 2C (from upper left to lower right representing G_1, S, and G_2/M phases). Vertical axis represents number of cells in each fraction and R represents DNA fluorescence signal peaks from chicken erythrocytes included with each fraction as an internal standard for DNA quantitation. The R peak is invisible in fractions 9–14 due to superimposition with the G_1 peaks but reemerges in fraction 15 as the G_1 peak in the later fractions get smaller. (*B*) Northern blot hybridization with the CML33 cDNA insert of RNA from the total cell population C and from cells at different cell cycle phases in lanes 1- 8, isolated by pooling the original 15 fractions as follows. Lanes: 1, fractions 1–3 (early/mid G_1); 2, fractions 4–8 (mid/late G_1); 3, fractions 9 and 10 (late G_1/early S); 4, fraction 11 (mid S); 5, fraction 12 (mid/late S); 6, fraction 13 (late S/early G_2); 7, fraction 14 (early G_2); 8, fraction 15 (G_2/M). Note progressively higher expression of CML33 from G_1 through G_2/M phase. (*C*) The same blot hybridized with an 18S rDNA probe to demonstrate comparable RNA loading in each lane.

DISCUSSION

Although most class II tRNA synthetases have an α_2 quaternary structure, phenylalanyl-tRNA synthetases are typically $\alpha_2\beta_2$ proteins. The β chain in eukaryotes typically corresponds to the α chain in prokaryotes. In the crystal structure of the

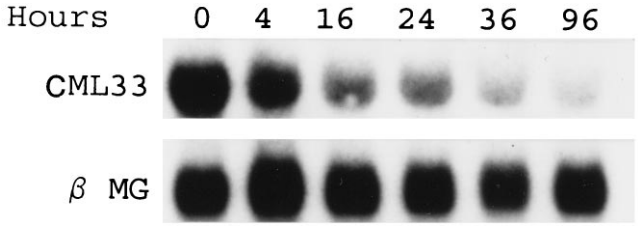

FIG. 6. Expression pattern of CML33 transcript in human myeloid leukemia cells HL-60 induced to undergo monocytic differentiation after PMA treatment for various intervals. Total RNA (10 μg) isolated from untreated exponentially growing cells (0) and cells treated for 4, 16, 24, 36, and 96 h were analyzed on Northern blots with the CML33 cDNA probe. Note marked reduction in the level of transcript with induced differentiation after prolonged treatment with PMA. The same blot was hybridized with a control β_2-microglobulin (β MG) probe to demonstrate comparable amount of RNA loading in each lane.

enzyme from *T. thermophilus,* the catalytic site is part of the α subunit whereas the noncatalytic β chain has the characteristic fold of the class II enzymes and some additional motifs. This work demonstrates that the CML33-encoded protein is a homolog of the α chain of *T. thermophilus* phenylalanyl-tRNA synthetase which, in turn, corresponds to the β chain of yeast phenylalanyl-tRNA synthetase. Thus, the CML33 protein corresponds to the catalytic subunit of human phenylalanyl-tRNA synthetase.

The alignment of the CML33 coding sequence with the sequence of the α chain of yeast phenylalanyl-tRNA synthetase suggests that the CML33 protein does not have the leader sequence that is characteristic of mitochondrial proteins. In addition, we did not find a significant match between the CML coding sequence and that of yeast mitochondrial phenylalanyl-tRNA synthetase (24). For these reasons, we believe that CML33 codes for a homolog of a subunit of the human cytoplasmic enzyme.

Although levels of tRNA synthetases such as the phenylalanine enzyme in *E. coli* vary under different growth conditions such as amino acid starvation (25), to our knowledge, the data presented are the first to demonstrate tumor-specific and cell cycle stage- and differentiation-specific expression of a member of the tRNA synthetase gene family in a mammalian cell. The reason why a cDNA for the β chain of phenylalanyl-tRNA synthetase should be isolated by our reiterative subtraction of cDNAs that are expressed in both tumorigenic and nontumorigenic variant is not clear. One possibility is that this chain has an additional cellular role that is distinct from that of a tRNA synthetase. Alternatively, if instead the free α chain had an additional cellular role, then overproduction of the β chain might sequester all of the α subunit and thereby regulate its alternative function. In this connection, Safro and Mosyak (26) pointed out that the *T. thermophilus* β chain (putative homologue of the mammalian α chain) has both a helix–turn–helix DNA binding domain and a Src homology (SH3) domain. Thus, if the free mammalian α chain functions in DNA binding and/or in a signal transduction pathway, then its activity for these cell regulatory events could be controlled by the levels of expression of the β subunit of phenylalanyl-tRNA synthetase. Therefore, a critical experiment is to isolate a cDNA probe for the α chain and, for example, determine whether its mRNA expression is regulated in the same way as that of the β subunit or whether it is expressed constitutively.

Abnormalities of human chromosome 19, the site of localization of CML33 gene, have frequently been reported in both hematologic and solid tumors (27). It would be interesting to know if CML33 locus is affected in some of these abnormalities.

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