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Expression of a Gene for Mouse Eucaryotic Elongation Factor Tu during Murine Erythroleukemic Cell Differentiation

WILLIAM W. ROTH, PAUL W. BRAGG, MARIA V. CORRIAS, N. S. REDDY, JAYDEV N. DHOLAKIA, AND ALBERT J. WAHBA*

Department of Biochemistry, The University of Mississippi Medical Center, Jackson, Mississippi 39216

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The eucaryotic elongation factor Tu (eEF-Tu) is a single polypeptide with an approximate M_r of 53,000. During protein synthesis eEF-Tu promotes the binding of aminoacyl-tRNA to the ribosome. To study the expression of the gene(s) for this factor, a genomic clone was isolated that contains a mouse eEF-Tu gene. We screened a phage genomic library with a synthetic oligonucleotide probe complementary to a region of the *Saccharomyces cerevisiae* and *Artemia* sp. eEF-Tu genes which codes for an area that is highly conserved between both yeast and *Artemia* sp. eEF-Tu. From approximately 75,000 phage plaques we obtained five isolates with apparently identical inserts. All five clones contained a 3.8-kilobase *Eco*RI fragment that hybridized to additional oligonucleotide probes corresponding to different conserved regions of eEF-Tu. We sequenced the 5′ end of one genomic clone and determined the length of the cloned fragment that was protected by eEF-Tu mRNA in S1 nuclease protection assays. A quantitative S1 nuclease protection assay was used to compare the relative steady-state levels of eEF-Tu mRNA in total RNA isolated from hexamethylene-bisacetamide-induced murine erythroleukemia cells. The results show a dramatic reduction in the steady-state level of eEF-Tu mRNA as differentiation proceeds. A similar reduction in transcription of eEF-Tu mRNA was observed in isolated nuclei. Finally, we examined the in vivo synthesis of eEF-Tu during differentiation and found that it declined in a manner parallel to the decline in the steady-state level of eEF-Tu mRNA. In addition, we have isolated and sequenced a cDNA clone for mouse eEF-Tu. The derived amino acid sequence is compared with sequences from other eucaryotes.

During protein synthesis in eucaryotic cells the binding of aminoacyl-tRNA to ribosomes is promoted by the eucaryotic elongation factor Tu (eEF-Tu) in the presence of GTP. Subsequent hydrolysis of GTP and release of the factor as eEF-Tu–GDP allows its reuse throughout the elongation process (reviewed reference in 13). The functional importance of eEF-Tu in protein synthesis has stimulated research on the structure and functional domains of the protein itself (12, 29, 30, 33) as well as on the identification and expression of the genes for eEF-Tu (2, 4, 14, 15, 19, 25, 31, 32).

Genomic and/or cDNA clones exist for eEF-Tu from *Saccharomyces cerevisiae* (4, 19, 25), *Artemia* sp. (14, 31), and humans (2). All exhibit a high degree of amino acid and nucleotide sequence conservation among these eucaryotes, especially in the amino-terminal and central two-thirds of the molecule (4). This suggests that functional constraints on the evolutionary modification of this protein exist, placing eEF-Tu within a group of evolutionarily conserved proteins.

Studies on the expression of eEF-Tu gene(s) have been limited thus far to the developing embryos of *Artemia* sp. Analysis of eEF-Tu mRNA levels by means of Northern blot (RNA blot) analysis with either a cDNA clone (15) or synthetic oligonucleotide-primed eEF-Tu cDNA (5) has demonstrated a sudden and dramatic increase in the expression of the eEF-Tu gene after hydration of dormant embryos. Such an increase is consistent with the requirement for renewed protein synthesis after cryptobiosis and parallels the increase in both general and specific protein synthesis previously reported (5, 35).

* Corresponding author.

Another system that demonstrates both general and specific changes in protein synthesis patterns is the Friend virus-transformed murine erythroleukemia (MEL) cell system (7, 17). Because of the alterations in protein synthesis in induced MEL cells and because of their well-characterized differentiation parameters, we chose to study the expression of a gene for eEF-Tu during MEL cell differentiation. The absolute requirement of this factor for protein synthesis and the possibility of its participation in control mechanisms make it an excellent candidate to help elucidate the molecular events which underlie the differentiation process. We have therefore isolated, identified, and partially characterized a genomic clone for mouse eEF-Tu. A quantitative S1 nuclease digestion assay (36) was then used to examine the steady-state levels of the mRNA for eEF-Tu during hexamethylene-bisacetamide (HMBA)-induced differentiation of MEL cells (17). We found that in spite of the reported gradual reduction in overall protein synthesis (21) and the selective increase in the synthesis of globin and other proteins (8, 10), the amount of mRNA for eEF-Tu declined dramatically. In vitro transcription experiments with isolated nuclei suggest that the decline in eEF-Tu mRNA is regulated at the level of transcription. Upon examination of the in vivo synthesis of eEF-Tu during differentiation, a decline was seen which paralleled the reduction in eEF-Tu mRNA. Both the steady-state eEF-Tu mRNA level and the synthesis of eEF-Tu reached identical values (as the percentage of their uninduced levels) by day 4 of differentiation. Implications of the effect of this reduction on continued protein synthesis are discussed.

MATERIALS AND METHODS

Oligonucleotide synthesis. All oligonucleotide probes were synthesized with an Applied Biosystems model 380A DNA synthesizer. Oligonucleotides were purified by polyacrylamide gel electrophoresis, electroelution, and dialysis. They were labeled at the 5′ end with polynucleotide kinase and [γ-^{32}P]ATP, followed by spun-column chromatography (16). For screening and identification of mouse genomic clones, probes were synthesized complementary to the coding strand of an *Artemia* sp. eEF-Tu cDNA clone (31) at nucleotide positions 416 through 457 (42-mer; 5′-TCTTAG AGATACCGGCTTCGAATTCACCGACACCAG CAGCAA-3′), 946 through 959 (14-mer; 5′-TC(A/G)TG(A/G)TGCAT(T/C)TC-3′) and 1347 through 1376 (30-mer; 5′-CCGACAGCGACTGTTTGTCTCATGTCACGG-3′).

Isolation and identification of a genomic clone for eEF-Tu. The mouse genomic library, which was supplied by L. Hood, is the result of an *Hae*III-*Alu*I partial digestion of isolated DNA cloned into λ Charon 4A as generally outlined previously (16). Library phage plating with host KH802 cells, plaque lifts, and hybridizations were performed essentially as previously described (1), with the following modifications. Filters were prehybridized and hybridized at 65°C in 6× SET (1× SET is 0.15 M NaCl–1 mM disodium EDTA–0.03 M Tris chloride, pH 8.0 at 20°C)–5× Denhardt solution (6)–200 μg of tRNA per ml–0.5% sodium dodecyl sulfate–0.1% sodium pyrophosphate. The probe used for screening was the 42-mer and its concentration during hybridization did not exceed 2 ng/ml. Probe specific activity usually exceeded 2.0×10^8 cpm/μg. DNA isolated from positive hybridizing plaques was shown to hybridize to all three *Artemia* sp.-specific synthetic oligonucleotides by using the hybridization buffer and conditions optimized for each probe as previously described (1). After subcloning into the plasmid pBR322 and then into the bacteriophage M13 mp8 and M13 mp9 (16), the DNA sequence was determined by the chain-termination method (24). High-stringency diagonal dot-matrix comparisons were performed on an IBM PC-XT computer with programs from IBI (New Haven, Conn.).

Cell culture and RNA isolations. A derived subclone (DS19E5) of murine erythroleukemia (MEL) cells (17) was cultured exactly as described by Brown et al. (3). Induction was initiated by the addition of HMBA to a final concentration of 5 mM. Cell viability, as measured by trypan blue exclusion, was always greater than 98%, and the percentage of induced cells by day 4 was also greater than 98% as measured by benzidine staining. MEL cell RNA preparations were prepared as previously described (3).

Quantitation of eEF-Tu mRNA levels during induction. A quantitative S1 nuclease protection assay (3, 36) was used to determine the steady-state level of both eEF-Tu and globin mRNAs in total RNA isolated from uninduced cells and from cells on each day of induction. RNA from the same preparation was used for both eEF-Tu and globin assays. The mouse β-major globin cDNA clone pCR1β (23) was digested with *Bam*HI, and the mouse eEF-Tu genomic clone pWR1 was digested with *Ava*I; both *Bam*HI and *Ava*I recognize sites within the coding regions. The digested globin clone was used without further manipulation. The pWR1 clone, which consists of a 3.8-kilobase fragment inserted into the *Eco*RI site of pBR322, was further digested with *Pst*I, and the desired fragment was purified by preparative agarose gel electrophoresis and electroelution (16). The eEF-Tu probe used for the S1 nuclease protection assays contained approximately 900 base pairs of cloned mouse DNA and 745 base pairs of pBR322 sequence (see Fig. 1B). After 5′-end labeling with [γ-^{32}P]ATP (16), the probes were hybridized for 16 h in a hybridization buffer containing 80% formamide, 40 mM PIPES [piperazine-*N*,*N*′-bis(2-ethanesulfonic acid)] (pH 6.4), 1 mM disodium EDTA, and 0.4 M NaCl (16) with total RNA from 2.5×10^4 cells at empirically determined optimal temperatures (52°C for globin; 50°C for eEF-Tu). After S1 nuclease digestion (3), the RNA-protected fragments were electrophoresed on 8% polyacrylamide–8.3 M urea sequencing gels (16) and exposed to Kodak XAR-5 film. The autoradiograms were scanned with a Transidyne General model 2510 densitometer with a model 2500 integrator. All assays were performed in triplicate and under conditions of excess DNA in the range of linear dependence on added RNA.

Transcription in isolated nuclei. Nuclei from uninduced cells and from 4-day-induced cells were prepared and incubated as described by Sittman et al. (27). Specifically, 5×10^6 nuclei were incubated in a 200-μl reaction mix. Incorporation of [α-^{32}P]GTP was linear during the 20-min incubation period. Reactions were terminated by treatment with DNase I followed by extraction with phenol at 55°C for 5 min. The RNA products of each reaction were separated from unincorporated nucleoside triphosphates by chromatography through Sephadex G-75.

Transcription products were hybridized to dot-blots prepared by the method of McKnight and Palmiter (18). Plasmids containing cloned sequences for eEF-Tu or β-globin (described above) were linearized by cleavage with appropriate restriction enzymes. Plasmid DNA was extracted with phenol and precipitated with ethanol. After alkaline denaturation the DNA was applied to nitrocellulose by means of a Hybri-Dot Manifold (Bethesda Research Laboratories, Gaithersburg, Md.). Each row contained dots of pBR322 (as a control), the eEF-Tu plasmid, and the β-globin plasmid (5 μg per dot). RNA samples were hybridized to the nitrocellulose filters for 3 days at 52°C as previously described (27), except that the hybridization buffer contained 50% (vol/vol) deionized formamide, 0.9 M NaCl, 0.09 M sodium citrate, 10 mM Tris hydrochloride (pH 7.5), 1 mM EDTA, 0.1% sodium dodecyl sulfate, 10 μg of poly(A) per ml, and 500 μg of heparin per ml. After hybridization the filters were washed as described above and exposed to Kodak XAR-5 film. The extent of hybridization of each dot was quantitated by densitometric scanning of the autoradiograms with a Bio-Rad model 620 video densitometer.

Analysis of in vivo eEF-Tu synthesis during induction. Uninduced MEL cells and cells on each day of differentiation were collected by centrifugation and suspended at 10^6 cells per ml in minimum essential medium lacking methionine and supplemented with 10% fetal bovine serum. The cells were incubated at 37°C for 1 h before the addition of [^{35}S]methionine (New England Nuclear Corp., Boston, Mass.; 1,140 Ci/mmol). Labeling was done at 37°C for 2 h at a specific activity of 25 μCi/ml. After labeling, a sample was removed and precipitated with trichloroacetic acid for determination of total radioactivity incorporated (37). The remainder of the cells diluted with 1 volume of ice-cold phosphate-buffered saline (150 mM NaCl, 66 mM sodium phosphate, pH 7.0) and collected by centrifugation at 700 × *g* for 10 min at 4°C. The cell pellet was washed twice with ice-cold phosphate-buffered saline and suspended in 10 mM Tris chloride (pH 7.6)–10 mM KCl–5 mM $MgCl_2$–4 mM dithiothreitol–1 mM phenylmethylsulfonyl fluoride at a con-

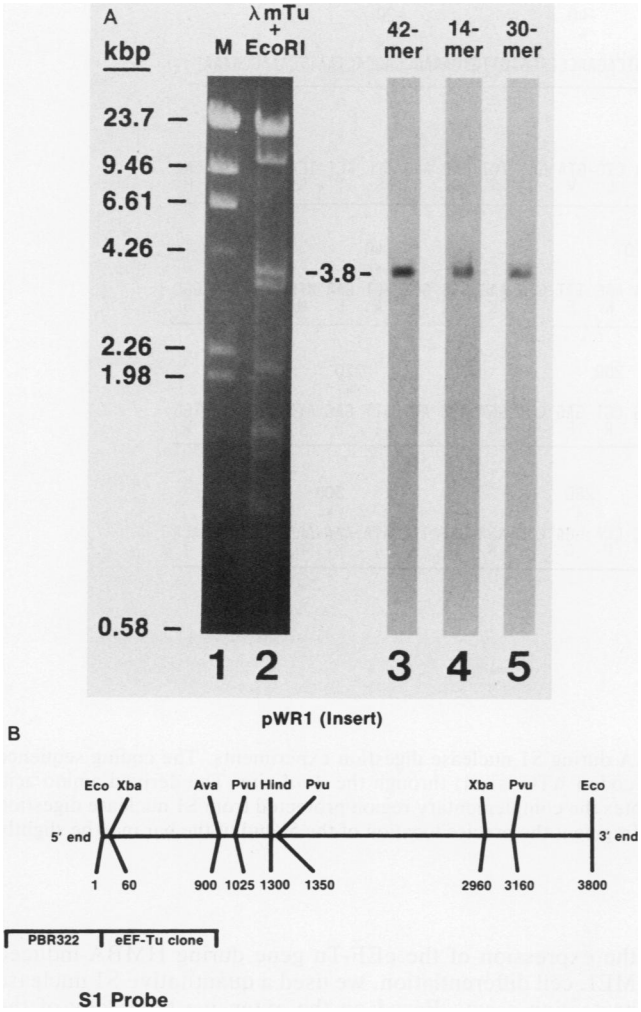

FIG. 1. Identification of a mouse eEF-Tu genomic clone (A) Hybridization with *Artemia* sp.-specific oligonucleotide probes. Shown is an ethidium bromide-stained gel of *Hin*dIII-digested lambda DNA fragments (lane 1) and *Eco*RI-digested genomic clone λmTu (lane 2). After transfer to nitrocellulose, filters containing the digested λmTu clone were hybridized to either the 42-mer (lane 3), the 14-mer (lane 4), or the 30-mer (lane 5). (B) Restriction map of the 3.8-kilobase fragment subcloned into pBR322. The ends are labeled with respect to the mRNA coding strand sequence. The bar below the map indicates the gel-purified fragment used for S1 nuclease digestion analysis, which contains 748 base pairs of pBR322 sequence in addition to 900 base pairs of the 5′ end of the genomic clone. Abbreviations: Eco, *Eco*RI; Xba, *Xba*I; Ava, *Ava*I; Pvu, *Pvu*II; Hind, *Hin*dIII.

centration of 10^8 cells per ml. The cells were then lysed by the addition of Tween 80 to a final concentration of 1% (vol/vol) and incubated on ice for 5 min. The nuclei were removed by centrifugation at 700 × *g* for 5 min at 4°C. After the removal of a second sample for trichloroacetic acid precipitation the cytoplasmic extracts were stored in liquid nitrogen until electrophoresis was performed.

Extracts from 10^6 cells during each day of differentiation were analyzed individually by nonequilibrium pH gradient gel electrophoresis in the first dimension, followed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis in the second dimension (5). Before electrophoresis 3 μg of purified eEF-Tu was added as an unlabeled marker. Fluorography of all gels was for 24 h at −70°C with Kodak XAR-5 film. Scanning densitometry was done as described above. The values reported are the averages of three individual gels for each day of differentiation.

Materials. Cell culture supplies were obtained from GIBCO Laboratories, Grand Island, N.Y. Enzymes were from Bethesda Research Laboratories and from Pharmacia Fine Chemicals, Piscataway, N.J. Radioactive nucleotides were from ICN Pharmaceuticals, Irvine, Calif. All other chemicals were reagent grade.

RESULTS

Isolation of the mouse eEF-Tu gene. Because of the considerable amino acid sequence homology observed between eEF-Tu in *S. cerevisiae* (4) and *Artemia* sp. (31), we decided that a probe synthesized complementary to the mRNA sequence of a mutually conserved region could be used to isolate the gene from another species. We therefore synthesized a 42-base oligodeoxyribonucleotide probe complementary to nucleotides 416 through 457 of an *Artemia* sp. cDNA clone (31). This probe was used to screen a mouse genomic library constructed in λ Charon 4A. After screening approximately 75,000 phage plaques in duplicate, we isolated five clones which hybridized on duplicate filters with this probe. DNA preparations from plaque-pure isolates of all five clones yielded inserts with identical cleavage patterns when digested with a variety of restriction endonucleases (data not shown). In addition, each phage clone contained a 3.8-kilobase *Eco*RI fragment which hybridized with the 42-base oligomer used to screen the library and with two other *Artemia* sp.-specific oligonucleotide probes (Fig. 1A), the 14-mer derived from the center of the amino acid sequence (5) and the 30-mer derived from the 3′ end of the *Artemia* sp. cDNA clone (31). All subsequent work was performed with one of these genomic clones, designated λmTu-1. The 3.8-kilobase *Eco*RI fragment was subcloned into pBR322 and designated pWR1. A restriction map of the insert is shown in Fig. 1B.

Preliminary analysis of the clone by hybridization with 5′- and 3′-specific oligonucleotide probes indicated that it contained the complete coding sequence for an eEF-Tu gene. By using sequence-specific oligonucleotides as primers we have completed the DNA sequence of both strands of the 5′ end, the region relevant to our analysis of eEF-Tu gene expression (Fig. 2). Although a discussion of the sequence homologies will be given below, one should note that comparison of the nucleotide sequence at the 5′ end (Fig. 2) to those of yeast and *Artemia* sp. eEF-Tu shows 82% homology overall, whereas the derived amino acid sequence homology is 91%. In addition, amino acid sequence homology with *Escherichia coli* EF-Tu within this region is 41%. After we isolated this genomic clone we isolated a cDNA clone for mouse eEF-Tu from a library supplied by Siegfried Ruppert. We sequenced the cDNA clone completely in both directions using the chain termination technique (see Materials and Methods). A high-stringency dot-matrix comparison of the derived amino acid sequence of this clone with both *Artemia* sp. and yeast eEF-Tu is shown in Fig. 3. The strongly conserved sequence homology is clearly evident. Furthermore, the derived amino acid sequence of mouse eEF-Tu differs from the human sequence (2) at only a single amino acid (human Asp-221 to mouse His-221), reinforcing the extremely conserved nature of this protein.

Expression of the eEF-Tu gene during induction. To follow

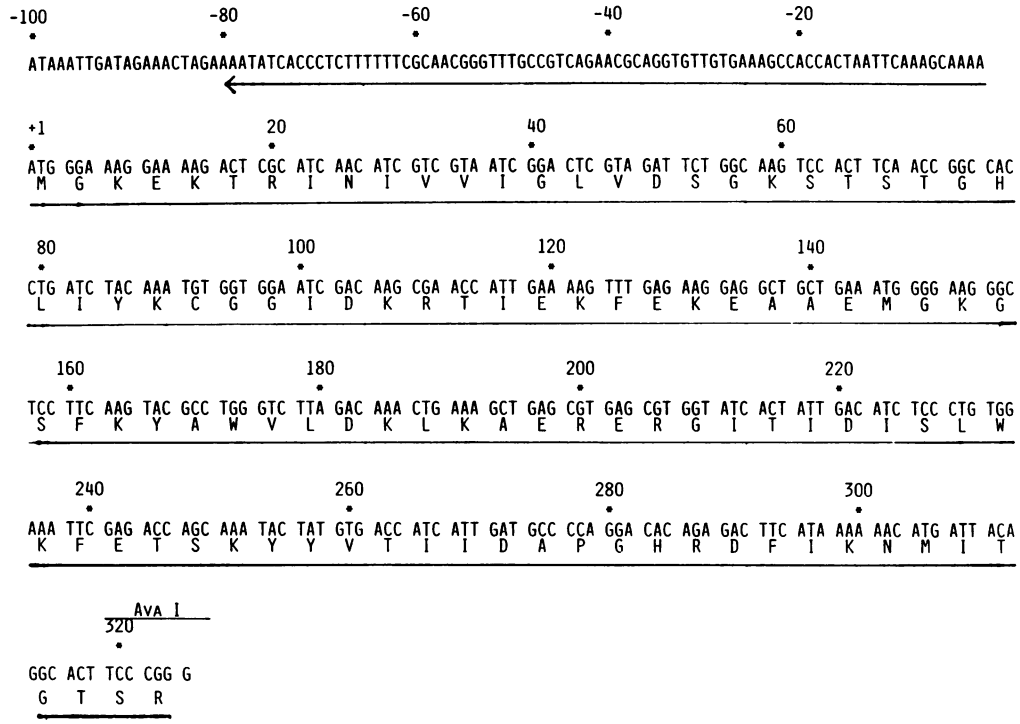

FIG. 2. Sequence of the cloned fragment protected by eEF-Tu mRNA during S1 nuclease digestion experiments. The coding sequence strand is shown from the base at position −100 (the A of the initiation codon ATG is +1) through the *Ava*I site. The derived amino acid sequence is shown in single-letter code. The bar below the sequence denotes the complementary region protected from S1 nuclease digestion by hybridization to eEF-Tu mRNA. Because of the size of the protected fragment the precise location of the 5′ end of the bar may be slightly variable.

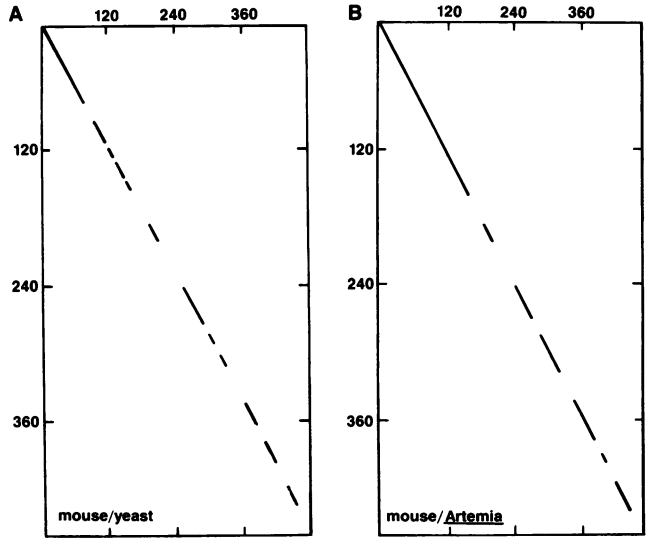

FIG. 3. High-stringency diagonal dot-matrix comparison of the derived amino acid sequence of mouse eEF-Tu with yeast and *Artemia* sp. eEF-Tu. The abscissa represents the sequence of mouse eEF-Tu derived from the cDNA sequence, and the ordinate represents the sequence from either yeast (A) or *Artemia* sp. (B). Numbering starts at the initiator methionine residue. Sequence homology was explored by looking for ca. 85% similarity over 11 contiguous amino acids.

the expression of the eEF-Tu gene during HMBA-induced MEL cell differentiation, we used a quantitative S1 nuclease protection assay. Based on the extensive homology of the derived amino acid sequence of the 5′ end of the mouse gene to both the yeast and *Artemia* sp. eEF-Tu peptides, we predicted that the end-labeled DNA probe used for this assay (Fig. 1B) should have been protected by 320 bases of eEF-Tu mRNA, from the first base of the *Ava*I restriction site to the initiation codon (Fig. 2), in addition to an unknown length of the probe protected by the 5′ nontranslated mRNA. After hybridization of the probe with total RNA and digestion with S1 nuclease, a single labeled band of approximately 400 bases was seen on denaturing polyacrylamide gels (Fig. 4). This band corresponds to the entire 5′ coding region and approximately 80 bases of 5′ nontranslated mRNA. Figure 2 shows the region of the probe protected by eEF-Tu mRNA in this assay. The specificity of the assay is demonstrated in lane 1 of Fig. 4, in which tRNA was substituted for mouse total RNA. No bands are present, indicating complete digestion of the labeled probe by S1 nuclease in the absence of protection by its cognate mRNA. The presence of the complementary DNA strand also does not protect the labeled probe under the stringent conditions of the assay, which allow only RNA-DNA hybrids to exist and therefore resist digestion by S1 nuclease.

Possible alterations in the expression of the eEF-Tu gene were followed by measuring the steady-state level of eEF-Tu mRNA in total RNA isolated from uninduced MEL cells and from cells on each day of differentiation as described above (Fig. 5). Both eEF-Tu mRNA levels and β-globin mRNA levels were determined in total RNA from 2.5×10^4 cells.

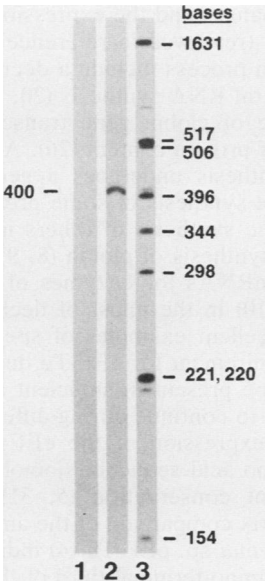

FIG. 4. S1 nuclease digestion of RNA-DNA hybrids. Autoradiograms of the cloned probe fragment (bar, Fig. 1B) protected from S1 digestion by hybridization to tRNA (lane 1) or to eEF-Tu mRNA (lane 2). Labeled size markers are a *Hin*fI digest of pBR322 (lane 3).

The level of eEF-Tu mRNA, as reflected in the 400-base protected band (Fig. 5A), declined within 24 h after induction with HMBA, and this decrease continued throughout the experiment (4 days).

As a comparison, and as a control for induction of the MEL-DS19 cells, we determined the steady-state levels of β-globin mRNA on each day during differentiation. Total RNA was hybridized with the labeled β-globin probe pCR1β, followed by S1 nuclease digestion of the hybrids as described above for the eEF-Tu assay. β-Globin mRNA synthesis increases sharply after induction, starting from undetectable levels (Fig. 5B). This reflects the commitment of the cells to erythropoiesis and the increase in both globin and globin mRNA previously reported (8, 9). By day 4, greater than 98% of the cells gave positive results to benzidine staining (indicating hemoglobin production) (3). The steady-state level of β-globin mRNA is shown expressed as a percentage of the maximum level observed (day 4). By the last day of differentiation employed in these experiments the level of globin mRNA had risen at least 15-fold over the earliest detectable mRNA level at day 1.

The results of scanning densitometry and peak integration of the S1 nuclease assay gels are presented in Fig. 5C. The decline in the steady-state level of eEF-Tu mRNA is expressed as a percentage of the uninduced value. The level underwent a sevenfold decrease, reaching 15% of uninduced levels by day 4 of differentiation. In contrast, the steady-state level of globin mRNA increased dramatically, reflecting the induction of the β-globin gene by HMBA and the progress into erythropoiesis.

In an effort to determine the components involved in the changing steady-state levels of mRNA species in MEL cells, we measured the transcription rates in isolated nuclei. Nuclei were incubated under conditions which allow the elongation of nascent RNA chains to measure the transcription rates during differentiation. Transcription of β-globin mRNA increased fourfold during differentiation as determined by hybridization to the globin-specific probe DNA (20.2 and 5.7 arbitrary densitometric units per 10^6 nuclei on days 0 and 4, respectively, for eEF-Tu and 6.5 and 26.3 units per 10^6 nuclei, respectively, for β-globin). This is comparable to the increased transcription previously reported in HMBA-induced MEL cells (8). In contrast, transcription of mRNA for eEF-Tu decreased during differentiation to approximately 28% of the level of transcription observed in nuclei from uninduced cells.

Uninduced MEL cells contain a level of eEF-Tu as high as 5% of total protein (26). Because of the decline in the steady-state level of eEF-Tu mRNA, we investigated the in vivo synthesis of eEF-Tu itself during differentiation. After labeling with [^{35}S]methionine, the total cytoplasmic proteins were analyzed by nonequilibrium pH gradient gel electrophoresis and dodecyl sulfate-polyacrylamide gel electrophoresis (5). Unlabeled eEF-Tu was included as a marker. This technique was used previously to quantitate eEF-Tu when poly(A)$^+$ RNA from *Artemia* sp. embryos at different stages of development was translated in vitro (5). The autoradiogram of a gel containing in vivo-synthesized proteins in MEL extracts from day 0 is shown for illustration (Fig. 6A). Scanning densitometry and integration of the eEF-Tu region of the gels (Fig. 6B) shows that the in vivo synthesis of eEF-Tu declines dramatically after induction with HMBA, reaching approximately 12% of the uninduced synthesis

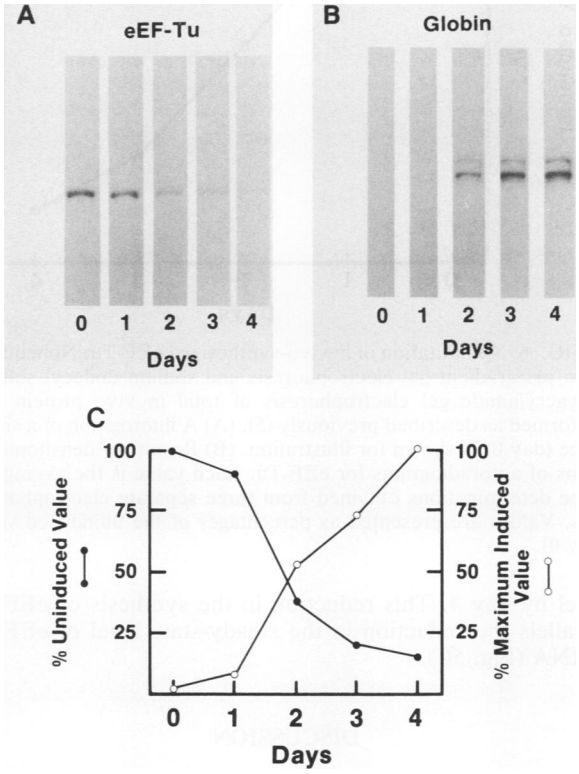

FIG. 5. S1 nuclease digestion analysis of steady-state levels of eEF-Tu and globin mRNAs during MEL cell differentiation. (A) Protection of the labeled eEF-Tu cloned fragment by specific transcripts in total RNA isolated on each day of induction. (B) Protection of cloned globin DNA by globin mRNA transcripts in total RNA from each day. (C) Results of densitometric scans of protected fragments of the eEF-Tu clone (●) and the globin clone (○). Each value is the average of at least three different determinations. Values of eEF-Tu mRNA are presented as percentages of the uninduced value (day 0). Values for globin mRNA are presented as percentages of the maximum measured value (day 4).

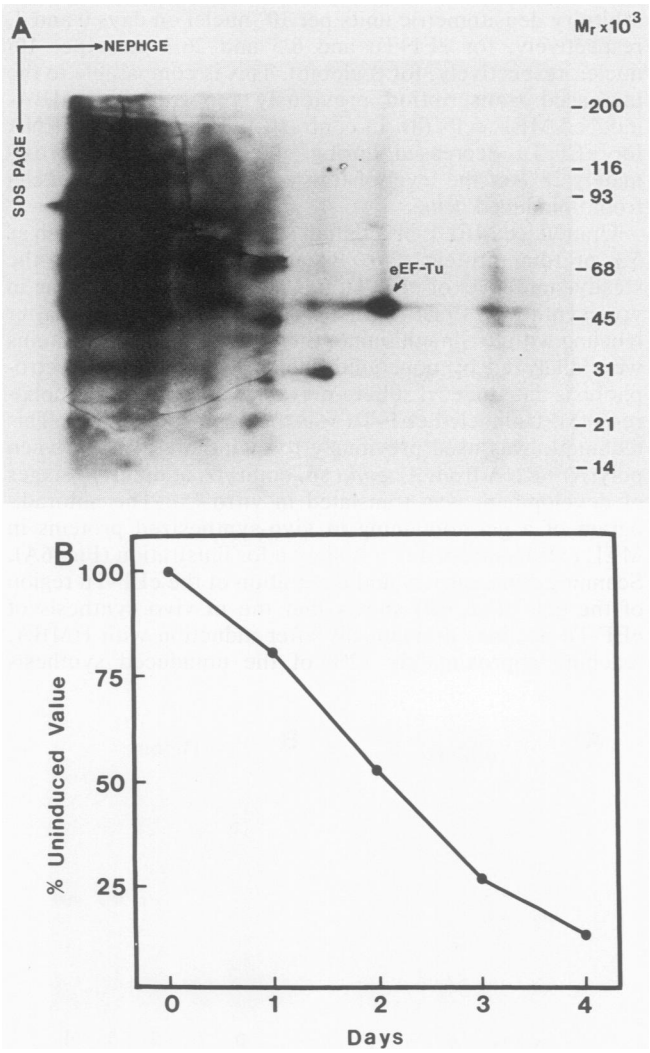

FIG. 6. Quantitation of in vivo-synthesized eEF-Tu. Nonequilibrium pH gradient gel electrophoresis and sodium dodecyl sulfate-polyacrylamide gel electrophoresis of total in vivo protein was performed as described previously (5). (A) A fluorograph of a single stage (day 0) is shown for illustration. (B) Results of densitometric scans of autoradiograms for eEF-Tu. Each value is the average of three determinations obtained from three separate electrophoretic gels. Values are presented as percentages of the uninduced value (day 0).

level by day 4. This reduction in the synthesis of eEF-Tu parallels the reduction in the steady-state level of eEF-Tu mRNA (Fig. 5C).

DISCUSSION

eEF-Tu is a central and essential element in protein synthesis. It becomes, therefore, a prime candidate in the regulation of general protein synthesis. The regulation of eEF-Tu could be accomplished in several ways and at several levels. We have chosen initially to study its regulation at the level of the expression of the gene. For this study the Friend virus complex-transformed MEL cell line (7) was used. Blocked in early erythrogenesis, these cells are inducible by a wide variety of agents to resume differentiation and proceed to terminal cell division accompanied by distinct morphological changes and the expression of genes specific to erythropoiesis (reviewed in reference 17). Hallmarks of the differentiation process include a decrease in total RNA content and general RNA synthesis (20, 26), an increase in the absolute rate of globin gene transcription (8), and a decline in cellular protein content (26). Although the rate of total protein synthesis undergoes a general reduction in induced cells, the synthesis of some proteins remains constant, whereas the synthesis of others increases (20). The virtual de novo synthesis of globin (8, 9) and the dramatic increase in the mRNAs for enzymes of the heme biosynthetic pathway (10) in the midst of declining total protein synthesis are excellent examples of specific gene control. The absolute requirement for eEF-Tu during these changes demands that it be present in sufficient amounts to enable protein synthesis to continue during differentiation.

To study the expression of the eEF-Tu gene we used interspecific amino acid sequence homologies to define regions of apparent conservation (5, 31). High-stringency diagonal dot matrix comparison of the amino acid sequence of yeast and *Artemia* sp. eEF-Tu (4) indicated high homology both at the amino-terminal third of the molecule and at a region within the central one-third of the molecule. In addition, scattered areas of homology existed in the carboxy-terminal region. Comparison at the nucleotide level revealed three areas of almost perfect homology, with one (the 42-mer) having only two nucleotide differences (one amino acid difference) over its entire length. We synthesized this oligonucleotide probe and used it to screen a mouse genomic library, selecting several phage clones which hybridized to the same plaque on duplicate plaque-lift filters. We synthesized two other oligonucleotide probes, one to a portion of the central homologous region, and one to the 3′ end of the *Artemia* sp. cDNA clone (see Materials and Methods). The hybridizations shown in Fig. 1 demonstrate that all three *Artemia* sp.-specific probes which correspond to the 5′ end, the central region, and the 3′ end of the eEF-Tu cDNA sequence hybridized with a single 3.8-kilobase *Eco*RI fragment of mouse genomic DNA. This reaffirms that the eEF-Tu gene retains considerable nucleotide sequence homology among several diverse species (5). Our subsequent isolation and sequencing of a mouse cDNA clone and the comparison of its derived amino acid sequence to that of yeast and *Artemia* sp. (Fig. 3) clearly demonstrates this remarkable sequence conservation of eEF-Tu.

Based on the mouse eEF-Tu sequence shown in Fig. 3 and its homology to other eEF-Tu clones (4, 31), we predicted that the complement of the sequence should be protected by mouse eEF-Tu mRNA from the *Ava*I site at which it was labeled to some unknown distance on the 5′ side of the initiation codon. The 400-base length of the protected fragment includes the 320 bases of predicted coding sequence and approximately 80 bases of 5′-nontranslated sequence (Fig. 3). This compares favorably with the 320 bases of homologous coding sequence in both *Artemia* sp. and human cDNA clones and the 70 bases of 5′-nontranslated sequence in the *Artemia* sp. cDNA clone (14, 31). The homologous region in the intronless yeast eEF-Tu gene is also 320 bases long, although the length of the 5′-nontranslated sequence is unknown (4).

The expression of the eEF-Tu gene was examined by measuring the steady-state level of eEF-Tu mRNA during induced differentiation in the MEL-DS19E5 cell line. The level of eEF-Tu mRNA declines approximately sevenfold by day 4 of differentiation (Fig. 5A and C). This reduction is as dramatic as the increase seen in globin mRNA (Fig. 4B and

C). Although other mechanisms such as a decrease in the half-life of the message could account for the loss in eEF-Tu mRNA, the most straightforward explanation is a reduction in rate or cessation of transcription of the eEF-Tu gene itself. This is consistent with the general decline in total RNA synthesis previously reported (26). The results of transcription assays in isolated nuclei indicate that this is the case. We observed a 72% decrease in eEF-Tu mRNA transcription during the differentiation process (see above), which reflects the dramatic decline seen in the steady-state mRNA level (Fig. 5C). During this same period globin mRNA synthesis was seen to increase fourfold, consistent with the results reported by Ganguly and Skoultchi (8). The most straightforward explanation of the reduction of eEF-Tu mRNA levels in differentiating MEL cells is that transcription is slowing down. This, combined with the normal degradation of eEF-Tu message, results in less eEF-Tu protein being synthesized (Fig. 6). Therefore, while the globin gene is being activated, it appears that the eEF-Tu gene is undergoing inactivation. Although general protein synthesis undergoes an overall decline in MEL cels (20), the estimate of a 60 to 70% reduction (26) may be excessive. For example, Parker and Housman (21) have recently reported that as many as 81% of proteins in three different abundance categories are either unchanged or more abundant in induced MEL cells. In fact, most if not all proteins continue to be synthesized, even though at different levels, throughout MEL cell differentiation (21, 22). A considerable amount of protein synthesis, therefore, continues during differentiation, which would require the presence of eEF-Tu. The severe reduction in the steady-state level of eEF-Tu mRNA is inconsistent with a sustained requirement for the factor for the protein synthesis which is continuing.

To begin to investigate the effect of this mRNA reduction on the continued synthesis of eEF-Tu, we examined synthesis of the protein itself in vivo. eEF-Tu synthesis declines in a manner parallel to the decline in the steady-state level of eEF-Tu mRNA, reaching virtually identical levels (as a percentage of the uninduced value) by day 4 of differentiation (Fig. 6). The in vivo synthesis of eEF-Tu, therefore, appears to be directly dependent upon the amoung of eEF-Tu mRNA present. Furthermore, unless the half-life of eEF-Tu is extended during the differentiation process (21), a reduction in eEF-Tu synthesis in the presence of a constant rate of turnover implies that there may be a reduction in the eEF-Tu level in the cell.

eEF-Tu is one of the most abundant proteins in eucaryotic cells (28), and this abundance may alone suffice for continued protein synthesis after the reduction in gene expression. It is possible, however, that other control mechanisms function to provide the cell with enough eEF-Tu to carry out its differentiation program. In addition to the possibility of an extended protein half-life, stored eEF-Tu, bound to nontranslating messenger ribonucleoprotein particles (11, 34), may be present as well as nonbound, aggregated eEF-Tu (13). It is also possible that the reduction in eEF-Tu gene expression functions as a control element itself, leading to preferred synthesis of certain proteins. We continue to study the expression of the eEF-Tu gene in MEL cells and to pursue the questions raised above.

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