Up-regulation of Nucleolin mRNA and Protein in Peripheral Blood Mononuclear Cells by Extracellular-regulated Kinase*

Cara J. Westmark and James S. Malter‡

From the Institute on Aging and Department of Pathology and Laboratory Medicine, University of Wisconsin Medical School, Madison, Wisconsin 53792

The signal transduction pathways regulating nucleolin mRNA and protein production have yet to be elucidated. Peripheral blood mononuclear cells treated with phorbol 12-myristate 13-acetate showed steady state levels of nucleolin mRNA that were 2–2.5-fold greater than untreated control cells. The up-regulation of nucleolin mRNA was substantially repressed by U0126, a specific inhibitor that blocks phosphorylation of extracellular-regulated kinase (ERK). Calcium ionophores A23187 and ionomycin also activated ERK and substantially elevated nucleolin mRNA levels, demonstrating phorbol 12-myristate 13-acetate and calcium signaling converge on ERK. Drugs that affected protein kinase C, protein kinase A, and phospholipase C signal transduction pathways did not alter nucleolin mRNA levels significantly. The half-life of nucleolin mRNA increased from 1.8 h in resting cells to 3.2 h with phorbol ester activation, suggesting ERK-mediated posttranscriptional regulation. Concomitantly, full-length nucleolin protein was increased. The higher levels of nucleolin protein were accompanied by increased binding of a 70-kDa nucleolin fragment to the 29-base instability element in the 3'-untranslated region of amyloid precursor protein (APP) mRNA in gel mobility shift assays. Supplementation of rabbit reticulocyte lysate with nucleolin decreased APP mRNA stability and protein production. These data suggest ERK up-regulates nucleolin posttranscriptionally thereby controlling APP production.

Alzheimer’s disease is characterized by the presence of senile plaques and neurofibrillary tangles in brain tissue. The major proteinaceous material in the senile plaques is β-amyloid, a 40–42-amino acid peptide derived from the amyloid precursor protein (APP).1 Investigation of the molecular regulation of APP mRNA and protein levels is vital to understanding β-amyloid accumulation and deposition in Alzheimer’s disease. Our laboratory has previously demonstrated that two RNA-binding proteins, nucleolin and heterogeneous nuclear ribonucleoprotein C (hnRNP C), bind to a 29-base instability element in the 3'-untranslated region (UTR) of APP mRNA (1). In rabbit reticulocyte lysate (RLR), hnRNP C binding to the 29-base element stabilized APP mRNA resulting in a 6-fold increase in APP protein production (2). The role of nucleolin was not determined in these experiments.

Nucleolin (C23) is a 110-kDa multifunctional phosphoprotein. It is an abundant nucleolar protein (3) found in the fibrillar centers and on organizer regions of metaphase chromosomes (4). Nucleolin plays a role in chromatin decondensation (5), the transcription and processing of rRNA (6–9), transcriptional regulation (10, 11), cell proliferation (12), differentiation and maintenance of neural tissue (13), apoptosis (14), nuclear/cytoplasmic shuttling (15), mRNA stability (16), and mRNP assembly and masking (17). Cell surface nucleolin has been reported to bind lipoproteins (18), laminin (19), growth factors (19), and the complement inhibitor, factor J (20). Central to nucleolin functions are RNA/DNA binding and helicase activities (21).

The cDNA for nucleolin has been cloned and codes for a 707-amino acid protein with at least three functional domains (3, 22). The 5'-flanking region and the first intron contain a high GC content similar to the housekeeping genes. The 5′ promoter has one atypical TATA box (GTGTA), one CCAAT box, three reverse complements of CCAAT (ATTGG), two pyrimidine-rich stretches, and numerous potential transcription factor-binding sites, whereas the 3′-UTR has five homology blocks in a 100-base region (23).

There are several distinct features in the amino acid sequence of nucleolin that enable this extraordinary protein to display such a vast array of functions (3, 7). At the amino-terminal end of the molecule, there are six (G/A/V)TP(G/A/V) repeats followed by several glutamic/aspartic acid stretches separated by basic sequences and four potential serine phosphorylation sites. The central region, a putative globular domain, contains alternating hydrophobic and hydrophilic stretches. There are four 90-residue repeats, each containing an RNP-like consensus sequence (24). The carboxyl terminus is glycine/arginine-rich with regularly spaced phenylalanine and dimethylarginine residues (25). The central 40-kDa domain of nucleolin containing the four RNA recognition motifs is responsible for the specificity of RNA binding, and the carboxyl-terminal domain enhances interaction but does not contribute to ligand specificity (26). The carboxyl-terminal domain contains an ATP-dependent duplex-

* This work was supported by National Institutes of Health Grant RO1AG10675 (to J. S. M.) and NIA Research Service Award AG00213 from the National Institutes of Health (to C. J. W.). 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.

‡ To whom correspondence should be addressed: University of Wisconsin Hospital and Clinics, Rm. K4/812, 600 Highland Ave., Madison, WI 53792. Tel.: 608-263-6043; Fax: 608-265-6215; E-mail: jsmalter@facstaff.wisc.edu.

The abbreviations used are: APP, amyloid precursor protein; CK2, casein kinase 2; D609, tricyclodecan-9-yl xanthogenate; DRB, 5,6-di-40–42-amino acid peptide derived from the amyloid precursor protein; ET-18-OCH3, 1-chlorobenzimidazole riboside; ECL, enhanced chemiluminescence; ERK, extracellular-regulated kinase; FBS, fetal bovine serum; hnRNP, heterogeneous nuclear ribonucleoprotein; MAPK, mitogen-activated protein kinase; MCA, methoxy carbamate; PBMC, peripheral blood mononuclear cells; PMA, phorbol 12-myristate 13-acetate; PKA, protein kinase A; PKC, protein kinase C; PLC, phospholipase C; PMA, phorbol 12-myristate 13-acetate; RRL, rabbit reticulocyte lysate; SC-9, OAG, 1-oleoyl-2-acetyl-3-(6-phenylhexyl)-5-chloro-1-naphthalenesulfonamide; U0126, bis[2-aminophenyIthio]butadiene; UTR, untranslated region.

Received for publication, October 16, 2000
Published, JBC Papers in Press, October 20, 2000, DOI 10.1074/jbc.M009435200
unwinding activity, and phosphorylation enhances this helicase activity (21, 27).

We were initially interested in determining how nucleolin regulation might affect APP mRNA levels and stability. Preliminary studies suggested cytokine-mediated signaling through protein kinases altered nucleolin mRNA levels in PBMC. We found that activation of the extracellular-regulated kinase (ERK)-specific mitogen-activated protein kinase (MAPK) pathway significantly up-regulated nucleolin mRNA levels independently of protein kinase C (PKC). Phorbol 12-myristate 13-acetate (PMA) treatment also resulted in higher levels of full-length nucleolin protein and the disappearance of the 47-kDa nucleolin cleavage fragment. In gel mobility shift assays, lysates from phorbol ester and ionomycin-treated peripheral blood mononuclear cells (PBMC) contained nucleolin that bound to the 29-base instability element of APP mRNA. In RRL supplemented with nucleolin, APP mRNA decayed with a shortened half-life of 105 min but was indefinitely stable in RRL supplemented with globin. The loss of APP mRNA stability resulted in decreased APP production. Therefore, ERK activation stabilizes nucleolin mRNA resulting in increased nucleolin levels and accelerated decay of APP mRNA.

EXPERIMENTAL PROCEDURES

Materials—Protease inhibitor mixture (catalog number P2714), Escherichia coli 1 RNA, RNase T1, 4-phenylboric acid, PMA, 1-oeyl-2-acetyl-sn-glycerol (C18:1,[9cis]-9(C2:0) (OAG), calcium ionophore A23187, iodo-mercaptopetanol, thioglycerol, dantrolene, forskolin, 1,9-dideoxyforskolin, cAMP, (R)-cAMPs, imipramine, wormthyn, 17β-estradiol, corticosterone, and 5,6-dichlorobenzimidazole riboside (DRB) were from Sigma. N-β-(6-Phenylethyl)-5-chloro-1-naphthalenesulfonamide (SC-9), tricycloclen-9-yl xanthogenate (D908), and 1-octadecyl-2-O-methylra-c-glycerol-3-phosphorylcholine (Et-18-OLCH) were from Calbiochem. Klenow enzyme, RNasin, bis[2-aminophenylthio]butadiene (U0126), skolin, cAMP, (-)tRNA, RNase TI, 4-AGCTTCT-3 and 5-TATAGGGAACTTGAATTAATCCACA-3 (APP cDNA(2415–2432) and 5-ACGCTAAATCTTCTAAGT-3' (APP cDNA(2520–2501)) by PCR (1 min at 94 °C, 1 min at 50 °C, and 10 s at 72 °C for 35 cycles). The 5' primer included a T7 RNA polymerase promoter sequence, which is underlined. The PCR product was extracted with Tris-saturated phenol/chloroform and precipitated with 0.1 volume of sodium acetate and 2 volumes of ethanol before gel purification on an 8% nondenaturing polyacrylamide gel. Radiolabeled RNA probes were prepared according to Promega's standard transcription protocol or a PhosphorImager screen.

Preparation of PBMC Cytoplasmic Lysates—Cytoplasmic lysates were purchased from Ambion, Inc. (Austin, TX). Peripheral blood was collected by phlebotomy from consented healthy donors. PBMC were isolated by Ficoll-Paque (Pharmacia, Piscataway, NJ) to generate pT7nuclcodingT90. The 3' UTR of nucleolin was amplified from Jurkat genomic DNA with the primers 5'-ACAGAGTTGAATGCTTC-3' (nucleolin cDNA(2211–2226)), and 5'-TGAGGAAAATGTTG-TGT-3' (nucleolin cDNA(2515–2529)) by PCR (1 min at 94 °C, 1 min at 50 °C, and 20 s at 72 °C for 35 cycles). The ends were blunted with T4 DNA polymerase and ligated with pTNovelT90 containing a T7 promoter and a 9-base poly(T) stretch.

Preparation of PBMC Cytoplasmic Lysates—Cytoplasmic lysates were prepared as described previously (29). Briefly, cultured PBMC (2 ml at 5 × 10^6 cells/ml) were scraped from tissue culture wells, spun at 2,000 × g for 30 s in a Stratagene picofuge microcentrifuge, washed three times with ice-cold phosphate-buffered saline (PBS), and resuspended in 50 μl of ice-cold buffer containing 25 mM Tris, pH 8, 0.1 mM EDTA, 0.5 mM EGTA, 3 mM PMSF, and 10 μg/ml aprotinin. The resuspended cells were lysed by five freeze–thaw cycles and spun at 15,000 × g for 15 min at 4 °C. The supernatants (cytoplasmic lysates) were transferred to fresh tubes and frozen at −80 °C. The protein concentration of the lysates was quantitatively determined with Bio-Rad protein assay dye reagent per the manufacturer's instructions.

Western Blot Analysis—Lysate (10 μg) in a 12-ml column was mixed with 4 μl (4X) SDS reducing buffer (62.5 mM Tris-HCl, pH 6.8, 10%, 2% SDS, 5% β-mercaptoethanol, 0.06% bromphenol blue), boiled for 5 min, and analyzed on a 12% SDS-polyacrylamide gel. The gel was equilibrated in Bjerrum and Schafer-Nielsen transfer buffer (48 mM Tris, 39 mM glycine, 20% methanol, 1.3 mM SDS, pH 9.2), and the proteins were transferred to 0.2-μm pure nitrocellulose membrane by semidry electrophoretic transfer. Bio-Rad Trans-Blot SD apparatus was used at 15 V for 45 min. The nitrocellulose membrane was blotted for 1 h in 5% nonfat dry milk and stained by ECL per the manufacturer's directions. The primary antibody was anti-nucleolin (1:1000) kindly provided by Dr. Raymond Petryshyn, Children's National Medical Center, Washington, D. C.) and the secondary antibody was anti-rabbit IgG (1:2000). The membrane was exposed to x-ray film for 1 min.

Preparation of DNA Template and In Vitro Transcription—APP106 template was prepared by amplification of nucleotides 2415–2520 of the plasmid pTAPP751wtHindIII190 (2) with the primers 5'-CACAATAGCTTACCATGAGAACTTGAATTAATCCACA-3' (APP cDNA(2415–2432)) and 5'-ACGCTAAATCTTCTAAGT-3' (APP cDNA(2520–2501)) by PCR (1 min at 94 °C, 1 min at 50 °C, and 10 s at 72 °C for 35 cycles). The 5' primer included a T7 RNA polymerase promoter sequence, which is underlined. The PCR product was extracted with Tris-saturated phenol/chloroform and precipitated with 0.1 volume of sodium acetate and 2 volumes of ethanol before gel purification on an 8% nondenaturing polyacrylamide gel. Radiolabeled RNA probes were prepared according to Promega's standard transcription protocol or a PhosphorImager screen.

Gel Mobility Shift Assays—The binding reactions were performed similarly to the procedures used in Ref. 28. Briefly, 2 μg of cytoplasmic lysates were incubated with 1 × 10^6 cpm of APP106 RNA probe in 10% glycerol, 15 mM HEpes, pH 8, 10 mM KCl, 1 mM dithiothreitol, 200 ng/μl E. coli tRNA, and 1 unit/μl RNasin in a total volume of 10 μl for 10 μl at 30 °C for 30 min. RNase T1 (20 units in 1-μl volume) was added, and samples were digested for 30 min at 37 °C. Reactions were collected on 4% agarose gels, and run under an automated system in 2× TBE buffer (90 mM Tris, 90 mM boric acid, 2 mM ethylenediamine tetraacetic acid [EDTA], pH 8.3) for 3 h at 15 V for 45 min. The nitrocellulose membrane was blotted for 1 h in 5% nonfat dry milk and stained by ECL per the manufacturer's directions. The primary antibody was anti-nucleolin (1:1000) (kindly provided by Dr. Raymond Petryshyn, Children's National Medical Center, Washington, D. C.) and the secondary antibody was anti-rabbit IgG (1:2000). The membrane was exposed to x-ray film for 1 min.

Received for publication April 1, 2003.

Copyright © 2003 the American Society for Biochemistry and Molecular Biology, Inc.
ERK Up-regulates Nucleolin mRNA and Protein

RESULTS

We examined the effect of several drugs that influence MAPK, PKC, protein kinase A (PKA), and phospholipase C (PLC) activity as well as calcium mobilization on the steady state level of nucleolin mRNA in PBMC. To investigate the role of PKC, cells were cultured for 3.25 h in the presence of the phorbol ester PMA. Compared with untreated controls, nucleolin mRNA levels increased by 2.5-fold (Fig. 1, lane 2). In all cases, nucleolin mRNA was a single 3.0-kilobase pair transcript.

The effect of several calcium mobilization drugs on nucleolin mRNA levels was next assessed. The ionophores A23187 and ionomycin, which transport calcium from the medium into cells, as well as thapsigargin, which causes the release of calcium from intracellular stores, all increased nucleolin mRNA levels. However, doxorubicin, which releases calcium from intracellular stores, had no effect on nucleolin mRNA levels. Therefore, nucleolin mRNA levels were up-regulated in response to drugs that increase intracellular calcium concentrations via release from intracellular stores or import from the extracellular environment.

We examined the influence of several activators and inhibitors of PKA and PLC signal transduction on the steady state level of nucleolin mRNA in PBMC. Cyclic AMP, a known activator of PKA, and (R) -cAMPs, an inhibitor of PKA, did not change the steady state level of nucleolin mRNA. Forskolin activates adenylate cyclase resulting in increased cAMP levels. 1,9-dideoxyforskolin, a negative control

![Image 1](http://www.jbc.org/)

![Image 2](http://www.jbc.org/)

FIG. 1. MAPK and calcium signaling, but not PKC, participate in the regulation of nucleolin mRNA. PBMC were treated with 20 ng/ml 4a-phorbol (lane 1), 20 ng/ml PMA (lane 2), 10 μM SC-9 (lane 3), 200 μM OAG (lane 4), 0.1% Me2SO (DMSO, lane 5), 5 μM A23187 (lane 6), 5 μM ionomycin (lane 7), 50 nM thapsigargin (lane 8), 10 μM dantrolene (lane 9), and 0.04% PBS (lane 10) for 3.25 h followed by a 15-min chase with DRB. Total RNA (6 μg) was analyzed by Northern blotting. A, representative Northern blot of the nucleolin-specific hybridization signals. B, representative Northern blot of the S26-specific hybridization signals. C, histogram depicting nucleolin mRNA levels normalized to S26 and plotted as a percentage of total nucleolin mRNA. The error bars represent the S.E. for triplicate samples. Student's t test results are as follows: PMA, p = 0.0012; SC-9, p = 0.17; OAG, p = 0.00040; A23187, p = 0.028; ionomycin, p = 0.0028; thapsigargin, p = 0.0092; and dantrolene, p = 0.089.  

![Image 3](http://www.jbc.org/)

FIG. 2. PKA and adenylate cyclase agonists do not affect nucleolin mRNA levels in PBMC. Cells were treated 3.5 h with 0.1% Me2SO (DMSO, lane 1), 13 μM 1,9-dideoxyforskolin (1,9-ddF, lane 2), 13 μM forskolin (lane 3), 142 μM cAMP (lane 4), 29 μM (R) -cAMPS (lane 5), 0.2% water (lane 6), and 20 ng/ml PMA (lane 7). After a 15-min chase with DRB, total RNA was isolated and analyzed by Northern blotting (6 μg of total RNA per lane). A, representative Northern blot of the nucleolin-specific hybridization signals. B, representative Northern blot of the 18 S-specific hybridization signals. C, histogram depicting nucleolin mRNA levels normalized to 18 S and plotted as a percentage of total nucleolin mRNA. The error bars represent the S.E. for triplicate samples. Student's t test results are as follows: 1,9-dideoxyforskolin, p = 0.22; forskolin, p = 0.29; cAMP, p = 0.96; (R) -cAMPS, p = 0.065; PMA, p = 0.063.
ERK Up-regulates Nucleolin mRNA and Protein

Fig. 3. PLC pathway agonists do not significantly alter nucleolin mRNA levels in PBMC. Cells were treated 4 h with 20 ng/ml PMA (lane 1), 19 μM D609 (lane 2), 15 μM ET-18-OCH₃ (lane 3), 100 μM imipramine (lane 4), 10 nM wortmannin (lane 5), 100 nM 17β-estradiol (lane 6), 1.5 μM corticosterone (lane 7), 100 nM 17β-estradiol with 1.5 μM corticosterone (est + cort, lane 8), and 0.1% PBS (lane 9) followed by a 15-min chase with DRB. Total RNA (10 μg per lane) was analyzed by Northern blotting. A, representative Northern blot of the nucleolin-specific hybridization signals. B, representative Northern blot of the 18S-specific hybridization signals. C, histogram depicting nucleolin mRNA levels normalized to 18S and plotted as a percentage of total nucleolin mRNA. The error bars represent the S.E. for triplicate samples. Student’s t test results are as follows: PMA, p = 0.0029; D609, p = 1.0; Et-18-OCH₃, p = 0.11; imipramine, p = 0.036; wortmannin, p = 0.17; 17β-estradiol, p = 0.23; corticosterone, p = 0.68; and 17β-estradiol + corticosterone, p = 0.16.

3.2 h after treatment for 3.5 h with PMA. Therefore, nucleolin mRNA accumulation after ERK activation can be accounted for by enhanced stability of the message.

As mentioned earlier, nucleolin protein has been implicated in many functions including APP mRNA stability. Thus, we examined cell lysates for nucleolin expression by Western blotting and nucleolin binding activity by RNA gel mobility shift assays. There was a time-dependent increase in nucleolin protein levels upon PMA treatment (Fig. 6). On ECL-stained Western blots probed with a polyclonal anti-nucleolin antibody, we observed two prominent nucleolin bands at 47 and 65 kDa in unstimulated PBMC. After PMA stimulation for 15 min, there was an increase in nucleolin fragments of ~70 kDa, and after 1 h, an increase in the 80-kDa nucleolin fragment. We observed full-length nucleolin protein (100 kDa) after 2 h of PMA treatment which continued to increase for several hours. As full-length nucleolin increased there was a decrease in the 47-kDa nucleolin cleavage product. RNA mobility shifts with radiolabeled APP RNA demonstrated a 2.8-fold increase in nucleolin binding after only 20 min of PMA treatment and a 5.1-fold increase by 135 min (Fig. 7). Our laboratory has previously demonstrated that the 70-kDa nucleolin polypeptide is responsible for the 84-kDa nucleolin/29-base element RNA-protein complex (1). In unstimulated cells, there were faint nucleolin-APP RNA complexes in the 60–70-kDa range (Fig. 7, lane 1) which were previously observed only in stimulated cells (16). These lower molecular weight complexes are not due to cell stimulation during the PBMC isolation procedure but rather are a consequence of including RNasin in the binding buffer. We have found an RNase A-like activity in PBMC that is inactivated by PMA or ionophore stimulation of the cells (data not shown). These complexes were specific for APP mRNA since...
ERK Up-regulates Nucleolin mRNA and Protein

**FIG. 5.** PMA up-regulates and stabilizes nucleolin mRNA. Cells were cultured in the presence of 0.002% ethanol (lanes 1–6) or 20 ng/ml PMA (lanes 7–12) for 3.5 h. Transcription was stopped by the addition of 20 μg/ml DRB, and after 15 min, total RNA was isolated at the indicated time points (0, 1, 2, 4, 6, and 8 h), and 5 μg of total RNA per lane was analyzed by Northern blotting. A, representative Northern blot of the nucleolin-specific hybridization signals. B, representative Northern blot of the 18S-specific hybridization signals. C, line graph depicting the percentage of nucleolin mRNA versus time. The circles represent nucleolin mRNA levels in the presence of ethanol alone (vehicle), and the triangles represent the nucleolin mRNA levels in the presence of PMA. The error bars represent the S.E. for triplicate samples. Student’s t test results are as follows: at 0 h, p = 0.015; at 1 h, p = 0.00026; at 2 h, p = 0.00013; at 4 h, p = 0.00029; at 6 h, p = 0.030; at 8 h, p = 0.00015.

**FIG. 6.** PMA stimulation increases the production of nucleolin protein in PBMC. Cells were treated for 15 min with 0.002% ethanol (lane 1) or 20 ng/ml PMA for 15 min (lane 2), 30 min (lane 3), 1 h (lane 4), 2 (lane 5), 3 (lane 6), and 4 h (lane 7). Cell lysates (10 μg per lane) were analyzed by SDS-polyacrylamide gel electrophoresis on a 12% gel, transferred to nitrocellulose membrane, and stained by ECL with anti-nucleolin serum.

They could not be detected in gel mobility shift assays with an APP RNA probe containing a randomized 29-base element (data not shown).

Cell activation with PMA, A23187, and ionomycin caused similar increases in the 84-kDa nucleolin-APP mRNA complexes (Fig. 8), whereas PKC, PKA, or PLC activation had no effect. Treatment of cells with U0126 prior to the addition of PMA blocked shift formation (Fig. 8E, lane 3). Thus drugs that activated ERK and calcium second messenger pathways increased nucleolin mRNA, protein, and APP RNA binding activity.

Nucleolin is a well-established RNA-binding protein with RNA helicase activity (21). We assessed the role nucleolin plays in APP mRNA stability and translation in an RRL translation system. Globin (control) or nucleolin mRNA was translated in RRL and subsequently incubated with APP mRNA as described under “Experimental Procedures.” Fresh RRL was then transferred to the preprogrammed APP mRNA, and translation and mRNA decay were measured by Northern blot analysis. Preincubation of the APP mRNA template with nucleolin increased decay of the message (t 1/2 = 105 min) (Fig. 9). Nucleolin also accelerated the decay of an APP mRNA containing a mutated 29-base element (APPmut mRNA) and decay of globin mRNA (data not shown). These mRNAs were all stable in RRL when preprogrammed with globin. Therefore, although nucleolin does bind to the 29-base instability element, it enhanced decay of APP mRNA independently of the element.

Nucleolin is a component of translation inhibitory particles (17). Translation of APP mRNA in RRL was measured by radioactive methionine incorporation. APP mRNA preprogrammed with nucleolin was translated at 52% of the level as template preprogrammed with globin (Fig. 10, 1 h). Similarly, translation of APPmut and globin mRNAs preprogrammed with nucleolin was also decreased (data not shown). Thus, APP production was compromised largely by accelerated decay of its coding mRNA.
**FIG. 8.** PMA and ionophores stimulate the ability of nucleolin to bind to the 3′-UTR of APP mRNA. Cells were treated with the indicated drugs at the concentrations noted in Fig. legends 1–3. A, gel mobility shift assays after PKC activators. Cells were stimulated with 4α-phorbol (lane 1), PMA (lane 2), SC-9 (lane 3), and OAG (lane 4). B, gel mobility shift assays after calcium mobilization drugs. Cells were stimulated with MeSO (lane 1), A23187 (lane 2), ionomycin (lane 3), dantrolene (lane 4), and PBS (lane 5). C, gel mobility shift assays after PKA/adenylate cyclase agonists. PBMC were treated with MeSO (lane 1), 1,9-dideoxyforskolin (lane 2), forskolin (lane 3), cAMP (lane 4), (R,3′)-cAMPs (lane 5), and water (lane 6). D, gel mobility shift assays after PLC effectors. Cells were treated with ethanol (lane 1), PMA (lane 2), D609 (lane 3), ET-18-OCH3 (lane 4), imipramine (lane 5), wortmannin (lane 6), 17β-estradiol (lane 7), corticosterone (lane 8), 17β-estradiol with corticosterone (lane 9), and PBS (lane 10). E, gel mobility shift assays after ERK agonists. Cells were pretreated with 10 µM U0126 for 15 min prior to the addition of PMA for 3 h with MeSO (lane 1), PMA (lane 2), U0126 + PMA (lane 3) and U0126 (lane 4). The arrow denotes the 84-kDa shift complex.

**DISCUSSION**

Our data indicate that activation of the MAPK or calcium second messenger pathways in PBMC results in a substantial accumulation of nucleolin mRNA. Drugs that activate/inhibit the PKA signal transduction pathway did not alter the steady state level of nucleolin mRNA, whereas PKC activation with OAG or PLC activation with imipramine had only modest effects. PMA stimulation of the cells resulted in posttranscriptional stabilization of the nucleolin message.

It was previously demonstrated that nucleolin mRNA levels increased in response to PHA in lymphocytes (30), serum stimulation in HeLa cells (31), vitamin A in monkey tracheobronchial epithelial cells (32), interleukin-2 in the C30.1 T cell line (33), and during liver regeneration in rat hepatocytes (34), whereas nucleolin mRNA levels were down-regulated during differentiation of human neuroblastoma cells with dibutyryl cAMP and/or retinoic acid (35) and by glucocorticoids in murine lymphosarcoma cells (36). In this study, we report that phorbol ester activation of ERK in PBMC increased nucleolin mRNA levels, and this increase was predominantly due to posttranscriptional stabilization of nucleolin mRNA. The half-life of nucleolin mRNA in resting PBMC was 1.8 h and increased to 3.2 h in PMA-treated cells. The cis acting domains responsible for regulated nucleolin mRNA stability are unknown. However, a comparison of the human, mouse, and hamster genomes reveals the presence of five homology blocks (nucleotides 2250–2258, 2268–2289, 2296–2302, 2305–2318, and 2321–2329) in the 3′-UTR within a 100-base region immediately following the stop codon (23). The first homology block is composed entirely of pyrimidine residues and is found within a uridine/cytidine-rich area. RNA-binding proteins with known specificities for pyrimidine-rich regions, such as pyrimidine tract binding protein, hnRNP C, or nucleolin itself, may bind to this region and protect the mRNA from RNase attack.

ERK signaling in PBMC resulted in increased nucleolin protein levels and binding activity to the 3′-UTR of APP mRNA. Nucleolin and hnRNP C form multiple RNA-protein complexes with the 29-base instability element situated ~200 bases downstream from the stop codon of APP mRNA (1). The 70-kDa nucleolin polypeptide is a constituent of previously identified 84-, 104-, and 140-kDa APP RNA-nucleolin complexes. The 47- and 48-kDa nucleolin peptides constitute the 65-, 73-, 90-, and 104-kDa complexes, suggesting protein homo- and heterodimers assemble on APP RNA (1). The 70-, 48-, and 47-kDa polypeptides all contain the carboxyl-terminal RNP domains. Since we observed increased binding of the 70-kDa nucleolin fragment to APP mRNA as more full-length nucleolin was produced by PMA-stimulated cells, and nucleolin exhibits increased susceptibility to proteolysis upon binding nucleic acid
Phosphorylation and cleavage likely regulate the activity of nucleolin. Amino-terminal phosphorylation enhanced binding to histone H1 and chromatin condensation (5). Nucleolin is phosphorylated on serine residues by casein kinase 2 (CK2) (38, 39) during interphase, suggesting phosphorylation plays a role in the control of rDNA transcription, but nucleolin is phosphorylated on threonine residues by cdc2 kinase during mitosis, which has been linked to mitotic reorganization of nucleolar chromatin (40, 41). The transition from the lower molecular weight nucleolin complexes we observe in unstimulated cells to the predominant 84-kDa complex in PMA-treated cells coincides with decreased stability of APP mRNA, suggesting that mRNA stability is dependent on which nucleolin polypeptides bind to the 29-base element. These data also support a model in which there is competition between nucleolin and hnRNP C for binding to the 5′-region of the cis element.

Phosphorylation and cleavage likely regulate the activity of nucleolin. Amino-terminal phosphorylation enhanced binding to histone H1 and chromatin condensation (5). Nucleolin is phosphorylated on serine residues by casein kinase 2 (CK2) (38, 39) during interphase, suggesting phosphorylation plays a role in the control of rDNA transcription, but nucleolin is phosphorylated on threonine residues by cdc2 kinase during mitosis, which has been linked to mitotic reorganization of nucleolar chromatin (40, 41). CK2 activity increases during cellular growth and declines when cells reach quiescence in nucleolar chromatin (40, 41). CK2 activity increases during mitosis, which has been linked to mitotic reorganization of nucleolar chromatin (40, 41). CK2 activity increases during mitosis, which has been linked to mitotic reorganization of nucleolar chromatin (40, 41).

Characterization of the nucleic acid-binding sites of nucleolin revealed a large array of DNA and RNA motifs including the pre-mRNA 3′ splice site sequence r(UUAG/G), the human telomeric DNA sequence d(TTAGGG) (49), and the 3′-UTR of poliovirus RNA (50). The exact poliovirus 3′-UTR sequence has not been determined but may involve nucleotides 7365–7373 (CAUUUAGU) which are very similar to the central portion of the APP 29-base element (16, 50). The nucleolin recognition element is an 18-base stem-loop containing the sequence UCCGAG (51). Mutations in this sequence prevented the specific interaction between nucleolin and the RNA target (52). These data suggest that nucleolin preferentially binds short stretches of C/U nucleotides.

Our laboratory has demonstrated that the proteins hnRNP C and nucleolin bind to the 29-base instability element in the 3′-UTR of APP mRNA (1, 16). We have also shown that hnRNP C binding to this element stabilizes the APP message and increases protein production in RRL (2). In PBMC, APP mRNA underwent biphasic decay upon ERK activation with a rapid, initial fall in message quantity followed by prolonged stability. We have proposed that the stable phase was a consequence of hnRNP C binding to the 29-base instability element that prevented RNase attack. In this paper, we demonstrate that nucleolin mRNA was up-regulated in PBMC via PMA or calcium ionophore treatment. The phorbol ester-mediated stabilization of nucleolin mRNA was accompanied by accumulation and decreased processing of nucleolin protein. The initial drop in the biphasic decay of APP mRNA corresponds temporally with increased nucleolin production. In RRL, APP mRNA pre-programmed with nucleolin decays with a half-life of 105 min but is stable in globin-supplemented RRL. Declines in APP message levels correspond with decreased translation.

The Gly-rich carboxyl-terminal domain of nucleolin contains DNA and RNA helicase activity that is modulated by phosphorylation (21). Another DNA and RNA helicase, G3BP, has sequence similarity with the carboxyl-terminal portion of nucleolin and is an element of the Ras signal transduction pathway (53). Collectively our data support a model in which nucleolin is up-regulated in response to ERK activation and then unwinds APP mRNA allowing RNase attack. Although nucleolin binds to the pyrimidine-rich 29-base element, the destabilizing effect of this protein on APP mRNA appears independent of the cis element since APPmut mRNA also decays rapidly.

These findings are meaningful in that APP undergoes processing by β- and γ-secretases to produce β-amyloid, a 40–42-amino acid peptide found in the senile plaques characteristic of Alzheimer’s disease and Down’s syndrome. Significant levels of nucleolin have been found in mature brain and in differentiating neuronal cells (13). Thus, dysregulation of this multifunctional protein could play an important role in regulating APP mRNA stability, APP levels, and β-amyloid production.

Acknowledgments—We thank Dr. Raymond Petryshyn (Children’s National Medical Center, Washington D.C.) for anti-nucleolin polyclonal antibody; Dr. Jeff Ross and Dr. Richard Spritz (University of Wisconsin, Madison, WI) for the plasmids pTT790 and pSPβ, respectively; Dr. Meera Srivastava (Georgetown University School of Medicine, Washington D.C.) for the plasmid pMAMnucleolin, the nursing staff (Infusion Center, University of Wisconsin Hospital) for drawing blood from volunteer donors; Dr. William Rehrauer (Molecular Diagnostics Laboratory, University of Wisconsin Hospital) for automated sequencing of plasmids; and members of the laboratory for their thoughtful comments.

REFERENCES
1. Zaidi, S. H., and Malter, J. S. (1995) J. Biol. Chem. 270, 17292–17298
2. Rajapakalan, L. E., Westmark, C. J., Jarzembowski, J. A., and Malter, J. S. (1998) Nucleic Acids Res. 26, 3418–3425
3. Lapeyre, B., Bourbon, H., and Amalric, F. (1987) Proc. Natl. Acad. Sci. U.S.A. 84, 1472–1476
4. Lisowczyk, M. A., Richards, R. L., Busch, R. K., and Busch, H. (1981) Exp. Cell...
ERK Up-regulates Nucleolin mRNA and Protein

Res. 136, 101–109
5. Erdar, M. S., Belenguer, P., Caizergues-Ferrer, M., Pantaloni, A., and Amalric, F. (1987) Eur. J. Biochem. 175, 329–335
6. Egyhazi, A., Pignon, A., Chang, J. H., Ghaffari, S. H., Dreessen, T. D., Wellman, S. E., Case, S. T., and Olson, M. O. (1988) Exp. Cell Res. 178, 264–272
7. Jordan, G. (1987) Nature 329, 489–496
8. Ginisty, H., Amalric, F., and Bouvet, P. (1998) EMBO J. 17, 1476–1486
9. Bouvet, P., Diaz, J. J., Kindbeiter, K., Madjar, J. J., and Amalric, F. (1998) J. Biol. Chem. 273, 19025–19029
10. Belenguer, P., Baldo, V., Mathieu, C., Prats, H., Bensaid, M., Bouche, G., and Amalric, F. (1989) Nucleic Acids Res. 17, 6625–6636
11. Yang, T. H., Tsai, W. H., Lee, Y. M., Lei, H. Y., Lai, M. Y., Chen, D. S., Yeh, J. W. H., and Lee, S. C. (1994) Mol. Cell. Biol. 14, 6068–6074
12. Derenzini, M., Sirri, V., Trere, D., and Ochs, R. L. (1995) Lab. Invest. 73, 497–502
13. Kibbey, M. C., Johnson, B., Petryshyn, R., Jucker, M., and Kleinman, H. K. (1995) J. Neurosci. Res. 42, 314–322
14. Brockstedt, E., Rickers, A., Kostka, S., Laubersheimer, A., Dorken, B., Wittmann-Liebold, B., Bommert, K., and Otto, A. (1998) J. Biol. Chem. 273, 28057–28064
15. Borer, R. A., Lehner, C. F., Eppenberger, H. M., and Nigg, E. A. (1989) Cell 56, 379–390
16. Zaidi, S. H., Denman, R., and Malter, J. S. (1994) J. Biol. Chem. 269, 24000–24006
17. Yurkova, M. S., and Murray, M. T. (1997) J. Biol. Chem. 272, 10870–10876
18. Semenovich, C. F., Ostlund, R. E., Jr., Olson, M. O., and Yang, J. W. (1990) Biochemistry 29, 9708–9713
19. Tak, M., Teutui, J., Ohama, H., Ozawa, M., Nakayama, T., Maruyama, I., Arima, T., and Muramatsu, T. (1994) J. Biochem. (Tokyo) 116, 1063–1068
20. Lapeyre, B., Amalric, F., Wallace, M. O., and Falaschi, A. (1995) Cell Proliferation 28, 267, 110–117
21. Tuteja, N., Huang, N. W., Skopac, D., Tuteja, R., Hristova, S., Zhang, J., Pongor, S., Juh, G., Faucher, C., Amalric, F., and Falaschi, A. (1995) Gene (Amst.) 160, 143–148
22. Srivastava, M., Fleming, P. J., Pollard, H. B., and Burns, A. L. (1989) FEBS Lett. 255, 39–43
23. Srivastava, M., McBride, O. W., Fleming, P. J., Pollard, H. B., and Burns, A. L. (1990) J. Biol. Chem. 265, 14922–14931
24. Bugler, B., Bourbon, H., Lapeyre, B., Wallace, M. O., Chang, J. H., Amalric, F., and Olson, M. O. (1987) J. Biol. Chem. 262, 10922–10925
25. Lapeyre, B., Amalric, F., Ghaffari, S. H., Rao, S. V., Dunbar, T. S., and Olson, M. O. (1986) J. Biol. Chem. 261, 9167–9173
26. Ghisolfi, L., Khouristi, A., Joseph, G., Amalric, F., and Erdar, M. (1992) Eur. J. Biochem. 209, 541–548
27. Ghisolfi, L., Joseph, G., Amalric, F., and Erdar, M. (1992) J. Biol. Chem. 267, 2955–2960
28. Malter, J. S. (1989) Science 246, 664–666
29. Fukuda, H., Nishida, A., Saito, H., Shimizu, M., and Yamawaki, S. (1994) Neurochem. Int. 25, 567–571
30. Mesas, G., and Pajor, L. (1995) Cell Proliferation 28, 329–336
31. Konishi, T., Karasaki, Y., Nomoto, M., Ohmori, H., Shibata, K., Abe, T., Shimizu, K., Itoh, H., and Higashi, K. (1995) J. Biochem. (Tokyo) 117, 1170–1177
32. Reddy, P. M., An, G., Di, Y. P., Zhao, Y. H., and Wu, R. (1996) Am. J. Respir. Cell Mol. Biol. 15, 388–403
33. Herblot, S., Chastagner, P., Samady, L., Moreau, J. L., Denais, C., Frousard, P., Liu, X., Bonnet, J., and Theze, J. (1999) J. Immunol. 162, 3280–3286
34. Ohmori, H., Murakami, T., Funatani, A., Higashi, K., Hirano, H., Gotoh, S., Kuroiwa, A., Masui, A., Nakamura, T., and Amalric, F. (1990) Exp. Cell Res. 189, 227–232
35. Murakami, T., Ohmori, H., Gotoh, S., Tsuda, T., Ohya, R., Akai, S., and Higashi, K. (1991) J. Biochem. (Tokyo) 110, 146–150
36. Meynais, O., Baldo, V., Bouche, G., and Amalric, F. (1990) Biochim. Biophys. Acta 1049, 35–44
37. Olson, M. O., Kirstein, M. N., and Wallace, M. O. (1990) Biochemistry 29, 5682–5686
38. Caizergues-Ferrer, M., Belenguer, P., Lapeyre, B., Amalric, F., Wallace, M. O., and Olson, M. O. (1987) Biochemistry 26, 7876–7883
39. Csermely, P., Schneider, T., Cheatham, B., Olson, M. O., and Kahn, C. R. (1993) J. Biol. Chem. 268, 9747–9752
40. Belenguer, P., Caizergues-Ferrer, M., Labbe, J. C., Doree, M., and Amalric, F. (1990) Mol. Cell. Biol. 10, 3607–3618
41. Peter, M., Nakagawa, J., Doree, M., Labbe, J. C., Nigg, E. A. (1990) Cell 60, 791–801
42. Schneider, H. R., and Issinger, O. G. (1989) Biochim. Biophys. Acta 1014, 98–100
43. Le, D. T., Leprow, G., and Krebs, E. G. (1996) J. Biol. Chem. 271, 15662–15668
44. Fang, S. H., and Yeh, N. H. (1993) Exp. Cell Res. 208, 48–53
45. Pasternack, M. S., Bleier, K. J., and McInerney, T. N. (1991) J. Biol. Chem. 266, 15662–15668
46. Bourbon, H. M., Bugler, B., Caizergues-Ferrer, M., Amalric, F., and Zalta, J. P. (1983) Mol. Biol. Rep. 9, 39–47
47. Warrenner, P., and Petryshyn, R. (1991) Biochem. Biophys. Res. Commun. 180, 716–723
48. Chen, C. M., Chiang, S. Y., and Yeh, N. H. (1991) J. Biol. Chem. 266, 7754–7758
49. Ishikawa, F., Matunis, M. J., Dreyfuss, G., and Cech, T. R. (1993) Mol. Cell. Biol. 13, 4301–4310
50. Waggoner, S., and Sarow, P. (1998) J. Virol. 72, 6699–6709
51. Serrit, G., Joseph, G., Faucher, C., Ghisolfi, L., Bouche, G., Amalric, F., and Bouvet, P. (1999) Biochimie (Paris) 81, 530–538
52. Ghisolfi-Nieto, L., Joseph, G., Puvion-Dutilleul, F., Amalric, F., and Bouvet, P. (1999) J. Mol. Biol. 290, 34–53
53. Costa, M., Ochem, A., Staab, A., and Falaschi, A. (1999) Nucleic Acids Res. 27, 817–821
Up-regulation of Nucleolin mRNA and Protein in Peripheral Blood Mononuclear Cells by Extracellular-regulated Kinase
Cara J. Westmark and James S. Malter

J. Biol. Chem. 2001, 276:1119-1126.
doi: 10.1074/jbc.M009435200 originally published online October 20, 2000

Access the most updated version of this article at doi: 10.1074/jbc.M009435200

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC’s e-mail alerts

This article cites 53 references, 22 of which can be accessed free at http://www.jbc.org/content/276/2/1119.full.html#ref-list-1