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RNA-Binding Protein HuR Is Required for Stabilization of *SLC11A1* mRNA and SLC11A1 Protein Expression

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The solute carrier family 11 member 1 (*SLC11A1*, formerly *NRAMP1*) gene is associated with infectious and autoimmune diseases and plays an important role in macrophage activation. Human *SLC11A1* mRNA contains an AU-rich element (ARE) within the 3′ untranslated region; however, its role in the regulation of *SLC11A1* gene expression has not been elucidated. Here we analyze the expression of SLC11A1 in human monocytes and HL-60 cells and then use HL-60 cells as a model to determine whether RNA-binding protein HuR is associated with the ARE and involved in *SLC11A1* mRNA turnover. Our results demonstrate a binding of HuR to the *SLC11A1* ARE in phorbol myristate acetate (PMA)-differentiated cells dramatically increased compared to that in undifferentiated cells. Interestingly, PMA-induced accumulation of cytoplasmic HuR occurs in parallel with an increase in the binding of HuR to *SLC11A1* ARE and with an increase in the *SLC11A1* mRNA level. This suggests that HuR's cytoplasmic localization plays an important role in the regulation of SLC11A1 expression. We also observe that down-regulation of HuR expression by RNA interference (RNAi) results in a decrease in SLC11A1 expression which can be restored by the addition of recombinant HuR protein to the RNAi-treated cells. Finally, we show that HuR overexpression in HL-60 cells significantly increases the *SLC11A1* mRNA stability. Taken together, our data demonstrate that HuR is a key mediator of posttranscriptional regulation and expression of the *SLC11A1* gene.

In mouse, natural resistance to infection with unrelated intracellular parasites such as *Salmonella enterica* serovar Typhimurium, *Leishmania donovani*, and various Mycobacterium species, including *Mycobacterium bovis* and *Mycobacterium lepraemurium*, is controlled by the solute carrier family 11 member 1 (*Slc11a1*) gene, also known as Ity/Lsh/BCG or *Nramp1* (6, 8, 44, 52, 56). In addition to restricting the growth of intracellular pathogens, the murine *Slc11a1* gene product was also shown to have many pleiotropic effects on macrophage activation, including regulation of the chemokine KC as well as several cytokines, such as interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α); induction of nitric oxide (NO) release; major histocompatibility complex class II molecule expression; and oxidative burst (7, 27). The human homologue (*SLC11A1*) gene has also been reported to be involved in susceptibility to infectious diseases, such as tuberculosis, leprosy, and human immunodeficiency virus infection, as well as to autoimmune diseases, such as rheumatoid arthritis, juvenile rheumatoid arthritis, sarcoidosis, and Crohn's diseases (7, 19). A large number of additional disorders with either known or suspected infectious etiologies have also been investigated for possible contributions of the *SLC11A1* gene to disease risk. In most examples, further studies are needed before firm conclusions can be reached.

The murine *Slc11a1* gene is expressed in monocytes/macrophages (28). Biochemical studies have shown that Slc11a1 is an integral membrane protein with a molecular mass of 90 to 110 kDa which is extensively glycosylated and phosphorylated in macrophages (4, 57). Interestingly, the expression of the *Slc11a1* gene in murine macrophages cells is up-regulated by bacterial lipopolysaccharide, interferon γ, granulocyte/macrophage colony-stimulating factor, and inflammatory stimuli (10, 28). In human, *SLC11A1* is expressed not only in monocytes/macrophages but also in polymorphonuclear neutrophils (11, 45). *SLC11A1* gene expression is undetectable in transformed human cell lines from either erythroid or lymphoid T or B lineages as well as in the progenitors of the monocyte/macrophage pathway (KG1, U937, THP) and the promyelocytic leukemia cell line HL-60. It can, however, be strongly induced in these cells when they are differentiated toward either the monocyte/macrophage or the granulocyte pathway (11). So far, little is known about how the *SLC1A1* gene is regulated by the above-mentioned stimuli. In differentiated HL-60 cells, the induction of SLC11A1 protein expression correlates with a high level of *SLC11A1* mRNA, suggesting that SLC11A1 expression may be controlled primarily at the level of transcription and/or mRNA stability (45).

A recent survey of the 3′ untranslated regions (3′UTR) of the human mRNA database showed that the *SLC11A1* message contains four AUUUA repeats, a typical AU-rich element (ARE). This sequence is homologous to the destabilizing sequence found in many short-lived mRNAs such as oncogene, cytokine, and growth hormone mRNAs (5, 9) and is involved in the regulation of the mRNA turnover of these genes. The role of this motif in the regulation of *SLC11A1* gene expression has

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not yet been elucidated. In general, mRNAs that contain AREs are rapidly degraded in unstimulated cells, but specific growth or stress conditions can modify the fate of specific messages. For example, IL-1α has been shown to enhance the stability of a variety of cytokine and chemokine mRNAs that otherwise exhibit short half-lives, and this depends, at least in part, upon the presence of ARE motifs in the 3′UTRs (30, 35). Similarly, eotaxin mRNA is stabilized following treatment with TNF-α and IL-4 (2), and treatment with phorbol esters, calcium ionophores, or interleukins increases the stability of lymphokine mRNA in lymphoid cells (31, 51, 64). Furthermore, multiple studies have shown that a specific mRNA/protein complex is formed or increased in response to extracellular stimulation, leading to changes in the half-lives of the target mRNAs (14, 48, 58, 65). In the case of *SLC11A1* mRNA, the possible implication of the ARE in its turnover comes from the fact that the murine counterpart does not contain any ARE and seems to be very stable with a long half-life (33). Indeed, this observation suggests that the presence of AUUUA repeats in the *SLC11A1* 3′UTR could indicate a posttranscriptional regulation affecting the stability and/or the mRNA export levels.

The AREs are known to bind RNA-binding proteins that positively or negatively regulate ARE-containing mRNA turnover (5, 9, 12, 38, 46, 47). A number of proteins that bind to 3′UTRs, in particular to AREs, have been identified (1, 25, 62). Among these proteins, AUF1 (also called heterogeneous nuclear ribonucleoprotein D [hnRNP D]) and HuR are well studied for their influence on mRNA turnover. AUF1 has been shown to promote mRNA degradation in most cases, while HuR stabilizes mRNA (9, 34). HuR is a member of the embryonic lethal abnormal vision family of mRNA-binding proteins. It is a 36-kDa protein, containing three RNA recognition motifs, which binds with high affinity to AREs and thus stabilizes specific ARE-containing mRNAs (43). Unlike the other three embryonic lethal abnormal vision proteins (Hel-N1, HuC, and HuD), which are developmentally regulated and expressed uniquely in the nervous system, HuR is ubiquitously expressed in all cell types (26). Although it is localized mainly in the nucleus, HuR can shuttle between the nucleus and cytoplasm by virtue of its shuttling signal, HNS (HuR nucleocytoplasmic shuttling domain), which is located in the hinge region between its second and third RNA recognition motifs. It is postulated that HuR binds to specific mRNAs in the nucleus, transporting them to the cytoplasm and protecting them from rapid degradation (3, 9). Studies have demonstrated that HuR's cytoplasmic localization increases under conditions of stress, such as heat shock (23), UV irradiation (59), amino acid starvation (65), chronic ethanol exposure (39), and T-cell receptor signaling (50). Furthermore, it was suggested that while HuR associates with its mRNA targets, thus stabilizing them (21), it serves as an adaptor for their rapid export to the cytoplasm (24). Thus, it is possible that HuR, through the $(AUUUA)_4$ element, regulates the posttranscriptional events of the *SLC11A1* gene, leading to its rapid expression upon extracellular stimulation.

To define the molecular mechanisms of the posttranscriptional regulation of *SLC11A1* genes as well as the role of its AREs, we chose the human promyelocytic leukemia HL-60 cell line as a model. We observed that the increased expression of the *SLC11A1* mRNA in phorbol myristate acetate (PMA)-differentiated HL-60 cells is in part due to its stabilization by the RNA-binding protein HuR. We present evidence supporting the implication of the above-described AREs in this process as mediators of HuR's association with *SLC11A1* mRNA turnover.

MATERIALS AND METHODS

Plasmid constructions. Vectors used for synthesis of RNA probes in electrophoretic mobility shift assays were constructed as follows. For the *SLC11A1* 3′UTR probe, human genomic DNA prepared from U937 cells was first amplified by PCR using the primers 5′-CCATCAACCTCTACTTCGTGGTCAGCT ATC-3′ and 5′-CTTGGGTTCCTTATGACCTGGTGCCTGA-3′. The PCR product was then amplified again using another set of primers: 5′-ATAAGAA TGCGGCCGCCCACACCAGGGCCTGGCT −3′ and 5′-GCTCTAGAGGTG GCTCACGCCTGTAATT-3′. The final PCR product (spanning nucleotides 1829 to 2540 of *SLC11A1* 3′UTR; NCBI GenBank accession number D50402) was gel purified and modified using the A-tailing procedure and then ligated into PGEM-T easy vector (Promega, Madison, WI). This construct was termed PGEM-T-3′UTR. For the 40-nucleotide ARE probe, the synthesized oligonucleotide (corresponding to positions 2248 to 2287 of *SLC11A1* 3′UTR) was cloned into PGEM-T easy vector and termed PGEM-T-ARE. The plasmids Gateway-AP-HuR-GST and Gateway-GST-AP, for expression of the recombinant proteins, and the pcDNA3.0-HuR-Flag plasmid, for overexpression of the recombinant human HuR in mammalian cells, were constructed as described previously (21, 55).

Protein expression and purification. The fusion proteins AP-HuR-GST and GST-HuR were expressed and purified as previously described (55). Briefly, *Escherichia coli* BL21(DE3) cells were transformed with the plasmid Gateway-AP-HuR-GST or Gateway-GST-AP, grown in LB medium (containing 50 μg/ml ampicillin) until the optical density at 600 nm was approximately equal to 0.6, and then induced with 0.3 mM IPTG (isopropyl-β-D-thiogalactopyranoside) for 4 h. After induction, cell pellets were washed with lysis buffer (50 mM Tris, pH 8.0, 200 mM NaCl, and 1 mM EDTA) and resuspended in lysis buffer containing 100 μg/ml lysosome for 15 min, followed by the addition of dithiothreitol, protease inhibitors (Roche, Laval, QC, Canada), and sarkosyl (1.5% final concentration) and then sonication. After sonication, the lysate was centrifuged and the supernatant was transferred to a new tube, a procedure followed by the addition of 10% Triton X-100 to a final concentration of 3%. The lysate was incubated with glutathione-Sepharose 4B (Amersham Biosciences, Baie d'Urfé, QC, Canada), and the recombinant proteins were eluted from the beads with glutathione elution buffer and dialyzed against phosphate-buffered saline (PBS) for the use described below.

Preparation of monocytes and cell culture. Human venous blood samples from healthy, medication-free volunteers were collected on citrate/dextrose/adenine. The peripheral blood mononuclear leukocytes were enriched by dextran sedimentation, layered over a Ficoll-Hypaque cushion, and centrifuged at $400 \times g$ for 20 min. Mononuclear leukocytes were then collected at the interface, washed twice with PBS, resuspended in RPMI 1640 medium with 10% heat-inactivated fetal bovine serum (FBS), 100 U/ml penicillin, and 100 ng/ml streptomycin and allowed to adhere to plastic petri dishes coated with defibrinized autologous serum for 1 h at 37°C. Nonadherent cells were removed by rinsing with PBS, and adherent cells (>90% monocytes) were detached with EDTA (0.01 M) in RPMI 1640. Mononuclear cells were further purified with anti-CD14 monoclonal antibody-coated microbeads by using MACS CD14 isolation kits from Miltenyi Biotec (Auburn, CA). Both monocytes and HL-60 cells were cultured in RPMI 1640 medium supplemented with 10% FBS, 2 mM L-glutamine, and 1% penicillin/streptomycin. For PMA treatment, the cells were seeded in six-well tissue culture plates at densities of 1×10^6 (monocytes) or 4×10^5 (HL-60) cells/ml and treated with 10 ng/ml PMA (Sigma, Oakville, ON, Canada).

Western blot analysis. After the appropriate treatment, total cell extracts as well as nuclear and cytoplasmic fractions were prepared as described previously (17, 20). Total cellular, cytoplasmic, and nuclear lysates were resolved on 4 to 12% Bis-Tris gel (Invitrogen, Burlington, ON, Canada) and electrophoretically transferred to polyvinylidene difluoride membranes (Millipore, Bedford, MA). The membranes were blocked with 5% milk in TBS-T (Tris-buffered saline–0.1% Tween 20) overnight, a procedure followed by incubation with primary antibody at room temperature for 1 h. A monoclonal antibody against HuR, 19F12, was used at a 1:3,000 dilution. A monoclonal antibody against SLC11A1 (Santa Cruz) was used at a 1:500 dilution. A monoclonal antibody against β-actin (Sigma, Saint Louis, Missouri) was used at a 1:5,000 dilution. A polyclonal antibody

against β-tubulin (Santa Cruz) was used at a 1:1,000 dilution, and a polyclonal antibody against HDAC1 (Santa Cruz) was used at a 1: 1,000 dilution. After washing, membranes were further incubated with appropriate secondary antibodies coupled to horseradish peroxidase and developed using enhanced chemiluminescence detection reagents.

Northern blot analysis. Total cellular RNA was extracted by using TRIzol reagent (Invitrogen, Burlington, ON, Canada). Equal amounts (30 μg/lane) of RNA were denatured in formamide/formaldehyde (65°C, 10 min), separated on 1.2% agarose/formaldehyde gels, and then transferred to Hybond-N^+ membranes (Amersham Biosciences, Baie d'Urfé, QC, Canada) and hybridized with a specific cDNA probe. To analyze *SLC11A1* RNA, a fragment of *SLC11A1* cDNA spanning positions 444 to 815 of the open reading frame (GenBank accession number D50402) was used. The PCR-amplified and gel-purified *SLC11A1* and G3PDH cDNAs were labeled with [α-^{32}P]dCTP by using the nick translation system (Invitrogen, Burlington, ON, Canada). The probe for the 18S rRNA was obtained by in vitro transcription of the pTRI RNA 18S antisense control template (Ambion, Austin, TX). After hybridization, membranes were washed and then exposed for 2 to 4 days to Bioflex MRI films (Clonex, Markham, ON, Canada).

Immunofluorescence. HL-60 cells were seeded on four-well culture slides (Becton Dickinson Labware, Franklin Lakes, NJ) and treated with PMA. Untreated HL-60 cells were collected by centrifugation at 500 × *g* for 5 min and placed on culture slides. Both treated and untreated cells were fixed for 15 min in PBS containing 3.7% paraformaldehyde and 0.18% Triton-X. After soaking in blocking buffer (PBS containing 1% goat serum) for 30 min, slides were then incubated with the primary antibodies (mouse anti-HuR [1:500] and rabbit anti-G3BP [Ras-GTPase-activating protein SH3 domain-binding protein] [1:1,000]) in blocking buffer for 1 h at room temperature. Following washes with blocking buffer, samples were incubated with a mixture of fluorescein isothiocyanate- and tetramethyl rhodamine isothiocyanate-labeled secondary antibodies, both at 1:500 dilutions in blocking buffer for 1 h. Finally, cells were stained with 1:20,000 DAPI (4′6′-diamidino-2-phenylindole) (1 mg/ml) for 5 min. After washing with blocking buffer, slides were mounted in Vectashield (Vector Laboratories, Burlingame, CA) and visualized with a Zeiss Axiovision 3.1 microscope using a 63× oil objective, and an Axiocam HR (Zeiss) digital camera was used for photography.

RNA-binding assay and supershift assay. Plasmid PGEM-T-3′UTR was linearized with the restriction endonuclease XbaI (New England Biolabs, Beverly, MA) and PGEM-T-ARE with SpeI (New England Biolabs, Beverly, MA) to generate the DNA template for in vitro transcription. RNA probes were prepared by using the SP6/T7 transcription kit according to the manufacturer's instruction (Roche, Laval, QC, Canada). Briefly, a 20-μl transcription reaction mixture containing 1 μg of template, 2.5 mM ATP, GTP, and CTP, 200 μM UTP, 5 μl [α-^{32}P]UTP, and 10 units of T7 RNA polymerase was incubated for 30 min at 37°C. Template DNA was removed by DNase I treatment, and the radiolabeled probe was purified through spin columns. The RNA-binding assay and the HuR supershift assay were performed as described previously (17).

Transfection of HL-60 cells. The electrotransfection of HL-60 cells has been described previously (36). Twenty μg of plasmid pcDNA3.0-HuR-Flag or pcDNA3.0-Flag was added to a 0.4-cm electroporation cuvette containing 0.5 ml of HL-60 cells (1×10^7 cells/ml) in RPMI 1640 medium supplemented with 20% fetal calf serum. Electroporation was performed at a setting of 240-V pulse and 960-μF capacitance by using a Bio-Rad gene pulser (Bio-Rad Laboratories, Hercules, CA). Cells were cultured in RPMI 1640 medium containing 20% FBS and allowed to recover for 3 days, a process followed by G418 (Geneticin, Invitrogen, Burlington, ON, Canada) selection for 10 days at a final concentration of 1.5 mg/ml. Clonal sublines were selected by plating on semisolid methyl cellulose Iscove's medium (Stem Cell Technologies, Paisley, Scotland) containing 1.5 mg/ml G418. The stably transfected colonies were grown in RPMI 1640 medium supplemented with 1 mg/ml G418 and screened for expression of the HuR-Flag recombinant protein.

Immunoprecipitation of mRNP complex and RT-PCR. To investigate the interaction of the *SLC11A1* mRNA and HuR protein, a combination of immunoprecipitation and reverse transcription (RT)-PCR analysis was used as previously described (54). HL-60 cells treated with PMA for different periods of time were lysed in 1 ml polysome lysis buffer (10 mM KCl, 5 mM $MgCl_2$, 10 mM HEPES [pH 7.0], 0.5% NP-40, 1 mM dithiothreitol, 100 units/ml RNase inhibitor, 0.2% vanadyl ribonucleoside complex, and protease inhibitors). Immunoglobulin G1 (IgG1) (Sigma, St. Louis, Missouri), anti-HuR antibody (3A2)- and anti-Flag antibody (Sigma, St. Louis, Missouri)-coated protein A beads were used to immunoprecipitate and separate mRNA-HuR complexes. RNA was extracted using TRIzol reagent. RT was performed using a SuperScript first-strand synthesis system for RT-PCR (Invitrogen, Burlington, ON, Canada), and the produced template was amplified by PCR using the sense primer 5′-TCAA ACTTCTCTGGGTGCTGCTCT-3′ and the antisense primer 5′-ACTCATAG CCAAAGGTCAAGGCCA-3′. PCR products (372 bp) were resolved on ethidium bromide-stained 1.5% agarose gel.

Protein labeling. AP-HuR-GST was conjugated with Alexa Fluor 594 using the Alexa Fluor 594 monoclonal antibody labeling kit (Molecular Probes, Eugene, OR) following the manufacturer's instructions. Briefly, 1 mg/ml AP-HuR-GST protein solution was prepared by adding 0.1 M sodium bicarbonate buffer to the lyophilized protein powder. One hundred μl of the protein solution was transferred to the vial of reactive dye, gently mixed, and incubated at room temperature for 1 h. The labeled protein was then purified through a spin column.

RNA interference experiment and protein transfection. The transfection of small interfering RNA (siRNA) into HL-60 cells was performed in six-well plates by using the Lipofectamine 2000 and Lipofectamine Plus reagents (Invitrogen, Burlington, ON, Canada) according to the manufacturer's instructions. The day before transfection, cells were split as necessary to achieve log growth by transfection time. On the day of transfection, the medium was removed and the cells were resuspended in fresh growth medium without antibiotics to a final density of 1×10^6 cells/ml. The siRNA duplexes used in the experiment are as follows. HuSi-1 (Dharmacon, Lafayette, CO) (5′-AAGCCUGUUCAGCAGCAUUGG-3′) targets to position 111 to 131 of nucleotides relative to the start codon of HuR mRNA, and HuSi-C (Dharmacon, Lafayette, CO) (5′-AAGCC AAUUCAUC AGCAAUGG-3′) is a mutant version of HuSi-1 (underlined nucleotides), which was used as a negative control. siRNA duplex (0.12 μM) was added to each well of cells seeded on six-well plates. Four h after transfection, PMA was added to the cells at a final concentration of 10 ng/ml. Twenty-four h later, the medium was changed with fresh growth medium containing PMA. Seventy-two h post-transfection, total cellular extracts were prepared. For protein transfection, recombinant AP-conjugated proteins were added to the HL-60 cells 4 h after siRNA treatment at concentrations of 30 or 50 nM, and then the HL-60 cells were induced to differentiation with PMA 6 h later. Twenty-four h after siRNA treatment, fresh medium containing PMA was added, and the transfected cells were kept in culture for another 48 h.

RESULTS

Effect of PMA and M-CSF treatment on the expression of SLC11A1 in human monocytes and HL-60 cells. To test the effect of PMA on the expression of SLC11A1, human monocytes were treated with the compound for 72 h and examined for their SLC11A1 protein levels. As shown in Fig. 1A and B, freshly isolated monocytes (Med, 0 h) did not express any detectable SLC11A1 protein. Even after 72 h of culturing (Med, 72 h) of monocytes, the SLC11A1 protein was barely detectable. However, when the monocytes were differentiated into macrophages by using PMA, the expression of SLC11A1 protein was dramatically increased. The increased expression of SLC11A1 protein was also observed when monocytes were differentiated by using macrophage colony-stimulating factor (M-CSF). Western blot analysis showed a band of 90- to 110-kDa electrophoretic mobility. As illustrated in Fig. 1C, we have also tested SLC11A1 protein expression in the HL-60 cell line with and without PMA treatment to assess whether this cell line can faithfully reproduce the modulation in SLC11A1 protein expression during the course of differentiation from promyelocytic cells to macrophages. As expected, differentiated HL-60 cells became adherent to plastic and spread in a manner characteristic of macrophages and they also lost their proliferation potential, as previously described by Harris and Ralph (29). Similarly, as we observed in monocytes, SLC11A1 protein expression was induced by the PMA treatment and was undetectable in protein extracts from untreated HL-60 cells. The SLC11A1 protein detected in the extracts from PMA-treated HL-60 cells showed an electrophoretic mobility the same as that observed in PMA-treated monocytes (Fig. 1A to C).

To determine whether the induction of SLC11A1 protein

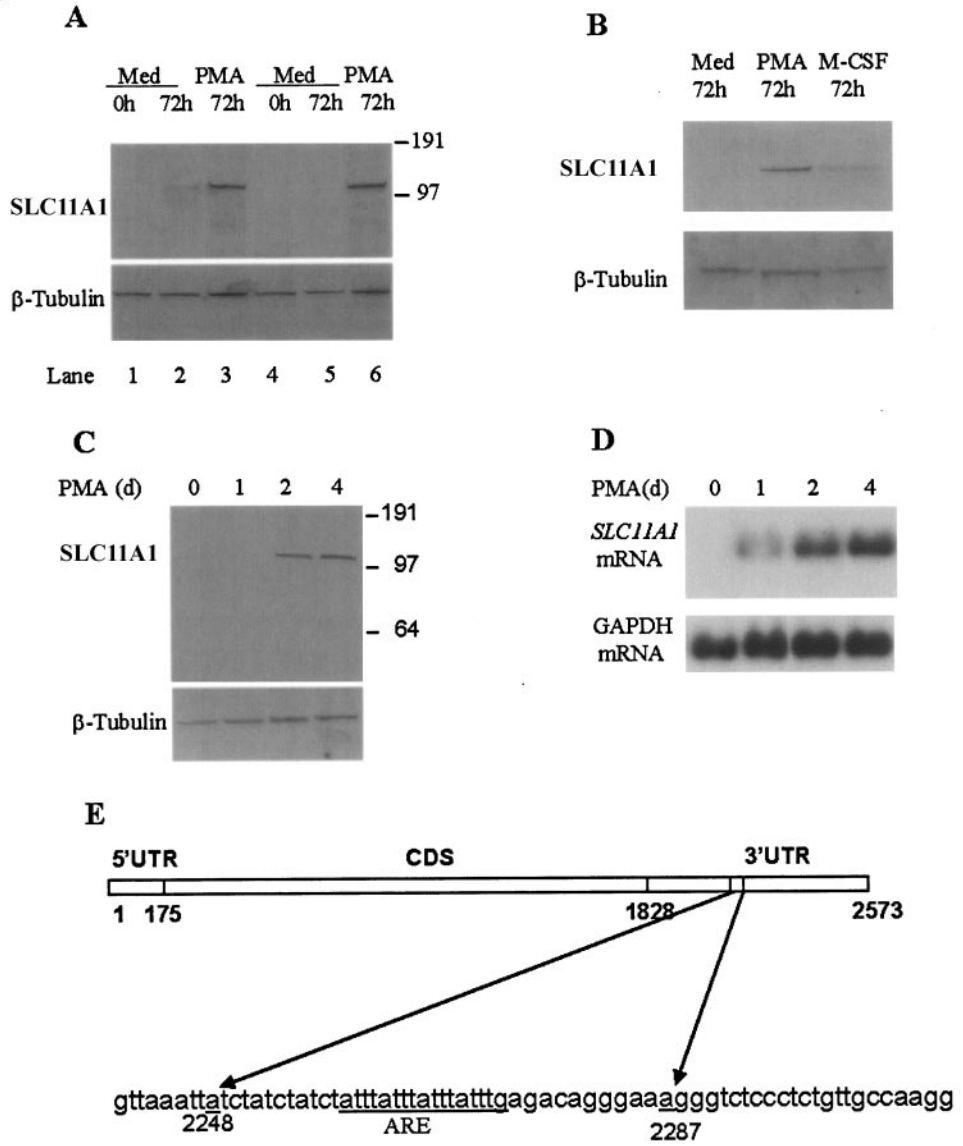

FIG. 1. PMA and M-CSF induce the expression of SLC11A1 in human monocytes and HL-60 cells. Human monocytes and HL-60 cells were either untreated (Med) or treated with PMA (10 ng/ml) or M-CSF (30 ng/ml). Total protein extracts were prepared by lysis of cells in radioimmunoprecipitation buffer (0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate, 1% NP-40 in 1× PBS). Total RNA was isolated by using TRIzol reagent. (A) SLC11A1 protein expression in human monocytes was assessed using Western blot analysis (20 μg of total protein extract/lane). The protein extracts were prepared from fresh monocytes (0 h; lanes 1 and 4), from monocytes cultured for 72 h (lanes 2 and 5), and from monocytes treated with PMA for 72 h (lanes 3 and 6). The protein extracts isolated from untreated and PMA-treated monocytes purified from the blood of volunteer 1 are shown in lanes 1 to 3, and those from volunteer 2 are shown in lanes 4 to 6. (B) Monocytes isolated from volunteer 3 were untreated or else treated with PMA or M-CSF for 72 h. The effect of PMA and M-CSF on SLC11A1 protein expression was assessed (20 μg of total protein/lane). (C) Twenty μg of each protein sample was subjected to Western blot analysis to assess SLC11A1 protein expression in HL-60 cells. (D) Thirty μg of total RNA from HL-60 cells was separated by electrophoresis in denaturing formaldehyde gel and transferred to Hybond-N^+ membrane. Blots were probed with cDNA probes corresponding to *SLC11A1* and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA. Days of PMA treatment [PMA (d)] are indicated at the tops of panels C and D. (E) Schematic representations of *SLC11A1* cDNA and probes used to study mRNA stability. 3′UTR and ARE probes spanning nucleotides 1829 to 2540 and 2248 to 2287 of human *SLC11A1* mRNA, respectively, are shown.

expression reflects an increase in the mRNA level, total RNA from HL-60 cells treated and untreated with PMA was detected by Northern blotting. As shown in Fig. 1D, *SLC11A1* mRNA levels were increased upon treatment with PMA. Overall, these results indicated that PMA-induced differentiation of promyelocytic cells towards macrophage lineage leads to an induction of *SLC11A1* mRNA and subsequently to the induction of SLC11A1 protein expression.

PMA treatment, which induces SLC11A1 expression in HL-60 cells, increases HuR's level in the cytoplasm. The above results, as well as the fact that the *SLC11A1* message presents a typical ARE in its 3′UTR (Fig. 1E), suggest that the PMA-

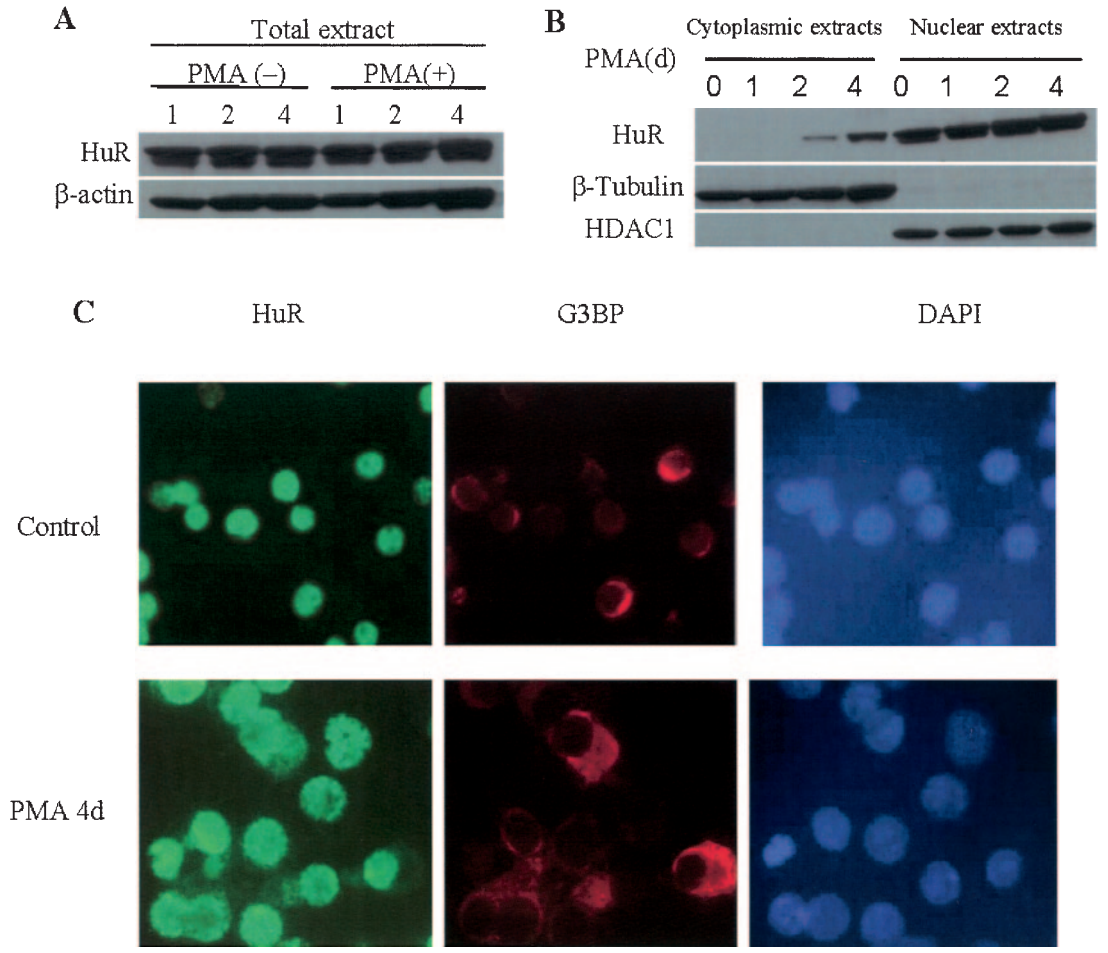

FIG. 2. Effect of PMA treatment on the subcellular localization of HuR. (A) HuR protein levels in total lysate (20 μg) prepared from untreated HL-60 cells (−) and HL-60 cells treated with PMA (10 ng/ml) (+) for 1, 2, and 4 days [PMA(d)] were monitored with Western blot analysis. (B) HuR protein levels in cytoplasm fractions (40 μg) and nuclear fractions (10 μg) prepared from both untreated HL-60 cells and HL-60 cells treated with PMA (10 ng/ml) were monitored using Western blot analysis. Expressions of the cytoplasmic marker β-tubulin and the nuclear marker HDAC1 were also monitored. (C) Immunofluorescence detection of HuR in HL-60 cells either untreated (control) or treated with PMA for 4 days. Left, HuR immunofluorescence; middle, G3BP immunofluorescence used to indicate cytoplasmic localization; right, DAPI staining used to localize nuclei. Pictures are representative of three separate experiments. Magnification, ×63.

induced expression of *SLC11A1* mRNA in HL-60 cells could be due, in part, to the increase in its stability. Furthermore, a repeat of AUUUA sequence was shown to mediate the association of ARE-containing messages with the stabilizing factor HuR (9). Based on this information, we performed experiments to determine whether HuR, through the $(AUUUA)_4$ elements, regulates the posttranscriptional events of the *SLC11A1* gene, leading to its rapid expression during the differentiation of HL-60 cells. Since HuR's cytoplasmic localization is associated with the nuclear export and stabilizing of its mRNA targets (9), it was of particular interest to first investigate the subcellular localization of HuR under conditions in which *SLC11A1* is induced. Western blot analysis using whole-cell lysates revealed no obvious increase in total HuR expression during PMA treatment of HL-60 cells (Fig. 2A), so we hypothesized that PMA could affect the cellular localization of HuR. Indeed, when subcellular fractions from untreated HL-60 cells were analyzed, no HuR protein was detected in the cytoplasm. Following treatment of HL-60 cells with PMA for 48 h, HuR could readily be detected in the cytoplasm. (Fig. 2B). Western blotting of the same membranes to detect nucleus- and cytoplasm-specific markers (histone deacetylase 1 and β-tubulin, respectively) verified that the nuclear protein did not leak into the cytoplasmic fractions during cell treatment or fractionation and further monitored the equal loading and transfer of the samples (Fig. 2B). These results demonstrated that the observed cytoplasmic accumulation of HuR in response to PMA treatment was not due to an overall increase of HuR expression but likely resulted from an elevated export of nuclear HuR, as previously reported (22, 32, 39). These observations were further confirmed by immunofluorescence experiments. In untreated cells, we observed that HuR is exclusively nuclear (Fig. 2C, control panel); however in PMA-treated HL-60 cells, HuR was also detectable in the cytoplasm (Fig. 2C, PMA-treated panel). These data confirmed the biochemical fractionation data (Fig. 2B) and strongly suggest the possibility that HuR escorts the *SLC11A1* mRNA from the nucleus to the cytoplasm, protecting it from the decay machinery.

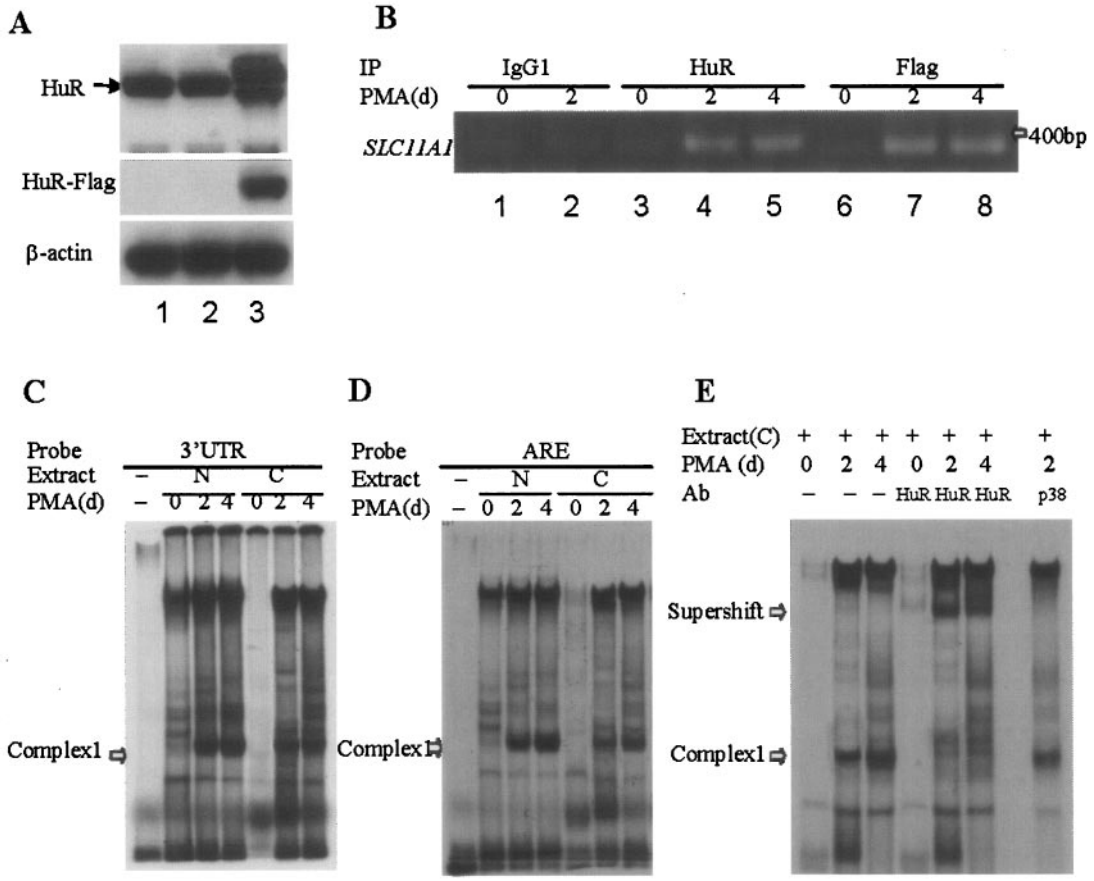

FIG. 3. HuR specifically binds to the *SLC11A1* 3′UTR ARE, and this binding increases after PMA treatment. (A) Western blot analysis of endogenous and Flag-tagged HuR expression. Total-cell extracts from wild-type HL-60 cells (lane 1), cells stably transfected with the empty expression plasmid pcDNA3.0-Flag (lane 2), and HuR expression plasmid pcDNA3.0-HuR-Flag (lane 3) were prepared and resolved using 4 to 12% Bis-Tris gel and immunoblotted using anti-HuR (top) or anti-Flag (middle) antibody. (B) Total-cell extracts were prepared from wild-type HL-60 cells (lanes 1 to 5) and stably transfected HL-60 cells (lanes 6 to 8) either untreated or treated with PMA (10 ng/ml) for 2 and 4 days [PMA(d)]. Protein-RNA complexes were immunoprecipitated using anti-HuR antibody, anti-Flag antibody, or IgG1. *SLC11A1* mRNAs present in the complexes were isolated and detected by using RT-PCR. PCR products were analyzed using 1.5% agarose gels and stained with ethidium bromide. (C) HL-60 cells were treated or not with 10 ng/ml PMA for 2 and 4 days. Cytoplasmic (C) and nuclear (N) extracts were prepared and incubated with α-^{32}P-labeled RNA probes containing 712-bp *SLC11A1* 3′UTR. Reaction mixtures were then resolved by electrophoresis on native polyacrylamide gel. (D) Cytoplasmic and nuclear extracts from HL-60 cells treated or untreated with 10 ng/ml PMA were incubated with α-^{32}P-labeled RNA probes containing 40-bp ARE in the *SLC11A1* 3′UTR. Reaction mixtures were then resolved by electrophoresis on a native polyacrylamide gel. (E) Cytoplasmic extracts [Extract(C)] from HL-60 cells treated or untreated with 10 ng/ml PMA were incubated with α-^{32}P-labeled RNA probes for 40-bp *SLC11A1* ARE. Complexes containing HuR were shifted using the monoclonal anti-HuR antibody 19F2 and anti-p38 kinase antibody. Ab, antibody.

HuR specifically binds to the *SLC11A1* 3′UTR-ARE following treatment with PMA. The stabilization effect of HuR on its mRNA targets was always associated with direct binding through specific *cis*-acting elements located in the 3′UTR. We already found a potential ARE in the 3′UTR of *SLC11A1*, which prompt us to verify the existence of *SLC11A1* mRNA-HuR complex in HL-60 cells before and after PMA treatment. Indeed, evidence for the in vivo association of endogenous *SLC11A1* mRNA and HuR in the cells was obtained through immunoprecipitation of endogenous or Flag-tagged HuR under conditions that preserved its association with target mRNAs by using a previously described method (54). For the analysis of Flag-tagged HuR binding to *SLC11A1* mRNA in vivo, plasmid pcDNA3.0-HuR-Flag was transfected into HL-60 cells, and stable transfectants of HL-60 cells that constitutively overexpress HuR were selected. As a control, HL-60 cells stably transfected with the empty expression plasmid pcDNA3.0-Flag were also generated. The relative amounts of endogenous HuR and Flag-tagged HuR were analyzed by Western blotting using anti-HuR and anti-Flag antibody, respectively (Fig. 3A). The level of HuR in the transfected cells increased by ~3.5-fold over that in nontransfected cells (Fig. 3A). The blot was stripped and probed with an anti-Flag monoclonal antibody, yielding a band at 37 kDa only in extracts from the cells transfected with pcDNA3.0-HuR-Flag (Fig. 3A). Total extracts prepared from untreated and PMA-treated HL-60 cells and cells stably transfected with the plasmid pcDNA3.0-HuR-Flag to overexpress HuR (Fig. 3A) were incubated with IgG1, anti-HuR antibody, and anti-Flag antibody, respectively. The precipitated RNAs were isolated and analyzed using primers spe-

cific for *SLC11A1* mRNAs (Fig. 3B). RT-PCR analysis revealed the absence of detectable *SLC11A1* mRNA in complexes immunoprecipitated with all three antibodies when the extracts of untreated HL-60 cells were used (Fig. 3B). However, after PMA treatment, endogenous *SLC11A1* mRNA was present when cells were immunoprecipitated using anti-HuR or anti-Flag antibody but not when a nonspecific isotype-matched antibody (IgG1) was used (Fig. 3B).

The *SLC11A1* gene was induced to express at both the mRNA and protein levels in the HL-60 cells treated with PMA. Under the same stimulations, we also observed that primarily nuclear HuR accumulates in the cytoplasm and HuR binds to endogenous *SLC11A1* mRNA. This data implies that the increase of *SLC11A1* mRNA is causally related to an increase in cytoplasmic HuR levels. To confirm this, RNA-binding assays were first performed on nuclear and cytoplasmic extracts from untreated and PMA-treated HL-60 cells by using the following two α-^{32}P-labeled RNA probes: the full-length *SLC11A1* 3′UTR probe (720 nucleotides in length, spanning nucleotides 1829 to 2540) and the *SLC11A1*-ARE probe, which contains an AU-rich element (40 nucleotides in length, comprising positions 2248 to 2287) (Fig. 1E). As shown in Fig. 3C and D, multiple protein-RNA complexes were formed when the nuclear or cytoplasmic extracts were incubated with either of the two labeled probes. The full-length *SLC11A1*-3′UTR and the *SLC11A1*-ARE probes display almost identical patterns of complex formation with one of the complexes (complex 1), which is dramatically increased following PMA treatment. To determine whether HuR is involved in complex 1, gel supershift assays were also performed using an antibody specific to HuR to supershift the protein-RNA complexes formed between the α-^{32}P-labeled ARE probe and the proteins present in the cytoplasmic extracts. As shown in Fig. 3E, the inclusion of antibodies specific to HuR resulted in a depletion of complex 1 and a strongly supershifted band, demonstrating that HuR is one of the components of this complex and that the PMA-induced redistribution of HuR from nuclei to cytoplasm is associated with the increased *SLC11A1* mRNA-binding activity. No supershifting was observed when the antibody specific to p38 kinase (Fig. 3E) or the anti-FXR antibody was used in the experiment (data not shown). Similar results were generated using nuclear extracts (data not shown).

HuR knockdown inhibits *SLC11A1* expression, which can be recovered by recombinant HuR transfection. To test whether HuR was essential for the induction of *SLC11A1* gene expression, we knocked down HuR by using a small RNA interference experiment. As shown in Fig. 4A, the transfection of specific HuR siRNAs into HL-60 cells led to a substantial down-regulation of HuR protein levels (almost 32% ± 4% of the amount of control), with no effect on actin expression. Transfection using a control HuR siRNA had no effect on either HuR or actin levels (Fig. 4A). Interestingly, *SLC11A1* gene expression was significantly inhibited in HuR knockdown cells in terms of both protein and mRNA levels (Fig. 4A and B, respectively). Statistical analysis of the results using the Mann-Whitney rank sum test gave a P value of 0.008. These results documented a causal relationship between the level of HuR protein and that of *SLC11A1* gene expression.

To further confirm that the observed down-regulation of *SLC11A1* gene expression was due specifically to the HuR knockdown, AP-HuR-GST and GST-AP (negative control) recombinant proteins were prepared (Fig. 4C) and transfected into HuR siRNA-treated HL-60 cells. These AP-conjugated proteins were added to the HL-60 cells 4 h after siRNA treatment. As shown in Fig. 4D and E, the AP-HuR-GST protein was effectively taken up by the cells. Six h after the addition of recombinant proteins, the HL-60 cells were treated with PMA and allowed to differentiate. After 72 h of the HuR siRNA treatment, we observed that addition of AP-HuR-GST, but not of GST-AP, was required to induce a normal level of *SLC11A1* expression in HuR siRNA-treated cells (Fig. 4E). The expression of β-actin protein was not affected by the addition of either of the recombinant proteins (Fig. 4E).

HuR overexpression stabilizes PMA-induced *SLC11A1* mRNA. The studies described above demonstrate that HuR is critical for PMA-induced *SLC11A1* expression; however, they do not explain the molecular mechanism responsible for the HuR-mediated augmentation of *SLC11A1* gene expression. In order to determine the role of HuR in *SLC11A1* gene expression, we performed actinomycin D chase experiments using HL-60 cells transfected with the plasmid pcDNA3-HuR-Flag or a control plasmid, pcDNA3.0-Flag (Fig. 5A). We determined that after PMA treatment for 48 h, the half-lives of *SLC11A1* mRNA were similar in both pcDNA3.0-Flag-transfected cells (mean, 4.7 ± 0.3 h) and wild-type cells (mean, 4.1 ± 0.4 h). However, the half-life of *SLC11A1* mRNA in pcDNA3-HuR-Flag-transfected cells was much longer (mean, 8.8 ± 0.4 h) than those observed in cells transfected with control vector or in wild-type cells (Fig. 5A and B). When using the t test, there is a significant difference in the mRNA half-lives for wild-type and HuR-overexpressing cell lines ($P < 0.001$). These results clearly demonstrated that HuR stabilizes mRNA, and therefore it plays an important role in the regulation of *SLC11A1* gene expression.

DISCUSSION

The *SLC11A1* gene plays an important role in macrophage activation and is linked to susceptibility to some infectious and autoimmune diseases (7, 19). Human SLC11A1 protein is expressed in monocytes, macrophages, and polymorphonuclear neutrophils. Here we found that following the treatment of human monocytes with PMA for 72 h, the expression of SLC11A1 protein was dramatically increased in monocytes derived from tested healthy medication-free volunteers. SLC11A1 protein was detectable, albeit at a very low level, in the monocytes cultured for 72 h in the absence of PMA from one of the two volunteers but was undetectable in monocytes isolated from the other volunteer. This difference in the basal SLC11A1 protein expression levels of the two tested volunteers might reflect individual variability. HL-60 cells were shown to be a useful model in the study of the regulation of *SLC11A1* gene expression during experimentally induced granulocytic and monocytic differentiation. Previous work (11, 45) and our present study have shown that both *SLC11A1* mRNA and protein are undetectable in untreated HL-60 cells. However, when the cells are induced with PMA to become macrophage-like, both steady-state mRNA and protein for *SLC11A1* are expressed (Fig. 1B and C). So far, the molecular mechanisms involved in regulating *SLC11A1* gene expression are not

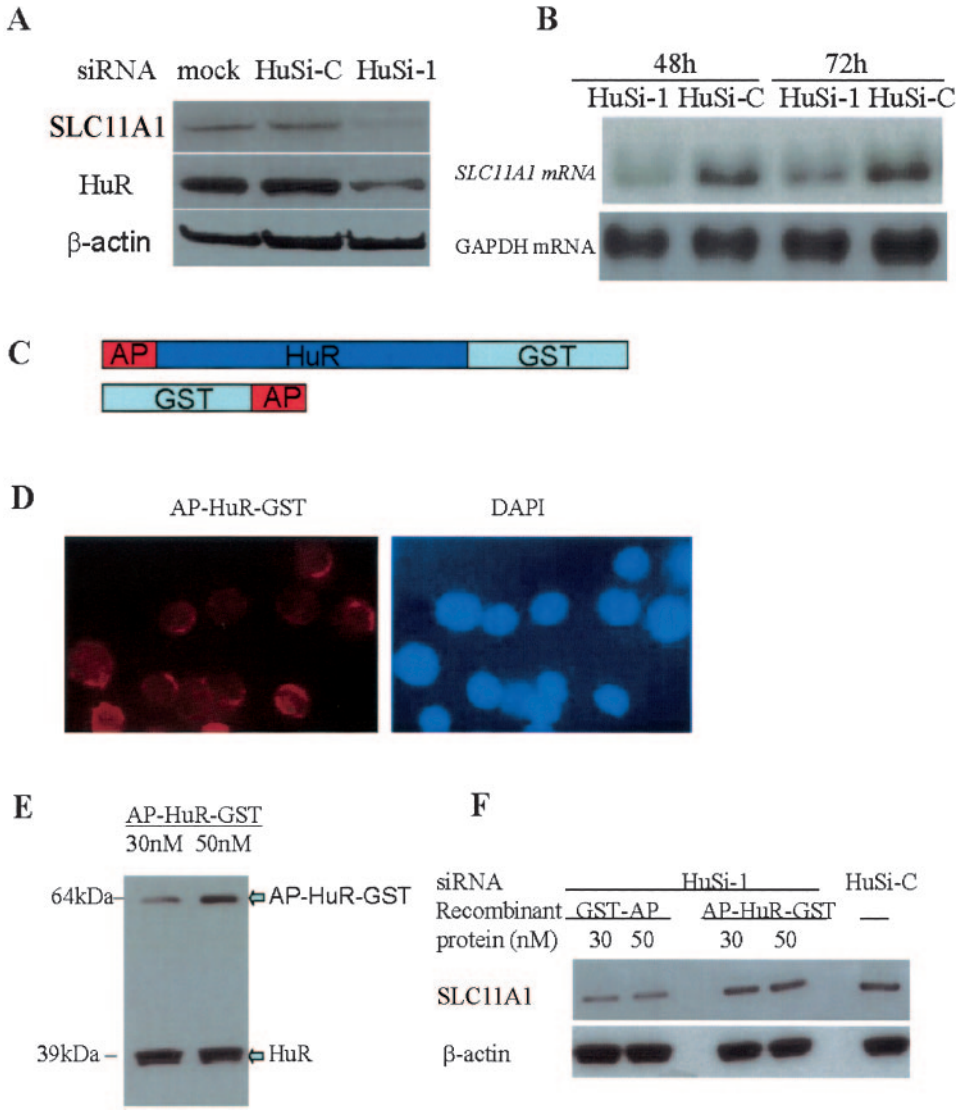

FIG. 4. SLC11A1 expression is inhibited and can be restored in HuR knockdown cells. (A) HL-60 cells were transiently transfected with siRNA targets HuR (HuSi-1), control siRNA (HuSi-C, a mutant of HuSi-1), or mock (transfection reagents only). Four h after transfection, HL-60 cells were treated with PMA (10 ng/ml) for 68 h. Protein extracts of mock-transfected HL-60 cells, as well as those of HuSi-C- and HuSi-1-transfected HL-60 cells, were prepared and analyzed by Western blotting with antibodies specific to SLC11A1, HuR, and β-actin. (B) HL-60 cells were transfected with siRNA and then treated with PMA as described above. Forty-eight and 72 h after PMA treatment, total RNA from HL-60 cells transfected with HuSi-1, HuSi-C, or mock were isolated, and Northern blot analysis was performed to determine *SLC11A1* mRNA levels. (C) Schematic representations of fusion proteins AP-HuR-GST and GST-HuR (a control) used to rescue HuR siRNA-treated HL-60 cells. (D) The Alexa Fluor 594-labeled AP-HuR-GST was added to HL-60 cells 4 h after transfection with HuSi-1. Six h after the addition of labeled AP-HuR-GST, the cells were washed, fixed (4% paraformaldehyde; 15 min), and visualized with a Zeiss Axiovision 3.1 microscope. (E) HL-60 cells were transfected with HuSi-1. The recombinant protein AP-HuR-GST was added to cells 4 h posttransfection, and total protein extracts were prepared 6 h after the addition of AP-HuR-GST recombinant protein. Western blot analysis was performed to detect the absorbance of AP-HuR-GST by HL-60 cells. (F) HL-60 cells were transfected with HuSi-1 or HuSi-C, and 4 h after transfection, recombinant proteins GST-AP and AP-HuR-GST were added to the cells. Six h later, cells were treated with PMA (10 ng/ml). Seventy-two h after HuR siRNA treatment, total protein extracts were prepared. Western blot analysis was performed using antibodies specific to SLC11A1 and β-actin.

well characterized. The present study provides evidence that during PMA-induced differentiation of HL-60 cells towards the monocytic/macrophage pathway, the RNA-binding protein HuR shuttles from nucleus to cytoplasm, binds to the ARE within the 3′UTR of human *SLC11A1* mRNA, and plays an important role in regulating its stability. To our knowledge, this is the first report demonstrating that HuR is essential for the regulation of *SLC11A1* mRNA stability and the induction of *SLC11A1* gene expression in human macrophages.

So far, little is known about the mechanism by which HuR shuttles between the nucleus and cytoplasm after induction by stimuli such as heat shock (23), UV irradiation (59), and amino acid starvation (65). Although protein kinase C, as well as several protein kinases involved in the mitogen-activated pro-

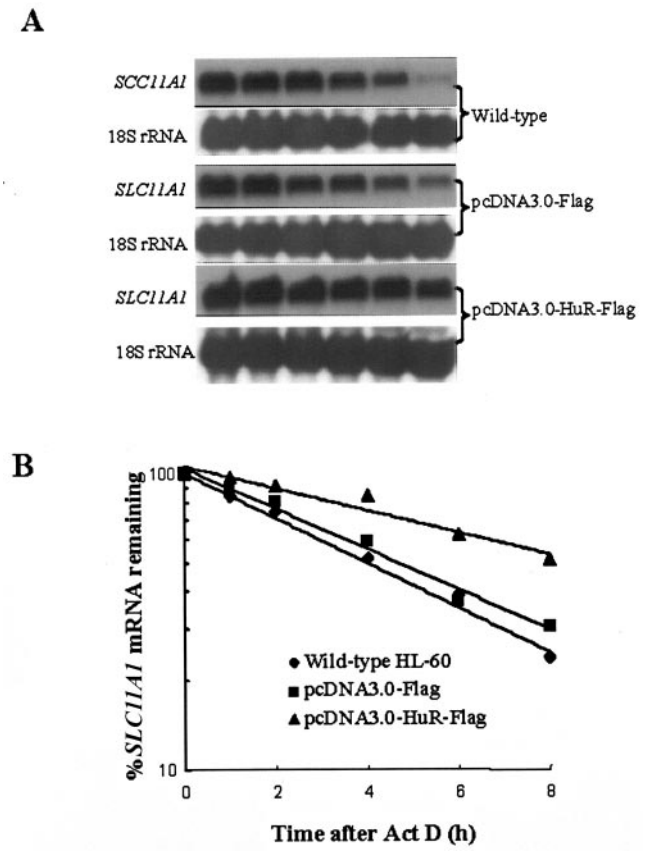

FIG. 5. Overexpression of HuR stabilizes PMA-induced *SLC11A1* mRNA. (A) HL-60 cells stably transfected with either plasmid pcDNA3.0-HuR-Flag or pcDAN3.0-Flag, as well as wild-type HL-60 cells, were treated with PMA for 3 days, a procedure followed by the addition of actinomycin D (Act D) (2 μg/ml), and RNA was isolated 0, 1, 2, 4, 6, and 8 h later. Northern blotting was performed by using *SLC11A1* and 18S rRNA cDNA probes. (B) *SLC11A1* mRNA decay curves derived from a densitometry analysis of the Northern blot hybridization. The half-life of *SLC11A1* mRNA in pcDNA3-HuR-Flag-transfected cells was much longer (mean, 8.8 ± 0.4 h) than those observed in pcDNA3.0-Flag-transfected cells (mean, 4.7 ± 0.3 h) and in wild-type cells (mean, 4.1 ± 0.4 h). There was a significant difference between the half-life of *SLC11A1* mRNA in the wild type and that in HuR-overexpressing cell lines (*t* test; $P < 0.001$).

tein kinase pathway, have been implicated in regulating mRNA turnover (13, 41, 42, 60, 61, 63), a previous study showed that none of them affects the subcellular localization of HuR, and the cytoplasmic HuR level is regulated only by the activity of AMP-activated kinase, an enzyme involved in response to metabolic stresses (58). In this study, we report that the levels of cytoplasmic HuR are potently influenced by the treatment of cells with PMA. Although HL-60 cells express substantial levels of the HuR protein, we did not detect any HuR in the cytoplasm of untreated cells. Differentiation of HL-60 cells for at least 2 days with PMA resulted in the appearance of HuR in the cytoplasm, and its level in the cytoplasm increased over time. A number of cell signaling pathways can be activated by PMA treatment, but no report has shown whether PMA treatment has an effect on AMP-activated kinase activity. Therefore, the mechanisms whereby PMA regulates the translocation of HuR from nuclei to cytoplasm remain to be elucidated.

Here we found, both in vitro and in vivo, that HuR shuttles from the nucleus to the cytoplasm and binds to the 3′ARE-*SLC11A1* mRNA during PMA treatment. There exists an association between the level of cytoplasmic HuR and the ability of cytoplasmic extracts to form the HuR-containing RNA-protein complex 1. Therefore, the increased cytoplasmic HuR during PMA treatment most likely contributes to the increased binding between HuR and *SLC11A1* mRNA. We propose that PMA-induced *SLC11A1* expression may be partly due to increased *SLC11A1* stability mediated by the binding of HuR to the ARE. However, increased *SLC11A1*-mRNA stability may not be exclusively dependent on cytoplasmic HuR levels. The basal HuR abundance in the nuclear extracts from untreated cells is almost the same as that in PMA-treated cells, but the level of complex 1 formed between the 3′UTR/ARE and nuclear extracts from untreated cells is much lower than that from PMA-treated cells. It is possible that PMA treatment could cause covalent modification of HuR (methylation or phosphorylation of HuR) or changes in the levels of other proteins that bind to the *SLC11A1* mRNA along with HuR and affect the formation of HuR-containing complex 1. Previously published studies have shown that lipopolysaccharide stimulation increased the methylation of HuR by coactivator-associated arginine methyltransferase as well as the formation of HuR-containing complexes on tumor necrosis factor α mRNA in macrophages (37, 39), but it is unclear whether there is a correlation between the methylation of HuR and its ability to stabilize labile mRNA.

It is well established that HuR binds to ARE-containing mRNAs and stabilizes them (9), and here we have revealed an interaction between HuR and *SLC11A1* mRNA; hence, we hypothesized that the knockdown of HuR in HL-60 cells could result in the inhibition of PMA-induced *SLC11A1* gene expression. Our data confirmed that the disruption of endogenous HuR levels by using small RNA interference led to the concomitant decrease of PMA-induced *SLC11A1* expression in terms of both protein and mRNA levels. The causal relationship between the HuR protein and *SLC11A1* gene expression was further supported by the restoration of *SLC11A1* gene expression by using recombinant HuR protein in HuR siRNA-treated HL-60 cells. Overall, our data imply that an appropriate level of HuR expression is required for the stabilization of *SLC11A1* mRNA and the induction of SLC11A1 expression.

To further explore the biological consequences of HuR binding to the *SLC11A1*-ARE, we analyzed the effect of HuR overexpression on *SLC11A1* mRNA stability. Actinomycin D chase experiments performed on stably transfected HL-60 cells overexpressing HuR provide us with interesting observations. In untreated HuR-overexpressing HL-60 cells, no *SLC11A1* mRNA could be detected by Northern blot analysis (data not shown), as observed similarly in untreated wild-type HL-60 cells. Therefore, HuR on its own is unable to activate the human *SLC11A1* promoter unless HL-60 cells are differentiated with PMA. HuR overexpression did increase *SLC11A1* mRNA stability, suggesting that HuR participates in the PMA-induced *SLC11A1* expression and that this mechanism takes place at the posttranscriptional level. Several studies have shown that overexpression of HuR is associated with increased

cyclooxygenase 2 expression in tumor tissues such as those from colon cancers, ovarian carcinomas, and breast carcinomas (15, 16, 18). Therefore, the overexpression of HuR may be part of a regulatory pathway to control the mRNA stability of several important target genes.

Since HuR is involved in the stabilization of a number of short-lived mRNAs containing ARE, PMA-induced changes in HuR may also contribute to the increased expression of other genes during the differentiation of monocytes. Immunoprecipitation of mRNP complex with HuR antibody combined with RT-PCR analysis revealed that HuR binds to the TNF-α mRNA but not to phospholipid scramblase 1 (RLSCR1) mRNA during the differentiation of HL-60 cells (data not shown). Both TNF-α and RLSCR1 gene expression were shown to increase during monocyte differentiation (66). Since TNF-α mRNA contains ARE, whereas RLSCR1 mRNA is devoid of ARE, these findings are suggestive of the importance of ARE as a target sequence for HuR protein binding.

It seems clear that *SLC11A1* polymorphisms are associated with susceptibility to infectious and autoimmune diseases in human (7, 40, 53). In the promoter region of *SLC11A1*, seven different alleles (alleles 1 to 7) of the polymorphism containing a functional Z-DNA-forming repeat have been reported for different populations. Interestingly, a study showed that the polymorphic repeat acts as a functional polymorphism, influencing *SLC11A1* gene expression. The high-promoter-activity-level sequence, allele 3, shows an association with susceptibility to autoimmune diseases, whereas the low-promoter-activity-level sequence, allele 2, is associated with susceptibility to infectious diseases (49). Therefore, host susceptibility to a disease may be linked to differential protein expression resulting from a genetic variant. In the present study, our results demonstrate that the ARE present in the 3′UTR of *SLC11A1* mRNA has an important biological function in posttranscriptional regulation and indicate that HuR, which binds to the ARE, is essential for PMA-induced SLC11A1 expression. Therefore, further studies on the mechanism of HuR regulation will contribute to a better understanding of the regulation of *SLC11A1* gene expression and disease susceptibility and may help us to develop new ways for treating infectious and autoimmune diseases.

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REFERENCES

1. **Adams, D. J., D. J. Beveridge, L. van der Wyden, H. Mangs, P. J. Leedman, and B. J. Morris.** 2003. HADHB, HuR, and CP1 bind to the distal 3′-untranslated region of human renin mRNA and differentially modulate renin expression. J. Biol. Chem. **278:**44894–44903.
2. **Atasoy, U., S. L. Curry, I. Lopez de Silanes, A. B. Shyu, V. Casolaro, M. Gorospe, and C. Stellato.** 2003. Regulation of eotaxin gene expression by TNF-alpha and IL-4 through mRNA stabilization: involvement of the RNA-binding protein HuR. J. Immunol. **171:**4369–4378.
3. **Atasoy, U., J. Watson, D. Patel, and J. D. Keene.** 1998. ELAV protein HuA (HuR) can redistribute between nucleus and cytoplasm and is upregulated during serum stimulation and T cell activation. J. Cell Sci. **111:**3145–3156.
4. **Atkinson, P. G., and C. H. Barton.** 1998. Ectopic expression of Nramp1 in COS-1 cells modulates iron accumulation. FEBS Lett. **425:**239–242.
5. **Bakheet, T., M. Frevel, B. R. Williams, W. Greer, and K. S. Khabar.** 2001. ARED: human AU-rich element-containing mRNA database reveals an unexpectedly diverse functional repertoire of encoded proteins. Nucleic Acids Res. **29:**246–254.
6. **Blackwell, J. M., C. H. Barton, J. K. White, T. I. Roach, M. A. Shaw, S. H. Whitehead, B. A. Mock, S. Searle, H. Williams, and A. M. Baker.** 1994. Genetic regulation of leishmanial and mycobacterial infections: the Lsh/Ity/Bcg gene story continues. Immunol. Lett. **43:**99–107.
7. **Blackwell, J. M., T. Goswami, C. A. Evans, D. Sibthorpe, N. Papo, J. K. White, S. Searle, E. N. Miller, C. S. Peacock, H. Mohammed, and M. Ibrahim.** 2001. SLC11A1 (formerly NRAMP1) and disease resistance. Cell. Microbiol. **3:**773–784.
8. **Bradley, D. J.** 1977. Regulation of Leishmania populations within the host. II. Genetic control of acute susceptibility of mice to Leishmania donovani infection. Clin. Exp. Immunol. **30:**130–140.
9. **Brennan, C. M., and J. A. Steitz.** 2001. HuR and mRNA stability. Cell. Mol. Life Sci. **58:**266–277.
10. **Brown, D. H., W. LaFuse, and B. S. Zwilling.** 1995. Cytokine-mediated activation of macrophages from *Mycobacterium bovis* BCG-resistant and -susceptible mice: differential effects of corticosterone on antimycobacterial activity and expression of the *Bcg* gene (candidate *Nramp*). Infect. Immun. **63:**2983–2988.
11. **Cellier, M., C. Shustik, W. Dalton, E. Rich, J. Hu, D. Malo, E. Schurr, and P. Gros.** 1997. Expression of the human NRAMP1 gene in professional primary phagocytes: studies in blood cells and in HL-60 promyelocytic leukemia. J. Leukoc. Biol. **61:**96–105.
12. **Chen, C. Y., and A. B. Shyu.** 1995. AU-rich elements: characterization and importance in mRNA degradation. Trends Biochem. Sci. **20:**465–470.
13. **Chen, C. Y., F. Gatto-Konczak, Z. Wu, and M. Karin.** 1998. Stabilization of interleukin-2 mRNA by the c-Jun NH2-terminal kinase pathway. Science **280:**1945–1949.
14. **Dean, J. L., R. Wait, K. R. Mahtani, G. Sully, A. R. Clark, and J. Saklatvala.** 2001. The 3′ untranslated region of tumor necrosis factor alpha mRNA is a target of the mRNA-stabilizing factor HuR. Mol. Cell. Biol. **21:**721–730.
15. **Denkert, C., W. Weichert, K. J. Winzer, B. M. Muller, A. Noske, S. Niesporek, G. Kristiansen, H. Guski, M. Dietel, and S. Hauptmann.** 2004. Expression of the ELAV-like protein HuR is associated with higher tumor grade and increased cyclooxygenase-2 expression in human breast carcinoma. Clin. Cancer Res. **10:**5580–5586.
16. **Denkert, C., W. Weichert, S. Pest, I. Koch, D. Licht, M. Kobel, A. Reles, J. Sehouli, M. Dietel, and S. Hauptmann.** 2004. Overexpression of the embryonic-lethal abnormal vision-like protein HuR in ovarian carcinoma is a prognostic factor and is associated with increased cyclooxygenase 2 expression. Cancer Res. **64:**189–195.
17. **Di Marco, S., Z. Hel, C. Lachance, H. Furneaux, and D. Radzioch.** 2001. Polymorphism in the 3′-untranslated region of TNFalpha mRNA impairs binding of the post-transcriptional regulatory protein HuR to TNFalpha mRNA. Nucleic Acids Res. **29:**863–871.
18. **Dixon, D. A., N. D. Tolley, P. H. King, L. B. Nabors, T. M. McIntyre, G. A. Zimmerman, and S. M. Prescott.** 2001. Altered expression of the mRNA stability factor HuR promotes cyclooxygenase-2 expression in colon cancer cells. J. Clin. Investig. **108:**1657–1665.
19. **Donninger, H., T. J. Cashmore, T. Scriba, D. C. Petersen, V. R. Janse, and V. M. Hayes.** 2004. Functional analysis of novel SLC11A1 (NRAMP1) promoter variants in susceptibility to HIV-1. J. Med. Genet. **41:**e49.
20. **Estrada-Chavez, C., A. L. Pereira-Suarez, M. A. Meraz, C. Arriaga, A. Garcia-Carranca, C. Sanchez-Rodriguez, and R. Mancilla.** 2001. High-level expression of NRAMP1 in peripheral blood cells and tuberculous granulomas from *Mycobacterium bovis*-infected bovines. Infect. Immun. **69:**7165–7168.
21. **Fan, X. C., and J. A. Steitz.** 1998. Overexpression of HuR, a nuclear-cytoplasmic shuttling protein, increases the in vivo stability of ARE-containing mRNAs. EMBO J. **17:**3448–3460.
22. **Figueroa, A., A. Cuadrado, J. Fan, U. Atasoy, G. E. Muscat, P. Munoz-Canoves, M. Gorospe, and A. Munoz.** 2003. Role of HuR in skeletal myogenesis through coordinate regulation of muscle differentiation genes. Mol. Cell. Biol. **23:**4991–5004.
23. **Gallouzi, I. E., C. M. Brennan, M. G. Stenberg, M. S. Swanson, A. Eversole, N. Maizels, and J. A. Steitz.** 2000. HuR binding to cytoplasmic mRNA is perturbed by heat shock. Proc. Natl. Acad. Sci. USA **97:**3073–3078.
24. **Gallouzi, I. E., and J. A. Steitz.** 2001. Delineation of mRNA export pathways by the use of cell-permeable peptides. Science **294:**1895–1901.
25. **Garnon, J., C. Lachance, M. S. Di, Z. Hel, D. Marion, M. C. Ruiz, M. M. Newkirk, E. W. Khandjian, and D. Radzioch.** 2005. Fragile X-related protein FXR1P regulates proinflammatory cytokine tumor necrosis factor expression at the post-transcriptional level. J. Biol. Chem. **280:**5750–5763.
26. **Good, P. J.** 1995. A conserved family of elav-like genes in vertebrates. Proc. Natl. Acad. Sci. USA **92:**4557–4561.
27. **Govoni, G., and P. Gros.** 1998. Macrophage NRAMP1 and its role in resistance to microbial infections. Inflamm. Res. **47:**277–284.

28. **Govoni, G., S. Gauthier, F. Billia, N. N. Iscove, and P. Gros.** 1997. Cell-specific and inducible Nramp1 gene expression in mouse macrophages in vitro and in vivo. J. Leukoc. Biol. **62:**277–286.
29. **Harris, P., and P. Ralph.** 1985. Human leukemic models of myelomonocytic development: a review of the HL-60 and U937 cell lines. J. Leukoc. Biol. **37:**407–422.
30. **Holtmann, H., R. Winzen, P. Holland, S. Eickemeier, E. Hoffmann, D. Wallach, N. L. Malinin, J. A. Cooper, K. Resch, and M. Kracht.** 1999. Induction of interleukin-8 synthesis integrates effects on transcription and mRNA degradation from at least three different cytokine- or stress-activated signal transduction pathways. Mol. Cell. Biol. **19:**6742–6753.
31. **Iwai, Y., K. Akahane, D. H. Pluznik, and R. B. Cohen.** 1993. Ca2+ ionophore A23187-dependent stabilization of granulocyte-macrophage colony-stimulating factor messenger RNA in murine thymoma EL-4 cells is mediated through two distinct regions in the 3′-untranslated region. J. Immunol. **150:** 4386–4394.
32. **Keene, J. D.** 1999. Why is Hu where? Shuttling of early-response-gene messenger RNA subsets. Proc. Natl. Acad. Sci. USA **96:**5–7.
33. **Lafuse, W. P., G. R. Alvarez, and B. S. Zwilling.** 2000. Regulation of Nramp1 mRNA stability by oxidants and protein kinase C in RAW264.7 macrophages expressing Nramp1(Gly169). Biochem. J. **351:**687–696.
34. **Lal, A., K. Mazan-Mamczarz, T. Kawai, X. Yang, J. L. Martindale, and M. Gorospe.** 2004. Concurrent versus individual binding of HuR and AUF1 to common labile target mRNAs. EMBO J. **23:**3092–3102.
35. **Lasa, M., K. R. Mahtani, A. Finch, G. Brewer, J. Saklatvala, and A. R. Clark.** 2000. Regulation of cyclooxygenase 2 mRNA stability by the mitogen-activated protein kinase p38 signaling cascade. Mol. Cell. Biol. **20:**4265–4274.
36. **Le, C., V, J. B. Cowland, J. Calafat, and N. Borregaard.** 1996. Targeting of proteins to granule subsets is determined by timing and not by sorting: the specific granule protein NGAL is localized to azurophil granules when expressed in HL-60 cells. Proc. Natl. Acad. Sci. USA **93:**6454–6457.
37. **Li, H., S. Park, B. Kilburn, M. A. Jelinek, A. Henschen-Edman, D. W. Aswad, M. R. Stallcup, and I. A. Laird-Offringa.** 2002. Lipopolysaccharide-induced methylation of HuR, an mRNA-stabilizing protein, by CARM1. Coactivator-associated arginine methyltransferase. J. Biol. Chem. **277:**44623–44630.
38. **Lindstein, T., C. H. June, J. A. Ledbetter, G. Stella, and C. B. Thompson.** 1989. Regulation of lymphokine messenger RNA stability by a surface-mediated T cell activation pathway. Science **244:**339–343.
39. **McMullen, M. R., E. Cocuzzi, M. Hatzoglou, and L. E. Nagy.** 2003. Chronic ethanol exposure increases the binding of HuR to the TNFalpha 3′-untranslated region in macrophages. J. Biol. Chem. **278:**38333–38341.
40. **Mohamed, H. S., M. E. Ibrahim, E. N. Miller, J. K. White, H. J. Cordell, J. M. Howson, C. S. Peacock, E. A. Khalil, A. M. El Hassan, and J. M. Blackwell.** 2004. SLC11A1 (formerly NRAMP1) and susceptibility to visceral leishmaniasis in The Sudan. Eur. J. Hum. Genet. **12:**66–74.
41. **Pages, G., E. Berra, J. Milanini, A. P. Levy, and J. Pouyssegur.** 2000. Stress-activated protein kinases (JNK and p38/HOG) are essential for vascular endothelial growth factor mRNA stability. J. Biol. Chem. **275:**26484–26491.
42. **Park, J. W., M. A. Jang, Y. H. Lee, A. Passaniti, and T. K. Kwon.** 2001. p53-independent elevation of p21 expression by PMA results from PKC-mediated mRNA stabilization. Biochem. Biophys. Res. Commun. **280:**244–248.
43. **Peng, S. S., C. Y. Chen, N. Xu, and A. B. Shyu.** 1998. RNA stabilization by the AU-rich element binding protein, HuR, an ELAV protein. EMBO J. **17:**3461–3470.
44. **Plant, J., and A. A. Glynn.** 1976. Genetics of resistance to infection with Salmonella typhimurium in mice. J. Infect. Dis. **133:**72–78.
45. **Roig, E. A., E. Richer, F. Canonne-Hergaux, P. Gros, and M. F. Cellier.** 2002. Regulation of NRAMP1 gene expression by 1alpha,25-dihydroxy-vitamin D(3) in HL-60 phagocytes. J. Leukoc. Biol. **71:**890–904.
46. **Ross, J.** 1995. mRNA stability in mammalian cells. Microbiol. Rev. **59:**423–450.
47. **Ross, J.** 1996. Control of messenger RNA stability in higher eukaryotes. Trends Genet. **12:**171–175.
48. **Rousseau, S., N. Morrice, M. Peggie, D. G. Campbell, M. Gaestel, and P. Cohen.** 2002. Inhibition of SAPK2a/p38 prevents hnRNP A0 phosphorylation by MAPKAP-K2 and its interaction with cytokine mRNAs. EMBO J. **21:**6505–6514.
49. **Searle, S., and J. M. Blackwell.** 1999. Evidence for a functional repeat polymorphism in the promoter of the human NRAMP1 gene that correlates with autoimmune versus infectious disease susceptibility. J. Med. Genet. **36:**295–299.
50. **Seko, Y., H. Azmi, R. Fariss, and J. A. Ragheb.** 2004. Selective cytoplasmic translocation of HuR and site-specific binding to the interleukin-2 mRNA are not sufficient for CD28-mediated stabilization of the mRNA. J. Biol. Chem. **279:**33359–33367.
51. **Shaw, G., and R. Kamen.** 1986. A conserved AU sequence from the 3′ untranslated region of GM-CSF mRNA mediates selective mRNA degradation. Cell **46:**659–667.
52. **Skamene, E., P. Gros, A. Forget, P. A. Kongshavn, C. St Charles, and B. A. Taylor.** 1982. Genetic regulation of resistance to intracellular pathogens. Nature **297:**506–509.
53. **Takahashi, K., J. Satoh, Y. Kojima, K. Negoro, M. Hirai, Y. Hinokio, Y. Kinouchi, S. Suzuki, N. Matsuura, T. Shimosegawa, and Y. Oka.** 2004. Promoter polymorphism of SLC11A1 (formerly NRAMP1) confers susceptibility to autoimmune type 1 diabetes mellitus in Japanese. Tissue Antigens **63:**231–236.
54. **Tenenbaum, S. A., P. J. Lager, C. C. Carson, and J. D. Keene.** 2002. Ribonomics: identifying mRNA subsets in mRNP complexes using antibodies to RNA-binding proteins and genomic arrays. Methods **26:**191–198.
55. **van der Giessen, K., S. Di Marco, E. Clair, and I. E. Gallouzi.** 2003. RNAi-mediated HuR depletion leads to the inhibition of muscle cell differentiation. J. Biol. Chem. **278:**47119–47128.
56. **Vidal, S. M., D. Malo, K. Vogan, E. Skamene, and P. Gros.** 1993. Natural resistance to infection with intracellular parasites: isolation of a candidate for Bcg. Cell **73:**469–485.
57. **Vidal, S. M., E. Pinner, P. Lepage, S. Gauthier, and P. Gros.** 1996. Natural resistance to intracellular infections: Nramp1 encodes a membrane phosphoglycoprotein absent in macrophages from susceptible (Nramp1 D169) mouse strains. J. Immunol. **157:**3559–3568.
58. **Wang, W., J. Fan, X. Yang, S. Fürer-Galban, I. Lopez de Silanes, C. von Kobbe, J. Guo, S. N. Georas, F. Foufelle, D. G. Hardie, D. Carling, and M. Gorospe.** 2002. AMP-activated kinase regulates cytoplasmic HuR. Mol. Cell. Biol. **22:**3425–3436.
59. **Wang, W., H. Furneaux, H. Cheng, M. C. Caldwell, D. Hutter, Y. Liu, N. Holbrook, and M. Gorospe.** 2000. HuR regulates p21 mRNA stabilization by UV light. Mol. Cell. Biol. **20:**760–769.
60. **Westmark, C. J., and J. S. Malter.** 2001. Up-regulation of nucleolin mRNA and protein in peripheral blood mononuclear cells by extracellular-regulated kinase. J. Biol. Chem. **276:**1119–1126.
61. **Westmark, C. J., and J. S. Malter.** 2001. Extracellular-regulated kinase controls beta-amyloid precursor protein mRNA decay. Brain Res. Mol. Brain Res. **90:**193–201.
62. **Wilusz, C. J., M. Wormington, and S. W. Peltz.** 2001. The cap-to-tail guide to mRNA turnover. Nat. Rev. Mol. Cell Biol. **2:**237–246.
63. **Winzen, R., M. Kracht, B. Ritter, A. Wilhelm, C. Y. Chen, A. B. Shyu, M. Muller, M. Gaestel, K. Resch, and H. Holtmann.** 1999. The p38 MAP kinase pathway signals for cytokine-induced mRNA stabilization via MAP kinase-activated protein kinase 2 and an AU-rich region-targeted mechanism. EMBO J. **18:**4969–4980.
64. **Wodnar-Filipowicz, A., and C. Moroni.** 1990. Regulation of interleukin 3 mRNA expression in mast cells occurs at the posttranscriptional level and is mediated by calcium ions. Proc. Natl. Acad. Sci. USA **87:**777–781.
65. **Yaman, I., J. Fernandez, B. Sarkar, R. J. Schneider, M. D. Snider, L. E. Nagy, and M. Hatzoglou.** 2002. Nutritional control of mRNA stability is mediated by a conserved AU-rich element that binds the cytoplasmic shuttling protein HuR. J. Biol. Chem. **277:**41539–41546.
66. **Zhao, K. W., X. Li, Q. Zhao, Y. Huang, D. Li, Z. G. Peng, W. Z. Shen, J. Zhao, Q. Zhou, Z. Chen, P. J. Sims, T. Wiedmer, and G. Q. Chen.** 2004. Protein kinase Cdelta mediates retinoic acid and phorbol myristate acetate-induced phospholipid scramblase 1 gene expression: its role in leukemic cell differentiation. Blood **104:**3731–3738.