Expression of CD83 Is Regulated by HuR via a Novel cis-Active Coding Region RNA Element*

Alexander T. Prechtel†1, Jan Chemnitz†1, Susann Schirmer‡, Christina Ehlers1, Ines Langbein-Detsch2, Jörg Stülke4, Marie-Christine Dabauvalle3, Ralph H. Kehlenbach**, and Joachim Hauber†2

From the †Heinrich Pette Institute for Experimental Virology and Immunology, D-20251 Hamburg, Germany, the ‡Institute of Microbiology, Biochemistry, and Genetics, University Erlangen-Nürnberg, D-91054 Erlangen, Germany, the §Institute for Microbiology and Genetics, University of Göttingen, D-37077 Göttingen, Germany, the ¶Department of Cell and Developmental Biology, Biocenter of the University of Würzburg, D-97074 Würzburg, Germany, and the **Center of Biochemistry and Molecular Cell Biology, University of Göttingen, D-37073 Göttingen, Germany

Dendritic cells are the most potent of the antigen-presenting cells and are characterized by surface expression of CD83. Here, we show that the coding region of CD83 mRNA contains a novel cis-acting structured RNA element that binds to HuR, a member of the ELAV family of AU-rich element RNA-binding proteins. Transient transfection of mammalian cells demonstrated that this CD83 mRNA-derived element acts as a post-transcriptional regulatory element in cells overexpressing HuR. Notably, binding of HuR to the CD83 post-transcriptional regulatory element did not affect mRNA stability. Using RNA interference, we show that HuR mediated efficient expression of CD83. In particular, HuR was required for cytoplasmic accumulation of CD83 transcripts. Likewise, inhibition of the CRM1 nuclear export pathway by leptomycin B or overexpression of a defective form of the nucleoporin Nup214/CAN diminished cytoplasmic CD83 mRNA levels. In summary, the data presented demonstrate that the HuR-CRM1 axis affects the nucleocytoplasmic translocation of CD83 mRNA under regular physiological conditions.

Dendritic cells (DCs) are the most potent antigen-presenting cells of the immune system and are specialized to sensitize helper and killer T cells during the induction of T cell-mediated immunity (1, 2). In fact, DCs are the only antigen-presenting cells that are able to stimulate naive CD4+ and CD8+ T cells and are therefore referred to as “nature’s adjuvant.”

The CD83 molecule is to date the best known marker for fully mature DCs because CD83 is predominantly expressed on the surface of dendritic lineage cells and cannot be detected on immature DC precursors (3–5). Although its exact function remains to be determined, the fact that CD83 expression is activated during DC maturation, together with co-stimulatory molecules such as CD80 and CD86, suggests a functionally important role for CD83 in DC-mediated T cell immunity (6, 7). This notion is also supported by the fact that inhibition of CD83 expression during DC maturation reduces the T cell stimulatory capacity of these DCs in allo-mixed leukocyte reactions (8). A study using the soluble extracellular domain of CD83 has provided the first direct evidence that this specific surface molecule is indeed functionally important for T cell activation; the soluble CD83 protein completely inhibited DC-mediated T cell stimulation in a concentration-dependent manner in vitro (9). These data were subsequently confirmed in an independent study by using CD83-Ig fusion protein (10). Thus, CD83 appears to play an important functional role in the regulation of DC-mediated T cell-specific immune responses. Therefore, the investigation of the regulation of CD83 expression may provide novel opportunities to modulate DC activity and subsequently DC-mediated immune responses.

Regulation of the decay rates of mRNA is considered an important mechanism by which cells control gene expression at the post-transcriptional level (reviewed in Refs. 11–13). The relative stability of cellular mRNAs can differ significantly, ranging from a few minutes to up to several hours (14, 15). Although transcripts encoding housekeeping proteins are highly stable, so-called early response gene (ERG) mRNAs subsets are extremely unstable. These short-lived ERG mRNAs encode functionally important proteins such as proto-oncoproteins, cytokines, and lymphokines. Their intrinsic instability depends on the presence of cis-active sequence elements termed AU-rich elements (AREs), which enhance deadenylation rates as well as mRNA degradation (16–18). AREs are commonly located in the 3′-untranslated region (UTR) and consist of stretches of adenylate and uridylate residues, which often contain variable copies of the typical AUUA pentamer motif (19–21). Studies from different laboratories have described various cellular proteins that bind selectively to AREs (summarized in Refs. 11, 22, and 23). For example, binding of heterogeneous nuclear ribonucleoprotein (RNP) D/AUF1 to AREs results in rapid degradation of the ARE-containing transcript (24–27). In contrast, binding of HuR to AREs has the opposite effect and significantly stabilizes labile mRNAs (16, 17, 28, 29). The HuR protein is a ubiquitously expressed member of a family of human RNA-binding proteins that is related to the Drosophile ELAV (embryonic lethal abnormal vision) protein (30–32). It therefore appears that the RNP composition on a given ARE (e.g. containing either heterogeneous nuclear RNP D/AUF1 or HuR) determines the fate of the respective mRNA, resulting either in rapid degradation or in stabilization and eventual translation.

Interestingly, both heterogeneous nuclear RNP D/AUF1 and HuR have been shown to migrate between the nucleus and cytoplasm in mammalian cells (28, 33, 34). The shuttling activity of HuR raised particular interest because it suggested that HuR binds to nascent mRNAs in the nucleus and may protect them from degradation by actively participating in their nucleocytoplasmic transport (reviewed in Refs. 23 and 35). Conceivably, this leads to elevated ERG mRNA levels in the cyto-

* This work was supported by Wilhelm Sander Stiftung Grant 2003.033.1. 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: Heinrich Pette Inst. for Experimental Virology and Immunology, Martinistrasse 22, D-20251 Hamburg, Germany. Tel: 49-40-4805-1241; Fax: 49-40-4805-1184; E-mail: joachim.hauber@hpi.uni-hamburg.de.

‡ The abbreviations used are: DCL, dendritic cells; ERG, early response gene; AREs, AU-rich elements; UTR, untranslated region; RNP, ribonucleoprotein; TNF-α, tumor necrosis factor-α; HIV-1, human immunodeficiency virus type 1; GST, glutathione S-transferase; siRNA, small interfering RNA; rt, nucleotides; SL, stem-loop; FACS, fluorescence-activated cell sorter; PMCA, phospholipid-12-myristate-13-acetate; RT, reverse transcription; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; PRE, post-transcriptional regulatory element.
plasm, which may indirectly promote their subsequent translation. Therefore, HuR appears to be an important regulator that is involved in the post-transcriptional processing of some cellular mRNAs.

In this work, we analyzed the potential effect of HuR on CD83 mRNA. We demonstrate that the coding region of CD83 mRNA contains a novel cis-active RNA element that binds to HuR and regulates the cytoplasmic accumulation of this specific message.

MATERIALS AND METHODS

Molecular Clones—Wild-type and mutant genes were cloned into various vectors using standard methods and synthetic double-strand oligonucleotides or PCR technology. For in vitro transcription, the full-length human CD83 mRNA coding region (3) and derivatives thereof were ligated between the HindIII and EcoRI sites of the vector pcDNA3 (Invitrogen). Likewise, the tumor necrosis factor-α (TNF-α) ARE (36) was inserted between the HindIII and Apal sites of the pcDNA3 vector. For in vitro transcription of polyadenylated RNAs, the full-length human CD83 and HuR (30) cDNAs were ligated between the HindIII and Aval sites and the HindIII and Xbal sites, respectively, of the vector pSP64-Poly(A) (Promega Corp.). The plasmid template pGEM-RRE, which is a derivative of the vector pGEM-3Zf(+) (Promega Corp.), was used for synthesis of the human immunodeficiency virus type 1 (HIV-1) Rev response element and has been described previously (37). A vector constitutively expressing green fluorescent protein was constructed by inserting an enhanced green fluorescent protein cDNA between the HindIII and BamHI sites of pcDNA3. The plasmid pGEX-HuR was a bacterial expression vector in which the human HuR cDNA is fused in-frame to the 3'-untranslated intronic sequences in the pGEX-5X-1 (Amersham Biosciences). The small interfering RNA (siRNA)-expressing vector psiHuR was constructed by inserting a synthetic double-strand oligonucleotide between the BglII and HindIII sites of the polymerase III gene promoter-containing vector pSUPER (38), targeting nucleotides 166–184 (5'-GGCTTTCTGGGCAAGAGC-3') in the human HuR cDNA. For reporter gene analyses, the interlinkerin-2-specific sequences in pBIC2/CMV/IL-2 (39) were substituted with a 1653-bp HindIII-BamHI fragment encoding the luciferase indicator gene. Subsequently, the 3'-untranslated intronic sequences in the pBIC2/CMV vector backbone were replaced with various BamHI-Xmal fragments derived from the CD83 cDNA (3), resulting in the following pBIC2/CMV/luc plasmid series: pBIC2/CMV/luc/SL1 + 2 (CD83 cDNA nucleotides (nt) 412–615), pBIC2/CMV/luc/SL1 (CD83 cDNA nt 412–465), and pBIC2/CMV/luc/SL2 (CD83 cDNA nt 466–615). The mammalian expression vectors pBIC2/CMV/HuR and pBIC2/CMV/CAT were created by inserting the respective genes between the HindIII and BamHI sites of pBIC2/CMV (39). The vector pBIC2/CMV/HuR-FLAG expresses C-terminally FLAG-tagged HuR. The parental vector pBIC2/CMV also served as a negative control plasmid in the transfection experiments. The expression plasmid p3CANC encodes the Nup214/CAN C terminus, also termed ΔCAN (40), and has been described previously (41). The plasmid p3UTR-CD83 contains an expression cassette that is inserted between the HindIII and Xhol sites of the vector pcDNA3 and that consists of the CD83 cDNA flanked by the CD83 mRNA 5'-UTR and 3'-UTR (42, 43). The plasmid pUHC-UTR-CD83 is a derivative of the vector pUHDL13-3 (44) and expresses the CD83 5'-UTR/cDNA/3'-UTR cassette from a chimeric promoter, comprising a minimal promoter sequence derived from the human cytomegalovirus immediate early promoter and seven copies of the tetracycline operator sequence, which allow regulatable gene expression in mammalian cells by addition of tetracycline analogs (e.g. doxycycline) to the culture medium. Likewise, pUHC-UTR-CD83ΔPRE is a derivative of pUHC-UTR-CD83 in which the CD83 stem-loop (SL) 2 sequence (nt 466–615) has been deleted.

In Vitro Synthesis of RNA—RNA was labeled by in vitro transcription with [α-32P]UTP (Amersham Biosciences) using a commercial T7 RNA polymerase-based kit (Promega Corp.) following the manufacturer's instructions. Likewise, polyadenylated and capped transcripts were synthesized in vitro using an Sp6 RNA polymerase-based kit (Promega Corp.) in combination with a Ribo m7G cap analog according to the manufacturer's instructions.

Purification of Recombinant Fusion Proteins and RNA Binding Assay—GST fusion proteins were expressed in Escherichia coli BL21(DE3) and purified from crude lysates by affinity chromatography on glutathione-Sepharose 4B (Amersham Biosciences) as described previously (45). RNA gel retardation assays were performed using in vitro transcribed 32P-labeled CD83-derived probes, MS2 competitor RNA, and GST fusion protein as described previously (46), except that RNA-protein complexes were resolved on 4 or 6% native polyacrylamide gels. Supershift assays were performed using anti-HuR (clone 19F12, Invitrogen) and anti-FLAG (Sigma) monoclonal antibodies.

Surface Plasmon Resonance (Biacore) Analysis—A Biacore X system and CM5 sensor chips (research grade; Biacore International AB) were used for all binding studies. GST or GST-HuR fusion protein was immobilized on the chip surface using the Biacore GST kit for fusion capture (Biacore International AB) according to the manufacturer's instructions. For this, proteins were diluted to concentrations of 5–10 μg/ml in Hapes-buffered saline/EP buffer (10 mM Hapes (pH 7.4), 150 mM NaCl, 3 mM EDTA, and 0.005% polysorbate 20) and were captured on the chip at a flow rate of 5 μl/min until a change of at least 1.000–1.500 resonance units was detectable. The resonance unit base line was then allowed to stabilize for at least 15 min.

For RNA binding, equal molar amounts of the in vitro transcribed RNAs were diluted in Hapes-buffered saline/EP buffer to a final volume of 80 μl and subsequently injected over both flow cells (flow cell 1; GST, and flow cell 2; GST-HuR) at a flow rate of 50 μl/min. The injected RNAs were allowed to dissociate from the proteins during a wash step in which Hapes-buffered saline/EP buffer was injected for an additional 60–90 s. Bound RNAs were removed with 30 μl of 0.5 mM NaCl at a flow rate of 20 μl/min. This step was repeated until the resonance unit change was <3 as compared with the resonance unit change prior to injection. To assess the stability of the protein surface and to determine the reproducibility of the analyses, each experiment was repeated at least three times.

Equilibrium binding experiments were performed under conditions similar to those described previously. Base-line data were collected for at least 15 min prior to injecting the respective amount of RNA at a flow rate of 50 μl/min, followed by a dissociation period of 300 s. The CD83 SL2 RNA was used at concentrations of 65, 129, 258, 388, 516, and 775 nM.

Upon completion of the binding profiles, the responses from all flow cells were base line-corrected. The response from the reference cell (flow cell 1, GST) was subtracted from the response from flow cell 2 (GST-HuR) to correct for refractive index changes, nonspecific binding, and instrument drift. The Kd value was determined using BIAevaluation Version 3.1 software (Biacore International AB) and applying the 1:1 Langmuir fitting model as described previously (47, 48).

Cell Culture, Transfections, Reporter Gene Assays, and Radioimmunoprecipitation Analysis—The cell lines COS, 293T, Jurkat, and HeLa-TA, which constitutively expresses a Tet repressor/VP16 transactivator fusion protein (44), were maintained as described previously (49, 50). For analysis of HuR-mediated reporter gene activation, COS cells (2.5 ×
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$10^8$ cells were cotransfected with 250 ng of either pBC12/CMV (negative control) or pBC12/CMV/HuR in combination with 125 ng of pBC12/CMV/CAT (internal control) and 250 ng of pBC12/CMV/luc expression vector using DEAE-dextran and chloroquine as described previously (51). At ~60 h post-transfection, cell lysates were prepared, and the levels of chloramphenicol acetyltransferase activity were measured using an enzyme-linked immunosorbent assay (Roche Applied Science). These values were subsequently used to determine the amount of cell extract to be assayed for luciferase activity using a commercially available assay system (Promega Corp.). DNA input levels were kept constant in all of these experiments by inclusion of pBC12/CMV vector DNA.

For analysis of CD83 expression in Jurkat T cells, 5 × 10⁶ cells were transiently cotransfected using Lipofectamine 2000 (Invitrogen) according to the manufacturer’s instructions with 10 µg of p3eGFP and 30 µg of either psiHuR or pSUPER (negative control). At ~120 h post-transfection, cells were enhanced green fluorescent protein–sorted and subjected to CD83–specific fluorescence–activated cell sorter (FACS) or Western blot analysis. In some experiments, the Jurkat cultures were activated by serum depletion for 12 h and by addition of 10% serum for 3 h, followed by addition of 50 ng/ml phorbol 12-myristate 13-acetate (PMA) and 1 µM ionomycin (both from Sigma) for an additional 3 h prior to further analysis. For UV light–induced cross-linking experiments, 2.5 × 10⁶ 293T cells were cotransfected with 14 µg of either pBC12/CMV/luc or pBC12/CMV/luc/SL2 in combination with 7 µg of pBC12/CMV/HuR–FLAG or pBC12/CMV (negative control) expression vector using the calcium phosphate method (51).

For analyses of RNA stability, the transcriptional pulsing strategy was employed (52, 53). Briefly, 2.5 × 10⁶ HeLa-TA cells were cotransfected with 10 µg of pUHC-UTR-CD83 or pUHC-UTR-CD83ΔPRE DNA together with 15 µg of parental control plasmid (pBC12/CMV or pSUPER) or expression vector (pBC12/CMV/HuR or psiHuR) using Lipofectamine 2000 according to the manufacturer’s instructions. The next day (in the case of HuR overexpression) or at 3 days post-transfection (in the case of HuR silencing), the transfected cells were trypsinized and seeded into 35-mm dishes. To these cultures was added 80 ng/ml doxycycline to inhibit expression in the absence of doxycycline as measured by Northern analysis, RNA pellets were lyophilized and subsequently dissolved in water for further analysis.

For analyses of subcellular accumulation of CD83 mRNAs, 2.5 × 10⁵ COS cells were transfected with 250 ng of p3UTR-CD83 alone (for leptomycin B experiments) or in combination with 400 ng of either psiHuR or pSUPER (negative control) using DEAE-dextran and chloroquine as described above. Similarly, COS monolayers were cotransfected with 250 ng of p3UTR-CD83 in combination with 400 ng of either p3CANC or pCDNA3 (negative control). At ~60 h post-transfection, cellular RNAs were isolated from the transfected cultures for reverse transcription (RT)–PCR or real-time PCR analysis.

De novo CD83 synthesis was analyzed in COS cell cultures (2.5 × 10⁶ cells) that were mock-transfected or cotransfected with 5 µg of either psiHuR or pSUPER (negative control) in combination with 2 µg of p3UTR-CD83 expression vector. At ~60 h post-transfection, cell cultures were washed with cytoine/methionine-free medium containing 10% dialyzed fetal calf serum and then incubated in the same medium for 1 h. The cells were metabolically labeled using 200 µCi of Tran35S-label (1175 Ci/mmool; MP Biomedicals). Cells were pelleted, washed twice with phosphate-buffered saline, and lysed in Nonidet buffer (0.1% Nonidet P-40, 150 mM NaCl, and 50 mM Hepes at pH 7.3). For deglycosylation, the lysates were incubated with peptide N-glycosidase F (New England Biolabs) according to the manufacturer’s instructions. Protein concentrations were determined by colorimetric protein assay (BioRad), and equal amounts of the various radiolabeled cell extracts were subjected to CD83–specific immunoprecipitation analyses using anti-CD83 monoclonal antibody (clone HB115a, Acris Antibodies) and analyzed by 12% SDS-PAGE and autoradiography.

Immunoblot Analysis—Western blot analyses of cell extracts was performed as described previously (54) using rabbit anti-HuR polyclonal antisera (kindly provided by Dr. Hermann Gram, Novartis Pharma AG), anti-tubulin monoclonal antibody (clone DM1A, Sigma), and anti-CD83 monoclonal antibody (clone HB115a).

FACS Analyses—For flow cytometry analyses, phycoerythrin-conjugated anti-CD83 monoclonal antibody (Pharmingen) was used. The phycocerythrin–conjugated IgG1x isotype control was obtained from Pharmingen and was run in parallel. Cell populations were analyzed on a FACSscan™ (BD Biosciences). Nonviable cells were gated out on the basis of their light scattering properties.

In Vivo UV Light Cross-linking—UV light cross-linking was performed on 293T cells that had been transiently cotransfected using the calcium phosphate method with the pBC12/CMV/luc or pBC12/CMV/luc/SL2 reporter gene construct in combination with the pBC12/CMV/HuR–FLAG plasmid or the pBC12/CMV parental vector as a negative control. At ~60 h post-transfection, cells were washed with phosphate-buffered saline and UV light–cross-linked three times in a Stratalinker 1800 (Stratagene) using the autocross-link function. Total cell lysates were prepared as described previously (55). To 1500 µl of cell lysate were added the following four luciferase–specific 5′–biotin–labeled oligonucleotides (end concentration of 70 nM each; MWG-Biotech AG): 5′–CAGTGCTACAGCAGAT-TCTG–3′, 5′–CTTGTGCCAGCGAGAGAATTG–3′, and 5′–GCTACATCACGTATTGTATTA–3′. Luciferase–specific mRNA–protein complexes were isolated on streptavidin–coated magnetic beads (Roche Applied Science) according to the manufacturer’s protocol. Samples were then analyzed by SDS-PAGE, followed by Western blotting with anti-FLAG monoclonal antibody (Sigma).

Likewise, UV light cross-linking was performed in Jurkat T cells using the following CD83–specific 5′–biotin–labeled oligonucleotides: 5′–GAGCAAGCCACCTTCCATCAGCG–3′, 5′–TCCCCCTGATTGGCTCTCT–3′, and 5′–CAGGGAATAGGGCCCTTTATTGGG–3′. For non-specific binding, the luciferase–specific oligonucleotides described above were used.

Translation in Vitro—A commercial nuclease–treated rabbit reticulocyte lysate system (Promega Corp.) was programmed in the presence of 30 µCi of [35S]cysteine (600 Ci/mmol; Hartmann Analytic GmbH) with 3 µg of each capped and polyadenylated HuR– and/or CD83–specific RNA template according to the manufacturer’s protocol.

RNA Isolation and PCR Analyses—Total cellular RNAs were isolated using TRIzol reagent according to the protocol recommended by Invitrogen. For isolation of cytoplasmic and nuclear RNAs, 2 × 10⁶ cells were lysed on ice for 1 min using 100 µl of Nonidet P-40 buffer (10 mM
Hepes-KOH (pH 7.8), 10 mM KCl, 20% glycerol, 1 mM dithiothreitol, and 0.25% Nonidet P-40). Subsequently, the lysates were cleared by centrifugation at 470 \times g for 5 min at 4°C. Cytoplasmic RNA was isolated from 80 μl of the supernatant using TRIzol reagent as described above. The nuclei were washed again with 100 μl of Nonidet P-40 buffer to deplete residual cytoplasmic RNA. Afterward, the nuclear RNA was prepared using TRIzol reagent.

Selected RNA samples were reverse-transcribed using the first strand cDNA (avian myeloblastosis virus) synthesis kit for RT-PCR (Roche Applied Science) according to the manufacturer's instructions. Subsequently, RT products were assayed by PCR. For detection of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) sequences, the following primers were used: 5'-TGAAGGTCGAGTCAACGGATTTGGT-3' (forward) and 5'-CATGTGGCCATGAGGTCCACCAC-3' (reverse). The amplification profile involved 25 cycles of denaturation at 95°C for 1 min, primer annealing at 56°C for 1 min, and primer extension at 72°C for 1 min. HuR messages were detected using the following primer pairs: 5'-CAGAAGAGGCATTTACCCATG-3' (forward) and 5'-GCTTCTGATTTGCTATGTCGC-3' (reverse). The amplification profile involved 30 cycles of denaturation at 95°C for 1 min, primer annealing at 56°C for 1 min, and primer extension at 72°C for 1 min.

Real-time PCR was performed in an ABI PRISM 7700 detector (PerkinElmer Life Sciences). Amplification was monitored by SYBR green fluorescence. Standard curves were derived by serial dilution of p3UTR-CD83 and pGAPDH. RNAs were normalized by amplification of GAPDH.

RESULTS

CD83 mRNA Contains a Defined HuR-binding Site—In a previous study using DCs, we provided indirect evidence that CD83 mRNA is transported from the nucleus to the cytoplasm via a specific nuclear export pathway (8). Because the RNA-binding shuttle protein HuR appears to be involved in the intracellular transport of some cellular mRNAs (23, 35), we hypothesized that CD83 mRNA may also be a HuR target. Therefore, we first assessed the binding of HuR to the CD83 mRNA in vitro by RNA gel retardation analyses (Fig. 1). For this, HuR was expressed and purified in the context of a fusion to GST and then analyzed in combination with various in vitro transcribed radiolabeled CD83-specific RNA probes. Addition of GST-HuR to the CD83 coding region (nt 1–615) (see Ref. 3) resulted in the appearance of an RNA-protein complex with slower mobility upon nondenaturing gel electrophoresis compared with the migration of the uncomplexed RNA probe (Fig. 1A, lanes 1 and 3). This HuR-CD83 RNA complex could be further retarded by addition of HuR-specific antibodies to the binding reaction (Fig. 1B, lanes 3–5), and the complex was not detected when GST alone was used in a control experiment (Fig. 1, A, lane 2; and B, lane 1). A competition experiment in which a constant level of the radiolabeled CD83 RNA probe was incubated in the presence of increasing amounts of the corresponding unlabeled RNA demonstrated dose-dependent competition with respect to HuR-RNA complex formation, thereby indicating a degree of binding specificity (Fig. 1C). Additional specificity controls showed that unrelated RNA-binding shuttle proteins such as human ribosomal protein L5 and the HIV-1 Rev transactivator did not interact with the CD83 mRNA, and HuR did not bind to the heterologous HIV-1 Rev response element RNA sequence in this type of experiment (data not shown). The subsequent analysis of data obtained with various CD83-derived RNA subfragments revealed that RNA probes derived from the 5'-half of the CD83 coding region failed to interact with HuR (Fig. 1D, lanes 3–6). In contrast, inclusion of an RNA probe comprising 3'-sequences of the CD83 coding region allowed us to detect a complex with lower mobility (Fig. 1D, lanes 7 and 8). Analysis of the combined data from these RNA mobility shift assays indicated the presence of a HuR-binding site in the 3'-half of the coding region of CD83 mRNA. The subsequent in silico analysis of this region suggested that this CD83-derived sequence is folded into a theoretical RNA secondary structure located at nt 412–615 of the CD83 coding region and comprising two adjacent stem-loop elements, termed SL1 and SL2 for convenience (Fig. 1E). We also would like to note that we were unsuccessful in detecting any interaction between HuR and the 3'-UTR of the CD83 mRNA (data not shown), which appears to be devoid of typical AU-rich sequence elements (3, 21, 43).

Next, we wanted to analyze the HuR-CD83 mRNA interaction described above in a more quantitative way. We therefore employed surface plasmon resonance using a Biacore X optical sensor, which allows the observation of association and dissociation interactions in real time and the determination of binding affinities ($K_d$ values). For this, the GST reference (binding control) and recombinant GST-HuR were immobilized on a sensor chip, which was placed into the Biacore flow cells. The sequential exposure of both flow cells to various RNAs allowed correction for refractive index changes and nonspecific binding (47). As shown in the sensorgrams (Fig. 2A), injection of SL1 RNA (nt 412–465) did not result in binding of this RNA to HuR. In sharp contrast, however, injection of an equal molar amount of SL2 RNA (nt 466–615) resulted in a distinct binding signal during the association phase, as represented by an immediate and continuous increase in the resonance signal over the injection period. Moreover, the subsequent injection of buffer alone (spike at ~30 s) during the dissociation phase demonstrated that this HuR-RNA complex is relatively stable (Fig. 2A). Next, we compared the kinetics of binding of HuR to RNA elements of different origin. As shown in Fig. 2B, HuR bound comparably well to the CD83 SL2 RNA element and 3'-UTR sequences of TNF-α mRNA, which contains a classical ARE (36, 56). Interestingly, the association of SL2 RNA with HuR occurred with slightly increased kinetics compared with the association of HuR with an equal molar amount of SL2 RNA (nt 466–615) resulting in a distinct binding signal during the association phase, as represented by an immediate and continuous increase in the resonance signal over the injection period. Moreover, the subsequent injection of buffer alone (spike at ~30 s) during the dissociation phase demonstrated that this HuR-RNA complex is relatively stable (Fig. 2A). Next, we compared the kinetics of binding of HuR to RNA elements of different origin. As shown in Fig. 2B, HuR bound comparably well to the CD83 SL2 RNA element and 3'-UTR sequences of TNF-α mRNA, which contains a classical ARE (36, 56). Interestingly, the association of SL2 RNA with HuR occurred with slightly increased kinetics compared with the association of HuR with an equal molar amount of the TNF-α mRNA-derived sequences. Furthermore, the dissociation kinetics revealed that the complex formed between HuR and the TNF-α ARE might be a bit more stable over time compared with the complex formed by HuR on the CD83 SL2 RNA element. As expected, HuR did not interact with the highly structured Rev response element sequence from HIV-1 (57). Finally, analysis of the binding kinetics using sequential dilutions of CD83 SL2 RNA allowed us to determine a $K_d$ of $2.0 \times 10^{-9}$ M for this RNA-protein interaction (Fig. 2C), which is in the range of the value previously determined by Biacore analysis for binding of the neuronal ELAV-like protein HuD to a typical ARE ($K_d = 0.7 \times 10^{-9}$ M) (58).

Taken together, the equilibrium measurements obtained by RNA gel retardation analyses and the real-time interaction studies performed using surface plasmon resonance revealed that a specific HuR-binding site resides in the coding region of CD83 mRNA and maps to nt 466–615. This RNA sequence is encoded by exon 4 (nt 383–488) and exon 5 (nt 489–615) of the CD83 gene (3) and potentially forms a secondary stem-loop structure with three-pronged morphology (see SL2 in Fig. 1E). When this RNA element was analyzed using the MFOLD program of the GCG Wisconsin package and a folding temperature of 37°C, an
energy of $-29.7 \text{ kcal/mol}$ was calculated. In light of the data presented below, we will refer to the SL2 region as a cis-active post-transcriptional regulatory element (PRE).

HuR Enhances CD83 Expression in Mammalian Cells—Next, we wanted to investigate the effect of HuR on the CD83 SL2 RNA element in mammalian cells. For this, we first inserted several CD83-derived sequen-
FIGURE 2. Kinetic analysis of HuR-RNA interactions by surface plasmon resonance. A, HuR binds specifically to CD83 SL2 RNA (nt 466–615; red line) and fails to interact with CD83 SL1 RNA (nt 412–465; blue line). GST and GST-HuR were immobilized on a CM5 sensor chip and placed into a Biacore X optical sensor device (flow cell 1, GST; and flow cell 2, GST-HuR). The various in vitro transcribed RNAs were subsequently injected at time 0 at a flow rate of 50 μl/min, followed by a 60–90-s flow of buffer, during which dissociation could be observed. (Buffer injection is marked by a spike at the ~30-s time point.) Each experiment was repeated at least three times. B, interaction of HuR with various RNA elements. HuR bound to CD83 SL2 RNA (red line) and to the classical TNF-α-derived ARE sequence (green line) with comparable binding kinetics. No binding of HuR to the HIV-1 Rev response element (RRE) RNA sequence (blue line) was observed. C, K_d value determination of the HuR-CD83 SL2 RNA interaction. The CD83 SL2 RNA was injected into the Biacore flow cells at a flow rate of 50 μl/min, followed by a dissociation period of 300 s. The injected RNA was used at concentrations of 775, 516, 388, 258, 129, and 65 nm. A K_d of 2.0 × 10^{-9} M was determined using BIA-evaluation Version 3.1 software and applying the 1:1 Langmuir fitting model. Resp. Diff., response difference.
ces into the 3′-UTR of the luciferase reporter gene (Fig. 3A). The respective constructs were transfected transiently into COS cells, and reporter gene assays were performed at ~60 h post-transfection. All experiments were internally controlled for transfection efficiencies by inclusion of an additional reporter plasmid constitutively expressing chloramphenicol acetyltransferase. Cotransfection of either a plasmid overexpressing HuR or the respective parental vector alone (negative control) allowed us to monitor HuR effects by assaying luciferase activity.

FIGURE 3. The CD83 SL2 element mediates a biological response to HuR overexpression in mammalian cells. A, shown is a schematic diagram of the pBC12/CMV/luc reporter plasmids used to monitor HuR activity. The luciferase indicator gene is expressed from the cytomegalovirus immediate early (CMV-IE) promoter. As indicated, CD83-derived sequences (SL1 and SL2) were inserted immediately 3′ of the luciferase gene into the UTR. pA, polyadenylation site. B, COS cells were cotransfected with the indicated reporter plasmids together with a vector expressing HuR or the respective parental vector (negative control). In addition, a vector constitutively expressing chloramphenicol acetyltransferase was included in all transfections to serve as an internal control for transfection efficiency. The relative luciferase activities of three independent experiments (normalized to the internal chloramphenicol acetyltransferase control) are shown. The indicated cultures were supplemented with 5 nM leptomycin B (LMB) 16 h prior to reporter gene analyses. Error bars indicate the S.E. of three independent experiments. w/o, without. C, the HuR effect is enhanced by repeated copies of the CD83 SL2 element. Reporter constructs containing single, double, or triple repeats of the SL2 sequence were analyzed for luciferase activity as described for B. D, HuR associates with the CD83 SL2 RNA element in vivo. 293T cells were cotransfected with the reporter gene construct pBC12/CMV/luc/luc (lanes 1 and 2) or pBC12/CMV/luc/SL2 (luc/SL2; lanes 3 and 4) in combination with an expression plasmid encoding HuR-FLAG (lanes 2 and 4) or the pBC12/CMV parental vector (negative control (neg.)). The mRNA-protein complexes were isolated from UV light-cross-linked (upper panel) and non-cross-linked (middle panel) cultures using biotin-labeled oligonucleotides complementary to luciferase mRNA sequences and streptavidin-coated magnetic beads. Samples were analyzed by SDS-PAGE, followed by Western blotting using anti-FLAG monoclonal antibody. In addition, HuR-FLAG expression was verified by standard Western blot analysis (lower panel).
As shown in Fig. 3B, HuR overexpression did not enhance luciferase activity in a reporter construct devoid of CD83 sequences. Likewise, insertion of the CD83 SL1 sequence into the luciferase 3′-UTR failed to confer responsiveness to HuR cotransfection. However, addition of SL2 sequences to this construct (SL1 +2) or insertion of the SL2 sequence alone caused increased luciferase activity in cells overexpressing HuR (Fig. 3B). Due to the fact that COS cells contain already a level of endogenous HuR protein, only moderately enhanced levels of reporter activity as a result of HuR overexpression could be expected in this type of experiment (28). Notwithstanding this, the reproducible HuR-specific effect observed correlated perfectly with the presence of the CD83 mRNA-derived HuR-binding site in our reporter constructs and was ablated when this sequence was in antisense orientation (data not shown). Interestingly, treatment of the cell cultures with 5 nM leptomyosin B, an inhibitor of the nuclear export receptor CRM1 (59), still clearly abrogated its SL2-dependent responsiveness to HuR overexpression. Moreover, the HuR effect was enhanced upon the insertion of increasing numbers of tandemly repeated copies of the SL2 element (Fig. 3C), indicating synergistic activity. This effect was observed only when HuR was overexpressed, which may indicate that the activity of HuR depends on the formation of multimeric complexes on its RNA target. In fact, it has been shown recently that Hu proteins can multimerize and retain their RNA binding properties in mammalian cells (60).

To examine whether HuR can associate with the SL2 RNA element in vivo, we transfected human 293T cells with the pBC12/CMV/luc (negative control) or pBC12/CMV/luc/SL2 reporter gene construct in combination with an expression plasmid encoding a C-terminally FLAG-tagged form of HuR. At ~60 h post-transfection, cells were UV light-irradiated, and luciferase-specific mRNA-protein complexes were isolated from total cell lysates prepared from both UV light-cross-linked and non-cross-linked cultures using biotin-labeled oligonucleotides and streptavidin-coated magnetic beads. Samples were analyzed by SDS-PAGE, followed by Western blotting using anti-FLAG monoclonal antibody. In addition, HuR-FLAG expression was verified by standard Western blot analysis. As shown in Fig. 3D (lane 4), the HuR-FLAG protein was complexed with luciferase-specific mRNA only when the CD83 SL2 element was present in the reporter construct and when the cells were exposed to UV light. These experiments show that the CD83 SL2 RNA element interacts with HuR in vivo.

As HuR clearly activated the expression of a heterologous reporter via the SL2 sequence, we questioned whether HuR also affects the expression of authentic endogenous CD83. If so, the knockdown of HuR by RNA interference should impair CD83 synthesis. To test this hypothesis, we generated an H1 RNA polymerase III gene promoter vector that expresses an siRNA targeted against HuR transcripts. In control experiments, the effect of the HuR-specific siRNA on endogenous HuR mRNA and protein levels was analyzed. It has to be noted, however, that human DCs are not suitable for this type of experiment because only very small amounts of DCs are routinely available (DCs are generated from primary CD14+ progenitor cells (8)), and unfortunately, any transfection protocol appears to negatively affect the metabolism of these extremely delicate cells. Therefore, we analyzed the effect of HuR on CD83 expression in Jurkat T cells, which express, upon stimulation with PMA and ionomycin, noticeable levels of CD83 (50). As shown by RT-PCR, transfection of stimulated Jurkat cells with the HuR-specific siRNA vector (psiHuR) decreased the level of the corresponding transcript in these cells (Fig. 4A, lane 2); in contrast, the negative parental control vector did not negatively affect the abundance of HuR messages (lane 1). Moreover, the inhibitory effect of the siRNA was also observed at the protein level as demonstrated by HuR-specific Western blot analysis (Fig. 4B). Remarkably, CD83-specific FACS analyses demonstrated that the silencing of HuR clearly inhibited surface expression of endogenously expressed CD83 (Fig. 4C). This was also confirmed by corresponding Western blot experiments (Fig. 4D, compare lanes 2 and 4), showing that also the total amount of CD83 was strongly reduced in stimulated T cells upon transfection of the HuR-specific siRNA vector.

The interaction of HuR with endogenous CD83 transcripts was demonstrated as described above by UV light cross-linking of activated Jurkat cells, followed by isolation of RNA-protein complexes using biotin-labeled oligonucleotides and streptavidin-coated magnetic beads. Subsequent Western blot analysis of the samples revealed that HuR indeed interacted with native CD83 messages (Fig. 4E).

We next cotransfected COS cells with a vector that expresses the human CD83 cDNA flanked by the entire homologous 5′- and 3′-UTRs (depicted in Fig. 4F) together with the psiHuR vector or the negative control plasmid. At ~60 h post-transfection, cells were pulsed using Tran35S-label for 10, 20, or 40 min. Subsequently, CD83 synthesis was monitored by immunoprecipitation analysis of equal amounts (as determined by the Bradford assay) of the respective radiolabeled cell extracts using CD83-specific antibody. The specificity of this antibody was confirmed by analysis of extracts derived from mock-transfected cells (data not shown). As shown in Fig. 4F (lanes 2, 4, and 6), the silencing of HuR clearly also inhibited the de novo synthesis of CD83 in these transfected cells. The quantification of the signals obtained is shown in Fig. 4G and revealed an average residual protein level of 38% compared with the level in the cultures that were transfected with the negative control plasmid.

In summary, these data confirm the notion that HuR regulates the expression of CD83 in mammalian cells. The combined experiments demonstrated that the CD83 SL2 RNA element is able to confer a HuR effect on CD83 synthesis by acting as a cis-active PRE.

**HuR Does Not Affect CD83 mRNA Stability**—Previous studies have shown that HuR overexpression has a pronounced stabilizing effect on otherwise highly labile ARE-containing mRNAs in vivo (16, 28). This suggested that the effect of HuR on the CD83 PRE observed in our functional assays may also have been due to increased stability of the respective messages. We therefore examined the effect of HuR on the stability of CD83 mRNAs in transfected mammalian cells. For these experiments, we employed the transcriptional pulsing approach using the tetracycline-regulated promoter system (52, 53). This experimental system allows assessment of the stability of specific mRNAs in the absence of general transcription inhibitors such as actinomycin D, which may interfere with mRNA degradation and/or subcellular HuR localization (16, 34).

HeLa cells constitutively expressing the tetracycline-sensitive transactivator (44) were transiently transfected with vectors containing CD83 coding sequences and the wild-type UTRs (depicted in Fig. 4F) under the control of the tetracycline-sensitive transactivator-responsive promoter. As detected by real-time PCR, the transcript containing the complete CD83 cDNA displayed comparable RNA stability upon doxycycline-induced transcriptional shutoff irrespective of whether or not HuR was overexpressed from a cotransfected plasmid (Fig. 5A). Clearly, when the PRE sequence (SL2, nt 466 – 615) was deleted from the CD83 coding sequence (CD83ΔPRE), the overall half-life of the respective message decreased (Fig. 5B). As expected, HuR overexpression again did not display any significant stabilizing effect on this specific transcript.
FIGURE 4. Silencing of HuR by RNA interference negatively affects CD83 expression. A, transient expression of an siRNA directed against HuR transcripts (siHuR) results in down-regulation of endogenous HuR-specific messages in Jurkat T cells as shown by RT-PCR. Control, negative control experiment. B, likewise, endogenous HuR protein levels are significantly reduced upon expression of HuR-specific siRNA as demonstrated by Western blot analysis. C, CD83-specific FACS analysis of PMA/ionomycin-stimulated Jurkat T cells transfected with the negative control vector (Control) or the HuR-specific siRNA vector (siHuR). The mean fluorescence of enhanced green fluorescent protein-positive cells was 168.47 (Control) and 77.85 (siHuR). PE, phycoerythrin. D, corresponding Western blot analyses of untreated (−) and PMA/ionomycin-treated (+) Jurkat T cell cultures. Stimulation of these cells with PMA/ionomycin induced CD83 expression (lower panel, lane 1 versus lane 2). HuR-specific siRNA significantly impaired CD83 expression in the PMA/ionomycin-induced cultures (lower panel, lane 2 versus lane 4). The effect of siRNA expression on the cellular HuR level is shown (middle panel, lanes 1 and 2 versus lanes 3 and 4). The detection of α-tubulin served as gel loading control (upper panel). E, HuR interacts with native CD83 mRNA. The mRNA-protein complexes were isolated from UV light-cross-linked (+ UV) and non-cross-linked (− UV) Jurkat T cell cultures (PMA/ionomycin-treated) as described for Fig. 3D using biotin-labeled oligonucleotides complementary to luciferase-specific (luc-oligo; negative control) or CD83-specific (CD83-oligo) sequence. The respective samples were analyzed by HuR-specific Western blot analysis. F, upper, schematic diagram of the p3UTR-CD83 expression plasmid used to monitor a potential HuR effect on CD83 expression in transfected COS cells. The CD83 expression cassette (composed of the wild-type 5′-UTR, followed by the CD83 cDNA and the 3′-UTR) is expressed from the cytomegalovirus immediate early (CMV-IE) promoter. CDS, CD83 coding sequence; pA, polyadenylation site. Lower, silencing of HuR impairs CD83 de novo synthesis. This was demonstrated by CD83-specific immunoprecipitation analysis of radiolabeled COS cell extracts that were obtained by pulsing the transfected cell cultures with Tran35S-label for 10, 20, or 40 min. G, quantification of the data shown in F. The CD83 signals obtained in the absence of HuR-specific siRNA were arbitrarily set to 100% (lanes 1, 3, and 5).
FIGURE 5. Analysis of CD83 mRNA stability in mammalian cells using the transcriptional pulsing approach. A, HeLa-tTA cells were cotransfected with the pUHC-UTR-CD83 construct, which contains the wild-type CD83 coding sequence, and with pBC12/CMV/HuR (＞HuR) or pBC12/CMV (Control). Addition of a low level of doxycycline (80 ng/ml) to the transfected cultures for 12 h followed by complete removal of the drug caused a burst of mRNA synthesis originating from the Tet<sup>Off</sup> tetracycline (doxycycline)-regulated promoter in pUHC-UTR-CD83. After 5 h, transcription was turned off by addition of a high level of doxycycline (1 μg/ml), and total RNA was isolated from the cultures at the indicated time points thereafter. The RNAs isolated were then subjected to real-time PCR. The CD83-specific results (normalized to GAPDH) are shown. The respective RNA half-lives (t<sub>1/2</sub>) are indicated below the panels.

B, messages derived from the construct pUHC-UTR-CD83∆PRE, in which the CD83 PRE sequence has been deleted, were analyzed for RNA stability as described for A. C, analysis of messages expressed from the vector pUHC-UTR-CD83 in HeLa-tTA cells in which endogenous HuR was silenced by siRNA (siHuR).
Although HuR overexpression has been reported to significantly stabilize ARE-containing transcripts in this type of assay (for examples, see Refs. 16, 61, and 62), at this point, we could not entirely rule out the possibility that the endogenous amount of HuR is already sufficient to fully stabilize PRE-containing messages, and therefore, overexpression would not have any additional effect. To test this possibility, we again silenced HuR as described above and determined the stability of complete (PRE-containing) CD83 mRNA in HeLa-tTA cells. As shown in Fig. 5C, knockdown of endogenous HuR, which strongly reduces the protein expression level of CD83, clearly did not decrease the half-life of the CD83 transcript, thereby supporting the data presented in Fig. 5A.

In sum, these data indicate that the PRE sequence affects the overall stability of the CD83 transcript, which may be caused by a generally different structure of PRE-deficient messages. The effect of HuR on CD83 expression appears, however, not to be based on protection of
the CD83 mRNA from rapid degradation due to HuR-PRE RNA interaction.

**HuR Affects the Cytoplasmic Accumulation of CD83 mRNA**—Considering that HuR effects on protein translation have been observed in previous studies (63, 64) and our inability to detect HuR-specific stabilization of CD83 transcripts (Fig. 5), the observed HuR effect on CD83 synthesis may be due either to an elevated initiation rate of CD83 translation or, alternatively, to HuR-mediated accelerated nuclear export of CD83 transcripts, which, in turn, would result in an increased rate of protein synthesis. To examine these two possibilities, we first analyzed protein translation by programming rabbit reticulocyte lysates with capped and polyadenylated in vitro transcribed RNAs that encode HuR and/or CD83. In these experiments, we reproducibly observed a HuR-specific double band (Fig. 6A, lane 2), which may be explained by the fact that rabbit reticulocyte lysates have been shown to sometimes bypass bona fide start codons and initiate at downstream AUG codons (65). More important, however, we clearly failed to demonstrate any significant effect of HuR on the initiation rate of CD83 synthesis (Fig. 6A, lane 1 versus lane 2).

We next analyzed the cytoplasmic accumulation of CD83-specific transcripts in COS cells transiently transfected with p3UTR-CD83 DNA. HuR has been shown previously to constantly shuttle between the nucleus and the cytoplasm (16, 34). Moreover, evidence had been provided that HuR mediates nuclear export of some cellular mRNAs such as c-fos mRNA via the specific CRM1 nuclear export pathway (66, 67). We therefore again knocked down HuR by RNA interference and also included various established CRM1 inhibitors in the following experiments. Total, cytoplasmic, and nuclear RNAs from the transfected cell cultures were prepared and subjected to real-time PCR (Fig. 6B). The data obtained revealed that the silencing of HuR had only minimal effects on total cellular and nuclear CD83 mRNA levels. In contrast, however, the level of cytoplasmic CD83 mRNA was significantly diminished in cells expressing HuR-specific siRNA, corresponding to ~48% of the level observed when HuR was not depleted (which was arbitrarily set to 100%) (Fig. 6B). It is important to note that this residual cytoplasmic RNA level approximates the level of CD83 protein synthesis observed in HuR-depleted cells (Fig. 4G). Thus, the main effect of HuR on CD83 expression can be explained by efficient HuR-dependent cytoplasmic accumulation of CD83-specific transcripts.

At this point, our data suggested that HuR may mediate the nucleocytoplasmic translocation of CD83 mRNAs via the CRM1 nuclear export pathway. We therefore analyzed two specific but mechanistically different CRM1 inhibitors with respect to the cytoplasmic accumulation of CD83-specific transcripts. As observed before by applying HuR-specific siRNA, no significant influence on the overall total CD83 mRNA level was observed when the transfected cultures were either treated with the CRM1-specific inhibitor leptomycin B (Fig. 6C, lanes 1 and 2) or cotransfected with a vector expressing the C-terminal CRM1-binding repeat region of the leucine zipper Nup214/CAN (Fig. 6D, lanes 1 and 2), which has been shown previously to efficiently block the CRM1 export pathway (40). In contrast, however, the isolation of cytoplasmic mRNAs revealed that inactivation of CRM1, by either leptomycin B (Fig. 6C, lanes 3 and 4) or coexpression of the CAN C terminus (Fig. 6D, lanes 3 and 4), profoundly abolished the accumulation of CD83 mRNAs in the cytoplasm. The detection of the exclusively nuclear U6 small nuclear RNA served as control of the cellular fractionation method (data not shown). In summary, these data indicate that HuR affects the cytoplasmic accumulation of CD83 mRNAs and that CRM1 is operational in the nucleocytoplasmic translocation of these cellular transcripts. The finding that the silencing of HuR did not significantly increase the level of CD83 mRNAs in the nucleus (Fig. 6B) does not invalidate this notion. In fact, the investigation of the HIV-1 Rev mRNA transport system repeatedly showed that interference with the Rev-CRM1 axis profoundly affects the cytoplasmic (but not or just barely the nuclear) level of Rev-regulated mRNAs (for examples, see Refs. 57 and 68–71).

**DISCUSSION**

The CD83 surface molecule is widely used as a surrogate marker that defines mature immunostimulatory DCs (3, 4). More recent data have provided evidence that CD83 is also required for DC-mediated T cell stimulation and hence appears to fulfill an important functional role in inducing T cell-mediated immunity (9, 10). Thus, the CD83 mRNA can be considered an important transcript that has to be efficiently processed during an immune response akin to some ERG mRNAs that encode, for example, cytokines. However, the CD83 transcript does not appear to contain an ARE sequence, which is a hallmark feature of ERG messages (19–21). Instead, in this study, we have identified a novel sequence element in the CD83 coding region (referred to as the PRE) that forms a structured RNA element that binds with specificity to HuR and that regulates the cytoplasmic accumulation of CD83 mRNAs in a HuR-dependent fashion.

Over recent years, multiple studies have provided evidence that the formation of messenger RNP complexes couples transcription and translation in eukaryotic cells (72–74). Thus, the dynamic recruitment of varying RNA-binding proteins to individual mRNAs determines the fate of each transcript with respect to splicing, nuclear export, stability, and ultimately translation (72). Moreover, RNA-binding proteins appear to regulate discrete classes or unique subpopulations of mRNAs at the post-transcriptional level in a coordinated fashion (75, 76). This predicts that functionally related genes contain common cis-active sequence elements that serve as targets of specific RNA-binding proteins (75). In this context, the RNA-binding shuttle protein HuR has been described to play an important role in the post-transcriptional regulation of the specific subset of ARE-containing cellular transcripts by particularly regulating their stability and/or nuclear export (15, 23, 35).

The nuclear export of different classes of RNA, including tRNAs, rRNAs, U small nuclear RNAs, and mRNAs, proceeds in the form of messenger RNP complexes that are translocated through the nuclear pore complex, which is a large supramolecular structure that perforates the nuclear envelope (for review, see Refs. 77–79). These various RNAs are exported from the nucleus to the cytoplasm via distinct and sometimes partially overlapping pathways (for details, see Refs. 80–82). The nuclear export of noncoding RNAs depends on soluble transport receptors of the karyopherin family and associated factors. Particularly the CRM1 export receptor is a key factor for the nucleocytoplasmic translocation of rRNAs and U small nuclear RNAs. In addition, CRM1 also mediates the nuclear export of incompletely spliced retroviral mRNAs such as, for example, HIV-1 messages that encode the viral structural proteins and enzymes Gag, Pol, and Env. In sharp contrast, however, the nuclear export of the vast majority of cellular mRNAs is mediated in metazoans by the conserved heterodimeric transport receptor NFXF1/Tip-associated protein-Nxt1/p15, which is structurally unrelated to the karyopherin export receptor family and bridges the interaction of export-competent messenger RNPs and the nuclear pore complex (summarized in Refs. 83 and 84). Because NFXF1/Tip-associated protein itself exhibits low affinity for RNA, its interaction with mRNAs is accomplished through adaptor proteins such as, for example, ALY and SR splicing factors (reviewed in Refs. 85 and 86).

It has become evident that the regulation of mRNA turnover is an important mechanism by which cells are able to quickly respond to developmental or environmental signals (reviewed in Refs. 11–13). To
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date, one of the most extensively investigated specific RNA-stabilizing factors is HuR, which binds to the ARE sequences that are intrinsic components of highly unstable ERG mRNAs (19–21). AREs have been shown to increase the in vivo rate of deadenylation and RNA turnover (87, 88) and are potent stimulators of decapping in vitro (18). Binding of HuR to ARE sequences protects the RNA body from degradation, but appears to have little effect on deadenylation (16, 17). Notably, the finding that HuR is a nucleocytoplasmic shuttle protein triggered the notion that HuR binds to ARE-containing transcripts in the nucleus and protects them from degradation during their transit to the cytoplasm (23, 35).

HuR consists of three RNA recognition motifs and a basic hinge region that separates RNA recognition motifs 2 and 3 (30, 34). Data were originally presented showing that the hinge region constitutes a bidirectional transport signal (34, 67). However, more recent studies demonstrated that the hinge region mediates primarily the nuclear import of HuR by interacting with the transportin-1 or transportin-2 nuclear transport receptor (89, 90). It has also been reported that, upon activation of AMP-activated protein kinase, the importin-transport receptor (89, 90). While more recent studies demonstrated that the hinge region mediates primarily the nuclear import of HuR by interacting with the transportin-1 or transportin-2 nuclear transport receptor (89, 90). It has also been reported that, upon activation of AMP-activated protein kinase, the importin-transport receptor (89, 90). While more recent studies demonstrated that the hinge region mediates primarily the nuclear import of HuR by interacting with the transportin-1 or transportin-2 nuclear transport receptor (89, 90). It has also been reported that, upon activation of AMP-activated protein kinase, the importin-transport receptor (89, 90). While more recent studies demonstrated that the hinge region mediates primarily the nuclear import of HuR by interacting with the transportin-1 or transportin-2 nuclear transport receptor (89, 90). It has also been reported that, upon activation of AMP-activated protein kinase, the importin-transport receptor (89, 90).

For example, the specific CRM1 inhibitor leptomycin B inhibits expression of cyclooxygenase-2 (95) and was shown to cause nuclear retention of c-fos mRNA, whereas general mRNA export appeared not to be affected (66). Furthermore, it was shown that CRM1 mediates the nuclear export of the transcript encoding interferon-α (96). Notably, the truncation of the 3′-UTR that contains ARE sequences does not negatively affect the CRM1-dependent nuclear export of the interferon-α mRNA. It is therefore conceivable that these cellular messages may also contain (as does the CD83 transcript) novel cis-active sequence elements that mediate their efficient cytoplasmic accumulation in a HuR-dependent fashion. It appears that the mode of action of HuR in higher eukaryotes is rather complex and, depending on its RNA target sequence, includes distinct activities at the level of RNA stability, nucleocytoplasmic transport, and translation.

Acknowledgments—We thank Cordula Grütter, Bettina Abel, and Nicole Studttrucker for excellent technical assistance. We are most grateful to Dr. Ilona Hauber for providing the p3UTR-CD83 plasmid, Dr. Hermann Gram for the TNF-α ARE and anti-HuR antiserum, Dr. Alexander Steinkasserer (University Erlangen-Nürnberg) for the CD83 cDNA, and Dr. Wolfgang Hillen (University Erlangen-Nürnberg) for HeLa-tTA cells and the pLHD13-3 vector. We are indebted to Drs. Hermann Gram and Alexander Steinkasserer for many helpful suggestions during the course of this study. We thank Dr. Sarah L. Thomas for critical comments on the manuscript.

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