Translational Repression by RNA-Binding Protein TIAR†

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Received 2 December 2005/Returned for modification 27 December 2005/Accepted 3 January 2006

The RNA-binding protein TIAR has been proposed to inhibit protein synthesis transiently by promoting the formation of translationally silent stress granules. Here, we report the selective binding of TIAR to several mRNAs encoding translation factors such as eukaryotic initiation factor 4A (eIF4A) and eIF4E (translation initiation factors), eEF1B (a translation elongation factor), and c-Myc (which transcriptionally controls the expression of numerous translation regulatory proteins). TIAR bound the 3'-untranslated regions of these mRNAs and potentially suppressed their translation, particularly in response to low levels of short-wavelength UV (UVC) irradiation. The UVC-imposed global inhibition of the cellular translation machinery was significantly relieved after silencing of TIAR expression. We propose that the TIAR-mediated inhibition of translation factor expression elicits a sustained repression of protein biosynthesis in cells responding to stress.

In mammalian cells, stress stimuli trigger alterations in gene expression patterns via transcriptional and posttranscriptional mechanisms. Among the latter processes, mRNA stability and translation are tightly regulated by the association of the mRNA with specific RNA-binding proteins (RBPs) (5, 43, 46). Messenger RNAs that contain adenine/uridine- or uridine-rich regions (collectively named AU-rich elements [AREs]) are the targets of specialized RBPs that govern their half-life and translation rates (5, 11, 43). Such ARE-RBPs include proteins that promote mRNA decay (including AU-binding factor 1 [AUF1], tristetraprolin, K-homology splicing regulatory protein, and butyrate response factor 1 [BRF1]), proteins that promote mRNA stabilization and modulate translation (such as the Hu proteins HuR, HuB, HuC, and HuD), and proteins that suppress translation, including the T-cell-restricted intracellular antigen 1 (TIA-1) and the TIA-1-related protein TIAR (4, 8, 10, 18, 28, 35, 39, 47).

TIA-1 and TIAR have been proposed to play a general role as translational repressors (1, 2). In the absence of stress, eukaryotic translation initiation factor 2 (eIF2)-GTP-tRNA^{Met} ternary complexes are available in the cell, and a canonical preinitiation complex (comprising eIF1, eIF2, eIF3, eIF5, and the 40S ribosomal subunit) forms at the 5' end of capped mRNAs. Following the recognition of an initiation codon, the 60S subunit is assembled, displacing the initiation factors and forming a functional ribosome to initiate translation. In cells exposed to a variety of environmental stress agents, phosphorylation of eIF2 α by a family of kinases (PKR, PERK, GCN2, and HRI) prevents the exchange of GTP, thereby lowering the concentration of the eIF2-GTP-tRNA^{Met} ternary complex (recently reviewed in reference 19). Under conditions of cellular damage, TIAR and TIA-1 have been postulated to function as translational repressors by associating with eIF1, eIF3, and the 40S ribosomal subunit and forming noncanonical preinitiation

complexes that are translationally inactive (2). The self-aggregating properties of TIA-1 and TIAR were further proposed to facilitate the accumulation of the translationally inactive preinitiation complexes into discrete cytoplasmic foci called stress granules (SGs). Given the presence of RBPs that influence mRNA stability (such as tristetraprolin and HuR) and translation (TIA proteins) at SGs, these foci have been hypothesized to function as dynamic sites of mRNA triage during stress, wherein molecular decisions are made regarding the composition of mRNA ribonucleoprotein (RNP) complexes and their subsequent engagement with the translation or degradation machineries (22, 24).

A related but distinct role for TIA-1 and TIAR has been suggested to specifically mediate the translational silencing of ARE-containing mRNAs, including those that encode tumor necrosis factor α (TNF- α), matrix metalloproteinase 13 (MMP13), cyclooxygenase 2, and β_2 -adrenergic receptor (12, 18, 20, 45). For example, TIA-1 was demonstrated to inhibit the translation of TNF- α (2, 35), as lipopolysaccharide-stimulated macrophages that either lacked or expressed TIA-1 displayed similar steady-state levels of total TNF- α mRNA, but the relative abundance of the TNF- α mRNA in the actively translating polyribosomes was severalfold higher in TIA-1^{-/-} macrophages than in wild-type macrophages, selectively elevating TNF- α production (35). Similarly, a recent search for global TIA-1 target mRNAs revealed that TIA-1 suppressed the translation of specific subsets of bound transcripts (30). According to Anderson and coworkers, the specific translational suppression of ARE-containing TIA-1/TIAR target mRNAs is likely based upon the increased chance that these RBPs will assemble a translationally silent complex on such mRNAs (3).

Recently, we sought to identify TIAR target mRNAs at a transcriptome-wide level. During the course of these studies, we observed that TIAR silencing resulted in a global increase in protein translation. In addition, the marked decline in protein biosynthesis seen in control populations following exposure to low-level UVC (short-wavelength UV light) irradiation was largely relieved in the TIAR-silenced populations, wherein UVC-irradiated cells displayed significantly elevated translation rates. The low dose of UVC irradiation employed here

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† Supplemental material for this article may be found at <http://mcb.asm.org/>.

failed to significantly increase the phosphorylation levels of eIF2 α , in keeping with the dose dependence of the UVC-triggered phosphorylation of eIF2 α (14, 44). Subsequent analyses to elucidate global subsets of TIAR target mRNAs, based on the immunoprecipitation of RNP complexes from colon cancer cells, revealed the putative association of TIAR with many mRNAs encoding translation factors and other proteins involved in translation. We directly examined these RNP interactions and investigated the consequent influence of TIAR on global protein translation. Based on our findings, we propose that the TIAR-mediated inhibition of translation factor expression contributes to implementing a translationally repressed state following stress stimulation.

MATERIALS AND METHODS

Cell culture, treatment, transfections, and polysome preparation. Human RKO colorectal carcinoma cells were cultured in minimum essential medium (Gibco). The dose of UVC irradiation was 20 J/m². The small interfering RNA (siRNA) sequence targeting TIAR was AAGGGCTATTTCATTTGTCAGA; the control siRNA was AATTCTCCGAACGTGTCACGT. siRNAs (100 nM; QIAGEN) were sequentially transfected with Oligofectamine (Invitrogen) on days 0 and 3, and cells were harvested on day 6. For polysome analysis, RKO cells (5×10^6 cells per sample) were collected, the cytoplasmic material was fractionated through 10 to 50% continuous sucrose gradients, and RNA from each fraction was extracted as described previously (16). For quantitative mRNA analyses in the polysome fractions, heparin and cycloheximide were excluded from the gradient. Pooled fractions 1 to 3 represented the nontranslating component; pooled fractions 5 to 11 represented the translating component.

Northern and Western blot analyses. For Northern blot analysis, oligonucleotides complementary to EIF4E2, EIF1B2, or 18S (CAGGACGTACCATTGTGGCTATAAAACCTCCAGAACTGCTCC, CCCACTGGAACCTAGTTTGTAGATGAGCCCACTAAGCCGT, and ACGGTATCTGATCGTCTTCGAACC, respectively) were end labeled using [α -³²P]dATP and terminal transferase, while a c-Myc cDNA fragment was labeled using random primers, [α -³²P]dATP, and Klenow enzyme. The resulting signals were visualized with a PhosphorImager (Molecular Dynamics).

For Western blot analysis, cytoplasmic lysates (20 μ g) and nuclear lysates (10 μ g) (prepared using digitonin, as previously described [26]), as well as whole-cell lysates (10 μ g), were size separated by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) and transferred onto polyvinylidene difluoride (PVDF) membranes. Blots were probed with monoclonal antibodies recognizing p-eIF2 α (Cell Signaling), c-Myc (Sigma), β -tubulin, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), or α -tubulin (Santa Cruz Biotechnology); β -actin (Abcam); or polyclonal antibodies recognizing eEF1B (ProteinTech Group, Inc.), eIF4E (Cell Signaling), TIAR, TIA-1, or hnRNP C1/C2 (Santa Cruz Biotechnology). No antibodies were available to detect eIF4A. Following incubation with the appropriate secondary antibodies, signals were detected with the ECL reagent (Amersham).

RT-PCR. RNA was purified from immunoprecipitation (IP) material or sucrose gradient fractions and was reverse transcribed using oligo(dT) and SSII RT (Invitrogen). The resulting material was amplified by either conventional PCR or quantitative ("real-time") PCR (qPCR) analysis using gene-specific primer pairs (see below); the resulting fragments were all 150 to 300 bp in length. An Applied Biosystems 7300 instrument and SYBR green PCR master mix (Applied Biosystems, Foster City, CA) were used to carry out the qPCR analysis.

Oligonucleotides used for conventional PCR- and qPCR-mediated detection of reverse-transcribed mRNAs in IP material and in pooled fractions from sucrose gradients. Oligonucleotides used for conventional PCR- and qPCR-mediated detection of reverse-transcribed mRNAs in IP material and in pooled fractions from sucrose gradients are as follows: GAGCACTTGTGCGGAAACG and CCAAAGTCCAATTGAGGAGT for MYC mRNA, AAGCAGATGTTG GCCATGAA and TGTGTTTCCAAAATGTAAGTCACC for EIF3SE, GCT CAATCTCTGGGGGCTGA and CCTTCTTTTGCCTCAAAAAGTGAC for EIF4A1, GTGCCTCCACTGATGCAGGA and CCTAGGGCGCAAGGTGG CTTT for EIF4EBP1, GAAGCTGGCGTCATCGGAGT and CTGCTGCGTG TCAGCTGTT for EIF4EL3, CCGGCACACAAAGTGGGTGA and GACA GCTCATGTGTTGCCCTTT for E2F3, TCCGGTCAGGGTCAAGTCGT and CCCCTCTGCCAATTCTGTG for EIF4A2, GCCGGAACCTCTCAG CTCT and TTGTGGCAAGCCAGATGTCC for SUI1, TGCTTGTGGGATG

CAGTGGT and ACTGGGAAGGGGTCCCTCAG for ARF3, CCAGCTCCT TGTTCCCAAGTT and CCGGTGAAGTCTCCACGTT for ETF1, ATCTG GGGCCCACTACTGGTT and GAGGGGGACAAGGCTGTAGAA for PLK, CCCAGGACCTCATCTCCAAA and GGAGGTAGAAAACAGATAAGGG AACA for STK12, CGTGGACTTCGTACCGCTTC and AAAACCAACGGG GATCTAGC for ENO1, ACTGCGCCCTTAACCTGCATC and AGAAGCAC CAAACGTGACCA for ID1, and CTCCCTAGGCGCTGTTCTT and GAG GCAAGAGGAGGGGAGAA for SFN.

Binding assays: biotin pull-down assay and IP followed by reverse transcription (RT)-PCR. For biotin pull-down assays, PCR fragments containing the T7 RNA polymerase promoter sequence (T₇) were used as templates for *in vitro* transcription of the GAPDH 3'-untranslated region (3'UTR) and the MYC, EIF4A, EIF4E, and EEF1B coding region (CR) and 3'UTR using biotinylated CTP (below). Four micrograms of biotinylated transcripts was then incubated with 80 μ g of total lysate for 30 min at 25°C, complexes were isolated with paramagnetic streptavidin-conjugated Dynabeads (Dyna), and the pull-down material was analyzed by Western blotting.

IP of RNP complexes of endogenous TIAR and endogenous mRNAs was carried out using methodologies previously described for other RNA-binding proteins (29, 40). In brief, 20 million RKO cells were collected per time point, and lysates were used for IP for 2 h at 25°C in the presence of excess (30 μ g) IP antibody (either a goat polyclonal anti-TIAR antibody [C-18; Santa Cruz Biotechnology] or control goat immunoglobulin G [IgG] [BD Life Sciences]). RNA in the IP material was reverse transcribed and subjected to both conventional PCR and qPCR to assess the abundance of the products listed in Fig. 2 (the oligomer pairs are listed below).

Oligonucleotides used for pull-down assay. The T7 RNA polymerase promoter sequence (T₇) is CCAAGCTTCTAATACGACTCACTATAGGGAGA. To prepare the EIF4A1 coding region template, oligonucleotides (T₇)CGAAG GCGTCATCGAGAGTA and CTATGACCTTCCCAACACAG were used; to prepare the 3'UTR template, oligonucleotides (T₇)ACCTCATCTGAGGGG CTGT and TGTCACCTTTTGGAGGCAAAAGA were used. To prepare the EIF4E coding region template, oligonucleotides (T₇)ATGATGACAGTGGGG ACCAT and GTTGAATGTGCCATGACCTT were used; to prepare the 3'UTR template, oligonucleotides (T₇)CATGACCTCTCCCTCTCTG and GCTGAGATCACTTAATAAATGGTGC were used. To prepare the eEF1B2 coding region template, oligonucleotides (T₇)TTTCGGAGACCTGAAAAGC and GATGTGGCTGCTTCAACAA were used; to prepare the 3'UTR template, oligonucleotides (T₇)CGGAATTAAGAACTTCAAATACAGT and GGA TCATGGCATTTAATAAAGATTG were used. To prepare the MYC coding region template, oligonucleotides (T₇)TTCGGGTAGTGAAAACACAG and TTT CCGCAACAAGTCTCTT were used; to prepare the 3'UTR template, oligonucleotides (T₇)AAGAGGACTTGTGCGGAAA and GGCTCAATGATATATTT GCCAGT were used.

Analysis of newly translated protein. To quantify whole-cell translation, 100,000 cells that were either left untreated or irradiated with 20 J/m² UVC were incubated at various time points with 50 μ Ci L-[³⁵S]methionine and L-[³⁵S]cysteine (Easy Tag EXPRESS; NEN/Perkin-Elmer) per 60-mm plate for 20 min; radiolabel incorporation was then monitored by both size fractionation of lysates (by using SDS-PAGE followed by visualization of the transferred lysates with a PhosphorImager) and trichloroacetic acid (TCA) precipitation. To quantify the translation of specific proteins, newly translated c-Myc, eIF4E, and eEF1B were investigated by incubating 10⁶ cells with 1 mCi L-[³⁵S]methionine and L-[³⁵S]cysteine per 60-mm plate for 20 min, whereupon cells were lysed using TSD lysis buffer (50 mM Tris [pH 7.5], 1% SDS, and 5 mM dithiothreitol). IP reactions were carried out using 300 μ g of lysate per sample for 1 h at 4°C using appropriate antibodies and IgG as a control; the methodology was described previously (31, 32). Following extensive washes in TNN buffer (50 mM Tris [pH 7.5], 250 mM NaCl, 5 mM EDTA, 0.5% NP-40), the IP material was resolved by 12% SDS-PAGE, transferred onto PVDF filters, and visualized and quantified using a PhosphorImager (Molecular Dynamics).

cDNA array data for TIAR target transcripts in IP material. cDNA array data for TIAR target transcripts in IP material are available at <http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE1433>.

Immunofluorescence. RKO cells cultured on coverslips were fixed in 2% paraformaldehyde and permeabilized in 0.4% Triton X-100 (each 15 min). After incubation in blocking buffer (1 h in phosphate-buffered saline containing 2% bovine serum albumin and 0.1% Tween 20), coverslips were incubated with either goat anti-TIAR or goat anti-TIA-1 (Santa Cruz Biotechnology) in blocking buffer (16 h, 1:100 dilution), washed with blocking buffer, and further incubated with Alexa Fluor 568 donkey anti-goat IgG (heavy plus light chains) (Molecular Probes) (1 h, 1:500 dilution). After washes with blocking buffer, coverslips were mounted in Vectashield (Vector Laboratories) and visualized

with a Zeiss LSM410 confocal microscope. Representative photographs from three independent experiments are shown in the figures.

RESULTS

Silencing of TIAR elevates basal and UVC-suppressed translation in cultured cells. In order to investigate the function of the RNA-binding protein TIAR, we downregulated TIAR levels in human colorectal carcinoma RKO cells by RNA interference (using siRNA). As shown in Fig. 1A, TIAR abundance in the TIAR siRNA transfection group was effectively reduced to less than 10% of the TIAR levels seen in control cultures (see Materials and Methods); importantly, TIA-1 expression levels remained unaltered (or increased slightly) in the TIAR siRNA group, despite the high homology between TIAR and TIA-1. In the course of experiments to characterize these cell populations, global protein synthesis was found to be significantly elevated in TIAR siRNA cultures relative to the control groups (Fig. 1B). Furthermore, while treatment with low levels of UVC (20 J/m^2) potentially lowered protein biosynthesis in control transfection groups (to 40% of the levels of unirradiated cells by 12 h following UVC irradiation), TIAR-silenced cells displayed significantly elevated levels of protein biosynthesis following UVC irradiation (60% of the levels seen in unirradiated, TIAR siRNA cells); in keeping with this trend, the translation of a general reporter (enhanced green fluorescent protein) was also elevated in TIAR siRNA cultures (see Fig. S1 in the supplemental material).

Given the central role of eIF2 α phosphorylation in the inhibition of global protein translation, we hypothesized that the levels of phosphorylated eIF2 α might differ between these transfection groups in a UVC-dependent manner. Unexpectedly, however, while eIF2 α phosphorylation was readily detectable in these cultures, it did not appear to be substantially elevated in either culture, whether left unirradiated or exposed to 20 J/m^2 UVC (Fig. 1C); these findings are in keeping with previous observations that exposure to low levels of UVC did not perceptibly increase eIF2 α phosphorylation ($>50 \text{ J/m}^2$ was needed to attain measurable increases in eIF2 α phosphorylation) (14, 44). Embryo fibroblasts derived from either wild-type or TIAR knockout mice also revealed no increase in eIF2 α phosphorylation following 20 J/m^2 UVC irradiation (see Fig. S2 in the supplemental material). These findings suggested that TIAR was capable of influencing global protein translation without an overt increase in eIF2 α phosphorylation and prompted efforts to identify endogenous TIAR target mRNAs.

TIAR specifically associates with mRNAs encoding translation factors and translation-related proteins. First, we immunoprecipitated TIAR RNP complexes from RKO cell lysates and performed microarray-based identification of TIAR target mRNAs (<http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE1433>). These experiments revealed the putative association of TIAR with many transcripts encoding translation factors and other proteins involved in translation; as anticipated, these transcripts contained AREs in their 3'UTRs (Fig. 2A; see Table S1 in the supplemental material). Figure 2B depicts the levels of TIAR, as detected by Western blotting of the IP material (lysate input was even; see Fig. S3 in the supplemental material). To validate these interactions, the presence of putative TIAR target mRNAs in the IP material was tested by

FIG. 1. TIAR silencing affects global translation. (A) Forty-eight hours after transfection of RKO cells with either a control siRNA ("Ctrl. siRNA") or an siRNA directed to TIAR ("TIAR siRNA"), cells were irradiated (20 J/m^2 UVC). Six hours later, the levels of expression of TIAR, TIA-1, and the control protein β -tubulin were assessed by Western blotting ($20 \mu\text{g}$ per lane). Untr., untreated. (B) Cells transfected as described in A were irradiated with UVC (20 J/m^2), whereupon they were incubated with L-[^{35}S]methionine and L-[^{35}S]cysteine for 20 min and lysed; whole-cell ^{35}S -labeled amino acid incorporation into 100,000 cells was quantified by TCA precipitation and scintillation counting (described in Materials and Methods). Data represent the means and standard errors of the means (SEM) from three independent experiments. (C) RKO cells transfected as described in A were irradiated with UVC, and whole-cell lysates were collected at the times shown. The levels of phosphorylated eIF2 α (p-eIF2 α) and GAPDH (loading control) were assessed by Western blot analysis. Tn, tunicamycin-treated mouse embryo fibroblasts ($2 \mu\text{g/ml}$, 6 h) included as a positive control for eIF2 α phosphorylation.

performing RT-PCR amplification using gene-specific primers. As shown, the PCR products corresponding to TIAR "target mRNAs" were considerably more abundant in the TIAR IP lanes than in the IgG lanes (Fig. 2C), supporting the specificity of the interaction between TIAR and such mRNAs within TIAR RNP complexes. Furthermore, control mRNAs that were not TIAR targets ("nontarget mRNAs") revealed no amplification in either IP (as with PLK and ID1) or showed background amplification that was comparable between the two IP groups (as seen with GAPDH and STK12, which were found as low-level contaminating mRNAs).

FIG. 2. Association of TIAR with target transcripts encoding translation factors and proteins involved in translation. (A) TIAR target transcripts were identified following IP analysis of TIAR, elution of bound RNA, reverse transcription, and hybridization of cDNA microarrays (see Materials and Methods). Among the ~300 transcripts identified on the array as being putatively associated with TIAR (<http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE1433>), several were found to encode translation factors as well as protein components of the translational machinery; the presence of class I and/or class III AREs in the respective 3'UTRs is indicated. (B) IP assays using either IgG (negative control) or anti-TIAR antibodies were followed by Western blot analysis to detect TIAR in the IP material. "Lys," whole-cell lysate (positive control, 10 μ g); "H.C.," heavy IgG chain; "L.C.," light IgG chain; MWM, molecular weight markers (in thousands). (C) The association of TIAR with individual target mRNAs was assessed by RT-PCR amplification (using sequence-specific primers) of the RNA isolated from samples that were obtained after IP assays employing either control (IgG) or anti-TIAR (TIAR) antibodies (see Materials and Methods). Left and center columns, validation of mRNAs predicted to be TIAR targets; right column, mRNAs that were predicted not to be TIAR targets.

TIAR contributes to the UVC-imposed translational repression of target mRNAs. The interaction of TIAR with target transcripts was further investigated after exposure of cells to UVC, a stimulus that triggered the formation of SGs (2, 23). SGs appeared in the cytoplasm by 1 h after UVC irradiation of RKO cells, were largest by 3 h, and virtually disappeared by 6 h (Fig. 3A; see Fig. S4 in the supplemental material). The TIAR-related protein TIA-1 has been reported to undergo dynamic shuttling into and out of SGs (21), and TIAR is likely subject to similar subcellular transport. However, whole-cell TIAR as well as overall TIAR in the cytoplasm and the nucleus appeared unchanged following UVC irradiation (Fig. 3B).

Messenger RNAs encoding c-Myc, the translation initiation factors eIF4A and eIF4E, and the translation elongation factor eEF1B were chosen for subsequent experiments. c-Myc is a transcription factor that transcriptionally upregulates the expression of many translation factors (reviewed in reference 36). eIF4E binds the cap structure (m^7 GpppN) at the 5' end of

mRNAs; eIF4E and the large adaptor protein eIF4G are essential for the recruitment of mRNAs to ribosomes and the commencement of protein synthesis (38). eIF4A is a DEAD family RNA helicase that exhibits an RNA-dependent ATPase activity necessary to unwind the secondary structure of the 5'UTR and thus facilitates ribosome binding and scanning of the initiation codon (6, 17). eEF1B is a guanine nucleotide exchange factor for eEF1A, a protein responsible for delivering aminoacyl-tRNA to the elongating ribosome (7). eEF1A and eEF1B are critically involved in recruiting aminoacyl tRNAs to the ribosome for translation elongation in mammals (9); in a recent report, *Saccharomyces cerevisiae* eEF1B was also shown to promote resistance to oxidative cellular stress (34). As shown in Fig. 4A, the association of TIAR with these target transcripts increased in a time-dependent manner following UVC irradiation, as assessed both by conventional PCR (top) and by qPCR (bottom) analyses of the reverse-transcribed RNA present in the IP samples. The changes in mRNA

FIG. 3. Detection of SGs in UVC-irradiated RKO cells. (A) At the times shown following UVC irradiation (20 J/m^2), TIAR levels and subcellular localization were monitored by immunofluorescence using confocal microscopy (see Materials and Methods) and by gross quantification (see Fig. S4 in the supplemental material). (B) Western blot analysis of TIAR expression in whole-cell ("Total") ($10 \mu\text{g}$), cytoplasmic ("Cytopl.") ($20 \mu\text{g}$), and nuclear ("Nuc.") ($10 \mu\text{g}$) lysates prepared from RKO cells 3 h after UVC irradiation. The levels of α -tubulin (a cytoplasmic protein), hnRNP C1/C2 (a nuclear protein), and β -actin in the same protein preparations were also assessed by Western blotting in order to monitor the quality of the fractionation and to detect any loading differences. Untr., untreated.

abundance in the IP material were not due to changes in overall mRNA levels, since these remained unaltered following UVC treatment (see Fig. 6A). These associations were further tested by synthesizing biotinylated transcripts corresponding to the CRs and 3'UTRs of these mRNAs and incubating them with whole-cell lysates prepared from RKO cells that were either left untreated or treated with UVC and collected at the times indicated. Pull-down of the biotinylated RNP complexes (see Materials and Methods) followed by Western blot analysis of TIAR in the pull-down material further revealed a time-dependent association of TIAR with the 3'UTRs of the transcripts in question, where the AREs reside (see Table S1 in the supplemental material), but showed no interaction with any of the CR transcripts or with the 3'UTR of GAPDH, which is not a target of TIAR and lacks AREs (Fig. 4B). Together, these observations suggest that the bind-

ing of TIAR to the 3'UTRs of several mRNAs encoding translation factors increases in a UVC-dependent manner.

Outcome of TIAR target mRNAs. In order to assess the functional consequences of the formation of these RNP complexes, RKO cells expressing endogenous or silenced TIAR levels were irradiated with UVC, an intervention that did not alter the cytoplasmic abundance (Fig. 3B) or the whole-cell levels of TIAR or TIA-1 (Fig. 1A). As anticipated, RT-PCR-amplified products corresponding to MYC, EIF4A1, EIF4E2, and EEF1B2 in the TIAR siRNA populations were poorly detectable from the TIAR siRNA populations but were readily amplified from the control siRNA group, particularly after UVC irradiation (Fig. 5A). These data indicated that the $\sim 10\%$ of TIAR remaining in the silenced cultures (Fig. 1A) was insufficient to recapitulate all of the binding activity seen in the presence of normal TIAR levels (Fig. 4A). As expected, little or no product

FIG. 4. UVC-dependent association of TIAR with transcripts encoding translational regulatory factors. (A) At the times indicated following UVC irradiation of RKO cells, lysates were prepared and IP assays were carried out using either IgG or TIAR-specific antibodies. The presence of MYC, EIF4A, EIF4E, and EEF1B mRNAs as well as the negative control GAPDH mRNA (a highly abundant mRNA that is present as a low-level contaminant in the IP materials, thus serving to monitor the equal input of lysate) was assessed by reverse transcription followed by either conventional PCR (visualized after electrophoresis in ethidium bromide-stained agarose gels) (top) or qPCR

was amplified in the IgG IP lanes, indicating that there was minimal background contamination of mRNA in the IP material (Fig. 5A). The reduction in cellular and SG-associated TIAR signals was also readily detectable by immunofluorescence (Fig. 5B). TIAR siRNA potentially lowered TIAR expression in both the nucleus and the cytoplasm, although SGs still formed in response to UVC irradiation, as detected by immunofluorescence using a TIA-1-reactive antibody (Fig. 5B; see Fig. S3 in the supplemental material).

The expression levels of mRNAs encoding MYC, EIF4E2, and EEF1B2 (as well as EIF4A1 [not shown]), as assessed by Northern blotting, did not change following UVC irradiation (Fig. 6A). By contrast, Western blot analysis of c-Myc, eIF4E, and eEF1B (no antibodies were available for detecting eIF4A) revealed that UVC treatment potentially inhibited the steady-state levels of these proteins at the late time points examined. Importantly, this inhibition was more pronounced under normal TIAR expression levels ("control siRNA" group), as the "TIAR siRNA" populations expressed higher levels of these proteins both before and after UVC irradiation (Fig. 6B). The finding that the basal levels of c-Myc, eIF4E, and eEF1B were elevated in the TIAR-silenced cultures at time zero (before exposure to UVC) supported the notion that TIAR repressed to some extent the translation of these proteins in unstimulated cultures, not only after UVC irradiation.

Since changes in both nascent translation and proteolysis could account for the observed differences in steady-state protein levels (Fig. 6B), we directly monitored protein translation by assessing nascent protein synthesis. To this end, cells were subjected to a brief (20-min-long) incubation in the presence of ^{35}S -labeled amino acids; immediately after labeling, cells were collected for IP, as previously described (see references 31 and 32), using antibodies that specifically recognized c-Myc, eIF4E, and eEF1B. While this labeling procedure is inefficient (lasting only 20 min) and hence yields relatively weak signals, it uniquely allows the measurement of true nascent translation, since any contributions from proteolysis are generally negligible during the short incubation period. As shown in Fig. 6C, the incorporation of radiolabeled amino acids was significantly lower for the three proteins after UVC irradiation in cells expressing normal TIAR levels ("control siRNA" group) but remained elevated in UVC-treated cells expressing reduced TIAR ("TIAR siRNA" group). It is important that the reduction in translation seen at 6 h (Fig. 6C) was not yet reflected in changes in steady-state protein levels on Western blots (6 h following UVC) (Fig. 6B), as the preexisting pool of c-Myc, eIF4E, and eEF1B had not yet been depleted.

The translational status of mRNAs encoding of MYC, EIF4A1, EIF4E2, EEF1B2, and GAPDH was also monitored by carrying

(bottom) (depicting the means and SEM from three independent experiments). (B) Schematic of the MYC, EIF4A, EIF4E, EEF1B, and GAPDH mRNAs, depicting the 5'UTR, CR (gray), and 3'UTR of each transcript. CR and 3'UTR biotinylated transcripts of each mRNA (heavy lines) were synthesized for testing in pull-down assays using whole-cell material prepared from RKO cultures at the times shown after UVC irradiation (see Materials and Methods). The association of TIAR to the biotinylated transcripts tested was detected by Western blotting; the biotinylated GAPDH 3'UTR was included as a negative control.

FIG. 5. TIAR levels after UVC irradiation. (A) After preparing lysates from RKO cells treated as described in the legend of Fig. 1A, IP assays (using either IgG or anti-TIAR antibodies) followed by RT and sequence-specific PCR amplification were used to assess the abundance of the mRNAs indicated (MYC, EIF4A, EIF4E, EEF1B, and GAPDH). Untr., untreated. (B) After transfection and treatment of cells as explained above (A), the expression and subcellular localization of TIAR and TIA-1 were studied by immunofluorescence using a confocal microscope; representative fields from three independent experiments are shown; SGs per cell in TIAR siRNA populations were quantified (see Fig. S4 in the supplemental material).

out sucrose gradient fractionations (see Materials and Methods) (see Fig. 8A) of control and TIAR-silenced cells that were either left untreated or UVC irradiated. After pooling fractions 1 to 3, representing the mRNA that was not associated with the

translation machinery ("nontranslating"), and fractions 5 to 11, representing the mRNA that was bound to the translation machinery ("translating"), qPCR was used to measure the abundance of specific mRNAs in each translation group (Fig. 7A) and verified when possible by Northern blotting (Fig. 7B). When the distribution of these mRNAs in the siRNA transfection groups was compared, MYC, EIF4A1, EIF4E2, and EEF1B2 mRNAs were significantly more abundant in the translating than in the nontranslating fractions, particularly after UVC irradiation; control GAPDH mRNA levels were virtually unchanged (Fig. 7A). To further document the changes in the relative distribution of these mRNAs along sucrose gradients, the MYC and EEF1B2 mRNAs in each gradient fraction of each transfection and treatment group were monitored by Northern blotting (Fig. 7B). Despite the intrinsic variability in the analysis of RNA data derived from polysome gradients (particularly coming from transfected and stress-treated populations), the inhibitory influence of TIAR upon its target mRNAs was also readily observed by Northern blotting (Fig. 7B). Collectively, these findings indicate that TIAR contributed to the translational repression of target mRNAs encoding c-Myc, eIF4E, and eEF1B.

Contribution of TIAR to global translational repression. Given the TIAR-mediated inhibition of the expression of c-Myc and translation factors eIF4E and eEF1B (and likely also eIF4A and other factors encoded by TIAR target mRNAs), we hypothesized that TIAR might elicit a global inhibition of translation lasting beyond the transient suppression that is brought upon by the formation of SGs. To test this possibility, polysome distribution profiles were prepared from cultures 6 h after UVC irradiation, when SGs were no longer detectable (Fig. 3 and data not shown). Indeed, such profiles were consistent with a translational repression by 6 h after UVC irradiation in cultures expressing normal TIAR levels (Fig. 8A). By contrast, cultures with reduced TIAR levels displayed a higher content of polysomes, consistent with a higher rate of protein translation (Fig. 8A). Moreover, assessment of the incorporation of ^{35} S-labeled amino acids into nascent protein, another method to monitor protein biosynthesis, further confirmed that both the basal translation and the UVC-inhibited translation were higher in cells expressing low TIAR levels (Fig. 8B), lending additional support to the notion that TIAR suppresses general protein translation in both unstressed and stress-treated cells. Notably, the inhibition of translation factor expression (Fig. 6B) occurred later than the inhibition of general cellular translation (Fig. 8B), suggesting that other UVC-triggered changes in the translational machinery would elicit the early inhibition of protein translation. In sum, our findings support the notion that TIAR contributes to maintaining a translationally repressed state in cells exposed to stress agents. We propose that the prolonged inhibition of protein translation is implemented, at least in part, through a TIAR-mediated decrease of the expression of translation factors.

DISCUSSION

By enhancing the association of noncanonical 48S ribosomal subunits onto mRNAs, TIAR has been postulated to repress translation under two distinct scenarios: in the absence of

FIG. 6. TIAR influence on the expression of c-Myc and translation factors. RKO cells in each siRNA transfection group (control [Ctrl.] and TIAR) were irradiated (20 J/m² UVC), and the expression levels of c-Myc and translation factors were assessed. (A) Northern blot analysis of the levels of MYC, EIF4E, and EEF1B mRNAs. GAPDH mRNA and 18S rRNA signals were also tested in order to monitor differences in loading and transfer of samples; shown are representative signals from two independent experiments. (B) At the times shown following UVC irradiation, the levels of c-Myc, eIF4E, eEF1B, and β-actin were detected by Western blot analysis in RKO cells expressing either normal levels of TIAR ("Ctrl. siRNA") or reduced TIAR levels ("TIAR siRNA"). Shown are representative signals from several experiments. (C) Assessment of nascent translation of c-Myc, eIF4E, and eEF1B by 6 h after UVC irradiation; signals indicate the relative intensity of the corresponding ³⁵S-labeled proteins after incubating the cells with L-[³⁵S]methionine and L-[³⁵S]cysteine for 20 min, preparing lysates, and carrying out IP with the corresponding antibodies (representative control "IgG" IP assays are indicated). Representative radiolabeled signals from three independent experiments are shown. In B and C, quantification of the signals is expressed as the percentage of the signal intensity relative to that in untreated cells in the control siRNA group.

stress, it prevents the translation of target ARE-containing mRNAs (such as those encoding TNF-α, MMP13, Cox-2, and β₂-adrenergic receptor) (12, 18, 20, 45), whereas following stress, it transiently suppresses the translation of general mRNA pools (1). The latter set of events is triggered when stress-activated kinases phosphorylate eIF2α and thereby reduce the eIF2-GTP-tRNA^{Met} that is required to load tRNA^{Met} onto the 40S ribosomal subunit to initiate protein synthesis. Under such conditions, TIA-1 and TIAR have been proposed to promote the assembly of translationally inactive preinitiation complexes (containing the small ribosomal subunit but lacking eIF2 and eIF5) and aggregate in SGs.

Our findings presented here provide experimental support for these two proposed functions of TIAR and expand its role to one of extension of general translational repression following stress, well after the disappearance of SGs. These functions are supported by evidence provided in this study that (i) SGs were readily visible by 3 h after UVC irradiation but disappeared almost completely by 6 h after UVC irradiation (Fig. 3A); (ii) global translation was repressed following UVC irradiation, while eIF2α phosphorylation, although readily detectable in both untreated and UVC-irradiated cells, was not noticeably elevated by UVC treatment and hence did not appear to significantly repress translation (Fig. 1C); (iii) silencing of TIAR caused translation to remain elevated, both in untreated and in UVC-irradiated cells (Fig. 1 and 7); and (iv) TIAR associated with mRNAs encoding several translation factors, components of the translation machinery, and other proteins that regulate translation (including eIF4A, eIF4E, eEF1B, and c-Myc) in a UVC-dependent manner and repressed their translation (Fig. 2, 4, and 6). Based on these observations, we postulate that in response to cellular stress, TIAR contributes to a global repression of translation via two sequential sets of events. First, TIAR would promote the general repression of translation through the transient assembly of SGs; these effects might occur in tandem with eIF2α phosphorylation, which reportedly increase after UVC phosphorylation (14, 44) and need not be strongly elevated in order to cause translational repression (33). Afterwards, despite the disappearance of SGs and the unchanged levels of eIF2α phosphorylation, the increased association of TIAR with target mRNAs encoding c-Myc, which regulates many translation control proteins, as well as translation factors (including eIF4E, eEF1B, and likely also eIF4A and other factors) (Fig. 2), would specifically block the translation of these proteins, causing their levels to drop. In turn, the reduced levels of translational initiation and elongation factors would contribute to the extension of a translationally repressed state until the cell has repaired the damage brought upon by the stress agent (Fig. 8B).

In the aforementioned study by Wu et al. (44), UVC irradiation was reported to inhibit global translation in various human and rodent cell lines, an effect that was shown to be specifically dependent on the phosphorylation of eIF2α by the endoplasmic reticulum-localized kinase PERK. However, the PERK-elicited inhibition was incomplete, since expression of a dominant negative variant (PERKΔC) only partially restored protein synthesis; accordingly, 24 h after UVC irradiation, translation was suppressed by 18% in the presence of wild-type PERK, but it was relieved to 50% of

FIG. 7. Relative distribution of TIAR target mRNAs on polysome gradients. (A) Cytoplasmic lysates from RKO cells that had been either UVC irradiated 6 h earlier or left unirradiated were size fractionated through sucrose gradients, as described in the legend of Fig. 8A. RNA was prepared from all 11 fractions and pooled: fractions 1 to 3 comprised the nontranslating RNA, and fractions 5 to 11 comprised the translating RNA. The levels of mRNAs encoding MYC, EIF4A1, EIF4E2, EEF1B2, and housekeeping GAPDH in the pooled RNA groups were calculated by RT of the pooled RNA followed by qPCR. The data are represented as percent mRNA in nontranslating and translating fractions. (B) Cells in each siRNA transfection group (control [Ctrl.] and TIAR) were either left untreated (Untr.) or irradiated with UVC and collected 6 h later, whereupon cell lysates were prepared and fractionated. RNA was isolated from each fraction in each transfection and treatment group, and Northern blot analysis was performed to determine the relative distribution of MYC (top) and EEF1B2 (bottom) mRNAs along the gradients; shown are representative Northern blots from two independent experiments. For the graphs, the Northern signals were quantified, and the relative mRNA abundance in each fraction was represented as a percentage of the total signal for that group (all fractions combined).

translation in cells expressing PERKΔC (44). While those investigators employed different cell types and higher doses of UVC than we used, it is plausible that TIAR-dependent events (i.e., suppressed expression of eIF4E, eEF1B, eIF4A, and c-Myc) contribute, at least in part, to the fraction of translational inhibition that was PERK independent, particularly at late time points following UVC treatment. A formal analysis of these possibilities awaits further study.

FIG. 8. TIAR influence on global translation. (A) Six hours after irradiation (20 J/m^2 UVC) of RKO cells in each siRNA transfection group (control [Ctrl.] and TIAR), cytoplasmic lysates were prepared and fractionated over sucrose gradients. From left to right, the distribution of free mRNA (not bound to ribosomes or ribosome subunits), ribosome subunits 40S and 60S, as well as monosomes (single ribosomes) and polysomes of increasing molecular weight is indicated. RNA was prepared from each fraction, and the relative intensity of the rRNA molecules is shown. Untr., untreated. (B) At the times shown following UVC irradiation, RKO cells were incubated for 20 min with ^{35}S -labeled amino acids. Lysate aliquots corresponding to the same cell numbers were size fractionated by SDS-PAGE, transferred onto PVDF membranes, and visualized and quantified using a PhosphorImager (top) or they were subjected to TCA precipitation, and the ^{35}S -amino acid incorporation was quantified (graph) and is presented as percent signal intensity relative to time zero in the control siRNA group; the data shown are the means and SEM from five independent experiments.

Much remains to be learned about SGs, including their genesis, function, and disassembly. It is formally possible that the aggregated preinitiation complexes that give rise to SGs in response to cell damage (peaking in size by about 3 h after UVC irradiation, as determined by immunofluorescence) (Fig. 3A) subsequently disassemble but remain as noncanonical, translationally silent 48S associations that are undetectable by fluorescence microscopy. Nonetheless, the finding that lowering of the TIAR levels effectively increased global translation in both untreated and UVC-stimulated cells (Fig. 1 and 7) suggests that TIAR is involved in translational repression both constitutively and in response to cellular injury. The inhibitory influence of TIAR on the expression of the translation factors identified here (eIF4E and eEF1B) as well as c-Myc (Fig. 4 and 6) likely contributes to the global translational repression of TIAR. The expression levels of proteins encoded by additional TIAR target mRNAs (Fig. 2 and additional transcripts identified on the microarrays [<http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE1433>]) and their possible involvement in the TIAR-mediated translational repression are also under investigation.

The precise mechanisms whereby TIAR binds to and represses the translation of specific target ARE-containing mRNAs also remain to be fully elucidated. The findings that TIAR abundance (whole-cell and cytoplasmic levels) (Fig. 3) remained unchanged after UVC irradiation while binding of cytoplasmic TIAR to target transcripts increased dramatically following stress (Fig. 4) suggest that TIAR may function in a manner different from that of HuR, another ARE-RBP that also modulates the translation of target mRNAs. In the case of HuR, which is almost exclusively nuclear in unstimulated cells, both its cytoplasmic abundance and its association with many target transcripts increase in response to stress agents (41). These findings have prompted researchers in the field to propose a model whereby HuR binds target mRNAs in the nucleus and contributes to their export to the cytoplasm, where it modulates their stability and translation (8, 25). Although no changes in cytoplasmic TIAR levels were seen here following UVC irradiation, a possible dynamic shuttle of TIAR between the nucleus and cytoplasm could effectively prevent a net increase of TIAR in either compartment. Thus, it has yet to be determined whether TIAR associates with target mRNAs destined for translational suppression while the mRNA is still in the nucleus, possibly during splicing (a process in which TIAR and TIA-1 actively participate) (15, 27, 37) of a pre-mRNA precursor, and whether it plays a further role in the export of target mRNAs. The alternative possibility that TIAR associates with target mRNAs only in the cytoplasm also awaits further study. Given that the TIAR target ARE-mRNAs previously described (such as cyclooxygenase 2, MMP13, and TNF- α) are also targets of other ARE-RBPs, it will be particularly interesting to examine whether TIAR competes or cooperates with other ARE-RBPs associating with common target mRNAs and whether stress stimuli influence these interactions. Finally, the analysis of possible posttranslational modifications of TIAR that modulate its association with target mRNAs is likely to be a fruitful area of future pursuit.

It is important that in populations in which TIAR was knocked down, SGs were still detected when an anti-TIA-1

antibody was used, although there appeared to be a slight delay in SG formation (see Fig. S4 in the supplemental material), suggesting that both proteins are required for optimal assembly of SGs. Whereas TIA-1 and TIAR appear to have somewhat interchangeable roles in SG formation, their relative affinity for target mRNAs has not been studied systematically. En masse analyses to identify TIAR and TIA-1 target mRNAs under way in our laboratory suggest the existence of both shared and specific target ARE-containing transcripts (30; data not shown), in keeping with the notion that TIAR and TIA-1 are functionally distinct (13, 42). Further efforts to elucidate the composition and function of RNP complexes involving TIAR, TIA-1, and other ARE-RBPs will decisively advance our understanding of the translational control of gene expression triggered by cell damage.

ACKNOWLEDGMENTS

We thank K. G. Becker and the NIA Array Facility for providing cDNA arrays for analysis and S. Galbán and R. van Huizen for valuable discussions.

This research was supported by the Intramural Research Program of the NIA, NIH.

REFERENCES

- Anderson, P., and N. Kedersha. 2002. Visibly stressed: the role of eIF2, TIA-1, and stress granules in protein translation. *Cell Stress Chaperones* 7:213–221.
- Anderson, P., and N. Kedersha. 2002. Stressful initiations. *J. Cell Sci.* 115: 3227–3234.
- Anderson, P., K. Phillips, G. Stoecklin, and N. Kedersha. 2004. Post-transcriptional regulation of proinflammatory proteins. *J. Leukoc. Biol.* 76:42–47.
- Antic, D., and J. D. Keene. 1997. Embryonic lethal abnormal visual RNA-binding proteins involved in growth, differentiation, and posttranscriptional gene expression. *Am. J. Hum. Genet.* 61:273–278.
- Bevilacqua, A., M. C. Ceriani, S. Capaccioli, and A. Nicolini. 2003. Post-transcriptional regulation of gene expression by degradation of messenger RNAs. *J. Cell Physiol.* 195:356–372.
- Bordeleau, M.-E., J. Matthews, J. M. Wojnar, L. Lindqvist, O. Novac, E. Jankowsky, N. Sonenberg, P. Northcote, P. Teesdale-Spittle, and J. Pelletier. 2005. Stimulation of mammalian translation initiation factor eIF4A activity by a small molecule inhibitor of eukaryotic translation. *Proc. Natl. Acad. Sci. USA* 102:10460–10465.
- Bourne, H. R., D. A. Sanders, and F. McCormick. 1990. The GTPase superfamily: a conserved switch for diverse cell functions. *Nature* 348:125–132.
- Brennan, C. M., and J. A. Steitz. 2001. HuR and mRNA stability. *Cell. Mol. Life Sci.* 58:266–277.
- Browne, G. J., and C. G. Proud. 2002. Regulation of peptide-chain elongation in mammalian cells. *Eur. J. Biochem.* 269:5360–5368.
- Carballo, E., W. S. Lai, and P. J. Blackshear. 1998. Feedback inhibition of macrophage tumor necrosis factor- α production by tristetraprolin. *Science* 281:1001–1005.
- Chen, C.-Y., and A.-B. Shyu. 1995. AU-rich elements: characterization and importance in mRNA degradation. *Trends Biochem. Sci.* 20:465–470.
- Cok, S. J., S. J. Acton, and A. R. Morrison. 2003. The proximal region of the 3'-untranslated region of cyclooxygenase-2 is recognized by a multimeric protein complex containing HuR, TIA-1, TIAR, and the heterogeneous nuclear ribonucleoprotein U. *J. Biol. Chem.* 278:36157–36162.
- Dember, L. M., N. D. Kim, K. Q. Liu, and P. Anderson. 1996. Individual RNA recognition motifs of TIA-1 and TIAR have different RNA binding specificities. *J. Biol. Chem.* 271:2783–2788.
- Deng, J., H. P. Harding, B. Raught, A. C. Gingras, J. J. Berlanga, D. Scheuner, R. J. Kaufman, D. Ron, and N. Sonenberg. 2002. Activation of GCN2 in UV-irradiated cells inhibits translation. *Curr. Biol.* 12:1279–1286.
- Forch, P., and J. Valcarcel. 2001. Molecular mechanisms of gene expression regulation by the apoptosis-promoting protein TIA-1. *Apoptosis* 6:463–468.
- Galbán, S., J. L. Martindale, K. Mazan-Mamczarz, I. López de Silanes, J. Fan, W. Wang, J. Decker, and M. Gorospe. 2003. Influence of the RNA-binding protein HuR in pVHL-regulated p53 expression in renal carcinoma cells. *Mol. Cell. Biol.* 23:7083–7095.
- Grifo, J. A., R. D. Abramson, C. A. Satler, and W. C. Merrick. 1984. RNA-stimulated ATPase activity of eukaryotic initiation factors. *J. Biol. Chem.* 259:8648–8654.
- Gueydan, C., L. Droogmans, P. Chalon, G. Huez, D. Caput, and V. Kruys. 1999. Identification of TIAR as a protein binding to the translational regulatory AU-rich element of tumor necrosis factor α mRNA. *J. Biol. Chem.* 274:2322–2326.
- Holcik, M., and N. Sonenberg. 2005. Translational control in stress and apoptosis. *Nat. Rev. Mol. Cell Biol.* 6:318–327.
- Kandasamy, K., K. Joseph, K. Subramaniam, J. R. Raymond, and B. G. Tholanikunnel. 2005. Translational control of beta2-adrenergic receptor mRNA by T-cell-restricted intracellular antigen-related protein. *J. Biol. Chem.* 280:1931–1943.
- Kedersha, N., M. R. Cho, W. Li, P. W. Yacono, S. Chen, N. Gilks, D. E. Golan, and P. Anderson. 2000. Dynamic shuttling of TIA-1 accompanies the recruitment of mRNA to mammalian stress granules. *J. Cell Biol.* 151:1257–1268.
- Kedersha, N., G. Stoecklin, M. Ayodele, P. Yacono, J. Lykke-Andersen, M. J. Fitzler, D. Scheuner, R. J. Kaufman, D. E. Golan, and P. Anderson. 2005. Stress granules and processing bodies are dynamically linked sites of mRNP remodeling. *J. Cell Biol.* 169:871–884.
- Kedersha, N. L., M. Gupta, W. Li, I. Miller, and P. Anderson. 1999. RNA-binding proteins TIA-1 and TIAR link the phosphorylation of eIF-2 α to the assembly of mammalian stress granules. *J. Cell Biol.* 147:1431–1442.
- Kedersha, N. L., and P. Anderson. 2002. Stress granules: sites of mRNA triage that regulate mRNA stability and translatability. *Biochem. Soc. Trans.* 30:963–969.
- Keene, J. D. 1999. Why is Hu where? Shuttling of early-response-gene messenger RNA subsets. *Proc. Natl. Acad. Sci. USA* 96:5–7.
- Lal, A., T. Kawai, X. Yang, K. Mazan-Mamczarz, and M. Gorospe. 2005. Antiapoptotic function of RNA-binding protein HuR effected through prothymosin α . *EMBO J.* 24:1852–1862.
- Le Guiner, C., F. Lejeune, D. Galiana, L. Kister, R. Breathnach, J. Stevenin, and F. Del Gatto-Konczak. 2001. TIA-1 and TIAR activate splicing of alternative exons with weak 5' splice sites followed by a U-rich stretch on their own pre-mRNAs. *J. Biol. Chem.* 276:40638–40646.
- Loflin, P., C. Y. Chen, and A.-B. Shyu. 1999. Unraveling a cytoplasmic role for hnRNP D in the in vivo mRNA destabilization directed by the AU-rich element. *Genes Dev.* 13:1884–1897.
- López de Silanes, I., M. Zhan, A. Lal, X. Yang, and M. Gorospe. 2004. Identification of a target RNA motif for RNA-binding protein HuR. *Proc. Natl. Acad. Sci. USA* 101:2987–2992.
- López de Silanes, I., S. Galbán, J. L. Martindale, X. Yang, K. Mazan-Mamczarz, F. E. Indig, G. Falco, M. Zhan, and M. Gorospe. 2005. Identification and functional outcome of mRNAs associated with TIA-1 ribonucleoprotein complexes. *Mol. Cell. Biol.* 25:9520–9531.
- Mazan-Mamczarz, K., S. Galbán, I. López de Silanes, J. L. Martindale, U. Atasoy, J. D. Keene, and M. Gorospe. 2003. RNA-binding protein HuR enhances p53 translation in response to ultraviolet light irradiation. *Proc. Natl. Acad. Sci. USA* 100:8354–8359.
- Mazan-Mamczarz, K., T. Kawai, J. L. Martindale, and M. Gorospe. 2005. *En masse* analysis of nascent translation using microarrays. *BioTechniques* 39: 61–67.
- Merrick, W. C. 1992. Mechanism and regulation of eukaryotic protein synthesis. *Microbiol. Rev.* 56:291–315.
- Olarewaju, O., P. A. Ortiz, W. Q. Chowdhury, I. Chatterjee, and T. G. Kinzy. 2004. The translation elongation factor eEF1B plays a role in the oxidative stress response pathway. *RNA Biol.* 1:89–94.
- Piecyk, M., S. Wax, A. R. Beck, N. Kedersha, M. Gupta, B. Maritim, S. Chen, C. Gueydan, V. Kruys, M. Streuli, and P. Anderson. 2000. TIA-1 is a translational silencer that selectively regulates the expression of TNF- α . *EMBO J.* 19:4154–4163.
- Schmidt, E. V. 2004. The role of c-myc in regulation of translation initiation. *Oncogene* 23:3217–3221.
- Shukla, S., W. P. Dirksen, K. M. Joyce, C. Le Guiner-Blanvillain, R. Breathnach, and S. A. Fisher. 2004. TIA proteins are necessary but not sufficient for the tissue-specific splicing of the myosin phosphatase targeting subunit 1. *J. Biol. Chem.* 279:13668–13676.
- Sonenberg, N., and T. E. Dever. 2003. Eukaryotic translation initiation factors and regulators. *Curr. Opin. Struct. Biol.* 13:56–63.
- Stoecklin, G., M. Colombi, I. Rainieri, S. Leuenberger, M. Mallaun, M. Schmidlin, B. Gross, M. Lu, T. Kitamura, and C. Moroni. 2002. Functional cloning of BRF1, a regulator of ARE-dependent mRNA turnover. *EMBO J.* 21:4709–4718.
- Tenenbaum, S. A., P. J. Lager, C. C. Carson, and J. D. Keene. 2002. Ribonucleosomes: identifying mRNA subsets in mRNP complexes using antibodies to RNA-binding proteins and genomic arrays. *Methods* 26:191–198.
- Wang, W., H. Furneaux, H. Cheng, M. C. Caldwell, D. Hutter, Y. Liu, N. J. Holbrook, and M. Gorospe. 2000. HuR regulates p21 mRNA stabilization by ultraviolet light. *Mol. Cell. Biol.* 20:760–769.
- Wilkie, G. S., K. S. Dickson, and N. K. Gray. 2003. Regulation of mRNA translation by 5'- and 3'-UTR-binding factors. *Trends Biochem. Sci.* 28:182–188.
- Wilusz, C. J., and J. Wilusz. 2004. Bringing the role of mRNA decay in the control of gene expression into focus. *Trends Genet.* 20:491–497.
- Wu, S., Y. Hu, J.-L. Wang, M. Chatterjee, Y. Shi, and R. J. Kaufman. 2002.

- Ultraviolet light inhibits translation through activation of the unfolded protein response kinase PERK in the lumen of the endoplasmic reticulum. *J. Biol. Chem.* **277**:18077–18083.
45. **Yu, Q., S. J. Cok, C. Zeng, and A. R. Morrison.** 2003. Translational repression of human matrix metalloproteinase-13 by an alternatively spliced form of T-cell-restricted intracellular antigen-related protein (TIAR). *J. Biol. Chem.* **278**:1579–1584.
46. **Zhang, T., V. Kruys, G. Huez, and C. Gueydan.** 2002. AU-rich element-mediated translational control: complexity and multiple activities of trans-activating factors. *Biochem. Soc. Trans.* **30**:952–958.
47. **Zhang, W., B. J. Wagner, K. Ehrenman, A. W. Schaefer, C. T. DeMaria, D. Crater, K. DeHaven, L. Long, and G. Brewer.** 1993. Purification, characterization, and cDNA cloning of an AU-rich element RNA-binding protein, AUF1. *Mol. Cell. Biol.* **13**:7652–7665.