T-cell Intracellular Antigen-1 (TIA-1)-induced Translational Silencing Promotes the Decay of Selected mRNAs*

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Gene array analysis revealed that a subset of mRNAs overexpressed in macrophages lacking the destabilizing factor TTP are also overexpressed in macrophages lacking the translational silencer TIA-1. We confirmed that a representative transcript, apobec-1, is significantly stabilized in cells lacking TIA-1. Tethering TIA-1 to a reporter transcript also promotes mRNA decay, suggesting that TIA-1-mediated translational silencing can render mRNA susceptible to the decay machinery. TIA-1-mediated decay is inhibited by small interfering RNAs targeting components of either the 5′-3′ (e.g. DCP2) or the 3′-5′ (e.g. exosome component Rrp46) decay pathways, suggesting that TIA-1 renders mRNA susceptible to both major decay pathways. TIA-1-mediated decay is inhibited by cycloheximide and emetine, drugs that stabilize polysomes, but is unaffected by puromycin, a drug that disassembles polysomes. These results suggest that TIA-1-induced polysome disassembly is required for enhanced mRNA decay and that TIA-1-induced translational silencing promotes the decay of selected mRNAs.

TIA-1 and TIAR are related proteins that bind to cis elements found in the 3′-untranslated region of selected mRNAs (1). TIA-1/R bind to at least three different sequence motifs: the adenine/uridine-rich elements (AREs) found in a variety of unstable transcripts (1); cytosine/uridine-rich elements identified using SELEX analysis (2); and a hairpin consensus motif identified in gene array studies (3). Interactions between TIA-1/R and ARE-containing transcripts have demonstrated physiological importance in inflammation, as TIA-1/R targets include several ARE-containing transcripts that encode pro-inflammatory proteins (4, 5). Macrophages obtained from mice lacking TIA-1 or TIP overexpress pro-inflammatory proteins such as TNFα, interleukin-1β, interleukin-6, matrix metalloproteinase, and cyclooxygenase-2 (5–10). Studies using mutant mice reveal that TIA-1 and TIAR dampen the expression of proteins encoded by ARE-containing transcripts without affecting mRNA levels (5). TIA-1 and TIAR reduce the expression of these proteins by holding their mRNAs in a translationally repressed state. Mice lacking TIA-1 develop mild arthritis, a reflection of their hyper-inflammatory state (11).

TIA-1 and TIAR also participate in the stress-induced translational arrest observed in cells subjected to noxious stimuli. Eukaryotic cells exposed to heat, UV irradiation, or oxidative conditions reprogram mRNA translation to allow the selective synthesis of stress response and repair proteins (12). Under these conditions, the translation of housekeeping proteins is turned off and the mRNAs encoding these proteins move from polysomes to discrete cytoplasmic foci known as stress granules (SGs) (13). TIA-1 and TIAR promote the assembly of a non-canonical 48S preinitiation complex that is the core component of SGs (14). SG assembly and disassembly play a central role in reprogramming mRNA translation and decay both in stressed cells and in cells that are recovering from stress (13).

SGs are spatially and compositionally linked to processing bodies (P Bs), related RNA granules that regulate mRNA translation and decay in both stressed and unstressed cells (15). SGs and PBs coordinately regulate mRNA translation and decay, thus emphasizing the connection between these processes (13). The ability of mRNA to move from polysomes to either SGs (16) or PBs (17) suggests that mRNA exists in a dynamic equilibrium between a translating state (i.e. polysomes) and a translationally repressed state (e.g. SGs and PBs). Several factors that repress translation by disassembling polysomes promote delivery of mRNA into SGs and/or PBs. In yeast, Dhh1 and Pat1p have been shown to drive mRNA from polysomes to PBs (18). In mammalian cells, drugs such as puromycin disassemble polysomes and deliver mRNA to SGs (16). This equilibrium between the translated and the repressed state predicts that translational silencers such as TIA-1 and TIAR disassemble polysomes and thus make untranslated mRNAs available for degradation at PBs. Consistent with this hypothesis, we have found that macrophages lacking either TIA-1 or TTP overexpress a common subset of mRNAs. Here we provide evidence that TIA-1-induced translational silencing concomitantly promotes the decay of selected mRNAs.

EXPERIMENTAL PROCEDURES

Microarray Analysis—Thioglycolate-elicited peritoneal macrophages were collected from wild type, TIA-1 knock-out (TIA-1−/−) (5), and TTP knock-out (TTP−/−) (19) mice by peritoneal lavage and purified by magnetic cell sorting (CD11b microbeads; Miltenyi Biotec). Isolated cells contain >95% mac-
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Northern Blot Analysis—Total RNA was extracted from subconfluent 10-cm dishes using TRIzol (Invitrogen), and 10 μg of RNA was resolved using 1.1% agarose/2% formaldehyde MOPS gel electrophoresis, blotted onto Nytran Supercharge membranes (Schleicher and Schuell) using 8X SSC, and hybridized overnight at 50 °C with digoxigenin-labeled DNA probes in digoxigenin Easy Hyb solution (Roche Applied Science). After washing at 60 °C with 2X SSC/0.1% SDS (10 min) and 0.5X SSC/0.1% SDS (20 min, 2 times), the membranes were blocked in blocking reagent (Roche Applied Science) for 30 min at room temperature, probed with alkaline phosphatase-labeled anti-digoxigenin antibody (Roche Applied Science) for 30 min, and washed for 30 min with 130 mM Tris-HCl, pH 7.5/100 mM NaCl/0.3% Tween 20. Signals were visualized with CDP-Star (Roche Applied Science). Probes were generated by PCR using digoxigenin-labeled nucleotides (Roche Applied Science) globin and nucleolin cDNAs and the primer pairs G29/G30 and G83/G84, respectively.

Fluorescence Microscopy—Immunofluorescence was performed as described previously (16). Cells grown on coverslips were fixed for 10 min in 4% paraformaldehyde in phosphate-buffered saline, pH 7.4, followed by post-fixation for 10 min at −20 °C in methanol. The fixed cells were stored in 70% ethanol at 4 °C until use. Coverslips were rinsed two times for 5 min in 2X SSC on an orbital shaker. The cells were hybridized with probes complementary to globin in situ hybridization buffer (Ambion) at 45 °C for 4 h. After washing with 4X SSC for 5 min, the coverslips were washed twice more with 2X SSC for 5 min and then incubated with gentle shaking for 1 h at room temperature in 2X SSC containing 0.1% Triton X-100 and the indicated antibody. Coverslips were then washed with 2X SSC three times for 5 min each and incubated with gentle shaking for 1 h at room temperature in 2X SSC containing 0.1% Triton X and the developing antibody (Jackson ImmunoResearch Laboratories). After washing with 2X SSC three times for 5 min each, coverslips were mounted in a polyvinyl-based mounting medium. Cells were observed using a Nikon Eclipse 800 microscope, and images were digitally captured using a CDD-SPOT RT digital camera and compiled using Adobe® Photoshop® software (v6.0). Human anti-GW182 (Advanced Diagnostics Laboratory) (15) and mouse anti-HA were used to visualize processing bodies and recombinant HA-TIA-1, respectively.

RNA Interference—HeLa cells were transfected with 40 nm siRNA duplexes using Lipofectamine 2000 (Invitrogen). After 24 h, cells were re-seeded and transfected with 20 nm siRNA again for another 24 h. In decay experiments, pTet-T7-βglobin reporters and pcDNA3-HA-TIA with or without PP7cp plasmids in presence of Tet-off plasmids were co-transfected with the second siRNA. siRNAs were purchased from Ambion. The following target sequences (sense strand) were chosen.

U0 (control): 5′-GAAUGCUAUGUUAGAUCA-3′; D0 (control), 5′-GCAUUCAGUAGAAAGUA-3′; C1 (dcp1), 5′-GCAAGCUGUGAUAUAAU-3′; D2 (dcp2), 5′-GAA-AUGCCCUAGUAUAGA-3′; D3 (dcp2), 5′-GUAACAGUU-AACCUAU-A-3′; X1 (xrn1), 5′-GUAUGAUGUUCACUUUGA-3′; X2 (xrn1), 5′-AGAUGAACUUACCCUGAGAA-3′; P6 (rrp46), 5′-GCAAAGAGAUUUUCAACAA-3′; P7 (rrp46), 5′-CAACACGCUUCGCUUUC-3′.

Cell Culture and Transfection—HeLa cells or COS cells were cultured in Dulbecco’s modified Eagle’s medium (Invitrogen) supplemented with 10% fetal calf serum (Sigma), penicillin (100 units/ml), and streptomycin (100 μg/ml). Lipofectamine 2000 (Invitrogen) and OptiMem medium (Invitrogen) were used for transfection of plasmids and/or small interfering RNAs. In reporter mRNA decay analysis, HeLa cells were cultured in Dulbecco’s modified Eagle’s medium containing 5% Tet system-approved fetal bovine serum (BD Biosciences) for 24 h prior to the addition of doxycycline (10 μg/ml; Sigma). Sodium arsenite (0.5 mM; Sigma) was added in conditioned medium to induce stress granules and processing bodies. TIA-1−/− and TIA-1+/+ cell lines were generated in our laboratory as described previously (20). 5,6-Dichlorobenzimidazole 1-arsenite (0.5 mM; Sigma) was added in conditioned medium to induce stress granules and processing bodies and recombinant HA-TIA-1, respectively.

Plasmid Constructs—For pcDNA3-HA-PP7 coat protein, cDNA was amplified by PCR using primers G87 and G88 and ligated into the KpnI-EcoRI sites of pcDNA3-HA. TIA-1 was excised from pcDNA3-HA-TIA-1 with BamH1 and Xhol and inserted into the BamHI-XhoI sites of pcDNA3-HA-PP7cp. For pTet-T7-βglobin-PP7bs, six repeats of the PP7bs contained in globin-PP7bs were ligated into the KpnI-EcoRI sites of pcDNA3-HA. TIA-1 was cDNA was amplified by PCR using primers G83/G84 and then incubated with gentle shaking for 1 h at room temperature in 2X SSC containing 0.1% Triton X-100 and the indicated antibody. Coverslips were then washed with 2X SSC three times for 5 min each and incubated with gentle shaking for 1 h at room temperature in 2X SSC containing 0.1% Triton X and the developing antibody (Jackson ImmunoResearch Laboratories). After washing with 2X SSC three times for 5 min each, coverslips were mounted in a polyvinyl-based mounting medium. Cells were observed using a Nikon Eclipse 800 microscope, and images were digitally captured using a CDD-SPOT RT digital camera and compiled using Adobe® Photoshop® software (v6.0). Human anti-GW182 (Advanced Diagnostics Laboratory) (15) and mouse anti-HA were used to visualize processing bodies and recombinant HA-TIA-1, respectively.

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Western Blot Analysis—Proteins were separated on 4–20% polyacrylamide gradient Tris-glycine gels and Western blots performed as described previously (16). Primary antibodies used were as follows: mouse anti-HA (HA.11; Covance), mouse anti-T7 tag (Novagen), rabbit anti-Dcp1, rabbit anti-Xrn1 (22), and rabbit anti-Dcp2 (23). Anti-Rrp46 antibody was kindly provided by Dr. Geurt Schilders (Nijmegen Center for Molecular Life Sciences).
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**RESULTS**

**Gene Array Analysis**—Cooperative interactions between the ARE-binding proteins TIA-1 and TTP regulate the expression of pro-inflammatory proteins in activated macrophages (1). TIA-1 inhibits the translation of ARE-containing transcripts (5), whereas TTP promotes the degradation of ARE-containing transcripts (24). In yeast, several proteins that inhibit mRNA translation coordinate proactively promote mRNA decay (25), suggesting that TIA-1-induced translational silencing might promote the decay of some transcripts. To test this hypothesis, we compared mRNA expression profiles in unactivated or LPS-activated macrophages derived from wild type, TIA-1−/−, and TTP−/− mice. Analysis of both unactivated and activated macrophages allowed us to control for the LPS-induced induction of TTP expression in these cells. The relative expression (TIA-1−/− versus WT; TTP−/− versus WT) of the genes most highly overexpressed in unactivated macrophages is plotted in Fig. 1A, red circles in both unactivated and LPS-activated macrophages are provided in Fig. 1B. We then used real-time PCR to quantify the relative expression of individual transcripts normalized to β-actin, a transcript expressed at similar levels in WT, TIA-1−/− or TTP−/− macrophages, (Fig. 1C). This analysis confirms that some transcripts (e.g. apobec-1, edr, and acs14) identified in the array analysis are overexpressed in TIA-1−/− macrophages compared with wild type controls, whereas others (e.g. eps8, hp, otud4, fmr1, and hiatt11) are not. The most highly overexpressed transcript encodes apobec-1, an RNA-editing enzyme that regulates the expression of lipoproteins (26). The 3′-untranslated region of this transcript includes an “AUUUA” pentamer, a minimal element for the recruitment of ARE-binding proteins. We confirmed that the average relative expression (n = three independent mice) of apobec-1 (compared with β-actin) is significantly greater (p < 0.05) in TIA-1−/− compared with TIA-1+/− macrophages (Fig. 1D), suggesting that TIA-1 can regulate the expression of this transcript. We next compared the half-life of apobec-1 mRNA in MEFs derived from wild type or TIA-1−/− mice. Cells were cultured for the indicated times in the presence of DRB (Fig. 2, A and B) or actinomycin D (Fig. 2, C and D) prior to processing for real-time PCR. The expression of apobec-1 mRNA was quantified using real-time PCR. The apobec-1 signals were normalized to β-actin signals, and the 2-h (A) or 0.5-h (C) value was set to 100%. The half-life of apobec-1 mRNA in unactivated wild type or TIA-1−/− macrophages determined by real-time PCR. The graph shows average ± S.E. from three independent experiments. *, p < 0.05.

**Primers**—Primers used were as follows. SY130, 5′-GACAGGCCCCTGTAGCTGTTG-3′; SY131, 5′-CGTGGGTGTGGTGTGTTTGG-3′; SY189, 5′-GCTCTGGCTACATTGACC-3′; M190, 5′-GCCACCGATCCACACCCGGT-3′; G87, 5′-ATTGCTACATGCCAAGACTACATGGG-3′; G88, 5′-TAGAATTCCGATCCACGGCCAGC-3′; G29, 5′-GTTGTGCTTACCCATGGAC-3′; G30, 5′-GGTGGATTTTGAGACCAAG-3′; G83, 5′-CTTAAATGTCATCCAGGATG-3′; G84, 5′-AGCTTCTTTAGCGTCTTCG-3′.
time PCR to quantify the expression of *apobec-1* mRNA (normalized to β-actin). The half-life of *apobec-1* mRNA determined from several independent experiments is plotted in Fig. 2, B and D. In both cases, the half-life of *apobec-1* mRNA is significantly greater (*, \( p < 0.05 \)) in TIA-1−/− MEFs compared with wild type controls. Although altered mRNA decay may not fully explain the increased expression of *apobec-1* mRNA in cells lacking TIA-1, this analysis reveals that TIA-1 directly or indirectly promotes the decay of *apobec-1* mRNA in these cells.

**TIA-1 Tethering Analysis**—To determine whether TIA-1 can directly promote mRNA decay, we tethered TIA-1 to a reporter transcript using the *Pseudomonas aeruginosa* PP7 phage coat protein (28). The system contains two components: 1) pcDNA3-HA-PP7cp-TIA1, a construct expressing a fusion protein between the PP7 coat protein and TIA-1; and 2) pTet-T7-βglobin-PP7bs, a construct expressing globin mRNA with a binding site for the PP7 coat protein in its 3′-untranslated region (see schematic, supplemental Fig. S1). The PP7 coat protein (PP7cp)/PP7 binding site (PP7bs) is analogous to the MS2 coat protein/MS2 binding site that is widely used to tether fusion proteins to selected mRNAs (31). COS cells were transfected with the indicated constructs, solubilized, and immunoprecipitated with anti-HA antibody to bring down HA-PP7cp and associated mRNAs. Cellular lysates (Fig. 3A, input) and immunoprecipitates (Fig. 3A, IP:anti-HA) were processed for Northern blotting (NB) to quantify reporter transcripts (glo) and endogenous nucleolin (ncl) mRNA or for Western blotting (WB) to quantify HA-PP7cp protein in the immunoprecipitates. This analysis confirmed that HA-PP7cp specifically binds to globin transcripts containing the PP7bs but not the MS2bs (Fig. 3A). *In situ* evidence for tethering of HA-PP7cp to globin-PP7bs transcripts was obtained by transfecting HeLa cells with the indicated combinations of proteins (HA−TIA-1 or HA-PP7-TIA-1) and mRNAs (globin or globin-PP7bs) prior to processing for immunofluorescence microscopy and *in situ* hybridization. In cells subjected to arsenite-induced oxidative stress, both HA−TIA-1 and HA-PP7-TIA-1 accumulate at cytoplasmic stress granules, whereas cotransfected untethered globin transcripts remain diffusely localized in the cytoplasm (supplemental Fig. S1). Reporter transcripts including the PP7bs are selectively recruited to SGs when cotransfected with PP7cp-TIA-1, providing morphological confirmation of the tethering of HA-PP7-TIA-1 to globin-PP7bs transcripts (supplemental Fig. S1). We next determined whether tethering of TIA-1 reduces the expression of globin mRNA or protein. HeLa cells transfected with pTet-T7-βglobin-PP7bs and pcDNA3-HA-PP7cp-TIA1 were found to express reduced amounts of both

**FIGURE 3.** Tethering assay. A, PP7cp binds to globin reporter mRNA containing the PP7bs, but not the MS2bs. COS cells were transfected with pcDNA3-HA-PP7cp and the indicated reporters. After 24 h, HA-PP7cp was immunoprecipitated from cytoplasmic lysates using anti-HA. Input fractions (upper panels) and immunoprecipitates (lower panels) were processed for Western blotting (WB, HA-PP7cp, middle panel) and Northern blotting (NB, ncl, glo) to quantify expression of HA-PP7cp and globin mRNA. B, HeLa cells were transfected with pTet-T7-βglobin and pcDNA3-HA-PP7cp-TIA1 (lane 1), pTet-T7-βglobin-PP7bs (lane 2), pTet-T7-βglobin-PP7bs and pcDNA3-HA-PP7cp-TIA1 (lane 3) in the presence of pTet-Off plasmid. Western blot by anti-HA (TIA-1) and anti-T7 (Globin) was carried out using whole cell lysates (upper panel). The expression of globin mRNA (glo or glo-PP7bs) in total RNA (10 μg/lane) was determined by Northern blot analysis (lower panel).
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globin protein and mRNA (Fig. 3B, lane 3) compared with control cells transfected with untethered combinations of pTet-T7-βglobin + pcDNA3-HA-PP7cp-TIA1 (lane 1) or pTet-T7-βglobin-PP7bs + pcDNA3-HA-TIA1 (lane 2). Thus, the tethering of PP7cp-TIA1 to the reporter transcript significantly reduces the expression of both mRNA and protein. We also confirmed that PP7cp-TIA1 reduces the expression of globin-PP7bs in a dose-dependent manner (Fig. 3C). TIA-1 has previously been shown to bind to AU-rich elements found in the 3′-untranslated region of TNFa transcripts (4). We therefore compared the ability of HA-TIA1 and HA-PP7cp-TIA1 to reduce the expression of reporters encoding the TNFa ARE and/or PP7bs. Consistent with previous results, inclusion of the TNFa ARE reduces the expression of globin reporter mRNA (Fig. 3D, Northern blot, lower panel, lanes 4–6) and protein (Fig. 3D, Western blot, upper panel, lanes 4–6). In the absence of PP7bs, neither HA-TIA1 (lane 5) nor HA-PP7cp-TIA1 (lane 6) further reduces the expression of this reporter. This result suggests that factors other than TIA-1 are responsible for ARE-mediated decay or that endogenous TIA-1 is sufficient for this process. In contrast, HA-PP7cp-TIA1 reduces the expression of reporter mRNA (Fig. 3D, lower panel, lanes 9 and 12) and protein (upper panel, lanes 9 and 12) in the absence or presence of the ARE. In this system, the ARE and tethered TIA-1 have an additive effect on the reduced expression of reporter mRNA and protein (compare lanes 9 and 12). If TIA1-mediated decay involves the recruitment of destabilizing factors, this event does not require the presence of an ARE. To confirm that the tethering of PP7cp-TIA1 to the reporter transcript promotes mRNA decay, we compared the half-life of the globin reporter transcripts in the presence of HA-TIA1 or HA-PP7cp-TIA1. HeLa cells were transfected with the indicated plasmids and then cultured in the presence of doxycycline to turn off transcription of the reporter transcripts. Cells harvested at the indicated times were processed for Northern blots to quantify the expression of the globin reporter and endogenous nucleolin mRNA (Fig. 4A). Decay curves are plotted in Fig. 4B, and mRNA half-lives are plotted in Fig. 4C. This analysis reveals that HA-PP7cp-TIA1, but not HA-TIA1, enhances the decay of the globin-PP7bs reporter, but not the globin reporter. This result indicates that tethering of TIA1 to the 3′-untranslated region promotes mRNA decay.

Mechanism of mRNA Decay—In mammalian cells, mRNA decay can be initiated from either the 5′-end or the 3′-end. Degradation from the 5′-end requires the decapping enzymes DCP1/2 and the 5′-3′-exonuclease XRN1 (27), whereas degradation from the 3′-end is mediated by a complex of exonucleases known as the exosome (28). To determine whether one or both of these mRNA decay pathways is used during TIA1-mediated decay, the expression of key components specific to each pathway was reduced using siRNA. The effectiveness of individual siRNAs was confirmed by quantifying the expression of endogenous proteins in cells treated with the indicated siRNAs (supplemental Fig. S2). As shown in Fig. 5A, control siRNAs (D0 and U0) or siRNAs targeting DCP1 do not affect the rate of globin-PP7bs decay. In contrast, siRNAs targeting DCP2 and Rrp46 significantly inhibit TIA1-mediated decay. Surprisingly, siRNAs targeting the 5′-3′-exonuclease XRN1 do not inhibit TIA1-mediated decay. Half-life determinations from several independent experiments are plotted in Fig. 5B, which also indicates the mean and standard error for each treatment. These data indicate that components of both decay pathways can affect the rate of TIA1-mediated decay.

Requirement for Polysome Disassembly—We next determined the effect of cycloheximide, emetine, and puromycin on TIA1-induced mRNA decay using the tethering system. Cycloheximide and emetine stabilize polysomes, whereas puromycin destabilizes polysomes. As shown in Fig. 6, cycloheximide and emetine inhibit TIA1-mediated globin-PP7bs decay. In contrast, puromycin does not inhibit TIA1-mediated decay. The average half-life from three independent experiments using vehicle control, puromycin, cycloheximide, and emetine was 2.67 ± 0.32, 2.63 ± 0.37, 4.40 ± 0.92, and 4.87 ± 1.09 h, respectively. The fact that puromycin does not enhance the decay rate suggests that tethering of TIA1 is sufficient for polysome disassembly.

FIGURE 4. Tethering analysis: half-life determination. A. HeLa cells were transfected with pTet-T7-βglobin and pcDNA3-HA-PP7cp-TIA1 (left panels, non-tethered), pTet-T7-βglobin-PP7bs and pcDNA3-HA-TIA1 (middle panels, non-tethered), pTet-T7-βglobin-PP7bs and pcDNA3-HA-PP7cp-TIA1 (right panels, tethered) in the presence of pTet-Off plasmid. Transfectants were treated with doxycycline (Dox) (10 μg/ml) to block transcription, and total RNA was isolated after 0.5, 2, and 3.5 h. Northern blots were hybridized using digoxigenin-labeled globin (glo) and nucleolin (ncl) probes. B, quantification of Northern blotting analysis. Signal intensities were quantified using the Image J 1.34s system. The globin signals were normalized to nucleolin signals, and the 0.5-h value was set at 100%. The percentage of the remaining mRNA (average ± S.E., four independent experiments) was plotted against time, and exponential decay curves were calculated using best-fit criteria. C, plots of half-lives of TIA1 tethered or non-tethered globin mRNAs. Bars represent average ± S.E. * p < 0.05.
stimulated macrophages lacking either TIA-1 or TIAR overexpress TNFα (5). In the absence of TIA-1, the percentage of TNFα transcripts associated with polysomes is significantly increased (5). This provided the first evidence that TIA-1 inhibits the translation of selected mRNAs. Because the steady state levels and the half-life of TNFα transcripts are similar in wild type and TIA-1−/− macrophages, TIA-1 was classified as a translational silencer (5).

Subsequent studies showed that TIA-1 and TIAR similarly inhibit the translation of several other proteins. TIA-1 and TIAR bind to the 3′-untranslated region of the cyclooxygenase-2 mRNA (33, 34), and TIA-1−/− MEFs express significantly more cyclooxygenase-2 protein than wild type MEFs (34). TIA-1 inhibits the translation of cytochrome c mRNA (35), and TIAR inhibits the translation of β2 adrenergic receptor protein mRNA (36), eIF4A, eIF4E, eEF1B, and c-Myc (6). Finally, a peptide derived from an alternatively spliced exon of TIAAR increases the expression of MMP-13, also without altering steady state mRNA levels (10). Thus, TIA-1 and TIAR regulate the translation of diverse classes of proteins, including inducible proinflammatory cytokines, constitutive survival factors, and growth-associated proteins such as eIF4E, eIF4A, and c-Myc.

Gene array analysis has also been used to identify mRNA sequences that are targets of TIA-1 and TIAR. Analysis of mRNAs that co-precipitate with TIA-1 identified a uridine-rich stem-loop (URSL) structure in the 3′-untranslated region of ~40% of selected transcripts (3). The URSL allows TIA-1 to bind to a heterologous reporter transcript, but it is not known whether this motif is sufficient to confer translational silencing or alter mRNA stability. Nevertheless, the expression of protein, but not mRNA, from four different transcripts bearing the URSL was increased by siRNA-mediated knock down of endogenous TIA-1, suggesting that TIA-1 binding is required for translational silencing. Both TNFα and cyclooxygenase-2 transcripts include the URSL, in addition to their respective AREs, suggesting that the URSL may participate in the TIA-1-mediated translational silencing of these transcripts.

**DISCUSSION**

TIA-1 and TIAR are related RNA-binding proteins that regulate alternative splicing (29, 30), mRNA translation (3, 5), and stress-induced translational arrest (12). Cells lacking both TIA-1 and TIAR are not viable, indicating that these proteins are essential for survival (31). Mutant mice lacking either TIA-1 or TIAR have a hyperinflammatory phenotype because of the overexpression of specific cytokines (5, 11), while mice lacking TIAAR also exhibit defective germ cell development (32). Thus, TIA-1 and TIAR regulate multiple processes that are essential for cellular and organismal survival.

The ability of TIA-1 and TIAR to dampen the expression of inflammatory proteins has been extensively documented. LPS-

**FIGURE 5. Identification of decay factors required for TIA-1-mediated decay.** A, HeLa cells were treated with control (D0 or U0) or dcp1 (C1), dcp2 (D2, D3), xrn1 (X1, X2), mp46 (P6, P7) siRNA (40 nM) for 48 h. The treated cells were transfected with pTet-T7-globin-PP7bs and pcDNA3-HA-PP7cp-TIA-1 and the indicated siRNA (20 nM) for an additional 24 h. The expression of target proteins was quantified by Western blotting analysis using antibodies specific for each protein (see supplemental Fig. S4). Transfectants were then treated with doxycycline (Dox) (10 μg/ml) to arrest transcription, and total RNA was isolated after 0.5, 2, and 3.5 h. Northern blots were hybridized to digoxigenin-labeled probes complementary to globin (glo) and nucleolin (ncl). Expression of each mRNA was quantified by densitometry, and the normalized percentage of globin mRNA (average ± S.E. from at least three independent experiments) was plotted against time. *, p < 0.05 compared with control siRNA.

B, half-life of globin-PP7bs mRNA in cells treated with the indicated siRNAs. Bars depict average ± S.E. **B**, p < 0.05 compared with control siRNA.
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Our results indicate that TIA-1 can also promote the decay of reporter transcripts to which it is tethered as well as selected endogenous mRNAs. The decay of transcripts to which TIA-1 is tethered requires polysome disassembly. Drugs that stabilize polysomes inhibit TIA-1-induced mRNA decay whereas a drug that destabilizes polysomes does not. Interestingly, puromycin-induced polysome disassembly does not enhance the rate of mRNA decay induced by tethering of TIA-1, which implies that TIA-1 disassembles specific polysomes as efficiently as puromycin disassembles all polysomes. Results using globin reporter transcripts with or without tethered TIA-1 and the TNF-ARE show that these elements cooperatively promote mRNA decay (Fig. 3D). In the absence of the ARE, tethering of TIA-1 promotes decay that is dependent upon Rrp46 (Fig. 5), implicating the exosome in this process. XRN1 is not required for the decay of TIA-1-tethered transcripts, making it unlikely that the 5′-3′- decay pathway is of major importance. Surprisingly, DCP2, but not DCP1, is required for TIA-1-mediated decay. Although DCP1 and DCP2 are required to remove the 7-methyl guanine cap, it is possible that these proteins have additional roles in the 5′-3′-and/or 3′-5′-decay pathways. Just as DcpS, the decapping enzyme required for exosome-dependent 3′-5′- decay (37), also regulates 5′-3′- decay (38), it is possible that DCP2 is required for efficient exosome-dependent decay in this system. Further work will be required to resolve this issue.

The discovery of a subset of transcripts that are overexpressed in macrophages lacking either TIA-1 or TTP suggests a role for both ARE-binding proteins in the decay of these mRNAs. The 3′-untranslated regions of these transcripts (i.e. apobec1, edr, and acs14, Fig. 1C) lack the URSL.3 Apobec-1 has a minimal “AUUUA” pentamer, but its role in the recruitment of TIA-1 or TTP remains to be determined. Whether the type of TIA-1 binding cis element is sufficient to determine the functional response to TIA-1 binding (e.g. silencing versus decay) is unclear. As multiple proteins can bind to adjacent elements on the same transcript or compete for the same binding site (39), TIA-1-mediated decay may require collaboration with other proteins in either a cooperative or competitive manner. For example, the decay of mRNAs containing separate TIA-1 and TTP binding sites may require coordinate (TIA-1-mediated) polysome disassembly and (TTP-mediated) recruitment of the mRNA decay machinery. Loss of either TIA-1 or TTP would stabilize this class of transcript. Alternatively, the decay of mRNAs containing overlapping TIA-1 and TTP binding sites may require sequential binding of these proteins to bring about polysome disassembly and recruitment of the decay machinery. In this case, the avidity of TIA-1 binding to the ARE could influence the functional outcome. Recent results reveal that the avidity of TIA-1 for the TNF-ARE is enhanced by SRC-3, a protein that binds to the carboxyl terminus of TIA-1 (40). As a consequence, SRC-3 promotes TIA-1-mediated translational silencing of TNFα transcripts without affecting mRNA stability (40). By enhancing the binding between TIA-1 and the TNF-ARE, the SRC3-TIA-1 complex may prevent TIA-1/TTP exchange and mRNA decay. The exchange of TTP for TIA-1 may also require the activity of an RNA helicase such as RCK/DHH1, which is required for both polysome disassembly and activation of mRNA decay (18). Moreover, additional protein-protein interactions involving TIA-1 may regulate the silencing/decay decision, such as its interaction with the endoribonuclease PMR1 (41).

The ability of TIA-1 to promote the decay of selected mRNA transcripts underscores the link between mRNA translation and decay. Our results suggest that TIA-1-mediated polysome disassembly makes some specific mRNAs available for degradation via the 3′-5′- or 5′-3′-decay pathways but suggest that other proteins may be involved in this process. It is particularly interesting that TIA-1 promotes the decay of some mRNAs, but not others. It is likely that proteins that make up the messenger ribonucleoprotein complex will collaborate to determine whether a given mRNA interacts with the translational machinery or the decay machinery and thus resides in polysomes, P-bodies, or elsewhere in the cytoplasm.

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