The Protein Zfand5 Binds and Stabilizes mRNAs with AU-rich Elements in Their 3′-Untranslated Regions*

Guoan He, Dongxu Sun, Zhiying Ou, and Aihao Ding

From the Department of Microbiology and Immunology, Weill Cornell Medical College, and the Graduate Program in Immunology and Microbial pathogenesis, Weill Graduate School of Medical Sciences, Cornell University, New York, New York 10065, the Department of Immunology, Lerner Research Institute, Cleveland Clinic, Cleveland, Ohio 44195, and the Guangzhou Women and Children’s Medical Center, Guangzhou 510623, China

Received for publication, March 13, 2012, and in revised form, May 25, 2012 Published, JBC Papers in Press, June 4, 2012, DOI 10.1074/jbc.M112.362020

Abstract

Background: AU-rich elements (AREs) in the 3′-UTR of mRNA confer instability; the mechanisms are incompletely understood.
Results: A genomic screen identified Zfand5 as a stabilizer of ARE-RNA.
Conclusion: Zfand5 enhances ARE-RNA stability by competing with TTP for mRNA binding.
Significance: A better understanding of ARE-RNAs regulation is of clinical significance because many inflammatory mediator transcripts contain ARE.

AU-rich elements (AREs) in the 3′-UTR of unstable transcripts play a vital role in the regulation of many inflammatory mediators. To identify novel ARE-dependent gene regulators, we screened a human leukocyte cDNA library for candidates that enhanced the activity of a luciferase reporter bearing the ARE sequence from TNF (ARETyne). Among 171 hits, we focused on Zfand5 (zinc finger, AN1-type domain 5), a 23-kDa protein containing two zinc finger domains. Zfand5 expression was induced in macrophages in response to IFNγ and Toll-like receptor ligands. Knockdown of Zfand5 in macrophages decreased expression of ARE class II transcripts TNF and COX2, whereas overexpression stabilized TNF mRNA by suppressing deadenylation. Zfand5 specifically bound to ARETyne mRNA and competed with tristetraprolin, a protein known to bind and destabilize class II ARE-containing RNAs. Truncation studies indicated that both zinc fingers of Zfand5 contributed to its mRNA-stabilizing function. These findings add Zfand5 to the growing list of RNA-binding proteins and suggest that Zfand5 can enhance ARE-containing mRNA stability by competing with tristetraprolin for mRNA binding.

The expression of inflammatory mediators, including cytokines, chemokines, and enzymes must be finely tuned such that these mediators do not cause more damage to the host than the microbial infection they evolved to defeat (1). One mechanism used by cells to shut down expression of genes whose functions are no longer needed is to decompose their mRNA (2). Intrinsic signals for the decay of individual mRNA are often embedded in their 3′-UTR as cis-acting elements (3, 4). Interactions of these elements with specific microRNA sequences or proteins provide fine-tune control over the half-lives of individual mRNAs (5–7).

One of the best-studied signatures for mRNA instability is an adenylyl/uridine-rich sequence element (ARE)2 AUUUA within the 3′-UTR of target mRNA molecules (3, 8, 9). More than 4000 human mRNAs contain AREs, which represent as much as 8–16% of the human protein-coding genes (10, 11). AREs are more complex than most cis elements in that there is no single consensus sequence for an ARE, and its number varies among ARE-containing RNAs (ARE-RNAs). Based on the number and the distribution of AUUUA pentamers, AREs have been grouped into three classes (12, 13). Class I AREs contain several dispersed copies of the AUUUA motif within U-rich regions. Class III AREs are much less well defined; they are U-rich regions but contain no AUUUA motif. The AREs of most inflammatory mediators belong to Class II, which typically consist of two or more overlapping UUAUUUA(U/A)(U/A) nonamers. Many inflammatory cytokines and chemokines belong to this class. For example, TNF, a major contributing factor in the systemic inflammatory response syndrome, rheumatoid arthritis, and inflammatory bowel disease (14), possesses a well defined ARE (13). Despite significant progress in the discovery of the ARE-regulatory proteins, the list of molecules participating in this process is far from complete. Identification of novel factors that are involved in regulating cytokine mRNA stability and capable of attenuating host inflammatory responses offers potentially important insights into the pathological basis of diverse inflammatory diseases and could aid in the development of therapeutic strategies.

Zfand5 is a 23-kDa cytosolic protein with one A20 zinc finger domain and one AN1-type zinc finger domain. Human Zfand5 was first identified from the Morton fetal cochlea library as a novel cochlear-expressed protein called ZNF216 (15). The Zfand5 gene is highly conserved, with 99% sequence conservation in humans and mice. The signature of two unique zinc finger domains at both the N and C termini of this protein can be also found in a group of plant stress-associated proteins that

* This work was supported, in whole or in part, by National Institutes of Health Grant AI030165 (to A. D.). The Department of Microbiology and Immunology is supported by the William Randolph Hearst Foundation.

† To whom correspondence should be addressed: Dept. of Microbiology and Immunology, Weill Cornell Medical College, New York, NY 10065. Tel.: 212-746-4551; Fax: 212-746-8536; E-mail: ahding@med.cornell.edu.

The abbreviations used are: ARE, AU-rich element; ARE-RNA, ARE-containing RNA; Act. D, actinomycin D; TLR, Toll-like receptor; TTP, tristetraprolin.
play an important role in abiotic responses in the plants (16). Zfand5 is highly expressed in the brain and skeletal muscle (15) and has been implicated in muscle atrophy and the differentiation of osteoclasts (15, 17, 18). Little is known about its role in immune responses, with conflicting reports on its role in NF-κB activation (17, 19).

In this study, we undertook an unbiased screen, using a leukocyte cDNA library, to identify genes whose expression led to enhanced activity of a luciferase reporter construct bearing an ARE\textsubscript{TNF} within its 3′-UTR. We identified several such proteins. One such protein is Zfand5. Our study reveals that Zfand5 stabilizes class II ARE-RNAs by binding directly to the ARE-RNA and competing with tristetraprolin (TTP), a zinc-finger-containing protein that destabilizes mRNAs with Class II AREs.

**EXPERIMENTAL PROCEDURES**

*Mice and Macrophages—*C57BL/6 mice were purchased from the Jackson Laboratories. RAW cells were from ATCC. Bone marrow-derived macrophages were prepared as described (20). Animal studies were approved by the Institutional Animal Care and Use Committee of Weill Cornell Medical College.

*Reagents—*Reagents were obtained as follows: human peripheral blood leukocyte cDNA library panels from Origene Technologies; the Dual-Luciferase reporter assay system from Promega; recombinant mouse IFNγ from Genentech; LPS (isolated from *Escherichia coli* 0111:B4) and actinomycin D from Sigma; superscript III reverse transcriptase, RNase inhibitor and TRIZOL (Ambion). 100 ng of RNA was subjected to reverse transcription and cDNA synthesis with the manufacturer’s protocol.

*Gene Library Screening—*As illustrated in Fig. 1A, a human leukocyte cDNA library contains ~480,000 cDNAs in a 96-well plate, ~5,000 cDNAs/well. The cDNAs from a single well were transformed into XL10 Gold ultracompetent cells (Stratagene) and plated onto 50 Luria broth (LB) agar-ampicillin plates at a density of ~100 colonies/plate. After 20 h of growth on the plates, colonies were scraped, and a small portion of the pooled bacteria was stored at ~80 °C for future screening. Remaining bacteria were used to prepare plasmid DNA using the plasmid 96 Miniprep Kit (EdgeBiosystems). RAW cells were transfected for 24 h with 0.5 μg of pooled DNA together with 5 ng of pGL3-Luc-3′-UTR\textsubscript{TNF}, pGL3-Luc-3′-UTR\textsubscript{IP10} or pGL3-Luc-3′-UTR\textsubscript{actin} together with 5 ng of pGL4.74[\textit{hRluc}/TK] plasmid (Promega). After 24 h, cells were lysed with 100 μl of lysis buffer (25 mM Tris buffer, pH 7.8, 2 mM DTT, 2 mM 1,2-diaminocyclohexane-N,N,N′,N′-tetraacetic acid, 10% glycerol, and 1% Triton X-100). 50 μl of cell lysates were assayed for luciferase activity using the Dual-Luciferase\textsuperscript{®} reporter assay system (Promega). Luciferase reporter activity was calculated by normalizing the firefly luciferase activity to *Renilla* luciferase activity.

**cDNA Library Screening—**—The levels of cytokines in the cultured media were measured with ELISA kits from R&D Systems according to the manufacturer’s protocol.

**Semiquantitative RT-PCR—**—Total cell RNA was prepared with TRIzol (Ambion). 100 ng of RNA was subjected to reverse transcription using random primers and superscript III reverse transcriptase. PCR was performed in a total volume of 20 μl containing 10 ng of cDNA, 1.5 mM MgCl\textsubscript{2}, 0.2 μM of each primer, and 1 unit of AmpliTaq Gold DNA polymerase (PerkinElmer Life Sciences). PCR products were separated on 2% agarose gels and visualized with ethidium bromide staining.
transcription with SuperScript III reverse transcriptase to generate template cDNAs. The PCR was performed in a total volume of 20 μl with a 2-μl input of RT products as templates (94 °C for 5 min; 94 °C for 30 s, 60 °C for 30 s, and 72 °C for 1 min for 28 cycles; followed by a 72 °C for 10 min extension). Sequences of primers for RT-PCR detection were 5′-gtcgacagtacgacctagc (antisense)/5′-tattctctggattttttcagccacaac (sense) for TNF, 5′-gctcaccggagccccctgaa (sense)/5′-tattctctggattttttcagccacaac (antisense) for β-actin, 5′-cgctgctcgatgtgattgc (sense)/5′-tattctctggattttttcagccacaac (antisense) for GAPDH, 5′-ctcaggagactaaccagacccca (sense)/5′-ggcttgcccctggaagtttc (antisense) for COX2, 5′-atgctcttccgagctgtgctgc (sense)/5′-atggcttgcccctggaagtttc (antisense) for p53.

**Knockdown of Zfand5 in RAW Cells with shRNA**—The construct expressing shRNA for mouse Zfand5 and the control constructs were purchased from OriGene Technologies. Sequences for shRNA were 5′-TTGCTACAAAGAATCTTCTG-3′ (ID: G6100218) for Zfand5 and 5′-GACCTACAGAGCTAACTCAGATAGTACT-3′ (TR30013) for CR2. CR1 is a shRNA that targets pGFP-V-RS vector (ID: TR300007, OriGene). RAW cells were plated on a 6-well plate 1 day before transfection. Controls and the shRNA construct were transfected into RAW cells (1 μg/2 × 105 cells). Cells were cultured for 24 h and treated with 100 ng/ml LPS for 1 h or 100 ng/ml Pam3 for 6 h before extraction of cell-associated RNA. 100 ng of total RNAs were used for RT-PCR for detection of internal Zfand5 mRNA level induced by LPS.

**Zfand5 Expression in Response to Microbial Stimuli in Vitro**—RAW cells (2 × 105) or bone marrow-derived macrophages (105) were seeded on 6-well plates for 24 h and then treated with LPS, poly(I:C), CpG, or IFNγ, as indicated. Total RNA from cells was extracted using TRIzol®. The content and Zfand5 was determined by semiquantitative RT-PCR.

**Generation of Recombinant Zfand5 and Its Mutants**—To generate recombinant proteins, pTriEx-Zfand5-ΔA20, pTriEx-Zfand5-ΔAN1, and pTriEx-Zfand5-ΔA20ΔAN1 were transformed into BL21-CodonPlus (DE3)-RIL competent cells (Stratagene). One liter of single cell-derived culture was treated with 1 mM IPTG for 6 h at 37 °C. Proteins were purified using an AKTA FPLC system with 5 ml of HisTrap™ FF columns (GE Healthcare) following the manual provided.

**TFN mRNA Stability Assay**—RAW cells plated on a 6-well plate (6 × 105 cells/well) were transfected with 1 μg of plasmid DNA. 24 h later, cells were exposed to 100 ng/ml LPS for 1 h before the addition of 10 μg/ml actinomycin D. Total RNA was prepared at different time points after actinomycin D treatment. TFN mRNA was determined by semiquantitative RT-PCR.

**Quantitative Real-time RT-PCR**—Total RNA was extracted with an RNeasy mini kit (Qiagen), and real-time PCR was performed on the ABI PRISM 7900HT sequence detection system (PerkinElmer Life Sciences). The primer and probe sequences for TFN and GAPDH were as described (20).

RNA EMSA—Cy5-labeled RNA probe (1 pmol) was incubated with purified Zfand5, TTP, or SAA, 10 pmol of poly(U)20 (Microsynth, Balgach, Schweiz), plus 1 μl of RNase inhibitor mixture in a total volume of 10 μl in a binding buffer (10 mM Tris, 50 mM KCl, 2.5 mM DTT, 0.25% Tween 20, pH 7.5) at room temperature for different times. The reaction mixtures were separated on a 5% native polyacrylamide gel as described (21) and visualized with the Odyssey Infrared Imaging System (LI-COR Biotechnology). Sequences for ARETNF and its mutant are indicated in Fig. 4A.

**Determination of Dissociation Constant (Kd)**—The Kd of Zfand5-ARETNF and TTP-ARETNF was determined using the BLItz system (ForteBio Inc.) according to the user guide and was calculated as dissociation rate (Kd) versus association rate (Ka). Biotin-labeled ARETNF RNA was from Biosearch Technologies, Inc. Streptavidin biosensors (catalog no. 18-5019, ForteBio Inc.) were hydrated for 10 min prior to the experiment in sample diluents (10 mM Tris-HCl, pH 8.0, 150 mM NaCl, 0.05% surfactant P20, 62.5 μg/ml BSA, 125 μg/ml tRNA, 1 mM dithiothreitol, 5% glycerol) (22). Biotin-labeled ARETNF RNA was at 40 μg/ml, and both purified Zfand5 and TTP were at 2.5, 5, 10, and 20 μg/ml. The settings were as follows: initial base line for 30 s, loading for 120 s, base line for 30 s, association for 240 s, and dissociation for 300 s. Kd values were generated by BLItz Pro software analysis as an inadvanced kinetics experiment.

**Deadenylation Assay**—Deadenylation activity of cell lysates was determined using a synthetic substrate that contains a 34-base ARE found in the 3’UTR of TNF mRNA (23) followed by 60-base poly(A) tail at its 3’end as described (24). A control plasmid was generated by removing the ARE sequence (ΔARE). S100 (100,000 × g, 4 °C, 60 min) cytoplasmic extracts were made from RAW cells 24 h after transfection with vector only (mock), Zfand5, TTP, or both Zfand5 and TTP. RNA substrates were trancribed in vitro as described (25) but with biotin-11-GTP (PerkinElmer Life Sciences) instead of [α-32P]GTP using linearized pGEM-ARE-A60 as a template. As a control, poly(A)-less RNA (A0) containing only the same 34-base AU-rich element as in A60 was transcribed similarly. Biotinylated A60 and A0 were gel-purified on a 5% denaturing (7 M urea) polyacrylamide gel. To determine deadenylation activity, A60 was incubated with cytoplasmic extracts at room temperature for different times before the addition of a stop solution (25 mM Tris-HCl, pH 7.5, 400 mM NaCl, 0.1% SDS). After phenol/chloroform extraction and ethanol precipitation, the recovered biotin-labeled RNAs were separated on 5% denaturing polyacrylamide gel and then incubated with Streptavidin-IRDy 800CW conjugate (1:10,000, 15 min) followed by a brief wash with phosphate-buffered saline plus 0.1% Tween 20 and visualized using the Odyssey Infrared Imaging System (LI-COR Biotechnology).

**Quantification of Image Signals**—Image signals were quantified using the software ImageJ version 1.410.

Statistical analyses were done using a two-tailed Student’s t test for independent samples. p values of <0.05 were considered statistically significant. All of the data are presented as means ± S.E.
RESULTS

Screening of a Leukocyte cDNA Expression Library for Gene Products Capable of Activating Luciferase-ARE^{TNF} Reporter—The first functional demonstration of ARE-dependent mRNA degradation was obtained by studying the stability of a globin mRNA chimera that contained the ARE from the granulocyte-macrophage colony-stimulating factor (ARE^{GM-CSF}) mRNA. This insertion reduced the half-life of globin mRNA from 17 h to less than 30 min (3, 8). We used a similar strategy with a chimeric luciferase reporter construct containing the ARE sequence of TNF (ARE^{TNF}) and observed that luciferase activity in RAW cells was greatly reduced as compared with cells carrying the same reporter with 3'UTR from actin (26). Co-transfection of cDNA expression plasmids for ARE-stabilizing molecules, such as myeloid differentiation primary response gene 88 (MyD88), MLK3, MEK3, P38, and MK2, was found to enhance luciferase reporter activity (26).

Using this chimeric reporter, we screened a cDNA expression library from human peripheral blood leukocytes for genes whose expression led to stabilization of ARE-RNAs. To increase the efficiency of the screening, we first divided a portion of the library into ∼50 pools of ∼100 cDNAs each (Fig. 1A, left) and transected them into RAW cells with the luciferase-ARE^{TNF} reporter construct. A control pGL4.74 hRluc/TK plasmid was included to record the transfection efficiency. Positive pools were selected if their luciferase activities were more than 3-fold above the mean activity among 50 pools. Of the 2,200 pools assayed in this fashion, 67 pools were positive based on the above criteria, ranging from a 3- to 7-fold increase in luciferase activity.

To identify the cDNA responsible for a pool’s activity, bacteria derived from the original stock were replated on an agar dish. Individual colonies were picked and expanded in LB medium in 96-well plates. Portions of bacteria from each well in one row (12 wells in total) were pooled, and the resulting mixed subpool DNAs were assayed for reporter activity in the secondary screen. Positive cDNAs were then identified by transfection of RAW cells with individual cDNA from positive subpools. Representative results of primary, secondary, and final screens from one representative group of cDNA pools are shown in Fig. 1A (right). When screening for promoter-activating factors, enhanced reporter activities sometimes reach magnitudes of >100-fold (27). In contrast, the -fold increase for Luc-ARE^{TNF} activity afforded by mRNA-stabilizing factors is often seen in the single-digit range. In addition, activity often plateaued before clonal purification. This may be due to a saturation of the

FIGURE 1. Identification and confirmation of Zfand5 as an ARE-RNA stabilizer. A, schematic display of the screening strategy (left). Right, representative results of primary, secondary, and final screens from one pool of cDNAs, expressed as -fold induction above the mean activity for the entire group. Green, E. coli culture; red, reporter activity-positive cDNA; yellow, reporter activity-negative cDNA. B, expression of Zfand5 resulted in enhanced reporter activities for luciferase reporter appended with ARE sequences. RAW cells were transfected with the indicated reporter constructs without (blank) or with an empty vector pCMV6 (mock) or pCMV6-Zfand5 (Zfand5). The results are calculated as the ratio of activities of firefly luciferase versus Renilla luciferase, expressed as -fold increase as compared with the blank and are mean ± S.E. (error bars) from three experiments. *, p < 0.01, Student’s t test. C, expression of Zfand5 enhanced LPS-induced release of TNF and IL-6. RAW cells were transfected as in 8 without luciferase reporters for 24 h before the addition of 100 ng/ml LPS, TNF, IL-6, and IL-10 contents in the media were determined 24 h later by ELISA. *, p < 0.05, Student’s t test. D, expression of Zfand5 retarded the decay of TNF mRNA. RAW cells were transfected as in 8 for 24 h, and then cells were treated with LPS (100 ng/ml) for 1 h before the addition of 10 μg/ml Act. D. RNA samples were prepared 0, 2, or 4 h thereafter. Transcripts for β-actin and TNF were determined by semiquantitative RT-PCR. Arrow, TNF amplicon; open arrowhead, β-actin amplicon. One of four similar experiments is shown.
The identities of the full-length cDNA isolates are shown in Table 1. These isolates reveal cellular proteins with diverse cellular gene products, corresponding to 38 unique sequences. Their expression was then confirmed through various assays, including reporter activity, which showed that Zfand5 increased the activity of reporters containing ARE sequences (28).

Screening of 220,000 cDNAs from this leukocyte library yielded 171 hits. Although the majority of hits (147 hits) belong to annotated genes, fewer than 32% (54 hits) coded for complete sequences of genes, either with (67 hits) or without (33 hits) an accompanying ORF. For hits containing partial ORF, most of them were out of frame. This indicates that the effects on the reporter activity were probably due to their transcripts rather than gene products. In fact, many of these hits encoded for ARE-containing genes subject to regulation by ARE-mediated RNA decay machinery. It is possible that an increased abundance of ARE-containing transcripts could deplete limited intracellular components of ARE-mediated mRNA decay machineries, thus stabilizing the reporter mRNA. Many 3’-UTRs also contain microRNA binding sites, their interaction with microRNA being prerequisite for microRNA-mediated RNA decay. Thus, overproducing transcripts containing such binding sites could deplete the available microRNAs and, by the same token, lead to a reduction in RNA decay and enhancement in RNA stability.

Isolation and Confirmation of Zfand5 as a Stabilizer for ARE-RNAs—One full-length candidate gene encoded for Zfand5, a small zinc finger protein known to participate in osteoclast differentiation (17). To confirm that the effects of Zfand5 on luciferase activity were dependent on its 3’ ARE sequence, we tested the effect of Zfand5 on a modified reporter carrying either 3’-UTR from actin or another ARE sequence from IP-10. Fig. 1B shows that forced expression of Zfand5 increased activities of luciferase appended with either ARETNF or AREIP-10 but not with 3’-UTR from actin, as compared with cells transfected with an empty vector (mock) or reporter only (blank, Fig. 1B).

We next tested culture media from RAW cells transfected with Zfand5 for the levels of TNF and IL-6. We reasoned that if expression of Zfand5 could stabilize ARE-RNAs, this should lead to enhanced levels of endogenous ARE-containing TNF and IL-6 mRNAs. Indeed, Zfand5 expression in RAW cells increased levels of TNF and IL-6 that were released into the conditioned media as determined by ELISA (Fig. 1C). The level of IL-10, whose transcript does not carry a similar UUAU-UUAAU sequence in its 3’-UTR, was not affected by Zfand5 expression.

Up to this point, all assays relied on ARE-containing reporter activities or the protein levels of cytokines encoded by ARE-containing transcripts. To ascertain that expression of Zfand5 could stabilize ARE-RNAs, RAW cells were transfected with Zfand5 or a positive control MyD88 (26) or vector only. Cells were then exposed to LPS for 1 h to boost the basal level of TNF mRNA before the addition of actinomycin D (Act. D), an inhibitor of mRNA synthesis. In non-transfected or mock-transfected samples, residual TNF mRNA was abundant when Act. D was added but became undetectable 2 h after treatment with Act. D (Fig. 1D). This is consistent with the short half-life of this molecule (37). TNF mRNA, however, was easily detected 2 h after Act. D treatment, when Zfand5 or MyD88 was expressed. These results confirm the stabilizing effect of Zfand5 on TNF mRNA.

Induction of Zfand5 by Microbial Products in Vitro—For the effect of Zfand5 on TNF mRNA to be physiologically relevant, Zfand5 expression should be induced in response to inflammatory stimuli that induce TNF. Hishiya et al. (17) reported that Zfand5 expression was induced in RAW cells after exposure to TLR4 agonists, including LPS, a ligand for Toll-like receptor 4 (TLR4). We found that LPS-induced Zfand5 expression in RAW cells or primary bone marrow-derived macrophages was concentration- and time-dependent (Fig. 2, A and B). To determine whether other TLR ligands also induce Zfand5, we exposed RAW cells to the lipopeptide Pam3CysSerLys4 (Pam3; a ligand of TLR2), poly(I:C) (a ligand of TLR3), and hypomethylated bacterial DNA (CpG; a ligand of TLR9) for 1 or 6 h. Zfand5 expression increased in response to each of these stimuli, suggesting that Zfand5 can be induced by various microbial ligands.

### Table 1: Identities of the Full-Length cDNA Isolates

| GenBank™ ID | Name          | No. of isolates |
|-------------|---------------|----------------|
| NM_002676   | ABHD6        | 1              |
| NM_001724   | BPGM         | 1              |
| NM_001731   | BTG1         | 1              |
| NM_002984   | CCL4         | 3              |
| NM_006110   | CD2BP2       | 3              |
| NM_203331   | CD59         | 1              |
| NM_005194   | CEBPB        | 1              |
| NM_080387   | CLEC4D       | 1              |
| NM_172245   | CSF2RA       | 1              |
| NM_203311   | CXC7         | 1              |
| NM_001402   | EEFA1        | 1              |
| NM_005801   | EIF1         | 1              |
| NM_001428   | EN01         | 1              |
| NM_002032   | FTH1         | 4              |
| NM_015675   | GADD45B      | 1              |
| NM_002046   | GAPDH        | 1              |
| NM_012203   | GRHP         | 1              |
| NM_000517   | HBA2         | 2              |
| NM_000576   | ILIB         | 3              |
| NM_000584   | IIL           | 2              |
| NM_000239   | ILZ          | 2              |
| NM_1454109  | MAPK3        | 1              |
| NM_005124   | NUP153       | 1              |
| NM_006813   | PNRC1        | 2              |
| NM_005729   | PP1F         | 1              |
| NM_006103   | RPL10        | 1              |
| NM_000982   | RPL21        | 1              |
| NM_000992   | RPL29        | 1              |
| NM_001693294| RP521        | 1              |
| NM_0010323  | RPS29        | 1              |
| NM_001010   | RPS6         | 1              |
| NM_002575   | SERPINB2     | 3              |
| BC047696    | STK17A       | 1              |
| NM_001099   | STOM         | 1              |
| NM_032796   | SYAP1        | 1              |
| NM_004295   | TRAF4        | 1              |
| NM_005252   | v-FOS        | 3              |
| NM_00102420 | ZFAND5       | 2              |
Zfand5 Binds and Stabilizes ARE-RNA

among which LPS and Pam3 were more effective (Fig. 2C). The induction of Zfand5 expression by LPS and Pam3 was higher after 1 h than after 6-h exposure. IFNγ also induced Zfand5 expression, albeit with a slow kinetics, similar to that of CpG (Fig. 2C). Thus, Zfand5 expression can be induced by inflammatory stimuli that induce many cytokines encoded by ARE-containing messengers.

Knockdown of Zfand5 with shRNA Down-regulates Transcripts for TNF and COX2 but Not NOS2 and p53—If expression of Zfand5 is important for TNF mRNA stabilization, then knockdown of endogenous Zfand5 should reverse this effect. To test this, we transfected RAW cells with a plasmid expressing an shRNA specific for Zfand5. Control cells were transfected with two different shRNAs; one targeted the pGFP-V-RS vector, and the other carried an irrelevant sequence. 24 h later, the cells were treated with Pam3 for 6 h to increase endogenous Zfand5 and TNF levels. shRNA for Zfand5 effectively reduced its transcripts as well as protein (Fig. 3A). The TNF level was also reduced following Zfand5 knockdown, whereas expression of β-actin remained unaffected. To test whether the stabilizing effect of Zfand5 on TNF mRNA extended to other classes of ARE-containing transcripts, we also measured the levels of other ARE-RNAs in Zfand5 knockdown cells. These include COX2 (class II ARE), NOS2 (class I ARE), and p53 (class III ARE). Silencing of Zfand5 selectively affected transcript stabilities of TNF and COX2 but not NOS2 or p53, suggesting that Zfand5 may specifically stabilize class II ARE-containing genes.

Zfand5 Binds Directly to ARE_{TNF}—ARE-mediated mRNA stability is regulated by a variety of mRNA binding partners. A number of ARE-binding proteins have been identified, including human antigen R (38), A+U-rich RNA-binding factor 1 (39), T-cell intracytoplasmic antigen 1 (40, 41), and TTP (42). TTP with ARE_{TNF} RNA in the presence of Zfand5. TTP-ARE binding to reduce the destabilizing effect of TTP on its target mRNAs (43–45). We hypothesized that Zfand5 may bind directly to the class II AREs and accelerate breakdown of niscent of a well studied ARE-binding protein, TTP (42). TTP—ARE-mediated mRNA stability is regulated by a variety of mRNA binding partners. A number of ARE-binding proteins have been identified, including human antigen R (38), A+U-rich RNA-binding factor 1 (39), T-cell intracytoplasmic antigen 1 (40, 41), and TTP (42). TTP with ARE_{TNF} RNA in the presence of Zfand5. TTP-ARE binding to reduce the destabilizing effect of TTP on its target mRNAs (43–45). We hypothesized that Zfand5 may bind directly to the class II AREs and accelerate breakdown of its target mRNAs (43–45). We hypothesized that Zfand5 may bind directly to the class II AREs and accelerate breakdown of its target mRNAs (43–45). We hypothesized that Zfand5 may bind directly to the class II AREs and accelerate breakdown of its target mRNAs (43–45). We hypothesized that Zfand5 may bind directly to the class II AREs and accelerate breakdown of its target mRNAs (43–45). We hypothesized that Zfand5 may bind directly to the class II AREs and accelerate breakdown of its target mRNAs (43–45).
Zfand5 Binds and Stabilizes ARE-RNA

A

ARE\textsuperscript{TNF}

Cy5-gcactaatattttatattttattttattttgcctaatgatatttt

ARE\textsuperscript{TNF-mut}

Cy5-gcactaatattttatattttattttattttgcctaatgatatttt

B

\begin{tabular}{|c|c|c|c|}
\hline
 & ARE\textsuperscript{TNF} & ARE\textsuperscript{TNF-mut} & probe \\
\hline
- BSA & His-Zfand5 & BSA & - protein \\
\hline
- 1 & 0.1 & 0.05 & 0.1 & 0.1 & 0.1 & 1 & - & \mu g \\
\hline
\end{tabular}

FIGURE 4. Zfand5 binds to ARE of TNF. A, sequences for the ARE\textsuperscript{TNF} and ARE\textsuperscript{TNF-mut} mutant probes with mutated nucleotides in gray. B, Zfand5 binds specifically to ARE\textsuperscript{TNF}. Recombinant His-Zfand5 or BSA was incubated with 1 pmol of ARE\textsuperscript{TNF} or ARE\textsuperscript{TNF-mut} for 30 min at room temperature and fractionated on a native gel as described under “Experimental Procedures.” Supershift was demonstrated by incubating anti-His tag or control antibody with recombinant His-Zfand5 for 10 min before the addition of the probe. One of four similar experiments is shown.

shifted toward the position for ARE\textsuperscript{TNF}/Zfand5 in a concentration-dependent fashion (Fig. 5A). To determine whether Zfand5 replaced TTP in the TTP-ARE\textsuperscript{TNF} complex or formed a Zfand5-TTP-ARE\textsuperscript{TNF} triple complex, we transferred proteins on the EMSA gel to a membrane and detected TTP and Zfand5 by Western analyses. TTP appeared on the same position with increasing concentrations of Zfand5 with the same signal intensity, with an exception when a large amount of BSA was present. No TTP was detected in the position of Zfand5-ARE complex (Fig. 5A). We also performed supershift assays with antibody against either TTP or His-tagged Zfand5. Fig. 5B shows that anti-TTP could supershift the band corresponding to TTP-ARE\textsuperscript{TNF} but not the slow migrating band that appeared when Zfand5 was added. This slowly migrating band, however, was readily supershifted with anti-His antibody for Zfand5. We also measured the dissociation constants ($K_D$) of RNA-protein by the BLItz system and found that the $K_{D(Zfand5)}$ (11.4 nM) was similar to the $K_{D(TTP)}$ (11.5 nM). These results suggest that at equal or greater concentrations, Zfand5 could compete with TTP in binding to ARE\textsuperscript{TNF}.

To test whether Zfand5 expression inhibited TTP-facilitated TNF mRNA decay, we transfected RAW cells with TTP plasmid and increasing concentrations of Zfand5 plasmid and measured the LPS-induced TNF mRNA levels in the presence of actinomycin D. Fig. 5C shows that expression of recombinant TTP reduced the level of TNF mRNA only slightly, perhaps because expression of endogenous TTP was already nearly optimal. Expression of Zfand5 reversed this inhibition and led to a Zfand5-dependent increase in TNF mRNA levels, as compared with mock transfectants. Taken together, these findings suggest that Zfand5 is able to compete with TTP binding to the TNF mRNA and antagonize the destabilizing effects of TTP on TNF mRNA.

Zfand5 Stabilizes ARE-RNA by Delaying Its Deadenylation—The destabilizing effect of TTP on TNF mRNA is in part due to the fact that TTP promotes deadenylation of the TNF mRNA (46), a requisite step in degradation of the majority of mRNA by endogenous 3’-5’ exonucleases (47). To test whether Zfand5 affects deadenylation of ARE-RNA, we prepared cytosolic extracts from the S100 fraction of RAW cells that had been transfected with Zfand5 or TTP for 24 h and then incubated with a biotin-labeled synthetic RNA substrate with or without ARE from TNF mRNA followed by 60-base poly(A) tail (A60). Deadenylation of the substrate was determined by fractionation of recovered RNA substrate on a denaturing gel followed by visualization of a streptavidin-conjugated infrared dye. Fig. 6A shows that expression of TTP greatly enhanced the de-adenylation rate with an ARE sequence with the fastest disappearance of A60 and accumulation of A0, a fully deadenylated poly(A)-less substrate. Expression of Zfand5, in contrast, retarded the conversion of A60 to A0, as compared with the mock-transfected sample. The deadenylation rates were greatly reduced when ARE sequence was deleted from the substrate (ΔARE) and were similar regardless of whether TTP or Zfand5 was expressed. The diffused migration patterns on the native gel reflect the heterogeneous nature of these biotin-labeled RNA substrates. Nevertheless, this isotope-free assay provides a useful window to assess deadenylation activity in cells. Fig. 6B quantifies the results in Fig. 6A that are expressed as the percentage of A60 remaining at each time point. The results suggest that Zfand5 inhibited, whereas TTP promoted, deadenylation of only ARE-containing mRNA.

Because Zfand5 can inhibit TTP-mediated TNF mRNA decay, we next tested whether Zfand5 could interfere with the effect of TTP on ARE-RNA deadenylation. We transfected RAW cells with TTP, Zfand5, or different ratios of both and compared their deadenylation activities. Consumption of A60 was highest in cells expressing TTP alone and lowest in cells expressing Zfand5 alone (Fig. 6C). Increases in the Zfand5/TTP ratio from 0.3 to 3 led to inhibition of deadenylation of ARE-RNA, resulting in less consumption of A60 in the same period (Fig. 6C). Thus, the antagonization by Zfand5 of the destabilizing effects of TTP on ARE-containing mRNA may be, at least in part, due to its inhibition of the deadenylation process.

Both Zinc Finger Domains of Zfand5 Contribute to Its mRNA-stabilizing Effect—The tandem zinc finger domain of TTP is required for its binding to ARE\textsuperscript{TNF} (45, 46). Zfand5 contains two different zinc finger domains, AN1 and A20. To evaluate the role of each of these two zinc fingers in Zfand5-mediated mRNA stabilization, we generated truncated mutants of Zfand5 in which either one or both zinc fingers were deleted (Fig. 7A). His-tagged Zfand5 and its mutants were expressed in E. coli and purified (Fig. 7B). First, we tested the ability of these truncation mutants to bind to ARE\textsuperscript{TNF} as compared with the full-length Zfand5 using RNA EMSA. Mutants with a single zinc finger deletion showed a weaker binding to ARE\textsuperscript{TNF} than the full-length Zfand5 (Fig. 7C). The AN1 deletion mutant-ARE\textsuperscript{TNF} complex migrated faster in the native gel than the
parental protein-RNA complex. The double deletion mutant Z5ΔA20ΔAN1, on the other hand, completely failed to bind to ARE
tNF (Fig. 7C).

Next, we tested whether expression of these mutants could stabilize TNF mRNA in RAW cells. RAW cells were transfected
with Zfand5 or its truncation mutants. 24 h later, cells were
exposed to LPS for 1 h to boost the basal level of TNF before the
addition of actinomycin D to block de novo mRNA synthesis.
The decay of TNF mRNA was determined by semiquantitative
RT-PCR at different times thereafter. Fig. 7D shows that dele-
tion of either zinc finger from Zfand5 reduced but did not abol-
ish its ability to stabilize TNF mRNA, as evidenced by the visible
TNF transcripts from cells transfected with single zinc finger
deletion mutants 60 min after the addition of actinomycin D. In
comparison, no TNF transcripts were detected in samples
transfected with vector only or the double deletion mutant.

When cells were transfected with full-length Zfand5, TNF tran-
script could be detected even 120 min after the addition of actinomycin D. In
comparison, no TNF transcripts were detected in samples
transfected with vector only or the double deletion mutant.

DISCUSSION
The half-lives of mammalian mRNAs generally range from a
few min to more than 10 h (48). Proinflammatory proteins,
however, are often encoded by the least stable mRNAs (2, 7).
Genome-wide analysis of mRNA stability revealed that tran-
scriptionally inducible genes, such as inflammatory cytokines
and chemokines, are disproportionately overrepresented in the
class of genes characterized by rapid mRNA turnover and often
share various versions of AREs in the 3′-UTR of their trans-
cripts (10, 49). In the immune system, rapid decay of inflam-
matory mediator transcripts promotes the resolution of
inflammation and helps prevent development of chronic
inflammation, a major driver of disease (1). Although there is
now an enormous literature on how RNA stability is controlled
by ARE-mediated signals, the picture is far from complete. A
better understanding of the mechanisms underlying rapid
decay of inflammatory mediators could help suggest therapeu-
tic strategies for resolving inflammation.

Here we report the identification of Zfand5 as a positive
modulator of ARE-RNA stability using an unbiased reporter
activity-based screen. We presented three lines of evidence
supporting the role of Zfand5 as an ARE-RNA stabilizer. First,
expression of Zfand5 in macrophages activated reporter activ-
ity only for transcripts with appended ARE-containing
sequences at their 3′-UTR. Second, expression of Zfand5 in
macrophages prolonged the half-life of TNF mRNA and
increased the release of TNF and IL-6 into the medium. Third,
knockdown of endogenous Zfand5 via short hairpin RNA spe-
cifically down-regulated transcripts containing ARE, such as
TNF and COX2. Zfand5 thus joins a growing list of ARE-RNA
regulators that collectively control the stability and translation
of an impressive number of inflammatory mediators with
3′-UTR AREs in their transcripts (3, 5).

The fate of mRNA, from its generation, processing, nuclear
export, and translation to its decay, is generally governed by the
proteins associated with it. Destabilizing signals carried by ARE-RNAs are transduced by RNA-binding proteins specifically targeting this motif (6). More than 20 ARE-RNA-binding proteins have been identified, some with positive and others with negative impacts on the half-lives of the target mRNAs (50). We show here that Zfand5 is a bona fide ARE-RNA-binding protein. Its $K_D$ with $TTP^{TNF}$ is similar to the $K_D$ of another ARE-RNA-binding protein, TTP. Consequently, expression of Zfand5 can reverse TTP-facilitated TNF mRNA decay. To our knowledge, this is the first evidence that the action of TTP on ARE-RNA can be antagonized by another protein that competes with its binding to ARE-RNA.

TTP, a member of the zinc finger protein-36 family, is the best-characterized ARE-destabilizing protein. TTP destabilizes a number of inflammatory mediators via its zinc finger-mediated interaction with AREs (44, 45). TTP knock-out mice develop an autoinflammatory syndrome marked by cachexia, arthritis, and dermatitis attributable to the apparently spontaneous expression of high levels of TNF (42, 51). The destabilizing action of TTP is known to be subject to several layers of regulation. First, TTP is induced by many inflammatory stimuli, including TNF, making rapid decay of ARE-RNA a routine phenomenon during inflammation (42). Second, phosphorylation of TTP by the MAPK/MK2 cascade decreases its affinity for ARE, promoting the association of TTP and adaptor protein 14-3-3. This excludes TTP from stress granules, leading to a restraint on TTP destabilizing action on ARE-RNAs (52) and allowing for their higher accumulation. On the other hand, TTP is physically associated with phosphatase 2A, which keeps restraint on TTP destabilizing action on ARE-RNA, protects the potential of TTP as an ARE-RNA destabilizer (53). Our current finding adds another layer of regulation to this already sophisticated modulation system. Zfand5 competes with TTP for association with ARE-RNA, suppresses TTP-induced deadenylation, and interferes with the destabilizing effect of TTP toward ARE-RNA.

Like TTP, Zfand5 can be induced by many inflammatory stimuli, including microbial or host products, although at first glance, it seems paradoxical that TTP and Zfand5, two proteins with opposite effects on TNF mRNA stability, would both be induced by LPS. Analysis of their induction revealed major differences in the kinetics. Whereas TTP exhibits a sustained increase in its level once induced (42), Zfand5 induction is transient (Fig. 2B). This is consistent with the kinetics of TNF mRNA, which is rapidly induced by LPS but declines after 90 min. Transient elevation of TNF levels is presumably beneficial for the host to respond to microbial challenge while avoiding prolonged inflammation. The asynchronous induction of TTP and Zfand5 by inflammatory stimuli demonstrates the complexity of ARE-mediated regulation of mRNA decay and underscores its physiologic importance.

Binding of TTP to ARE-RNA is mediated by its two CCCH zinc fingers (44, 45). The binding of Zfand5 to ARE-RNA is
Zfand5 Binds and Stabilizes ARE-RNA

similarly mediated by its two zinc fingers, A20 and AN1. However, these are structurally distinct from the CCCH type in TTP. Both A20 and AN1 domains seem to contribute to the stabilizing action of Zfand5 on ARE-RNA. Deletion of both zinc fingers from Zfand5 prevented its binding to ARE-TNF and eliminated its ability to stabilize TNF transcripts. Zfand5 belongs to a protein family with six mammalian members; all contain one A20 and one AN1 zinc finger domain. Whether other members of the family share RNA binding and stabilizing effects with Zfand5 remains to be determined.

In summary, a genetic screen allowed us to identify a small zinc finger protein, Zfand5, as an ARE-RNA-binding protein. Association of Zfand5 and ARE-RNA prevents formation of the TTP-ARE complex and antagonizes the destabilizing action of TTP on target mRNAs. Zfand5 and TTP coordinately regulate many ARE-encoding proinflammatory genes at the level of mRNA decay.

Acknowledgments—We thank C. J. Wilusz for reagents, Q. Yin and H. Li for technical assistance, and C. Nathan and K. Rhee for discussion and critical reading of the manuscript.

REFERENCES

1. Nathan, C., and Ding, A. (2010) Nonresolving inflammation. Cell 140, 871–882
2. Hao, S., and Baltimore, D. (2009) The stability of mRNA influences the temporal order of the induction of genes encoding inflammatory molecules. Nat. Immunol. 10, 281–288
3. Shaw, G., and Kamen, R. (1986) A conserved AU sequence from the 3’-untranslated region of GM-CSF mRNA mediates selective mRNA degragation. Cell 66, 659–667
4. Koehler, D. M., Casey, J. L., Hentze, M. W., Gerhardt, E. M., Chen, L. N., Klausner, R. D., and Harford, J. B. (1989) A cytosolic protein binds to structural elements within the iron regulatory region of the transferrin receptor mRNA. Proc. Natl. Acad. Sci. U.S.A. 86, 3574–3578
5. von Roretz, C., and Gallouzi, I. E. (2008) Decoding ARE-mediated decay. Is mRNA transcripta the equation? J. Cell Biol. 181, 189–194
6. Glisovic, T., Bachorik, J. L., Yong, J., and Dreyfuss, G. (2008) RNA-binding proteins and post-transcriptional gene regulation. FEBS Lett. 582, 1977–1986
7. Anderson, P. (2010) Post-transcriptional regulons coordinate the initiation and resolution of inflammation. Nat. Rev. Immunol. 10, 24–35
8. Treisman, R. (1985) Transient accumulation of c-fos RNA following serum stimulation requires a conserved 5’ element and c-fos 3’ sequences. Cell 42, 889–902
9. Gingerich, T. J., Feige, J. J., and LaMarre, J. (2004) AU-rich elements and their control of gene expression through regulated mRNA stability. Annu. Rev. Biochem. 73, 49–63
10. Bakheet, T., Frevel, M., Williams, B. R., Greer, W., and Khabar, K. S. (2001) ARED. Human AU-rich element-containing mRNA database reveals an unexpectedly diverse functional repertoire of encoded proteins. Nucleic Acids Res. 29, 246–254
11. Gruber, A. R., Fallmann, J., Kratochvill, F., Kovarik, P., and Hofacker, I. L. (2011) AREsite. A database for the comprehensive investigation of AU-rich elements. Nucleic Acids Res. 39, D66–D69
12. Chen, C. Y., and Shyu, A. B. (1995) AU-rich elements. Characterization and importance in mRNA degradation. Trends Biochem. Sci. 20, 465–470
13. Xu, N., Chen, C. Y., and Shyu, A. B. (1997) Modulation of the fate of cytoplasmic mRNA by AU-rich elements. Key sequence features controlling mRNA deadenylation and decay. Mol. Cell. Biol. 17, 4611–4621
14. Aggarwal, B. B. (2003) Signaling pathways of the TNF superfamily. A double-edged sword. Nat. Rev. Immunol. 3, 745–756
15. Scott, D. A., Greinwald, J. H., Jr., Marietta, J. R., Drury, S., Swiderski, R. E., Viitas, A., DeAngelis, M. M., Carmi, R., Ramesh, A., Kraft, M. L., Elbedour, K., Skoworak, A. B., Friedman, R. A., Srikumar Sarasirapaty, C. R., Verboven, K., Van Gamp, G., Lovett, M., Deininger, P. L., Batzer, M. A., Morton, C. C., Beals, I. R., Smith, R. J., and Sheffield, V. C. (1998) Identification and mutation analysis of a cooehlar-expressed, zinc finger protein gene at the DBK7/11 and dn hearing loss loci on human chromosome 9q and mouse chromosome 19. Gene 215, 461–469
16. Dixit, A. R., and Dhanekha, O. P. (2011) A novel stress-associated protein “ATSAP10” from Arabidopsis thaliana confers tolerance to nickel, manganese, zinc, and high temperature stress. PLoS One 6, e20921
17. Hishiya, A., Ikeda, K., and Watanabe, K. (2005) A RANKL-induced gene Zn216 in osteosteat formation. J. Recept. Signal. Transduct. Res. 25, 199–216
18. Hishiya, A., Iemura, S., Natsume, T., Takayama, S., Ikeda, K., and Watanabe, K. (2006) A novel ubiquitin-binding protein ZNF216 functioning in muscle atrophy. EMBO J. 25, 554–564
19. Huang, J., Teng, L., Li, L., Liu, T., Li, L., Chen, D., Xu, L. G., Zhai, Z., and Shu, H. B. (2004) ZNF216 Is an A20-like and IxB kinase γ-interacting inhibitor of NFκB activation. J. Biol. Chem. 279, 16847–16853
20. Yin, F., Banerjee, R., Thomas, B., Zhou, P., Qian, L., Ia, T., Ma, X., Ma, Y., Iadecola, C., Beal, M. F., Nathan, C., and Ding, A. (2010) Exaggerated inflammation, impaired host defense, and neuropathology in progranulin-deficient mice. J. Exp. Med. 207, 117–128
21. Ludwig, L. B., Hughes, B. J., and Schwartz, S. A. (1995) Biotinylated probes in the electrophoretic mobility shift assay to examine specific dsDNA, ssDNA, or RNA-protein interactions. Nucleic Acids Res. 23, 3792–3793
22. Katsamba, P. S., Park, S., and Laird-Offringa, I. A. (2002) Kinetic studies of RNA-protein interactions using surface plasmon resonance. Methods 26, 95–104
23. Ford, L. P., Watson, J., Keene, J. D., and Wilusz, J. (1999) ELAV proteins stabilize deadenylated intermediates in a novel in vitro mRNA deadenylation/degradation system. Genes Dev. 13, 188–201
24. Milone, J., Wilusz, J., and Beloﬀatto, V. (2004) Characterization of deadenylation in trypanosomes extracts and its inhibition by poly(A)-binding protein Pab1p. RNA 10, 448–457
25. Zhang, S., Williams, C. J., Wormington, M., Stevens, A., and Peltz, S. W. (1999) Monitoring mRNA deacapping activity. Methods 17, 46–51
26. Sun, D., and Ding, A. (2006) MyD88-mediated stabilization of interferon-γ-induced cytokine and chemokine mRNA. Nat. Immunol. 7, 375–381
27. Pomerantz, J. L., Denny, E. M., and Baltimore, D. (2002) CARD11 mediates factor-specific activation of NF-kB by the T cell receptor complex. EMBO J. 21, 5184–5194
28. Xu, N., Lollin, P., Chen, C. Y., and Shyu, A. B. (1998) A broader role for AU-rich-element-mediated mRNA turnover revealed by a new transcriptional pulse strategy. Nucleic Acids Res. 26, 558–565
29. Oh, Y. K., Lee, H. J., Jeong, M. H., Rhee, M., Mo, J. W., Song, E. H., Lim, J. Y., Choi, K. H., Jo, I., Park, S. I., Gao, B., Kwon, Y., and Kim, W. H. (2008) Role of activating transcription factor 3 on TAp73 stability and apoptosis in paclitaxel-treated cervical cancer cells. Mol. Cancer Res. 6, 1232–1249
30. Wang, W., Chen, J. X., Liao, R., Deng, Q., Zhou, J. H., Huang, S., and Sun, P. (2002) Sequential activation of the MEK-extracellular signal-regulated kinase and MKK3/6-p38 mitogen-activated protein kinase pathways mediates oncogenic ras-induced premature senescence. Mol. Cell. Biol. 22, 3389–3403
31. Han, Q., Leng, J., Bian, D., Mahanivong, C., Carpenter, K. A., Pan, Z. K., Han, J., and Huang, S. (2002) Rac1-MKK3-p38-MAPKAPK2 pathway promotes uokinase plasminogen activator mRNA stability in invasive breast cancer cells. J. Biol. Chem. 277, 48379–48385
32. Koehler, D. M., Horowitz, J. A., Casey, I. L., Klausner, R. D., and Harford, J. B. (1991) Translation and the stability of mRNAs encoding the transferrin receptor and c-fos. Proc. Natl. Acad. Sci. U.S.A. 88, 7778–7782
33. Ghosh, A. K., Steele, R., and Ray, R. B. (1999) Functional domains of c-myc promoter binding protein 1 involved in transcriptional repression and cell growth regulation. Mol. Cell. Biol. 19, 2880–2886
34. Takekawa, M., and Saito, H. (2008) A family of stress-inducible GADD45-like proteins mediate activation of the stress-responsive MTK1/AEK4 MAPK/P. Cell 95, 521–530
35. Takekawa, M., Tatebayashi, K., Itoh, F., Adachi, M., Imai, K., and Saito, H.
(2002) Smad-dependent GADD45β expression mediates delayed activation of p38 MAP kinase by TGF-β. EMBO J. 21, 6473–6482
36. Michael, W. M., Choi, M., and Dreyfuss, G. (1995) A nuclear export signal in hnRNP A1. A signal-mediated, temperature-dependent nuclear protein export pathway. Cell 83, 415–422
37. Han, J. H., Beutler, B., and Hueg, G. (1991) Complex regulation of tumor necrosis factor mRNA turnover in lipopolysaccharide-activated macrophages. Biochim. Biophys. Acta 1090, 22–28
38. Good, P. J. (1995) A conserved family of elav-like genes in vertebrates. Proc. Natl. Acad. Sci. U.S.A. 92, 4557–4561
39. Zhang, W., Wagner, B. J., Ehrenman, K., Schaefer, A. W., DeMaria, C. T., Crater, D., DeHaven, K., Long, L., and Brewer, G. (1993) Purification, characterization, and cDNA cloning of an AU-rich element RNA-binding protein, AUFI. Mol. Cell. Biol. 13, 7652–7665
40. Dember, L. M., Kim, N. D., Liu, Q. Q., and Anderson, P. (1996) Individual RNA recognition motifs of TIA-1 and TIAR have different RNA binding specificities. J. Biol. Chem. 271, 2783–2788
41. Gueydan, C., Droogmans, L., Chalon, P., Hueg, G., Caput, D., and Kruys, V. (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
42. Carballo, E., Lai, W. S., and Blackshear, P. J. (1998) Feedback inhibition of macrophage tumor necrosis factor-α production by tristetraprolin. Science 281, 1001–1005
43. Blackshear, P. J., Lai, W. S., Kennington, E. A., Brewer, G., Wilson, G. M., Guan, X., and Zhou, P. (2003) Characteristics of the interaction of a synthetic human tristetraprolin tandem zinc finger peptide with AU-rich element-containing RNA substrates. J. Biol. Chem. 278, 19947–19955
44. Lai, W. S., Kennington, E. A., and Blackshear, P. J. (2002) Interactions of CCCH zinc finger proteins with mRNA. Non-binding tristetraprolin mutants exert an inhibitory effect on degradation of AU-rich element-containing mRNAs. J. Biol. Chem. 277, 9606–9613
45. Hudson, B. P., Martinez-Yamout, M. A., Dyson, H. J., and Wright, P. E. (2004) Recognition of the mRNA AU-rich element by the zinc finger domain of TIS11d. Nat. Struct. Mol. Biol. 11, 257–264
46. Lai, W. S., Carballo, E., Strum, J. R., Kim, E. A., Phillips, R. S., and Blackshear, P. J. (1999) Evidence that tristetraprolin binds to AU-rich elements and promotes the deadenylation and destabilization of tumor necrosis factor α mRNA. Mol. Cell. Biol. 19, 4311–4323
47. Decker, C. J., and Parker, R. (1993) A turnover pathway for both stable and unstable mRNAs in yeast. Evidence for a requirement for deadenylation. Genes Dev. 7, 1632–1643
48. Wilusz, C. J., Wormington, M., and Peltz, S. W. (2001) The cap-to-tail guide to mRNA turnover. Nat. Rev. Mol. Cell Biol. 2, 237–246
49. Grigull, J., Mnaimneh, S., Pootoolal, J., Robinson, M. D., and Hughes, T. R. (2004) Genome-wide analysis of mRNA stability using transcription inhibitors and microarrays reveals posttranscriptional control of ribosome biogenesis factors. Mol. Cell. Biol. 24, 5534–5547
50. Chang, S. H., and Hla, T. (2011) Gene regulation by RNA binding proteins and microRNAs in angiogenesis. Trends Mol. Med. 17, 650–658
51. Taylor, G. A., Carballo, E., Lee, D. M., Lai, W. S., Thompson, M. J., Patel, D. D., Schenkenman, D. I., Gilkeson, G. S., Broxmeyer, H. E., Haynes, B. F., and Blackshear, P. J. (1996) A pathogenic role for TNF-α in the syndrome of cachexia, arthritis, and autoimmunity resulting from tristetraprolin (TTP) deficiency. Immunity 4, 445–454
52. Stoecklin, G., Stubbs, T., Kedersha, N., Wax, S., Rigby, W. F., Blackwell, T. K., and Anderson, P. (2004) MK2-induced tristetraprolin-14-3-3 complexes prevent stress granule association and ARE-mRNA decay. EMBO J. 23, 1313–1324
53. Sun, L., Stoecklin, G., Van Way, S., Hinkovska-Galcheva, V., Guo, R. F., Anderson, P., and Shawley, T. P. (2007) Tristetraprolin (TTP)-14-3-3 complex formation protects TTP from dephosphorylation by protein phosphatase 2a and stabilizes tumor necrosis factor-α mRNA. J. Biol. Chem. 282, 3766–3777