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Inflammation: Cytokines and RNA-based Regulation

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Abstract

The outcome of an inflammatory response depends upon the coordinated regulation of a variety of both pro-inflammatory and anti-inflammatory cytokines and other proteins. Regulation of these inflammation mediators can occur at multiple levels, including transcription, mRNA translation, post-translational modifications, and mRNA degradation. Post-transcriptional regulation has been shown to play an important role in controlling the expression of these mediators, allowing for normal initiation and resolution of the inflammatory response. Many inflammatory mediators have unstable mRNAs due, in part, to the presence of AU-rich elements in their 3′-untranslated regions. Increasing numbers of RNA-binding proteins have been identified that can bind to these AU-rich elements and then regulate the stability and/or translation of the mRNA. This review summarizes current knowledge about the role of several RNA-binding proteins that act through AU-rich elements to post-transcriptionally regulate the biosynthesis of proteins involved in inflammation.

Keywords

Cytokines; inflammation; AU-rich element; RNA-binding protein

Introduction

Inflammation is a normal defense mechanism that the body uses to protect itself from tissue injury. Regardless of the nature of the agent that caused the injury (physical, chemical, or microbial), the inflammatory response follows the same regulated process [1]. In the early or acute stage of inflammation, there is an initial increase in blood flow to the site of injury as well as increased vascular permeability. A variety of signaling molecules are released, including chemokines, cytokines, eicosanoids and adhesion molecules, resulting in the recruitment of polymorphonuclear leukocytes to the site of injury. As the inflammatory response progresses, monocytes, which differentiate locally into macrophages, and lymphocytes accumulate, assisting in the neutralization of the injurious agent. In the late stage of inflammation, tissue remodeling processes take over, leading to a resolution of the inflammatory response and a return to normal tissue physiology [2, 3, 4, 5, 6].

During acute inflammation, leukocytes that are recruited to the site of inflammation release pro-inflammatory mediators that initiate and enhance the acute phase of the response. The production of pro-inflammatory mediators (e.g., interleukin-1 [IL-1] and tumor necrosis factor alpha [TNF]) is counterbalanced by the local release of anti-inflammatory mediators such as interleukin-10 (IL-10) as well as endogenous anti-inflammatory molecules such as corticosteroids. The initiation and resolution of the inflammatory response involves the

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complex and coordinated expression of many factors, including cytokines, chemokines, growth factors, proteases, lipids, etc. Dysregulation of the normal regulatory pathways involved in the resolution of the inflammatory response can lead to the development of chronic inflammatory diseases such as rheumatoid arthritis and inflammatory bowel disease.

The production of both pro- and anti-inflammatory mediators of inflammation can be regulated at many levels, including gene transcription, mRNA translation, and mRNA degradation [7, 8, 9, 10, 11, 12]. Transcriptional activation is required to initiate the inflammatory response, but post-transcriptional mechanisms play important roles in controlling the expression of potentially harmful pro-inflammatory mediators.

In this review, we will discuss the post-transcriptional regulation of pro-inflammatory protein production, focusing on several mRNA-binding proteins that have been shown to participate in this regulation.

The role of AU-rich elements in mRNA stability

Many inflammatory mediators, such as cytokines and chemokines, have unstable mRNAs. This instability often results in part from the presence of *cis*-acting adenine and uridine-rich (AU-rich) elements, or AREs, in their 3′ untranslated (UTR) regions. These AU-rich elements provide binding sites for *trans*-acting factors, such as RNA-binding proteins, that can subsequently regulate the stability and/or translation of the mRNA. AREs were first identified as conserved regions within the 3′ UTRs of several human and mouse mRNAs encoding inflammatory-related proteins [13], including TNF, IL-1, granulocyte-macrophage colony-stimulating factor (GM-CSF), and interferon-alpha (IFN-α). Because 3′ UTRs are relatively poorly conserved compared to protein coding regions, it was proposed that the identified consensus sequence, UUAUUUAU, may play an important regulatory role. This regulatory function was demonstrated by Shaw and Kamen [14] when they showed that the placement of the ARE from the GM-CSF mRNA into the 3′ UTR of the relatively stable β-globin transcript caused the β-globin mRNA to become highly unstable.

The pro-inflammatory cytokine TNF has been known to play an important role in the pathogenesis of many human inflammatory diseases [15]. The use of anti-TNF agents has proven to be successful in the treatment of several inflammatory conditions including rheumatoid arthritis, Crohn's disease, and psoriasis [16, 17], reinforcing the central role that TNF plays in chronic inflammation. TNF expression is regulated both at the transcriptional level as well as post-transcriptionally. Post-transcriptional control has been shown to occur both at the level of mRNA stability as well as translation [18]. The importance of the 3′ UTR of TNF in the development of arthritis was demonstrated in studies using mice that overexpressed a TNF/β-globin 3′ UTR transgene [19]. These mice expressed a human TNF gene driven by its own promoter and protein coding region, but the 3′ UTR from the human β-globin mRNA replaced the 3′ UTR of the human TNF transcript. These mice developed chronic inflammatory polyarthritis [19]. Treatment with an antibody to human TNF was able to prevent the development of disease, demonstrating the connection between TNF and the pathogenesis of the arthritis.

Subsequent animal studies established the role of the TNF 3′ UTR ARE in the development of inflammatory disease [20]. In these studies, the 69 bp ARE was deleted by homologous recombination "knockin" technology. Mice lacking this ARE (TNFΔARE mice) had increased constitutive and LPS-induced levels of circulating TNF protein and were more susceptible to the endotoxic effects of LPS [20]. Both peritoneal macrophages and bone marrow-derived macrophages (BMDM) expressed increased levels of TNF mRNA in the TNFΔARE mice compared to control mice although the level of TNF gene transcription was

the same [20]. Actinomycin D studies showed that cells from the TNFΔARE mice accumulated more TNF mRNA than those from control mice, due to an increased TNF mRNA half-life [20]. Other studies have shown that the ARE in the 3′ UTR of TNF is also responsible for translational repression of TNF [20, 21, 22, 23]. This translational repression could be released upon treatment with lipopolysaccharide (LPS) [21]. Thus, the TNF ARE appeared to contribute to both TNF mRNA stability and translation. In the absence on the ARE, the TNFΔARE mice developed both chronic inflammatory arthritis and a Crohn's-like inflammatory bowel disease, suggesting a link between excess TNF production and the analogous diseases in humans [20].

AREs within mRNAs are often between 50 and 150 nt in length and range greatly in composition. One classification was based on the number and distribution of AUUUA pentamers [24]. Class I AREs contain 1 to 3 AUUUA pentamer motifs scattered throughout the 3′ UTR within or near a U-rich region (e.g., c-myc and c-fos). Class II AREs contain at least two overlapping copies of the nonamer UAUUUA(U/A)(U/A) within a U-rich region (e.g., cytokine mRNAs such as TNF and GM-CSF). Class III AREs contain U-rich regions but no AUUUA pentamers (e.g., c-jun). Bakheet et al. [25] have created a database of human ARE mRNAs (ARED; http://brp.kfshrc.edu.sa/ARED/) based on the pattern of AUUUA pentamers. Group I contains 5 pentameric repeats, AUUUAUUUAUUUAUUUAUUUA; group II contains 4, AUUUAUUUAUUUAUUUA; group III contains 3 (A/U)AUUUAUUUAUUUA(A/U); group IV contains 2, $(A/U)_2AUUUAUUUA(A/U)_2$; and group V contains 1, $(A/U)_4AUUUA(A/U)_4$. Based on their analysis, the percentage of mRNAs within the human genome that contain AREs is about 8%. This database has been updated several times and the most recent update includes human, mouse and rat ARE mRNAs [26, 27, 28].

Pathways of ARE-mediated mRNA decay

Deadenylation, or the processive removal of the poly(A) tail, is thought to be the first and rate-limiting step in the degradation of mRNA in eukaryotes. Several eukaryotic deadenylases have been shown to participate in this process, including poly(A)-specific ribonuclease (PARN) and the CCR4/POP2/NOT1 complex (a complex of 9 proteins). AREs have been shown to enhance the rate of deadenylation [14, 29]. Following deadenylation, the mRNA "body" can be degraded further by decapping followed by 5′→3′ exonucleolytic decay; 3′→5′ exonucleolytic degradation; or by endonucleolytic degradation [12, 30, 31]. The 3′→5′ exonucleolytic decay pathway has been shown to require the exosome, a large multi-unit complex of 3′→5′ exonucleases [32, 33]. A component of the exosome, PM-SCL75, has been shown to interact directly with AREs [32]. In the decapping and 5′→3′ exonucleolytic decay pathway, the LSM 1–7 complex binds to the 3′ end of the mRNA and promotes decapping by the DCP1/DCP2 complex. Following decapping, the mRNA can be degraded by the 5′→3′ exonuclease XRN1 [12, 30, 31]. ARE-containing mRNAs have been shown to stimulate RNA decapping, suggesting that ARE-mediated decay can also occur through the 5′→3′ exonucleolytic decay pathway [34].

Several components of the 5′→3′ decay machinery, including deadenylases, the decapping enzymes DCP1/DCP2, the LSM 1–7 complex, XRN1 and others are enriched in granular cytoplasmic foci called processing bodies or P bodies [30, 31, 35, 36]. Translationally silent mRNA is also present in P bodies and mRNA decay intermediates have been detected in P bodies, leading to the proposal that P bodies are cellular sites of mRNA decay [37, 38]. P bodies form when mRNA translation is impaired or the 5′→3′ decay pathway gets overloaded. mRNA within P bodies can be degraded or held in a translationally inactive state until it is released to associate with polysomes for translation. Another type of RNA granule that can be found in eukaryotic cells is the stress granule or SG [39, 40]. Stress

granules form in response to environmental stress (e.g., heat shock, oxidative stress, and UV irradiation) and contain stalled initiation complexes. Stress granule formation requires the phosphorylation of the initiation factor eIF2α. Phosphorylated eIF2α reduces the availability of the eIF2α-GTP-$tRNA_i^{Met}$ ternary complex, resulting in blocked translation initiation and polysome disassembly [41, 42]. The components of SGs rapidly shuttle in and out of the SG [43]. Studies using live-cell microscopy demonstrated that P bodies and SGs can interact with each other [44]. It has been proposed that following polysome disassembly, released mRNA is sorted within SGs and some mRNAs are selected for storage or reinitiation while other mRNAs are selected to be delivered to P bodies where they are degraded [44]. A number of ARE-binding proteins have been shown (discussed below) to associate with components of the exosome and the 5′→3′ decay pathway as well as P bodies, suggesting that both degradation pathways are involved in ARE-mediated mRNA decay [33, 44, 45, 46, 47].

Post-transcriptional regulation via ARE-binding proteins

A growing number of ARE-binding proteins have been identified over the past 20 years [48]. Table I lists ARE-binding proteins that have been shown to regulate their target mRNAs at the post-transcriptional level. These proteins bind to AREs in their target RNAs via specific protein domains, including the RNA-recognition motif (RRM), the CCCH tandem zinc finger domain, and the K-homology domain (KH). The interactions between ARE-binding proteins and their target mRNAs have been shown using a variety of methods, including UV crosslinking, RNA electrophoretic mobility shift assay, co-transfection functional assays, RNA immunoprecipitation (RIP) assays, siRNA knockdown assays, or mRNA decay in cells isolated from ARE-binding protein knockout mice. To date, only relatively few ARE-binding proteins have been shown to be involved in inflammatory processes in intact animals.

The Tristetraprolin (TTP) family of proteins

Tristetraprolin (TTP), zinc finger protein 36 or ZFP36 (also known as Tis11, Nup475, and Gos24), is the prototype of a small family of four mammalian CCCH tandem zinc finger proteins capable of binding to RNA. TTP and two other family members, ZFP36L1 (TIS11B, cMG1, BRF1, ERF1, Berg36) and ZFP36L2 (TIS11D, BRF2, ERF2) are expressed in a variety of species, including man [49, 50, 51]. The most recently identified member, ZFP36L3, has only been found in rodents and has been detected only in placenta and extraembryonic tissues [52, 53]. The CCCH tandem zinc finger domain of all family member proteins is characterized by 2 zinc fingers, spaced 18 amino acids apart, each containing 3 cysteines and one histidine with a strictly defined internal spacing, $C\text{-}X_8\text{-}C\text{-}X_5\text{-}C\text{-}X_3\text{-}H$ [49]. TTP itself is the most extensively studied member of this family of proteins. It was first identified as the product of an immediate early response gene, *Zfp36*, in fibroblasts and other cells stimulated with insulin, phorbol esters, or serum [49, 54, 55, 56]. TTP is localized in the nucleus of serum-deprived quiescent fibroblasts and is rapidly translocated to the cytoplasm in response to mitogens or serum [57]. Translocation is accompanied by serine phosphorylation of the protein [58]. In macrophages, TTP is dramatically induced by LPS, and is then located primarily in the cytoplasm [59, 60].

Cell transfection and cell-free experiments have shown that TTP, as well as the three other mouse family members, ZFP36L1, ZFP36L2 and ZFP36L3, bind via their conserved tandem zinc finger domains to AREs in mRNAs at a consensus nonamer site, UUAUUUAUU. Binding of synthetic random zinc finger domain peptides to this sequence has been shown to occur with low nanomolar affinity [61]. This initial RNA binding leads to the deadenylation and subsequent degradation of the transcript [52, 53, 62, 63, 64, 65, 66, 67].

Several studies have implicated TTP in deadenylation as well as both 5′→3′ and 3′→5′ exonucleolytic decay. We have shown that TTP, as well as its other family members, can promote ARE-containing mRNA deadenylation in a cell-free assay [52, 65]. Co-transfection studies with PARN demonstrated that TTP's ability to promote deadenylation was enhanced by adding increasing concentrations of PARN [65]. Chen et al. [33] demonstrated that the ARE-binding proteins AUF1 (AU-rich binding factor 1), KSRP (KH-type splicing regulatory protein) and TTP co-purified with human exosomes. Subsequent cell-free studies showed that the addition of KSRP or TTP to isolated exosomes stimulated ARE-mediated mRNA decay [33]. TTP and ZFP36L1 (BRF1) have been shown to interact with several components of the mRNA decay pathways including the deadenylase CCR4, the decapping enzymes DCP1 and DCP2, two components of the exosome, RRP4 and PM-SCL75, and the 5′→3′ exonuclease XRN1 [45, 46, 47]. These studies did not show an interaction between TTP or ZFP36L1 and PARN [45]. The N terminal domain of TTP and ZFP36L1was found to be the region responsible for interacting with the mRNA decay proteins [45], while both the N- and C-terminal domains were capable of activating mRNA decay when linked to a heterologous RNA-binding protein [45]. The N-terminal domain of TTP that was shown to interact with DCP2 was able to activate decapping of an ARE substrate in an *in vitro* decapping assay [46].

TTP and ZFP36L1 have also been shown to co-localize to P bodies along with components of the 5′→3′ mRNA decay pathway [44]. Subsequent studies demonstrated that TTP and ZFP36L1 were able to target a linked heterologous RNA-binding protein to P bodies [68]. The N- and C-terminal domains of TTP functioned as redundant P body localization domains [68]. These studies have lead to the hypothesis that TTP may exert its effect on ARE-mediated mRNA decay by recruiting the exosome to the bound transcript [33, 47]. TTP may also deliver bound transcripts to P bodies, where mRNA decay can proceed by the 5′→3′ pathway, or TTP may promote the assembly of P bodies through the recruitment of mRNA decay enzymes [9, 44, 68]. TTP has also been linked to the RNA-induced silencing complex (RISC). Jing et al. [69] have shown that TTP interacts with Argonaute/eiF2C2 and eiF2C4, components of the RISC complex. MiR16, a miRNA containing an UAAAUAUU sequence that is complimentary to the ARE in TNF mRNA, was required for ARE-mediated mRNA decay, and miR16 required the presence of TTP in order to have an effect on ARE-mediated decay [69]. It has been proposed that TTP, through binding to Argonaute proteins, assists miR16 targeting to the ARE, resulting in mRNA decay [69]. Argonaute proteins localize to P bodies and mRNAs that are targeted by miRNAs accumulate within P bodies [70].

The physiological role of TTP in intact animals was first suggested by studies involving TTP knockout (KO) mice. These mice appeared normal at birth but soon developed a complex syndrome that included inflammatory arthritis, cachexia, autoimmunity, dermatitis and myeloid hyperplasia [71]. Because some aspects of the TTP KO phenotype resembled mouse models of TNF overexpression [19, 72, 73], we treated newborn mice with a monoclonal antibody directed against mouse TNF [74]. This resulted in prevention of most aspects of the KO phenotype. Subsequent breeding experiments with mice deficient in both types of TNF receptor also alleviated most aspects of the KO phenotype [75]. Taken together, these studies indicated that TTP played a role in the production or clearance of TNF [75]. Subsequent studies done in macrophages isolated from TTP KO mice demonstrated that TTP could bind directly to the ARE within the TNF mRNA, and that TTP was capable of de-stabilizing the TNF mRNA [59]. These studies also showed that the tandem zinc finger domain of TTP was the region that interacted with the ARE of TNF. Thus, the increased production of both TNF protein and mRNA in the TTP KO mice was the result of an increase in the stability of TNF mRNA [59], resulting in overproduction of TNF protein.

Subsequent experiments identified a second physiological target of TTP, the GM-CSF mRNA, in bone marrow-derived stromal cells isolated from TTP KO mice. As was seen for TNF, the absence of TTP resulted in an increased production of GM-CSF due to a marked stabilization of GM-CSF mRNA [76]. Similar results were seen in stromal cells isolated from TTP KO mice that were also deficient in both TNF receptors, ruling out the possibility that increased circulating TNF was responsible for the observed effect on GM-CSF mRNA [76].

An important clue concerning TTP's mechanism of action came out of these studies. Northern blot analysis of bone marrow-derived stromal cells demonstrated that, in cells from wild type (WT) mice, the GM-CSF mRNA exists as two species of similar abundance but differing in size by about 200 bases. This size difference was shown to be the result of the presence or absence of a poly(A) tail on the RNA [76]. The larger of the two species, the polyadenylated version, was almost the only transcript detectable in the cells from the TTP KO mice [76]. Actinomycin D mRNA turnover studies indicated that while both species of GM-CSF mRNA were unstable in wild type stromal cells, the fully polyadenylated transcript seen in the TTP KO stromal cells barely decayed at all during the course of the experiment [76]. Thus, the absence of TTP led to the failure of removal of the poly(A) tail (deadenylation), resulting in the accumulation of the fully polyadenylated GM-CSF mRNA and overproduction of the GM-CSF protein.

Many potential mRNA targets for TTP have since been described in the literature, using a variety of techniques (see Table II). For example, TTP overexpression studies have identified a number of mRNAs that were negatively regulated, and, in some cases, mRNA stability was shown to be decreased, including IL-3 [59, 77, 78, 79], plasminogen activator inhibitor type 2 or PAI-2 [80], cyclooxygenase-2 or Cox-2 [81], paired-like homeodomain transcription factor 2 or Pitx2 [82], IL-2 [78], IL-6 [78], IL-8 [83], vascular endothelial growth factor or VEGF [83], and coxsackie- and adenovirus-receptor-like membrane protein or CLMP [84]. A RIP-CHIP (immunoprecipitation with TTP antibody followed by RNA isolation and hybridization to Affymetrix GeneChips) approach was used to identify potential TTP targets in human dendritic cells [85]. More than 300 genes were identified by this approach of which 37 were present in the ARED2 database. Six of these identified targets were verified by real-time PCR and functional transfection studies. These targets include dual specificity phosphatase 1 (DUSP1, also known as mitogen-activated protein kinase phosphatase 1 or MKP-1), indoleamine 2,3-dioxygenase (IDO), superoxide dismutase 2 (SOD2), CD86, and MHC class I-B and F [85]. CD86 and MHC class I mRNAs were not in the ARED database, although CD86 contains two ARE-like sequences (UAUUUAU and UUAUUUUAU) in its 3′ UTR. Two previously identified targets of TTP, Cox-2 and DUSP1, were found to be transiently induced during the early stages of differentiation of 3T3-L1 preadipocytes [86]. Both of these transcripts contain AU-rich elements in the 3′ UTR and both were found to co-immunoprecipitate with TTP [86].

Knockdown of TTP by siRNA has been performed in a variety of cell types, permitting the identification of mRNAs whose stability is regulated by TTP. These include IL-8 [87], VEGF [88], E2a-encoded transcription factor E47 [89], c-fos [90], cyclin-dependent kinase inhibitor 1A (p21; [90]), IL-6 [91], IL-12 [91], Cxcl2 or macrophage inflammatory protein 2-alpha (MIP-2; [91]), cyclin D1 [92], c-myc [92], and 1,4-galactosyltransferase [93]. All of these transcripts were found to be negatively affected by TTP, i.e., TTP caused the destabilization of these mRNAs. In some cases mRNA (p21, c-fos) or protein (IL-12, MIP-2) levels were measured but mRNA stability was not assessed [90, 91].

In contrast to the general theme of TTP as an mRNA *destabilizing* factor, two mRNAs have been found to be *stabilized* by TTP using siRNA knockdown approaches [91, 94]. In one

case, involving inducible nitric-oxide synthase (iNOS) mRNA [94], TTP did not interact with the iNOS mRNA itself (based on UV crosslinking), but instead interacted with the KH-type splicing regulatory protein, KSRP [94], which has been shown to destabilize iNOS mRNA through binding to the ARE in the 3′UTR of the transcript [95]. Knockdown of TTP in a mouse macrophage cell line resulted in decreased production of MIP-3α, also known as CC chemokine ligand 20 (CCL20) [91]. MIP-3α does not contain classical nonamer binding sites for TTP, but 5 AUUUA pentamers are present in the 3′ UTR. Binding studies were not performed in this study nor were mRNA levels or stability measured [91]. These studies suggest that TTP may sometimes regulate mRNAs by indirect mechanisms, for example, involving protein-protein interactions.

Since the identification of TNF and GM-CSF as the first documented “physiological” targets of TTP, a number of target mRNAs have been validated as physiologically relevant, i.e., meeting the strict criterion that they are stabilized in cells from TTP KO mice (see Table II, bold entries).

For example, Ogilvie et al. [96] examined the role of TTP in the regulation of IL-2 mRNA in T cells isolated from TTP KO mice. First, they demonstrated that in activated human T cells, TTP is induced and can bind to the ARE in the 3′ UTR of IL-2 [96]. Activated total splenocytes or CD-3 positive T cells from TTP KO mice produced more IL-2 protein and mRNA compared to WT cells [96]. Subsequent actinomycin D decay assays demonstrated that lack of TTP resulted in stabilization of IL-2 mRNA [96]. In a subsequent study, Ogilvie et al. [97] showed that activated T cells purified from TTP KO spleens produced more interferon-γ (IFN-γ) than WT cells, again associated with increased mRNA stability seen in the absence of TTP [97]. Both exogenous and endogenous TTP could bind to a 70 nucleotide ARE present in the 3′ UTR of IFN-γ [97].

Cxcl1 (also known as KC, growth-related oncogene α, Gro-α, and melanoma growth stimulatory activity) is a member of the CXC subfamily of chemokines and acts as a functional homologue of human interleukin-8 in the mouse [98]. The 3′ UTR of Cxcl1 contains a cluster of several overlapping AUUUA pentamers as well as isolated pentamers. These AREs confer instability to the Cxcl1 mRNA [99, 100]. Co-transfection studies showed that TTP could interact with the AREs in the 3′ UTR of the Cxcl1 mRNA, resulting in a decreased mRNA half-life [99]. Cxcl1 mRNA was found to be stabilized in peritoneal macrophages isolated from TTP KO mice [99].

Two different “global” approaches aimed at identifying new targets of TTP have recently been published. In one approach, microarray analysis of RNA isolated from WT and TTP KO fibroblasts was used to identify transcripts that had different decay rates following serum stimulation and actinomycin D treatment [101, 102, 103]. Lai et al. [101] identified 250 transcripts apparently stabilized in the TTP knockout fibroblasts. Of those, 23 had two or more UAUUUAU binding sites that were conserved in the human transcript, and nine of those were confirmed to be stabilized by northern blot analysis. One of the nine, Ier3, is a protein that has been implicated in the physiological control of blood pressure [104]. Another is Plk3, a serine-threonine kinase that has been implicated in many cellular processes, including cell cycle progression, mitosis, cytokinesis and the response to DNA damage [105, 106, 107]. Actinomycin D mRNA decay assays done in WT and TTP KO fibroblasts verified that Ier3 and Plk3 mRNAs were stabilized in the absence of TTP [101, 102]. One of the TTP family members, ZFP36L1, was found not to have an effect on either Ier3 or Plk3 mRNA stability [101, 102]. As expected, the AREs from Ier3 and Plk3 could bind TTP, and could mediate TTP-dependent mRNA decay in co-transfection experiments [101, 102].

In a second "global" experiment, microarray analysis was used to identify transcripts that could be co-immunoprecipitated with TTP in LPS treated RAW264.7 macrophages [103]. This approach identified a number of potential TTP-associated mRNAs, of which only a subset contained AUUUA pentamers or UUAUUUAUU nonamers. One of these, IL-10 was identified as a physiological target of TTP using BMDM and splenocytes from WT and TTP KO mice [103]. Actinomycin D studies demonstrated that lack of TTP decreased the rate of IL-10 mRNA decay [103]. In both "global" experiments, many transcripts were identified that did not contain an obvious ARE, raising the possibility that TTP could regulate some transcripts indirectly.

These studies have demonstrated that TTP can regulate both pro-inflammatory and anti-inflammatory mRNA decay, which may give TTP a way to "fine tune" the inflammatory response and prevent the development of chronic inflammation. It will be important to understand the timing of these countervailing responses in the acute response to an inflammatory stimulus, and the return to homeostasis.

As mentioned earlier, mammalian members of the TTP family of proteins behave like TTP in cell-free RNA binding assays, co-transfection assays evaluating the turnover of ARE-containing reporter transcripts, and cell-free deadenylation assays [52, 53, 62, 63, 64, 65, 66, 67]. Studies using TTP KO fibroblasts have demonstrated at least two examples of known TTP target transcripts whose stability was not affected by ZFP36L1 (Ier3 and Plk3 [101, 102]) or ZFP36L2 (Plk3 [102]), at least in this experimental system. Although there may be overlapping biochemical functions among the different TTP family members, their different physiological roles in the intact mouse probably depend on differences in regulated and tissue-specific expression, as well as probable differences in post-translational modifications, binding protein association, subcellular localization, etc.

The different physiological roles are illustrated by the different phenotypes of the *Zfp36l1* [50, 108] and *Zfp36l2* knockout mice [51]. *Zfp36l1* [50] knockout embryos died between embryonic days 8 and 10, primarily due to failure of chorioallantoic fusion [50]. When fusion did occur, the KO placentas exhibited decreased cell proliferation and atrophy of the trophoblast layers [50]. No physiological target transcripts were identified in this study, but we postulated that ZFP36L1 may regulate one or more mRNAs whose encoded proteins may be involved in placental development. Bell et al. [108] also saw universal embryonic lethality at mid-gestation, with trophoblast layer defects in the placenta. These authors identified VEGF-A as a target mRNA for ZFP36L1 [108]. VEGF-A contains a prominent ARE in its 3′ UTR; previous studies had shown that ZFP36L1 could bind to this ARE, and that overexpression of ZFP36L1 caused decreases stability of VEGF-A mRNA [109]. Subsequent experiments demonstrated that there was no change in the stability of VEGF-A mRNA but rather there was enhanced association with polysomes, suggesting that ZFP36L1 regulated VEGF-A at the level of translation [108]. It appears from both reports that the determination of the role of ZFP36L1 in the adult animal will require the use of conditional KO mice.

Overexpression of ZFP36L1, like TTP, can result in increased turnover of TNF, GM-CSF and IL-3 mRNA [63, 64, 110]. More recently, ZFP36L1 has been shown to regulate the transcript for steroidogenic acute regulatory (STAR) protein. ZFP36L1 interacted with the ARE nonamer repeats present in the 3′ UTR, and ZFP36L1 overexpression and siRNA knockdown resulted in decreased and increased levels of STAR mRNA, respectively [111]. Interestingly, the effect of ZFP36L1 overexpression and knockdown had the opposite effect on protein levels, i.e., overexpression increased STAR protein levels and knockdown decreased protein levels [111]. The authors suggested that ZFP36L1 was capable of

decreasing the stability of STAR mRNA while simultaneously enhancing translation, leading to increased cholesterol metabolism [111].

A partial *Zfp36l2* KO mouse was described, in which the amino terminal 29 amino acids were deleted [112]. These mutant mice, ΔN-ZFP36L2, expressed decreased levels of an amino-terminal truncated protein that still contained an intact, functional tandem zinc finger domain. These mice displayed complete female infertility due to a defect in early embryonic development [112], presumably because of a defect in stored maternal mRNA and protein in the fertilized egg and early embryo. We recently described a full *Zfp36l2* KO mouse with a very different phenotype [51]. Mice completely lacking ZFP36L2 exhibited profound and lethal hematopoietic defects. There were decreased definitive hematopoietic progenitors in fetal livers and yolk sac, and fetal liver hematopoietic stem cells were unable to reconstitute the hematopoietic system of lethally irradiated recipients [51]. Microarray analysis of RNA from fetal livers identified the transcript for the chemokine Cxcl1, among others, as being upregulated in the KO mice [51]. Actinomycin D mRNA decay assays showed no apparent change in the turnover of Cxcl1 mRNA in KO mouse fibroblasts; however, fibroblasts may not be the appropriate cell source in which to study Cxcl1 mRNA stability [51]. In addition, it may be that translation rather than mRNA stability is affected, as suggested for VEGF-A in *Zfp36l1* KO fibroblasts [108]. Future studies to elucidate ZFP36L2 target transcripts will involve analysis of gene expression in fetal liver hematopoietic stem cells as well as conditional KO strategies. It should be noted that overexpression of Zfp36L2, like TTP and ZFP36L1, can promote the turnover of TNF, GM-CSF and IL-3 mRNA [63, 64], and that ZFP36L2, like TTP and ZFP36L1, can bind to 14-3-3 proteins [113] and can co-localize in P bodies with decapping enzymes [36].

AUF1

AUF1 (ARE/poly-(U) binding degradation factor 1 or heterogeneous nuclear ribonucleoprotein D, HNRNPD) was the first identified ARE-binding protein [114, 115, 116]. AUF1 has been shown to have both destabilizing effects on mRNA based on overexpression and knockdown experiments (transcripts of c-myc [115]), c-fos and GM-CSF [117], IL-3 [118], p21 and cyclin D1 [119], and iNOS [120]), as well as stabilizing effects based on overexpression studies (c-myc, c-fos, GM-CSF, TNF [121], parathyroid hormone [122]). AUF1 exists in 4 different isoforms (p37, p40, p42 and p45) resulting from alternative splicing, and each isoform apparently shows a different affinity for ARE-containing mRNAs, p37>p42>p45>p40 [123]. All four isoforms contain two nonidentical RNA recognition motifs, RRM1 and RRM2 [123]. There is differential expression of the different isoforms in some cell types, and it has been hypothesized that the opposing effects of AUF1 on mRNA stability may result from the relative levels of expression of each isoform in a given cell type or in response to a given stimulus [118, 124, 125]. The p37 isoform has been shown to interact with the exosome [33] and to exhibit the greatest destabilizing activity towards ARE-containing mRNAs [126]. Like the TTP family members, AUF1 can shuttle between the nucleus and cytoplasm [116, 119, 127, 128]. AUF1 KO mice are more sensitive to LPS-induced endotoxemia than WT mice [129], and serum levels of the pro-inflammatory cytokines, TNF and IL-1β, were found to be increased in the KO mice. Studies in LPS-stimulated peritoneal macrophages demonstrated that this increased production of TNF and IL-1β was the result of increased mRNA stability [129]. Treatment with neutralizing antibodies to both TNF and IL-1β together prior to the LPS challenge was able to protect the KO mice from lethal endotoxic shock [129]. These studies showed that AUF1 exerted post-transcriptional control over pro-inflammatory cytokine expression. A more recent report demonstrated that the AUF1 KO mice developed chronic pruritic dermatitis with age that resembles some aspects of human atopic dermatitis [130]. AUF1 KO mice also exhibited increased contact hypersensitivity characterized by increased

T cell and macrophage infiltration and an increase in the production of pro-inflammatory cytokines TNF, IL-2 and IL-1β, and the monocyte chemoattractant protein 1 (MCP1; CCL2) [130]). These studies link AUF1 and its regulation of pro-inflammatory mediator mRNAs with the inflammatory response seen in skin.

Hur

Hur (Hu antigen R, HuA, ELAVL1) is the only ubiquitously expressed member of the mammalian embryonic lethal abnormal vision (ELAV) family of RNA-binding proteins [131, 132]. Expression of the other 3 members of this family, HuB (Hel-N1, ELAVL2), HuC (ELAVL3), and HuD (ELAVL4), is primarily restricted to neuronal cells [133]. HuR contains 3 RRMs and, like AUF1, is primarily nuclear but can shuttle between the nucleus and cytoplasm [131, 134, 135]. Numerous studies involving overexpression and RNA knockdown of HuR have implicated HuR in the stabilization many mRNAs, including TNF [136], VEGF [137, 138], IL-8 [138], Cox-2 [139], GM-CSF, c-fos [140, 141], p21 [119, 142], Cyclin A [143], Cyclin B1 [143], Cyclin D1 [119], iNOS [144], activating transcription factor-2 or ATF-2 [145], and the X chromosome-linked inhibitor of apoptosis protein, XIAP [146]. HuR has also been implicated in enhancing the translation of several mRNAs, including p21 [119], p53 [147, 148] and hypoxia-inducible factor 1α or HIF-1α [149]. Overexpression of HuR *in vivo* resulted in translational silencing of some select pro-inflammatory mediators. Katsanou et al. [150] established conditional myeloid-specific overexpressing HuR transgenic mice. They demonstrated in LPS-stimulated peritoneal macrophages that the myeloid-specific expression of HuR resulted in increased mRNA stability for TNF and Cox-2 but actually inhibited translation of the stabilized mRNA [150]. They also saw translational silencing of transforming growth factor β1 (TGFβ1) mRNA [150]. Administration of concanavalin A to control mice resulted in inflammatory hepatitis and liver damage due to the release of inflammatory mediators; similar treatment of mice overexpressing HuR did not result in inflammatory hepatitis or liver damage [150]. These studies suggest a link between HuR's translational silencing of pro-inflammatory mediators and the suppression of inflammatory responses in the mouse.

Two descriptions of HuR KO mice were recently published. In the first report, Katsanou et al. [151] demonstrated that HuR was essential for normal mouse embryonic development, and that HuR KO mice died at mid-gestation due to placental defects. A second KO has been described in which HuR deficiency was also embryonic lethal [152]. The postnatal role of HuR was investigated using a tamoxifen-inducible Cre-mediated gene deletion strategy. Postnatal deletion of HuR resulted in atrophy of hematopoietic and lymphoid tissues, accompanied by decreased cellularity as a result of increased apoptosis in progenitor populations [152]. KO animals developed cachexia and died within 10 days of treatment with tamoxifen. Subsequent studies with embryonic fibroblasts demonstrated that, in the absence of HuR, p53 levels were increased due to decreased levels of the p53 upstream regulator, Mdm2 [152]. HuR binds to the 3′ UTR ARE in Mdm2, resulting in stabilization of the mRNA; thus, in HuR KO fibroblasts, Mdm2 mRNA is destabilized [152]. HuR is highly expressed in tissues undergoing active proliferation and the authors suggest that HuR may play an important survival role in actively dividing cell populations. Studies done so far with this conditional KO have not identified a connection between HuR and inflammatory processes, but tissue- or cell-specific KO of HuR, as opposed to a global KO, may shed more light on the post-transcriptional action of HuR on pro-inflammatory mediators.

Translational regulation by ARE-binding proteins

TIA-1/TIAR

The T-cell restricted intracellular antigen 1 (TIA-1 or TIA1) and the TIA-1-related protein (TIAR or TIAL1) are closely related RNA-binding proteins containing 3 RRMs that have been implicated in the apoptotic process as well as RNA binding [153, 154, 155, 156]. TIA-1 and TIAR have been shown to repress the translation of both pro-inflammatory [157, 158, 159, 160, 161] as well as anti-inflammatory mRNAs [162]. The physiological relevance of TIA-1 translational silencing of target mRNAs was demonstrated in TIA-1 KO mice. Mice deficient in TIA-1 were phenotypically normal in a BALB/c background [158]. LPS-stimulated peritoneal macrophages from TIA-1 KO mice expressed more TNF protein than did WT macrophages, although there was no difference in the levels or stability of TNF mRNA [158]. The absence of TIA-1 resulted in an increase in the proportion of TNF transcripts associated with polysomes, suggesting that TIA-1 functions as a translational silencer [158]. The translational silencing effect of TIA-1 appeared to be relatively selective for TNF, as no effect was seen for GM-CSF, IL-1β, or IFN-γ [158]. TIA-1 KO mice were more sensitive to endotoxic shock induced by LPS than WT mice [158].

The TIA-1 KO phenotype was different in a C57Bl/6 background. Macrophages from TIA-1 KO mice in a C57Bl/6 background produced significantly more TNF protein in response to LPS than was seen in a BALB/c background [161, 163]. It was observed that TIA-1 had no translational silencing effect on TNF in activated T cells suggesting that TIA-1 exerts its post-transcriptional effect in a cell-specific manner [163]. C57Bl/6 TIA-1 KO mice developed mild arthritis, which was not seen in a BALB/c background [161]. TIA-1/TTP double KO mice developed a more severe arthritis than was seen in either the TIA-1 or TTP KO mice, suggesting that both proteins have an influence on the severity of arthritis that develops [161]. Interestingly, bone marrow neutrophils and not macrophages were the main source of the increased production of TNF protein seen in the double KO mice [161]. These studies showed that TIA-1 and TTP could act as genetic modifiers of inflammatory arthritis that, through different post-transcriptional mechanisms, mRNA stability and mRNA translation, can regulate the abundance of pro-inflammatory mediators.

In addition to selectively interacting with ARE-containing mRNAs, TIA-1 and TIAR are thought to be involved in the general repression of translation seen during the stress response [164]. TIA-1 and TIAR shuttle between the nucleus and cytoplasm, and under stress conditions they can promote the assembly of translationally inactive preinitiation complexes and the formation of stress granules within the cytoplasm [41]. The mechanism by which TIA-1 and TIAR regulate the translation of selective ARE-containing mRNAs is thought to occur by the same mechanism as used for general translational repression in response to stress [165].

Conclusions

Post-transcriptional regulation of the many pro- and anti-inflammatory proteins involved in the initiation and subsequent resolution of the inflammatory response can influence the severity of the response. A major determining factor in the post-transcriptional control of many cytokines is the presence of a 3′ UTR AU-rich region in their transcripts. Both mRNA stability/instability and translational suppression can be mediated by ARE-binding proteins, several of which have been discussed in this review. Many ARE-binding proteins have been identified since the cloning of the first ARE-binding protein, AUF1 (see Table I, [48]). *In vitro* assays have identified target mRNAs for many ARE-binding proteins and also revealed many aspects of the mechanisms by which ARE-binding proteins regulate their target mRNAs. Studies in intact animals have begun to elucidate the roles of specific ARE-binding

proteins and their target mRNAs in physiological and pathological inflammation. To our knowledge, TTP was the first ARE-binding protein demonstrated to have an effect on inflammation in intact animals, due to its effect on the regulation of TNF mRNA stability [59, 71]. Since then, several other ARE-binding proteins have been shown to be involved in the inflammatory response *in vivo* [130, 150, 161]. Due to the deleterious effect of the complete absence of some of the ARE-binding proteins on embryonic development, the demonstration of their possible involvement in inflammation and the identification of physiologically relevant target mRNAs in the adult will have to wait for conditional cell- or tissue-specific KO targeting approaches.

Given the large number of ARE-binding proteins, and the number of target mRNAs that can be regulated by multiple ARE-binding proteins, it seems likely that any given mRNA will be regulated by multiple ARE-binding proteins in intact animals. It will be fascinating to determine how these multiple ARE-binding proteins interact with AREs and other ARE-binding proteins to bring about the degree of gene regulation needed to respond to an inflammatory stimulus.

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Table I

ARE-binding Proteins

ARE-binding protein	RNA-binding domain	Function	References
APOBEC1	Unknown	Destabilize mRNA	[166, 167, 168, 169]
AUF1 (hnRNPD)	RRM	Destabilize mRNA Stabilize mRNA	[115, 117, 118, 119, 140] [121, 122]
AUF2 (CBF-A)	RRM	Stabilize mRNA	[170]
CUGBP2	RRM	Translational silencing Stabilize mRNA	[171] [172]
FXR1P	K homology; RGG	Translational silencing	[173]
HuR (ELAVL1)	RRM	Stabilize mRNA Translational activation Translational silencing	[119, 135, 136, 137, 138, 139, 140, 142, 143, 144, 145, 146] [119, 147, 148, 149] [150]
KSRP	K homology	Destabilize mRNA	[33, 95, 174, 175, 176, 177, 178]
NF90	DRBD	Stabilize mRNA Translational silencing	[179, 180, 181, 182, 183, 184] [184]
RHAU	Novel N-terminal RNA-binding domain	Destabilize mRNA	[185, 186]
YB1	CSD	Stabilize mRNA	[187, 188]
TIA-1 (TIA1)	RRM	Translational silencing	[158, 159, 160, 161]
TIAR (TIAL1)	RRM	Translational silencing	[157, 160, 162, 189, 190]
ZFP36 (TTP)	CCCH zinc finger	Destabilize mRNA Stabilize mRNA	[59, 63, 64, 76, 77, 78, 79, 81, 82, 83, 84, 87, 88, 89, 92, 93, 96, 97, 99, 101, 102, 103, 191] [94]
ZFP36L1 (BRF1)	CCCH zinc finger	Destabilize mRNA Translational silencing	[63, 64, 82, 110, 111] [108]
ZFP36L2 (BRF2)	CCCH zinc finger	Destabilize mRNA	[51, 63, 64]
ZFP36L3	CCCH zinc finger	Destabilize mRNA	[52, 53]

APOBEC1, apolipoprotein B mRNA editing enzyme, catalytic polypeptide 1; AUF1/hnRNPD, AU-rich binding factor 1/heterogeneous nuclear ribonucleoprotein D; AUF-2/CBF-A, AU-rich binding factor 2/CArG-box-binding factor-A; CCCH, cysteine-cysteine-cysteine-histidine; CSD, cold shock domain; CUGBP2, CUG triplet repeat, RNA binding protein 2; DRBD, dsRNA-binding domain; FXR1P, fragile X-related protein 1; HuR/ELAVL1, Hu antigen R/embryonic lethal abnormal vision-like 1; KSRP, KH-type splicing regulatory protein; NF90, nuclear factor 90; RGG, arginine-glycine-glycine; RHAU, RNA helicase associated with AU-rich element; RRM, RNA recognition motif; YB1, Y-box-binding protein 1; TIA-1, T-cell restricted intracellular antigen 1; TIAR, TIA-1-related; ZFP36/TTP, zinc finger protein 36/tristetraprolin; ZFP36L1/BRF1, zinc finger protein 36-like 1/butyrate response factor 1; ZFP36L2/BRF2, zinc finger protein 36-like 2/butyrate response factor 2; ZFP36L3, zinc finger protein 36-like 3

Table II

TTP Family Members and Identified Target mRNAs

Protein	mRNA Target	Mechanism	Binding	Co-transfection	Deadenylation	siRNA knockdown	KO cells	References
TTP (ZFP36)	**TNF**[a]	mRNA stability	REMSA; UVCL	RNA; mRNA stability	Cell-free		RNA; Protein; mRNA stability	[59, 62, 63, 64, 65, 66, 192]
	GM-CSF	mRNA stability	REMSA; UVCL	mRNA stability	Cell-free; RNase H		RNA; Protein; mRNA stability	[64, 65, 76, 193]
	IL-2	mRNA stability	REMSA; UVCL	mRNA stability			RNA; Protein; mRNA stability	[96]
	Immediate early response gene 3 (Ier3)	mRNA stability	REMSA				mRNA stability	[101]
	IL-10	mRNA stability	RIP	mRNA stability	Cell-free		RNA; Protein; mRNA stability	[103, 191]
	CXCL1	mRNA stability		mRNA stability			RNA; mRNA stability	[99]
	Polo-like kinase 3 (Plk3)	mRNA stability	REMSA				mRNA stability	[101, 102]
	Interferon-α (IFN-α)	mRNA stability	REMSA; UVCL	mRNA stability			RNA; Protein; mRNA stability	[97]
	Cyclooxygenase -2 (Cox-2)	mRNA stability	UVCL; RIP				RNA; Protein; mRNA stability	[81, 191]
	Myeloid/lymph oid or mixed lineage leukemia (trithorax) (Mllt11)	mRNA stability					mRNA stability	[101]
	Leukemia inhibitory factor (Lif)	mRNA stability	RIP	mRNA stability			mRNA stability	[101, 103]
	B double-prime 1 (Bdp1)	mRNA stability					mRNA stability	[101]
	Proviral integration site 3 (Pim3)	mRNA stability					mRNA stability	[101]
	RUN-and SH3 domain-containing 2 (Rusc2)	mRNA stability					mRNA stability	[101]
	Pleckstrin homology-like domain, family A, member 1 (Phlda1)	mRNA stability					mRNA stability	[101]
	IL-1α	mRNA stability	REMSA; UVCL				RNA; mRNA stability	[191]
	IL-3	mRNA stability	REMSA	RNA; Protein; mRNA stability	Rnase H			[59, 64, 77, 78, 79, 193]
	IL-6	mRNA stability		RNA; Protein; mRNA stability		Protein	RNA; Protein; mRNA stability	[78, 91, 191, 194]

Protein	mRNA Target	Mechanism	Binding	Co-transfection	Deadenylation	siRNA knockdown	KO cells	References
	Plasminogen activator inhibitor type 2 (PAI-2)	mRNA stability	UVCL	RNA; Protein; mRNA stability				[80]
	Paired-like homeodomain transcription factor 2 (Pitx2)	mRNA stability	UVCL; RIP	mRNA stability[b]				[82]
	1,4-galactosyltransferase	mRNA stability	UVCL; RIP	mRNA stability		RNA; mRNA stability		[93]
	Inducible nitric-oxide synthase (iNOS)	mRNA stability	UVCL[c]	RNA; mRNA stability		RNA; Protein		[94]
	Cyclin D1	mRNA stability	REMSA			mRNA stability		[92]
	Myc	mRNA stability	REMSA			mRNA stability		[92]
	Cyclin-dependent kinase inhibitor 1A (P21)	ND	RIP			RNA		[90]
	Fos	ND	RIP	RNA		RNA		[90]
	IL-12	ND				Protein		[91]
	Macrophage inflammatory protein 2α (MIP2)	ND				Protein		[91]
	Chemokine (C-C) motif ligand 2 (CCL2) or monocyte chemotactic protein-1 (MCP1)	ND					RNA; Protein	[194]
	Chemokine (C-C) motif ligand 3 (CCL3) or macrophage inflammatory protein-1 (MIP1α)	ND					RNA; Protein	[194]
	Macrophage inflammatory protein-3α (MIP3α)	ND				Protein		[91]
	Dual specificity phosphatase 1 (DUSP1)	ND	RIP	RNA				[85]
	VEGF	mRNA stability	REMSA; RIP; UVCL	Protein; mRNA stability		RNA		[83, 88]
	E2a-encoded transcription factor E47 (E47)	ND	REMSA			RNA		[89]
	IL-8	mRNA stability	UVCL	Protein; mRNA stability	Cell-free	mRNA stability		[83, 87]
	Indolamine 2,3-dioxygenase (IDO)	ND	RIP	RNA				[85]
	Superoxide dismutase (SOD2)	ND	RIP	RNA				[85]
	CD86	ND	RIP	RNA				[85]
	MHC class I-B and F	ND	RIP	RNA				[85]
	Coxsackie-and adenovirus-receptor-like membrane protein (CLMP)	ND	REMSA	RNA				[84]

Protein	mRNA Target	Mechanism	Binding	Co-transfection	Deadenylation	siRNA knockdown	KO cells	References
ZFP36L1	**VEGF**	translation	REMSA	mRNA stability		mRNA stability	RNA; Protein; mRNA stability; polysomes	[108, 109]
	TNF	mRNA stability	REMSA; UVCL	RNA; mRNA stability	Cell-free			[63, 64, 65, 66]
	GM-CSF	mRNA stability		mRNA stability	Cell-free			[64, 65]
	IL-3	mRNA stability	REMSA	mRNA stability	Cell-free	RNA		[64, 110]
	Pitx2	mRNA stability	UVCL; RIP	mRNA stability[b]				[82]
	Human inhibitor of apoptosis protein-2 (c-IAP2)	ND		RNA				[195]
	Steroidogenic acute regulatory protein (STAR)	mRNA stability	UVCL; RIP	RNA		RNA; Protein		[111]
ZFP36L2	TNF	mRNA stability	REMSA; UVCL	RNA; mRNA stability	Cell-free			[63, 64, 65, 66]
	GM-CSF	mRNA stability		mRNA stability				[64]
	IL-3	mRNA stability		mRNA stability				[64]
	CXCL1	ND					RNA	[51]
ZFP36L3	TNF	mRNA stability	REMSA	mRNA stability	Cell-free			[52, 53]

[a] Confirmed “physiological” targets are listed in bold; mRNA stability has been demonstrated in cells derived from knockout animals

[b] In vitro RNA decay system

[c] No direct binding observed between TTP and iNOS mRNA

ND, Not determined

Cell-free, cell-free deadenylation assay; KO cells, assays performed in cells isolated from knockout animals; Polysomes, mRNA association with polysomes; Protein, protein measured by western or ELISA; REMSA, RNA electrophoretic mobility shift assay; RIP, RNA immunoprecipitation; RNA; RNA measured by northern, RT-PCR, or RNase protection; RNase H; removal of poly(A) tail measured by hybridization to oligo(dT) followed by RNase H digestion; UVCL, UV cross linking assay