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The roles of TTP and BRF proteins in regulated mRNA decay

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Abstract

AU-rich element (ARE) motifs are *cis*-acting elements present in the 3'UTR of mRNA transcripts that encode many inflammation- and cancer-associated genes. The TIS11 family of RNA-binding proteins, composed of TTP, BRF-1, and BRF-2 play a critical role in regulating the expression of ARE-containing mRNAs. Through their ability to bind and target ARE-containing mRNAs for rapid degradation, this class of RNA-binding proteins serves a fundamental role in limiting the expression of a number of critical genes, thereby exerting anti-inflammatory and anti-cancer effects. Regulation of TIS11 family members occurs on a number of levels through cellular signaling events to control their transcription, mRNA turnover, phosphorylation status, cellular localization, association with other proteins, and proteosomal degradation, all of which impact TIS11 members' ability to promote ARE-mediated mRNA decay along with decay-independent functions. This review summarizes our current understanding of post-transcriptional regulation of ARE-containing gene expression by TIS11 family members and discuss their role in maintaining normal physiological processes and the pathological consequences in their absence.

Keywords

Tristetraprolin; Butyrate Response Factor; AU-rich element; mRNA decay; post-transcriptional regulation

Messenger RNA turnover is a tightly regulated process that is critical in controlling mammalian gene expression. The importance of this level of regulation is evident in a variety of diseases where loss of post-transcriptional gene regulation directly contributes to the overexpression of many genes encoding growth factors, inflammatory cytokines, and proto-oncogenes [1,2]. A characteristic feature present within the 3' untranslated region (3'UTR) of these mRNAs is the adenylate- and uridylate (AU)-rich element (ARE). The significance of this conserved *cis*-acting RNA element is apparent in its frequency since it is currently estimated that approximately 8% of the human transcriptome contains AREs [3]. A primary function of the ARE is to target specific mRNAs for rapid decay through interaction with *trans*-acting RNA-binding proteins. Initially discovered in 1989 [4], the tristetraprolin (TTP) protein and its related family members butyrate response factors 1 and 2 (BRF-1 and

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Cross-References

Overview – RNA decay as a major mediator of gene expression and QC
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Cis acting elements that regulate mRNA decay
 Networking between mRNA decay and other cellular processes
 RNA-binding domains-Zn-F
 Mechanisms of deadenylation-dependent decay
 Stress granules and P bodies

BRF-2) are among the best characterized RNA-binding proteins that are directly involved in regulating the expression of many inflammation and cancer-associated transcripts. Through their ability to bind ARE sequences, the TTP and BRF proteins target ARE-containing transcripts and promote their rapid decay. This review summarizes the current advances in understanding the roles of TTP and BRF proteins in post-transcriptional regulation, focusing largely on the importance these RNA-binding proteins have in maintaining normal physiology and the dramatic pathological consequences that occur when expression of these RNA-binding regulatory proteins is lost.

The TIS11 Family of RNA-Binding Proteins

The founding member of the TIS11 family of RNA-binding proteins, TTP, was originally identified as TPA (12-O-tetradecanoylphorbol-13-acetate) inducible sequence-11 and concurrent work had also identified TTP as a gene expressed in response to growth factor and insulin treatment of cells [5-8]. Within this protein's primary sequence three Pro-Pro-Pro repeats were observed, giving rise to its more commonly used name of tristetraprolin or TTP [7]. At a similar time as the identification of TTP, two other cDNA sequences commonly called BRF (butyrate response factor) -1 and 2 were identified that displayed >70% amino acid identity to TTP within the region of two tandem zinc-finger (Cys₃His) domains [5,9]. Currently, the human TIS11 family consists of three members that include TTP (TIS11, ZFP36), BRF-1 (TIS11b, ZFP36L1), and BRF-2 (TIS11d, ZFP36L2). The rodent TIS11 family has an additional member, Zfp36l3, which is expressed exclusively in the placental tissue [10] (Table 1).

Characteristic to many immediate-early response gene expression patterns, TTP levels are low-to-undetectable in a wide variety of cell types that are rendered quiescent or serum starved. When stimulated with growth or proinflammatory factors, rapid induction of TTP transcription is mediated through activation of conventional responsive gene-promoter elements as well as sequence elements present in the first intron, leading to a rapid increase in mRNA levels as soon as 15 min after stimulation and returning to basal levels within 2 hours [7,11,12]. One aspect that separates TTP expression from other immediate-early response genes is its inducible expression in response to factors normally associated with regulating growth. Potent growth-inhibitory cytokines such as TGF- β and interferons, along with anti-inflammatory compounds and natural products such as glucocorticoids, cinnamon, and green tea can promote TTP transcription, indicating that their role in modulating immune responses and inflammation is in part through TTP-dependent control of various proinflammatory genes [13-18]. Similar to that of TTP, rapid transcriptional induction of BRF-1 has also been observed *via* a number of differing stimuli and the observed differences in the kinetics of TIS11 family member induction appears to be dependent upon the cell type and stimulus [19]. Additionally, the circadian clock regulates BRF-1 gene expression in peripheral organs such as the heart and liver and this may be responsible for imposing circadian expression on target transcripts [20,21]. Interestingly, the mRNA of TTP has been demonstrated to be quite labile, yielding half-lives of 17 min and 45 min in unstimulated and serum-stimulated fibroblasts, respectively [22]. Based on the presence of an ARE within the TTP mRNA 3'UTR, TTP has been demonstrated to auto-regulate its expression through a negative feedback loop by binding its own ARE and promoting decay [23,24]. Although not functionally defined, both BRF-1 and BRF-2 mRNAs also contain AREs in their respective 3'UTRs [3], and both BRF-1 and BRF-2 mRNAs were found to be part of the TTP-associated mRNA pool, suggesting the possibility of cross-regulation between TTP and its paralogs [25].

The inducible nature of TIS11 family members suggests that their expression might be limited in most human tissues, although examination of their expression patterns indicates

otherwise. RNA analysis of TTP, BRF-1, and BRF-2 expression in various human tissues demonstrated a broad range of constitutive transcript abundance [26]. In general, the TIS11 members maintained similar mRNA levels within differing tissue types, with low-level expression seen in testicle, skeletal muscle, stomach, liver, spleen, heart, kidney, and brain; a significant increase in BRF-1 and 2 mRNA as compared to TTP was observed in the pancreas and thymus. Tissues displaying increased TIS11 members mRNA levels were small intestine, ovary, bladder, colon, lung, and uterine cervix. Interestingly, TTP mRNA expression in cervical tissue was the highest level of any TIS11 member and was approximately 6-times greater than BRF-1 or 2. With regard to their respective protein expression, recent work has examined TTP protein expression histologically in normal human tissue samples across various ages [27]. In most tissues, TTP protein displayed a decreasing pattern of expression that was dependant upon age. In contrast, TTP expression was shown to be increased with age in reproductive tissues; in gastrointestinal and endocrine tissues, TTP levels were constant across all age groups. Although the basis for tissue-specific constitutive expression of TIS11 family members is not entirely clear, these findings point to a role in maintaining tissue homeostasis particularly in tissues that may be under specific physiological stresses.

Phosphorylation of TIS11 proteins

A critical aspect controlling the ability of TIS11 family members to function as RNA decay factors occurs through protein phosphorylation, and current evidence has demonstrated that this post-translational modification can impact many aspects of TTP function. Early studies had implicated TTP to be a phospho-protein, based on observed changes in TTP's molecular mass [28]. More current work has demonstrated TTP to be extensively phosphorylated, primarily at a number of serine residues [29], and predicted to be modified at threonine and tyrosine residues, with a number of these residues located in conserved sequence blocks of BRF-1 and 2 [30]. TTP has been shown to be a downstream target of phosphorylation through the major signaling pathways such as the ERK MAPK, p38 MAPK, JNK, GSK3 β , PKA, PKB/AKT, and PKC pathways [30-35], many of which can also induce the transcription of TTP. Within BRF-1 and 2 there exists amino acid sequences that are well conserved to specific phosphorylation sites in TTP [36]. Accordingly, BRF-1 has been shown to be phosphorylated through the p38 MAPK/MAPK-activated protein kinase 2 (MK2) and PKB/AKT pathways [37-39]. While significant information is known about the signaling pathways promoting TIS11 family members phosphorylation, current findings have identified the serine-threonine phosphatase PP2A to promote dephosphorylation of TTP, and through this activity, PP2A can promote decay of proinflammatory mRNAs that are stabilized through activation of p38 MAPK/MK2 pathway-mediated phosphorylation of TTP [40].

The effect of phosphorylation on controlling TTP's ability to target ARE-containing mRNA for degradation occurs on many levels indicating the importance of this post-translational modification. *In vitro* studies have shown that unphosphorylated or dephosphorylated TTP displays a heightened affinity for ARE RNA sequences than native, phosphorylated TTP [33,34,41], suggesting that the unphosphorylated form of TTP is active in targeting mRNAs for rapid decay. However, contrasting data does indicate that the mRNA binding activity of TTP is not impacted by phosphorylation occurring through ERK MAPK, p38 MAPK/MK2, or JNK kinase activity [31,35,42]. Similarly, phosphorylation of BRF-1 did not affect its ability to bind AREs [37,39].

A key feature in which phosphorylation modulates TIS11 family members activity is through enhanced complex formation with 14-3-3 proteins which are multifunctional adaptor proteins that specifically interact with partner proteins containing phosphorylated

serine/threonine residues [43]. Phosphorylation of TTP and BRF-1 at conserved serine residues increases their interaction with 14-3-3 proteins [31,37,38,40,42,44]. This interaction protects TIS11 members from dephosphorylation, promotes enhanced cytoplasmic localization, and protects it from proteosomal degradation [38,40,45]. Taken together, these seemingly unrelated aspects have been assembled in similar models to explain the coordinated regulation of TIS11 members [36,38,41,46,47]. In the unphosphorylated state, TTP and BRF-1 binds to ARE-containing mRNAs targeting them for decay. In this state, these proteins are also subject to proteosomal degradation, thereby limiting their activity. Upon signal-dependant activation of pathways associated with controlling ARE-mediated mRNA decay (i.e., p38 MAPK/MK2 or PKB/AKT), protein phosphorylation of TTP and BRF-1 occurs and this event allows for increased association with 14-3-3 proteins. This complex is now inactive with regard to promoting mRNA decay and is resistant to degradation *via* the proteasome leading to their cytoplasmic accumulation. When dissipation of the stabilizing signal occurs, the phosphatase PP2A can promote dephosphorylation of TTP, allowing it to resume its activity of targeting critical transcripts for degradation and limiting their expression levels.

TTP and ARE-Mediated mRNA Decay

The function of TIS11 family members was unknown at the time of their discovery and these early studies had implicated a role for TTP as a transcription factor. In a series of seminal experiments using inflammatory cells derived from TTP-deficient mice, it was discovered that TTP acts on a post-transcriptional level to promote rapid decay of ARE-containing mRNAs by directly binding to the ARE [48]. A number of studies have determined that TIS11 members specifically bind the sequence UUAUUUAUU, which can be considered the core destabilizing element of many ARE-containing mRNAs [49-54]. The binding of TIS11 members to ARE regions of mRNA transcripts depends on the integrity of the (Cys₃His) residues of TTP's two zinc-finger domains [54-56]. To this extent, a single mutation from cysteine to arginine in either of the zinc-finger domains attenuated ARE binding, and the non-binding mutant version of TTP exerted a dominant-negative effect over wild-type TTP with regard to promoting ARE-mediated decay [57].

The initial work demonstrating the function of TTP as an mRNA decay factor arose from findings that showed TTP's ability to inhibit TNF- α production in macrophages *via* its binding to the ARE present in the TNF- α mRNA transcript [48]. Since this initial discovery, TTP has been identified to bind the AREs present in many immune and inflammatory mediators and limit their expression *via* ARE-mediated decay (Table 2). Furthermore, the role of TTP as a potential tumor suppressor is coming into light by virtue of its ability to mediate rapid decay of factors associated with tumorigenesis. More recent genome-wide assessment of TTP targets has identified novel mRNA targets and brought to light some of the complexities associated with TTP-mediated mRNA decay. In studies using TTP-deficient mouse embryo fibroblasts, over 250 mRNAs were stabilized in the absence of TTP, notably the ARE-containing gene *Ier3* was identified and this finding points to a role for TTP in controlling blood pressure and cardiac hypertrophy [22]. In similar microarray-based studies using macrophages, >100 mRNAs were identified to directly be associated with TTP and the 3'UTRs of these genes were enriched in ARE motifs, one in particular being interleukin-10 (IL-10) [25]. An unexpected finding in this study demonstrated that in TTP-deficient macrophages the expression levels of some TTP-associated mRNAs were not increased, which may reflect the underlying redundancy among TIS11 family members in their ARE mRNA targets. Alternatively, it may imply that these mRNAs are resistant to TTP-mediated mRNA degradation through an unidentified mechanism.

A key step in the initiation of ARE-mediated decay lies within TTP's capacity to promote deadenylation of ARE-containing transcripts. This was initially demonstrated through experiments showing the ability of TTP to cause a shortening of the poly(A) tail to the TNF- α and GM-CSF transcripts [56,58]. Efficient TTP-mediated deadenylation is strongly dependent on the core binding sequence UUAUUUAUU and it being bound by the two zinc finger domains of TTP and BRF members [53] [54,57]. To this extent, three major deadenylation complexes have been reported in vertebrates, the Ccr4/Caf1/Not complex, the Pan2/Pan3 complex, and the poly A-specific ribonuclease (PARN) complex [59-61]. Co-immunoprecipitation experiments have shown direct association of Ccr4 with TTP and BRF-1 which leads to decay of ARE-containing reporter mRNAs [62], and depletion of Caf1 allows for stabilization of ARE-containing transcripts [59]. Moreover, *in vitro* studies have demonstrated that TIS11 family members can stimulate PARN activity to promote deadenylation of ARE-containing, polyadenylated mRNAs [63]. Interestingly, the association between TTP and PARN appears to be indirect, since these two factors do not physically associate [62,63], thus it is possible that other factors yet to be identified may facilitate bridging between TTP and PARN in order to activate deadenylation [63].

The process of deadenylation leaves the mRNA body susceptible to rapid decay, and in mammalian cells TIS11 family members can participate in two primary decay pathways: 5'-3' decay that occurs in processing (P)-bodies which are small cytoplasmic foci that contain many of the enzymes required for mRNA decay, and 3'-5' decay is mediated through a complex of exonucleases known as the exosome [64,65]. Which pathway TTP preferentially directs ARE-containing mRNAs into is not currently known, thus it is unclear what determines the prevailing decay pathway. One possibility is that 5'-3' mRNA decay in P-bodies may prevail under conditions of cellular stress. Cells exposed to various stresses such as heat shock, oxidative stress, or glucose deprivation promote the assembly of stress granules which are small cytoplasmic foci that harbor translationally arrested mRNAs, stalled translation initiation factors, and the ARE-binding proteins TIA-1 and TIAR [66]. Under conditions of stress, TTP and BRF-1 can be recruited to stress granules where they have been shown to target and sequester ARE-containing mRNAs and, as visualized using immunofluorescence microscopy, stress granules and P-bodies make frequent yet transient contacts [67,68]. This evidence indicates the dynamic relationship between stress granules and P-bodies to be dependant upon TIS11 family members where they may facilitate delivery of selected mRNAs from stress granules to P-bodies for degradation.

Another feature contributing to TIS11 family members directing ARE-containing mRNA to P-bodies is the ability of TTP and BRF-1 proteins to bind and deliver ARE-containing mRNAs to P-bodies [69]. In this complex, TTP associates with P-body components involved with decapping such as Dcp1a, Dcp2, and Hedls, along with the 5'-3' exonuclease Xrn1 to promote ARE-containing mRNA decapping and exonucleolytic degradation in a 5'-3' direction [47,62,70,71]. Additionally, central components of the RNA-induced silencing complex, Argonaute proteins, primarily reside in P-bodies [72] and current work has implicated TTP as a novel mediator of microRNA-dependent mRNA decay and translational repression. Through its interaction with Ago2 and Ago4, TTP has been shown to facilitate targeting of an ARE-specific microRNA (miR16) to the TNF- α ARE [73]. Although TTP does not directly bind miR16, they interact through association with Argonaute proteins to mediate ARE-containing mRNA silencing.

A main component involved in 3'-5' mRNA degradation is the exosome. This multiprotein complex of exonucleases and RNA binding proteins is required for 3'-5' decay [74]. TTP and BRF-1 have been shown to interact with two components of the exosome PM-scl75 and Rrp4 [62,71,75]. Although the exosome is not localized to P-bodies, these findings indicate that TIS11 family members can act outside cellular RNA decay centers and provide a

functional link recruiting the exosome to ARE-containing mRNAs. Taken together, the TIS11 family member's ability to regulate gene expression by targeting ARE-containing mRNAs for degradation is a complex interplay of various decay enzymes comprising a number of decay pathways that are functionally housed in unique cellular compartments. The fact that TTP and BRF proteins serve as a molecular link between ARE-containing mRNAs and these pathways indicates their significance in post-transcriptional gene regulation (Figure 1).

Decay Independent Functions of TTP

In addition to its role as a post-transcriptional regulator, recent studies have identified a novel function of TTP regulating NF- κ B-dependent transcription [76,77]. In both of these studies it was shown that the expression of TTP inhibited NF- κ B-dependent transcription and this attenuation was independent of the ability of TTP to bind RNA. TTP can also physically interact with the p65 subunit of NF- κ B [76], and this interaction provides a functional basis for the observation that TTP interferes with the nuclear import of the p65 subunit resulting in attenuation of NF- κ B activity [77]. Another mechanism by which TTP can influence NF- κ B is through TTP's association with histone deacetylases (HDACs). HDACs can serve as transcriptional co-repressors to inhibit NF- κ B activity [78] and findings demonstrating the physical interaction between HDACs-1, -3, and -7 with TTP, indicates in part the inhibitory effect of TTP on NF- κ B activity to be mediated through recruitment of HDACs [76].

TTP has also been shown to interact with other proteins that are not directly associated with mRNA decay. The nuclear pore protein Nup214 can interact with TTP and this interaction may regulate intracellular trafficking of TTP [79]. TTP also can bind the retroviral Tax oncoprotein that serves as a transcriptional regulator of viral gene expression [80]. By virtue of this interaction, TTP can inhibit the transcriptional activating function of Tax and Tax can inhibit the mRNA destabilizing function of TTP. This interaction supports increased cytokine expression in virus-infected cells which is central to pathogenesis. Most recently, TTP has been shown to interact with the CIN85 protein (CBL-interacting protein of 85 kDa) [81]. This protein, which can regulate receptor endocytosis and apoptotic signaling, appears to only interact with human TTP and not other TIS11 family members and its association with TTP results in MEKK4-dependant phosphorylation of TTP. The functional consequences of this interaction remain to be determined, since TTP/CIN85 interaction did not influence TTP's binding to AREs or impact TTP-mediated mRNA decay.

The Role of TIS11 Family Members in Disease

Targeted disruption of TIS11 family members in mice

The physiological role of TTP was first investigated by Taylor and colleagues by targeted disruption of the *Zfp36* gene in mice [82]. TTP^{-/-} mice appeared normal at birth; however, their post-natal rate of weight gain was significantly decreased as compared to littermates from 1-8 weeks of age. Additionally, TTP^{-/-} mice developed cachexia, dermatitis, patchy alopecia, erosive arthritis, conjunctivitis, and myeloid hyperplasia. Occurring with the systemic inflammatory syndrome exhibited in TTP^{-/-} mice were several hematopoietic abnormalities. These mice exhibited extensive extramedullary hematopoiesis, small and hypoplastic thymuses, loss of thymic cortical/medullary organization, and enlarged hyperplastic spleens and lymph nodes. Inflammatory abscesses in the liver and the heart were also observed [82]. Recent findings have also shown that TTP^{-/-} mice are prone to develop severe left-sided cardiac valvulitis resulting in thickening of the mitral and aortic valves [83]. Although these systemic syndromes occurred with an incidence of 100%, the severity of each condition was variable. To this extent, 34% of these animals died before

reaching 7 months of age and the longest observed survival time of TTP^{-/-} mice was of 16 months [82], [84].

The hyper-inflammatory phenotype of TTP^{-/-} mice is attributed to the overproduction of the pro-inflammatory cytokine TNF- α . Studies with liver-derived macrophages, bone marrow precursors, as well as resident peritoneal macrophages from adult TTP^{-/-} mice revealed a 5-fold increase in TNF- α secretion following LPS stimulation, as compared to wild-type controls. Additionally, TTP-deficient cells exhibited a 2-fold increase in TNF- α mRNA [85]. To validate that this pathology was dependent on the overexpression of TNF- α in TTP-deficient inflammatory cells, transplantation of bone marrow obtained from TTP^{-/-} mice into recombination activating gene (RAG)-2-deficient mice resulted in development of the full syndrome observed in the TTP^{-/-} mice [85]. Furthermore, weekly injections of anti-TNF- α antibodies into TTP^{-/-} mice prevented weight loss and the development of the various inflammatory syndromes observed in TTP^{-/-} mice [86]; [82]. Taken together, these findings strongly implicated a physiological role for TTP in the regulation of TNF- α expression *in vivo*.

Evidence of a mechanistic role for TTP in the control of TNF- α expression was initially shown in bone marrow-derived macrophages from TTP^{-/-} mice where these cells displayed a significantly increased TNF- α mRNA half-life [48]. Similarly, reporter gene constructs bearing the ARE-containing 3'UTR of TNF- α were destabilized by TTP [48]. RNA-binding studies demonstrated a functional interaction between the TNF- α ARE and TTP [48], establishing the link between TTP and TNF- α to occur on the post-transcriptional level through regulation of ARE-mediated mRNA decay.

Subsequent studies using bone marrow stromal cells (BMSC) derived from TTP^{-/-} mice revealed that the granulocyte macrophage-colony stimulating factor (GM-CSF) mRNA was another physiological target of TTP [58]. To this extent, stimulation of TTP-deficient BMSCs resulted in a significant increase of GM-CSF mRNA levels resulting from increased mRNA stability. Although the pathologies of the TTP^{-/-} mice are primarily attributed to excessive TNF- α production, loss of TTP-dependent post-transcriptional regulation of GM-CSF exerts a secondary effect by contributing to the development of myeloid hyperplasia in the TTP^{-/-} mice [58].

Further characterization of the physiological role of other TIS11 family members was accomplished using similar knockout mouse models. Targeted disruption of *Zfp361l* (BRF-1) resulted in embryonic lethality by approximately day 11 (E11) [87,88]. BRF-1-null embryos exhibited intraembryonic and extraembryonic vascular abnormalities and heart defects. In two-thirds of the cases chorioallantoic fusion failed to occur and, in cases where fusion did occur, deficient placental function was observed [88]. Associated with this phenotype was a significant increase in vascular endothelial growth factor (VEGF)-A in the embryos [87]. Interestingly, BRF-1 appears to repress VEGF-A mRNA translation and not promote mRNA decay, indicating a novel function of BRF-1 in translational regulation. In BRF-1-null fibroblasts no changes in VEGF-A mRNA levels were observed as compared to wild-type controls, whereas an enhanced association with polyribosomes was observed, suggesting that increased VEGF-A expression resulted from loss of BRF-1-dependent translational regulation [87].

Initial attempts to disrupt *Zfp3612* (BRF-2) resulted in the expression of truncated BRF-2 protein (Δ N-Zfp3612) that was missing the first 29 amino acids but retained the functional tandem zinc finger domains [89]. The expression of Δ N-Zfp3612 resulted in complete female infertility. Despite normal reproductive tract and menstrual behavior, post-fertilization embryos failed to grow beyond the two-cell stage possibly due to defects in

maternal mRNA turnover [89]. More recent work using a BRF-2 knockout model in which the majority of the BRF-2 coding region, including tandem zinc finger domains, was deleted revealed a more dramatic phenotype [90]. BRF-2-deficient mice survive for approximately 2 weeks after birth, but then die suddenly as a result of intestinal hemorrhage. Blood analysis indicated BRF-2 deficiency resulted in pancytopenia, as indicated in decreased red and white cells, hemoglobin, hematocrit, and platelet counts [90]. This can be attributed to markedly reduced numbers of definitive multilineage and lineage-committed hematopoietic progenitors in BRF-2-deficient mice. Although differing in their phenotypes when absent, these studies illustrate critical physiological roles of BRF-1 and BRF-2 in regulating normal fetoplacental development and modulating hematopoiesis, respectively.

The anti-inflammatory role of TIS11 family members

As evidenced from the knockout mouse model, TTP is a critical physiological regulator of TNF- α and other pro-inflammatory cytokines, and its loss of expression can be argued to influence the onset and severity of inflammatory syndromes in humans such as rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), and ulcerative colitis (UC). However, histological examination of synovial tissues of patients with rheumatoid arthritis revealed apparent cytoplasmic expression of TTP protein [91] and the magnitude of expression was greater in rheumatoid arthritis tissues as compared to osteoarthritic tissues [92]; whether TTP gene expression is elevated in arthritic synovium compared to healthy synovium is not currently known. Additionally, TTP levels were lower in patients that displayed heightened serum C-reactive protein (CRP), an inflammation marker used to monitor arthritis activity [92]. Interestingly, elevated TTP expression has also been observed in macrophages associated with atherosclerotic lesions [93]. Although these distinct inflammatory diseases appear to induce TTP expression at sites of inflammation, it is not known if during disease progression that TTP expression levels become limited. Work suggesting that this may be evident has shown that TTP overexpression in diseased tissue *via* adenovirus-based delivery is beneficial in preventing inflammatory bone loss associated with periodontitis [94].

The association of TTP deficiency with systemic autoimmune inflammatory syndrome and severe arthritis in mice prompted studies to identify genetic polymorphisms in TIS11 family members correlating with autoimmune disease in humans. Initial work had identified 13 polymorphisms in the protein-coding regions of these three genes and six of these polymorphisms result in amino acid changes [95]. Although the functional significance of these genetic changes has yet to be evaluated, one particular allele of BRF-1, which was a dinucleotide substitution that would prevent proper splicing, led to a 50% decrease in BRF-1 mRNA expression in lymphoblasts compared to cells without the mutation [95]. Using a larger population base, further efforts had expanded on polymorphism identification in TTP and detected 28 polymorphisms [96]. From this study, a novel polymorphism (SNP ZFP36*8) was identified to be significantly associated with a higher incidence of rheumatoid arthritis in African-American individuals. The SNP ZFP36*8 which is a C to T transition, lies in the region of exon 2 encoding the first arginine of the first tandem zinc finger domain. Even though the ZFP36*8 SNP is not predicted to alter the amino acid sequence of TTP, it has been argued to engage in the rare codon phenomenon, thereby slowing TTP translation and allowing for increased cytokine production. Alternatively, the presence of this SNP could influence transcription or the stability of TTP mRNA [96]. More recently a single nucleotide polymorphism (SNP359A->G) was identified within the promoter region of TTP [97]. The functional consequence of this SNP was demonstrated to impact TTP promoter activity with the presence of the G allele resulting in approximately a 2-fold decrease in TTP promoter activity [97]. Although no significant differences were observed in the allele frequencies of SNP359A->G between healthy individuals and RA patients, a trend, although not statistically significant, was observed in patients with the GG

genotype to have a younger age of disease onset compared to those with genotypes AA/AG [97]. These findings indicate that this SNP could possibly modulate disease activity of RA due to a subtle defect in the promoter activity of *ZFP36* leading to attenuation of TTP expression on a transcriptional level.

The anti-cancer role of TIS11 family members

Normal cellular growth is associated with rapid decay of ARE-containing mRNAs and targeted mRNA decay is an essential way of controlling their pathogenic overexpression. Interestingly, a number of observations have implicated the loss of ARE-mediated post-transcriptional regulation in the neoplastic transformation of cells [1,2]. This loss of ARE-mediated decay is evident as recent findings have demonstrated an enrichment of this subset of transcripts to occur during colon tumorigenesis (Figure 2). Gene expression profiling comparing adenomas to late stage adenocarcinomas show a 3- to 4-fold enrichment in ARE-containing genes compared to the genome as a whole and a similar enrichment is observed as early as stage I tumors [98], indicating that ARE-mediated decay is lost during the early stages of tumorigenesis. Based upon the inherent genetic instability of tumor cells, it might be expected that mutations in AREs are a frequent event. However, few mutations in AREs have been described [99], implying that loss of ARE function in tumor cells is primarily due to defects in *trans*-acting regulatory factors.

TIS11 family members' ability to play a central role in regulating ARE-mRNA turnover indicate a probable link between their expression and cancer. In studies examining the gene expression signatures of a large human cancer compendium spanning 22 tumor types, it was found that all TIS11 family members were coordinately downregulated in multiple epithelial tumor types [100]. Similarly, examination of TIS11 members in the NCI 60 panel of human cancer cell lines appeared to show comparable results, however heterogeneity in expression levels among the various cell types was observed most likely reflecting cell-specific differences in gene promoter and/or mRNA decay activities [26]. Recent reports examining TTP expression in a variety of human cancer cell lines and tumors, such as breast, colon, cervix, prostate, and lung has shown a consistent loss of TTP expression to occur in tumor samples as compared to normal tissue [101-103] and suppressed TTP expression can serve as a negative prognostic indicator in breast cancer [101]. Furthermore, our recent work examining TTP expression in colorectal cancer has shown the loss of TTP expression to occur at an early stage of tumorigenesis [103]. Concomitant with loss of TTP expression was elevated expression of the ARE-mRNA stability factor HuR, implying that both loss-of-TTP and gain-of-HuR function are required events for ARE-mediated mRNA stabilization in colorectal cancer [103].

The loss of TTP expression appears to be an early event in the process of tumorigenesis, thereby suggesting the possibility that TTP can function as a tumor suppressor. This novel ability of TTP should reflect the ARE-containing mRNAs needed for enhanced tumor cell growth and survival. This was first demonstrated using a v-H-Ras-transformed mast cell tumor model where TTP expression potently attenuated cell growth and tumorigenic phenotype through downregulation of IL-3 [104]. Similarly, expression of TTP in colon cancer cells inhibited their growth and tumorigenesis, in part to TTP-mediated downregulation of COX-2 and VEGF [103,105]. In recent studies, we and others have demonstrated that TTP expression in human papillomavirus (HPV) 18 positive HeLa cells induced morphological changes and lead to inhibition of cell proliferation and tumorigenesis [101,102] and constitutive expression of TTP led to the induction of cellular senescence [102]. The mechanism by which HPV transforms cervical cells is by maintaining a dormant p53 pathway and elevating telomerase (hTERT) activity. TTP expression in HeLa cells counteracted both these effects by stabilizing p53 protein and inhibiting hTERT expression and this occurred *via* targeting the ARE-containing mRNA of the cellular ubiquitin ligase

E6-associated protein (E6-AP) for rapid decay [102]. Furthermore, previous findings have indicated that TTP overexpression can sensitize cells to TNF- α -mediated apoptosis [106] and staurosporine-induced apoptosis [101]. Similarly, BRF-1 can enhance the cell death of squamous cell carcinoma cells in response to cisplatin treatment [107]. Although the mechanism underlying these effects remains to be determined, it is plausible that TIS11 members selectively target ARE-containing anti-apoptotic mRNAs such as the human inhibitor of apoptosis protein-2 (cIAP2) [107].

Although loss of TTP expression is evident in tumors, the mechanism that accounts for loss of TTP expression remains elusive. As aforementioned, the single nucleotide polymorphisms reported in *ZFP36* may contribute to decreased expression levels [95-97]. Recent findings revealed that loss of TTP expression in breast tumors may occur *via* a microRNA-based mechanism. MicroRNA profiling of human breast cancer cell lines and tissue specimens revealed that miR29-a was expressed in high levels in cells where TTP expression was lost and overexpression of this microRNA in breast epithelial cells resulted in suppression of TTP expression [108]. In addition to these mechanisms, TTP has been recently shown to be regulated by epigenetic modifications. Examination of the human TTP promoter (accession no. AY771351) has identified putative CpG islands present in the proximal 3' region of the promoter, suggesting that TTP may be epigenetically silenced in tumors [103] and evidence of increased DNA methylation of the TTP promoter in hepatocellular carcinoma (HCC) has been recently shown [109]. Interestingly, the silencing of TTP expression in HCC was dependent upon a single CpG methylation site and inhibition of DNA methylation restored TGF- β -mediated transcriptional induction of TTP [109].

Conclusion

Since their discovery over 20 years ago, the TIS11 family members have established themselves as key players in post-transcriptional gene regulation, particularly with regard to the regulation of ARE-mediated decay. A considerable amount of genetic and biochemical evidence has unraveled many of the key aspects of this distinct family of RNA-binding proteins and demonstrated their involvement in controlling a wide range of physiological and pathological processes. Yet, there are still many other interesting questions that will most likely be forthcoming. A more complete understanding of the role of phosphorylation and its functional consequences on TIS11 members' ability to regulate ARE-mediated decay will give greater insight into how this dynamic modification impacts the ability of diverse cellular pathways to control gene expression. On these same lines, we have limited knowledge of the direct and indirect mRNA targets of TIS11 family members and what mRNA targets may be distinct to one particular member or those with overlapping specificity. Expanding our knowledge of the role of TIS11 members in regulating other aspects of post-transcriptional regulation such as microRNA-mediated regulation will identify other key regulatory events these proteins participate in to impact global gene regulation. An area of active interest involves understanding the diverse expression patterns of TIS11 family members in various cell and tissue types and current work has established their role in controlling inflammatory syndromes and their involvement in reproductive and developmental processes. Finally, establishing the ability of TIS11 members to serve in a tumor suppressor capacity by attenuating ARE-containing gene expression will shed new light in the area of cancer etiology and provide new therapeutic avenues to pursue that will allow the nascent cellular RNA degradation machinery to counteract the oncogenic effects of mRNA stabilization.

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Figure 1. TIS11 plays a central role in post-transcriptional regulation of gene expression
 TTP and the other TIS11 family members BRF-1 and BRF-2 bind to AU-rich elements in the 3'UTR of mRNA transcripts. Through their direct and indirect association with components of the deadenylase complex such as Ccr4, Caf1, and PARN, TIS11 family members promote rapid mRNA deadenylation. TTP and BRF proteins target the deadenylated mRNA transcript for storage or decay in P-bodies. Once in P-bodies, TTP interacts with the mRNA decapping complex and activates mRNA decapping. After decapping, the mRNA body undergoes 5'-3' exonucleolytic decay. P-bodies are closely associated with cellular stress granules, and TTP possibly mediates the transport of translationally repressed ARE-containing mRNAs from stress granules to be decayed in P-bodies. With regard to 3'-5' mediated decay, TTP associates with components of the exosome and targets the mRNA for 3'-5' exosome-mediated degradation.

Figure 2. Loss of TTP expression promotes chronic inflammation and progression of tumorigenesis

In normal cells, expression of TTP allows for the controlled expression of a variety of cancer- and inflammation-associated genes by selectively targeting these ARE-containing mRNA transcripts for rapid decay. Loss of TTP expression promotes chronic inflammation and is observed during tumorigenesis, thereby allowing for stabilization of ARE-containing transcripts. This selective ARE mRNA stabilization contributes to overexpression of factors such as COX-2, TNF- α , cyclin D1, and VEGF that promote chronic inflammation, resistance to apoptosis, enhanced cell growth and proliferation, and increased angiogenesis and metastasis.

Table 1

The TIS11 Gene Family

Gene	Alternative names	Species	Chromosomal location	Size	
				Gene (kb)	Protein (kD)
<i>ZFP36</i>	TTP, TIS11, NUP475, GOS24	Human Mouse Rat	19q13.1 7qA3 1q21	2.5 2.4 2.5	34.0 33.6 33.7
<i>ZFP36L1</i>	BRF-1, TIS11b, ERF-1, cMG1, Berg36	Human Mouse Rat	14q22-q24 12qC3 6q24	5.4 5.2 5.0	36.3 36.4 36.4
<i>ZFP36L2</i>	BRF-2, TIS11d, ERF-2	Human Mouse Rat	2p22.3-p21 17qE4 6q12	4.2 4.0 4.0	51.0 50.0 50.0
<i>Zfp36l3</i>	AY661338, RGD1559581	Mouse Rat	XqA5 Xq3 6	3.7 N.A.	72.3 72.2*

NA, not annotated

* Predicted molecular weight

Table 2**Reported TTP mRNA Targets**

Cellular Process	mRNA Target	Reference
<i>Immune Response and Inflammation</i>	Ccl2 Ccl3 CD86 COX-2 DUSP 1 E47 GM-CSF** IDO Interleukins (IL-2, IL-3**, IL-6, IL-8, IL-10, IL-12) IFN- γ MHC (Class 1B and F) PAI-2 SOD2 TNF- α ** TIS11 (TTP)	[17] [17] [110] [111] [110] [112] [58,113] [110] [25,55,113-118] [119] [110] [120] [110] [48,54] [24]
<i>Cell Cycle</i>	Cyclin D1 MIP-2 p21 Plk3	[44] [118] [93] [121]
<i>Carcinogenesis</i>	c-myc c-fos E6-AP	[44] [93] [102]
<i>Angiogenesis</i>	VEGF*	[87,101,122]
<i>Development</i>	PITX2	[123]
<i>Protein Glycosylation</i>	1,4-galactosyltransferase	[124]

* Reported target of BRF-1

** Reported Target of BRF-1 and BRF-2