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Review

Tristetraprolin (TTP): Interactions with mRNA and proteins, and current thoughts on mechanisms of action ☆

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ABSTRACT

Changes in mRNA stability and translation are critical control points in the regulation of gene expression, particularly genes encoding growth factors, inflammatory mediators, and proto-oncogenes. Adenosine and uridine (AU)-rich elements (ARE), often located in the 3′ untranslated regions (3′UTR) of mRNAs, are known to target transcripts for rapid decay. They are also involved in the regulation of mRNA stability and translation in response to extracellular cues. This review focuses on one of the best characterized ARE binding proteins, tristetraprolin (TTP), the founding member of a small family of CCCH tandem zinc finger proteins. In this survey, we have reviewed the current status of TTP interactions with mRNA and proteins, and discussed current thinking about TTP's mechanism of action to promote mRNA decay. We also review the proposed regulation of TTP's functions by phosphorylation. Finally, we have discussed emerging evidence for TTP operating as a translational regulator. This article is part of a Special Issue entitled: RNA Decay mechanisms.

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1. Introduction

Gene expression is a highly regulated process that begins with transcriptional initiation and ends with translation of a mature mRNA into protein. In between these two points are a linked series of events including processing and splicing of the pre-mRNA, export of the message from the nucleus to the cytoplasm, quality control assessment of the mRNA through the pioneer round of translation, message decay and stabilization, and translational repression and de-repression. All of these events from initiation of transcription by transcription factors to the stability of the message to effective translation of the message are controlled by the presence of specific nucleotide sequences which are bound by specific *trans*-acting proteins. As a message is transcribed, proteins bind to form a messenger ribonucleoprotein complex (mRNP) and the composition of the mRNP controls all aspects of the life of the mRNA, from pre-mRNA processing to mRNA localization to translation and degradation. Transitions between these events are accompanied by mRNP remodeling and exchange of mRNP proteins.

2. Posttranscriptional regulation

Posttranscriptional control of gene expression, particularly mRNA stability and translation, allows for rapid changes in mRNA levels. Dysregulated mRNA stability and translation underly a number of diseases, directly contributing to the overexpression of many genes encoding growth factors, inflammatory cytokines, and proto-oncogenes [1–4]. Among the best studied *cis*-elements regulating posttranscriptional control are the adenosine and uridine (AU)-rich elements (ARE), located in the 3′ untranslated region (3′UTR) of many unstable mRNAs. AREs are most often arranged in repeated and/or overlapping pentamers of the sequence AUUUA, although there can be variability [5]. The relevance of these conserved *cis*-acting sequence elements is apparent in their frequency, currently estimated at approximately 8% of the human transcriptome [6,7]. AREs target mRNAs for rapid decay but also allow the stability and translation of mRNAs to be regulated in response to extracellular cues. These effects of an ARE on the posttranscriptional fate of a message depend on the interaction of an ever increasing list of *trans*-acting RNA binding proteins [4,8]. The focus of this review is on one of the best characterized ARE binding proteins, tristetraprolin (TTP).

3. Tristetraprolin

TTP, also known as Nup475, G0S24, and TIS11, is the founding member of a small family of proteins containing tandem CCCH zinc fingers. One of the major purposes of this review was to survey the current

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literature for reports of mRNA binding "targets" for TTP. As described below, we tried to include as many reports as we could find, but we did not attempt to determine the likelihood that a given report described a "true", physiologically relevant interaction. Accordingly, we performed a literature search on August 15, 2012, searching PubMed, Scopus, and Web of Science for the terms tristetraprolin, G0S24, and Nup475, and tris-tetraprolin; and we also searched with the combined terms "TTP and RNA". After the removal of abstracts, duplicates and papers on unrelated subjects, this search yielded 293 papers under those search terms. During revision of this review, the search was updated for papers published in 2012 on 2/7/13.

The original description of full-length TTP was in 1990 [9], which describes the cloning of the mouse cDNA from insulin-stimulated 3T3-L1 fibroblasts; this was followed within six weeks by the description of the same sequence as Nup475, cloned from serum-stimulated fibroblasts [10]. A partial fusion sequence had been published earlier from phorbol ester-stimulated fibroblasts [11]; this sequence was later corrected [12]. These initial descriptions were followed by descriptions of the human sequence [13]; in a later description, this was labeled G0S24 [14]. These represent the earliest descriptions of what we now know as tristetraprolin or TTP.

Fig. 1 shows the number of publications from the above search and their year of publication. It shows that there was very little activity for several years following the initial cloning of the human and mouse cDNAs, although the laboratory of one of us (PJB) made contributions during that time on the hormone and growth factor stimulated phosphorylation of the protein [15], the hormone and growth factor stimulated nuclear export of the protein [16], the influence of metal ions on its mRNA stability [17], and characteristics of the *Zfp36* promoter from mice and man [18].

4. Tristetraprolin and tumor necrosis factor alpha (TNF)

The modern era of TTP research can be said to have begun with the development and evaluation of a TTP knockout mouse, which exhibited a severe syndrome of growth retardation, cachexia, arthritis, inflammation and autoimmunity [19]. Most importantly, this paper established the first connection between TTP and the potent pro-inflammatory cytokine, tumor necrosis factor alpha (TNF). Treatment of the TTP-deficient mice, soon after birth, with anti-TNF antibodies prevented the development of virtually all aspects of the TTP deficiency syndrome.

This observation was followed by the demonstration that the syndrome could be transplanted with whole bone marrow into lethally irradiated immunodeficient mice. A lag of several months was necessary for disease development, suggesting that hematopoietic cells were required but that lymphocytes were unlikely to be the major sources of excess TNF; rather myeloid cells were the most likely source of TNF [20]. This paper also showed that TNF mRNA accumulated abnormally in the TTP KO macrophages, isolated from several body sites, after stimulation with various concentrations of lipopolysaccharide (LPS), and that TNF protein was hypersecreted from the same cells after LPS stimulation.

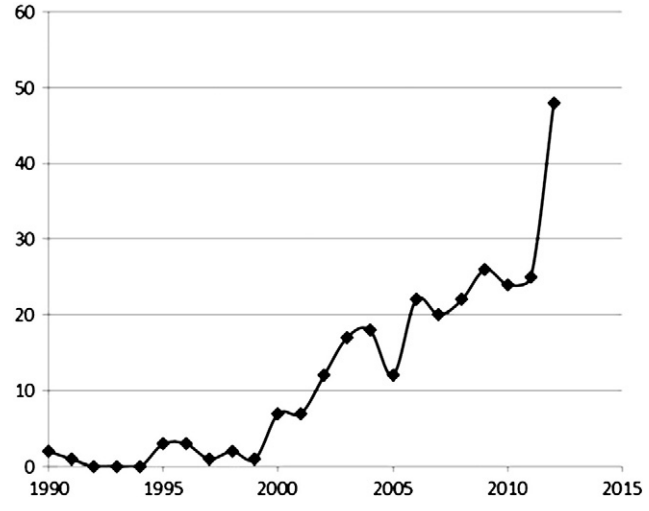

Fig. 1. Papers published on TTP since 1990. The figures show the results of the literature search described in the text; abstracts were not included.

Finally, a physical connection between TTP protein and the TNF mRNA was made in a subsequent paper [21]. This work showed that the TNF mRNA decayed more slowly in the TTP KO macrophages after stimulation with LPS and inhibition of transcription with actinomycin D; in these experiments, conducted after 4 h of LPS stimulation, the half-life of the TNF mRNA was increased from about 39 min to about 85 min. These experiments demonstrated for the first time that TTP protein could bind directly to the TNF mRNA, as assayed by RNA gel shift experiments. Thus, in the absence of TTP, the TNF mRNA accumulated abnormally due to its increased stability, leading in turn to enhanced TNF secretion, and presumably elevated local and systemic levels of the TNF protein.

Although a detailed discussion of this and other animal models is beyond the scope of this review, there are many important questions regarding the role of TTP in the intact mouse that remain unanswered. For example, in the "TNF delta ARE" mice described by Kontoyiannis et al. [22], the mice not only develop the cachexia and arthritis characteristic of the TTP KO mice, but also severe colitis; this has not been seen in the TTP KO mice to our knowledge. One possible explanation is the presence of the other TTP family members in the cells of the intestine, possibly regulating locally produced TNF or other cytokines, but this remains to be determined. Another interesting model is the myeloid-specific TTP KO mouse, which does not exhibit cachexia or arthritis, but is hypersensitive to low doses of LPS [23,24]. These data suggest that TTP-dependent pathways need to be dysregulated in one or more cell types other than myeloid cells to produce the full TTP-deficiency phenotype, but the identity of these cells is currently unknown.

5. TTP binding to AU-rich elements

Subsequent work characterized the binding sites on the TNF mRNA as one of several conserved instances of the simple linear RNA motif, UUAUUUAUU, in the 3′UTR of the message [25]. A similar conclusion had been reached from a completely different avenue of experimentation, using different selection procedures, but arriving at the same optimal binding site [26]. The site of RNA binding was the central tandem zinc finger domain (TZF), and it was striking that a single mutation of any of the eight cysteines or histidines in the TZF domain completely abrogated RNA binding [27]. Quantitative analysis of TZF–RNA interaction was later performed using fluorescence anisotropy, in a solution with pure components, in which the TZF domain was represented by a pure 75 amino acid totally synthetic peptide derived from the human TTP TZF domain sequence, and the "targets" were fluorescently labeled RNA oligonucleotides [28]. A selection of the dissociation constants from the interactions described in that paper is shown in Table 1. One of the key features of this interaction is that the previously determined optimal binding sequence, UUAUUUAUU, bound to the synthetic TZF peptide with a K_d of 3.0–3.2 nM at room temperature. This compares, for example, to a K_d of 280 nM for a polyU sequence, or 56 nM for polyU containing a single A residue. However, one point that remains a subject of ongoing research is the physiological importance of different numbers of U residues between the two As. As shown in the table, increasing this number to four increases the K_d to 6.4 nM, and increasing the internal Us to five increases the K_d to 17 nM. Conversely, decreasing the number of Us to two increases the K_d to 18 nM, while decreasing the number of Us to one increases the