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DOI: 10.1042/BST20160166

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Document Version
Publisher's PDF, also known as Version of record

Citation for published version (Harvard):
Clark, A & Dean, JLE 2016, 'The control of inflammation via the phosphorylation and dephosphorylation of tristetraprolin: a tale of two phosphatases', Biochemical Society Transactions, vol. 44, no. 5, pp. 1321-1337. https://doi.org/10.1042/BST20160166

Link to publication on Research at Birmingham portal
The control of inflammation via the phosphorylation and dephosphorylation of tristetraprolin: a tale of two phosphatases

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Twenty years ago, the first description of a tristetraprolin (TTP) knockout mouse highlighted the fundamental role of TTP in the restraint of inflammation. Since then, work from several groups has generated a detailed picture of the expression and function of TTP. It is a sequence-specific RNA-binding protein that orchestrates the deadenylation and degradation of several mRNAs encoding inflammatory mediators. It is very extensively post-translationally modified, with more than 30 phosphorylations that are supported by at least two independent lines of evidence. The phosphorylation of two particular residues, serines 52 and 178 of mouse TTP (serines 60 and 186 of the human orthologue), has profound effects on the expression, function and localisation of TTP. Here, we discuss the control of TTP biology via its phosphorylation and dephosphorylation, with a particular focus on recent advances and on questions that remain unanswered.

Introduction
Tristetraprolin (TTP) belongs to a small family of RNA-binding proteins, which has three members in most mammalian species but four in the mouse and rat. Its name derives from three dispersed stretches of four consecutive proline residues (Figure 1). Reflecting its independent discovery by several laboratories, it has also been named as 12-O-tetradecanoyl phorbol 13 acetate-inducible sequence 11a, G0/G1 switch gene 24, nuclear protein 475 and ZFP36 (zinc finger protein of 36 kDa). The protein is now almost universally known as TTP, whereas the correct systematic name for the corresponding gene is Zfp36 in the mouse, ZFP36 in man. The biology of TTP is very well reviewed elsewhere [1]. This review focuses on the role of phosphorylation and dephosphorylation of TTP in the regulation of inflammatory responses, unresolved controversies and questions that remain to be answered.

The first Zfp36 gene knockout revealed a fundamental role of TTP in the constraint of inflammation [1–3]. Mice lacking TTP developed a spontaneous and pervasive inflammatory syndrome, including cachexia, dermatitis and erosive joint inflammation resembling rheumatoid arthritis (RA). Most symptoms were ascribed to increased stability of tumour necrosis factor (Tnf) mRNA and expression of TNF protein. Consequently, much research on TTP has focussed on myeloid cells, which are the principal sources of TNF in vivo. However, myeloid-specific disruption of the Zfp36 gene did not reproduce the same pervasive syndrome. Mice lacking TTP in myeloid cells were healthy under normal conditions, but developed excessive inflammatory responses to challenge with lipopolysaccharide (LPS) [4,5]. These findings clearly establish that TTP functions in non-myeloid cells to inhibit inflammation. Although activation-induced TTP expression can be detected in several different cell types, relatively little is known about its function outside the myeloid lineage, other than in fibroblasts [6–8]. TNF sustains inflammation in part via its actions on fibroblasts [9], an effect that is modulated by fibroblast TTP [8,9a].
TTP is a sequence-specific mRNA-binding protein with a preferred binding site consisting of the heptameric sequence UAUUUAU [1]. The heptamer is a particular example of an element known as an adenylate/uridylate-rich element or ARE. Binding of TTP to RNA substrates is mediated by a central zinc finger domain (ZFD), in which two tandem zinc fingers each co-ordinate a single zinc ion via three cysteine and one histidine residues. A crystal structure has been solved for the RNA heptamer in complex with the ZFD of the TTP family member ZFP36L2 (ZFP36-like protein 2, otherwise known as butyrate response factor 2 or BRF-2) [10].

As the ZFD is highly conserved between the members of the family, it is highly likely that TTP recognises its targets in the same manner. The UAUUUAU heptamer and closely related sequences are frequently found in the 3'-untranslated regions (3'-UTRs) of mRNAs encoding cytokines, chemokines and other mediators of inflammation, growth factors, regulators of apoptosis and the cell cycle. A growing number of such mRNAs have been shown to be recognised and regulated by TTP [1]. However, *in silico* prediction of TTP targets remains difficult. Transcriptome-wide identification of targets has been performed using two related methods including PAR-CLIP (photoactivatable ribonucleoside-enhanced cross-linking and immunoprecipitation) and
i-CLIP (individual nucleotide resolution cross-linking and immunoprecipitation) [11–13]. A user-friendly website integrates PAR-CLIP and i-CLIP data sets, providing rapid visualisation of transcriptome-wide TTP-binding sites, coupled with the analysis of differential mRNA expression in Zfp36−/− macrophages (http://ttp-atlas.univie.ac.at/index.php). Particularly where high confidence hits are selected, these two methods support the consensus heptamer as a preferred binding site for TTP in vivo. The absence of secondary structure appears to be an important determinant of binding [12], consistent with the structure of the ZFD in complex with an RNA substrate [10]. The presence of more than one consensus site also favours binding, perhaps reflecting co-operative interactions between TTP molecules [1,14]. TTP interactions were enriched near to the poly-(A) tail [11,13], possibly related to interactions between TTP and poly-(A)-binding proteins [15,16].

Like other members of its family, TTP is able to recruit several proteins or protein complexes that participate in mRNA turnover [15,17,18]. These include deadenylases, which catalyse the shortening of the 3′-poly-(A) tail; decapping enzymes, which remove the 7-methylguanylate cap from the 5′-end of mRNA and exonucleases that catalyse the degradation of the mRNA body from either the 5′-end or the 3′-end. In most cases, an obligate and rate-limiting step in the destruction of an mRNA is the removal of the poly-(A) tail [19]. Therefore, the interactions between TTP and deadenylases are likely to be highly relevant. In a simplified outline, binding of TTP to cognate sites in the 3′-UTRs of target mRNAs is followed by recruitment of deadenylases and shortening of the poly-(A) tail. When the poly-(A) tail becomes too short to support high-affinity binding of poly-(A)-binding proteins, destruction of the mRNA body is initiated by decapping of the 5′-end and rapid exonucleolytic degradation of the mRNA body. Hence, TTP target mRNAs tend to be abnormally stable in Zfp36−/− cells that lack TTP and highly unstable in Zfp36aa/aa cells expressing a constitutively active form of TTP known as TTP-AA, in which serines 52 and 178 (Ser-52 and Ser-178) are substituted by alanine residues [20] (see below). Some investigators have found TTP to regulate the expression of target mRNAs principally at the level of translation rather than mRNA stability [21–23]. More recently, TTP target mRNA abundance and translation were systematically studied using iCLIP, RNASeq and ribosome footprinting (RiboSeq) in Zfp36−/− macrophages reconstituted with GFP-TTP or GFP-TTP-AA [13]. This analysis suggested that TTP phosphorylation has greatest effects at the level of target mRNA stability. Relatively few transcripts were differentially expressed at the level of translation without evident changes of mRNA abundance. The reasons for the discrepancies between these observations are far from clear. It should be noted that similar controversy has surrounded the mitogen-activated protein kinase (MAPK) p38 pathway, and whether its post-transcriptional effects are chiefly at the level of mRNA stability or translation [24]. It may be relevant that the poly-(A) tail not only protects mRNA against degradation, but also promotes efficient mRNA translation via interactions between poly-(A)-binding proteins and 5′-cap-binding proteins [25]. One hypothesis is that target mRNA deadenylation may be uncoupled from degradation of the mRNA body under some conditions, or in a transcript-specific manner, such that the principal effects of MAPK p38 and TTP are to modulate translation rather than degradation. However, experimental evidence in support of this hypothesis is so far lacking.

**TTP as a phosphoprotein**

In denaturing polyacrylamide gel electrophoresis, TTP appears either as a broad smear or as a ladder of discrete bands with apparent molecular mass between 40 and 55 kDa (the actual molecular mass of murine TTP being 33.6 kDa). Most or all of this variation in electrophoretic mobility is a consequence of phosphorylation, since phosphatase treatment of cell lysates collapsed the multiple forms of TTP to one or two bands of high mobility [26–29]. Conversely, in lysates from cells treated with phosphatase inhibitors, such as okadaic acid or calyculin A, TTP migrates with an apparent molecular mass of up to 62 kDa [27,30,31]. Only a fraction of this change in electrophoretic mobility can be attributed to the mere addition of mass. Even the phosphorylation of 30 residues of TTP would increase its mass by <3 kDa. However, it is evident that TTP can be very extensively phosphorylated in vivo.

Information about sites of phosphorylation of TTP has emerged both from high-throughput phosphoproteomic studies of tumour cells, macrophages and other cells [28,32–34] and from focussed experiments in which epitope-tagged human or mouse TTP was stably expressed in HEK293 human kidney cells [35–37], mouse 3T3 fibroblasts, hamster kidney [38] or a mouse macrophage cell line [20]. This information is very helpfully summarised on the PhosphositePlus web site (www.phosphosite.org) [39] and illustrated graphically in Figure 1. If all of the documented phosphorylations are accepted at face value, TTP is in the top 0.1% of phosphorylated proteins in the proteome in terms of the number of phosphorylations per unit mass. Making allowance for species divergence and technical differences, such as proteolytic cleavage strategies, targeted and untargeted
approaches, generate a quite consistent picture of TTP phosphorylation in different cellular contexts. For consistency and clarity, we use murine TTP co-ordinates, even where the relevant experiments were performed using human TTP. The corresponding human co-ordinates can be read from PhosphositePlus. Phosphorylation of Ser-52 and Ser-178 is extremely well documented, although the former was not detected in focused studies of transfected HEK293 cells [35,37], perhaps for technical reasons. As discussed below, TTP protein is stabilised via phosphorylation of Ser-52 and Ser-178. Therefore, TTP lacking these two phosphorylations may be low in abundance and consequently under-represented in phosphoproteomic analyses. A serine-, threonine- and proline-rich region N-terminal to the first zinc finger can be phosphorylated at Ser-80, Ser-82, Ser-85 and Thr-87. Additional clusters of phosphorylation sites are found in the region of the second and third tetraprolin motifs, and within the C-terminal domain of the protein. Mass spectrometric data indicate high stoichiometry of phosphorylation within these regions [20,36] and our unpublished data. Both targeted and untargeted approaches provide evidence for phosphorylation of Ser-316, very close to the C-terminus of TTP [20,28,32,37]. Finally, there is some evidence for phosphorylation of Tyr-112, Tyr-150 and Tyr-277. The majority of the sites discussed above are highly conserved between mammalian TTP orthologues, and several are also present in other members of the TTP family [35] (Figure 1B, discussed below).

**TTP function is modulated by phosphorylation**

MAPK p38 plays a central role in the expression of many mediators of inflammatory responses [40]. To a large extent, it operates via the downstream kinase MAPK-activated protein kinase 2 (MK2), regulating target genes at a post-transcriptional level, inhibiting deadenylation, increasing mRNA stability and/or increasing its translational efficiency [41]. Tight regulation of MAPK p38 signalling is critical to prevent ectopic, excessive or unprovoked inflammation. An important negative feedback mechanism involves dual-specificity phosphatase 1 (DUSP1, also known as MAPK phosphatase 1 or MKP-1) [42–44]. DUSP1 is normally expressed at very low levels in resting cells. Pro-inflammatory stimuli increase its expression in an MAPK p38-dependent manner. It then dephosphorylates and inactivates MAPK p38 (as well as other MAPKs), helping to bring about the termination of the inflammatory response [42,43]. *Dusp1*−/− mice are healthy under normal conditions, but their dysregulated MAPK p38 responses to pro-inflammatory challenges are often fatal.

There is a considerable overlap between transcripts that are targeted by TTP and those that are post-transcriptionally regulated by MAPK p38. The pivotal role of TTP in MAPK p38-mediated post-transcriptional regulation of inflammatory responses was demonstrated by the failure of MAPK p38 inhibitors to decrease gene expression, or to destabilise target mRNAs, in *Zfp36−/−* cells [45,46]. MK2 efficiently phosphorylated TTP *in vitro* [26], and the major sites were subsequently identified as Ser-52, Ser-178 and Ser-316 [38]. A landmark paper then demonstrated that the MK2-mediated phosphorylation of Ser-52 and Ser-178 impaired the mRNA-destabilising activity of TTP [47]. This mRNA-stabilising effect was accompanied by an interaction of phosphorylated TTP with 14-3-3 proteins. These are a family of abundant, dimeric adaptor proteins that specifically recognise certain client phosphoproteins, helping to bring about changes in their structure, stability, function or subcellular localisation [48,49]. Phosphorylation of TTP and recruitment of 14-3-3 proteins could be enhanced by the treatment of cells with the somewhat unspecific phosphatase inhibitor, okadaic acid [27]. The identity of the cellular phosphatase or phosphatases responsible for the dephosphorylation of Ser-52 and Ser-178 is rather important. The best evidence to date implicates protein phosphatase 2A (PP2A), since siRNA-mediated knockdown of a catalytic subunit of PP2A increased TTP phosphorylation, 14-3-3 protein recruitment, expression of TNF and of a TNF 3′-UTR reporter mRNA [27]. The recruitment of 14-3-3 proteins is thought to antagonise PP2A-mediated dephosphorylation of TTP [27], with consequences that are discussed below.

Conflicting, although not mutually exclusive, mechanisms have been suggested to mediate the control of mRNA stability via MK2-mediated phosphorylation of TTP. According to one school of thought, TTP competes for RNA binding with HuR (human antigen R), a member of the embryonic lethal abnormal vision family of RNA-binding proteins [50–53]. HuR is generally considered as an mRNA-stabilising factor [54] and binds to RNA with specificity overlapping rather than identical with that of TTP. Phosphorylation of TTP is proposed to decrease its affinity for RNA and favour its displacement by HuR [23,55]. However, there is disagreement about the extent to which binding sites for TTP and HuR overlap [11,12]. High-resolution mapping by PAR-CLIP suggested that instances of direct competition between the two RNA-binding proteins may be rare [12]. Comparison of transcriptome-wide-binding sites of GFP-TTP and GFP-TTP-AA suggested that phosphorylation may decrease the affinity and/or specificity with which TTP binds to RNA [13]. However, the non-canonical TTP-binding sites described in that study were generally not found in another study using
actions of TTP are proposed, which do not involve its binding to RNA, it is important to rule out indirect phenomena that would not necessarily require TTP to be present in the nucleus. Where non-canonical hormone receptors or nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) function by preventing nuclear translocation of the p65 subunit [73–75], a phenomenon that would not necessarily require TTP to be present in the nucleus. Where non-canonical actions of TTP are proposed, which do not involve its binding to RNA, it is important to rule out indirect

Several independent groups have reported that mammalian TTP promotes deadenylation of target mRNA by recruiting the carbon catabolite repressor protein 4-negative on TATA-less (CCR4–NOT) deadenylase complex, a large (1 mDa) complex containing at least 10 subunits [31,56–59]. The Drosophila melanogaster orthologue of TTP employs a similar mechanism to regulate the expression of anti-microbial products [60,61]. As the MAPK p38 pathway regulates mRNA stability at the level of poly-(A) tail length [62,63], an obvious hypothesis is that MK2-mediated phosphorylation of TTP impairs CCR4–NOT recruitment. TTP-dependent in vitro deadenylation and degradation of an ARE-containing reporter RNA were prevented by MK2-mediated phosphorylation of Ser-52 and Ser-178, which also inhibited the recruitment of the CCR4–NOT complex [56]. A 14-3-3 sequestering agent prevented the binding of 14-3-3 proteins to phosphorylated TTP, but did not destabilise the reporter RNA. This suggests that MK2-mediated phosphorylation of TTP may impair deadenylation recruitment directly rather than via binding of 14-3-3 proteins. However, another group also described inhibition of CCR4–NOT recruitment via the phosphorylation of Ser-52 and Ser-178, and implicated 14-3-3 binding in this process [31]. Two other groups described recruitment of CCR4–NOT1 via the C-terminal domain of TTP [57,58]. MK2-mediated phosphorylation of Ser-316 was shown to impair the interaction between TTP and CNOT1, a scaffold protein of the CCR4–NOT1 complex, thereby inhibiting deadenylation and degradation of TTP target transcripts [57,58]. These observations are not necessarily conflicting. As the CCR4–NOT1 complex is extremely large, there is potential for multiple contacts with TTP, and redundant, 14-3-3-dependent or independent mechanisms for regulation of the interaction by phosphorylation. Elucidation of the mechanisms and modulation of TTPs interaction with CCR4–NOT1 is not a trivial undertaking.

TTP subcellular localisation is modulated by phosphorylation

Like many RNA-binding proteins, TTP shuttles between the nucleus and the cytoplasm. Nuclear export is mediated by CRM1 and dependent on a Leu-rich N-terminal region [64,65]. Both 14-3-3-dependent and -independent mechanisms maintain TTP in the cytoplasm [66]. The description of TTP as an almost exclusively cytoplasmic protein requires some qualification. At least in macrophages, TTP is likely to be phosphorylated at Ser-52 and Ser-178 as soon as it is generated. Although low amounts of endogenous, nuclear TTP can be detected [12], nuclear localisation has been most clearly demonstrated under somewhat artificial circumstances: ectopic expression in the absence of MAPK p38 activity [30,67,68], acute inhibition of MAPK p38 at the peak of TTP expression [30] or alanine substitution of Ser-52 and Ser-178 of endogenous TTP (ARC, in preparation). Together, these observations suggest that MAPK p38-dependent phosphorylation of Ser-52 and Ser-178 contributes to, but is not indispensable for, the localisation of TTP in the cytoplasm, at least in macrophages.

It remains unclear what biological function, if any, is served by TTP in the nucleus. A role in the regulation of nuclear polyadenylation of ARE-containing mRNAs was described in one report [69]. Large numbers of TTP-binding sites were identified by i-CLIP or PAR-CLIP in intronic RNA, and conventional RNA-IP confirmed binding of TTP to an excised intron, presumably in the nucleus [11,12]. The consequences of such interactions are not known. TTP has also been proposed to function as a transcriptional corepressor of nuclear hormone receptors or nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) [70–72]. Alternatively, TTP may impair NF-κB function by preventing nuclear translocation of the p65 subunit [73–75], a phenomenon that would not necessarily require TTP to be present in the nucleus. Where non-canonical actions of TTP are proposed, which do not involve its binding to RNA, it is important to rule out indirect
effects. For example, TTP may regulate the expression of components of the NF-κB signalling pathway or the expression of p65 itself [13].

Interruption of translation leads to the formation (or accretion) of granular cytoplasmic structures known as stress granules (SGs) and processing bodies (P bodies). Detailed description of these structures is beyond the scope of the present study, but reviewed extensively elsewhere [76–78]. SGs contain components of the translation machinery and are thought to be formed via aggregation of translationally stalled ribonucleoprotein complexes. P bodies are crucial sites of mRNA turnover that contain many components of the cellular mRNA degradation machinery, including the CCR4-NOT complex. The two discrete structures are dynamically linked and sometimes in contact with one another. TTP has been implicated in the traffic of ARE-containing mRNAs between P bodies and SGs, a process that may either determine or reflect decisions between mRNA storage, destruction and re-initiation of translation [78,79]. MK2-mediated phosphorylation of Ser-52 and Ser-178 was demonstrated to result in exclusion of TTP from SGs, accompanied by recruitment of 14-3-3 proteins and stabilisation of target mRNAs [47]. One might predict that phosphorylation of Ser-52 and Ser-178 also prevents localisation of TTP to P bodies, but to our knowledge this has not yet been demonstrated.

TTP protein stability is modulated by phosphorylation

TTP protein is also stabilised in response to phosphorylation of Ser-52 and Ser-178 [21,30,80]. The description of TTP as a relatively stable protein [81] is therefore accurate under most circumstances, where TTP expression is accompanied by (indeed, dependent on) MAPK p38 activation. Lacking two sites of MK2-mediated phosphorylation, TTP-AA is constitutively degraded by the proteasome and therefore expressed at low levels [20,21,30]. If primary macrophages or RAW264.7 cells are stimulated with LPS for 2 h prior to the addition of an MAPK p38 inhibitor, pre-existing TTP is rapidly degraded in a manner that requires the activity of both phosphatase(s) and the proteasome [30].

In the majority of cases, targeting of proteins for destruction by the proteasome depends on the covalent addition of polyubiquitin chains to lysine residues. Unexpectedly, ubiquitination of TTP could not be detected, nor was TTP protected from proteasomal degradation by mutation of all five lysine residues [82]. These observations suggest an atypical mode of degradation of TTP protein. The only 3D structure of TTP protein solved to date is for the highly conserved central zinc finger region [83], and secondary structure prediction programmes all fail to identify any stable structure in the N-terminal and C-terminal domains [82] (JLED, unpublished data). The unstructured nature of the majority of TTP protein is consistent with proteasomal degradation via a default degradation pathway shared with other largely unstructured proteins [84]. A key question is how the phosphorylation of Ser-52 and Ser-178 acts to stabilise TTP protein. Since a common feature of 14-3-3 proteins is that they impose secondary structure upon client phosphoproteins [48,49], an obvious hypothesis is that 14-3-3-mediated imposition of stable structure allows TTP protein to escape degradation-by-default. Gueydan and colleagues report that the addition of recombinant 14-3-3 proteins failed to prevent degradation of purified TTP in vitro [82]. It would arguably be more relevant (though less easy) to ask whether 14-3-3 proteins are necessary for the stabilisation of phosphorylated TTP.

A working model and an experimental validation

The regulation of both TTP expression and function by the MAPK p38 pathway effectively couples the activation and resolution phases of an inflammatory response. A working model of this process is presented in Figure 2. During the early phase of the response to a pro-inflammatory agonist, such as LPS, strong MAPK p38 activity promotes the expression of TTP at the levels of transcription, mRNA stability, mRNA translation and protein stability. Although direct experimental evidence is lacking, the TTP that accumulates under these conditions is thought to be phosphorylated at Ser-52, Ser-178 and Ser-316, therefore inactive as an mRNA destabiliser or translation suppressor. Efficient expression of inflammatory mediators is therefore possible. As MAPK p38 activity declines, the balance between phosphorylation and dephosphorylation of these sites shifts in favour of the latter. The accumulated pool of inert TTP then becomes active and can promote the off-phase of the response, blocking further translation, promoting mRNA decay or both. This signal-dependent post-transcriptional regulation of ARE-containing mRNAs contributes to the complex programme of temporally tuned gene expression, in which the influence of TTP increases with time [4,12,14,20].

Illustrating the physiological significance of MAPK p38-mediated regulation of TTP function, we recently used homologous recombination to generate a knockin mouse strain, in which Ser-52 and Ser-178 codons of the endogenous Zfp36 locus were substituted by alanine codons [20]. We refer to the mutated locus as Zfp36aa...
and the altered protein product as TTP-AA. Homozygous Zfp36aa/aa mice proved healthy and fertile with no
evident phenotype under standard maintenance conditions. As mentioned above, TTP-AA was expressed
weakly (being constitutively degraded by the proteasome), but it constitutively destabilised target mRNAs and
strongly inhibited the expression of several inflammatory mediators. When challenged by intraperitoneal injec-
tion of LPS, Zfp36aa/aa mice were protected against the subsequent cytokine storm and organ damage, re-
flecting dramatically reduced serum levels of many inflammatory cytokines. For example, in these experiments, the
expression of interleukin 6 (IL-6) was ∼200-fold lower in Zfp36aa/aa than in Zfp36+/+ mice (ARC, in prepar-
ation). In a highly robust experimental model of inflammatory arthritis, Zfp36aa/aa mice were completely pro-
tected, developing absolutely no symptoms of disease [9a]. They also demonstrated decreased pathogenic
responses in some experimental models of pulmonary inflammation (Phil Hansbro and Alaina Ammit,
personal communication). Other experimental models of inflammatory pathology remain to be tested.

We hypothesised that DUSP1 regulates inflammatory responses by modulating the phosphorylation state, and
hence the activity, of TTP. By combining Dusp1−/− and Zfp36aa/aa genotypes, it was demonstrated that
harmful, dysregulated inflammatory responses in the absence of DUSP1 were largely dependent on intact Ser-52
and Ser-178 residues of TTP [85]. Certain genes, for example, Tnf, Cxcl1 and Cxcl2, were regulated by DUSP1
exclusively via TTP phosphorylation. Their expression was strongly elevated in Dusp1−/− macrophages and
equally strongly diminished in Zfp36aa/aa macrophages. In double genetically modified macrophages, expression
of these genes remained low, indicating that dysregulated MAPK p38 signalling could enhance gene expression
only if TTP could be phosphorylated and inactivated. DUSP1 controlled many other genes in part via TTP and
in part via other mechanisms that have not been identified, but probably include effects on transcription. The

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**Figure 2. Schematic model of the regulation of TTP expression and function by the MAPK p38 signalling pathway.**
The dynamic equilibrium between phosphorylation and dephosphorylation of TTP controls the switch between the on- and
time off-phases of pro-inflammatory gene expression. Only Ser-52 and Ser-178 phosphorylations are indicated.
regulation of TTP function by the two phosphatases, DUSP1 and PP2A, is illustrated schematically in Figure 2. DUSP1 indirectly regulates the inactivation of TTP, whereas PP2A directly mediates the activation of TTP.

Several groups have reported that TTP is not only regulated by DUSP1, but also regulates the expression of DUSP1 [13,86–91]. Interaction of TTP with the Dusp1 3′-UTR was also confirmed in iCLIP and PAR-CLIP studies [12,13]. This suggests the existence of a homeostatic feedback mechanism by which TTP may regulate its own function. Elevated TTP activity would be predicted to decrease DUSP1 expression and enhance the activity of MAPK p38, ultimately promoting TTP inactivation. Consistent with this concept, the kinetics of MAPK p38 activation were altered in retrovirally transduced macrophages expressing GFP-TTP-AA [13]. In contrast, no changes in MAPK p38 activation profile were observed in macrophages expressing TTP-AA from the endogenous Zfp36 promoter [20]. Nevertheless, this putative homeostatic mechanism may be physiologically relevant and merits further investigation.

There is a great deal more that remains puzzling or unknown about the phosphorylation and dephosphorylation of TTP. The remainder of this review focuses on a few unanswered questions.

**Are phosphorylation and dephosphorylation of TTP linked to pathogenesis?**

TTP protein was strongly expressed at sites of inflammation in cardiovascular disease and a mouse model thereof, where it was suggested to play an anti-inflammatory role at both transcriptional and post-transcriptional levels [75]. TTP was also abundant in the inflamed RA synovium, prompting the authors to ask why it failed to down-regulate the expression of TNF and other inflammatory mediators [92]. Such observations might be re-interpreted in light of the coupled stabilisation and inactivation of TTP due to phosphorylation of Ser-52 and Ser-178. We hypothesise that TTP can accumulate in a phosphorylated and inactive form at sites of prolonged inflammation, and that its inactivity contributes to the establishment of chronicity. In support of this hypothesis, we found TTP to be co-localised with active MK2 in the cytoplasm of RA synovial macrophages [9a]. Constitutive MAPK p38 activation in tumour-associated macrophages was accompanied by accumulation of inactive TTP, contributing to overexpression of several inflammatory mediators [22]. However, the phosphorylation state of Ser-52 and Ser-178 at inflammatory sites has not yet been directly demonstrated. Both we [20] and others [47] have generated phospho-specific antibodies against TTP, but so far these have not proved amenable to approaches such as immunofluorescence. More sophisticated methods may be required to determine whether TTP is phosphorylated and inactivated at sites of chronic inflammation.

TTP and other members of its family are putative tumour suppressors, whose expression is diminished or absent from various cancers [53,93–98]. Inhibition of tumourigenesis may occur at several different levels; for example, down-regulation of cell cycle regulators and proto-oncogenes, growth factors, inflammatory cytokines and proteases that support tumour growth, vascularisation or metastasis. Phosphorylation and inactivation rather than loss of TTP has also been implicated in tumour development [99]. Potent anti-proliferative effects were ascribed to a mutant form of TTP, in which eight phospho-acceptor sites, including Ser-52 and Ser-178, were substituted by non-phosphorylatable alanines [100]. It would be interesting to test responses of the Zfp36aa/aa mouse in experimental models of tumourigenesis, particularly those in which TTP targets, such as cyclooxygenase 2, have been implicated as pathogenic factors.

**Why are Ser-52 and Ser-178 evolutionarily conserved?**

As described above, Zfp36aa/aa mice were protected against excessive inflammatory responses in experimental models of endotoxemia, RA and pulmonary inflammation. Moreover, their ability to mount protective immune responses against a model pathogen was not significantly impaired [20]. Yet in the real world outside the laboratory, the phosphorylation of TTP appears to be under strong selective pressure. The sequences surrounding serines 52 and 178 are highly conserved amongst mammals (Figure 3). Although there is predictably greater sequence divergence in fish, amphibian and reptile TTP, the phospho-acceptor sites themselves are conserved. They are predicted to be recognised and phosphorylated by MK2, although this remains to be experimentally tested. The implication is that the control of TTP function via phosphorylation arose early during vertebrate evolution and has been maintained by natural selection. In turn, this implies that Zfp36aa/aa mice have some selective disadvantages that have not yet come to light under laboratory conditions. We speculate that this relates to the innate immune response to pathogens more virulent than those tested so far, organisms that generate the strongest possible selective pressure. Interestingly, a virulent strain of the intracellular
pathogen Francisella tularensis promotes increased apoptosis of infected macrophages, accompanied by increased stability of \( \text{Il1b} \) mRNA and secretion of IL-1\( \beta \) protein. This response was linked to sustained phosphorylation of serine 178 of TTP [34]. Bacillus anthracis has a more evasive strategy, using lethal toxin (Le-Tx) to cleave MAPK kinases, silence MAPK signalling cascades and reduce innate immune responses. Le-Tx was shown to favour the formation of P bodies and promote destabilisation of IL-8 mRNA in a TTP-dependent manner [101]. These observations suggest that TTP and its phosphorylation sites are at the battlefront of an evolutionary war between hosts and pathogens, finely balanced between excessive and insufficient innate immune responses. It would be interesting to determine whether \( \text{Zfp36aa/aa} \) macrophages are more or less susceptible than wild-type macrophages to infection by these two pathogens.

**What are the mediators and the consequences of other phosphorylations of TTP?**

Surprisingly, little is known about sites of phosphorylation of TTP other than Ser-52 and Ser-178. Several of the sites are followed by proline residues and are candidates for phosphorylation by MAPKs or other proline-directed kinases. Indeed, recombinant TTP was found to be efficiently phosphorylated by extracellular signal-
regulated kinase (ERK), c-Jun N-terminal kinase (JNK) or MAPK p38 in vitro [102,103]. We found that ERK phosphorylated several sites of recombinant TTP in vitro, and that an inhibitor of the ERK pathway decreased the phosphorylation of these sites in LPS-treated RAW264.7 cells without diminishing TTP protein levels (ARC, unpublished). These observations may be significant because ERK is thought to exert post-transcriptional effects via TTP [30,104,105]. Glycogen synthase kinase 3β, protein kinases A and ζ also phosphorylated recombinant TTP in vitro, but sites were not identified [103]. Mediators of tyrosine phosphorylations have not been identified.

The electrophoretic mobility of TTP was strongly influenced by phosphorylation of serines 189, 210 and 220 [37]. As discussed above, such changes in mobility cannot be attributed to simple acquisition of mass. It is thought that large, phosphorylation-dependent changes in electrophoretic mobility generally involve cis-trans-isomerisation at proline residues adjacent to phospho-acceptor sites, which are catalysed by Pin1 (protein interacting with never in mitosis 1) or other prolyl isomerases [106]. Although there is no obvious link between electrophoretic mobility and cellular function, prolyl isomerisation can impose significant reorganisation of protein structure, affecting interaction with partner proteins, nucleic acids or other substrates. There is therefore potential for these mobility-associated phosphorylations to impact TTP function. Pin1 modulates the function of other RNA-binding proteins [107], but physical and functional interactions with TTP have not yet been described. Finally, it is tempting to speculate that the addition of multiple phosphates adjacent to or within the ZFD of TTP alters its affinity for RNA substrates.

How and where is the dephosphorylation of TTP performed?

Even if PP2A is correctly identified as the phosphatase solely responsible for dephosphorylation of Ser-52 and Ser-178, the matter does not rest there. PP2A functions as a heterotrimer between a structural subunit, a regulatory subunit and a catalytic subunit [108,109]. Catalytic and structural subunits can each be encoded by two different genes. Regulatory subunits are encoded by at least 24 different genes, many of which give rise to different proteins due to alternative splicing or translation initiation. It is the B subunit that dictates the specificity for RNA substrates.

Therapeutic implications of the regulation of TTP by PP2A and DUSP1

The MAPK p38 signalling pathway plays a fundamental role in the regulation of inflammatory responses and was long considered as a promising target for novel anti-inflammatory drugs [40]. In fact, the first MAPK p38 inhibitors were discovered in a screen for compounds that inhibited macrophage expression of TNF [110]. However, clinical trials of several different classes of MAPK p38 inhibitors yielded disappointing results, including anti-inflammatory effects that were not sustained [111]. The unanticipated negative results have almost put a halt to this line of translational enterprise, although the underlying reasons are not clear [112]. In macrophages, prolonged inhibition of MAPK p38 prevented the expression of TTP protein [26] and failed to destabilise TTP target mRNAs [46,113]. Therefore, the complex role of MAPK p38 in controlling both the expression and the activity of TTP may help to explain why chronic inhibition of this pathway does not exert the expected anti-inflammatory effects.

An intriguing question is whether PP2A might be therapeutically targeted to promote the dephosphorylation and activation of TTP. Several compounds have been shown to promote PP2A function, usually by disrupting interactions between the phosphatase and its inhibitory protein partners [108,109]. One such compound, a sphingolipid known as AAL(s), exerted TTP-dependent anti-inflammatory effects in an airway epithelial cell line [114,115]. AAL(s) and another PP2A agonist, the apolipoprotein E-derived peptide COG1410, exerted protective effects and prevented bone erosion in an experimental model of RA [9a]. In vitro, COG1410 decreased the expression of TNF in Zfp36+/+ macrophages, but not in Zfp36aa/aa macrophages. It also increased the
electrophoretic mobility and decreased the expression of wild-type TTP protein, but had neither of these effects on TTP-AA [9a]. These observations are consistent with COG1410 exerting anti-inflammatory effects by promoting the dephosphorylation and increasing the activity of TTP. They constitute no more than proof of principle for the concept of therapeutic targeting of PP2A in inflammation. Because of its multiple cellular functions, indiscriminate activation of PP2A is a risky strategy. For this reason, it is important to understand the physical and functional interaction between PP2A and TTP in greater detail. An added complication is that elevated PP2A activity and a consequent increase in TTP function has been implicated in age-related impairment of immunoglobulin class switching in B cells [116,117]. It is therefore possible that stimulation of PP2A could have undesired effects on adaptive immunity.

Reflecting its central role in the regulation of innate immune responses, DUSP1 is targeted by many endogenous immunoregulators as a means of promoting or suppressing inflammatory responses [42,118]. For example, glucocorticoids (GCs) exert anti-inflammatory effects in part by enhancing and prolonging the expression of DUSP1, and thereby curtailing MAPK p38 activity [119–124]. GCs [125] and a variety of other anti-inflammatory agonists have been reported to increase the expression of TTP. An emerging concept is that anti-inflammatory agonists may exert their effects not only by increasing TTP expression, but also by targeting DUSP1 to promote the activation of TTP [126]. Both Dusp1−/− and Zfp36aa/aa mouse strains will be useful for further exploration of this concept.

Lessons from relatives of TTP
Two broadly expressed TTP family members are ZFP36L1 (otherwise known as butyrate response factor 1 or BRF-1; TPA-inducible sequence 11B or TIS11B; B-cell early response gene of 36 kDa or Berg36) and ZFP36L2 (also known as BRF-2 or TIS11D). A fourth family member, ZFP36L3, appears to be expressed only in rodent placenta and is not discussed here [127]. Similarity between TTP, ZFP36L1 and ZFP36L2 is highest (>70%) within the zinc finger RNA-binding domains and drops to 10–24% outside of these domains (Figure 1B). Two additional short stretches of high similarity are discussed below. The members of the ZFP36 family have very similar RNA-binding specificities, and they recruit the same complexes of enzymes to regulate the degradation and/or translation of their targets [17,18,128]. However, disruption of the murine Zfp36, Zfp36l1 or Zfp36l2 genes has very different consequences. The pro-inflammatory effects of Zfp36 gene disruption have been discussed above. Zfp36l1 gene disruption is embryonic lethal due to defects in placental function [129,130]. Zfp36l2 gene disruption causes perinatal mortality associated with defective haematopoiesis [131]. Conditional knockouts have demonstrated critical roles of ZFP36L1 and ZFP36L2 in the development of both B- and T-cell lineages [132,133]. It is unclear how much overlap exists between functions and mRNA targets of ZFP36 family members. To some extent, differences in the phenotypes of knockouts may be explained by different tissue-specific and developmental patterns of expression or different kinetics of expression in response to cell stimulation [91]. However, there are circumstances in which two or more family members are present. For example, both TTP and ZFP36L1 are expressed and phosphorylated in LPS-treated macrophages [28]. The pro- and anti-inflammatory macrophage phenotypes arising from the absence of TTP or the expression of a constitutively active form are striking. In contrast, there is little evidence that ZFP36L1 plays an important role in restraining macrophage inflammatory responses [134]. The nature and extent of redundancy between these proteins is puzzling.

Another intriguing question is whether members of the ZFP36 family differ in their regulation by phosphorylation. Although there are no published phosphoproteomic studies focussing on ZFP36L1 or ZFP36L2, some information on their phosphorylation can be obtained from high-throughput studies. As a rule, phosphorylations of TTP seem not to be shared by its relatives. In some cases, the sites themselves are not conserved. For example, the extensively phosphorylated, proline-rich domain between residues 80 and 90 of TTP is absent from ZFP36L1 and ZFP36L2. In other cases, potential phosphorylation sites are conserved but adjacent residues may not favour their phosphorylation. An example is serine 220, which is followed by a proline residue in TTP. In ZFP36L1 or ZFP36L2, the equivalent serine residue is followed by alanine or serine, which will preclude phosphorylation by proline-directed kinases. The sequence surrounding Ser-52 is not well conserved in ZFP36L1 and ZFP36L2 (Figure 1B). On the other hand, certain prominent phosphorylations appear to be specific to ZFP36L1 and ZFP36L2. These are at Ser-54 and Ser-92 of ZFP36L1 and at Ser-57 and Ser-127 of ZFP36L2. The phospo-acceptor sites and/or surrounding residues are not conserved in TTP (Figure 1B). There are two protein regions in which both amino acid sequence and phosphorylation are conserved between TTP and its relatives. The first is centred around Ser-178 (Figure 1B). There is good evidence of phosphorylation of the corresponding residues Ser-203 in ZFP36L1 and Ser-263 in ZFP36L2. The second conserved
phosphorylation domain is at the C-terminus (Figure 1B). ZFP36L1 can be phosphorylated at Ser-334 and Ser-336, ZFP36L2 at Ser-480 and Ser-482, which correspond to Ser-316 and Ser-318 of TTP.

The phosphorylation of ZFP36L1 at Ser-92 and Ser-203 promotes recruitment of 14-3-3 proteins and regulates both ZFP36L1 protein stability and mRNA-destabilising activity [135–137]. This is remarkably similar to the regulation of TTP stability and function via the phosphorylation of Ser-52 and Ser-178. However, while Ser-203 of ZFP36L1 corresponds to Ser-178 of TTP, Ser-92 does not correspond to Ser-52 (Figure 1B). We speculate that evolutionary acquisition of novel phospho-acceptor sites has allowed the proteins to diverge in terms of the exact location of the sites used, while retaining the same basic mechanism of regulation by phosphorylation. While one study implicated MK2 in the phosphorylation of ZFP36L1 at Ser-92 and Ser-203 [136], others implicated the kinase Akt, downstream of phosphatidylinositol 3-kinase (PI3K) [135,137,138]. The fact that these two distinct kinases may phosphorylate the same residues in ZFP36L1 is unsurprising, given that their substrate specificities overlap. It is likely that MK2 and/or Akt regulate the function of ZFP36L2, although to our knowledge this has not been demonstrated. Another intriguing question is whether the expression and function of TTP may also be regulated by PI3K-Akt.

MK2-mediated phosphorylation of Ser-316 and Ser-318 in the C-terminus of TTP is thought to stabilise TTP targets by impairing the recruitment of CCR4–NOT [58]. The C-terminal domain has been strongly conserved throughout the duplication and evolution of the ZFP36 family, and is clearly recognisable even in oyster and lamprey orthologues [139]. Regulation of function via phosphorylation of the C-terminus may therefore have been a property of the ancestral ZFP36 protein. In the cases of ZFP36L1 and ZFP36L2, phosphorylation can be mediated by p90 ribosomal S6 kinase, which is downstream of ERK [140]. The emerging picture of the relationship between kinases and ZFP36 family members appears increasingly complex, as individual phospho-acceptor sites may be targeted by more than one kinase pathway, and more than one family member may be subjected to phosphorylation in a given cell type.

Conclusion

TTP plays a vital role in orchestrating the finely tuned, temporally precise responses of macrophages to pro-inflammatory stimuli. Work from several laboratories has contributed to a detailed understanding of certain elements of TTP function. It has been demonstrated, using cell transfections, in vitro assays and genetically modified mouse strains, that the MAPK p38 pathway mediates the phosphorylation of key residues of TTP to modulate both its expression and its function. This constitutes an elegant system for linking the initiation of an inflammatory response to its resolution. Stronger or more prolonged activation of MAPK p38 will generate a more robust induction of inflammatory mediators, but it will also generate a larger pool of dormant TTP, which is then ready to promote the destruction of inflammatory mRNAs as soon as MAPK p38 activity declines. While we may admire the elegance of the biology, we need to remember quite how much remains unknown about TTP. For example, most of the above discussion has centred on just two sites of phosphorylation of TTP. We do not yet understand the influences of other signalling pathways via the same sites or the importance of the phosphorylation of orthologous sites on other members of the TTP family. Most importantly, there are at least 28 additional sites of TTP phosphorylation about which we know next to nothing.

Abbreviations

3′-UTRs, 3′-untranslated regions; ARE, adenylate-/uridylate-rich element; CCR4-NOT, carbon catabolite repressor protein 4-negative on TATA-less; DUSP1, dual-specificity phosphatase 1; ERK, extracellular signal-regulated kinase; GCs, glucocorticoids; HuR, human antigen R; i-CLIP, individual nucleotide resolution cross-linking and immunoprecipitation; Le-Tx, lethal toxin; LPS, lipopolysaccharide; MAPK, mitogen-activated protein kinase; MK2, MAPK-activated protein kinase 2; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; P bodies, processing bodies; PAR-CLIP, photoactivatable ribonucleoside-enhanced cross-linking and immunoprecipitation; PI3K, phosphatidylinositol 3-kinase; Pin1, protein interacting with never in mitosis 1; PP2A, protein phosphatase 2A; RA, rheumatoid arthritis; Ser-52 and Ser-178, serines 52 and 178; SGs, stress granules; TNF, tumour necrosis factor; TTP, tristetraprolin; ZFD, zinc finger domain; ZFP36L2, ZFP36-like protein 2.

Funding

Andy Clark’s research on TTP was funded by Arthritis Research UK [Programme Grant 19614] and the Medical Research Council [Project Grant G0800207].
Competing Interests
The authors declare that there are no competing interests associated with the manuscript.

References

1 Brooks, S.A. and Blackshear, P.J. (2013) Tristetraprolin (TTP): interactions with mRNA and proteins, and current thoughts on mechanisms of action. Biochim. Biophys. Acta, Gene Regul. Mech. 1829, 666–679 doi:10.1016/j.bbagen.2013.02.003

2 Taylor, G.A., Carballo, E., Lee, D.M., Lai, W.S., Thompson, M.J., Patel, D.D. et al. (1996) A pathogenic role for TNF in the syndrome of cachexia, arthritis, and autoimmunity resulting from tristetraprolin (TTP) deficiency. Immunology 4, 445–454 doi:10.1016/S0174-7613(96)00841-2

3 Carballo, E., Giksen, G.S. and Blackshear, P.J. (1997) Bone marrow transplantation reproduces the tristetraprolin-deficiency syndrome in recombination activating gene-2 (−/−) mice. Evidence that monocyte/macrophage progenitors may be responsible for TNFalpha overproduction. J. Clin. Invest. 100, 986–995 doi:10.1172/JCI119649

4 Kratcchvil, F., Machacke, C., Vogl, C., Ebner, F., Sediyarv, V., Gruber, A.R. et al. (2011) Tristetraprolin-driven regulatory circuit controls quality and timing of mRNA decay in inflammation. Mol. Syst. Biol. 7, 560 doi:10.1038/msb.2011.93

5 Qiu, L.-Q., Stumpo, D.J. and Blackshear, P.J. (2012) Myeloid-specific tristetraprolin deficiency in mice results in extreme lipopolysaccharide sensitivity in an otherwise minimal phenotype. J. Immunol. 188, 5150–5159 doi:10.4049/jimmunol.1103700

6 Lai, W.S., Parker, J.S., Grissom, S.F., Stumpo, D.J. and Blackshear, P.J. (2006) Novel mRNA targets for tristetraprolin (TTP) identified by global analysis of stabilized transcripts in TTP-deficient fibroblasts. Mol. Cell. Biol. 26, 9196–9208 doi:10.1128/MCB.00945-06

7 Chen, X., Wei, Z., Wang, W., Yan, R., Xu, X. and Cai, Q. (2012) Role of RNA-binding protein tristetraprolin in tumor necrosis factor-α mediated gene expression. Biochem. Biophys. Res. Commun. 428, 327–332 doi:10.1016/j.bbrc.2012.09.033

8 Tiedje, C., Lai, W.S., Bradbury, A., Zeldin, D.C. and Blackshear, P.J. (2015) Cellular tristetraprolin (TTP) (A) binding protein.

9 Li, E., Blackshear, P.J. and Lai, W.S. (2014) Recruitment and activation of mRNA decay enzymes by two ARE-mediated decay activation domains in the RNA-binding protein ZFP36. Mol. Syst. Biol. 10, R2288 doi:10.6010/mst.2014.15-112

10 Tiedje, C., Diaz-Muñoz, M.D., Trullen, P., Ahlfors, H., Laali, K., Blackshear, P.J. et al. (2016) The RNA-binding protein TTP is a global post-transcriptional regulator of feedback control in influenza. Nucleic Acids Res. doi:10.1093/nar/gkw474

11 Rabani, M., Raychowdhury, R., Jovanovic, M., Rooney, M., Stumpo, D.J., Pauli, A. et al. (2014) High-resolution sequencing and modeling identifies distinct dynamic RNA regulatory strategies. Cell 159, 1698–1710 doi:10.1016/j.cell.2014.11.015

12 Tiedje, C., Kotlyarov, A. and Gaestel, M. (2010) Molecular mechanisms of phosphorylation-regulated TTP (tristetraprolin) action and screening for further TTP-interacting proteins. Biochim. Biophys. Acta, Gene Regul. Mech. 1802, 1632–1637 doi:10.1016/j.bbagen.2010.03.006

13 Rowlett, R.M., Chrestensen, C.A., Schroeder, M.J., Harp, M.G., Pelo, J.W., Shabanowitz, J. et al. (2008) Inhibition of tristetraprolin deadenylation by poly(A) binding protein. J. Am. Physiol. Gastrointest. Liver Physiol. 295, 5421–5430 doi:10.1152/jag.00508.2007

14 Lykke-Andersen, J. and Wagner, E. (2005) Recruitment and activation of mRNA decay enzymes by two ARE-mediated decay activation domains in the proteins TTP and BRF-1. J. Exp. Med. 205, 331–337 doi:10.1084/jem.20070906

15 Ross, E.A., Naylor, A.J., O’Neil, J.D., Crowley, T., Ridley, M.L., Crowe, J. et al. (2016) Treatment of inflammatory arthritis via targeting of Tristetraprolin, a master regulator of pro-inflammatory gene expression. Ann. Rheum. Dis. in press.

16 Garneau, N.L., Wilusz, J., Wilusz, C.J. (2007) The highways and byways of mRNA decay. Mol. Cell. Biol. 27, 3766–3777 doi:10.1128/MCB.26.6.2399-2407.2006

17 Fredriksson, A., Nyström, E., Thulin, E., Engfeldt, S., Zurcher, E., Shirakawa, M. et al. (2016) A global protein–protein interactome survey of human T defends the relationship between the protein–protein interaction network and the human disease. Bioinformatics 32, 295–304 doi:10.1093/bioinformatics/btv418

18 Sun, L., Stoecklin, G., Van Way, S., Hinkovska-Galcheva, V., Guo, R.-F., Anderson, P. et al. (2007) Tristetraprolin (TTP)-14-3-3 complex formation protects TTP from dephosphorylation by protein phosphatase 2a and stabilizes tumor necrosis factor-α mRNA. J. Biol. Chem. 282, 3766–3777 doi:10.1074/jbc.M607347200
31 Clement, S.L., Scheckel, C., Stoecklin, G. and Lykke-Andersen, J. (2011) Phosphorylation of tristetraprolin by MK2 impairs AU-rich element mRNA localization and protein stability by p38 mitogen-activated protein kinase and extracellular signal-regulated kinase pathways. Mol. Cell. Biol. 26, 2408–2418 doi:10.1128/MCB.26.2408-2418.2006

32 Cao, H. (2004) Expression, purification, and biochemical characterization of the antiinflammatory tristetraprolin: a zinc-dependent mRNA binding protein affected by posttranslational modifications. Biochemistry 43, 13724–13738 doi:10.1021/bi049014y

33 Brook, M., Tchen, C.R., Santalucia, T., Mcifiable, J., Arthur, J.S.C., Sakathvala, J. et al. (2006) Posttranslational regulation of tristetraprolin subcellular localization and protein stability by p38 mitogen-activated protein kinase and extracellular signal-regulated kinase pathways. Mol. Cell. Biol. 26, 2408–2418 doi:10.1128/MCB.26.2408-2418.2006

34 Cao, H., Deterding, L.J., Venable, J.D., Kennington, E.A., Yates, III, J.R., Tomer, K.B. et al. (2006) Identification of a major phosphopeptide in human tristetraprolin by phosphopeptide mapping and mass spectrometry. PLoS ONE 9, e1000977 doi:10.1371/journal.pone.0100097

35 Cao, H., Deterding, L.J., Venable, J.D., Kennington, E.A., Yates, III, J.R., Tomer, K.B. et al. (2006) Identification of the anti-inflammatory protein tristetraprolin as a hyperphosphorylated protein by mass spectrometry and site-directed mutagenesis. Biochem. J. 394, 285–297 doi:10.1042/BJ20051316

36 Christensen, C.A., Schroeder, M.J., Shabanowitz, J., Hunt, D.F., Pelo, J.W., Worthington, M.T. et al. (2004) MAPKAP kinase 2 phosphorylates tristetraprolin on in vivo sites including Ser178, a site required for 14-3-3 binding. J. Biol. Chem. 279, 10176–10184 doi:10.1074/jbc.M310486200

37 Hornbeck, P.Y., Zhang, B., Murray, B., Bomhard, J.M., Latham, V. and Skrypek, E. (2015) PhosphoPepList, 2014: mutations, PTMs and recalibrations. Nucleic Acids Res. 43, D512–D520 doi:10.1093/nar/gku1267

38 Arthur, J.S. and Loy, S.C. (2013) Mitogen-activated protein kinases in innate immunity. Nat. Rev. Immunol. 13, 679–682 doi:10.1038/nri3495

39 Gaestel, M. (2013) What goes up must come down: molecular basis of MAPKAP kinase 2/3-dependent regulation of the inflammatory response and its inhibition. Biol. Chem. 394, 1301–1315 doi:10.1515/hbz-2013-0197

40 Abraham, S.M. and Clark, A.R. (2006) Dual-specificity phosphatase 1: a regulator of innate immune responses. Biochem. Soc. Trans. 34, 1018–1023 doi:10.1042/BST0341018

41 Lang, R., Hammer, M. and Maggs, J. (2006) DUSP meet immunology: dual specificity MAPK phosphatases in control of the inflammatory response. J. Immunol. 177, 7497–7504 doi:10.4049/jimmunol.177.11.7497

42 Bode, J.G., Ehring, C. and Haussinger, D. (2012) The macrophage response towards LPS and its control through the p38MAPK–STAT3 axis. Cell. Signal. 24, 1185–1194 doi:10.1016/j.cellsignal.2012.01.018

43 Carballa, E., Cao, H., Lai, W.S., Kennington, E.A., Campbell, D. and Blackshear, P.J. (2001) Decreased sensitivity of tristetraprolin-deficient cells to p38 inhibitors suggests the involvement of tristetraprolin in the p38 signaling pathway. J. Biol. Chem. 276, 42580–42587 doi:10.1074/jbc.M104953200

44 Tudor, C., Marchese, F.P., Hitti, E., Aubareda, A., Rawlinson, L., Gaestel, M. et al. (2009) The p38 MAPK pathway inhibits tristetraprolin-directed decay of interleukin-10 and pro-inflammatory mediator mRNAs in murine macrophages. FEBS Lett. 583, 1933–1938 doi:10.1016/j.febslet.2009.04.039

45 Stockelin, G., Stubbs, T., Kedersha, N., Xiao, S., Rigby, W.F.C., Blackwell, T.K. et al. (2004) MK2-induced tristetraprolin:14-3-3 complexes prevent stress granule association and ARE-mRNA decay. EMBO J. 23, 1313–1324 doi:10.1093/emboj/cdh108

46 Yaffe, M.B. (2002) How do 14-3-3 proteins work? – Gatekeeper phosphorylation and the molecular anvil hypothesis. FEBS Lett. 513, 53–57 doi:10.1016/S0014-5793(01)02384-4

47 Bustos, D.M. (2012) The role of protein disorder in the 14-3-3 interaction network. Mol. Biosyst. 8, 178–184 doi:10.1039/C1MB05216K

48 Al-Souhibani, N., Al-Ghamdi, M., Al-Ahmadi, W. and Khabar, K.S.A. (2014) Posttranscriptional control of the chemokine receptor CXCR4 expression in cancer cells. Carcinogenesis 35, 1983–1993 doi:10.1093/carcin/bgu080

49 Grisi, P. and Pagès, G. (2014) Control of pro-inflammatory cytokine mRNA half-life in cancer: the role of AU-rich elements and associated proteins. J. Interferon Cytokine Res. 34, 242–254 doi:10.1089/jir.2014.0070

50 Soler, D.M., Ghosh, A., Chen, F. and Shneider, B.L. (2014) A single element in the 3′-UTR of the apical sodium-dependent bile acid transporter controls both stabilization and destabilization of mRNA. Biochem. J. 462, 547–553 doi:10.1042/BJ20140070

51 Young, L.E., Sanduja, S., Bernis-standoll, K., Pena, E.A., Price, R.L. and Dixon, D.A. (2009) The mRNA binding proteins HuR and tristetraprolin regulate cyclooxygenase 2 expression during colon carcinogenesis. Gastroenterology 136, 1669–1679 doi:10.1053/j.gastro.2009.01.010

52 Brennan, C.M. and Stolz, J.A. (2001) HuR and mRNA stability. Cell. Mol. Life Sci. 58, 266–277 doi:10.1007/PL000000854

53 Zhao, W., Liu, M., D'Silva, N.J. and Kirkwood, K.L. (2011) Tristetraprolin regulates interleukin-6 expression through p38-dependent affinity changes with mRNA 3′ untranslated region. J. Interferon Cytokine Res. 31, 629–637 doi:10.1089/jir.2010.0154

54 Marchese, F.P., Aubareda, A., Tudor, C., Sakathvala, J., Clark, A.R. and Dean, J.L. (2010) MAPKAP kinase 2 blocks tristetraprolin-directed mRNA decay by inhibiting CAF1 deadenylation recruitment. J. Biol. Chem. 285, 27590–27600 doi:10.1074/jbc.M110.136473

55 Sandier, H., Keeth, J., Timmers, H.T.M. and Stockelin, G. (2011) Nol1 mediates recruitment of the deadenylase Caf1 to mRNAs targeted for degradation by tristetraprolin. Nucleic Acids Res. 39, 4373–4386 doi:10.1093/nar/gkr501

56 Fabian, M.R., Frank, F., Rouya, C., Siddiqui, N., Lai, W.S., Karthikov, A. et al. (2013) Structural basis for the recruitment of the human CC4-NOT deadenylase complex by tristetraprolin. Nat. Struct. Mol. Biol. 20, 735–739 doi:10.1038/nsmb.2572

57 Shi, J.-X., Li, J.-S., Hu, R., Shi, Y., Su, X., Li, G. et al. (2014) CNOT7/CAF1 is involved in ICAM-1 and IL-8 regulation by tristetraprolin. Cell. Signal. 26, 2390–2396 doi:10.1016/j.cellsig.2014.07.020
120 Bhattacharyya, S., Brown, D.E., Brewer, J.A., Vogt, S.K. and Muglia, L.J. (2007) Macrophage glucocorticoid receptors regulate Toll-like receptor 4-mediated inflammatory responses by selective inhibition of p38 MAP kinase. *Blood* **109**, 4313–4319 doi:10.1182/blood-2006-10-048215

121 Furst, R., Schroeder, T., Elken, H.M., Bulak, M.F., Kiemer, A.K., Zahler, S. et al. (2007) MAP kinase phosphatase-1 represents a novel anti-inflammatory target of glucocorticoids in the human endothelium. *FASEB J.* **21**, 74–80 doi:10.1096/fj.06-6752com

122 Joanny, E., Ding, Q., Gong, L., Kong, P., Saklatvala, J. and Clark, A.R. (2012) Anti-inflammatory effects of selective glucocorticoid receptor modulators (SGRMs) are partially dependent on upregulation of dual specificity phosphatase 1 (DUSP1). *Br. J. Pharmacol.* **165**, 1124–1136 doi:10.1111/j.1476-5381.2011.01574.x

123 Quante, T., Ng, Y.C., Ramsay, E.E., Hennessy, S., Allen, J.C., Parmentier, J. et al. (2008) Corticosteroids reduce IL-6 in ASM cells via up-regulation of MKP-1. *Am. J. Respir. Cell Mol. Biol.* **39**, 208–217 doi:10.1165/rcmb.2007-00140C

124 Wang, X., Nelin, L.D., Kuhlman, J.R., Meng, X., Welty, S.E. and Liu, Y. (2008) The role of MAP kinase phosphatase-1 in the protective mechanism of MKP-1. *FASEB J.* **21**, 4196–5381.2011.01574.x

125 Smoak, K. and Cidlowski, J.A. (2006) Glucocorticoids regulate tristetraprolin synthesis and posttranscriptionally regulate tumor necrosis factor alpha inflammatory signaling. *Mol. Cell. Biol.* **26**, 9126–9135 doi:10.1128/MCB.00679-06

126 Prabha, P., Bunge, K., Ge, Q. and Ammit, A.J. (2016) Corticosteroid-induced MKP-1 represses pro-inflammatory cytokine secretion by enhancing activity of tristetraprolin (TIP) in ASM cells. *J. Cell. Physiol.* **231**, 2153–2158 doi:10.1002/jcp.25327

127 Blackshear, P.J., Phillips, R.S., Ghosh, S., Ramos, S.V., Richtfeld, E.K. and Lai, W.S. (2005) Zfp36l3, a rodent X chromosome gene encoding a placentaspecific member of the tristetraprolin family of tandem CCCH zinc finger proteins. *Biol. Reprod.* **73**, 297–307 doi:10.1095/biolreprod.105.040527

128 Clement, S.L. and Lykke-Andersen, J. (2008) A tethering approach to study proteins that activate mRNA turnover in human cells. *Methods Mol. Biol.* **419**, 121–133 doi:10.1007/978-1-59745-033-1_8

129 Bell, S.E., Sanchez, M.J., Spasic-Boskovic, O., Santalucia, T., Gambardella, L., Burton, G.J. et al. (2006) The RNA binding protein ZFP36L1 regulates by phosphatidylinositol 3-kinase signaling. *Mol. Cell. Biol.* **26**, 10037–10049 doi:10.1128/MCB.01099-06

130 Stumpo, D.J., Byrd, N.A., Phillips, R.S., Ghosh, S., Maronpot, R.R., Castranio, T. et al. (2004) Chorioallantoic fusion defects and embryonic lethality for normal vascularisation and post-transcriptionally regulates VEGF expression. *FASEB J.* **18**, 2549–2554 doi:10.1096/fj.04-2283fie

131 Stumpo, D.J., Broxmeyer, H.E., Ward, T., Cooper, S., Hangoc, G., Chung, Y.J. et al. (2009) Targeted disruption of Zfp36l2, encoding a CCCH tandem zinc finger RNA-binding protein, results in defective hematopoiesis. *Blood* **114**, 2401–2410 doi:10.1182/blood-2009-04-214619

132 Galloway, A., Saveliev, A., Lukasiak, S., Hodson, D.J., Bolland, D., Balmanno, K. et al. (2016) RNA-binding proteins ZFP36L1 and ZFP36L2 promote cell quiescence. *Science* **352**, 453–459 doi:10.1126/science.aad5978

133 Hodson, D.J., Janas, M.L., Galloway, A., Bell, S.E., Andrews, S., Li, C.M. et al. (2010) Deletion of the RNA-binding proteins ZFP36L1 and ZFP36L2 leads to perturbed thymic development and T lymphoblastic leukemia. *Nat. Immunol.* **11**, 717–724 doi:10.1038/nmol.1901

134 Hyatt, L.D., Wasserman, G.A., Rah, Y.J., Matsuura, K.Y., Coleman, F.T., Hilliard, K.L. et al. (2014) Myeloid ZFP36L1 does not regulate inflammation or host defense in mouse models of acute bacterial infection. *PLoS ONE* **9**, e109072 doi:10.1371/journal.pone.0109072

135 Benjamin, D., Schmidlin, M., Min, L., Gross, B. and Moroni, C. (2006) BRF1 protein turnover and mRNA decay activity are regulated by protein kinase B at the same phosphorylation sites. *Mol. Cell. Biol.* **26**, 9497–9507 doi:10.1128/MCB.01199-06

136 Maitra, S., Chou, C.-F., Luber, C.A., Lee, K.-Y., Mann, M. and Chen, C.-Y. (2008) The AU-rich element mRNA decay-promoting activity of BRF1 is regulated by mitogen-activated protein kinase-activated protein kinase 2. *RNA* **14**, 950–959 doi:10.1261/rna.983708

137 Schmidlin, M., Lu, M., Leuenberger, S.A., Stoecklin, G., Mallaun, M., Gross, B. et al. (2004) The ARE-dependent mRNA destabilizing activity of BRF1 is regulated by protein kinase B. *EMBO J.* **23**, 4760–4769 doi:10.1038/sj.emboj.7600477

138 Graham, J.R., Hendershot, M.C., Terragni, J. and Cooper, G.M. (2010) mRNA degradation plays a significant role in the program of gene expression regulated by phosphatidylinositol 3-kinase signaling. *Mol. Cell. Biol.* **30**, 5295–5305 doi:10.1128/MCB.00303-10

139 Blackshear, P.J., Phillips, R.S., Ghosh, S., Ramos, S.V., Richtfeld, E.K. and Lai, W.S. (2005) Zfp36l3, a rodent X chromosome gene encoding a placentaspecific member of the tristetraprolin family of tandem CCCH zinc finger proteins. *J. Interferon Cytokine Res.* **24**, 6445–6455 doi:10.1089/jir.2013.0150

140 Adachi, S., Homoto, M., Tanaka, R., Hoki, Y., Murakami, H., Suga, H. et al. (2014) ZFP36L1 and ZFP36L2 control LDLR mRNA stability via the ERK-RSK pathway. *Nucleic Acids Res.* **42**, 10037–10049 doi:10.1093/nar/gku652