Minireview

# Post-transcriptional regulation of gene expression by mitogen-activated protein kinase p38

Andrew R. Clark*, Jonathan L.E. Dean, Jeremy Saklatvala

*Kennedy Institute of Rheumatology Division, Faculty of Medicine, Imperial College London, 1 Aspenlea Road, Hammersmith, London W6 8LH, UK*

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**Abstract The mitogen-activated protein kinase p38 pathway was originally identified as a signalling cascade activated by pro-inflammatory stimuli and cellular stresses, and playing a critical role in the translational regulation of pro-inflammatory cytokine synthesis. In almost a decade since this discovery, a great deal has been learned about the role of the p38 pathway in the post-transcriptional regulation of pro-inflammatory gene expression. However, important questions remain to be answered concerning the specificity and mechanism or mechanisms of action of p38. This review describes recent progress and remaining puzzles in the field of post-transcriptional regulation by p38.**


*Key words:* Adenosine/uridine rich element; MAPK p38; MAPKAPK-2; mRNA stability; Translation; Inflammation

## 1. MAPK p38

The three principal mitogen-activated protein kinase (MAPK) pathways of mammalian cells mediate changes in cellular gene expression in response to extracellular stimuli [1]. In many cases the extracellular signal-regulated kinase (ERK) pathway is activated by mitogenic stimuli, whereas the cJun N-terminal kinase (JNK) and p38 pathways are activated by pro-inflammatory or stressful stimuli (although there are exceptions to this generalisation). The pathways are organised as discrete, parallel signalling cascades in which a MAPK kinase kinase (MKKK) phosphorylates and activates a dual-specificity MAPK kinase (MKK), which then activates a MAPK by phosphorylating both threonine and tyrosine residues in a Thr-Xxx-Tyr motif. The central amino acid (Xxx) is a defining characteristic of the particular MAPK family, and is glutamic acid in the case of the ERKs, proline in the case of the JNK family and glycine in the case of the p38 family. Phosphorylation of both threonine and tyrosine residues is essential for full MAPK activation, hence the activity of MAPKs may be negatively regulated by members of three phosphatase families, serine/threonine-specific, tyrosine-specific or dual specificity. The latter family contains about 12 phosphatases, which efficiently dephosphorylate both threonine and tyrosine residues, and display differing patterns of expression and specificities for MAPK substrates. Strength and duration of MAPK activation determine cellular responses, and are critically regulated by the action of these phosphatases [2]. Activated MAPKs exert their effects directly by phosphorylating substrates such as transcription factors, or indirectly by activating downstream kinases, which in turn phosphorylate their own substrates. At the post-transcriptional level many of the effects of MAPK p38 appear to be mediated by its substrate, MAPK-activated protein kinase 2 (MAPKAPK-2; see Fig. 1).

The MAPK p38 pathway was independently discovered by several laboratories [3]. In one study it was identified as the target of a novel class of anti-inflammatory drugs, which blocked the expression of interleukin (IL-) 1 and tumour necrosis factor α (TNFα) in myeloid cells stimulated with bacterial lipopolysaccharide (LPS) [4,5]. These novel pyridinyl imidazole drugs inhibit p38 with sub-micromolar $IC_{50}$ values

*Corresponding author. Fax: (44)-20-8383 4499. *E-mail address:* andy.clark@imperial.ac.uk (A.R. Clark).

*Abbreviations:* ARE, adenosine/uridine-rich element; AREBP, ARE binding protein; ASK1, apoptosis signal-regulating kinase 1; AUF1, ARE/polu-(U)-binding/degradation factor 1; BCL2, B-cell CLL/lymphoma factor 2; BRF1, butyrate response factor 1; CCL, chemokine (C-C motif) ligand; COX-2, cyclooxygenase 2; CPSF6, cleavage and polyadenylation specific factor 6; CXCL, chemokine (C-X-C motif) ligand; DAF, decay accelerating factor for complement; DTR, diphtheria toxin receptor; eIF, eukaryotic initiation factor; ERK, extracellular signal-regulated kinase; FGF9, fibroblast growth factor 9; GAD1, glutamate decarboxylase 1; GC, glucocorticoid; GCH1, GTP cyclohydrolase 1; GM-CSF, granulocyte/macrophage colony stimulating factor; GRO, growth related oncogene; $hnRNPA_0$, heterogeneous nuclear ribonucleoprotein $A_0$; hsp27, 27 kDa heat shock protein; IL, interleukin; IRF1, interferon regulatory factor 1; JNK, cJun N-terminal kinase; LPS, lipopolysaccharide; MAPK, mitogen-activated protein kinase; MAPKAPK-2, MAPK-activated protein kinase 2; MIP, macrophage inflammatory protein; MCP, monocyte chemotactic protein; MEKK4, MAPK or ERK kinase kinase 4; MKK, MAPK kinase; MKKK, MAPK kinase kinase; MKP-1, MAPK phosphatase 1; MLK2, mixed lineage kinase 2; MMP, matrix metalloproteinase; MNK1, MAPK-interacting kinase 1; MSK1, mitogen- and stress-activated kinase; PABPc1, cytoplasmic poly-(A)-binding protein 1; PMAIP1, phorbol-12-myristate-13-acetate-induced protein 1; RBM7, RNA-binding motif 7 protein; SOX9, sex determining region Y box 9 transcription factor; TAK1, TGFβ-activated kinase; TGFβ, transforming growth factor β; TIA-1, T-cell-restricted intracellular antigen 1; TIAR, T-cell-restricted intracellular antigen related protein; TNFα, tumour necrosis factor α; TNFAIP3, TNFα induced protein 3; TTP, tristetraprolin; UNG2, uracil-DNA glycosylase 2; UPA, urokinase plasminogen activator; UTR, untranslated region; VCAM-1, vascular cell adhesion molecule 1; VEGF, vascular endothelial growth factor

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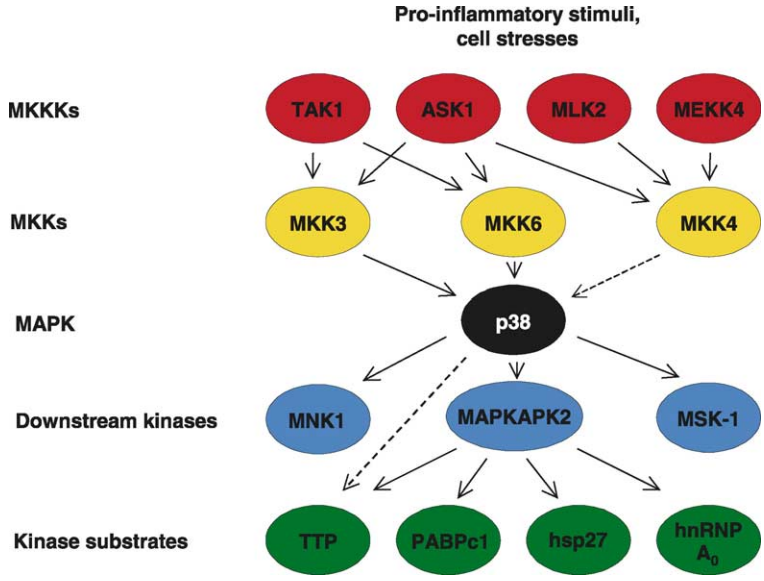


Fig. 1. The MAPK p38 pathway. Some aspects of the MAPK p38 signal transduction pathway are illustrated schematically. Upstream (membrane proximal) signalling events, several kinases and substrates including transcription factors are omitted for the sake of clarity. Adapted from [1].

by blocking the access of ATP to the catalytic site of the kinase. Carefully used, they are invaluable tools for the investigation of p38 function, although at higher concentrations the inhibition of other kinase pathways may become significant. To date four members of the p38 MAPK family have been cloned: p38-α, -β, -γ and -δ. The γ and δ isoforms are insensitive to the pyridinyl imidazole inhibitors, for which reason their function has been difficult to explore. This review concentrates on MAPKs p38-α and -β, which appear similar in function, and are intimately linked to both inflammation and post-transcriptional regulation.

## 2. Post-transcriptional regulation of gene expression

Mechanisms of post-transcriptional regulation of gene expression are very well reviewed elsewhere [6–8] and will be described here only in outline. Eukaryotic mRNA is modified at the 5′ end by addition of a methyl guanosine cap, and at the 3′ end by addition of a poly-(A) tail. The poly-(A) tail is recognised by the highly abundant cytoplasmic poly-(A)-binding protein 1 (PABPc1), and the 5′ cap is bound by eukaryotic initiation factor 4F (eIF4F), a high molecular-weight complex consisting of eIF4E (the direct cap-binding component), eIF4G and eIF4A. Direct or indirect interactions between cap-binding and poly-(A)-binding factors effectively circularise the mRNA, increasing its stability and efficiency of translation [9]. Degradation of mRNA is influenced by both 5′ cap and 3′ poly-(A) tail, and by sequences within the 5′ untranslated region (UTR), 3′ UTR or coding sequence. Recently, most attention has been given to the regulation of mRNA decay by adenosine- and uridine-rich elements (AREs), located within the 3′ UTRs of unstable mRNAs and generally containing a number of copies of the pentamer sequence AUUUA [10,11]. According to the classification proposed by Shyu and his colleagues, class I AREs contain dispersed pentamers within a uridine-rich context, whereas class II AREs contain clustered, often overlapping copies of the pentamer, and class III AREs do not possess canonical AUUUA motifs [12]. AREs are thought to control mRNA degradation via interactions with specific binding proteins (AREBPs). Several such proteins have been identified, although only a few have been clearly implicated in the regulation of mRNA stability. HuR is believed to stabilise transcripts to which it binds, whereas mRNA destabilisation is a property of the zinc finger RNA binding proteins tristetraprolin (TTP) and butyrate response factor 1 (BRF-1), as well as the ARE/poly-(U)-binding/degradation factor (AUF1) [6–8,13].

Degradation of mRNA may be initiated by endonucleolytic cleavage within AREs or by removal of the 5′ cap and exonucleolytic digestion in the 5′ to 3′ direction [14,15]. However, 3′ to 5′ exonucleolytic digestion is believed to be the major pathway for degradation of mammalian mRNAs. The poly-(A) tail protects against 3′ to 5′ degradation, therefore poly-(A) removal is thought to be an obligate first step in mRNA decay. Both deadenylation and 3′ to 5′ decay of the mRNA body are stimulated by AREs in vitro or in vivo [12,16–21]. The control of 3′ to 5′ degradation involves the recruitment by AREBPs of a multi-component exonucleolytic complex, the exosome [17,21]. The regulation of deadenylation is less well understood; however, some AREBPs have been shown to interact with PABPc1, or directly with the poly-(A) tail itself, which may mediate crosstalk between the ARE and the regulation of poly-(A) tail length [22–24]. The processes of translation and mRNA degradation are closely coupled, and may be regulated by common ARE-dependent mechanisms, as discussed below.

## 3. MAPK p38 and the post-transcriptional regulation of pro-inflammatory gene expression

In early experiments the blockade of TNFα and IL-1β protein synthesis by p38 inhibitors was accompanied by little or no change in the corresponding steady-state mRNA levels, suggesting that p38 activity is essential for translation of these transcripts [25,26]. Consistent with this hypothesis, ribosomal density-gradient profiling showed that TNFα mRNA was

mainly found in monosomal rather than actively translated polysomal fractions in the presence of a p38 inhibitor [26,27]. Furthermore, a mouse knockout of MAPKAPK-2 resulted in impairment of TNFα protein synthesis, with no discernible change in TNFα mRNA steady-state levels or stability [28,29]. Another informative mouse strain carries a targeted genomic deletion of a conserved ARE in the TNFα 3′ UTR [30]. This deletion caused overproduction of TNFα and loss of sensitivity to the p38 inhibitors. Hence p38 is thought to act through its substrate MAPKAPK-2 to release TNFα mRNA from a state of translational arrest imposed by the ARE. ARE-mediated translational arrest of TNFα biosynthesis is also a feature of LPS tolerance, a state in which myeloid cells pretreated with low doses of LPS are refractory to further stimulation with this agonist [31,32]. Glucocorticoids (GCs) may similarly inhibit the expression of TNFα protein but not mRNA [33,34]. Both of these phenomena are likely to involve suppression of p38 activity. The basis of the inhibition of translation and the mechanism of derepression are not known. T-cell-restricted intracellular antigen 1 (TIA-1) and T-cell-restricted intracellular antigen-related protein (TIAR) have been identified as TNFα 3′ UTR-binding translational silencers, but do not appear to be targets of the p38 pathway [27].

A role for MAPK p38 in the control of pro-inflammatory mRNA translation initially seemed clear, and was borne out by other studies [35]. However, p38 inhibitors blocked the expression of another pro-inflammatory mediator, cyclooxygenase 2 (COX-2), at both protein and steady-state mRNA levels, suggesting a function other than translational regulation [36]. In IL-1-stimulated HeLa cells [37] or LPS-stimulated primary human monocytes [38] a p38 inhibitor profoundly destabilised COX-2 mRNA. Regulation of COX-2 mRNA stability by the p38 pathway has also been demonstrated in several other cell systems (see Fig. 2). Similarly, in primary human monocytes the expression of TNFα was blocked at both protein and mRNA levels by a p38 inhibitor [38]. The blockade of TNFα expression by p38 inhibitors was shown to involve mRNA destabilisation in primary human monocytes [39], a human monocytic cell line [40] and a mouse macrophage-like cell line [41]. Many other pro-inflammatory mediators are regulated by p38 at the level of mRNA stability (references in Fig. 2). One target of the MAPK p38 pathway was identified as IL-6 mRNA, which showed markedly decreased stability in macrophages from the MAPKAPK-2 knockout mouse [42], and was destabilised by a p38 inhibitor in human synoviocytes [43]. Reporter assays and other experiments confirmed that stabilisation of COX-2, IL-6, IL-8 and TNFα mRNAs was mediated by MAPKAPK-2, and was dependent upon ARE sequences [41,42,44–46]. Hence the p38 pathway may regulate either translation or mRNA stability in a single cell type, and a particular mRNA may be regulated at either of these two post-transcriptional levels, depending upon cell type or species. Furthermore, both phenomena are MAPKAPK-2-mediated and dependent upon AREs. The close coupling of translation and mRNA stability [47] suggests that

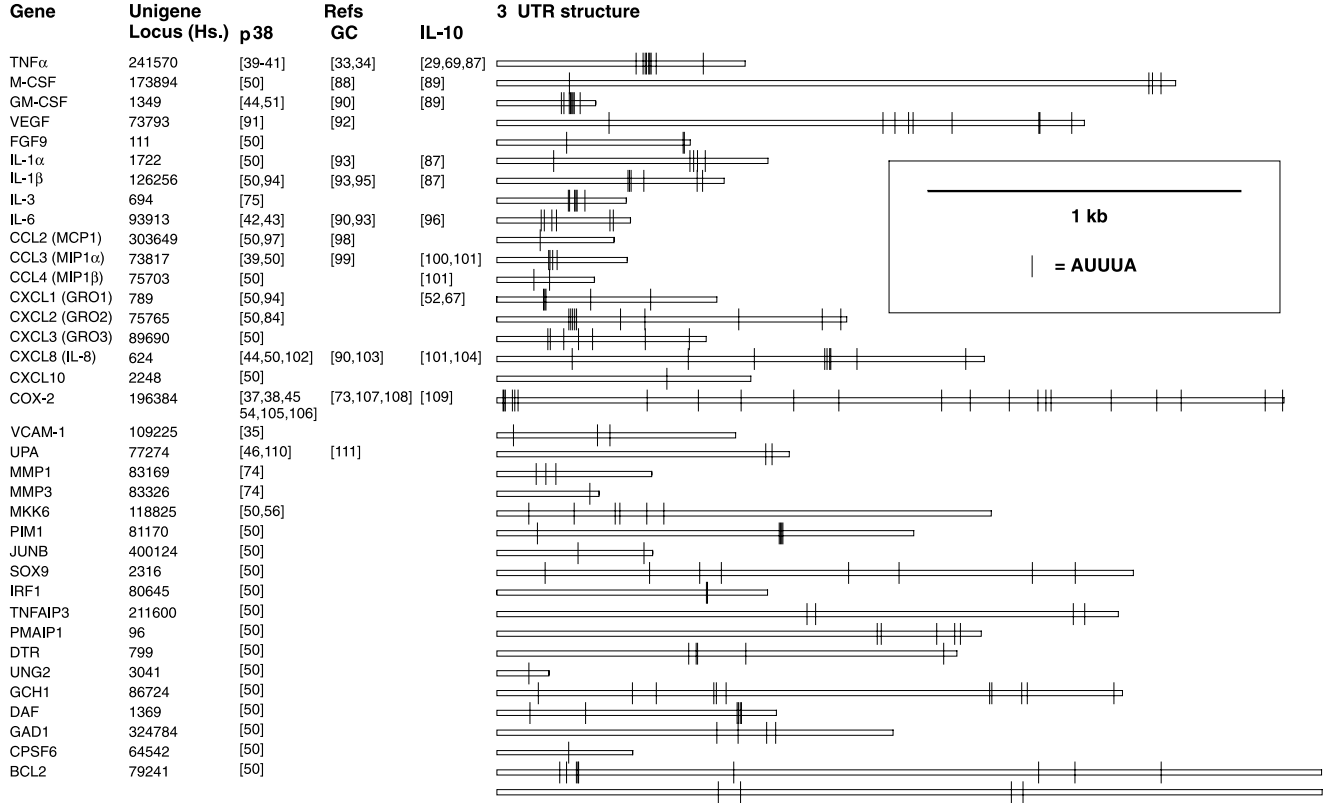


Fig. 2. Post-transcriptional targets of MAPK p38, GCs and IL-10 [29,33-35,37-46,52,54,56,67,69,73-75,84,87-111]. The 3′ UTRs of several p38-sensitive, GC-sensitive and/or IL-10-sensitive mRNAs are illustrated to scale, and the positions of AUUUA motifs indicated. Selected mRNAs from [50] are included, namely those which were both induced by LPS treatment of THP-1 cells and destabilised by a p38 inhibitor. Several of the observations from that study have not yet been confirmed by Northern blotting or reverse-transcription polymerase chain reaction.

these two facets of post-transcriptional regulation by p38 share a common mechanistic core (see below and [48]).

In vitro differentiation of primary human monocytes has been shown to alter the stability of TNFα mRNA and the kinetics of its accumulation [49]. This differentiation is mimicked by treatment of the human monocytic cell line THP-1 with PMA (phorbol-12-myristate-13-acetate). In differentiated THP-1 cells TNFα mRNA stability was sensitive to p38 inhibition [40] whereas in undifferentiated cells it was not [50], hence p38 sensitivity may be a developmentally acquired state. Other inconsistencies in the control of mRNA stability by p38 have been noted. For example, the stability of the chemokine CXCL3 mRNA was sensitive to p38 inhibitors in THP-1 cells [50] but not in the myeloid T98G glioblastoma cell line [51], whereas the reverse was true of granulocyte/macrophage colony stimulating factor (GM-CSF) mRNA. Effects on protein synthesis were not reported; for example, it is not known whether CXCL3 expression in T98G or GM-CSF expression in THP-1 cells is translationally regulated by p38. Nevertheless, these inconsistencies may provide clues to the mechanisms of post-transcriptional regulation by the MAPK p38 pathway. In T98G glioblastoma cells p38-dependent stabilisation of GM-CSF mRNA was not apparent until 2 h after stimulation with LPS, and was dependent upon ongoing protein synthesis [51]. At least two interpretations are possible: firstly, that sensitivity to p38 requires the synthesis of a regulatory protein, which is expressed in response to the activating stimulus; secondly, that sensitivity to p38 requires the target mRNA to be in a translationally active compartment (see also [52]). Again, further analysis of this system may provide insights into mechanisms of regulation by p38.

In LPS-stimulated RAW264.7 cells [41] or in IL-1-stimulated HeLa cells [53] a strong, rapid activation of p38 was followed by a sustained phase of lower, yet significant activity. Inhibition of p38 during this sustained second phase destabilised TNFα [41] or COX-2 [37] mRNA, and profoundly inhibited the expression of COX-2 protein [53]. This suggests that a quantitatively modest though sustained activation of p38 may be essential for the maintenance of mRNA stability and efficient expression of certain pro-inflammatory genes. Although the pro-inflammatory cytokines IL-1 and TNFα are generally similar in their abilities to activate signal transduction pathways, differential regulation of mRNA stability by these two agonists has been described [51,54,55]. In microvascular endothelial cells the differential induction of COX-2 mRNA by IL-1 and TNFα was explained by the ability of IL-1 but not TNFα to cause p38 activation and COX-2 mRNA stabilisation [54]. Other possible explanations of differential regulation of mRNA stability by IL-1 and TNFα include dissimilar kinetics of p38 activation (particularly in the sustained phase) [51] or different abilities to induce the expression of regulatory proteins. These possibilities remain to be explored.

The stability of MKK6 mRNA (encoding an upstream activator of p38) was positively regulated by p38 in THP-1 cells [50], suggesting a positive feedback loop which may modulate p38 signalling. Curiously, p38 appeared to destabilise MKK6 mRNA in mouse cardiac myocytes and human HEK293 cells, suggesting a negative feedback loop [56]. To our knowledge this is the first description of p38-dependent mRNA destabilisation, and it will be interesting to determine whether this is also MAPKAPK-2 dependent and ARE-mediated.

## 4. MAPK p38 as a target of anti-inflammatory agonists

MAPK p38 is strongly activated by pro-inflammatory stimuli, and has been studied largely in the context of inflammation [3,57]. Very many mRNAs stabilised by p38 encode inflammatory mediators or other immunomodulators (Fig. 2), suggesting that this kinase pathway plays a major role in the regulation of the inflammatory response. If the regulation of mRNA translation or stability by the p38 pathway plays a physiologically significant role in the expression of pro-inflammatory genes it follows that inhibition of this pathway may be a versatile anti-inflammatory strategy. This accounts for the intense interest of the pharmaceutical industry in the development of p38 or MAPKAPK-2 inhibitors [58].

It has recently been shown that GCs, which are powerful anti-inflammatory agents, induce rapid and prolonged expression of MAPK phosphatase 1 (MKP-1) [53,59–62], a dual-specificity phosphatase which potently inactivates p38 [63]. In the macrophage cell line RAW264.7 the expression of MKP-1 was associated with decreased expression of IL-6 and TNFα [60], which are known post-transcriptional targets of the p38 pathway. In HeLa cells the induction of MKP-1 and inhibition of p38 by GCs resulted in the destabilisation of a reporter mRNA containing a COX-2 ARE [53]. Furthermore, GCs destabilised COX-2 mRNA even when added to cells long after the activating stimulus [53,73], presumably by targeting the sustained phase of p38 activity. This late effect may be relevant to the role of GCs in the resolution of inflammation. Many pro-inflammatory genes are post-transcriptionally repressed by GCs, possibly by means of the same mechanism (Fig. 2). Interestingly, the expression of MKP-1 is induced by other anti-inflammatory agonists such as transforming growth factor β (TGFβ) [64], the B subunit of cholera toxin [60] and cAMP elevating agents [65,66].

The potent anti-inflammatory cytokine IL-10 also destabilised a number of ARE-containing transcripts (see Fig. 2). The destabilisation of KC mRNA (encoding a mouse CXCL2 homologue) was dependent upon an ARE within its 3′ UTR [67,68]. The inhibition of TNFα biosynthesis was similarly mediated by its 3′ UTR [69]. Like p38 inhibitors, IL-10 inhibited the translation of TNFα in primary mouse macrophages, and this translational blockade was impaired in mice which expressed an internally deleted TNFα mRNA, lacking the ARE [29]. The IL-10-dependent inhibition of TNFα biosynthesis was impaired in mouse macrophages lacking MAPKAPK-2 [29]. These observations are consistent with post-transcriptional inhibition of gene expression through the inhibition of p38 function by IL-10. However, such an effect remains controversial, having been observed by some groups [29,70,71] but not by others [69,72].

## 5. Mechanism, specificity and functional targets of the MAPK p38 pathway

As illustrated in Fig. 3, the p38 pathway could post-transcriptionally regulate gene expression at any of several stages. Regulation at the level of polyadenylation and/or the interactions between 5′ and 3′ termini could account for the effects of p38 upon both translation and mRNA stability. In RAW-264.7 cells destabilisation of TNFα mRNA by SB203580 was preceded by apparent shortening of the transcript [41]; however, it has not been demonstrated that this corresponds

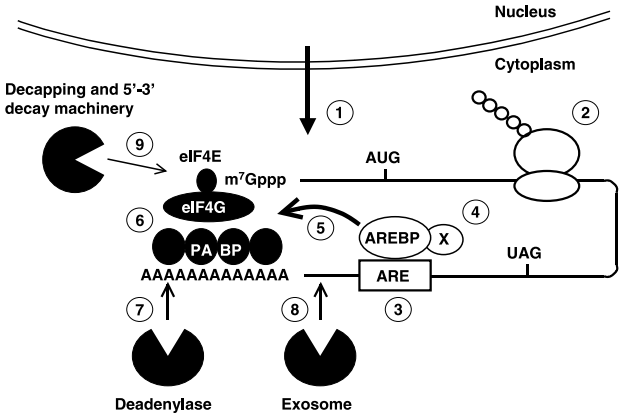


Fig. 3. Hypothetical mechanisms of post-transcriptional regulation by MAPK p38. This figure illustrates post-transcriptional events or processes which might be regulated by p38. This is not an exhaustive list, nor is it implied that different mechanisms are mutually exclusive. 1, Export of mRNA from the nucleus to the cytoplasm, or to particular sites of translation in the cytoplasm. 2, mRNA translation (or initiation). 3, Binding of AREBPs to AREs. 4, Interaction of other RNA binding proteins with AREBPs and/or directly with RNA. 5, Interaction of AREBPs with cap binding or poly-(A)-binding factors. 6, Interactions between cap binding and poly-(A)-binding factors. 7, Recruitment or activity of deadenylating complexes. 8, Recruitment or activity of the exosome. 9, Recruitment or activity of 5′ decapping and/or 5′ to 3′ degradation machinery.

to deadenylation. In A594 cells destabilisation of COX-2 mRNA by the synthetic GC dexamethasone was deadenylation-mediated [73]. Although dexamethasone induces MKP-1 expression in A549 cells (R. Newton, personal communication), a causal link between p38 inhibition and deadenylation has not been proven. In conclusion, circumstantial evidence suggests a role for p38 in the regulation of the poly-(A) tail length; however, all of the theoretical mechanisms illustrated in Fig. 3 remain possible, and are not mutually exclusive.

Transcript-specific regulation of mRNA stability or translation is presumably critical to the biological effects of the p38 pathway, yet is poorly understood. For example, many proto-oncogene mRNAs are unstable and contain AREs, but are not responsive to the p38 pathway [40,45,50,51]. Specificity cannot be explained simply in terms of AUUUA pentamers, although these are clearly involved in responses to p38. A GM-CSF ARE consisting of five overlapping pentamers conferred neither instability nor p38-mediated stabilisation, but required flanking sequences to function as a p38-responsive instability determinant [41,44]. A 76 nucleotide p38 responsive region of the COX-2 3′ UTR contains two discrete clusters of three pentamers. Mutation of pentamer motifs within either cluster resulted in loss of destabilising function, consequently stabilisation in response to p38 could not be measured. The shortest sequence element we have found to confer p38-mediated mRNA stabilisation was a 44 nucleotide TNFα ARE, containing six pentamers in 1-2-3 configuration (i.e. single, two overlapping, three overlapping) [41]. These observations suggest that p38-regulated mRNA decay may be associated with a complex arrangement of several pentamer motifs. However, matrix metalloproteinase (MMP-) 3 [74] and several other p38 targets identified by a microarray-based analysis of global mRNA decay rates [50] possess only one 3′ AUUUA motif (Fig. 2). The latter study could find no clear correlation of p38 sensitivity with the presence of a particular class of ARE (I or II). To understand specificity it may be necessary to consider the contexts of RNA sequence or secondary structure in which AUUUA motifs are found.

We have performed a detailed mutational analysis of a single short p38-responsive element of the COX-2 3′ UTR and correlated changes in function with changes in the binding of AREBPs (Dean et al., in preparation). Mutations which resulted in loss of HuR binding in vitro, but did not alter the binding of AUF-1 and other AREBPs, caused the stabilisation of a reporter mRNA. The p38-responsiveness of the stable mutant transcript could, of course, not be investigated. Another study implicated HuR as a functional target of the p38 pathway [75]. A reporter mRNA containing the IL-3 3′UTR was destabilised by coexpression of TTP. This effect was counteracted by coexpression of HuR and constitutively active MKK6, but not by expression of either of these alone. On the other hand, the expression of either HuR or MKK6 alone was capable of stabilising reporter mRNAs in the absence of TTP, implying p38-independent actions of HuR [75,76]. Several of the post-transcriptional targets of the p38 pathway are known to bind to HuR [7]; however, this could simply reflect the important role of HuR in the post-transcriptional metabolism of unstable mRNAs rather than a direct involvement in p38-mediated regulation.

Knockout mouse, reporter gene and other experiments suggest that major post-transcriptional effects of the MAPK p38 pathway are likely to be mediated by MAPKAPK-2 [28,42,44–46]. For this reason several labs have attempted to identify regulators of mRNA stability which are phosphorylated by MAPKAPK-2. Phosphomimetic mutants of the small (27 kDa) heat shock protein hsp27, the best-known substrate of MAPKAPK-2, stabilised a β-globin-COX-2 reporter mRNA [45]; however, the specificity of this effect is not yet clear. Under some conditions hsp27 was found in stress granules, which also contained polyadenylated mRNAs, PABPc1 and the translational inhibitors TIA-1 and TIAR [77]. However, the formation of these translationally inert granules appears to be a response to extraphysiological stress, and may not be related to the normal regulation of pro-inflammatory gene expression.

One study suggested that TTP may be phosphorylated by MAPKAPK-2 [78], whereas others pointed to phosphorylation of TTP by MAPK p38 itself [79,80]. It has not yet been demonstrated that either kinase phosphorylates TTP in vivo, therefore the physiological relevance of these observations is uncertain. However, it is clear that TTP is not required for the stabilisation of mRNAs by the p38 pathway, as HeLa cells are capable of p38-mediated mRNA stabilisation but do not express TTP (our unpublished observations). The phenotype of the TTP knockout suggests a myeloid- or lymphoid-specific role in the regulation of the off-phase of cytokine synthesis [29,81–83]. Finally, the expression of TTP in RAW264.7 cells was blocked by a p38 inhibitor [78], therefore MAPK p38 may regulate both on and off phases of TNFα synthesis by distinct mechanisms.

Two recent publications describe affinity purification methods to identify MAPKAPK-2 substrates which interact with ARE-containing mRNAs. This and the EMSA (electrophoretic mobility shift assay) method may detect distinct ARE–AREBP interactions since they differ greatly in terms of protein concentration, quantity of RNA substrate and other pa-

rameters. In one study [84], TNFα ARE-binding proteins were affinity purified from RAW264.7 cells and incubated in vitro with recombinant MAPKAPK-2. Of the many proteins bound to the affinity column only hnRNPA$_0$ and a second protein tentatively identified as RBM7 (RNA-binding motif 7 protein) were phosphorylated. In vitro, recombinant GST-hnRNPA$_0$ was phosphorylated by MAPKAPK-2 at serine 84, which lies within a consensus phosphorylation sequence. Phosphorylation of the same residue in vivo was stimulated by LPS, and dependent upon the MAPK p38 pathway. Finally, ARE-containing mRNAs COX-2, TNFα and CXCL2 (otherwise known as GRO2 (growth related oncogene 2) or MIP2 (macrophage inflammatory protein 2)) co-immunoprecipitated with hnRNPA$_0$ in a p38-dependent manner. HnRNPA$_0$ was originally cloned by affinity purification with an ARE substrate, but did not correspond to a high-affinity ARE-binding protein which was sought in that study and later identified as HuR [85]. Further studies are required to determine the precise binding specificity of hnRNPA$_0$ and its role in the post-transcriptional regulation of gene expression by MAPK p38.

In a similar approach, GM-CSF ARE-binding proteins were affinity-purified using a biotinylated RNA, then analysed by mass spectrometry [86]. This study also identified hnRNPA$_0$ as an ARE-binding protein; however, the ARE-binding protein most strongly phosphorylated by recombinant MAPKAPK-2 was PABPc1. PABPc1 is an extremely abundant cytoplasmic protein, which could bind non-specifically under conditions of high RNA and protein concentrations. However, it did not bind to a control RNA lacking an ARE, and its interaction with the GM-CSF AREs was resistant to high salt concentrations, suggesting high affinity. It will be interesting to learn whether PABPc1 interacts with other p38-sensitive AREs, whether such interactions are direct or indirect, whether MAPKAPK-2-mediated phosphorylation of PABPc1 occurs in vivo, and if so, what fraction of the cellular pool is modified. In theory the phosphorylation of PABPc1 by MAPKAPK-2 could mediate changes in poly-(A) tail length and/or the higher order structure of an mRNA. However, the intriguing question is how selective post-transcriptional regulation could be achieved, since PABPc1 binds unselectively to all polyadenylated mRNAs. One possibility is that a secondary interaction of PABPc1 with AREs or AREBPs mediates selectivity. Another is that MAPKAPK-2 is specifically recruited to certain mRNAs, for example through interaction with ARE-binding proteins, and then mediates transcript-specific phosphorylation of PABPc1. This would be analogous to mechanisms of transcriptional regulation, in which transcription factors recruit chromatin modifying enzymes in a sequence-specific and/or activation-specific manner to bring about promoter-targeted modifications of histone proteins, which themselves possess no promoter specificity.

## 6. Summary

A major function of the MAPK p38 pathway is the regulation of pro-inflammatory gene expression, although microarray-based analyses of mRNA turnover suggest additional targets. The maintenance of stability or translatability of pro-inflammatory mRNAs appears to require sustained, albeit quantitatively modest p38 activity. Anti-inflammatory agonists may exert their effects partly by targetting this sustained phase of p38 activity, bringing about a rapid and specific post-transcriptional shutdown of gene expression. Post-transcriptional effects of the p38 pathway are mediated by the kinase MAPKAPK-2 and by AREs within the 3′ UTRs of certain mRNAs. Beyond this, the mechanism of action of p38 is not well understood. Although a number of AREBPs have been shown to be phosphorylated by MAPKAPK-2, much further work is required to establish the role of these phosphorylations in post-transcriptional regulation. A related challenge is to understand the basis of transcript-selective regulation by the p38 pathway, since target mRNAs are extremely diverse in the sequence and structure of their 3′ UTRs. A more detailed understanding of these processes may result in the development of novel methods of intervention in inflammatory processes.

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