

Commentary

Post-transcriptional regulation of pro-inflammatory gene expression

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Abstract

The cytokine tumour necrosis factor (TNF) α is a vital mediator of the innate immune response, and a pleiotropic regulator of cellular function. Its involvement in rheumatoid arthritis is illustrated by the clinical benefits of TNF α blockade. Post-transcriptional regulation (the control of mRNA stability and translation) appears to play a critical role in the regulation of TNF α expression by mitogen activated protein kinase signal transduction pathways and by anti-inflammatory agents. The aim of this article is to review some recent advances in our understanding of these processes, and to speculate on mechanisms of regulation of TNF α and other pro-inflammatory genes.

Keywords: adenosine/uridine-rich element, dexamethasone, inflammation, mRNA stability, translation, tumour necrosis factor α

Post-transcriptional regulation of gene expression is generally mediated by the 5' or 3' untranslated regions (UTRs) of the mature mRNA, which flank the protein coding sequence. It is assumed that regulation is mediated by proteins that specifically interact with these regions. Much recent attention has focused on adenosine/uridine-rich elements (AREs), particularly repeats of the sequence AUUUA, which are present in the 3' UTRs of many transiently expressed cytokine and growth factor mRNAs, including TNF α , IL 1 α , 1 β , 6 and 8, IFN γ and granulocyte macrophage colony stimulating factor (GM-CSF) [1,2]. Several AREs have been shown to destabilise heterologous transcripts into which they are inserted [3]. Mammalian TNF α mRNAs possess a 3' UTR of almost 800 nucleotides, within which is a well-conserved ARE containing seven or eight copies of the AUUUA sequence. Among factors known to interact with the TNF α ARE [4–6] is tristetraprolin (TTP), an unusual zinc finger protein whose expression is induced by both lipopolysaccha-

ride (LPS) and TNF α itself. In mice deficient in TTP, TNF α protein is overexpressed, and the stability of TNF α mRNA is increased [6]. When coexpressed with a mimic TNF α mRNA, TTP is able to promote shortening of the poly(A) tail, normally an early step in mRNA degradation [7]. This property of TTP is dependent upon a (more or less) intact TNF α ARE. Therefore, TTP may participate in a negative feedback loop whose effect is to limit the magnitude or duration of a burst of TNF α expression by destabilising its mRNA in an ARE-dependent manner.

Mimic transcripts and other 'post-transcriptional reporters' for the study of TNF α gene regulation do not faithfully reproduce all features of endogenous gene expression. A more subtle approach has recently been used by Kollias *et al*, who precisely deleted the ARE region from the mouse TNF α genomic locus [8]. In the resulting (Δ ARE) strain, the dynamics of LPS-stimulated TNF α mRNA accumulation were

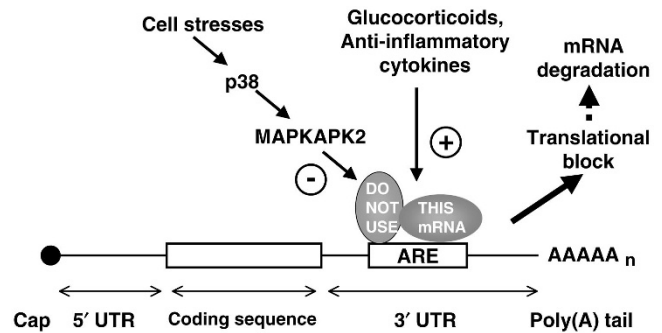
altered, with a larger, slower and more sustained induction. Altered TNF α mRNA stability was strongly implicated in this change, but not directly demonstrated. Other workers have shown that TNF α mRNA stability is regulated by LPS, and is sensitive to protein kinase inhibitors in myeloid cell lines [9]. These studies should be interpreted with caution because of their dependence upon toxic transcriptional inhibitors like actinomycin D, which may give rise to artefacts.

Previous studies established that the mitogen activated protein kinase (MAPK) p38 regulates TNF α production at the translational level [10–12]. Specific inhibitors of p38 blocked TNF α protein synthesis, while downregulating TNF α mRNA by 50% at most. The deletion of the TNF α ARE resulted in the loss of sensitivity to a p38 inhibitor in the mouse strain of Kollias *et al* [8]. A recent mouse knockout study suggests that the effects of MAPK p38 may be mediated by mitogen activated protein kinase-activated protein kinase 2 (MAPKAPK2), a kinase that is phosphorylated and activated by p38. In the absence of MAPKAPK2 activity, reduced quantities of TNF α protein were synthesised following an LPS challenge, while the induction of TNF α mRNA was unaffected [13]. Together, these findings suggest that p38 regulates TNF α mRNA translation via MAPKAPK2, and by a mechanism involving the TNF α ARE. In contrast, p38 and MAPKAPK2 regulate the stability rather than the translation of IL-6, IL-8, GM-CSF and cyclooxygenase-2 (Cox-2) mRNAs in the human HeLa cell line [14] (Lasa *et al*, submitted). Regulation of IL-8 and Cox-2 mRNA stability is mediated by 3' AU-rich elements that closely resemble the TNF α ARE. It is significant that these studies do not rely upon pharmacological inhibitors of transcription or of p38, and provide independent confirmation of earlier reports that p38 is able to regulate mRNA stability.

The parallels and differences between TNF α and IL-8 or Cox-2 raise several questions. Do AREs such as those in the TNF α and Cox-2 transcripts regulate translation, stability, or both? What of the p38 signalling pathway? Is it conceivable that distinct mechanisms have evolved for the regulation of both translation and stability by mitogen activated protein kinases, through extremely similar RNA sequence motifs? Perhaps the apparently different consequences of p38 inhibition reflect an underlying common mechanism. It is suggested that both HeLa cells and monocytes/macrophages can label mRNAs as 'Not For Use', most probably by means of specific protein–mRNA interactions that involve AREs and are sensitive to p38 (Fig. 1). The immediate consequence of this might be a cessation of translation, with indirect consequences such as mRNA degradation modulated in a species, cell type or transcript specific manner.

Studies employing specific inhibitors have implicated MAPK p38 in the post-transcriptional regulation of a

Figure 1



Convergence of regulatory signals at the 3' untranslated region of a pro-inflammatory mRNA. ARE, Adenosine/uridine-rich elements; UTR, untranslated region; MAPKAPK2, mitogen activated protein kinase-activated protein kinase 2.

number of genes that could be broadly characterised as pro-inflammatory. These include TNF α , IL-6, -8 and -1 β , GM-CSF, Cox-2 and the vascular cell adhesion molecule VCAM-1 [12,15–17]. In the MAPKAPK2 knockout mouse strain, LPS-induced expression of IL-6 was blocked at both protein and mRNA levels, while expression of both TNF α and IFN γ was blocked at the protein but not the mRNA level [13]. The defective expression of TNF α and IFN γ suggests translational regulation by MAPKAPK2 within both myeloid and T cell lineages.

Dexamethasone, a synthetic glucocorticoid and a potent anti-inflammatory reagent, regulates the expression of TNF α and Cox-2 partly at the post-transcriptional level. Post-transcriptional reporter studies have implicated the TNF α 3' UTR in dexamethasone sensitivity [18], and the deletion of the ARE diminishes the inhibitory effect of dexamethasone upon TNF α gene expression *in vivo* [8]. In a human pulmonary epithelial cell line, dexamethasone destabilises Cox-2 mRNA by a mechanism that involves shortening of the poly(A) tail, although it has not yet been possible to map the RNA sequences that mediate this effect [19]. Both TNF α and Cox-2 are subject to negative regulation by anti-inflammatory cytokines such as IL-10, which has been shown to regulate the stability of ARE-containing transcripts [20,21], and preliminary evidence indicates that its effects upon TNF α expression may be partly mediated by the TNF α 3' UTR (B. Foxwell, personal communication). The responses of the mouse Δ ARE strain to anti-inflammatory cytokines would be interesting to observe.

It is an intriguing possibility that these disparate observations reflect a common mechanism for the post-transcriptional control of several pro-inflammatory genes. In other words, the ARE represents a point of convergence of pro-inflammatory signals (such as MAPK p38 activation) and

anti-inflammatory signals (such as activation of IL-10 or glucocorticoid signalling pathways; Fig. 1). In theory, this might allow a cell to modulate its sensitivity to pro- or anti-inflammatory stimuli through changes in the expression of ARE binding factors, and to rapidly and coordinately shut down the expression of several inflammatory mediators by means of phosphorylation-regulated changes in protein-RNA interactions. In the simplest model, all of these regulatory processes could be mediated by a single ARE binding factor; however, in reality, the situation is likely to be rather more complex. One can imagine post-transcriptional regulation involving competition between positive and negative regulatory RNA binding proteins, and other subtleties such as cell-specific differences in the levels of expression of these factors. The assessment of this hypothesis requires the characterisation of putative post-transcriptional regulators, and their functional and physical interactions. It may also be instructive to look for additional targets of suspected regulatory factors such as TTP.

Both the TTP knockout and the Δ ARE mouse strains display striking inflammatory pathologies, including an erosive arthritis with features of rheumatoid arthritis (RA) in man [8,22]. Thus, the disruption of either cis-acting (ARE) or trans-acting (TTP) components of negative regulatory control of TNF α synthesis can cause joint disease. One wonders whether 'off phase' or negative regulatory mechanisms for the restraint of TNF α biosynthesis are perturbed in RA. Even if not, post-transcriptional control may represent a target for novel anti-inflammatory therapies. For example, a recent paper reports disease modifying activity of a p38 inhibitor in an animal model of RA, with downregulation of both TNF α and IL-6 expression [23]. In the event of unwanted side effects of intervention with MAPK or glucocorticoid signal transduction pathways, it might be possible to target downstream effectors, the putative RNA binding coordinators of pro- and anti-inflammatory post-transcriptional regulation. It is conceivable that reactivation or reinforcement of 'off phase' regulatory mechanisms may have quite broad anti-inflammatory effects. A recent paper [24] reports that TTP regulates the stability of GM-CSF mRNA.

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