Post-transcriptional regulation of tumour necrosis factor α production

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Tumour necrosis factor α (TNF α) is a proinflammatory cytokine produced by activated macrophages, lymphocytes and other cells.¹⁻³ The production of TNF α is under transcriptional and post-transcriptional control.⁴⁻¹⁰ Post-transcriptional control of TNF α expression is achieved by regulating translational initiation, mRNA stability, and polyadenylation.⁶⁻⁷⁻¹⁰ An adenine and uridine (AU)-rich element (ARE) in the 3' untranslated region (3' UTR) of TNF α transcripts¹¹⁻¹³ is an important determinant of post-transcriptional control. Transfer of this element to heterologous reporter transcripts changes the expression of the reporter protein. Transacting factors that bind to the ARE and participate in post-transcriptional control have recently been identified. The ARE binding proteins expressed in a given cell are thought to determine the level of protein expression.

AU-rich elements (AREs)

The 3' untranslated region of mRNAs encoding short lived immediate early genes (for example, fos, jun) as well as selected cytokines (for example, TNF α , GM-CSF) possess AU-rich elements that regulate protein expression. Class I AREs consist of one or more pentamer repeats (that is, AUUUA), whereas class II AREs consist of one or more nonamer repeats (that is, AUUUAUUUA). Whereas class I AREs are sufficient to promote the degradation of transcripts encoding immediate early proteins, both class I and class II AREs regulate the production of cytokines such as TNF α , GM-CSF, interleukin 3 (IL3) and interferon α (IFN α). The 3' untranslated region of TNF α transcripts contains both class I and class II AREs. Electrophoretic mobility shift assays have identified two distinct regulatory complexes that assemble on these adjacent cis elements in HeLa cells.¹⁴⁻¹⁵ The complex assembled on the class II ARE (designated complex 1) includes the related RNA binding proteins TIA-1 and TIAR.¹⁴ The complex assembled on the class I ARE (designated complex 2) includes an unidentified 55 kDa protein. Whereas complex 1 forms with extracts derived from activated and unactivated macrophages, complex 2 is only formed using extracts derived from activated macrophages.

The importance of the TNF α ARE is underscored by the severe phenotype of transgenic knock-in mice expressing TNF α transcripts that lack the ARE.¹⁶⁻¹⁷ Overexpression of TNF α by these animals results in chronic inflammatory arthritis and inflammatory bowel disease. The overexpression of TNF α is a consequence of increased transcript stability combined with

a loss of translational silencing. Furthermore, the inhibition of TNF α production by cytokine suppressive anti-inflammatory drugs (CSAIDs) was absent in these animals, indicating that the TNF α ARE mediates the translational silencing that is abrogated by inhibition of p38 MAP kinase. As a result of these defects, synovial fibroblasts spontaneously produce TNF α in the mutant mouse.

ARE binding proteins

Transacting factors that bind to the TNF α ARE are essential for post-transcriptional control of TNF α expression. For example, the zinc-finger protein tristetraprolin (TTP) binds to the TNF α ARE and promotes the degradation of TNF α transcripts.¹⁸⁻²⁰ Mutant mice lacking TTP develop cachexia, arthritis and autoimmunity as a consequence of overexpressed TNF α mRNA and protein.²⁰ This clinical syndrome can be prevented by the repeated injection of neutralising antibodies reactive with TNF α .²⁰ In addition to TTP, the RNA binding proteins Hel-N1,²¹ HuR,²²⁻²⁵ AUF1 (hnRNP D),²⁶ and TIAR¹⁴ are ARE binding proteins that have been proposed to regulate the expression of TNF α .²⁷ Hel-N1²¹⁻²⁸⁻²⁹ and HuR²²⁻²⁵ stabilise ARE containing transcripts, whereas AUF1 destabilises these same transcripts.³⁰ It is therefore likely that the stability of ARE containing transcripts is determined by the relative expression of functionally antagonistic ARE binding proteins.

TIA-1 and TIAR

TIAR and its closely related homologue TIA-1 are members of the RNA recognition motif (RRM) family of RNA binding proteins.³¹⁻³⁵ Both proteins possess three RRM domains at their amino termini that confer high affinity binding to uridine-rich motifs.³² Like components of the general heteronuclear ribonucleoprotein complex (hnRNP), TIA-1 and TIAR continuously shuttle between the nucleus and the cytoplasm (N Kedersha and P Anderson, manuscript in preparation), suggesting that they might participate in the nucleocytoplasmic transport of selected mRNAs. These proteins also regulate the general translational arrest that accompanies environmental stress. After the stress induced phosphorylation of translation initiation factor eIF-2 α , TIA-1 and TIAR recruit most cytoplasmic mRNAs to discrete foci known as stress granules.³⁶ The TIA-1/R dependent sequestration of these mRNAs prevents their translational initiation. In this capacity, TIA-1 and TIAR function as translational silencers that seem to influence the duration of stress induced translational arrest.

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Mutant mice lacking TIAR exhibit partial embryonic lethality and defective germ cell maturation, implicating this protein in selective aspects of vertebrate development.³⁷

The discovery of TIAR as a component of the ARE associated complex that assembles on the 3' UTR of TNF α transcripts¹⁴ provided the first clue that TIA-1 and TIAR might specifically regulate the expression of TNF α . To test this hypothesis, we produced mutant mice lacking TIA-1 and compared the LPS induced expression of TNF α in wild type and TIA-1^{-/-} macrophages.³⁸ Our results indicate that LPS induced expression of TNF α is significantly increased in macrophages lacking TIA-1.³⁸ The functional effects of TIA-1 seem to result from translational silencing rather than regulation of mRNA stability.³⁸ Thus, the ARE binding protein TIA-1 represses the expression of TNF α by a mechanism that differs from that used by other known ARE binding proteins.

Although both TIA-1 and TIAR are concentrated in the nucleus at steady state, heterokaryon analysis shows that both proteins continuously shuttle between the nucleus and the cytoplasm (Kedersha and Anderson, manuscript in preparation). In this respect, TIA-1 and TIAR resemble the heteronuclear ribonucleoproteins (hnRNPs) that assemble around nascent RNA transcripts and facilitate transport from the nucleus to the cytoplasm.³⁹ Like the hnRNPs, TIA-1 and TIAR can function as general RNA binding proteins that interact with many, if not most, mRNAs in vitro.³² At the same time, these proteins can selectively interact with RNAs possessing uridine-rich motifs.³² In their ability to function as both general and specific RNA binding proteins, TIA-1 and TIAR resemble hnRNPs K and E1, proteins that participate in general RNA export and also bind to the 3' UTRs of 5-lipoxygenase transcripts to repress translational initiation.^{40, 41}

Conclusions

Our results introduce TIA-1 and TIAR as translational silencers that can independently and selectively regulate the production of TNF α . Previous studies using macrophage cell lines have clearly shown that translational silencing is important in the post-transcriptional control of TNF α production.^{4-7, 10} In the unstimulated macrophage cell line RAW 264.7, TNF α transcripts are expressed but excluded from polysomes and not translated.^{4, 6} Comparison of TNF α mRNA distribution into polysomes in wild type and TIA-1^{-/-} macrophages indicate that TIA-1 controls the association of TNF α mRNA with polysomes. It remains to be determined whether TIA-1/R induced translational silencing is achieved by regulation of translational initiation. In any case, the ability of TIA-1 and TIAR to inhibit TNF α mRNA translation suggests that these proteins might be targets of the stress kinase signalling cascade that is blocked by CSAIDS. CSAIDS block the LPS induced production of TNF α by preventing translational de-repression.^{8, 42} This is accomplished by inhibiting the p38-MK2 signalling

cascade,⁴³⁻⁴⁵ suggesting that these kinases phosphorylate a translational silencer that associates with TNF α transcripts. The ability of CSAIDS to similarly repress the expression of TNF α in wild type and TIA-1^{-/-} macrophages indicates that TIA-1 is not an essential target of these drugs. TIA-1 might thus act as a constitutive translational suppressor controlling excessive TNF α production. Alternatively, the functional redundancy of TIA-1 and TIAR leaves open the possibility that cells lacking both TIA-1 and TIAR might be resistant to the suppressive effects of CSAIDS.

Taken together, our results suggest that TIA-1 and TIAR are translational silencers that regulate the cellular and organismal response to stress. At the cellular level, these proteins contribute to the general translational arrest that accompanies environmental stress. By controlling the duration of translational arrest, TIA-1 and TIAR might determine whether stressed cells live to repair the stress induced damage or die by apoptosis. At the organismal level, these proteins regulate the expression of at least one inflammatory mediator that serves as a sentinel to signal the presence of microbial infection. It remains to be determined whether the translational control exerted by these proteins is limited to the stress response, or is a general feature of normal cellular metabolism.

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