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Pillars of Immunology

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A New Mechanism of Gene Regulation Mediated by Noncoding RNA

Martin Turner* and Peter D. Katsikis†

Cytokines play a critical role in innate and adaptive immunity, acting as messenger molecules that regulate the growth, survival, and function of cells. The revolution in recombinant DNA techniques in the late 1970s led to the molecular cloning of cDNAs encoding a number of cytokines. By the mid-1980s, many of these had been identified and were revealed to be relatively small secreted proteins with diverse primary structures. The pleiotropic actions of cytokines, which underpin the complex interaction between the immune system and other tissues, were only beginning to be appreciated. With the identification of their gene sequences, it was possible to examine how the expression of cytokines was regulated at the molecular level. It was widely appreciated that answering this question would give insights into how the immune response was coordinated and how altered regulation of cytokines might contribute to disease. The factors controlling gene expression at the molecular level were only starting to be understood (e.g., the identification of promoters and enhancers that regulate gene transcription in tissue-specific and inducible manners). However, the mechanisms by which these factors functioned remained poorly defined. Accordingly, the publication of the paper we highlight in this commentary coincided with the discovery of NF-κB and predated the identification of NFAT and STAT transcription factors, which are among the most often encountered regulators of cytokine gene transcription.

In addition to regulation at the level of gene transcription, regulating RNA stability offers the potential to control mRNA abundance, but whether such a mechanism existed for the physiological regulation of gene expression was uncertain. Work on oncogenes such as *myc* and *fos*, which encode mRNAs that are short lived in untransformed cells, demonstrated that these mRNAs were frequently stabilized in tumor cells (1–3). The principles that regulated changes in mRNA half-life were, however, unknown. The highlighted study by Gray Shaw and Robert Kamen (4) identified elements in the noncoding region of an mRNA that controlled its stability.

Shortly before Shaw and Kamen's study, a comparison between the sequences of human and mouse GM-CSF cDNAs had revealed a striking 93% homology within an adenine- and uridine-rich subregion of the 3′ untranslated region (UTR) (5). Anthony Cerami and colleagues (6) had also identified a UUAUUAU octamer motif in 3′UTRs of genes linked to the inflammatory response. The high degree of conservation of this motif implied a conserved function, but it was the Shaw and Kamen study that provided the first evidence for the function of this AU-rich element (ARE) (4).

Shaw and Kamen had observed that GM-CSF mRNA was unstable in human T lymphoblastoid cells activated with PHA, exhibiting a $t_{1/2}$ of ≤30 min. Remarkably, treatment of the cells with phorbol ester completely blocked mRNA decay over the 2-h period examined. The precise mechanism by which phorbol esters mediate this effect remains far from clear, but it was postulated by Shaw and Kamen to involve an mRNA degradation pathway. The effect on mRNA stability of the insertion of the conserved ARE derived from the GM-CSF 3′UTR was examined by generating a reporter construct. The presence of the ARE led to a 97% reduction in the abundance of the hybrid transcript.

A direct effect of the ARE on mRNA decay was demonstrated by treating transfected cells with actinomycin D to block transcription and measuring abundance of the reporter mRNA. The control β-globin transcript and the transcript encoded by a reference plasmid remained stable for several hours, but the globin transcript with the inserted ARE decayed with a $t_{1/2}$ of <30 min. Two years later, Wilson and Treisman demonstrated that the *fos* ARE triggered deadenylation (7). It is now known that ARE-containing mRNAs are selected for recruitment to the deadenylase complex (8, 9).

The Shaw and Kamen paper provided the foundation for understanding a fundamental mechanism of gene regulation relevant to many different cell types. The subsequent discovery of AREs within mRNA in species as diverse as yeast, *Drosophila*, and sea urchin suggests that this is an ancient and conserved regulatory process that contributes to the adaptation to environmental change. Several extensive database resources such as ARED (10) and ARE site (11) were created to assist in the identification of AREs in different genomes. The paper also had important implications for understanding the regulation of cytokine gene expression in health and disease. Deletion of the

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Abbreviations used in this article: ARE, AU-rich element; UTR, untranslated region.

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TNF ARE in the mouse germline led to a lethal inflammatory syndrome owing to loss of the ability to restrain TNF production (12). Furthermore, the paper can be seen as the starting point for the discovery of RNA-binding proteins that interact with AREs. Many such proteins with structurally diverse RNA-interacting domains have since been identified (13). Among these, tristetraprolin was shown to be an inhibitor of TNF and GM-CSF production (14) and to act posttranscriptionally to promote mRNA decay. By contrast, HuR could be shown to promote stabilization of mRNA (15). This finding led to a model in which the dynamic regulation of RNA decay and translation was mediated by the abundance and activation state of different ARE-binding proteins. Any such model now has to take into account the biological effects mediated by microRNAs, which increasingly are becoming connected to regulation of ARE-mediated mRNA fate through novel mechanisms (16–18).

Shaw and Kamen noted that treatment of transfected cells with cycloheximide, an inhibitor of protein synthesis known to cause "superinduction" of unstable mRNAs, stabilized the reporter transcript containing the ARE. This finding suggested that either ARE-dependent mRNA degradation required ongoing translation or a labile protein was required for decay of the transcript. Further links between the ARE and translation came from the ability of the ARE to cause translational blockade, but not mRNA decay, in *Xenopus* oocytes (19). It was subsequently reported by Savant-Bhonsale and colleagues (20) that the ARE promoted the translation-dependent assembly of a large mRNA-destabilizing complex, a study that could be considered a harbinger of the characterization of RNA granules such as processing bodies and stress granules (21). More recently, the formation of processing bodies has indeed been linked to ARE and their interacting proteins (22).

The tentative conclusion made by Shaw and Kamen that the transient accumulation of mRNA following stimuli arises from a temporary block in specific mRNA degradation pathways now underpins an important concept in gene expression relevant to the shaping of response kinetics and to the process by which gene expression can be switched off rapidly in response to intrinsic or extrinsic cues. Early evidence in support of this concept was found in studies of c-myb, an ARE-containing transcript found to have a $t_{1/2}$ of ~246 min at 2 h following serum stimulation but ~28 min at 9 h post stimulation (23). Later studies provided evidence for the differential regulation of ARE-containing proto-oncogene and cytokine mRNAs in the same cells, suggestive of selectivity in the regulation of ARE-mediated RNA destabilization (24–26). The temporal order of gene expression plays a decisive role in shaping innate and adaptive immune responses, and the application of methods for the global analysis of gene expression demonstrates that the ARE is a regulatory element that exerts a dominant effect on the temporal order of gene expression (27, 28). Identification of the functional activity of the ARE by Shaw and Kamen >25 y ago, beyond identifying a factor that controlled expression of an individual cytokine, laid the foundation for understanding the coordinated expression of cytokines in inflammatory networks.

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Disclosures

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