*Commentary*

# Misregulated Posttranscriptional Checkpoints: Inflammation and Tumorigenesis

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Our understanding of gene expression and regulation has undergone tremendous strides over the past several decades due to the successes of many laboratories in elucidating the control of transcription. But of course, the control of gene expression in mammalian cells extends to many levels beyond transcription and occurs in the nucleus and cytoplasm. For example, in the nucleus, pre-mRNA molecules undergo modifications involving the addition of a 5′ cap structure and for most, the addition of a 3′ poly(A) tract. These two modifications improve the translation and stability of mRNAs. Some genes encode alternative polyadenylation sites, the choice of which can determine the sequence of the 3′ noncoding region of the mRNA. Many pre-mRNA molecules must also be processed by the splicing machinery to remove introns. In some cases, pre-mRNAs transcribed from a single gene are alternatively spliced to create multiple mRNA species with differing exon compositions. Clearly, this can affect the coding and/or noncoding regions of mRNAs. Additionally, mRNA coding sequences can be altered by RNA editing processes, such as site-specific base modification of a C to a U (which introduces a stop codon in some mRNAs). Messenger RNA molecules must then be actively transported to the cytoplasm.

In the cytoplasm, mRNA degradation processes contribute to establishing suitable steady-state levels, which in turn impact protein levels. Protein levels are the result of the differences in their rates of synthesis by translation versus their rates of degradation. Translation of mRNAs can be regulated in several ways. For example, multiple AUGs in the 5′ noncoding region, short upstream open reading frames (uORFs), and structured 5′ noncoding regions can severely limit translation of the downstream protein coding region. These controls are often found in the mRNAs of protooncogenes and other genes important for cellular growth and differentiation. Messenger RNAs, such as those encoding proteins important for cell growth, can also contain internal ribosome entry sites (IRES), which as the name implies, permits initiating ribosomes to begin translation internally without prior scanning of the 5′ noncoding region. Messenger RNAs can also contain cis-acting sequences that control their translation rates. These control sequences include A + U–rich elements (AREs) in the 3′ noncoding region and 5′ terminal oligopyrimidine tracts (5′-TOPs). AREs are found in many mRNAs encoding proteins important for cell growth and immune function; 5′-TOPs are found in mRNAs encoding components of the translational machinery. For protein molecules themselves, posttranslational modifications may be required for proper functioning of the protein. Additionally, a protein's function may require that it be localized in the proper intracellular compartment or that it be secreted.

Clearly, posttranscriptional processes can contribute significantly both to the abundance of a protein and the timing of its expression, and they can provide multiple regulatory points to control each of these parameters. Alterations in the expression of some transcription factors, cytokines, growth factors, and signal transduction components can lead to cellular transformation (for a review, see reference 1). Loss of posttranscriptional controls for the syntheses of these proteins contributes to their inappropriate expression, leading to eventual transformation (for a review, see reference 2).

Several discoveries over the past several years have increased our appreciation of the roles that controlling mRNA degradation and translation can have for normal immune function and cell growth. Shaw and Kamen (3) and Caput et al. (4) discovered that many mRNAs encoding oncoproteins and cytokines contain AREs in their 3′ noncoding regions. AREs target these mRNAs for rapid degradation (3). However, in response to certain environmental stimuli, ARE-dependent decay of selected cytokine mRNAs is repressed, resulting in their stabilization and rapid increases in their abundance (5). Under some conditions, an ARE can also serve as a regulatory element for translation (6). Thus, AREs contribute to the tight regulation of proteins important for cell growth and immune function. For example, in mice with a targeted disruption of the ARE from TNF-α mRNA, the animals developed chronic inflammatory arthritis and Crohn's-like inflammatory bowel disease due to increased TNF-α synthesis (7).

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Additionally, translational control of TNF-α synthesis in these animals was no longer responsive to control by the p38 mitogen-activated protein (MAP) kinase and c-Jun $NH_2$-terminal kinase (JNK). These results suggest that the p38 and JNK signal transduction pathways can act through the TNF-α ARE, perhaps via proteins that bind the TNF-α ARE.

AREs appear to mediate their control functions through association with trans-acting proteins (for a review, see reference 8). The particular protein bound may influence whether the ARE controls mRNA degradation, mRNA stabilization, or translation. For example, the ARE-binding proteins AUF1 and tristetraprolin (TTP) appear to mediate mRNA destabilization (9, 10). Knockout of TTP causes increased TNF-α production by macrophages, leading to inflammatory arthritis, dermatitis, cachexia, autoimmunity, and myeloid hyperplasia (10). By contrast, binding of the protein HuR stabilizes ARE-containing mRNAs (11, 12). However, the ARE-binding proteins TIAR and TIA-1 confer ARE-dependent translational regulation to TNF-α mRNA (6). Knockout of the translational repressor TIA-1 in mice leads to elevated levels of TNF-α and confers hypersensitivity to LPS (13). Precisely how the ARE-binding proteins effect mRNA decay and translational control is unknown. Another important question for further investigation relates to the observation that many mRNAs contain AREs, yet not all ARE-containing mRNAs are coordinately regulated in response to an environmental stimulus. This would suggest that ARE-containing mRNAs likely possess multiple cis-acting elements, which could provide mRNA-specific regulation. For example, the AREs of IL-2 and c-*myc* mRNAs direct their rapid degradation in T cells. Activation of the JNK signal transduction pathway in T cells induces the stabilization of IL-2 mRNA. Binding of the proteins nucleolin and YB-1 to a JNK-response element (JRE) in the 5′ noncoding region of IL-2 mRNA prevents its ARE-directed decay, thus stabilizing the mRNA (14). By contrast, c-*myc* mRNA does not appear to contain a JRE. This might explain why c-*myc* mRNA remains unstable in stimulated T cells.

The idea of multiple cis-acting elements that permit mRNA-specific regulation can be extended to translational regulation of an mRNA as well. For example, it has been known for some time that diverse cell types synthesize IL-15 mRNA, but remarkably, the IL-15 protein was difficult to find by either activity assays or by ELISA (e.g., see reference 15). This observation led to the hypothesis that IL-15 mRNA is under stringent translational control(s). Indeed, the human T cell leukemia cell line HuT-102 was found to produce IL-15. Cloning of the IL-15 cDNA from these cells indicated that the mRNA is altered such that most of the 5′ noncoding region of the mRNA is replaced with the HTLV-I R region (16). This removes 8 of 10 AUGs located upstream of the start codon for IL-15 protein synthesis. Both transfection experiments and in vitro translation experiments revealed that the 5′ noncoding region of wild-type IL-15 mRNA can attenuate translation. Although it has been appreciated for some time that upstream AUGs can attenuate translation of downstream protein coding regions, all of the upstream AUGs in the 5′ noncoding region of IL-15 mRNA have an in-frame termination codon preceding the AUG start codon for IL-15 protein synthesis. This raises the possibility that one or more of the upstream AUGs may actually encode a small peptide that could control translation of IL-15 mRNA. Short, uORFs occur in several mRNAs ranging from viruses to humans (for a review, see reference 17). In mammals, translation of the mRNAs encoding $\beta_2$-adrenergic receptor, retinoic acid receptor-$\beta_2$, and S-adenosylmethionine decarboxylase (Ado MetDC) is regulated by uORFs encoding peptides of 19, 19, and 6 amino acids, respectively. In the case of AdoMetDC, the nascent peptide product of the uORF can interfere with the terminating ribosome, resulting in its stalling within the 5′ noncoding region, thus blocking downstream translation (i.e., synthesis of AdoMetDC). In any event, further work will be required to determine if the 5′ noncoding region of IL-15 mRNA actually encodes one or more peptides.

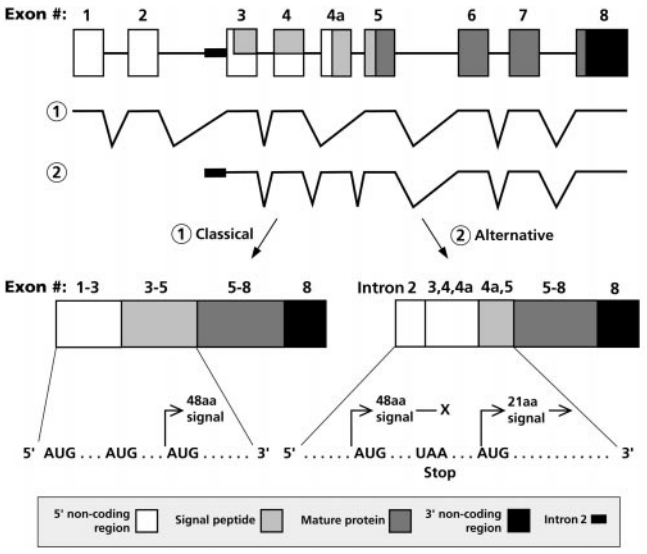


**Figure 1.** Diagram illustrating posttranscriptional control of IL-15 expression and the complexity of its regulation. The nine exons of the human IL-15 gene are shown at the top. The assignment of exons to 5′ noncoding region, signal peptide, mature protein, and 3′ noncoding region is depicted by shaded boxes labeled in the diagram. Two possible pre-mRNA splicing pathways are depicted, one for the classical IL-15 mRNA (pathway no. 1) and the other for an alternative pathway (no. 2). The alternative pathway includes exon 4a in the mRNA; however, exons 1 and 2 are not present, perhaps due to use of an alternate transcription start site. Exon numbers are shown above each drawing. At the bottom is an expanded diagram of the mRNA structures at the 5′ ends. For the classical IL-15 mRNA (left), which encodes a 48-amino acid (aa) signal peptide for IL-15 (depicted by the rightward arrow), translational efficiency is low due to the upstream AUGs. Its translational efficiency may also be low due to a region containing rare codons in the mRNA sequence encoding the 48-amino acid signal peptide. For the mRNA derived from the alternative pathway (right), it lacks the upstream AUGs. However, the start codon for the 48-amino acid signal peptide is still present (rightward arrow), but there is an in-frame stop codon that would terminate synthesis of this IL-15 isoform. Instead, ribosomes utilize the downstream AUG for synthesis of the IL-15 isoform containing the 21-amino acid signal sequence (rightward arrow). This isoform is not secreted.

Not surprising for a tightly regulated protein like IL-15, its translation and secretion are also linked in unexpected ways. Apparently, there are two isoforms of IL-15 that differ in the length and sequence of their respective signal peptides which affects secretion. One form has a signal peptide of 48 amino acids and is secreted, whereas the other has a shorter signal peptide of 21 amino acids and is not secreted (18, 19). These are synthesized in a cell type–specific fashion. The mRNAs differ in primary structure in two ways. First, exons 1 and 2, which contain the multiple upstream AUGs, are not present in the mRNA for the 21-amino acid isoform. Instead, it has intron 2 sequence, perhaps due to use of an alternative transcription start site (19, 20; Fig. 1). This could clearly impact the translational efficiency of the mRNA encoding the 21-amino acid isoform. Second, due to alternative pre-mRNA splicing, the mRNA encoding the 21-amino acid isoform contains an additional 119 nucleotides from exon 4a not present in the mRNA encoding the 48-amino acid isoform (Fig. 1). Exon 4a disrupts the 48-amino acid signal peptide by introducing a stop codon; it also introduces an alternative start codon, where synthesis of the 21-amino acid isoform begins (Fig. 1). Additionally, the 21-amino acid signal isoform is not secreted but remains intracellular in both the nucleus and cytoplasm. By contrast, the 48-amino acid signal isoform is secreted, but it is not as efficiently translated as the 21-amino acid isoform. The property of reduced translational efficiency can be conferred upon another mRNA (e.g., encoding green fluorescent protein) by linking it downstream of the coding sequence for the 48-amino acid leader peptide. Thus, synthesis of the 48-amino acid signal peptide itself seems to reduce the translational efficiency of the mRNA. Reduced translational efficiency might be due to codon usage within the region of the mRNA encoding the 48-amino acid signal peptide. Examination of the codons for this region reveals that within one 9-amino acid stretch, 5 of the 9 amino acids are encoded by rare codons (defined as appearing at a frequency of 10 or less times per 1,000 codons for humans). This might cause the ribosome to pause enough to affect translational efficiency. By contrast, this situation does not exist in the coding sequence for the 21-amino acid signal peptide. However, further work will be required to determine if these rare codons are in fact affecting translational efficiency of the 48-amino acid signal isoform of IL-15.

Loss of posttranscriptional control of IL-15 synthesis can have profound biological effects. In this issue, Fehniger et al. (21) describe transgenic mice expressing an IL-15 transgene they designed for optimal expression by eliminating IL-15's posttranscriptional controls. To achieve this they removed the 5′ noncoding region of IL-15 mRNA (containing the 10 upstream AUGs) and replaced the poorly translated, 48-amino acid signal peptide of IL-15 with the IL-2 signal peptide in the modified transgene. They also introduced sequence encoding an epitope tag to the 3′ end of IL-15's open reading frame in the transgene. This modification seems to enhance IL-15 expression by means that are not understood (15). These modifications led to overexpression of secreted IL-15 protein, which in turn led to early expansions in NK and memory phenotype $CD8^+$ T lymphocytes in the transgenic mice. The mice later developed fatal lymphocytic leukemia with a T-NK phenotype, similar to the leukemia of large granular lymphocytes in humans. Thus, loss of posttranscriptional controls that serve to limit IL-15 expression is deleterious to the host. The results of Fehniger et al. also show that lymphocytic leukemia, like some other cancers, can result from chronic stimulation by a proinflammatory cytokine, illustrating how essential posttranscriptional controls are for maintaining proper immune function. Undoubtedly, work in the coming years will unveil many disorders that arise in part due to posttranscriptional processes gone awry. A continuing challenge is to understand the molecular details of the diverse array of posttranscriptional control processes. Moreover, it is highly probable that we will find new, unimagined mechanisms of posttranscriptional control along the way.

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