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Revisiones

Aberrant mRNA Stability Regulation in Human Diseases

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ABSTRACT

mRNA stability is emerging as a fundamental and effective cellular tool to regulate gene expression at posttranscriptional levels. mRNA stability is controlled via orchestrated interactions between mRNA structural components (*cis*-elements) and specific *trans*-acting factors. The most widespread and efficient determinant of RNA stability are the adenylate and uridylate-rich elements (ARE) that, through binding of ARE-binding proteins (AUBPs), modulate the stability of transcripts and/or their translation. Alterations in any of these components can lead to disease. Here, we review the genetic alterations in 3'UTR regulatory sequences as well as the aberrant levels, subcellular localization, and posttranslational modifications of AUBPs that are linked to human diseases. A thorough understanding of these alterations and their impact on mRNA stability regulation will uncover promising new targets for therapeutic intervention.

Key words: Post-transcriptional gene regulation.—RNA-binding proteins.—AU-rich elements (ARE).—ARE-binding proteins (AUBPs).—Cancer.—Inflammation.—Thalassemia.—Alzheimer disease.

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RESUMEN

Regulación anormal de la estabilidad del ARNm en enfermedades humanas

La estabilidad del ARN mensajero está surgiendo como instrumento celular fundamental y efectivo para regular la expresión génica a nivel post-transcripcional. La estabilidad del ARNm se controla vía interacciones coordinadas entre componentes estructurales del ARNm (elementos *cis*) y factores *trans* específicos. Los determinantes de estabilidad de ARNm más conocidos y eficientes son los elementos ricos en adenina y uridina (ARE) que, a través de su unión con proteínas de unión a ARE (AUBPS), modulan la estabilidad de los transcritos y/o su traducción. Alteraciones en cualquiera de estos componentes puede dar lugar a enfermedades. Aquí revisamos las alteraciones genéticas en elementos regulatorios del 3'UTR, así como las aberraciones en los niveles, localización subcelular y modificaciones post-traslacionales de AUBPs que están asociadas a enfermedades humanas. Un conocimiento detallado de estas alteraciones y su impacto en la regulación de la estabilidad del ARNm revelará nuevas dianas para su aplicación terapéutica.

Palabras clave: Regulación génica post-transcripcional.—Proteínas de unión a ARN.—Elementos enriquecidos en AU (ARE).—Proteínas de unión a ARE (AUBPs).—Cáncer.—Inflamación.—Talasemia.—Enfermedad de Alzheimer.

INTRODUCTION

The regulation of gene expression is a fundamental cellular process that is controlled at multiple levels. While the study of gene regulation has traditionally focused on transcription as a major regulator of gene expression, it has recently become apparent that the posttranscriptional control of gene expression may play an equally important role. Posttranscriptional events comprise pre-mRNA processing, nucleo-cytoplasmic export, mRNA localization, mRNA stabilization and translational regulation (1). Due to the impact that each of these steps can have on gene expression, each of them is tightly regulated. The mechanisms underlying this regulation, still poorly understood, involve mRNA structural components (*cis* elements) and *trans*-acting factors [primarily RNA-binding proteins and non-coding RNAs (e.g., miRNAs)]. Therefore, alterations in any of these levels can have a profound impact on global RNA levels and have been related to numerous pathologies including cancer, inflammatory and autoimmune diseases, developmental defects, and neurodegenerative diseases (2-5).

The posttranscriptional processes that affect the mRNA are regulated by the orchestrated interactions between mRNA structural components (*cis* elements) and specific *trans*-acting factors. Well-characterized RNA sequence elements can be found throughout the body of mRNAs including the 5'-untranslated region (5'UTR), the coding region, and the 3'UTR (Fig. 1A). Most RNA sequence elements [e.g., the 5'-cap structure and the 3'poly(A) tail] are universally present in all mRNAs and direct constitutive processes without apparent selectivity of one mRNA relative to another. However, specific RNA elements have been identified which affect the stability and/or translation of given subsets of mRNAs. The vast majority of such specific RNA sequences are present in the 3'UTR; among them, the best characterized are regions rich in adenine and uridine residues known as AU-rich elements (ARE). AREs have been identified in the 3'UTR of a variety of short-lived mRNAs, including those encoding oncogenes and growth factors, cell-cycle regulatory proteins, cytokines, and inflammatory mediators. AREs frequently, though not always, contain a variable number of AUUUA pentamers, sometimes harbored within a U-rich region. The canonical motifs identified for RNA-biding proteins HuR and TIA-1 (6, 7) consist of a combination of primary sequence and secondary structure in the 3'UTR. AREs are well known to influence stability and are increasingly recognized to affect translation (8, 9). The RNA levels of many of ARE-containing mRNAs are altered in pathological situations such as cancer and inflammation due to abnormal mRNA stabilization and /or translation processes. It is crucial, therefore, to understand the regulation of these «ARE-containing genes» because of their demonstrated involvement in human diseases (2-5).

Cis-elements serve as binding sites for a variety of RNA-binding proteins that modulate posttrancriptionally mRNA levels. Of particular interest for this review is the family of RNA-binding proteins that associate to the ARE-determinants (AUBPs). AUBPs regulate, among other processes, the mRNA stability (in a positive way such as the mRNA-stabilizing HuR or negative, like the mRNA-destabilizing AUF1 and TTP) and translation (e.g., TIA inhibiting translation or HuR, enhancing mRNA translation) of ARE-containing mRNAs. At least 12 ARE-BPs (see Table 1) have been identified so far. Most AUBPs are predominantly nuclear proteins that shuttle

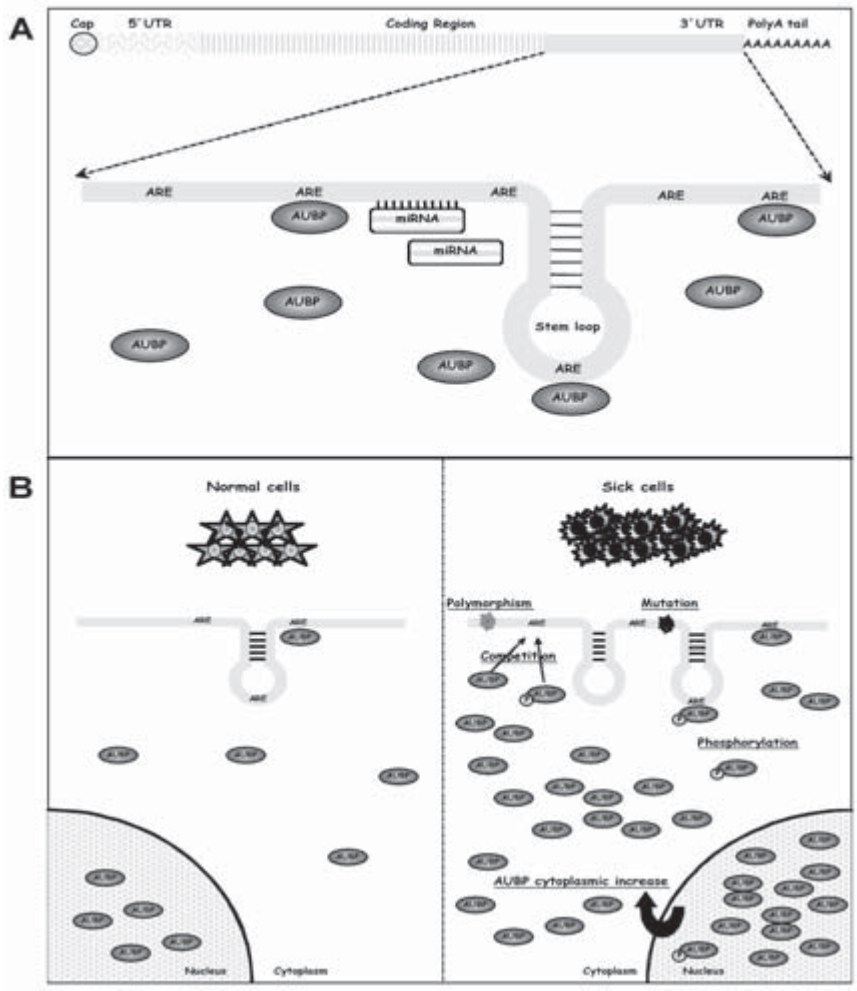

FIGURE 1. ***mRNA stability determinants and their alterations in human diseases. (A)*** *Schematic of the different regions in the mRNA. The 3'UTR is enlarged to show the cis-elements (e.g., AREs) that are found in this region and the trans-acting factors (e.g., AUBPs, and miRNA) that associate to them.* ***(B)*** *In defective cells, two main groups of alterations affect mRNA stability and/or translation: 1) Genetic alterations in ARE such as mutations, deletions, translocations and polymorphisms and 2) Altered regulation of AUBPS such as global increase of AUBS, changes in their subcellular localization (e.g., increased cytoplasmic levels of AUBPs in cancer), aberrant pattern of posttranslational modifications (e.g., phosphorylation) and altered competition for binding between AUBPs. AUBPs: AU-rich binding proteins. ARE: AU-rich elements. miRNA: microRNAs.*

between the nucleus and the cytoplasm. Their cytoplasmic presence appears to be intimately linked to their influence upon target mRNAs and hence AUBPs localization has been the subject of gene expression studies, specially in cancer. AUBS are also targeted for posttranslational modifications, influencing AUBP ability to bind to target mRNAs as well as their subcellular location. Increasing evidence supports the notion that several RNA-binding proteins can bind to a common ARE-containing target mRNA on both distict, nonoverlapping sites, and on common sites in a competitive fashion. It is becoming increasing apparent that the composition and fate (stability, translation) of ribonucleoprotein complexes depend on the target mRNA of interest, RNA-binding protein abundance, stress conditions, and subcellular compartment. While AUBPs regulate numerous posttranscriptional aspects of the mRNA (such as splicing, mRNA localization, and mRNA storage), this review will focus on the literature describing their influence on mRNA stability and translation (8-10).

Given the aforementioned involvement of 3'UTR *cis*-elements and *trans*-acting factors in dictating mRNA stability and translation, it is easy to envision how alterations in any of these components can have a major impact on mRNA half-life and/or translation. In turn, defective mRNA turnover can cause abnormal stabilization or decay of mRNAs, while disregulated translation can elevate or lower translation rates. Together, these anomalous processes will result in aberrant levels of expressed protein and hence metabolic changes leading to disease. Defective mRNA half-life and translation can arise from 1) Mutations in regulatory *cis*-elements (e.g., AREs) such as single-point mutations, large deletions/insertions and polymorphisms, and 2) alterations in *trans*-acting factors (e.g., AUBPs) such as defective expression and/or subcellular localization of *trans*-acting factor, altered pattern of posttranslational modifications, and aberrant competition among AUBPs which will ultimately influence their net influence upon the fate of the mRNA (stabilization, translation) (Fig. 1B). In human diseases, alterations in both *cis*-elements and *trans*-acting factors have been described (2-5). The purpose of this review is, therefore, to examine the altered mRNA stability and/or translation regulation mediated, mainly, by ARE determinants that can be found in human diseases. Identifying

such alterations and studying how they modify cell biology will help to better understand the mechanisms involved in human diseases and will facilitate the development of novel therapeutic modalities.

TABLE 1. *List of AU-rich binding proteins (AUBPs)*

RNA-binding protein	Official name	Gene Symbol & (Ref Seq)	Subcellular localization	Function	Target mRNAs (examples)
HuR	ELAV (embryonic lethal, abnormal vision, *Drosophila*)-like 1 (Hu antigen R)	ELAVL1 (NM_001419)	Mainly nuclear (ubiquitous)	mRNA stabilization (1), translational enhancer (2)	c-fos (1), c-myc (1), p21 (1), COX-2 (1), TNF-α (1), cyclin A, B1, D1 (1), iNOS (1), IL-3 (1), MyoD (1), p53 (2)
HuB	ELAV (embryonic lethal, abnormal vision, Drosophila)-like 2 (Hu antigen B)	ELAVL2 (NM_004432)	Mainly nuclear (neuronal and sex glands)	mRNA stabilization (1), translational enhancer (2)	GLUT1 (1 and 2), NF-M (2)
HuC	ELAV (embryonic lethal, abnormal vision, *Drosophila*)-like 3 (Hu antigen C)	ELAVL3 (2 isoforms: NM_001420; NM_032281)	Mainly nuclear (neuronal)	mRNA stabilization	VEGF, c-myc
HuD	ELAV (embryonic lethal, abnormal vision, *Drosophila*)-like 4 (Hu antigen D)	ELAVL4 (NM_021952)	Mainly nuclear (neuronal)	mRNA stabilization (1), translational enhancer (2)	GAP-43 (1), MARCKS (1), Msi-1 (1 and 2)
AUF1	heterogeneous nuclear ribonucleo-protein D	hnRNPD (4 isoforms: NM_031370; NM_031369; NM_002138; NM_001003810)	Isoforms p42 and p45 are nuclear; p37 and p40, nucleo-cytoplasmic	mRNA destabilizing	c-myc, GM-CSF, cyclin D1, GADD45, bcl-2, cyclin D1

Numbers in brackets of each individual target mRNAs refer to the matched function shown for each specific RNA-binding protein.

TABLE 1. *List of AU-rich binding proteins (AUBPs)* (cont.)

RNA-binding protein	Official name	Gene Symbol & (Ref Seq)	Subcellular localization	Function	Target mRNAs (examples)
TTP	Tristetraprolin	TTP (NM_003407)	Nuclear (20%) and cytoplasmic (80%)	mRNA destabilizing (1), decapping (2)	PAI-2 (1), TNF-α (1), COX-2 (1), GM-CSF (2)
TIA-1	cytotoxic granule-associated RNA binding protein	TIA1 (NM_022173)	Mainly nuclear	Translational represor (1), alternative RNA processing (2)	COX-2 (1), TNF-α (1), Fos (2)
TIAR	TIA1 cytotoxic granule-associated RNA binding protein-like 1	TIAL1 (NM_003252) KHSRP (NM_003685)	Mainly nuclear	Translational represor (1), alternative RNA processing (2)	IL-8 (1), iNOS (1), β2-AR (1), GADD45 (1), calcitonin/ CGRP (2)
KSRP	KH-type splicing regulatory protein		Mostly nuclear	mRNA destabilizing (1), RNA splicing (2)	c-fos (1), c-jun (1), IL-2 (1), TNF-α (1), iNOS (2)
BRF1	zinc finger protein 36, C3H type-like 1	ZFP36L1 (NM_004926)	Nuclear and cytoplasmic	mRNA destabilizing	cIAP2, IL-3
NF90	interleukin enhancer binding factor 3, 90kDa	ILF3 (3 isoforms: NM_004516; NM_012218; NM_153464)	Mainly nuclear	mRNA stabilization (1), Translational represor (2)	IL-2 (1), acid beta-glucosidase (2)
CUG-BP2	CUG triplet repeat, RNA binding protein 2	CUGBP2 (3 isoforms: M_001025076; NM_001025077; NM_006561)	Mainly nuclear	RNA editing (1), mRNA stabilization (2) and translational silencer (3)	Apolipoprotein B (1), COX-2 (2 and 3)

Numbers in brackets of each individual target mRNAs refer to the matched function shown for each specific RNA-binding protein.

DEFECTIVE mRNA STABILITY REGULATION IN HUMAN DISEASES

Several human diseases, outlined below, are associated to mutations in 3'UTR mRNA stability determinants or to alterations in the regulation of 3'UTR-binding regulatory proteins. Next, specifics examples will be given to illustrate both aspects.

Cancer

In cancer, most studies have sought to identify mutations in the coding region and very few naturally occurring mutations in noncoding areas have been described to date. Genetic alterations in 3'UTR sequences can modify the binding properties of *trans*-acting factors and lead to deregulation in protein production. The examples below illustrate the importance of the genetic alterations in AREs in promoting malignancy and its usefulness in determining cancer therapy. The first example described was for the oncogene c-fos and its viral counterpart v-fos. The coding region of both c-fos and v-fos is identical differing only in a missing 67-bp (that contains a ARE) in the v-fos 3'UTR. Consequently, v-fos mRNA is more stable than c-fos mRNA and this may account in part for its higher oncogenic potential (11). Other naturally alteration in AREs have been described such as the disruption of the 3'UTR during the integration of Human papillomavirus type 16 (HPV-16), 3'UTR rearrangement of Cyclin D1 and translocation and deletion of ARE in the proto-oncogene c-myc, all of them linked to malignant transformation. 3'UTR polymorphism also affect mRNA stability as it has been found for the thymidylate synthase (TYMS) gene. The polymorphism in the 3'UTR consists of the deletion (D)/insertion (I) of a 6-bp stretch (TTAAAG). The D allele showed decreased message stability compared to the I allele due to increased binding to the decay-promoting protein AUF1 (12). In agreement with this finding, colorectal tumors from D-allele carriers have decreased intratumoral TYMS mRNA levels (13) suggesting that the 3'UTR polymorphism can have an impact on the efficiency of TYMS-targeted chemotherapy treatment. Another relevant example is a novel single-nucleotide polymorphism (SNP) in the human dihydrofolate reductase (DHFR) gene that influences mRNA expression levels as well.

Other commonly found alteration in cancer is the frequently elevated levels of RNA-binding proteins as it has been documented for the mRNA-stabilizing protein HuR, and the destabilizing AUF1 and TTP in a wide variety of malignancies (6, 14-16). Depending on the type of tumor, AUBPs with similar functions (e.g., AUF1 and TTP) can have oncogenic or tumor suppressor activities (15, 16). These studies suggest that AUBP play a central role in cancer by

binding to mRNAs encoding proteins involved in malignant transformation, and inducing or repressing their expression by altering mRNA stability and/or translation rates. Moreover, the cytoplasmic abundance of AUBPs (e.g., HuR and AUF1) increases with malignancy (6, 17). This is particularly relevant since the cytoplamic presence of AUBPs seems to be linked to its stabilizing/destabilizing function. Wnt activation, the tumor suppressor protein von Hippel Lindau (VHL), and AMP-activated protein kinase (AMPK) are responsible for the cytoplasmic distribution of AUBPs and their pathways are also altered in cancer (18-20). Posttranslational modifications of RNA-binding proteins can affect their ability to bind to target mRNAs as well as their subcellular location as it has been shown with the phosphorylation of AUF1 and KSRP. Cell signaling events may also alter mRNA stability, translation and AUBP abundance. For instance, the MAPK pathway affects mRNA stability and translations through the differential phosphorylation of RNA-binding proteins (21). Importantly, several RNA-binding proteins can bind to a common ARE-containing target mRNA on both distict, nonoverlapping sites, and on common sites in a competitive fashion. For instance, IL-8 plays an integral role in promoting the malignant phenotype in breast cancer and its production is directly influenced by inflammatory cytokines in the tumor microenvironment. In keeping with this notion, activation of the IL-1 receptor on malignant breast cancer cells strongly induced IL-8 mRNA levels. HuR, KSRP and TIAR were found to bind to one or more locations within the IL-8 3'UTR although the association of the stabilizing factor HuR was 20-fold greater than that of the destabilizing factor KSRP (22).

Inflamation and autoimmunity

Several proteins that are encoded by ARE-containing transcripts are critical components of the effector phase of inflammatory and autoimmune diseases. Of particular importance is tumor necrosis factor α (TNF-α), one of the principal mediators of the inflammatory response in mammals. In addition to its well-known role in acute septic shock, it has been implicated in the pathogenesis of graft-versus-host disease, rheumatoid arthritis, Crohn's disease, and the cachexia that accompanies cancer and the acquired

immunodeficiency syndrome. Many evidences supports the impact of ARE sequences on the posttranscriptional regulation of TNF-α biosynthesis. Macrophages from mice with a genomic deletion of the TNF-α ARE exhibit spontaneous production of TNF-α and their TNF-α mRNA has a substantially prolonged half-life. Remarkably, they also spontaneously develop a chronic inflammatory arthritis and Crohn's disease-like intestinal inflammation. In these studies, it was shown that the absence of ARE-dependent translational control of TNF mRNA was associated with the inability of p38/SAPK and JNK/SAPK signalling pathways to regulate its translational activation. The binding of a number of AUBPs to the ARE sequences are directly responsible of the destabilization of the TNF-α mRNA. TIA-1 and TTP are AUBPs that prevent the pathological expression of tumor necroris factor alpha, TIA inhibiting its translation and TTP promoting the degradation of TNF-α transcripts and, in turn, functioning as arthritis suppressor genes (23). Therapies such as neutralizing antibodies to TNF-α and chimeric soluble TNF-α receptor have demonstrated efficacy against some of these conditions in clinical trials. Furthermore, the TNF-α ARE is also known to be a target for the mRNA stabilizing factor HuR, and mutations of this *cis*-element both impair HuR binding and decrease TNF-α protein production. Post-transcriptional mechanisms also regulate the production of other proteins involved in inflammatory responses such as cyclooxygenase-2 (COX-2) and matrixmetalloproteinase-13 (MMP-13). Pharmacologic inhibitors of COX-2 are potent, anti-inflammatory agents, which significantly reduce the severity of inflammatory arthritis.

Polymorphisms in the 3'UTR of multiple immune related mRNAs are also associated with the development of human autoimmune disease. For instance, deficient TCR ζ chain on the T cells of patients with systemic lupus erythematosus appears to be due to an alternatively spliced form of the ζ chain mRNA that has reduced stability. As a consequence, these cells display abnormal TCR-induced early signaling and have diminished IL-2 production (3).

Thalassemia

Thalassemia is a hereditary anemia resulting from defects in hemoglobin production. It is considered the most common genetic disorder worldwide. Thalassemia is clinically heterogeneous because multiple genetic lesions have been described to variably impair globin-chain synthesis. In the normal adult, hemoglobin A, which is composed of two alpha and two beta globins ($\alpha 2\beta 2$), is the most prevalent, comprising about 95% of all hemoglobin. The thalassemias are classified according to which chain of the globin molecule is affected. An α-globin gene variant, a constant spring (α^{cs}), is the most common cause of nondeletional α-thalassemia worldwide. The α^{cs} mutations harbors a single nucleotide substitution at the wild-type α-globin mRNA (α^{WT}) translational stop codon (UAA to CAA). This mutation allows the ribosomes to translate into the normally ribosome-free 3'UTR, causing a major decrease in α-globin mRNA half-life. Searching for the mechanisms responsible for the accelerated decay of the α^{cs} mRNA, Morales and colleagues found that the α^{cs} mRNA poly(A) tail was significantly shorter that the α^{WT} mRNA poly(A) tail. Therefore, reduced α^{cs} mRNA half-life appeared to be linked to accelerated 3' terminal deadenylation (24). Moreover, insertion of a stop codon into the α^{cs} immediately upstream of the α^{cs} mutation prevented ribosome entry into the 3'UTR and stabilized the α^{cs} mRNA. This data supported a model in which a stability determinant was present in the 3'UTR and could be affected by the elongating ribosome (25). In fact, analysis of this region identified three cytosine-rich (C-rich) segments [also known as pyrimidin-rich element (PRE)] that contributed to α-globin mRNA stability when studied in transfected erythroid cells. Subsequently *in vitro* studies demonstrated assembly of a sequence-specific ribonucleic-protein (RNP) complex at this site. Members of the α-globin poly(C)-binding protein (αCP) were identified as essential protein components of the α-complex. In patients who have the α^{cs} mutant, read through translation of the 3'UTR prevents the PCBPs from binding to the C-rich elements. *In vitro* studies suggested that additional proteins may also contribute to α-complex structure and/or function. Of particular interest was the identification of the ARE binding/degradation factor AUF1 and poly(A)-binding protein (PABP) as an interacting partners of αCPs (25).

Alzheimer disease

Dysregulated synthesis and deposition of extracellular amyloid-beta (Aβ) within the central nervous system is a major characteristic of Alzheimer disease (AD). Aβ is derived from proteolytic processing of one of multiple amyloid precursors protein (APP) isoforms. Cleavage of the APPs generates the β/A4 peptide, the major component of amyloid in senile plaque. In addition to the altered Aβ levels, APP mRNA levels are elevated in brain tissue of AD patients, and transgenic mice that overexpress APP mRNA and protein have accelerated Aβ deposition. The 3'UTR of APP mRNA contains a contiguous 29-base C + U-rich region that appears necessary and sufficient to control the degradation of the mRNA. Purification studies identified two RNA-binding proteins that recognize the APP sequence element (26). One is nucleolin and the other the heterogeneous nuclear ribonucleoprotein C (hnRNP C), a nuclear protein that binds U-rich RNA sequences. In cell-free mRNA decay assays, addition of nucleolin accelerates degradation of APP mRNA, while addition of hnRNP C stabilizes APP mRNA (27). Recently, immunohistochemical studies have found the presence of other AUBPs, hnRNP A2 and B1, in brains of patients with AD. In any event, like ARE-mRNAs, the cellular decay rate of APP mRNA may reflect a competition between destabilizing versus stabilizing proteins. One might envision that tipping the balance to stabilization might predispose neural tissue to APP overproduction and possibly AD (28).

Viral infections

The ability to regulate cellular gene expression is a key aspect of the life-cycles of a diverse array of viruses. In fact, viral infection often results in a global shutoff of host cellular gene expression, being host and viral mRNA stability control critical during viral infection. For instance, simple herpes virus (HSV-1) achieves host-shutoff through the complementary actions of two viral proteins, ICP27 and virion host shutoff (vhs), that inhibit cellular mRNA biogenesis and trigger global mRNA decay, respectively. Although most cellular mRNAs are thus depleted, some instead increase in

abundance after infection; perhaps surprisingly, some of these contain AU-rich instability elements (AREs) in their 3'-untranslated regions. ARE-containing mRNAs normally undergo rapid decay; however, their stability can increase in response to signals such as cytokines and virus infection that activate the p38/MK2 mitogen-activated protein kinase (MAPK) pathway. HSV-1 infection stabilizes the ARE mRNA encoding the stress-inducible IEX-1 mRNA. Whether the IEX-1 mRNA stabilization is carried out by vhs or through the activation of p38 by ICP27 remains to be clarified (29).

The presence of AU-rich elements in the 3'UTR of viral genomes adds an additional level of regulation. Thus, direct interactions between RNA-binding proteins and viral RNA can alter viral RNA stability and viral protein expression in a manner favorable for viral survival. AU-rich elements have been identified in the late 3'UTR of the human papilloma virus (HPV-1) and HPV31 genomes, in the E6 and E7 oncogenes of HPV-16, in the 3'UTR of hepatitis C virus and certain alpaviruses and in the 5'UTR AU-rich region of the gag gene of human immmunodeficiency virus (HIV-1). Several of these regions are binding sites for HuR and the stability of the RNAs is inversely proportional to the levels of cellular HuR expression (30).

Other diseases

Alterations in the regulation of mRNA stability processes in other types of diseases have been reported as well. For instance, mutations that increase mRNA degradation provoking the absence of factor VIII mRNA (F8) in haemophilia A (31); binding of the Y box-binding factor-1 (YB-1) to ARE in GM-CSF mRNA, enhancing GM-CSF-dependent survival of eosinophils that are central in the pathogenesis of asthma (32); polymorphisms between 2 ARE that increase protein binding to the allele ARE2, causing a faster degradation of PPP1R3 mRNA that, in turn, lowers the concentration of the protein implicated in insulin resistance, and thus increasing the risk for development of type 2 diabetes (33); and finally, it has been found reduced expression of sGC subunits in animal models of genetic hypertension because of reduced binding to the mRNA-stabilizing protein HuR compared to normal animals (34).

mRNA STABILITY AS A TARGET FOR THERAPY

By gaining a more detailed knowledge of the 3'UTR regulatory sequences and the *trans*-acting factors specifically binding to them it will be possible to design effective therapies. As noted earlier, a 6-bp polymorphism in the 3'UTR of thymidylate synthase decreases mRNA levels (13) and, thereby could be useful in predicting the efficacy of TYMS-targeted chemotherapy treatment. TNF-α is effective in the treatment of advanced solid tumors such as melanoma and soft tissue sarcoma. When analyzing mRNA levels of 22 genes in tumor biopsies from patients treated with doxorubicin alone or combined with TNF-α, TIA-1 was the only gene differentially expressed between the two groups. When TNF-α effects were tested *in vitro* in endothelial cells, fibroblasts, CTLs and NK cells, TIA-1 became upregulated only in endothelial and NK cells. These findings could indicate that TNF-α -induced TIA-1 overexpression might sensitize endothelial cells to proapoptotic stimuli present in the tumor microenvironment and enhance NK cell cytotoxic activity against cancer cells (35). The chemotherapeutic agent Prostaglandin A_2 (PGA_2) causes growth arrest associated with decreased cyclin D1 in several cell lines. PGA_2 leads to the destabilization of cyclin D1 mRNA via a 3'UTR element that binds the RNA-binding protein AUF1 (36). These studies underscore the potential importance of understanding 3'UTR regulation in cancer therapy.

Alteration on mRNA stability rates might possibly be cured by pharmacological approach by correcting the stabilization/ degradation mRNA rates. The specific approach, eventually, would be targeting the correct mRNA since AUBPs bind to a wide variety of mRNA targets and exogenous compound might affect a broad array of genes. If there is an increased in mRNA stabilization, in order to deacrease the RNA levels, the ARE subregion can be targeted by antisense oligodeoxynucleotides (ODNs) or ribozymes. By contrast, when the mRNA is destabilized by mutations or altered binding to RNA degradation-promoting AUBPs, synthetic oligoribonucleotides (ORNs) are worth considering since they do not activate catabolic processes like ODNs. The hybridization of ORN to ARE sequences might insulate/protect ARE from «contact» with cellular *trans*-acting factors (AUBPs) and the exosome (machinery

responsible for mRNA degradation). More interesting will be the use of sense-oriented ORNs that are no supposed to interact with the homologous RNA but might compete for the sequestration of *trans*-acting factor and exosome, inhibiting in this way the target mRNA degradation (37).

The p38 MAPK signaling pathway plays an important role in inflammation and other physiological processes. There are four p38 MAPKs: α and β, which are 75% homologous, and γ and δ wich are more distant relatives. Inflamatory stimuli activate four major intracellular signaling pathways: the nuclear factor-κB (NFκB) pathways and the three MAPK pathways (ERK, JNK and p38). All four drive transcription of inflammatory genes. The p38 pathway is also involved in posttranscriptional regulation, and stabilizes inflammatory response mRNAs and promotes their translation through ARE in the 3'UTR of the mRNAs. Posttranscriptional regulation of TNF-α and cyclooxygenase-2 mRNAs by p38 have been extensively investigated and several examples were already shown in this review. Because inhibiting p38 MAPK suppresses production of key inflammatory mediators, it was obvious to search for inhibitors of p38 MAPK for therapy of chronic inflammatory diseases such as rheumatorid arthritis, Crohn's disease, chronic obstructive pulmonary disease and psoriasis. The most widely used are pyridinyl imidazole compounds such as SB203580 and SB202190. The potential of p38 as a drug target has led to development of large collection of inhibitors by pharmaceutical companies. Preclinical studies have largely used model of arthritis, and several inhibitors are now in human trials. Adverse effects of inhibition are hard to predict but immunosupression is likely. Targets upstream or downstream could also be used to block the MAPK pathway although to date they have not been reported (38).

Furthermore, a number of approaches that exploit RNA's structural dynamics and sequence-specific binding abilities (RNA interference, antisense RNA) are already in place to modulate gene expression. However, there is increasing need for developing synthetic riboregulators that can be integrated into biological networks to function with a wide array of genes and yield insights into RNA-based cellular processes. Isaacs and colleagues were able to engineer riboregulators that both repress and activate translation

in vivo, enabling precise control of gene expression through highly specific RNA-RNA interactions (39). Moreover, the administration of riboregulators (3'UTR frаments) such as the one from the prohibitin RNA, effectively controlled tumor cellular proliferation *in vivo* and induced systemic antitumor immunity in rat models (40).

FUTURE PERSPECTIVES

The examples given above represent compelling evidences for the involvement of alterations in 3'UTR mediated functions in the pathogenesis of different diseases and emphasize the notion that control exerted via the 3'UTR mRNA is crucial for the correct regulation of gene expression. A systematic search for «3'UTR-mediated diseases» is needed since, in the past, virtually all efforts were focused on the coding region. For instance, the involvement of the 3'UTR in controlling mRNA translation during both male and female gametogenesis and in early embryonic development, and as such post-transcriptional controls are essential to these processes, it is a reasonable assumption that certain reproductive disorders will be found to belong to this class of diseases. Additionally, the identification of putative additional «3'UTR-mediated diseases» will require the improvement and development of new methods *in vitro* and *in vivo* to assess the interaction between *cis*-elements and *trans*-acting factors as well as their kinetics. A more detailed understanding of 3'UTR regulatory events through increased efforts directed towards the study of this region and the development of adequate analytical methods will aid in the design of novel chemotherapeutic venues.

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