



HAL
open science

AU-rich elements and associated factors: are there unifying principles?

Carine Barreau, Luc Paillard, Howard Beverley Osborne

► **To cite this version:**

Carine Barreau, Luc Paillard, Howard Beverley Osborne. AU-rich elements and associated factors: are there unifying principles?. *Nucleic Acids Research*, 2005, 33 (22), pp.7138-50. 10.1093/nar/gki1012 . inserm-00292935

HAL Id: inserm-00292935

<https://inserm.hal.science/inserm-00292935v1>

Submitted on 3 Jul 2008

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Nucleic Acids Research

AU-rich elements and associated factors: are there unifying principles?

Carine Barreau, Luc Paillard and H. Beverley Osborne

Nucleic Acids Res. 33:7138-7150, 2005.

doi:10.1093/nar/gki1012

Supplement/Special Issue	This article is part of the following issue: " <i>Supplementary Material</i> " http://nar.oxfordjournals.org/cgi/content/full/33/22/7138/DC1 The full text of this article, along with updated information and services is available online at http://nar.oxfordjournals.org/cgi/content/full/33/22/7138
References	This article cites 181 references, 130 of which can be accessed free at http://nar.oxfordjournals.org/cgi/content/full/33/22/7138#BIBL
Cited by	This article has been cited by 15 articles at 3 July 2008 . View these citations at http://nar.oxfordjournals.org/cgi/content/full/33/22/7138#otherarticles
Supplementary material	Data supplements for this article are available at http://nar.oxfordjournals.org/cgi/content/full/33/22/7138/DC1
Reprints	Reprints of this article can be ordered at http://www.oxfordjournals.org/corporate_services/reprints.html
Email and RSS alerting	Sign up for email alerts, and subscribe to this journal's RSS feeds at http://nar.oxfordjournals.org
PowerPoint® image downloads	Images from this journal can be downloaded with one click as a PowerPoint slide.
Journal information	Additional information about Nucleic Acids Research, including how to subscribe can be found at http://nar.oxfordjournals.org
Published on behalf of	Oxford University Press http://www.oxfordjournals.org

SURVEY AND SUMMARY

AU-rich elements and associated factors: are there unifying principles?

Carine Barreau, Luc Paillard and H. Beverley Osborne*

UMR 6061 CNRS Génétique et Développement, IFR 140 Génétique Fonctionnelle Agronomie et Santé, Université de Rennes 1, Faculté de Médecine, CS 34317, 35043 Rennes cedex, France

Received September 17, 2005; Revised November 5, 2005; Accepted November 25, 2005

ABSTRACT

The control of mRNA stability is an important process that allows cells to not only limit, but also rapidly adjust, the expression of regulatory factors whose over expression may be detrimental to the host organism. Sequence elements rich in A and U nucleotides or AU-rich elements (AREs) have been known for many years to target mRNAs for rapid degradation. In this survey, after briefly summarizing the data on the sequence characteristics of AREs, we present an analysis of the known ARE-binding proteins (ARE-BP) with respect to their mRNA targets and the consequences of their binding to the mRNA. In this analysis, both the changes in mRNA stability and the lesser studied effects on translation are considered. This analysis highlights the multitude of mRNAs bound by one ARE-BP and conversely the large number of ARE-BP that associate with any particular ARE-containing mRNA. This situation is discussed with respect to functional redundancies or antagonisms. The potential relationship between mRNA stability and translation is also discussed. Finally, we present several hypotheses that could unify the published data and suggest avenues for future research.

INTRODUCTION

The regulation of mRNA stability and translation are essential in the control of gene expression [reviewed in (1,2–4)] and regulation of these two processes allows a cell to rapidly respond to changes in intracellular and extracellular stimuli. A number of sequence elements control the half-life of a mRNA either by stimulating or inhibiting degradation. In mammalian cells the sequence elements rich in adenosine and uridine, called AU-rich elements (AREs), were identified

by their ability to target host mRNAs towards rapid degradation. In general, these mRNAs encode proteins that regulate either cell growth or the response of an organism to external factors such as micro-organisms, inflammatory stimuli and environmental factors. Such genes require a very precise control of their spatial and temporal expression patterns which is achieved, in addition to a transcriptional control, by a regulation of the translation and the stability of the mRNA (5–8). In resting or unstimulated cells the ARE-dependent degradation mechanism ensures a very low level of expression of these potent proteins. The importance of this repression in the resting state is testified by the observation that pathological states (cancers, chronic inflammations and auto-immune pathologies) are associated with a deregulation of the stability of ARE-containing mRNAs [(9–20), reviewed in (21,22)].

The sequence motifs that form an ARE were first identified within the 3'-untranslated regions (3'-UTR) of mRNAs encoding several cytokines or lymphokines (23) and, since, the list of mRNAs that contain such motifs has considerably lengthened. It has been estimated that 5–8% of human genes code for ARE-containing mRNAs; the corresponding proteins perform a variety of functions implicated in numerous transient biological processes (24,25). This gene-based analysis underscores the importance of AREs in the regulation of gene expression as only a subclass of AREs were taken into account.

DISCOVERY OF AREs AND SEQUENCE MOTIFS

AREs as mRNA destabilizing elements

AREs are sequence elements of 50–150 nt that are rich in adenosine and uridine bases. They are located in the 3'-UTRs of many but not all mRNAs that have a short half-life and have been identified by their capacity to provoke degradation of the host mRNA by a mechanism dependent on deadenylation [shortening of the poly(A) tail] [reviewed in (26,27)]. However, depending on the cellular context and the

*To whom correspondence should be addressed. Tel: +33 223 23 4523; Fax: +33 223 23 4478; Email: Beverley.osborne@univ-rennes1.fr

precise stimulus, the presence of an ARE can also lead to the stabilization of a mRNA (see below). For historical reasons it is generally considered that the term ARE is reserved for sequence elements that confer instability and/or contain AUUUA motifs. Consequently, there are regulatory RNA elements, rich in A and U nucleotides, that are not called AREs. The first functional demonstration of ARE-dependent mRNA degradation was obtained by studying, in transfected cells, the stability of a globin mRNA into which the ARE from the granulocyte-macrophage colony stimulating factor (GM-CSF) mRNA had been inserted (28). Since, inserting a putative ARE into the 3'-UTR of an otherwise stable reporter RNA has become the classical experimental approach to study cellular or artificial AREs. Many of these studies used Actinomycin D or 5,6-dichloro-1- β -D-ribofuranosyl-benzimidazole (DRB) to block transcription and thereby allow degradation rates to be measured. However, these drugs can cause a number of artefacts and even stabilize the mRNA (29). Wilson and Treisman (30) and Shyu *et al.* (31) have circumvented this problem by using the c-fos promoter to drive transient transcription of a reporter gene in transfected cells after serum stimulation. The use of the c-fos promoter to drive transient expression is however not amenable to all experimental situations. This led Xu *et al.* (32) to develop a protocol using the tetracycline-regulatory promoter system to produce a pulsed synthesis of the mRNA under study.

ARE sequence features

The first AREs identified highlighted the presence of AUUUA pentamers, often overlapping, and frequently found within U-rich regions of the 3'-UTR [reviewed in (26)]. Many studies have since shown that the AUUUA motif and a certain uridine enrichment are two important characteristics of an ARE, but also, that they cannot fully explain the destabilizing activity of an ARE (33–37). The minimal sequence motif necessary to increase the turnover of chimeric mRNAs is the nonamer UUAUUUA(U/A)(U/A) (36,37), but only a modest effect on the stability of a reporter mRNA is produced by the insertion of a single nonamer in the 3'-UTR (36,37). Systematic mutagenesis of the UUAUUUAUU motif in GM-CSF ARE confirmed that two or four copies of the nonamer motif were more efficient in promoting deadenylation and instability than a single copy of this motif (38). However, mutagenesis of the single UUAUUUAUU present in the c-fos ARE does not significantly decrease its ability to destabilize a mRNA (33) suggesting that the UUAUUUA(U/A)(U/A) motif may be essential for only a subset of AREs. This notion is reinforced by the observation that a consensus recognition sequence for the ARE-binding proteins (ARE-BPs) HuR and TIA-1, derived from large scale screens of the endogenous targets, although U-rich did not contain a UUAUUUAUU motif (39,40).

Classification of AREs

Based on the number and the distribution of AUUUA pentamers, AREs have been grouped into three classes (26). A number of these are listed in Table 1 and are grouped according to the two classification methods that have been proposed (24,26). Class I AREs contain several dispersed copies of the AUUUA motif within U-rich regions. Class II AREs possess at least 2 overlapping UUAUUUA(U/A)(U/A) nonamers.

Bakheet *et al.* (24,25) have constructed a database of mRNAs containing class II AREs and these regulatory elements were divided into five groups, three of which are presented in Table 1. The classification in this ARE database (<http://rc.kfshrc.edu.sa/ared/>) is based on the repetition pattern of the AUUUA pentamer.

Class III AREs are much less well defined, they are U-rich regions but contain no AUUUA motif. The best documented example of a type III ARE is that situated within the 3'-UTR of c-jun mRNA (35,41,42).

It is necessary to note that no real consensus sequence has yet been precisely defined for any class of ARE and, furthermore, these ARE classifications are based neither on the associated proteins nor on biological functions. However, it is interesting to note that most of the mRNAs containing class II AREs encode cytokines whereas mRNAs encoding transcription factors and cell cycle regulatory proteins mostly contain class I and occasionally class III AREs (Table 1). Also inhibition of transcription with Actinomycin D or DRB stabilized mRNAs containing class I or II AREs (29) but had only a modest effect on the decay kinetics of a mRNA with a class III ARE (41). Therefore, although the initial classification was not based on biological function it could be that they group together mRNAs controlled by similar pathways or encoding proteins with associated functions.

ROLE OF ARE-BPs IN RNA DEGRADATION

A great deal of attention has been given to cellular proteins that bind to AREs as regulators of mRNA stability. Identification of ARE-BPs and analysis of their contributions to the ARE-dependent degradation of targeted mRNAs started ~15 years ago and is still continuing [reviewed in (27,43,44)]. As would be expected for proteins involved in controlling the stability of proto-oncogenes or cytokines, changes in the expression level of certain ARE-BPs have been implicated in the development of cancers [reviewed in (22)].

Although the AREs present in the many individual mRNAs listed in Table 1 are different in sequence, it is obvious from the summarized data that most are able to bind more than one ARE-BP. In some instances [e.g. c-myc, c-fos, GM-CSF, tumour necrosis factor α (TNF- α) and cyclooxygenase 2 (Cox-2)] the same ARE-containing mRNA can bind to many of the known ARE-BPs. Furthermore, many of the ARE-BPs have been observed to bind to multiple mRNAs and this binding crosses the ARE classification limits. Therefore, at present no clear segregation of certain ARE-BPs or structurally related ARE-BPs with specific types of AREs appears possible. It should also be noted that in many cases listed in Table 1 where an association between an ARE-BP and multiple mRNAs has been reported, these demonstrations depended principally on *in vitro* analyses such as UV-crosslinking or electrophoretic mobility shift assays (EMSA) (see table in Supplementary Data). Only a few studies have analysed associations between endogenous mRNAs and proteins (indicated by an asterisk in Table 1). Despite this reserve, the multiplicity of possible associations between ARE-BPs and AREs brings forward the question of binding specificity and functional redundancy/additivity or antagonism that is discussed latter in this survey.

Table 1. ARE-binding proteins that associate with AREs in mammals

miRNAs Class	Motif	Examples	ARE-BPs AUFI hmRNP D	HuR HuA	Hel-N1 HuC HuB	HuD TTP	BRF1 TIA-1	TIAR KSRP AUH GAPDH Hsp70	Hsp110	hmRNP A1	hmRNP A2	hmRNP A3	hmRNP C	hmRNP L	CUG-BP1 CELF1 ETR-3	CUG-BP2 CELF2 ETR-3	Nucleolin TINO PAIP2		
I	1 to 5 dispersed AUUUA motifs, in an U-rich context	c-myc	(46)	(155)	(157)* (78)	(78)			(158) (163)	(159)									
			(47)	(156)															
		c-fos	(42,67)	(63)	(157)	(160)	(90)	(93)											
			(47)	(67)		(161)													
			(53)	(70)															
			(154)	(71)															
		Beta I-AR PTH Interferon-gamma MyoD p21	(162)	(103)															
			(162)	(155)															
			(162)	(56)															
			(48)*	(68)*															
(167)	(68)*			(164)															
(48)*	(69)																		
Cyclin A Cyclin B1 Cyclin D1	(48)*	(70)																	
	(165)	(70)																	
	(166)	(70)																	
	(102)	(64)																	
	(103)	(101)																	
	(138)	(101)		(157)															
GM-CSF	(42)	(84)																	
	(47)	(85)																	
	(54)*	(101)																	
	(167)	(85)																	
	(94)	(84)																	
	(167)	(85)																	
TNF-alpha	(169)	(86)																	
	(167)	(89)																	
	(171)	(87)																	
	(172)	(87)																	
	(139)*	(139)*																	
	(172)	(172)																	
IL-2 IL-3 bcl-2	(167)	(82)																	
	(174)	(82)																	
	(176)	(92)																	
	(159)	(159)																	
	(158)	(158)																	
	(175)	(175)																	
Interferon-beta VEGF c-jun GLUT1 p53	(171)	(87)																	
	(172)	(87)																	
	(139)*	(139)*																	
	(172)	(172)																	
	(173)	(172)																	
	(100)*	(82)																	
No AUUUA, U-rich region	(174)	(92)																	
	(176)	(92)																	
	(62)	(78)																	
	(72)	(78)																	
	(99)*	(93)																	
	(137)*	(93)																	
Id hsp70 Myogenin NF-M GAP-43	(181)*	(179)																	
	(68)*	(73)*																	
	(180)	(180)																	
	(177)*	(177)*																	
	(155)	(155)																	
	(178)	(178)																	

A matrix presentation has been used to represent the identified associations between ARE-BPs and ARE-containing mRNAs. The mRNAs containing identified functional AREs are listed vertically and are grouped according to the classifications proposed by (24) and (26). The ARE-BPs are displayed horizontally. Where appropriate the different names used to denote the same protein or mRNA are given. The lists of ARE-containing mRNAs and of ARE-BPs are not exhaustive and only direct interactions have been considered. Where the experimental methods used identified endogenous interactions, these are indicated by an asterisk. Data on the experimental methods are presented in the Supplementary data. Numbers correspond to listed references.

Table 2. Effect of ARE-BPs on the stability and translation of ARE-containing mRNAs

ARE-BPs	mRNA stability		Protein expression		Abundance Up regulated	Down regulated
	Increase	Decrease	Translational efficiency Increase	Decrease		
AUF1	c-myc (42) c-fos (42,67) PTH (56) GM-CSF (42) TNF-alpha (42)	c-myc (46) c-fos (53) p21 (48) Cyclin D1 (48) GM-CSF (53,54) IL-3 (55)				GM-CSF (55) IL-3 (55)
HuR	c-fos (59,63,67) MyoD (68) p21 (48,68,69) Cyclin A (70) Cyclin B1 (70) Cyclin D1 (48) NOS II/iNOS (64) GM-CSF (59) TNF-alpha (65,74,139) Cox-2 (71,139) IL-3 (55,66) VEGF (62) Myogenin (68)		p53 (99,137)	TNF-alpha (139) Cox-2 (139)	p21 (69) Cyclin A (70) Cyclin B1 (70) NOS II/iNOS (64) GM-CSF (55) Cox-2 (71,173) IL-3 (55) VEGF (173) p53 (99,137)	TNF-alpha (139)
Hel-N1	TNF-alpha (74) GLUT1 (72)		NF-M (73) GLUT1 (72)		NF-M (73) GLUT1 (72) GAP-43 (75,76)	
HuD	GAP-43 (75-77)					
TTP		c-fos (90) GM-CSF (18, 81,83-85, 91) TNF-alpha (18,81,83-86,89,90) Cox-2 (87) IL-2 (82,90) IL-3 (18, 66, 83,84, 88)				GM-CSF (81) TNF-alpha (80) IL-2 (82) IL-3 (88)
BRF1		TNF-alpha (89,93) IL-3 (55,92,93)				GM-CSF (55) IL-3 (55)
TIA-1				TNF-alpha (120) Cox-2 (121)		TNF-alpha (120) Cox-2 (121)
KSRP		c-fos (90,93) NOS II/iNOS (102) TNF-alpha (90,93) IL-2 (90,93) c-jun (93)				NOS II/iNOS (102)
CUG-BP2	Cox-2 (150)			Cox-2 (150)		Cox-2 (150)
Nucleolin	bcl-2 (175)					
TINO		bcl-2 (176)				
PAIP2	VEGF (177)				VEGF (177)	

The listed ARE-BPs correspond to those in Table 1 for which effects on mRNA stability and protein expression levels (translational efficiency or steady-state abundance) have been reported. The mRNAs whose stability is increased (stabilized) or decreased (degraded) are indicated for each ARE-BP. The stability of TNF- α and Cox-2 mRNA or NF-M mRNA were not modified by TIA-1 or Hel-N1, respectively. The several mRNAs for which increased or decreased expression of the encoded protein has been reported are similarly indicated. Increased or decreased translational efficiency was measured either by polysome analysis or methionine incorporation. Changes in protein expression classified as abundance were determined on steady-state levels either by western analysis or measurement of associated enzymatic activities. Numbers correspond to listed references.

In a number of cases, the consequences of ARE-BP binding to a particular mRNA have been studied either with respect to the changes in mRNA stability or translation, (Table 2). For a small proportion of the ARE-BPs the effects on mRNA stability and translation have been investigated simultaneously. The results from these studies are summarized below for AUF1, the Hu family, in particular HuR, and Tristetraprolin (TTP) that have been the most extensively studied.

AUF1

This protein was the first ARE-BP to be isolated and for which a role in controlling mRNA stability was demonstrated (45-47). AUF1, also named hnRNP D (heterogeneous nuclear ribonucleoprotein D), is essentially nuclear (47,48), but it can

shuttle between the nuclear and cytoplasmic compartments (49-52). It binds to both class I and II AREs but no example of a class III ARE associated with AUF1 has been reported (Table 1).

The causality of the correlation between AUF1-binding and decreased mRNA stability has been demonstrated by experiments using either over expression (53,54) or depletion (by siRNAs) (48,55) of AUF1 in cells or modulation of the AUF1 content in cytoplasmic extracts (46) (Table 2). In one study (55) the increased stability of IL-3 mRNA caused by a siRNA knock-down of AUF1 led to an increased expression of the encoded protein. This is coherent with AUF1 targeting these mRNAs for degradation and consequently decreasing the expression of the encoded proteins. However, AUF1-binding has also been associated with increased mRNA

stability (Table 2). In the case of Parathyroid hormone mRNA (56), which contains a class I ARE (Table 1), the effect is seen in a particular situation, cytoplasmic extracts of rat parathyroid. In the study by Xu *et al.* (42), the stabilizing effect of AUF1 over expression (all isoforms) in NIH-3T3 cells was most evident for class II AREs (TNF- α and GM-CSF), less pronounced for the class I AREs (c-myc and c-fos) and absent for class III ARE (c-jun and a synthetic non-AUUUA ARE). As indicated by Xu *et al.* (42) the effect of AUF1 on mRNA stability may be cell type specific: destabilizing in K562 proerythroblast cells (46,53) and stabilizing in NIH-3T3 fibroblast cells (42). However, Sarkar *et al.* (54) reported that over expression of the p37 isoform of AUF1 did not stabilize reporter mRNAs containing the GM-CSF ARE in a variety of cell type including NIH-3T3 cells. At present there is no clear explanation for this discrepancy.

Hu family

The mammalian genome encodes four closely related proteins (HuR/HuA, Hel-N1, HuC and HuD) that are part of a super family of elav-related proteins (57). HuR, which is expressed ubiquitously, has been the most extensively studied. The expression of the other Hu proteins is restricted to neurons except for Hel-N1 that is also expressed in gonads [reviewed in (58)]. HuR, like AUF1, has a predominantly nuclear localization and can shuttle between nucleus and cytoplasm (59,60). This shuttling may have a functional role as HuR has been shown to serve as an adaptor for the nuclear export of a class I ARE mRNA, c-fos (61).

HuR has been observed to bind to a large number of RNAs of all three ARE classes (Table 1). For a number of these mRNAs, the effect on their stability has been studied by modulating intracellular HuR either by ectopically expressing or over expressing HuR (59,62–68) or by siRNA or anti-sense RNA knock-down (48,55,62,64,69–71) (Table 2). In all these cases the data show that HuR-binding and increased mRNA stability are causally related.

Over expression of both Hel-N1 (72–74) and HuD (75–77) have also been correlated with an increased stability of the target mRNA and expression of the encoded protein (Table 2). The binding of HuC to c-myc and VEGF mRNAs was not correlated with a functional change to the mRNA (78).

TTP

In contrast to AUF1 and HuD the binding specificity of TTP appears to be restricted to mRNAs containing class II AREs (Table 1). Another difference is that TTP is predominantly cytoplasmic (18,79). The demonstration that TTP-binding can cause degradation of the target mRNA has been greatly helped by the use of TTP knockout mice. In TTP deficient mice increased protein production and/or mRNA was observed for TNF- α in foetal liver derived macrophages (80), for TNF- α and GM-CSF in bone marrow precursor cells and macrophages (18,80,81) and for IL-2 in splenocytes and T-cells (82). Further *in vitro* studies from several laboratories showed that the degradation of mRNAs encoding GM-CSF (18,83–85), TNF- α (18,83,86), Cox-2 (87) and IL-3 (18,66,83,88) was dependent on the amount of TTP expressed. Experiments using cell free extracts confirmed (83–85,89,90) and extended these observations to c-fos (90) mRNAs. TTP

mutants that do not bind to the target mRNA are ineffective in stimulating degradation (84,85) whereas tethering the domain of TTP required to activate degradation to a mRNA enhances the degradation of an otherwise stable reporter mRNA (91). By a systematic mutation of TTP-binding sites in GM-CSF ARE, Lai *et al.* (38) showed that maximum deadenylation and degradation rates requires the binding of two TTP molecules to the mRNA.

FUNCTIONAL RELATIONSHIPS BETWEEN ARE-BPs

Redundant or additive effects

As indicated in Table 2, most of the identified ARE-BPs, with the exception of the Elav-like proteins (HuR, Hel-N1 and HuD), CUG-BP2, Nucleolin and PAIP2, have been implicated in the degradation of mRNAs containing AREs. Some of these factors are co-expressed in the same cells and show overlapping binding affinities [reviewed in (27,44)]. However, the depletion of specific ARE-BPs can cause the stabilization of sub-populations of ARE-containing mRNAs in certain cell types (18,81,92,93). Nevertheless, it is not clear whether these proteins are functionally redundant or if each protein targets specific sub-populations of ARE-containing mRNAs for degradation. One possible example of a partial functional redundancy between two ARE-BPs is that of KSRP and BRF1. *In vitro* and *in vivo* studies have shown that a loss of either KSRP or BRF1 proteins from HT1080 or HeLa cells leads to a partial stabilization of a reporter mRNA containing an ARE. However, the simultaneous decrease of both proteins increases, additively, the stability of the reporter mRNA (93) indicating that the effect may be more additive than redundant. However, the relative importance of the two proteins, with respect to controlling mRNA stability, was cell type specific suggestive of a complex interplay with other factors.

Functional cooperation is also a possibility that needs to be taken into account. For example, the transition between a condensed and an open conformation of the RNA associated with AUF1 is dependent on the phosphorylation state of AUF1 (94). Potentially, this structural transition could affect the binding properties of other ARE-BPs.

Antagonistic effects

A study of the tissue specific expression of several ARE-BPs in the mouse showed that high levels of expression of AUF1 and HuR occurred in the same tissues (95). Some ARE-containing mRNAs, such as those encoding c-fos, GM-CSF, the cyclin dependent kinase inhibitor p21, IL-3 and cyclin D1 are destabilized in the presence of AUF1 and stabilized by HuR (Table 2). Consequently, it is possible that the relative cytoplasmic concentrations of functionally antagonistic ARE-BPs define the stability of a particular mRNA. Supporting this hypothesis is a RNA interference (RNAi) study in which the decreased half-life of IL-3 mRNA caused by HuR depletion is neutralized by co-depletion of another ARE-BP, BRF1, that is known to mediate the rapid degradation of several cytokines (55). Similarly, the increased expression of endogenous GM-CSF associated with the RNAi

mediated knock-down of BRF1 or certain isoforms of AUF1 is partially repressed by the co-transfection of siRNAs directed against HuR (55). Lal *et al.* (48) have directly investigated if functional interactions can exist between AUF1 and HuR. Using a micro-array based screen they showed that these two proteins can co-exist on the same mRNA when it is in the nuclear compartment. However, this association is no longer observed when the mRNA has been exported into the cytoplasm. HuR is found associated with mRNAs in polysomes and AUF1 is only found in polysome free fractions. This confirms earlier studies showing that HuR and AUF1 partition differently between polysomes and polysome free fractions (96,97). When the association of these two proteins with cyclin D1 and p21 mRNAs were studied both *in vitro* and *in vivo* (48), the binding sites were found to be distinct and non-overlapping which renders possible the simultaneous binding of these two proteins to the same mRNA. However, the depletion of either AUF1 or HuR caused a reciprocal increase in the association of the other protein with the mRNA, by binding to the site of the depleted factor.

Therefore, the degradation rate of a mRNA could be determined, at least in part or for certain mRNAs, by an equilibrium or a balance between stabilizing and destabilizing factors in the cytoplasm. This balance would be modulated by physiological stimuli that could, for instance, affect the nuclear/cytoplasmic distribution of the factors. In many cell types the majority of HuR is localized in the nucleus but it is observed to translocate to the cytoplasm in a cell cycle dependent manner (70,98) or after stimulation of the cells (69,71,99,100) and during skeletal myogenesis (68). AUF1 is also a predominantly nuclear protein and can shuttle between the nucleus and the cytoplasm (49,51,52). The sub-cellular localization of AUF1 is influenced by heat shock (50).

AUF1 and HuR are not the only factors whose relative amounts may be important. In the case of T-lymphocytes, after activation both HuR and TTP appear in the cytoplasm (101). According to a number of *in vitro* studies, these two proteins, that have opposing effects on ARE-dependent mRNA degradation (Table 2), present binding specificities that are distinct but overlapping, notably for GM-CSF, TNF- α , IL-2 and COX-2 mRNAs (Table 1). Furthermore, by co-transfection of NIH-3T3 cells with expression plasmids for TTP and HuR, Ming *et al.* (66) showed that TTP antagonises the HuR induced stabilisation of a reporter mRNA containing the IL-3 ARE.

Finally, a complex antagonistic interplay between KSRP, TTP and HuR has recently been reported by Linker *et al.* (102). They showed that KSRP targets the human inducible nitric oxide synthase (iNOS), also called nitric oxide synthase II (NOS II), mRNA for rapid degradation by binding to an ARE that is also recognized by HuR (64) and a competition between these two proteins for the ARE in the iNOS mRNA was demonstrated. Cytokine stimulation of DLD-1 cells, that increases the stability of iNOS mRNA, reduced KSRP-binding while HuR-binding increased. TTP is also an actor in this scenario. Contrary to the general picture, the over expression of TTP in DLD-1 cells stabilizes the iNOS mRNA (103). However, in this case TTP does not bind to iNOS mRNA but participates in the RNA-protein complex via an interaction with KSRP that appears to inhibit KSRP directed degradation of iNOS mRNA. Comparing the data in Tables 1 and 2 brings

to light several other ARE-BPs reported to regulate the stability of a mRNA but to which binding has not been demonstrated (AUF1/IL-3, HeI-N1/TNF- α , TTP/IL-3, BRF1/GM-CSF and KSRP/IL-2). This suggests that a re-evaluation of whether the observed ARE-BP induced changes are direct effects or due to the interaction with another ARE-BP is required.

Hence, there are significant data to support the notion that variations in the relative amounts and potentially the binding affinities of AUF1, KSRP, BRF1, TTP and HuR could determine both the identity of the targeted mRNAs and their cytoplasmic fate. By including the association with common or distinct co-factors, this regulation could be further fine tuned. We propose that the same scenario also applies to other combinations of ARE-BPs. The corollary to this is that understanding how a cell differentially regulates the stability of diverse ARE-containing mRNAs has become a question of how the relative cytoplasmic expression levels of the different ARE-BPs are controlled.

MECHANISMS AND PATHWAYS OF ARE-DEPENDENT DEGRADATION

Rapid deadenylation is a prelude to ARE-dependent degradation

In yeast, deadenylation [shortening of the 3' poly(A) tail] is followed by decapping (removal of the 5' cap structure) and degradation by 5'-3' exonucleases [reviewed in (2,43,104)]. In mammals, deadenylation is also the first and rate-limiting step in the degradation of many ARE-containing mRNAs both *in vivo* (30,105–108) and *in vitro* (109) and decapping can follow this initial event (110). Several *in vitro* studies have shown that the kinetics with which a mRNA is deadenylated varies between the ARE classes. Reporter mRNAs containing class I and III AREs are deadenylated in a synchronous manner, whereas for mRNAs containing class II AREs the poly(A) tails are shortened asynchronously (29,33,35). It is not clear at present what the biological significance of these differences may be as the overall stability of the mRNA does not appear to be affected.

Following deadenylation two separate cellular entities have been implicated in mRNA degradation, the exosome and GW or processing bodies (P-bodies). *In vitro* the degradation of the decapped mRNA occurs principally by the exosome associated 3'-5' exonucleases (111). However, the presence of the 5'-3' exonuclease Xrn1 in P-bodies implies that this route is also active in mammalian cells (112).

Exosome

The study of mRNA degradation using *in vitro* systems has shown that 3'-5' degradation of mRNAs, including those containing AREs, requires a large multi-protein complex called the exosome [reviewed in (113)]. Mukherjee *et al.* (111) suggested that certain subunits of the human exosome specifically bind to AREs causing an ARE-dependent degradation of mRNAs. Other studies have shown that several ARE-BPs, for instance TTP and KSRP, are physically associated *in vitro* with the exosome and are required so that the exosome can preferentially degrade ARE-containing mRNAs (90,93). Also TTP can stimulate the deadenylase [poly(A)

ribonuclease] (PARN) in cell extracts and this confers a specificity towards ARE-containing mRNAs (85). In cytoplasmic extracts, the ARE-BP KSRP simultaneously associates with PARN and the exosome (93). These results imply that both TTP and KSRP can recruit to a mRNA the cellular factors necessary for both deadenylation and degradation via the exosome, thereby ensuring a rapid and preferential elimination of the transcript.

P-bodies

P-bodies, first identified in yeast (114), contain mRNA decapping (Dcp1 and Dcp2) and degradation (Xrn1) factors. In mammalian cells, these same factors are found in GW bodies (112,115–117). It has been recently shown that, in mammalian cells, stress granules and P-bodies are dynamically linked and that mRNAs can be transferred between these two entities (112,118). Stress granules, that accumulate in response to particular physiological signals sequester untranslated mRNAs [reviewed in (119)] but they do not contain decapping factors (112). Therefore, stress granules act as an anti-chamber to P-bodies and hold mRNA in a translationally repressed state awaiting the decision to either recycle the mRNA towards polysomes or direct it to P-bodies for degradation (112,118). Several ARE-BPs are associated with either stress granules or P-bodies. TIA-1 and TIAR are an integral part of stress granules and HuR and TTP can be recruited to both [reviewed in (119)]. Interestingly, the translational repression of TNF- α and Cox-2 mRNAs has been correlated with TIA-1 expression (Table 2) (120,121). Furthermore, in HeLa cell extracts, the presence of an ARE in the mRNA stimulates decapping (122) and the proteins TTP and BRF1 are associated with decapping enzymes (91).

Coupling of ARE-dependent degradation and translation

The question of whether ARE-dependent degradation of mRNAs and translation are coupled is still controversial. Evidence both for and against this coupling have been reported for a number of cellular models. In some cases inhibiting translation of reporter RNAs containing the GM-CSF or c-fos ARE either pharmacologically [(28,123), reviewed in (124)] or by mutating the initiation codons (125) or inserting stable secondary structures (123,126,127), stabilized the mRNA. Also the deadenylation kinetics conferred by c-fos ARE was modified by blocking translation with a stable stem-loop structure (29). In contrast, in other studies inhibiting the translation of mRNAs containing c-fos, GM-CSF or c-jun AREs by introducing a stem-loop structure or an Iron Responsive Element in the 5'-UTR did not stabilize the mRNA (29,41,128). In addition to mRNAs, snRNA can also be targeted for rapid deadenylation by an ARE-dependent process (129), which implicitly supports the model that ARE-dependent deadenylation and decay does not require translation of the host mRNA.

It is now known that stress granules and P-bodies are affected by drugs that inhibit translation; cycloheximide and emetine that 'freeze' polysomes cause disassembly of stress granules and P-bodies whereas puromycin that dismantles polysomes promotes the assembly of stress granules (112,130). This indicates a need to re-evaluate at least some of the earlier data using drugs to inhibit translation.

TRANSLATIONAL REGULATION BY AREs AND ARE-BPs

In addition to a reduction in protein synthesis consequential to mRNA degradation, AREs can also repress the translation of host mRNA (Table 2). The 3'-UTR of Interferon- β , that contains an ARE, strongly inhibits the translation not only of the endogenous mRNA but also of reporter mRNAs containing this region in both rabbit reticulocyte lysates and in *Xenopus* oocytes (131,132). Injection of mRNAs into *Xenopus* oocytes, a biological model in which mRNAs are particularly stable, was also used to show that the AREs of GM-CSF and c-fos mRNAs inhibit the translation of the host mRNA in the absence of mRNA degradation (133). Another example of an ARE-containing mRNA that is regulated by many processes (stability, deadenylation and translational initiation) is that of TNF- α (134–136). In resting macrophages TNF- α mRNA is in a translationally inactive form (136). In response to endotoxins, the translational repression of a reporter mRNA containing the TNF- α 3'-UTR is relieved and expression of the reporter protein increases importantly; this is not due to an increase in the cytoplasmic concentration of the mRNA (135). Similarly, the translational repression conferred on a reporter mRNA by the AU-rich 3'-UTR of p53 mRNA is relieved by UV-irradiation (137). Finally, Grosset *et al.* (138) showed, also by using a reporter mRNA, that the ARE of GM-CSF mRNA can repress translation independently of the effect of this element on mRNA stability.

Among the various studies of ARE-BPs only a few have addressed the question of their role in the translational regulation of ARE-containing mRNAs (Table 2). The protein TIA-1 has been described as a translational inhibitor of some labile mRNAs such as TNF- α and Cox-2 (120,121). Using mRNAs identified in a large scale screen for TIA-1 associated mRNA, Lopez de Silanes *et al.* (40) extended this role for TIA-1 as a translational repressor to a large number of mRNAs. However, in this study the TIA-1-binding sites within the target mRNAs did not contain AUUUA motifs.

In contrast, HeL-N1 has been shown to activate translation of GLUT1 and NF-M mRNAs in 3T3L1 and hNT2 cells, respectively (72,73). Recently, HuR was reported to act as both a positive and a negative translational regulator. In carcinoma cells UV-irradiation (137) or expression of the tumour suppressor Von Hippel-Lindau (VHL) gene (99) causes an increase in HuR expression and binding to the 3'-UTR that are positively correlated with an increased translation (polysome recruitment or pulse-chase assay) of p53 mRNA. In contrast, over expression of HuR in stressed macrophages inhibited the polysome recruitment of TNF- α and Cox-2 (139) (Table 2). Interestingly, HuR induced changes in the expression of p53 (99,137) occurred in the absence of any change in the abundance of the corresponding mRNA whereas for TNF- α and Cox-2 mRNAs opposing changes in mRNA stability and translation were observed (139). It should be noted that in all these studies particular experimental conditions were used: UV-induced stress (137); over expression of the von Hippel-Lindau tumour suppressor protein (99); induced inflammatory response in macrophages (139).

The inhibitory effects of ARE-BPs on mRNA translation could be mediated by two mechanisms. In oocytes and early embryos a direct correlation has been established between the

adenylation status of a mRNA and its presence in polysomes; the polyadenylated mRNAs being translated more efficiently [reviewed in (140)]. This relationship has also been observed in many lower and higher eukaryotes [reviewed in (141)]. Cap-dependent recruitment of ribosomes is mediated by the translation initiation complex eIF4F composed of several proteins including eIF4E and eIF4G. The cap structure (m⁷GpppX), present at the 5' end of eukaryotic cellular mRNAs is recognised by eIF4E. eIF4G acts as a scaffold protein that binds both eIF4E, at the 5' extremity of the mRNA, and the poly(A) binding protein1 (PABP1) associated with the 3' poly(A) tail [reviewed in (142)]. The association between eIF4E/eIF4G and the 5' cap is stabilized when eIF4G also binds to PABP1 (143,144). Therefore, ARE-dependent inhibition of translation could ensue from the deadenylation of the target mRNA leading to a loss of PABP1 bound to the mRNA which, in turn, would destabilise the eIF4E-cap interaction. A variant of this model would be that the ARE-BP binds to eIF4E, competitively inhibiting the interaction of eIF4E with eIF4G. Such mechanisms have been discussed for *Xenopus* maskin and *Drosophila* Cup [reviewed in (145)], two proteins that are indirectly recruited to the 3'-UTR of specific mRNAs. Although no direct interaction between an ARE-BP and eIF4E has been reported to date, it is intriguing that AUF1 is part of a multimeric complex containing eIF4G (50).

An alternative mechanism brings into play stress granules. As described above stress granules contain untranslated mRNAs and are associated with several ARE-BPs. This suggests a route for translational arrest by an ARE-BP mediated targeting of specific mRNAs into stress granules. However, in the examples given above translation is repressed in the resting state and activated after a cellular stimulus that can be assimilated to an environmental stress. Therefore, it is necessary to posit that at least some ARE-containing mRNAs would be constitutively targeted to stress granules. Relief of TNF- α (135) or p53 (137) translational arrest by endotoxins or UV-irradiation, respectively would then correspond to an export of these mRNAs out of the stress granules. In this context it is interesting to note that stress-induced nascent transcripts are excluded from stress granules in both plants (146) and mammalian cells (119).

ARE THERE UNIFYING PRINCIPLES?

Despite the large amount of published work on AREs and ARE-BPs since the initial description of AREs in 1986 (23), we are still largely in the dark as to the precise molecular pathways to which these *cis*-acting sequence elements target mRNAs for degradation and/or translational arrest. This is not particular to ARE-dependent degradation and we would posit that part of the difficulty in describing these molecular machines is inherent in their nature. It is only recently that molecular data on the characteristics of localised factories implicated in the regulation of gene expression have been obtained. This is true for both cytoplasmic and nuclear processes. The co-localization of transcribed genes in the nucleus is now being documented (147) and the nuclear processing (capping, splicing and polyadenylation) of pre-mRNAs is also coordinated within particles [reviewed in (148,149)]. We have already described the cytoplasmic stress granules and P-bodies

that respectively sequester mRNAs in response to particular physiological signals or achieve particular processing events. Several ARE-BPs are associated with the exosome, stress granules and P-bodies (see above).

One very plausible role for ARE-BPs is to act as traffic markers that channel targeted mRNAs into specific pathways. This is easy to envisage for ARE-BPs implicated in enhancing the degradation or imposing a translational arrest on an mRNA. These observed effects could be two steps in the same pathway, first sequestering the mRNA away from the translation machinery and then shuttling it into a degradation pathway. The transfer of mRNAs into stress granules and then to P-bodies is precisely such a pathway. If control was exerted between these two steps then some mRNAs could be translationally arrested but not degraded. Cox-2 mRNA is an example of this (150).

Several models by which an ARE-BP could enhance protein expression can also be envisaged. Evidently, if the binding of the ARE-BP stabilizes the target mRNA then an increase in the synthesis of the encoded protein will probably ensue. Enhanced translation could also result from relieving repression. Consider a mRNA that is translationally repressed but not degraded. If following a particular cellular stimulus the mRNA leaves the inhibitory complex and enters into polysomes, translation will increase. For some mRNAs stress granules can achieve this task (112).

The functional analyses of ARE-BPs both *in vitro* and *in vivo* have led to the identification of a large number of target mRNAs (Tables 1 and 2). Many of the *in vitro* experiments are performed using the same cellular models. Hence the large number of ARE-BPs binding to the same mRNA suggests that many redundant factors have evolved which, although not impossible, is rather surprising. We favour an alternative hypothesis that, in fact, these proteins are not redundant but only appear to be so due the experimental design(s) that are feasible at present. As mentioned above, ARE-BPs are probably integral components of large functional complexes. In such complexes they will interact with some but probably not all of the other components. The functions of many regulatory proteins have been studied by either over expressing or depleting the factor(s) of interest, both of which can cause several 'unnatural' consequences. For instance, certain proteins when over expressed act as dominant negative mutants. However, such considerations should not be taken to exclude the possibility that in a physiological normal situation any one ARE-containing mRNA can associate with more than one ARE-BP. Indeed, several examples have already been discussed. Furthermore, the association of an ARE with several ARE-BP with either complementary or antagonistic consequences would allow targeting of these mRNA into different processing pathways. Furthermore, several ARE-BPs could combine together in a single complex and cooperatively define the functional outcome for a mRNA.

FUTURE DIRECTIONS

It is now obvious that further characterization of ARE-BPs and their functions will require a description of the cellular complexes in which they are normally found. Furthermore, the composition of these RNA-containing complexes is probably

not static but may be cell cycle dependent, change with localization of the associated mRNA or as a function of external stimuli. This adds a temporal aspect to ARE-dependent regulation. Accordingly, a first necessary step towards understanding ARE-dependent regulation is the isolation of ribonucleoprotein complexes containing ARE-BPs from unmodified cells. One interesting study in this vein is that of Lal *et al.* (48) who showed that HuR and AUF1 can simultaneously bind to many mRNAs in the nucleus but that they are associated with distinct mRNA populations in the cytoplasm. This brings to light a second facet of ARE-BPs that has not been systematically considered; the intracellular localization of these proteins. For the majority of ARE-BPs whose localization has been studied, this is predominantly nuclear. This is the case for HuR and AUF1 whose most well described functions are, respectively, to stabilize and destabilize mRNAs, presumably in the cytoplasm. In unperturbed cells how is this achieved? Do these ARE-BPs piggy-back on targeted mRNAs that are predestined to be stable or rapidly degraded and how is this destiny defined in the nucleus? Intriguingly, a 'nuclear history' is required for one of the AUF1 isoforms to exert its cytoplasmic function (151). Localization of ARE-BPs within the cytoplasm should also be high on the priority list for future research. HuR, TIA-1, TIAR and TTP are already identified as components of stress granules (112,152) and the interaction between stress granules and P-bodies is promoted by TTP and BRF1 (112). A complete study of stress granule and P-body dynamics, associated with precise measurements of ARE-dependent mRNA translation, deadenylation and decay and the localization of the related ARE-BPs is now possible and should yield results of high interest.

From this survey it is apparent that many questions remain to be answered and, as indicated above, light will probably only start to be shed by the conjunction of complementary approaches. For instance, highly specific biochemical analyses of the composition of ribonucleoprotein complexes containing ARE-BPs and analysis of the binding affinities of ARE-BPs for the various target AREs would certainly yield data on any redundant, antagonistic or cooperative binding between ARE and BPs. Finally, *in situ* localization methods coupled with the biochemical data on the composition of ARE-BP complexes and micro-array profiling of expressed and translated mRNAs would provide the data to develop a more overall functional map of the pathways into which AREs and the associated ARE-BPs direct specific mRNAs either constitutively or in response to particular cellular events or external stimuli.

SUPPLEMENTARY DATA

Supplementary Data are available at NAR Online.

ACKNOWLEDGEMENTS

We particularly thank Yann Audic and Vincent Legagneux for discussions during the preparation of this survey. Work in the authors' laboratory is supported by grants from the Association pour la Recherche sur le Cancer no. 4791, and the Ministère Délégué à la Recherche ACI BCMS314 to L.P. and the Ligue Contre le Cancer (comité d'Ille et Vilaine) and ACI BCMS 369

to H.B.O. The Open Access publication charges for this article were waived by Oxford University Press.

Conflict of interest statement. None declared.

REFERENCES

- Ross, J. (1995) mRNA stability in mammalian cells. *Microbiol. Rev.*, **59**, 423–450.
- Mitchell, P. and Tollervey, D. (2000) mRNA stability in eukaryotes. *Curr. Opin. Genet. Dev.*, **10**, 193–198.
- Mitchell, P. and Tollervey, D. (2001) mRNA turnover. *Curr. Opin. Cell. Biol.*, **13**, 320–325.
- Guhaniyogi, J. and Brewer, G. (2001) Regulation of mRNA stability in mammalian cells. *Gene*, **265**, 11–23.
- Herschman, H.R. (1991) Primary response genes induced by growth factors and tumor promoters. *Annu. Rev. Biochem.*, **60**, 281–319.
- Schiavi, S.C., Belasco, J.G. and Greenberg, M.E. (1992) Regulation of proto-oncogene mRNA stability. *Biochim. Biophys. Acta*, **1114**, 95–106.
- Espele, E., Garcia-Sanz, J.A., Aubert, V., Menoud, V., Sperisen, P., Fernandez, N. and Spertini, F. (1996) Transcriptional and translational control of TNF- α gene expression in human monocytes by major histocompatibility complex class II ligands. *Eur. J. Immunol.*, **26**, 2417–2424.
- Crawford, E.K., Ensor, J.E., Kalvakolanu, I. and Hasday, J.D. (1997) The role of 3' poly(A) tail metabolism in tumor necrosis factor- α regulation. *J. Biol. Chem.*, **272**, 21120–21127.
- Miller, A.D., Curran, T. and Verma, I.M. (1984) c-fos protein can induce cellular transformation: a novel mechanism of activation of a cellular oncogene. *Cell*, **36**, 51–60.
- Meijlink, F., Curran, T., Miller, A.D. and Verma, I.M. (1985) Removal of a 67-base-pair sequence in the noncoding region of protooncogene fos converts it to a transforming gene. *Proc. Natl Acad. Sci. USA*, **82**, 4987–4991.
- Lee, W.M., Lin, C. and Curran, T. (1988) Activation of the transforming potential of the human fos proto-oncogene requires message stabilization and results in increased amounts of partially modified fos protein. *Mol. Cell Biol.*, **8**, 5521–5527.
- Schuler, G.D. and Cole, M.D. (1988) GM-CSF and oncogene mRNA stabilities are independently regulated in trans in a mouse monocytic tumor. *Cell*, **55**, 1115–1122.
- Keffer, J., Probert, L., Cazlaris, H., Georgopoulos, S., Kaslaris, E., Kiuoussis, D. and Kollias, G. (1991) Transgenic mice expressing human tumour necrosis factor: a predictive genetic model of arthritis. *EMBO J.*, **10**, 4025–4031.
- Keyomarsi, K. and Pardee, A.B. (1993) Redundant cyclin overexpression and gene amplification in breast cancer cells. *Proc. Natl Acad. Sci. USA*, **90**, 1112–1116.
- Lebwohl, D.E., Muise-Helmericks, R., Sepp-Lorenzino, L., Serve, S., Timaul, M., Bol, R., Borgen, P. and Rosen, N. (1994) A truncated cyclin D1 gene encodes a stable mRNA in a human breast cancer cell line. *Oncogene*, **9**, 1925–1929.
- Nair, A.P., Hahn, S., Banholzer, R., Hirsch, H.H. and Moroni, C. (1994) Cyclosporin A inhibits growth of autocrine tumour cell lines by destabilizing interleukin-3 mRNA. *Nature*, **369**, 239–242.
- Jacob, C.O., Lee, S.K. and Strassmann, G. (1996) Mutational analysis of TNF- α gene reveals a regulatory role for the 3'-untranslated region in the genetic predisposition to lupus-like autoimmune disease. *J. Immunol.*, **156**, 3043–3050.
- Carballo, E., Lai, W.S. and Blakeshear, P.J. (1998) Feedback inhibition of macrophage tumor necrosis factor- α production by tristetraprolin. *Science*, **281**, 1001–1005.
- Kontoyiannis, D., Pasparakis, M., Pizarro, T.T., Cominelli, F. and Kollias, G. (1999) Impaired on/off regulation of TNF biosynthesis in mice lacking TNF AU-rich elements: implications for joint and gut-associated immunopathologies. *Immunity*, **10**, 387–398.
- Pullmann, R.Jr, Juhaszova, M., Lopez de Silanes, I., Kawai, T., Mazan-Mamczarz, K., Halushka, M.K. and Gorospe, M. (2005) Enhanced proliferation of cultured human vascular smooth muscle cells linked to increased function of RNA-binding protein HuR. *J. Biol. Chem.*, **280**, 22819–22826.

21. Conne,B., Stutz,A. and Vassalli,J.D. (2000) The 3' untranslated region of messenger RNA: A molecular 'hotspot' for pathology? *Nature Med.*, **6**, 637–641.
22. Audic,Y. and Hartley,R.S. (2004) Post-transcriptional regulation in cancer. *Biol. Cell*, **96**, 479–498.
23. Caput,D., Beutler,B., Hartog,K., Thayer,R., Brown-Shimer,S. and Cerami,A. (1986) Identification of a common nucleotide sequence in the 3'-untranslated region of mRNA molecules specifying inflammatory mediators. *Proc. Natl Acad. Sci. USA*, **83**, 1670–1674.
24. Bakheet,T., Frevel,M., Williams,B.R., Greer,W. and Khabar,K.S. (2001) ARED: human AU-rich element-containing mRNA database reveals an unexpectedly diverse functional repertoire of encoded proteins. *Nucleic Acids Res.*, **29**, 246–254.
25. Bakheet,T., Williams,B.R. and Khabar,K.S. (2003) ARED 2.0: an update of AU-rich element mRNA database. *Nucleic Acids Res.*, **31**, 421–423.
26. Chen,C.Y. and Shyu,A.B. (1995) AU-rich elements: characterization and importance in mRNA degradation. *Trends Biochem. Sci.*, **20**, 465–470.
27. Wilson,G.M. and Brewer,G. (1999) The search for *trans*-acting factors controlling messenger RNA decay. *Prog. Nucleic Acid Res. Mol. Biol.*, **62**, 257–291.
28. Shaw,G. and Kamen,R. (1986) A conserved AU sequence from the 3' untranslated region of GM-CSF mRNA mediates selective mRNA degradation. *Cell*, **46**, 659–667.
29. Chen,C.-Y.A., Xu,N. and Shyu,A.B. (1995) mRNA decay mediated by two distinct AU-rich elements from c-fos and granulocyte-macrophage colony-stimulating factor transcripts: different deadenylation kinetics and uncoupling from translation. *Mol. Cell Biol.*, **15**, 5777–5788.
30. Wilson,T. and Treisman,R. (1988) Removal of poly(A) and consequent degradation of c-fos mRNA facilitated by 3' AU-rich sequences. *Nature*, **336**, 396–399.
31. Shyu,A.B., Greenberg,M.E. and Belasco,J.G. (1989) The c-fos transcript is targeted for rapid decay by two distinct mRNA degradation pathways. *Genes Dev.*, **3**, 60–72.
32. Xu,N., Loflin,P., Chen,C.Y. and Shyu,A.B. (1998) A broader role for AU-rich element-mediated mRNA turnover revealed by a new transcriptional pulse strategy. *Nucleic Acids Res.*, **26**, 558–565.
33. Chen,C.-Y.A., Chen,T.-M. and Shyu,A.B. (1994) Interplay of two functionally and structurally distinct domains of the c-fos AU-rich element specifies its mRNA-destabilizing function. *Mol. Cell Biol.*, **14**, 416–426.
34. Stoecklin,G., Hahn,S. and Moroni,C. (1994) Functional Hierarchy of AUUUU motifs in mediating rapid interleukin-3 mRNA decay. *J. Biol. Chem.*, **269**, 28591–28597.
35. Chen,C.-Y.A. and Shyu,A.-B. (1994) Selective degradation of early-response-gene mRNAs: Functional analysis of sequence features of the AU-rich elements. *Mol. Cell Biol.*, **14**, 8471–8482.
36. Lagnado,C.A., Brown,C.Y. and Goodall,G.J. (1994) AUUUU is not sufficient to promote poly(A) shortening and degradation of an mRNA: the functional sequence within AU-rich elements may be UUAUUUA(U/A)(U/A). *Mol. Cell Biol.*, **14**, 7984–7995.
37. Zubiaga,A.M., Belasco,J.G. and Greenberg,M.E. (1995) The nonamer UUAUUUAUU is the key AU-rich sequence motif that mediates mRNA degradation. *Mol. Cell Biol.*, **15**, 2219–2230.
38. Lai,W.S., Carrick,D.M. and Blackshear,P.J. (2005) Influence of Nonameric AU-rich tristetraprolin-binding sites on mRNA deadenylation and turnover. *J. Biol. Chem.*, **280**, 34365–34377.
39. Lopez de Silanes,I., Zhan,M., Lal,A., Yang,X. and Gorospe,M. (2004) Identification of a target RNA motif for RNA-binding protein HuR. *Proc. Natl Acad. Sci. USA*, **101**, 2987–2992.
40. Lopez de Silanes,I., Galban,S., Martindale,J.L., Yang,X., Mazan-Mamczarz,K., Indig,F.E., Falco,G., Zhan,M. and Gorospe,M. (2005) Identification and Functional Outcome of mRNAs Associated with RNA-Binding Protein TIA-1. *Mol. Cell Biol.*, **25**, 9520–9531.
41. Peng,S.S., Chen,C.Y. and Shyu,A.B. (1996) Functional characterization of a non-AUUUA AU-rich element from the c-jun proto-oncogene mRNA: evidence for a novel class of AU-rich elements. *Mol. Cell Biol.*, **16**, 1490–1499.
42. Xu,N., Chen,C. and Shyu,A. (2001) Versatile role for hnRNP D isoforms in the differential regulation of cytoplasmic mRNA turnover. *Mol. Cell Biol.*, **21**, 6960–6971.
43. Wilusz,C.J., Wormington,M. and Peltz,S.W. (2001) The cap-to-tail guide to mRNA turnover. *Nature Rev. Mol. Cell Biol.*, **2**, 237–246.
44. Bevilacqua,A., Ceriani,M.C., Capaccioli,S. and Nicolini,A. (2003) Post-transcriptional regulation of gene expression by degradation of messenger RNAs. *J. Cell. Physiol.*, **195**, 356–372.
45. Brewer,G. and Ross,J. (1989) Regulation of c-myc mRNA stability *in vitro* by a labile destabilizer with an essential nucleic acid component. *Mol. Cell Biol.*, **9**, 1996–2006.
46. Brewer,G. (1991) An A+U-rich element RNA-binding factor regulates c-myc mRNA stability *in vitro*. *Mol. Cell Biol.*, **11**, 2460–2466.
47. Zhang,W., Wagner,B.J., Ehrenman,K., Schaefer,A.W., DeMaria,C.T., Crater,D., DeHaven,K., Long,L. and Brewer,G. (1993) Purification, characterization, and cDNA cloning of an AU-rich element RNA-binding protein, AUF1. *Mol. Cell Biol.*, **13**, 7652–7665.
48. Lal,A., Mazan-Mamczarz,K., Kawai,T., Yang,X., Martindale,J.L. and Gorospe,M. (2004) Concurrent versus individual binding of HuR and AUF1 to common labile target mRNAs. *EMBO J.*, **23**, 3092–3102.
49. Dreyfuss,G., Matunis,M.J., Pinol-Roma,S. and Burd,C.G. (1993) hnRNP proteins and the biogenesis of mRNA. *Annu. Rev. Biochem.*, **62**, 289–321.
50. Larroia,G., Cuesta,R., Brewer,G. and Schneider,R.J. (1999) Control of mRNA decay by heat shock-ubiquitin-proteasome pathway. *Science*, **284**, 499–502.
51. Shyu,A.B. and Wilkinson,M.F. (2000) The double lives of shuttling mRNA binding proteins. *Cell*, **102**, 135–138.
52. Sarkar,B., Lu,J.Y. and Schneider,R.J. (2003) Nuclear import and export functions in the different isoforms of the AUF1/heterogeneous nuclear ribonucleoprotein protein family. *J. Biol. Chem.*, **278**, 20700–20707.
53. Loflin,P., Chen,C.Y. and Shyu,A.B. (1999) Unraveling a cytoplasmic role for hnRNP D in the *in vivo* mRNA destabilization directed by the AU-rich element. *Genes Dev.*, **13**, 1884–1897.
54. Sarkar,B., Xi,Q., He,C. and Schneider,R.J. (2003) Selective degradation of AU-rich mRNAs promoted by the p37 AUF1 protein isoform. *Mol. Cell Biol.*, **23**, 6685–6693.
55. Raineri,L., Wegmueller,D., Gross,B., Certa,U. and Moroni,C. (2004) Roles of AUF1 isoforms, HuR and BRF1 in ARE-dependent mRNA turnover studied by RNA interference. *Nucleic Acids Res.*, **32**, 1279–1288.
56. Sela-Brown,A., Silver,J., Brewer,G. and Naveh-Manly,T. (2000) Identification of AUF1 as a parathyroid hormone mRNA 3'-untranslated region-binding protein that determines parathyroid hormone mRNA stability. *J. Biol. Chem.*, **275**, 7424–7429.
57. Good,P., Chen,O., Warner,S. and Herring,D. (2000) A family of human RNA-binding proteins related to the *Drosophila* Bruno translational regulator. *J. Biol. Chem.*, **275**, 28583–28592.
58. Antic,D. and Keene,J.D. (1997) Embryonic lethal abnormal visual RNA-binding proteins involved in growth, differentiation, and posttranscriptional gene expression. *Am. J. Hum. Genet.*, **61**, 273–278.
59. Fan,X.C. and Steitz,J.A. (1998) Overexpression of HuR, a nuclear-cytoplasmic shuttling protein, increases the *in vivo* stability of ARE-containing mRNAs. *EMBO J.*, **17**, 3448–3460.
60. Fan,X.C. and Steitz,J.A. (1998) HNS, a nuclear-cytoplasmic shuttling sequence in HuR. *Proc. Natl Acad. Sci. USA*, **95**, 15293–15298.
61. Gallouzi,I.E. and Steitz,J.A. (2001) Delineation of mRNA export pathways by the use of cell-permeable peptides. *Science*, **294**, 1895–1901.
62. Levy,N., Chung,S., Furneaux,H. and Levy,A. (1998) Hypoxic stabilization of vascular endothelial growth factor mRNA by the RNA-binding protein HuR. *J. Biol. Chem.*, **273**, 6417–6423.
63. Peng,S.S., Chen,C.Y., Xu,N. and Shyu,A.B. (1998) RNA stabilization by the AU-rich element binding protein, HuR, an ELAV protein. *EMBO J.*, **17**, 3461–3470.
64. Rodriguez-Pascual,F., Hausding,M., Ihrig-Biedert,I., Furneaux,H., Levy,A.P., Forstermann,U. and Kleinert,H. (2000) Complex contribution of the 3'-untranslated region to the expressional regulation of the human inducible nitric-oxide synthase gene. Involvement of the RNA-binding protein HuR. *J. Biol. Chem.*, **275**, 26040–26049.
65. Dean,J.L., Wait,R., Mahtani,K.R., Sully,G., Clark,A.R. and Saklatvala,J. (2001) The 3' untranslated region of tumor necrosis factor alpha mRNA is a target of the mRNA-stabilizing factor HuR. *Mol. Cell Biol.*, **21**, 721–730.
66. Ming,X.F., Stoecklin,G., Lu,M., Looser,R. and Moroni,C. (2001) Parallel and independent regulation of interleukin-3 mRNA turnover by phosphatidylinositol 3-kinase and p38 mitogen-activated protein kinase. *Mol. Cell Biol.*, **21**, 5778–5789.

67. Chen, C.Y., Xu, N. and Shyu, A.B. (2002) Highly selective actions of HuR in antagonizing AU-rich element-mediated mRNA destabilization. *Mol. Cell Biol.*, **22**, 7268–7278.
68. Figueroa, A., Cuadrado, A., Fan, J., Atasoy, U., Muscat, G.E., Munoz-Canoves, P., Gorospe, M. and Munoz, A. (2003) Role of HuR in skeletal myogenesis through coordinate regulation of muscle differentiation genes. *Mol. Cell Biol.*, **23**, 4991–5004.
69. Wang, W., Furneaux, H., Cheng, H., Caldwell, M.C., Hutter, D., Liu, Y., Holbrook, N. and Gorospe, M. (2000) HuR regulates p21 mRNA stabilization by UV light. *Mol. Cell Biol.*, **20**, 760–769.
70. Wang, W., Caldwell, M.C., Lin, S., Furneaux, H. and Gorospe, M. (2000) HuR regulates cyclin A and cyclin B1 mRNA stability during cell proliferation. *EMBO J.*, **19**, 2340–2350.
71. Sengupta, S., Jang, B.C., Wu, M.T., Paik, J.H., Furneaux, H. and Hla, T. (2003) The RNA-binding protein HuR regulates the expression of cyclooxygenase-2. *J. Biol. Chem.*, **278**, 25227–25233.
72. Jain, R.G., Andrews, L.G., McGowan, K.M., Pekala, P.H. and Keene, J.D. (1997) Ectopic expression of Hel-N1, an RNA-binding protein, increases glucose transporter (GLUT1) expression in 3T3-L1 adipocytes. *Mol. Cell Biol.*, **17**, 954–962.
73. Antic, D., Lu, N. and Keene, J.D. (1999) ELAV tumor antigen, Hel-N1, increases translation of neurofilament M mRNA and induces formation of neurites in human teratocarcinoma cells. *Genes Dev.*, **13**, 449–461.
74. Ford, L.P., Watson, J., Keene, J.D. and Wilusz, J. (1999) ELAV proteins stabilize deadenylated intermediates in a novel *in vitro* mRNA deadenylation/degradation system. *Genes Dev.*, **13**, 188–201.
75. Mobarak, C.D., Anderson, K.D., Morin, M., Beckel-Mitchener, A., Rogers, S.L., Furneaux, H., King, P. and Perrone-Bizzozero, N.I. (2000) The RNA-binding protein HuD is required for GAP-43 mRNA stability, GAP-43 gene expression, and PKC-dependent neurite outgrowth in PC12 cells. *Mol. Biol. Cell*, **11**, 3191–3203.
76. Anderson, K.D., Morin, M.A., Beckel-Mitchener, A., Mobarak, C.D., Neve, R.L., Furneaux, H.M., Burry, R. and Perrone-Bizzozero, N.I. (2000) Overexpression of HuD, but not of its truncated form HuD1+II, promotes GAP-43 gene expression and neurite outgrowth in PC12 cells in the absence of nerve growth factor. *J. Neurochem.*, **75**, 1103–1114.
77. Beckel-Mitchener, A.C., Miera, A., Keller, R. and Perrone-Bizzozero, N.I. (2002) Poly(A) tail length-dependent stabilization of GAP-43 mRNA by the RNA-binding protein HuD. *J. Biol. Chem.*, **277**, 27996–28002.
78. King, P.H. (2000) RNA-binding analyses of HuC and HuD with the VEGF and c-myc 3'-untranslated regions using a novel ELISA-based assay. *Nucleic Acids Res.*, **28**, e20.
79. Cao, H., Tuttle, J.S. and Blackshear, P.J. (2004) Immunological characterization of tristetraprolin as a low abundance, inducible, stable cytosolic protein. *J. Biol. Chem.*, **279**, 21489–21499.
80. Carballo, E., Gilkeson, G.S. and Blackshear, P.J. (1997) Bone marrow transplantation reproduces the tristetraprolin-deficiency syndrome in recombination activating gene-2 ($-/-$) mice. Evidence that monocyte/macrophage progenitors may be responsible for TNF α overproduction. *J. Clin. Invest.*, **100**, 986–995.
81. Carballo, E., Lai, W.S. and Blackshear, P.J. (2000) Evidence that tristetraprolin is a physiological regulator of granulocyte-macrophage colony-stimulating factor messenger RNA deadenylation and stability. *Blood*, **95**, 1891–1899.
82. Ogilvie, R.L., Abelson, M., Hau, H.H., Vlasova, I., Blackshear, P.J. and Bohjanen, P.R. (2005) Tristetraprolin down-regulates IL-2 gene expression through AU-rich element-mediated mRNA decay. *J. Immunol.*, **174**, 953–961.
83. Lai, W.S. and Blackshear, P.J. (2001) Interactions of CCCH zinc finger proteins with mRNA: tristetraprolin-mediated AU-rich element-dependent mRNA degradation can occur in the absence of a poly(A) tail. *J. Biol. Chem.*, **276**, 23144–23154.
84. Lai, W.S., Kennington, E.A. and Blackshear, P.J. (2002) Interactions of CCCH zinc finger proteins with mRNA: non-binding tristetraprolin mutants exert an inhibitory effect on degradation of AU-rich element-containing mRNAs. *J. Biol. Chem.*, **277**, 9606–9613.
85. Lai, W.S., Kennington, E.A. and Blackshear, P.J. (2003) Tristetraprolin and its family members can promote the cell-free deadenylation of AU-rich element-containing mRNAs by poly(A) ribonuclease. *Mol. Cell Biol.*, **23**, 3798–3812.
86. Lai, W.S., Carballo, E., Strum, J.R., Kennington, E.A., Phillips, R.S. and Blackshear, P.J. (1999) Evidence that tristetraprolin binds to AU-rich elements and promotes the deadenylation and destabilization of tumor necrosis factor alpha mRNA. *Mol. Cell Biol.*, **19**, 4311–4323.
87. Sawaoka, H., Dixon, D.A., Oates, J.A. and Boutaud, O. (2003) Tristetraprolin binds to the 3'-untranslated region of cyclooxygenase-2 mRNA. A polyadenylation variant in a cancer cell line lacks the binding site. *J. Biol. Chem.*, **278**, 13928–13935.
88. Stoecklin, G., Ming, X.F., Looser, R. and Moroni, C. (2000) Somatic mRNA turnover mutants implicate tristetraprolin in the interleukin-3 mRNA degradation pathway. *Mol. Cell Biol.*, **20**, 3753–3763.
89. Lai, W.S., Carballo, E., Thorn, J.M., Kennington, E.A. and Blackshear, P.J. (2000) Interactions of CCCH zinc finger proteins with mRNA. Binding of tristetraprolin-related zinc finger proteins to AU-rich elements and destabilization of mRNA. *J. Biol. Chem.*, **275**, 17827–17837.
90. Chen, C.Y., Gherzi, R., Ong, S.E., Chan, E.L., Raijmakers, R., Pruijn, G.J., Stoecklin, G., Moroni, C., Mann, M. and Karin, M. (2001) AU binding proteins recruit the exosome to degrade ARE-containing mRNAs. *Cell*, **107**, 451–464.
91. Lykke-Andersen, J. and Wagner, E. (2005) Recruitment and activation of mRNA decay enzymes by two ARE-mediated decay activation domains in the proteins TTP and BRF-1. *Genes Dev.*, **19**, 351–361.
92. Stoecklin, G., Colombi, M., Raineri, I., Leuenberger, S., Mallaun, M., Schmidlin, M., Gross, B., Lu, M., Kitamura, T. and Moroni, C. (2002) Functional cloning of BRF1, a regulator of ARE-dependent mRNA turnover. *EMBO J.*, **21**, 4709–4718.
93. Gherzi, R., Lee, K.Y., Briata, P., Wegmuller, D., Moroni, C., Karin, M. and Chen, C.Y. (2004) A KH domain RNA binding protein, KSRP, promotes ARE-directed mRNA turnover by recruiting the degradation machinery. *Mol. Cell*, **14**, 571–583.
94. Wilson, G.M., Lu, J., Sutphen, K., Suarez, Y., Sinha, S., Brewer, B., Villanueva-Feliciano, E.C., Ysla, R.M., Charles, S. and Brewer, G. (2003) Phosphorylation of p40AUF1 regulates binding to A + U-rich mRNA-destabilizing elements and protein-induced changes in ribonucleoprotein structure. *J. Biol. Chem.*, **278**, 33039–33048.
95. Lu, J.Y. and Schneider, R.J. (2004) Tissue distribution of AU-rich mRNA-binding proteins involved in regulation of mRNA decay. *J. Biol. Chem.*, **279**, 12974–12979.
96. Gallouzi, I.E., Brennan, C.M., Stenberg, M.G., Swanson, M.S., Eversole, A., Maizels, N. and Steitz, J.A. (2000) HuR binding to cytoplasmic mRNA is perturbed by heat shock. *Proc. Natl Acad. Sci. USA*, **97**, 3073–3078.
97. Grosset, C., Chen, C.Y., Xu, N., Sonenberg, N., Jacquemin-Sablon, H. and Shyu, A.B. (2000) A mechanism for translationally coupled mRNA turnover: interaction between the poly(A) tail and a c-fos RNA coding determinant via a protein complex. *Cell*, **103**, 29–40.
98. Atasoy, U., Watson, J., Patel, D. and Keene, J.D. (1998) ELAV protein HuA (HuR) can redistribute between nucleus and cytoplasm and is upregulated during serum stimulation and T cell activation. *J. Cell Sci.*, **111**, 3145–3156.
99. Galban, S., Martindale, J.L., Mazan-Mamczarz, K., Lopez de Silanes, I., Fan, J., Wang, W., Decker, J. and Gorospe, M. (2003) Influence of the RNA-binding protein HuR in pVHL-regulated p53 expression in renal carcinoma cells. *Mol. Cell Biol.*, **23**, 7083–7095.
100. Seko, Y., Azmi, H., Fariss, R. and Ragheb, J.A. (2004) Selective cytoplasmic translocation of HuR and site-specific binding to the interleukin-2 mRNA are not sufficient for CD28-mediated stabilization of the mRNA. *J. Biol. Chem.*, **279**, 33359–33367.
101. Raghavan, A., Robison, R.L., McNabb, J., Miller, C.R., Williams, D.A. and Bohjanen, P.R. (2001) HuA and tristetraprolin are induced following T cell activation and display distinct but overlapping RNA binding specificities. *J. Biol. Chem.*, **276**, 47958–47965.
102. Linker, K., Pautz, A., Fechir, M., Hubrich, T., Greeve, J. and Kleinert, H. (2005) Involvement of KSRP in the post-transcriptional regulation of human iNOS expression-complex interplay of KSRP with TTP and HuR. *Nucleic Acids Res.*, **33**, 4813–4827.
103. Fechir, M., Linker, K., Pautz, A., Hubrich, T., Forstermann, U., Rodriguez-Pascual, F. and Kleinert, H. (2005) Tristetraprolin regulates the expression of the human inducible nitric-oxide synthase gene. *Mol. Pharmacol.*, **67**, 2148–2161.
104. Beelman, C.A. and Parker, R. (1995) Degradation of mRNA in eukaryotes. *Cell*, **81**, 179–183.
105. Lieberman, A.P., Pitha, P.M. and Shin, M.L. (1992) Poly(A) removal is the kinase-regulated step in tumor necrosis factor mRNA decay. *J. Biol. Chem.*, **267**, 2123–2126.

106. Poppel, K. and Baglioni, C. (1991) Deadenylation and turnover of interferon-beta mRNA. *J. Biol. Chem.*, **266**, 6663–6666.
107. Laird-Offringa, I.A., De Wit, C.L., Elfferich, P. and Van der Eb, A.J. (1990) Poly(A) tail shortening is the translation-dependent step in c-myc mRNA degradation. *Mol. Cell Biol.*, **10**, 6132–6140.
108. Shyu, A.B., Belasco, J.G. and Greenberg, M.E. (1991) Two distinct destabilizing elements in the c-fos message trigger deadenylation as a first step in rapid mRNA decay. *Genes Dev.*, **5**, 221–231.
109. Brewer, G. and Ross, J. (1988) Poly(A) shortening and degradation of the 3' A+U-rich sequences of human c-myc mRNA in a cell-free system. *Mol. Cell Biol.*, **8**, 1697–1708.
110. Couttet, P., Fromont-Racine, M., Steel, D., Pictet, R. and Grange, T. (1997) Messenger RNA deadenylation precedes decapping in mammalian cells. *Proc. Natl Acad. Sci. USA*, **94**, 5628–5633.
111. Mukherjee, D., Gao, M., O'Connor, J.P., Rajmakers, R., Pruijn, G., Lutz, C.S. and Wilusz, J. (2002) The mammalian exosome mediates the efficient degradation of mRNAs that contain AU-rich elements. *EMBO J.*, **21**, 165–174.
112. Kedersha, N., Stoecklin, G., Ayodele, M., Yacono, P., Lykke-Andersen, J., Fitzler, M.J., Scheuner, D., Kaufman, R.J., Golan, D.E. and Anderson, P. (2005) Stress granules and processing bodies are dynamically linked sites of mRNP remodeling. *J. Cell Biol.*, **169**, 871–884.
113. Butler, J.S. (2002) The yin and yang of the exosome. *Trends Cell Biol.*, **12**, 90–96.
114. Sheth, U. and Parker, R. (2003) Decapping and decay of messenger RNA occur in cytoplasmic processing bodies. *Science*, **300**, 805–808.
115. Ingelfinger, D., Arndt-Jovin, D.J., Luhrmann, R. and Achsel, T. (2002) The human LSm1-7 proteins colocalize with the mRNA-degrading enzymes Dcp1/2 and Xrn1 in distinct cytoplasmic foci. *RNA*, **8**, 1489–1501.
116. Eystathiou, T., Jakymiw, A., Chan, E.K., Seraphin, B., Cougot, N. and Fritzler, M.J. (2003) The GW182 protein colocalizes with mRNA degradation associated proteins hDcp1 and hLSM4 in cytoplasmic GW bodies. *RNA*, **9**, 1171–1173.
117. Cougot, N., Babajko, S. and Seraphin, B. (2004) Cytoplasmic foci are sites of mRNA decay in human cells. *J. Cell Biol.*, **165**, 31–40.
118. Wilczynska, A., Aigueperse, C., Kress, M., Dautry, F. and Weil, D. (2005) The translational regulator CPEB1 provides a link between dcp1 bodies and stress granules. *J. Cell Sci.*, **118**, 981–992.
119. Kedersha, N. and Anderson, P. (2002) Stress granules: sites of mRNA triage that regulate mRNA stability and translatability. *Biochem. Soc. Trans.*, **30**, 963–969.
120. Piecyk, M., Wax, S., Beck, A.R., Kedersha, N., Gupta, M., Maritim, B., Chen, S., Gueydan, C., Krays, V., Streuli, M. et al. (2000) TIA-1 is a translational silencer that selectively regulates the expression of TNF-alpha. *EMBO J.*, **19**, 4154–4163.
121. Dixon, D.A., Balch, G.C., Kedersha, N., Anderson, P., Zimmerman, G.A., Beauchamp, R.D. and Prescott, S.M. (2003) Regulation of cyclooxygenase-2 expression by the translational silencer TIA-1. *J. Exp. Med.*, **198**, 475–481.
122. Gao, M., Wilusz, C.J., Peltz, S.W. and Wilusz, J. (2001) A novel mRNA-decapping activity in HeLa cytoplasmic extracts is regulated by AU-rich elements. *EMBO J.*, **20**, 1134–1143.
123. Winstall, E., Gamache, M. and Raymond, V. (1995) Rapid mRNA degradation mediated by the c-fos 3' AU-rich element and that mediated by the granulocyte-macrophage colony-stimulating factor 3' AU-rich element occur through similar polysome-associated mechanisms. *Mol. Cell Biol.*, **15**, 3796–3804.
124. Jacobson, A. and Peltz, S.W. (1996) Interrelationships of the pathways of mRNA decay and translation in eukaryotic cells. *Annu. Rev. Biochem.*, **65**, 693–739.
125. Savant-Bhonsale, S. and Cleveland, D.W. (1992) Evidence for instability of mRNAs containing AUUUA motifs mediated through translation-dependent assembly of a >20S degradation complex. *Genes Dev.*, **6**, 1927–1939.
126. Aharon, T. and Schneider, R.J. (1993) Selective destabilization of short-lived mRNAs with the granulocyte-macrophage colony-stimulating factor AU-rich 3' noncoding region is mediated by a cotranslational mechanism. *Mol. Cell Biol.*, **13**, 1971–1980.
127. Curatola, A.M., Nadal, M.S. and Schneider, R.J. (1995) Rapid degradation of AU-rich element (ARE) mRNAs is activated by ribosome transit and blocked by secondary structure at any position 5' to the ARE. *Mol. Cell Biol.*, **15**, 6331–6340.
128. Koeller, D.M., Horowitz, J.A., Casey, J.L., Klausner, R.D. and Harford, J.B. (1991) Translation and the stability of mRNAs encoding the transferrin receptor and c-fos. *Proc. Natl Acad. Sci. USA*, **88**, 7778–7782.
129. Fan, X.C., Myer, V.E. and Steitz, J.A. (1997) AU-rich elements target small nuclear RNAs as well as mRNAs for rapid degradation. *Genes Dev.*, **11**, 2557–2568.
130. Kedersha, N., Cho, M.R., Li, W., Yacono, P.W., Chen, S., Gilks, N., Golan, D.E. and Anderson, P. (2000) Dynamic shuttling of TIA-1 accompanies the recruitment of mRNA to mammalian stress granules. *J. Cell Biol.*, **151**, 1257–1268.
131. Krays, V., Wathlet, M., Poupard, P., Contreras, R., Fiers, W., Content, J. and Huez, G. (1987) The 3' untranslated region of the human interferon-beta mRNA has an inhibitory effect on translation. *Proc. Natl Acad. Sci. USA*, **84**, 6030–6034.
132. Graf, G., Sela, I. and Galili, G. (1993) Translational regulation of human beta interferon mRNA: association of the 3' AU-rich sequence with the poly(A) tail reduces translation efficiency *in vitro*. *Mol. Cell Biol.*, **13**, 3487–3493.
133. Krays, V., Marin, O., Shaw, G., Deschamps, J. and Huez, G. (1989) Translational blockade imposed by cytokine-derived UA-rich sequences. *Science*, **245**, 852–855.
134. Han, J. and Beutler, B. (1990) The essential role of the UA-rich sequence in endotoxin-induced cachectin/TNF synthesis. *Eur. Cytokine Netw.*, **1**, 71–75.
135. Han, J., Brown, T. and Beutler, B. (1990) Endotoxin-responsive sequences control cachectin/tumor necrosis factor biosynthesis at the translational level. *J. Exp. Med.*, **171**, 465–475.
136. Beutler, B., Krochin, N., Milsark, I.W., Luedke, C. and Cerami, A. (1986) Control of cachectin (tumor necrosis factor) synthesis: mechanisms of endotoxin resistance. *Science*, **232**, 977–980.
137. Mazan-Mamczarz, K., Galban, S., Lopez de Silanes, I., Martindale, J.L., Atasoy, U., Keene, J.D. and Gorospe, M. (2003) RNA-binding protein HuR enhances p53 translation in response to ultraviolet light irradiation. *Proc. Natl Acad. Sci. USA*, **100**, 8354–8359.
138. Grosset, C., Boniface, R., Duchez, P., Solanilla, A., Cosson, B. and Ripoche, J. (2004) *In vivo* studies of translational repression mediated by the granulocyte-macrophage colony-stimulating factor AU-rich element. *J. Biol. Chem.*, **279**, 13354–13362.
139. Katsanou, V., Papadaki, O., Milatos, S., Blackshear, P.J., Anderson, P., Kollias, G. and Kontoyiannis, D.L. (2005) HuR as a negative posttranscriptional modulator in inflammation. *Mol. Cell*, **19**, 777–789.
140. Paillard, L. and Osborne, H.B. (2003) East of EDEN was a poly(A) tail. *Biol. Cell*, **95**, 211–219.
141. Mangus, D.A., Evans, M.C. and Jacobson, A. (2003) Poly(A)-binding proteins: multifunctional scaffolds for the post-transcriptional control of gene expression. *Genome Biol.*, **4**, 223.
142. Gingras, A.C., Raught, B. and Sonenberg, N. (1999) eIF4 initiation factors: effectors of mRNA recruitment to ribosomes and regulators of translation. *Annu. Rev. Biochem.*, **68**, 913–963.
143. Borman, A.M., Michel, Y.M. and Kean, K.M. (2000) Biochemical characterization of cap-poly(A) synergy in rabbit reticulocyte lysates: the eIF4G-PABP interaction increases the functional affinity of eIF4E for the capped mRNA 5'-end. *Nucleic Acids Res.*, **28**, 4068–4075.
144. Kahvejian, A., Svitkin, Y.V., Sukarieh, R., M'Boutchou, M.N. and Sonenberg, N. (2005) Mammalian poly(A)-binding protein is a eukaryotic translation initiation factor, which acts via multiple mechanisms. *Genes Dev.*, **19**, 104–113.
145. de Moor, C.H., Meijer, H. and Lissenden, S. (2005) Mechanisms of translational control by the 3' UTR in development and differentiation. *Semin. Cell Dev. Biol.*, **16**, 49–58.
146. Nover, L., Scharf, K.D. and Neumann, D. (1989) Cytoplasmic heat shock granules are formed from precursor particles and are associated with a specific set of mRNAs. *Mol. Cell Biol.*, **9**, 1298–1308.
147. West, A.G. and Fraser, P. (2005) Remote control of gene transcription. *Hum. Mol. Genet.*, **14**, R101–R111.
148. Bentley, D. (2002) The mRNA assembly line: transcription and processing machines in the same factory. *Curr. Opin. Cell Biol.*, **14**, 336–342.
149. Lamond, A.I. and Spector, D.L. (2003) Nuclear speckles: a model for nuclear organelles. *Nature Rev. Mol. Cell Biol.*, **4**, 605–612.
150. Mukhopadhyay, D., Houchen, C.W., Kennedy, S., Dieckgraefe, B.K. and Anant, S. (2003) Coupled mRNA stabilization and translational silencing of cyclooxygenase-2 by a novel RNA binding protein, CUGBP2. *Mol. Cell*, **11**, 113–126.

151. Chen, C.Y., Xu, N., Zhu, W. and Shyu, A.B. (2004) Functional dissection of hnRNP D suggests that nuclear import is required before hnRNP D can modulate mRNA turnover in the cytoplasm. *RNA*, **10**, 669–680.
152. Kedersha, N.L., Gupta, M., Li, W., Miller, I. and Anderson, P. (1999) RNA-binding proteins TIA-1 and TIAR link the phosphorylation of eIF-2 alpha to the assembly of mammalian stress granules. *J. Cell. Biol.*, **147**, 1431–1442.
153. Paillard, L., Legagneux, V., Maniey, D. and Osborne, H.B. (2002) c-Jun ARE targets mRNA deadenylation by an EDEN-BP (embryo deadenylation element-binding protein)-dependent pathway. *J. Biol. Chem.*, **277**, 3232–3235.
154. DeMaria, C.T. and Brewer, G. (1996) AUF1 binding affinity to A+U-rich elements correlates with rapid mRNA degradation. *J. Biol. Chem.*, **271**, 12179–12184.
155. Ma, W.J., Cheng, S., Campbell, C., Wright, A. and Furneaux, H. (1996) Cloning and characterization of HuR, a ubiquitously expressed Elav-like protein. *J. Biol. Chem.*, **271**, 8144–8151.
156. Ma, W.J., Chung, S. and Furneaux, H. (1997) The Elav-like proteins bind to AU-rich elements and to the poly(A) tail of mRNA. *Nucleic Acids Res.*, **25**, 3564–3569.
157. Levine, T.D., Gao, F., King, P.H., Andrews, L.G. and Keene, J.D. (1993) Hel-N1: an autoimmune RNA-binding protein with specificity for 3' uridylate-rich untranslated regions of growth factor mRNAs. *Mol. Cell Biol.*, **13**, 3494–3504.
158. Nakagawa, J., Waldner, H., Meyer-Monard, S., Hofsteenge, J., Jenö, P. and Moroni, C. (1995) AUH, a gene encoding an AU-specific RNA binding protein with intrinsic enoyl-CoA hydratase activity. *Proc. Natl Acad. Sci. USA*, **92**, 2051–2055.
159. Henics, T., Nagy, E., Oh, H.J., Csermely, P., von Gabain, A. and Subjectk, J.R. (1999) Mammalian Hsp70 and Hsp110 proteins bind to RNA motifs involved in mRNA stability. *J. Biol. Chem.*, **274**, 17318–17324.
160. Liu, J., Dalmau, J., Szabo, A., Rosenfeld, M., Huber, J. and Furneaux, H. (1995) Paraneoplastic encephalomyelitis antigens bind to the AU-rich elements of mRNA. *Neurology*, **45**, 544–550.
161. Chung, S., Jiang, L., Cheng, S. and Furneaux, H. (1996) Purification and properties of HuD, a neuronal RNA-binding protein. *J. Biol. Chem.*, **271**, 11518–11524.
162. Pende, A., Tremmel, K.D., DeMaria, C.T., Blaxall, B.C., Minobe, W.A., Sherman, J.A., Bisognano, J.D., Bristow, M.R., Brewer, G. and Port, J. (1996) Regulation of the mRNA-binding protein AUF1 by activation of the beta-adrenergic receptor signal transduction pathway. *J. Biol. Chem.*, **271**, 8493–8501.
163. Nagy, E. and Rigby, W.F. (1995) Glyceraldehyde-3-phosphate dehydrogenase selectively binds AU-rich RNA in the NAD(+)-binding region (Rossmann fold). *J. Biol. Chem.*, **270**, 2755–2763.
164. Joseph, B., Orlian, M. and Furneaux, H. (1998) p21(waf1) mRNA contains a conserved element in its 3'-untranslated region that is bound by the Elav-like mRNA-stabilizing proteins. *J. Biol. Chem.*, **273**, 20511–20516.
165. Lin, S., Wang, W., Wilson, G.M., Yang, X., Brewer, G., Holbrook, N.J. and Gorospe, M. (2000) Down-regulation of cyclin D1 expression by prostaglandin A(2) is mediated by enhanced cyclin D1 mRNA turnover. *Mol. Cell Biol.*, **20**, 7903–7913.
166. Maurer, F., Tierney, M. and Medcalf, R.L. (1999) An AU-rich sequence in the 3'-UTR of plasminogen activator inhibitor type 2 (PAI-2) mRNA promotes PAI-2 mRNA decay and provides a binding site for nuclear HuR. *Nucleic Acids Res.*, **27**, 1664–1673.
167. Bhattacharya, S., Giordano, T., Brewer, G. and Malter, J.S. (1999) Identification of AUF-1 ligands reveals vast diversity of early response gene mRNAs. *Nucleic Acids Res.*, **27**, 1464–1472.
168. Hamilton, B.J., Nagy, E., Malter, J.S., Arrick, B.A. and Rigby, W.F. (1993) Association of heterogeneous nuclear ribonucleoprotein A1 and C proteins with reiterated AUUA sequences. *J. Biol. Chem.*, **268**, 8881–8887.
169. Wilson, G.M., Sun, Y., Lu, H. and Brewer, G. (1999) Assembly of AUF1 oligomers on U-rich RNA targets by sequential dimer association. *J. Biol. Chem.*, **274**, 33374–33381.
170. Gueydan, C., Droogmans, L., Chalou, P., Huez, G., Caput, D. and Krays, V. (1999) Identification of TIAR as a protein binding to the translational regulatory AU-rich element of tumor necrosis factor alpha mRNA. *J. Biol. Chem.*, **274**, 2322–2326.
171. Lasa, M., Mahtani, K.R., Finch, A., Brewer, G., Saklatvala, J. and Clark, A.R. (2000) Regulation of cyclooxygenase 2 mRNA stability by the mitogen-activated protein kinase p38 signaling cascade. *Mol. Cell Biol.*, **20**, 4265–4274.
172. Cok, S.J., Acton, S.J., Sexton, A.E. and Morrison, A.R. (2004) Identification of RNA-binding proteins in RAW 264.7 cells that recognize a lipopolysaccharide-responsive element in the 3'-untranslated region of the murine cyclooxygenase-2 mRNA. *J. Biol. Chem.*, **279**, 8196–8205.
173. Dixon, D.A., Tolley, N.D., King, P.H., Nabors, L.B., McIntyre, T.M., Zimmerman, G.A. and Prescott, S.M. (2001) Altered expression of the mRNA stability factor HuR promotes cyclooxygenase-2 expression in colon cancer cells. *J. Clin. Invest.*, **108**, 1657–1665.
174. Lapucci, A., Donnini, M., Papucci, L., Witort, E., Tempestini, A., Bevilacqua, A., Nicolini, A., Brewer, G., Schiavone, N. and Capaccioli, S. (2002) AUF1 is a bcl-2 A + U-rich element-binding protein involved in bcl-2 mRNA destabilization during apoptosis. *J. Biol. Chem.*, **277**, 16139–16146.
175. Sengupta, T.K., Bandyopadhyay, S., Fernandes, D.J. and Spicer, E.K. (2004) Identification of nucleolin as an AU-rich element binding protein involved in bcl-2 mRNA stabilization. *J. Biol. Chem.*, **279**, 10855–10863.
176. Donnini, M., Lapucci, A., Papucci, L., Witort, E., Jacquier, A., Brewer, G., Nicolini, A., Capaccioli, S. and Schiavone, N. (2004) Identification of TINO: a new evolutionarily conserved BCL-2 AU-rich element RNA-binding protein. *J. Biol. Chem.*, **279**, 20154–20166.
177. Onesto, C., Berra, E., Grepin, R. and Pages, G. (2004) Poly(A)-binding protein-interacting protein 2, a strong regulator of vascular endothelial growth factor mRNA. *J. Biol. Chem.*, **279**, 34217–34226.
178. Hamilton, B.J., Nichols, R.C., Tsukamoto, H., Boado, R.J., Partridge, W.M. and Rigby, W.F. (1999) hnRNP A2 and hnRNP L bind the 3'-UTR of glucose transporter 1 mRNA and exist as a complex *in vivo*. *Biochem. Biophys. Res. Commun.*, **261**, 646–651.
179. King, P.H., Levine, T.D., Fremeau, R.T. Jr and Keene, J.D. (1994) Mammalian homologs of *Drosophila* ELAV localized to a neuronal subset can bind *in vitro* to the 3' UTR of mRNA encoding the Id transcriptional repressor. *J. Neurosci.*, **14**, 1943–1952.
180. Chung, S., Eckrich, M., Perrone-Bizzozero, N., Kohn, D.T. and Furneaux, H. (1997) The Elav-like proteins bind to a conserved regulatory element in the 3'-untranslated region of GAP-43 mRNA. *J. Biol. Chem.*, **272**, 6593–6598.
181. Gallouzi, I.E., Brennan, C.M. and Steitz, J.A. (2001) Protein ligands mediate the CRM1-dependent export of HuR in response to heat shock. *RNA*, **7**, 1348–1361.