

Review

HuR and mRNA stability

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Abstract. An important mechanism of posttranscriptional gene regulation in mammalian cells is the rapid degradation of messenger RNAs (mRNAs) signaled by AU-rich elements (AREs) in their 3' untranslated regions. HuR, a ubiquitously expressed member of the Hu family of RNA-binding proteins related to *Drosophila* ELAV, selectively binds AREs and stabilizes ARE-containing mRNAs when overexpressed in cul-

tured cells. This review discusses mRNA decay as a general form of gene regulation, decay signaled by AREs, and the role of HuR and its Hu-family relatives in antagonizing this mRNA degradation pathway. The influence of newly identified protein ligands to HuR on HuR function in both normal and stressed cells may explain how ARE-mediated mRNA decay is regulated in response to environmental change.

Key words. AU-rich element; ELAV; Hu proteins; stress; nuclear export; mRNA stability.

Introduction

The growth and development of eukaryotic organisms require that gene expression be regulated. Typically, this regulation is considered to occur at the level of DNA (differential transcription) or protein (selective degradation). However, gene expression can also be regulated at the level of RNA. Posttranscriptional gene regulation occurs through alterations in translational efficiency (reviewed in [1]) and in messenger RNA (mRNA) stability (for review, see [2] and [3]). The stability of mRNA fluctuates tremendously in eukaryotes (reviewed in [4]). In yeast, message half-lives range from approximately 5 min to more than 60 min. In vertebrates, message half-lives vary from 20 min to more than 24 h. Consequently, 1000-fold differences in the cellular abundance of various mRNAs can result from seemingly minor differences in half-life (for re-

view, see [3]). These changes are ultimately reflected in the amount of protein produced.

Over the course of the last 15 years, many different types of mRNA decay have been described which alter the stability of particular mRNAs in eukaryotes. Both the signals and proteins participating in these processes have been identified. Some act to degrade, whereas others selectively stabilize mRNA. In this review, we provide a general background concerning these different forms of mRNA decay and the proteins implicated in determining message stability. We focus on one specific pathway of mRNA degradation, mediated by AU-rich elements (AREs), and a protein, HuR, which antagonizes this degradation.

Many different elements affect mRNA stability

Messenger RNA degradation is dependent upon both cis-elements in the RNA and trans-acting factors. The cis-elements that affect mRNA stability are numerous and vary in location (fig. 1). Some elements are ubiqui-

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tous, whereas others signal the degradation of specific messages.

Beginning at the 5' end of the molecule, the cap structure is an important determinant of the stability of all messages. Its 5'-5' triphosphate linkage renders messages resistant to general exonucleases. Only by removal of the cap structure can degradation occur in a 5' to 3' fashion. Studies in yeast have identified a decapping enzyme (DCP1; [5]) as well as a cytoplasmic 5' to 3' exonuclease (XRN1; [6]). Decapping activity requires at least two distinct proteins that interact directly or indirectly (DCP1 and DCP2; [7]). Yeast cells also contain a nuclear 5' to 3' exonuclease (Rat1), which is functionally interchangeable with the cytoplasmic 5' to 3' exonuclease [8]. Higher eukaryotic homologs of both the decapping enzyme and the 5' to 3' exonucleases remain to be identified.

At the opposite end of the molecule, the poly(A) tail plays an important role in mRNA stability (reviewed in [9]). Poly(A) shortening is the rate-limiting step in the turnover of many mRNAs. Several 3' to 5' exoribonuclease activities have been identified in yeast, including poly(A) nuclease (PAN) [10], mitochondrial NTP-dependent exoribonuclease (mtEXO) [11] and the exosome (reviewed in [12]). PAN activity requires two proteins, Pan2p [13] and Pan3p [14]; it is not clear which PAN protein is the ribonuclease. Likewise, whereas mtEXO contains three subunits, 75, 90 and 110 kDa, the nucleolytic subunit remains to be identified.

A higher eukaryotic deadenylating nuclease (DAN or PARN) has been purified from calf thymus [15] and the *Xenopus* oocyte [16]. PARN activity is altered (either stimulated or inhibited depending on salt concentration) in vitro by the cytoplasmic poly(A)-binding protein (PAB I). The microinjection of anti-PARN antibody into *Xenopus* oocytes inhibits default deadenylation during progesterone-induced maturation [16]. Similarly, the ectopic expression of a human PARN

homolog in enucleated *Xenopus* oocytes rescues maturation-specific deadenylation [16]. Interestingly, PARN has recently been discovered to bind the 5' cap structure and thereby enhance the rate of deadenylation [17, 18].

The yeast exosome is an ~300–400 kDa complex consisting of at least 11 proteins, many or all of which independently contain exoribonucleolytic activity [19, 20]. Ten of these proteins are essential for viability, and the absence of one results in temperature-sensitive lethality [20]. The exosome, like the proteasome, performs both partial and complete degradation of substrates. Human homologs exist for at least 9 of the 11 identified subunits [20]. Three of these human proteins complement mutations in their counterparts in *Saccharomyces cerevisiae* [19, 20]. Two of the human homologs are components of the PM-Scl particle, recognized by the sera of patients with the relatively rare autoimmune disease polymyositis-scleroderma overlap syndrome [20]. Therefore, it is likely that the PM-Scl particle is the functional human counterpart of the yeast exosome.

The degradation of mRNAs can be signaled by sequence elements in the 5' untranslated region (UTR), coding sequence and/or the 3' UTR (see fig. 1). For example, the 5' UTR and proximal coding sequence region are required for stabilization of the interleukin-2 message by the *c-jun* NH2-terminal kinase pathway [21]. The coding region of several messages, including the yeast MAT α 1 mRNA [22], mammalian *c-fos* [23] and *c-myc* mRNAs [24], contain specific destabilizing sequences. More generally, coding sequences can promote degradation whenever they contain premature nonsense codons upstream of special regions termed downstream elements in yeast (for review, see [25]) or of introns in vertebrates (reviewed in [26]).

3' UTRs often regulate decay. One well-studied example is the 3' UTR of transferrin mRNA, where an instability element is located adjacent to stem-loop structures

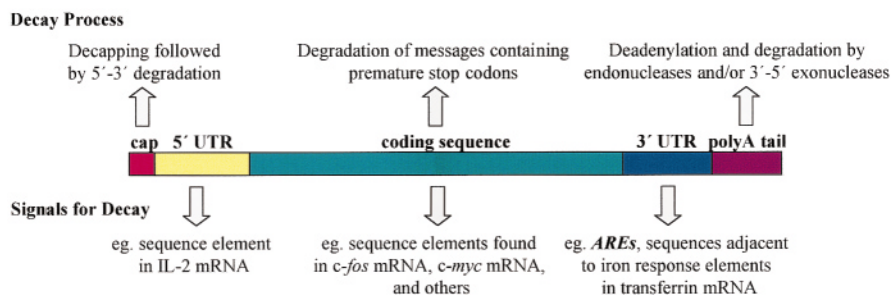


Figure 1. mRNA stability is determined by numerous cis-acting elements. Indicated above are decay processes that act on all mRNAs. Examples of decay signals found only in certain mRNAs are listed below. See text for references.

called iron-responsive elements (IREs), which are reversibly bound by an iron-regulatory protein (IRP) leading to mRNA stabilization at reduced levels of cellular iron (for review, see [27]). Hormones or cyclic nucleotides alter the stability of a number of specific mRNAs through sequences in their 3' UTRs (see [28] for the plasminogen activation inhibitor mRNA and a discussion of other examples). Another well-studied instance is the 3' UTR of α -globin mRNA, where the binding of a multiprotein complex confers stability, apparently by protecting the poly(A) tail [29]. Finally, since the poly(A) tail is important for mRNA stability, elements in the 3' UTR that regulate poly(A) lengthening (such as the cytoplasmic polyadenylation element [CPE] and AAUAAA [30, 31]) should not be ignored as potential determinants affecting mRNA decay.

AREs target mRNA for rapid degradation

The best-studied instability element in mammalian messages is the ARE [32]. AREs consist of multiple stretches of adenylate and uridylate residues and are present in the 3' UTRs of many mRNAs, including those of growth factors, cytokines and lymphokines. Because of the importance of proteins encoded by ARE-containing mRNAs to normal and neoplastic cell growth, this element must be considered a pivotal gene regulatory target in vertebrate cells.

The earliest account of an ARE came from Verma and colleagues [33]. Comparison of the mRNAs encoding *v-fos*, an oncogene carried by the FBJ murine osteosarcoma virus, and *c-fos*, its cellular protooncogene counterpart, revealed differences in the 3' UTR. In contrast to the *v-fos* 3' UTR, the *c-fos* 3' UTR contains an ARE. This difference is functionally significant. When the *c-fos* coding region was placed upstream of the *v-fos* 3' UTR (but not the *c-fos* 3' UTR) its overexpression led to the transformation of cultured cells. Further analysis revealed that the removal of a 67-nucleotide sequence in the 3' UTR converted *c-fos* into a transforming gene [34]. Based on these and additional data showing that the transient accumulation of *c-fos* mRNA following serum stimulation is mediated by a region contained in the 3' UTR, Treisman [35] speculated that these sequences act to destabilize the mRNA. This hypothesis was proven correct by Shaw and Kamen [36], who demonstrated that the insertion of a 51-nucleotide AU-rich sequence from the 3' UTR of human granulocyte monocyte-colony stimulating factor (GM-CSF) mRNA into the 3' UTR of the relatively stable β -globin mRNA signaled its degradation. Mutation of the ARE to include a higher G-C content abolished the destabilization of the β -globin reporter mRNA. ARE-directed mRNA degradation is initiated by rapid removal of the

poly(A) tail and followed by degradation of the remainder of the message [37].

Since the initial discovery of the *c-fos* ARE, the catalog of ARE-containing mRNAs has increased markedly. Sequences of these AREs and the decay patterns of the messages in which they reside have led to ARE classification. Currently, AREs have been assigned to three classes based on sequence and decay characteristics [32]. Class I AREs, such as the *c-fos* ARE, contain one to three scattered copies of the pentamer AUUUA embedded within U-rich regions. Class II AREs, like the GM-CSF ARE, consist of at least two overlapping copies of a critical nonamer UUAUUUA(U/A)(U/A) [38] also in the context of a U-rich region. Class III AREs, an example of which is the *c-jun* ARE, lack the hallmark AUUUA pentamer but signal degradation with U-rich (and possibly other unknown) sequences. mRNAs containing class III AREs, like those containing class I AREs, exhibit degradation intermediates with 30–60 nucleotides of their poly(A) tail remaining. Detectable degradation intermediates of messages containing class II AREs are poly(A)⁻.

In vivo and in vitro systems for the study of ARE-mediated decay

The study of ARE-mediated decay has been facilitated by the development of both in vivo and in vitro systems. Shyu and colleagues designed the first in vivo system [23]. It takes advantage of the rapid but transient response of the *c-fos* promoter to serum induction. The central construct contains the human *c-fos* promoter region fused to the 5' UTR, protein-coding region, 3' UTR and polyadenylation signal of the rabbit β -globin gene. Various AU-rich sequences can be inserted into the 3' UTR. The resulting plasmid is transfected into cells along with a control plasmid encoding a stable RNA, the cells are serum-starved (usually overnight) and serum is later added to stimulate transcription from the *c-fos* promoter. Alternatively, transcription can be induced by the addition of purified growth factors [39]. Transcription levels rise to a maximum approximately 30–45 min after cell stimulation [39], and mRNA stability is then assayed by either Northern blotting or RNase protection. Although this system has been extremely useful, it is not ideal. Serum starvation restricts observation to cells undergoing the G0–G1 transition. Moreover, the induction procedure can potentially complicate the analysis of regulatory mechanisms affecting ARE-directed mRNA decay since it relies on cell stimulation.

A second in vivo system reproduces the transcriptional pulse while avoiding the disadvantages of the *c-fos* promoter system [40]. This procedure utilizes the high-

affinity, regulatable interaction between the tetracycline transactivator (tTA) and the tetracycline regulatable element (TRE) (for review, see [41]). tTA is a fusion protein composed of the *Escherichia coli* tet repressor and the activation domain of the herpes simplex virus VP16 protein. The TRE contains seven copies of a 19-bp sequence derived from the tetracycline-resistance operon of the bacterial transposon Tn10. The rabbit β -globin 5' UTR, coding sequence and 3' UTR have been cloned downstream of a TRE and a human cytomegalovirus (CMV) minimal promoter. AREs are inserted into the rabbit β -globin 3' UTR, and the resulting plasmid is cotransfected into cells with a plasmid encoding a stable message. tTA must be coexpressed by either transient or stable transfection of a plasmid encoding this protein. In the absence of tetracycline, tTA interacts with the TRE and drives transcription; in the presence of tetracycline, a conformational change renders tTA incapable of binding the TRE and activating transcription. ARE-mediated decay is assessed by Northern blotting in reference to a stable mRNA. In principle, the opposite approach could be used in cells expressing the reverse tetracycline transactivator (rtTA). rtTA is a mutant version of tTA that allows transcription downstream of the TRE only in the presence of tetracycline or tetracycline-related compounds [42].

Three in vitro systems for investigating ARE-mediated mRNA degradation have also been established. A system derived from a human erythroleukemic K562 cell extract [43] has been used to characterize the 3' to 5' decay of *c-myc* mRNA [44, 45]. The degradation of this transcript is enhanced by hnRNP D (AUF1; [46]), which specifically binds the *c-myc* ARE (see below). The second degradation system utilizes S100 extracts either from PC12, H9c2 or WT-8 cells. A capped RNA containing the vascular endothelial growth factor 3' UTR and a poly(A) tail is stabilized when the extract is prepared from hypoxic cells [47] or when HuR is added to the extract [48] (see below). The third in vitro system uses HeLa cell S100 extract to investigate the decay of ARE-containing RNAs that range from 94 to 132 nucleotides in length, including a 60-residue poly(A) tail [49]. Curiously, large amounts of poly(A) must be added to the extract in both this [49] and the 3' to 5' decay system [44] in order to observe deadenylation/degradation. Degradation but not deadenylation requires ATP. The effect of an ARE in this system is to increase deadenylation, as observed in vivo. Moreover, the addition of a Hu-family protein (see below) inhibits degradation but not deadenylation in vitro, consistent with in vivo observations that overexpression of HuR slows decay of the mRNA body, not deadenylation (see below). This system has recently been employed to show that PARN interacts with the 5' cap and influences mRNA deadenylation rates [17].

ARE-mediated decay is a regulated phenomenon

There exists substantial evidence that ARE-mediated mRNA decay is subject to regulation. Cell stress [50], stimulation [51] and neoplastic transformation [52] have all been shown to stabilize ARE-containing mRNAs. Moreover, in some cases known signal transduction pathways have been implicated in the response to these stimuli. The treatment of quiescent primary T cells with antibodies directed against CD3/CD28 receptors stabilizes several mRNAs containing AREs [51]. Stabilization of ARE-containing mRNAs has been associated with the activation of *c-jun* N-terminal kinase (JNK), which is correlated with lower decay rates of interleukin (IL)-3 mRNA in mast cells [53]. Similarly, the activation of MAP kinase-activated protein kinase 2 has been associated with the stabilization of ARE-containing mRNA in HeLa cells [54, 55]. mRNA stability has also been linked to the activity of phosphatases. An antagonist of calcineurin (protein phosphatase 2B), cyclosporin A, destabilizes IL-3 mRNA in autocrine tumor cell lines [56]. At present, the mechanisms by which these kinases and phosphatases function to produce changes in mRNA stability remain unclear. Much work will be required to determine the cellular roles of the multiple players in these pathways.

The role of trans-acting factors in the stability of ARE-containing mRNA

In an effort to understand the mechanism and regulation of ARE-signaled mRNA degradation, a number of laboratories have looked for proteins that selectively bind AU- and U-rich sequences. At least 14, apparently distinct proteins have been identified in cell extracts by ultraviolet (UV)-crosslinking and gel-shift assays: AUBF [57], AU-A [58], AU-B [58], AU-C [59], Hel-N1 [60], hnRNP D (AUF1; [61]), hnRNP A1 [62], hnRNP C [62], AUH [63], GAPDH [64], hnRNP A0 [65], HuR [66, 67], tristetraprolin [68] and TIAR [69]. However, only two of these proteins, hnRNP D and HuR, have been demonstrated to alter the stability of ARE-containing mRNA in vivo.

HnRNP D has been reported to be a DNA- [70], as well as an RNA-binding protein [71]. It consists of four alternatively spliced isoforms: p37, p40, p42 and p45 [72]. All isoforms contain two RRM domains and are localized in both the nucleus and the cytoplasm [61]. The overexpression of hnRNP D (particularly the p37 and p42 isoforms) in hemin-induced human erythroleukemic K562 cells increased ARE-directed mRNA decay [73]. The triggering of degradation is consistent with changes in the cellular localization of hnRNP D. Blocking ARE-mediated mRNA decay by heat shock, downregulation of the ubiquitin-proteasome pathway or by inactivation

Table 1. RNAs bound by HuR.

RNA	ARE class	Reference
β -adrenergic receptor	III	[119]
Cyclin A	I	[82]
Cyclin B1	I	[82]
Cyclin D1	I	[82]
<i>c-fos</i>	I	[83]
<i>c-myc</i>	I	[83]
HPV-16 late	III	[120]
HPV-1 late	I	[121]
<i>Herpesvirus saimiri</i> -encoded U RNA-1	II	[122]
<i>Herpesvirus saimiri</i> -encoded U RNA-2	I	[122]
<i>Herpesvirus saimiri</i> -encoded U RNA-5	I	[122]
IL-3	II	[83]
<i>N-myc</i>	III	[83]
Neurofibromin	III	[123]
p21	I	[81]
Plasminogen activator inhibitor	I	[124]
Tumor necrosis factor- α	II	[49]
Vascular endothelial growth factor	III	[48]
GAP-43	III	[125]

of the E1 ubiquitinating enzyme all resulted in hnRNP D movement to the nucleus of human HeLa cells [74]. Interestingly, hnRNP D (all isoforms) is also part of a specific RNP complex which associates with the 3' UTR of α -globin and appears to regulate the accumulation of α -globin mRNA in erythrocytes [75]. The cellular factors and/or events involved in regulating these different activities for hnRNP D remain to be defined.

HuR stabilizes ARE-containing mRNA

HuR (or HuA) is a ubiquitously expressed member of the embryonic lethal abnormal vision (ELAV) family of RNA-binding proteins [66, 76], originally identified in *Drosophila melanogaster* as essential for neural development [77]. Both gel shift [67] and UV-crosslinking [78] experiments have provided evidence that HuR binding parallels the in vivo ability of ARE sequences to direct mRNA degradation. However, overexpression of this protein does not enhance degradation, but rather stabilizes messages containing class I and class II (and to a lesser extent class III) AREs in transient transfection experiments [79, 80]. Accordingly, most of the specific mRNAs bound by HuR in vitro (table 1) contain class I or class II AREs. HuR appears to act by protecting the body of the message from degradation, rather than slowing the rate of deadenylation, in overexpressing cells [80]. An alternative explanation for the stabilization observed in cells overexpressing HuR is that it is active in decay, but when overexpressed sequesters

other factors needed for degradation. A HuR gene knockout should clarify this question. The finding that expression of antisense RNA to HuR increases the decay of ARE-containing mRNAs [81, 82] is consistent with HuR's major role being to stabilize.

Like other Hu-family proteins (see below), HuR contains three classic RNA recognition motifs (RRMs). At least in the case of HuD, ARE recognition appears to be mediated by the first two RRM; the third RRM of HuD has been suggested to bind the poly(A) tail [83]. In transient transfection assays, deletion of RRM3 alone abolishes HuR's ability to stabilize ARE-containing reporter mRNAs [79].

Although predominantly nuclear, HuR shuttles between the nucleus and the cytoplasm via a novel shuttling sequence, HNS, located in the hinge region between its second and third RRM (see fig. 2) [79, 84]. HNS is similar to the M9 shuttling sequence of hnRNP A1 but differs in several critical residues. The nuclear export receptor for HNS is not yet known. HuR's ability to shuttle has led to the suggestion that HuR may initially bind mRNAs in the nucleus and accompany them into the cytoplasm, providing ongoing protection from the degradation machinery. Recent in vivo crosslinking experiments and gradient analyses established that HuR is capable of binding poly(A) + RNA in both the nucleus and the cytoplasm [85]. These data are consistent with the observation that a substantial fraction of cytoplasmic HuR (~15%) is associated with polysomes [85].

HuR binding proteins modulate the nucleocytoplasmic trafficking of HuR

In an effort to understand the intracellular interactions that regulate the activity of HuR in stabilizing ARE-containing mRNA, several protein ligands to HuR have been identified and characterized [86]. Four ligands were purified through affinity chromatography by passing RNase A-treated HeLa nuclear extract [87] over a column containing HuR fused to glutathione-S transferase. They are SET α/β [88, 89], pp32 [90] and acidic protein rich in leucine (APRIL; [91]). Coimmunoprecipitation experiments confirmed that interactions between these ligands and HuR can be detected in gently lysed cell extracts.

Three of these HuR ligands (SET α , SET β and pp32) had previously been identified as inhibitors of protein phosphatase 2A (PP2A) [92, 93]. PP2A is a multimeric serine/threonine phosphatase, affecting a diverse set of cellular functions including: cell cycle progression, DNA replication, transcription, splicing, development and morphogenesis [94]. Its diverse cellular roles reflect the enzyme's ability to convert between holoenzyme

forms in response to stimulation [95]. PP2A dephosphorylates both the substrates of kinases and kinases themselves, thereby regulating their activities. Its targets include several major protein-kinase families including those of the AGC subgroup (e.g. protein kinase B [96], protein kinase C [97] and p70 S6 kinase [98]), the calmodulin-dependent kinase [99] and members of the ERK MAP-kinase pathway [100, 101].

The four HuR-binding proteins exhibit striking structural similarity. All contain an acidic C-terminal tail of at least 50 amino acids [86]. Beyond this common acidic region, the HuR-binding proteins divide into two subsets: SET α and SET β , and pp32 and APRIL. SET α and SET β are identical over their 253 C-terminal amino acids, and are probably splice variants of one another [89]. In contrast, pp32 (249 amino acids) and APRIL (251 amino acids) exhibit 71% sequence identity and 81% sequence similarity and are clearly products of separate genes. They contain a second structural similarity: rev-like leucine-rich repeats in their N-terminal regions. Deletion experiments suggested that the acidic tail of at least pp32 (and possibly these other HuR ligands) is required for their interaction with a region spanning the hinge region and third RRM of HuR [86].

Confocal microscopy revealed that SET α/β are detected in both the nucleus and cytoplasm, whereas pp32 and APRIL are predominantly nuclear. Heterokaryon fusion experiments showed that like HuR, pp32 and APRIL shuttle between the nucleus and the cytoplasm [86]. As suggested by their rev-like leucine-rich repeats, coimmunoprecipitation experiments demonstrated that pp32 and APRIL interact with the nuclear export factor CRM1. Accordingly, inhibition of CRM1 with the antifungal and antitumor agent leptomycin B (LMB; [102–104]) resulted in the loss of shuttling and nuclear accumulation of pp32 and APRIL. Most important, CRM1 inhibition produced increased association of HuR with pp32 and with APRIL, and an increase in HuR's ability to bind nuclear poly(A) + RNA in vivo. Moreover, a specific ARE-containing mRNA, *c-fos*, becomes retained in the nucleus after LMB treatment. These data provide evidence for the in vivo interaction of HuR with its ligands and strongly suggest that these HuR ligands either increase HuR's affinity for its target mRNAs or modulate HuR export from the nucleus. The fact that three of these HuR-binding proteins are documented PP2A inhibitors argues that PP2A is involved either in signaling cascades that regulate the stability of ARE-containing mRNAs or directly in the mechanism of ARE-mediated mRNA decay.

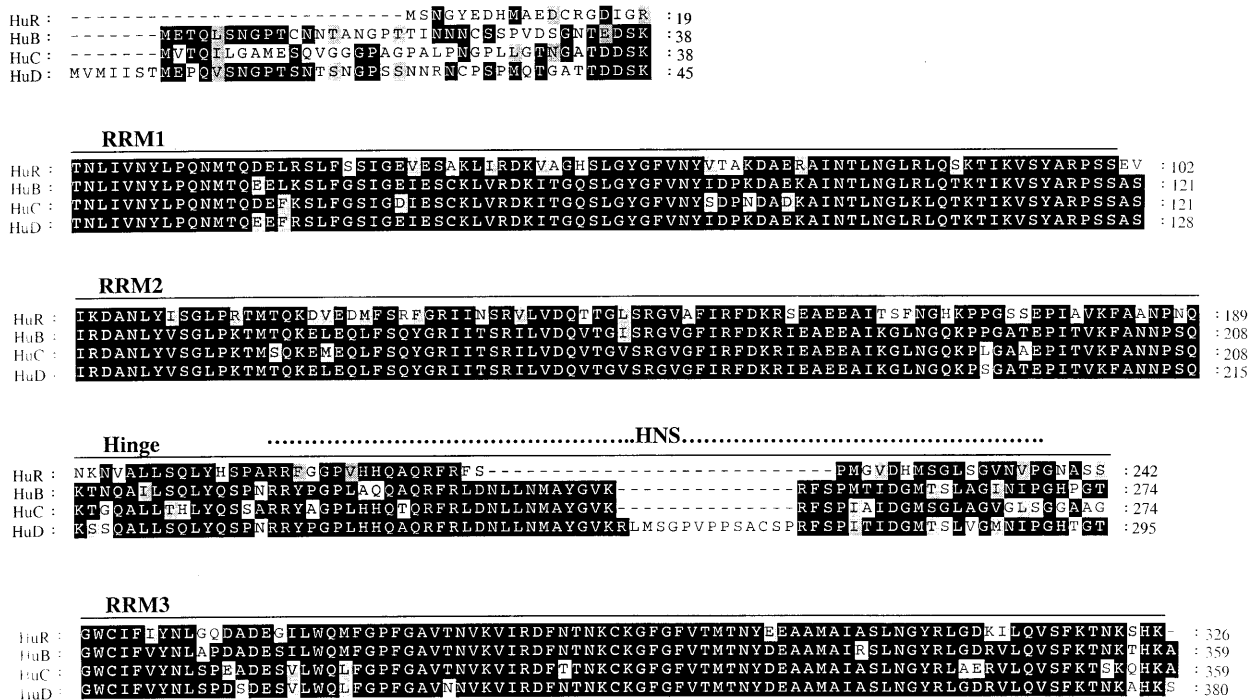


Figure 2. Sequence alignment of human Hu-family proteins. Identities appear in black, similarities appear in gray. The amino acids comprising the shuttling sequence of HuR, HNS' are indicated; it is not known whether the insertions and changes in this region of the other Hu proteins affect the functioning of HNS.

Hu-family proteins promote cellular differentiation

Genetic analyses performed in *D. melanogaster* have shown the ELAV locus to be essential for proper neural development [77, 105]. The expression patterns of Hu-family proteins hint that this is also true of ELAV's mammalian counterparts. There are two neural-specific Hu-family members in mammals: HuC [106], and HuD [107]. Another Hu-family member, HuB, is expressed in both neurons and sex organs ([108] and references therein). All four of the Hu-family proteins are target antigens in antibody-associated paraneoplastic encephalomyelitis sensory neuropathy (Hu syndrome) (reviewed in [109]). Like HuR, ELAV and the other Hu-family proteins all contain three RRM. Between RRM2 and 3 is a poorly conserved hinge region of ~50–80 amino acids. Recent NMR studies of the first two RRM of HuC show that the individual RRM bind weakly to AUUUA, whereas a didomain fragment (comprised of both RRM) binds more tightly to a longer ARE [110]. Several amino acid residues, which are conserved in the related ELAV family protein Sxl, are implicated in mRNA binding. HuB (Hel-N1) and its alternatively spliced isoform (Hel-N2) have been shown to stabilize deadenylated intermediates generated from the turnover of ARE-containing substrate mRNAs in vitro [49]. This result is consistent with the ectopic expression of HuB (Hel-N1) resulting in Glut1 (a class III-ARE-containing mRNA) stabilization [111]. Thus, Hu-family proteins, like HuR, may function by stabilizing their target mRNAs in vivo.

Recently published reports from several laboratories have confirmed the involvement of Hu-family members in neural development. The overexpression of HuB, HuC and HuD in PC12 cells has been shown to induce a neuronal phenotype even in the absence of nerve growth factor [106, 112]. Moreover, the misexpression of chicken HuD in cultured neural crest cells results in an increase in the proportion of cells exhibiting neuronal morphology [113]. Significantly, the overexpression of HuR in these same cells does not induce neurite outgrowth. Neurite formation can likewise be induced in human embryonic teratocarcinoma cells by transfection with HuB [114]. Moreover, the treatment of PC12 cells with antisense deoxynucleotides directed against HuD blocks the induction of neurite outgrowth in NGF-treated PC12 cells [115]. Interestingly, mutants of HuB and HuC lacking their third RRM fail to produce this phenotype upon overexpression [106]. Instead, the third RRM of HuB and HuC function as dominant-negative proteins when cotransfected with either wild-type HuB or wild-type HuC into PC12 cells [106]. These dominant-negative mutants also prevent mouse embryonic central nervous system maturation

when misexpressed, as judged by immunostaining for an early motor neuronal marker. RRM3 of HuD was also observed to be important for the neurite-inducing activity in PC12 cells [112]. The fact that the third RRM of HuB and HuC (and presumably HuD) retain only a marginal ability to bind RNA [106] suggests that they may function as dominant negatives by competing with their endogenous (complete) counterparts for intracellular interactions with essential ligands. Perhaps these are the same proteins as those characterized as HuR ligands [86]. Thus, the same signaling pathway may have different outputs in different tissues, simply because the end target (an mRNA required for neural differentiation) and its regulator (an Hu-family protein) are differentially expressed.

Cytoplasmic localization of Hu-family proteins is induced by stress, correlates with RNA stabilization and is required for cellular differentiation

The realization that HuR both stabilizes ARE-containing mRNAs and shuttles led to the idea that HuR might bind its target mRNAs in the nucleus and follow them to the cytoplasm to provide ongoing protection from the degradation machinery [79, 80]. Although this hypothesis remains unproven, much evidence has emerged to support the importance of HuR's cytoplasmic localization for mRNA stabilization (for review, see [116]). Gorospe and colleagues have recently shown that the increased cytoplasmic localization of HuR upon the treatment of cells with UV light is correlated with the stabilization of p21 mRNA, an ARE-containing mRNA [81]. Gorospe and colleagues have also correlated the increased cytoplasmic localization of HuR during late G1, S and G2 phases in colorectal carcinoma RKO cells with the stabilization of ARE-containing mRNA [82]. Similarly, an increased ability of epithelial cells containing relatively high amounts of cytoplasmic HuR to stabilize mRNA containing the human papillomavirus type 1 AU-rich element has been reported [117]. These data are consistent with the increased cytoplasmic localization of HuR following heat shock [85], a stress known to stabilize ARE-containing mRNA [74]. A model for HuR nucleocytoplasmic transport after heat shock is presented in figure 3.

Interestingly, the cytoplasmic localization of other mammalian Hu-family proteins appears to be crucial for their function [108]. Two deletion mutants of HuD containing its novel nuclear export sequence dominantly inhibit the wild-type protein's neurite-inducing activity in PC12 cells [112]. Comparable deletion mutants inhibit the neurite-inducing activity of HuB in PC12 cells [106].

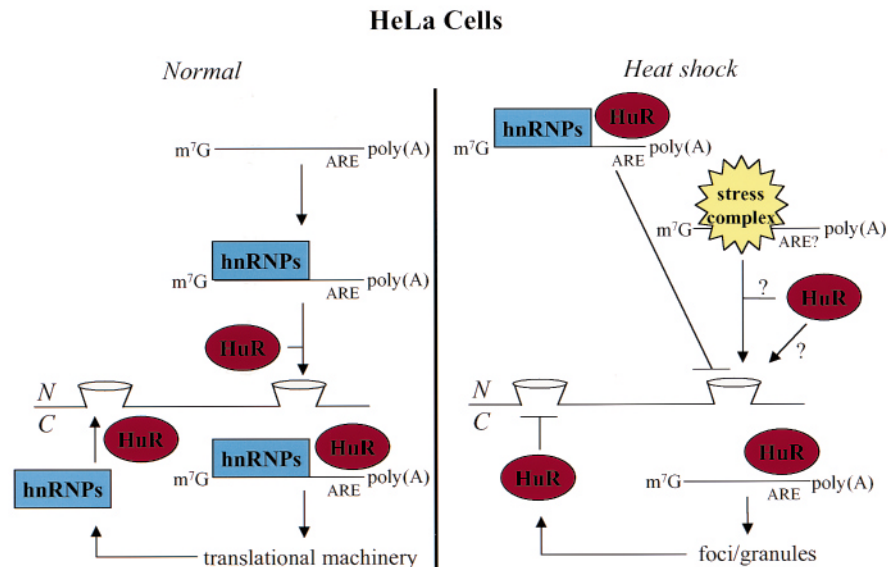


Figure 3. Model for HuR transport and hnRNP assembly in the presence and absence of stress, heat shock being the specific example. In vivo UV-crosslinking of proteins to poly(A) + RNA indicates that HuR normally binds nuclear mRNA shortly before export and well after hnRNP A1 binds. Upon heat shock, HuR is exported (whether with or without mRNA is not known) and accumulates in cytoplasmic foci [85] similar, if not identical, to stress granules [118].

Future challenges

Although much has been learned about the role HuR plays in ARE-mediated mRNA stability since it was first recognized 4 years ago, much more remains to be understood. First, how does HuR specifically recognize ARE-containing mRNAs? Does a single HuR molecule simultaneously bind the ARE (using RRM1 and 2) and the poly(A) tail (using RRM3)? Are the residues that contact the mRNA amino acids that are conserved between HuB, HuC, HuD and HuR? Second, the export and import receptor(s) for HuR remain to be identified. Are they the same or different for other Hu-family proteins? Is HuR a dominant player in the export of ARE-containing mRNAs? Third, do the HuR ligands increase the affinity of HuR for its target mRNAs or do they regulate HuR export, thereby modulating its ability to stabilize ARE-containing mRNAs? Fourth, does PP2A inhibition play a role in ARE-mediated mRNA stability in terms of either mechanism or regulation? Finally, how does hnRNP D, which appears to facilitate the degradation of ARE-containing mRNAs, fit into the puzzle? Do hnRNP D and HuR simply compete for the same binding site(s) in the 3' UTR of mRNAs? If hnRNP D were to triumph in this dynamic struggle, then decay might be instantaneous, consistent with in vivo crosslinking results in which hnRNP D binding to poly(A) + RNA was not detected [85]. What about all of the other ARE-binding proteins that have

been identified? Are they coconspirators in either HuR-mediated stabilization or hnRNP D-targeted degradation of this important class of cellular mRNAs? Insights from structural biology and biochemistry, as well as cell biological approaches, will be needed to achieve a molecular understanding of this critical aspect of gene regulation in mammalian cells.

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