Cell. Mol. Life Sci. 65 (2008) 3168 – 3181
1420-682X/08/203168-14
DOI 10.1007/s00018-008-8252-6

Cellular and Molecular Life Sciences

Review

Diverse molecular functions of Hu proteins

M. N. Hinman[a] and H. Lou[a,b,c,*]

[a] Department of Genetics, School of Medicine, Case Western Reserve University, 10900 Euclid Ave., Cleveland, Ohio 44106 (USA), Fax: +1 (216) 368 – 0491, e-mail: hxl47@case.edu
[b] Case Comprehensive Cancer Center, School of Medicine, Case Western Reserve University, 10900 Euclid Ave., Cleveland, Ohio 44106 (USA)
[c] Center for RNA Molecular Biology, School of Medicine, Case Western Reserve University, 10900 Euclid Ave., Cleveland, Ohio 44106 (USA)

Received 07 May 2008; received after revision 23 May 2008; accepted 27 May 2008
Online First 26 June 2008

Abstract. Hu proteins are RNA-binding proteins involved in diverse biological processes. The neuronal members of the Hu family, HuB, HuC, and HuD play important roles in neuronal differentiation and plasticity, while the ubiquitously expressed family member, HuR, has numerous functions mostly related to cellular stress response. The pivotal roles of Hu proteins are dictated by their molecular functions affecting a large number of target genes. Hu proteins affect many post-transcriptional aspects of RNA metabolism, from splicing to translation. In this communication, we will focus on these molecular events and review our current understanding of how Hu proteins mediate them. In particular, emphasis will be put on the nuclear functions of these proteins, which were recently discovered. Three examples including calcitonin/calcitonin gene-related peptide, neurofibromatosis type 1, and Ikaros will be discussed in detail. In addition, an intriguing theme of antagonism between Hu proteins and other AU-rich sequence binding proteins will be discussed.

Keywords. RBPs, Hu proteins, mRNA stability, translation, polyadenylation, splicing.

Introduction

Recent years have witnessed an increasing realization that post-transcriptional regulation is essential for ensuring tight and precise control of gene expression. It is now widely appreciated that each and every step of post-transcriptional processes, including splicing, polyadenylation, editing, nuclear export, RNA localization, RNA degradation, and translation, can serve as a regulation point to modulate protein production of a particular gene. RNA-binding proteins (RBPs) play essential roles in the maturation and function of mRNAs. A conservative estimate suggests that there are more than a thousand RBPs in the human genome, of which more than 300 contain one or more classical RNA-binding domains known as RNA recognition motifs (RRMs) [1, 2]. In their journeys starting as pre-mRNA molecules in the nucleus and ending as mRNA molecules in the cytoplasm, RNAs are always accompanied by RBPs, which provide constant guidance and protection [3]. Thus, RBPs play essential roles in regulating the function and fate of the RNA molecules with which they associate.

Hu proteins, the focus of this review, are a group of classical RBPs. Three of the four members of this protein family are highly enriched in neurons. The Drosophila homolog of these proteins, embryonic

* Corresponding author.

lethal abnormal vision (ELAV) protein, is exclusively expressed in neurons and plays an essential role in flies [4]. As discussed below, Hu proteins modulate many aspects of post-transcriptional regulatory events. Understanding how these proteins function at the molecular level will provide important insights into the complex and coordinated biological output of the nervous system.

RBPs in the brain

RBPs are expressed in many different tissue and cell types. A recent genome-wide study examined expression of 323 mouse RBPs, the functions of which are mostly uncharacterized, in developing brain using *in situ* hybridization [5]. The RBPs in this study include many important proteins that are ubiquitously involved in gene expression, such as spliceosomal proteins. The authors found that approximately two-thirds of these proteins show restricted expression to anatomically distinct brain regions, a rather surprising result considering that a number of the proteins in this category have ubiquitous functions [5]. This study highlights the importance of RBPs and the need to investigate the role of these RBPs in the nervous system.

The following characteristics of the highly specialized structure and function of neurons may explain the need for a large number of RBPs to support their normal function. First, many neuronal proteins are expressed in numerous isoforms through alternative splicing. These proteins include neurotransmitters, membrane receptors, cell adhesion molecules and components of signal transduction proteins. In fact, alternative splicing occurs more frequently in the nervous system compared to other tissues in vertebrates [6, 7]. The diversification of protein function through the generation of different isoforms from a single gene plays an essential role in fulfilling the complex needs of neuronal activities, including various aspects of cell–cell communication. Second, the structural features of a neuron make long-distance transport of mRNAs necessary. RBPs are required to protect these mRNAs, guide their localization and transport, and regulate their activities. In this regard, RBPs that regulate mRNA stability play a pivotal role in neurons. Third, *de novo* synthesis of proteins has been demonstrated to occur in dendrites and developing axons away from the cell body of a neuron [8, 9]. RBPs that regulate this specific type of translation are important for brain functions such as learning and memory.

The essential role of RBPs in brain development and function is also revealed by a number of neurological diseases that result from perturbed expression of RBPs. A severe form of retinitis pigmentosa is caused by mutations of PRPC8, which codes for PRP8, an integral protein of the spliceosomal U5 snRNP [10]. Fragile X mental retardation is caused by diminished expression of the RNA-binding protein FMRP, which is involved in regulation of synaptic plasticity through binding to G quartet-containing RNA targets and affecting their transport and translation [11]. Myotonic dystrophy type 1 (DM1) is caused by CTG triplet expansion that leads to abnormally up-regulated expression of two RBPs, muscleblind and CUGBP1, which are important regulators of a number of alternatively spliced RNAs [12]. Although the most prominent phenotype of DM1 is muscle wasting and myotonia, there are also clear symptoms of the nervous system including mental retardation and personality and behavioral abnormalities [13]. Intriguingly, tumor formation can sometimes trigger the ectopic expression of normally neuron-specific RBPs, which act as antigens in autoimmune diseases known as paraneoplastic neurologic degenerations (PNDs) [14]. Of this type of diseases, paraneoplastic opsoclonus-myoclonus ataxia (POMA) is caused by generation of antibodies against the RBPs Nova1/Nova2 triggered by ectopic expression of these proteins in breast cancers, while paraneoplastic syndrome, characterized by encephalomyelitis and sensory neuropathy, is caused by the generation of antibodies to the Hu family of RBPs, triggered by their ectopic expression in small cell lung cancers [14]. Mouse models have been generated for a number of neuron-specific RBPs. For example, Nova1 null mice die postnatally from motor deficits [15]. In another model, mutant mice that show a deficiency of CELF4 (Brunol4), a member of the CUG-BP and ETR-3 like factors (CELF) family, have a complex seizure phenotype [16].

These diseases demonstrate the biological significance of RBPs. To eventually discover cures for these diseases, it is important to understand how these RBPs function under normal physiological conditions. Very few brain-specific (or brain-enriched) RBPs have been investigated to achieve a deep understanding of the fundamental mechanisms by which these proteins function. The goal of this review is to illustrate recent advancements that provide comprehensive mechanistic insights into how a group of neuron-specific proteins, the Hu family, function as important RBPs in many biological processes. In particular, we will discuss in great detail the recently described nuclear functions of Hu proteins. Excellent reviews have been written on these proteins with different emphases, mostly on their biological function or on mRNA stability control, which is the most

studied molecular function of these proteins [17, 18, 19].

The Hu protein family

Hu proteins were first identified as target antigens of paraneoplastic neurological syndrome, described in the previous section [20]. There are four vertebrate members of this Hu RBP family: HuR (HuA), HuB (Hel-N1), HuC (PLE21), and HuD. As they share homology with the Drosophila ELAV protein, they are sometimes referred to as the ELAV family. HuR is widely expressed, whereas HuB, HuC, and HuD are expressed specifically in neurons, and are therefore called the neuronal Hu proteins. HuB is also expressed in gonads [21].

Hu proteins have been shown to contribute to an ever-growing list of biological functions. First, numerous studies indicate that neuronal Hu proteins play an important role in neuronal development. For instance, over-expression of HuD accelerates neurite outgrowth in E19 rat cortical neurons, PC12 cells, and retinoic acid-induced embryonic stem cells [22, 23, 24], while antisense-mediated inhibition of HuD expression in PC12 cells results in a failure to grow neurites upon nerve growth factor stimulation [25]. HuD deficient mice, though showing no apparent adult brain morphological defects, exhibit transient impairment of embryonic cranial nerve development, and primary neurospheres derived from these mice generate fewer neurons compared to wild type mice [26]. Second, neuronal Hu proteins are implicated in neuronal plasticity and memory. For example, mice and rats that have learned spatial discrimination paradigms exhibit an increase in expression of Hu proteins specifically in areas of the brain associated with spatial learning, and antisense-mediated knockdown of HuC in mice results in impaired spatial learning [27, 28]. Furthermore, over-expression of HuD in forebrain neurons of transgenic mice results in impaired acquisition and retention of memories [29]. For more information on the roles of neuronal Hu proteins in neuronal development and memory, we recommend two excellent reviews by Deschênes-Furry and colleagues [18] and Pascale and colleagues [19].

The ubiquitously expressed family member, HuR, has a variety of biological functions. Through its post-transcriptional regulation of targets, such as several genes controlling cell growth and proliferation, HuR is believed to mediate cellular response to DNA damage and other types of stress [30]. HuR has been shown to enhance the expression of various anti-apoptotic proteins, such as the apoptosome inhibitor prothymosin α [31]. Thus, some propose that HuR functions as an upstream coordinator of a pro-survival program [32]. However, a recent study suggests that while HuR has an anti-apoptotic function during early cell stress response, it may promote apoptosis when cell death is unavoidable. This pro-apoptotic function of HuR appears to be regulated by caspase-dependent cleavage of HuR [33]. Others propose that the post-transcriptional effects of HuR on the expression of various target genes may contribute to oncogenesis [34]. Studies also suggest that HuR may be important in diverse functions such as muscle differentiation, adipogenesis, suppression of inflammatory response and modulation of gene expression in response to chronic ethanol exposure and amino acid starvation [35, 36, 37, 38, 39].

All of the biological functions of Hu proteins are believed to be a result of their ability to bind to specific target mRNAs and affect their expression. Hu proteins recognize and bind to AU-rich RNA elements (AREs) and also show an empirical preference for U-rich sequences as well as some other RNA sequences [40, 41, 42, 43, 44, 45]. The amino acid sequence of Hu proteins reveals that they are classical RBPs (Fig. 1). Each Hu protein has three RNA recognition motifs (RRMs 1–3) that share greater than 90% amino acid sequence identity among family members [46]. The crystal structure of HuD RRM1 and RRM2 reveals that they form a cleft between them where RNA is bound between β-sheets, in a manner similar to other RBPs [47]. Likewise, NMR studies of HuC RRM1 and RRM2 indicate that they cooperate in binding to an ARE [48]. RRM3 helps to maintain the stability of the RNA-protein complex, and might also bind to poly(A) tails or be involved in protein-protein interactions [22, 49, 50, 51, 52]. In addition to the highly conserved RRMs, Hu proteins contain a less conserved basic hinge region between RRMs 1 and 2, and a divergent N-terminus (Fig. 1). The hinge region is believed to contain sequences that allow Hu proteins, like many other RBPs, to shuttle back and forth between the nucleus and cytoplasm. For example, HuR has a sequence in its hinge region, deemed the HNS (HuR nucleocytoplasmic shuttling sequence), which contains both a nuclear localization signal and a nuclear export signal [53]. Not surprisingly, the Hu proteins, like a number of other RBPs, are great multi-tasking proteins regulating various aspects of RNA metabolism in both cellular compartments [54].

As proposed by Keene, RBPs may coordinately regulate the expression of multiple mRNAs that encode functionally related proteins, termed RNA operons. Individual mRNAs can be members of

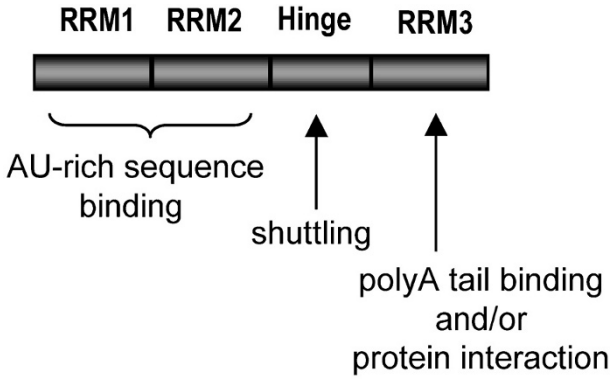

Figure 1. Hu protein domains and their functions. Protein domain structure of Hu proteins is shown in the diagram. The function of each domain is indicated below the diagram.

multiple operons, forming higher-order "RNA regulons" [55]. Thus, as RBPs, Hu proteins may perform their overall biological functions by coordinately regulating functionally related mRNAs. For example, the anti-apoptotic function of HuR may be mediated by its ability to bind to mRNAs and enhance the expression of several anti-apoptotic proteins, including $p21^{waf1}$, p53, and prothymosin α. [31, 56, 57]. Hu proteins affect the expression of their regulons through diverse mechanisms, from splicing to translation (Fig. 2), which are the molecular bases for the biological functions of Hu proteins described above. In the cytoplasm, Hu proteins are best known for stabilizing target mRNAs, such as GAP-43, VEGF, GLUT1, eotaxin and c-fos, by binding to AREs in their 3' untranslated regions (UTRs) [25, 49, 58, 59, 60, 61, 62]. They can also bind to target mRNAs, such as neurofilament M, GLUT1, and p27, and regulate their translation [58, 63, 64]. In the nucleus, Hu proteins serve as regulators of polyadenylation and alternative splicing [65, 66, 67, 68]. The remainder of this review will focus on what is known about the mechanisms by which Hu proteins perform their cytoplasmic and nuclear molecular functions.

Hu proteins and mRNA stability

The most well-known molecular function of Hu proteins is the regulation of mRNA stability. Hu proteins bind to the 3' UTRs of target mRNAs and prevent their degradation, thus indirectly enhancing protein production. Although numerous Hu mRNA stabilization targets have been identified (reviewed in [17, 19, 45, 69]), the mechanisms by which Hu proteins stabilize mRNAs are only partially characterized, with the most known about the ubiquitously expressed family member, HuR. The molecular mechanisms by which HuR regulates mRNA stability are likely to be similar to those of the closely related neuronal Hu proteins.

In most cases, Hu proteins bind to AREs in the 3'UTRs of target mRNAs. Approximately 5–8% of human genes code for mRNAs containing AREs [70, 71]. Numerous ARE binding proteins have been identified, and most, such as TTP and BRF1, decrease the stability of their target mRNAs (reviewed in [69]). Hu proteins, on the other hand, stabilize their target mRNAs [69]. AREs are divided into three classes based on rough sequence similarities [72]. Class I AREs contain multiple copies of an AUUUA motif within U-rich regions, class II AREs contain two or more overlapping nonamers containing the AUUUA motif, and class III AREs are U-rich regions without AUUUA pentamers. Although Hu proteins have been shown to bind to representative mRNAs from all three ARE classes [40, 58, 73], they do not bind to and stabilize all ARE-containing mRNAs indiscriminately. For example, HuR was shown to stabilize a reporter construct bearing the c-fos ARE, but not reporters containing the c-myc or GM-CSF AREs [44]. Determining exactly how Hu proteins recognize some AREs but not others remains an interesting challenge for future research. One possibility is that, depending on physiological conditions or cell types, Hu proteins may associate in complexes with different proteins, altering their binding specificity for AREs. The binding of Hu proteins can have different effects on the stability of mRNAs. HuD binding to the GAP-43 mRNA results in a decrease in the rate of deadenylation. Interestingly, the affinity of HuD for the GAP-43 mRNA weakens as the poly(A) tail is shortened [49]. In contrast, the binding of HuR to the c-fos mRNA has little effect on deadenylation, but inhibits the degradation of the RNA body [59]. The stabilization effects of Hu proteins on target mRNAs are believed to be mediated by competition with destabilizing ARE binding proteins (ARE-BPs). Destabilizing ARE-BPs can enhance the rate of degradation of mRNAs through multiple mechanisms. As has been shown for TTP, they may stimulate the deadenylase PARN (poly(A) ribonuclease), increasing the rate of removal of the 3′ poly(A) tail, often the rate-limiting step of mRNA degradation [74]. The ARE-BPs TTP and KSRP associate *in vitro* with the exosome, and therefore may stimulate the 3′-5′ degradation of mRNAs [75, 76]. Some ARE-BPs also associate with decapping enzymes, suggesting that they may enhance decapping-dependent 5′-3′ degradation of their target mRNAs [77].

One mechanism by which Hu proteins could potentially block association of destabilizing proteins is by forming oligomers on their target mRNAs. Indeed,

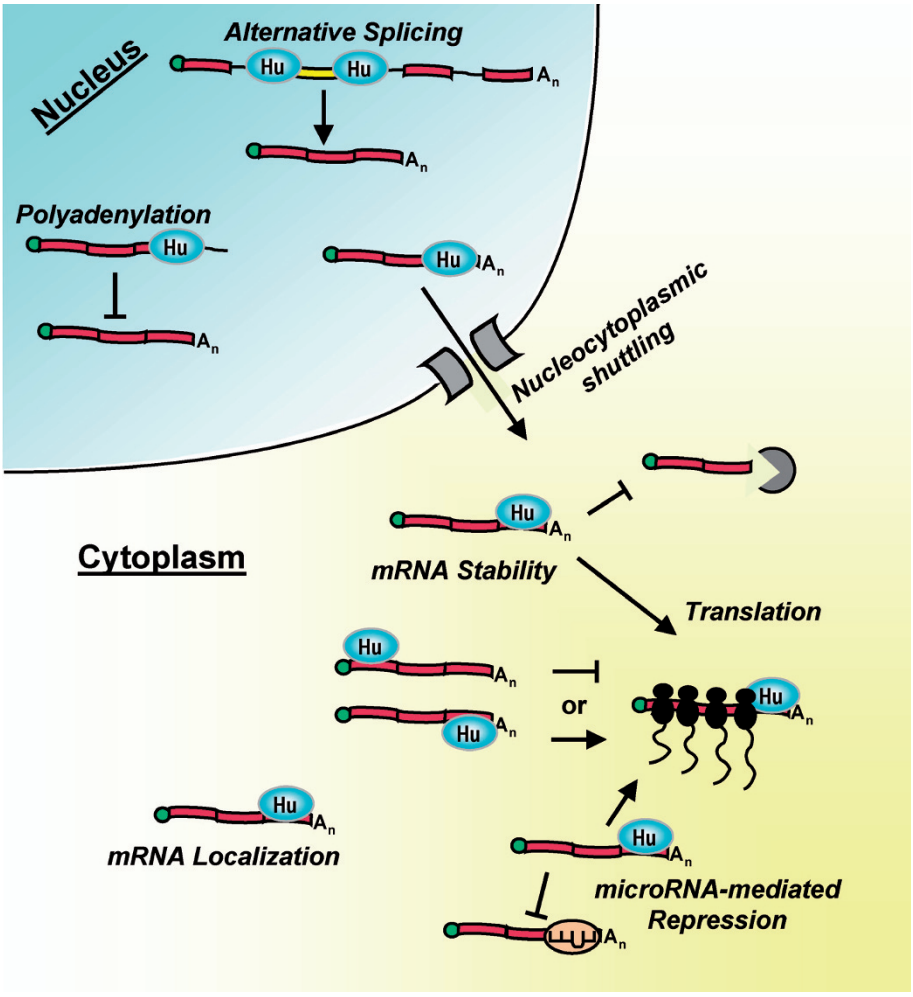

Figure 2. Diverse mechanisms of Hu protein functions. Hu proteins mediate many post-transcriptional processing events in both nucleus and cytoplasm.

HuR forms cooperative oligomeric protein complexes on an RNA substrate containing the tumor necrosis factor α (TNF α) ARE [78]. Binding of multiple HuR proteins requires an ARE substrate of at least 18 nucleotides in length. In addition, deletion analysis indicates that neither the hinge region nor RRM3 are required for ARE recognition, but both are important for assembly of HuR oligomers. Consistent with these observations, it has been shown that sequences within the RRM3 and a small adjacent portion of the hinge of ELAV, the Drosophila homolog of the Hu proteins, are required for its multimerization on the intron of one of its alternative splicing targets [79]. In addition, fluorescence resonance energy transfer (FRET) analysis indicates that HuR is capable of homodimerization [80]. A truncated form of HuD lacking RRM3 fails to stabilize one of its targets, the GAP-43 mRNA, and HuR without RRM3 fails to stabilize reporter mRNAs containing AREs [22, 53]. An interesting possibility is that without RRM3, HuD and HuR cannot oligomerize on their target mRNAs, weakening their ability to prevent mRNA decay.

Recent studies suggest that the mechanism by which Hu proteins antagonize the effects of destabilizing proteins may be more complicated than simple competition for binding to the same sites within AREs. Lal and colleagues provided evidence that HuR and AUF1 (hnRNP D) can bind simultaneously to shared target mRNAs, p21 and cyclin D1, in which HuR has a stabilizing effect and AUF1 a destabilizing effect [81]. Results of RNAi knockdown experiments suggest that competition between these proteins may exist for binding to these discrete sites. Although each protein preferred to bind to discrete sites within the AREs of targets when both proteins were present, upon knockdown of one protein, the other would bind to the preferred site of the knocked down protein. The simultaneous binding of HuR and AUF1 was observed mainly in the nucleus, and the authors propose a model in which either HuR or AUF1 dissociates from the mRNA once it reaches the cytoplasm, allowing the mRNA to enter either the stabilization or destabilization pathway [81]. In the cytoplasm, HuR colocalizes with the translational apparatus and AUF1 with the

exosome [31]. Using FRET analysis, David and colleagues obtained slightly different results. They showed that HuR and AUF1 could functionally interact both within the nucleus and in the cytoplasm [80]. These studies suggest that in some instances, HuR may be able to antagonize the effects of destabilizing proteins through some mechanism other than simply preventing their binding to mRNAs. For example, the presence of HuR may recruit mRNAs to the translation machinery and/or prevent destabilizing proteins from recruiting mRNAs to degradation machinery. In this regard, it is important to identify which proteins Hu proteins interact with in the cytoplasm under specific physiological conditions.

The ability of Hu proteins to stabilize their mRNA targets may be regulated by post-translational modifications of the Hu proteins themselves. For instance, the stabilization of the SIRT1 mRNA appears to be modulated by phosphorylation of HuR [82]. In general, HuR tends to enhance stabilization of its target mRNAs in response to cellular stress. However, in the case of SIRT1, cell stress induced by hydrogen peroxide leads to the dissociation of HuR/SIRT1 mRNA complexes and mRNA decay. The cell cycle checkpoint kinase Chk2 is activated by hydrogen peroxide and phosphorylates HuR at three residues. The phosphorylation of one of these residues, serine 100, appears to contribute to dissociation of HuR and the SIRT1 mRNA after hydrogen peroxide induced stress. In addition, both HuR and HuD are methylated by coactivator-associated arginine methyltransferase 1 (CARM1) [83, 84]. In the case of HuR, lipopolysaccharide stimulation of macrophages has been shown to cause increased methylation of arginine 217 within the hinge region. Lipopolysaccharide stimulation also leads to HuR-mediated stabilization of TNFα, suggesting that methylation of HuR may modulate its ability to stabilize mRNA targets [83]. HuD is methylated by CARM1 on arginine 236. In contrast to the effect of HuR methylation on the stabilization of its target, methylation of HuD decreases its ability to bind to and stabilize one of its mRNA targets, $p21^{cip1/waf1}$ [84]. It is likely that these and other post-translational modifications affect the stabilization of many Hu mRNA targets.

Hu proteins and translation

Though Hu proteins are best known for stabilizing mRNAs, they can also affect target protein expression at the level of translation. Unlike the effects of Hu proteins on mRNA stability, which generally enhance target protein expression, Hu proteins may serve as enhancers or repressors of translation. They regulate translation through diverse mechanisms, which are described below.

Hu proteins up-regulate the translation of many target mRNAs. In several of the cases that have been characterized, they do so by binding to the 3′UTRs of target mRNAs, similar to what occurs with mRNA stability targets. However, instead of affecting mRNA turnover, Hu binding to the 3′UTR in these cases results in increased recruitment of target mRNAs to polysomes, suggesting increased translation initiation. Examples of this are the enhancement of cytochrome c and p53 translation by HuR, and of neurofilament M translation by HuB [57, 63, 85]. However, unlike the regulation of mRNA stability, Hu proteins may also enhance translation by binding to the 5′ UTR of targets. For instance, it is believed that HuR promotes translation of hypoxia-inducible factor 1α by binding mainly to the 5′ UTR of the mRNA and enhancing its association with actively translating polysomes [86].

In most cases, the mechanisms by which the interaction of Hu proteins with the 5′ or 3′ UTR of target mRNAs leads to increased translation initiation are largely unknown. One intriguing example where the mechanism is partially understood is the up-regulation of cationic amino acid transporter 1 (CAT-1) translation in human hepatocarcinoma cells by binding of HuR to the 3′ UTR of its mRNA [87]. Under normal conditions, microRNA miR-122 base pairs with the 3′ UTR of CAT-1 mRNA and represses its translation, and the CAT-1 mRNA is largely sequestered in processing bodies (PBs). Under conditions of stress, miR-122-mediated repression of CAT-1 mRNA is relieved, and it is released from PBs to be recruited into polysomes. HuR is required for this derepression, as its knockdown prevents stress-mediated release of CAT-1 mRNA from PBs and its translational activation. The exact function of HuR in this derepression process is not clear. Although HuR was shown to bind specifically to a long ARE sequence of the CAT-1 3′UTR [38], binding per se could not explain the function of HuR in this translation regulation event, as a reporter that could be bound by HuR but not by miR-122 did not show increased translation [87]. It is tempting to speculate that an mRNP remodeling process that involves multiple components of translational machinery occurs when HuR interacts with the CAT-1 3′-UTR, which leads the mRNA to a different fate from when miR-122 binds. mRNP remodeling has been suggested as an important event in the miRNA-mediated regulation of mRNA stability and translation [88, 89]. An interesting question is whether other Hu targets are also regulated by relief of microRNA-mediated translational repression.

It is hypothesized that in some cases Hu proteins may enhance protein synthesis indirectly by binding to targets in the nucleus and influencing their export to the cytoplasm where they may be translated. The idea was first proposed in the late 1990s after it was demonstrated that Hu proteins can shuttle between the nucleus and cytoplasm [53, 90]. Recent studies support this hypothesis. For example, HuR enhances CD83 protein expression, yet has no apparent effect on its mRNA stability or translation initiation [91]. Instead, HuR binds to the CD83 mRNA in the nucleus (intriguingly, within the coding region rather than the 5′ or 3′ UTR) and shuttles it into the cytoplasm, thus increasing the abundance of cytoplasmic CD83 mRNA that is available for translation. Supporting this idea is the fact that when the CRM1 pathway needed for nuclear export of HuR is inhibited, cytoplasmic CD83 mRNA levels are diminished [91]. Similarly, HuR appears to enhance the expression of C/EBPβ by binding to its 3'UTR and transporting it from the nucleus to the cytoplasm in response to differentiation stimuli in preadipocytes [92, 93]. In addition, HuR may enhance protein synthesis from prothymosin α mRNA in part by increasing its abundance in the cytoplasm, though it also appears to affect its association with polysomes [31].

Hu proteins may also suppress the translation of targets. The best-characterized examples of this involve the interaction of Hu proteins with the 5′UTRs of mRNAs. HuR and HuD have been shown to bind to a U-rich sequence in the 5′UTR of the p27 mRNA and inhibit its translation [64, 94]. The 5′UTR of the p27 mRNA is complex, and its translation can be initiated through two different mechanisms: cap-dependent initiation in which the 43 s ribosome complex recognizes the 5′ cap of the mRNA and scans for the start codon, and cap-independent initiation, in which the ribosome recognizes a specialized sequence called the internal ribosome entry site (IRES). HuR and HuD suppress p27 translation by inhibiting IRES-mediated initiation [64]. Similarly, HuR represses insulin-like growth factor I receptor (IGF-IR) translation by inhibiting IRES-mediated initiation, but can also inhibit cap-dependent initiation. HuR permanently blocks IRES-mediated initiation, perhaps by inactivating the IRES-associated translation pre-initiation complex, but its inhibition of cap-dependent initiation is transient, as HuR is displaced from its binding site in the 5′UTR as a consequence of ribosomal scanning. The third RNA recognition motif of HuR is required for this translational suppression [95]. Whether Hu proteins might also be able to enhance IRES-mediated translation initiation through binding to 5′UTRs is unknown.

Binding of Hu proteins to 3′ UTRs can also lead to translational repression. For example, Wnt-5a translation is inhibited as a result of HuR associating with its 3'UTR. The precise mechanism by which this occurs is not understood [96].

Hu proteins may regulate the expression of some targets at both the mRNA stability and translational levels. For example, association of HuB with the glucose transporter (GLUT1) mRNA 3′UTR leads to both mRNA stabilization and accelerated formation of translation initiation complexes [58]. Likewise, neuronal HuD increases both stability and translation of the Nova1 mRNA [97], and under the stress of amino acid starvation, HuR promotes both events of the CAT-1 mRNA [87, 98]. Intriguingly, HuR appears to stabilize the TNF and Cox2 transcripts, yet suppresses their translation to have an overall negative effect on their expression [37].

An interesting question is whether Hu proteins might be able to combine other molecular functions to influence target expression. For instance, Hu proteins are negative regulators of neurofibromatosis type I (NF1) exon 23a inclusion (see below) [66], and have been shown to bind to the NF1 3'UTR, though the function of this interaction is unknown [99]. Whether Hu proteins regulate NF1 expression at both nuclear (splicing) and cytoplasmic (mRNA stability or translation) levels remains to be investigated.

Nuclear functions of Hu proteins

Perhaps one of the most important and exciting findings in the Hu protein field in recent years is the discovery of nuclear functions of these proteins as RNA processing factors. When the founding member of the Hu protein family, HuD, was cloned in 1991, it was postulated to play a role in neuron-specific alternative RNA processing [20]. The speculation was largely based on the high degree of homology between HuD and two Drosophila proteins, ELAV and sex-lethal (Sxl). Both Drosophila proteins have established functions as RNA processing regulators [100, 101]. It is also conceivable that Hu proteins have both nuclear and cytoplasmic functions since these proteins shuttle back and forth between the two cellular compartments [24, 53]. However, fifteen years passed before the first target of Hu proteins as RNA processing regulators was identified [65]. To date, it has been documented in the literature that RNA processing of at least three pre-mRNAs is regulated by Hu proteins at the level of polyadenylation or splicing [65, 66, 67, 68]. Although the number of these examples is still very small at present, these studies have greatly expanded the functional reper-

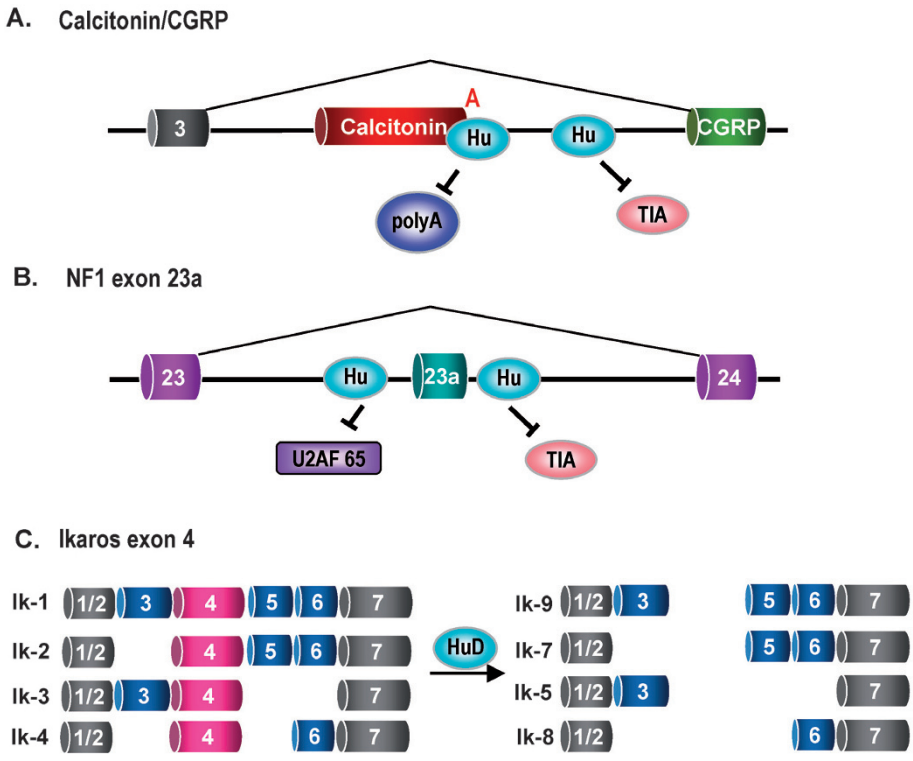

Figure 3. Nuclear functions of Hu proteins. (*A*) Diagram indicating how Hu proteins block inclusion of calcitonin exon 4. (*B*) Diagram indicating how Hu proteins block inclusion of neurofibromatosis type I exon 23a. (*C*) HuD protein promotes production of the Ikaros isoforms that do not contain exon 4.

toire of Hu proteins, from the cytoplasmic to nuclear territory, and thus will greatly help us to obtain a complete understanding of the biological functions of Hu proteins. In the following section, we will review these three examples in detail and illustrate the underlying mechanisms if known.

Calcitonin/calcitonin gene-related peptide (CGRP) pre-mRNA

Calcitonin/CGRP is one of the classical examples of alternative RNA processing [102]. As shown in Fig. 3A, this gene has the potential to encode either calcitonin or CGRP. In the C cells of the thyroid gland, 95–98% of the primary transcript from this gene is processed to produce mRNA encoding the peptide calcitonin, the most potent peptide inhibitor of osteoclast-mediated bone resorption. In neuronal cells, 99% of the primary transcript is processed to produce the peptide CGRP, the most potent endogenous vasodilator.

Previous studies demonstrate that recognition of exon 4 of the calcitonin/CGRP pre-mRNA is the key regulatory event of its alternative RNA processing [103]. Processing to include calcitonin exon 4 is the predominant phenotype seen in most cell types when the calcitonin/CGRP gene is constitutively expressed in transgenic mice, whereas exclusion of exon 4 is observed in only a subset of neuronal cells [104]. In common with other alternatively spliced exons, inclusion (non-neuronal pathway) and skipping (neuronal pathway) of this exon are both controlled by complex mechanisms involving multiple sequence elements and protein factors [105]. Two groups of neuron-specific RNA-binding proteins, Fox-1/Fox-2 and Hu proteins, contribute to skipping of exon 4 [65, 67, 105]. For the purpose of this review, we will focus on the Hu proteins.

Hu proteins promote the neuron-specific CGRP processing pathway of the calcitonin/CGRP pre-mRNA by binding to two target sequences (Fig. 3A). One target is also the binding site of TIA-1/TIAR, a pair of closely related proteins that are expressed in many tissues. It was previously demonstrated that TIA-1/TIAR proteins promote the non-neuronal pathway of calcitonin/CGRP pre-mRNA processing [106]. By binding to the sequence U_6AU_4, Hu proteins block the activity of TIA-1/TIAR in neuron-like cells. Targeting TIA-1 to the vicinity of the U-tract sequence through engineered RNA-protein binding increased CGRP-specific processing [65]. It appears that the major function of Hu proteins upon binding to this U-tract sequence is to block the activity of TIA-1/TIAR [65]. However, it remains to be determined how TIA-1/TIAR proteins promote inclusion of calcitonin exon 4.

The other target, $UUAU_5$, is located at the polyadenylation site of exon 4 immediately downstream of the AAUAAA polyadenylation signal [67]. *In vitro* polyadenylation analysis indicates that all of the four Hu proteins are capable of blocking both cleavage and poly(A) addition at this site containing the wild type AU-rich sequences but not at the mutant site in which the AU-rich sequences are disrupted. All three RRMs of the Hu proteins are required for this activity [67]. Interaction of this poly(A) site with CstF64, a critical component of the polyadenylation complex, is blocked by Hu proteins. GST pull-down experiments suggest that Hu proteins interact directly with at least two of the poly(A) factors, CstF64 and CPSF160 [67]. The polyadenylation regulatory activity of Hu proteins is very interesting because the U-rich sequence to which Hu proteins bind is one of the few enriched sequence motifs associated with poly(A) signals [107, 108]. The implication is that many more poly(A) sites may be regulated by Hu proteins. Indeed, the SV40 late poly(A) site was identified as an additional target for these proteins. In both *in vitro* and cell transfection analyses, polyadenylation of this poly(A) site that is surrounded by AU-rich sequences was blocked by Hu proteins [67]. We predict that additional targets for Hu-mediated polyadenylation regulation will be identified in the future.

Neurofibromatosis type 1 (NF1) exon 23a

In order to identify more Hu protein targets, a bioinformatic analysis was carried out to search for the well-characterized AU-rich Hu protein binding site in proximity to alternatively spliced exons that show neuron specificity, and more than seventy potential targets of Hu proteins were identified. Of these potential targets, exon 23a of the NF1 pre-mRNA was chosen to study the role of Hu proteins in splicing regulation because of the clear tissue specificity and biological importance of this particular alternative splicing event. Exon 23a is an in-frame exon encoding 21 amino acids in the NF1 GTPase activating protein (GAP) region. This exon is alternatively included, producing two NF1 isoforms (Fig. 3B) [109]. The type I isoform does not contain this exon, while the type II isoform does. The ratio of the two isoforms varies in different tissues and during development. The type I isoform is predominantly expressed in neurons of the adult central nervous system [110, 111] and shows ten times higher activity in down-regulating Ras activity than the type II isoform [109, 112].

NF1 exon 23a is surrounded by several AU-rich sequences. RNA immunoprecipitation with anti-Hu sera, which can recognize HuB, HuC, and HuD, showed that Hu proteins are associated with the NF1 pre-mRNA in CA77 cells, a rat medullary thyroid carcinoma cell line displaying numerous neuronal features [66]. To study splicing regulation of NF1 exon 23a, two cell lines, HeLa and CA77, were chosen to mimic the two splicing pathways. These cell lines are excellent models to study this alternative splicing event because the NF1 gene is endogenously expressed in both cell lines but its transcript is differentially processed. Exon 23a is predominantly included in HeLa cells to produce type II NF1 and excluded in CA77 cells to produce type I NF1 [66]. As the neuron-specific HuB, HuC or HuD proteins are also expressed in CA77 cells but not HeLa cells, these experiments indicate a correlation between neuron-specific Hu protein expression and exon 23a skipping. A reporter was generated containing NF1 exon 23a with part of its flanking intronic sequences inserted into the first intron of the human metallothioniein (HMT) gene. Transfection of this reporter into HeLA or CA77 cells generated a pre-mRNA that is processed similarly to endogenous NF1 [66]. In both cell lines, co-transfection of mouse HuC cDNA plasmid with the NF1 reporter decreased inclusion of exon 23a of the reporter. In contrast, over-expression of PTB, a general splicing suppressor that binds to pyrimidine-rich sequences, did not affect inclusion of this exon [66]. Over-expression of Hu proteins also decreased inclusion of exon 23a of the endogenous NF1 pre-mRNA in HeLa and PC12 cells [66].

The underlying mechanisms by which Hu proteins regulate skipping of exon 23a were subsequently investigated. Two binding sites for Hu proteins, located upstream and downstream of the regulated exon, were identified and biochemical evidence that Hu proteins specifically block exon definition by preventing binding of essential splicing factors was provided. At the upstream binding site, Hu proteins appear to decrease U2AF binding at the 3' splice site of exon 23a [66]. At the downstream site, inclusion of NF1 exon 23a is regulated in part by competition between Hu proteins and a group of previously characterized proteins, TIA-1/TIAR. Hu proteins prevent binding of U1 and U6 snRNPs to the 5' splice site, while TIAR increases binding [66]. This recurring theme of the competition between positive and negative regulators may represent an important aspect of neuron-specific alternative splicing regulation. Although these data demonstrate that Hu proteins interfere with the interaction of basic spliceosomal components with the 5' and 3' splice sites, it is not clear how the interference occurs. It is possible that Hu proteins directly interact with basic splicing factors, similarly to how they interact with polyade-

nylation factors. Future studies will be aimed at identifying these potential interactions and elucidating the underlying mechanisms.

Ikaros isoforms

Ikaros, also known as LyF-1, is a zinc-finger DNA-binding protein and plays an essential role during lymphocyte development [113, 114]. As shown in Fig. 3C, at least nine isoforms (IK-1-9) generated through alternative splicing have been reported [113, 114, 115]. Interestingly, these isoforms contain various numbers of zinc fingers and therefore have different DNA-binding capabilities. The IK-1-3 isoforms have at least three N-terminus zinc fingers and are capable of DNA binding, while the other isoforms cannot bind DNA and act like dominant-negative proteins [113, 114].

A switch of Ikaros isoforms from fully functional (IK-1-3) to dominant-negative short proteins (IK-5-9) has been observed in transgenic mice expressing the intracellular domain of the Notch3 receptor [116]. The loss of functional Ikaros proteins leads to leukemia/lymphoma in these animals [116]. A recent report links HuD expression with the altered alternative splicing of the Ikaros pre-mRNA. HuD was found to be up-regulated in the transgenic animals, which appears to be responsible for the splicing change [68]. The most convincing data came from over-expression and siRNA knockdown of HuD using pre- and immature T-cell lines, which led to increased and decreased formation of the dominant-negative isoforms, respectively [68].

What remains to be determined, however, is whether HuD is directly involved in this splicing control. A close inspection of the exon structure of the isoforms indicates that inclusion of exon 4 is the key regulatory event, as IK-1-4 isoforms contain this exon and the rest do not (Fig. 3C). Thus, it is possible that HuD blocks inclusion of this exon when up-regulated, analogous to exon 23a of the NF1 pre-mRNA. An examination of the sequences of exon 4 and its flanking introns gives few hints as to how HuD might regulate this exon. No significant AU-rich sequences are located within 100 nucleotides from either end of this exon, although some are present beyond 100 nucleotides from the exon. It is possible that HuD cooperates with another protein to block exon 4. It is also conceivable that HuD regulates expression of a splicing factor, which in turn regulates inclusion of exon 4. Additional experiments will need to be carried out to test these possibilities.

A unifying theme of Hu action?

Results from the limited mechanistic studies on Hu proteins discussed above start to reveal a possible theme of action for these proteins. They appear to function mainly by counteracting the actions of other proteins, or in one case a miRNA, that regulate the same target mRNAs. These antagonistic actions of Hu proteins may occur through direct competition for binding to AU-rich sequences. Once bound to target mRNAs, Hu proteins direct them to adopt a fate opposite from that directed by other regulatory proteins, possibly by causing them to interact with different protein complexes. This theme of action is most evident in Hu-mediated regulation of mRNA stability and splicing. For example, Hu proteins appear to stabilize target mRNAs by antagonizing the actions of destabilizing proteins, such as TTP or AUF1 (reviewed in [69]). Likewise, Hu proteins have been shown to inhibit alternative exon inclusion by competing with the positive splicing regulators TIA-1/TIAR for binding to pre-mRNA [66, 67]. The antagonistic actions may also occur between Hu proteins and other regulatory proteins that do not bind to the exact same AU-rich sequences as Hu proteins. In this case, a functional competition between the two proteins may be the result of mRNP remodeling involving different sets of proteins that interact with either protein. In the future, it will be interesting to see whether this emerging theme of antagonistic actions holds up as more is learned about the mechanisms by which Hu proteins regulate the many steps of RNA processing and metabolism.

Future directions

As discussed above, Hu proteins are involved in almost every type of post-transcriptional regulatory event. Since the biological output of Hu proteins is dictated by all of these molecular functions, it will be very important to identify additional targets of Hu proteins and understand how specificity is achieved. In particular, as we are only beginning to investigate the function of these proteins as RNA processing regulators, computational and other types of global approaches will be very helpful to identify nuclear targets of Hu proteins. To provide mechanistic insights into any of the functions described above, it will be critical to investigate what proteins are in the same complex as Hu proteins, both in the cytoplasm and nucleus. Powerful proteomic approaches will be very useful in this regard. Furthermore, little is known about the overlapping and non-redundant functions of the four members of the Hu protein family. Do they

have the same RNA targets and, if not, where does the specificity come from? Answers to questions like these will help us to fully understand the biological functions of individual Hu protein family members. Also, more emphasis will be put on the neuron-specific members of the Hu protein family in regards to how they function at the molecular level.

Acknowledgements. We thank Maria Hatzoglou (Case Western Reserve University), Phillip Pekala (East Carolina University) and Ann-Bin Shyu (University of Texas) for critical reading of the manuscript. We thank Hua Lou's group for helpful discussion. Hua Lou is supported by a NIH grant (NS-049103) and a DOD grant (NF060083) and Melissa Hinman is supported by a training grant from NIH (T32GM008613).

1 Keene J. D. (2001) Ribonucleoprotein infrastructure regulating the flow of genetic information between the genome and the proteome. Proc Natl Acad Sci U S A 98: 7018–7024.
2 2. Lander E. S., Linton L. M., Birren B., Nusbaum C., Zody M. C., Baldwin J., Devon K., Dewar K., Doyle M., FitzHugh W., Funke R., Gage D., Harris K. and Heaford A. et al. (2001) Initial sequencing and analysis of the human genome. Nature 409: 860–921.
3 Moore M. J. (2005) From birth to death: the complex lives of eukaryotic mRNAs. Science 309: 1514–1518.
4 Yao K. M., Samson M. L., Reeves R. and White K. (1993) Gene elav of Drosophila melanogaster: a prototype for neuronal-specific RNA binding protein gene family that is conserved in flies and humans. J Neurobiol 24: 723–739.
5 McKee A. E., Minet E., Stern C., Riahi S., Stiles C. D. and Silver P. A. (2005) A genome-wide in situ hybridization map of RNA-binding proteins reveals anatomically restricted expression in the developing mouse brain. BMC Dev Biol 5: 14.
6 Lipscombe D. (2005) Neuronal proteins custom designed by alternative splicing. Curr Opin Neurobiol 15: 358–363.
7 Lee C. J. and Irizarry K. (2003) Alternative splicing in the nervous system: an emerging source of diversity and regulation. Biol Psychiatry 54: 771–776.
8 Sutton M. A. and Schuman E. M. (2005) Local translational control in dendrites and its role in long-term synaptic plasticity. J Neurobiol 64: 116–131.
9 Hengst U. and Jaffrey S. R. (2007) Function and translational regulation of mRNA in developing axons. Semin Cell Dev Biol 18: 209–215.
10 McKie A. B., McHale J. C., Keen T. J., Tarttelin E. E., Goliath R., van Lith-Verhaeven J. J., Greenberg J., Ramesar R. S., Hoyng C. B., Cremers F. P., Mackey D. A., Bhattacharya S. S., Bird A. C., Markham A. F. and Inglehearn C. F. (2001) Mutations in the pre-mRNA splicing factor gene PRPC8 in autosomal dominant retinitis pigmentosa (RP13). Hum Mol Genet 10: 1555–1562.
11 Jin P., Alisch R. S. and Warren S. T. (2004) RNA and microRNAs in fragile X mental retardation. Nat Cell Biol 6: 1048–1053.
12 Kuyumcu-Martinez N. M. and Cooper T. A. (2006) Misregulation of alternative splicing causes pathogenesis in myotonic dystrophy. Prog Mol Subcell Biol 44: 133–159.
13 de Leon M. B. and Cisneros B. (2008) Myotonic dystrophy 1 in the nervous system: from the clinic to molecular mechanisms. J Neurosci Res 86: 18–26.
14 Albert M. L. and Darnell R. B. (2004) Paraneoplastic neurological degenerations: keys to tumour immunity. Nat Rev Cancer 4: 36–44.
15 Jensen K. B., Dredge B. K., Stefani G., Zhong R., Buckanovich R. J., Okano H. J., Yang Y. Y. and Darnell R. B. (2000) Nova-1 regulates neuron-specific alternative splicing and is essential for neuronal viability. Neuron 25: 359–371.
16 Yang Y., Mahaffey C. L., Berube N., Maddatu T. P., Cox G. A. and Frankel W. N. (2007) Complex seizure disorder caused by Brunol4 deficiency in mice. PLoS Genet 3: e124.
17 Brennan C. M. and Steitz J. A. (2001) HuR and mRNA stability. Cell Mol Life Sci 58: 266–277.
18 Deschenes-Furry J., Perrone-Bizzozero N. and Jasmin B. J. (2006) The RNA-binding protein HuD: a regulator of neuronal differentiation, maintenance and plasticity. Bioessays 28: 822–833.
19 Pascale A., Amadio M. and Quattrone A. (2008) Defining a neuron: neuronal ELAV proteins. Cell Mol Life Sci 65: 128–140.
20 Szabo A., Dalmau J., Manley G., Rosenfeld M., Wong E., Henson J., Posner J. B. and Furneaux H. M. (1991) HuD, a paraneoplastic encephalomyelitis antigen, contains RNA-binding domains and is homologous to Elav and Sex-lethal. Cell 67: 325–333.
21 Dalmau J., Furneaux H. M., Cordon-Cardo C. and Posner J. B. (1992) The expression of the Hu (paraneoplastic encephalomyelitis/sensory neuronopathy) antigen in human normal and tumor tissues. Am J Pathol 141: 881–886.
22 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 HuD I+II, promotes GAP-43 gene expression and neurite outgrowth in PC12 cells in the absence of nerve growth factor. J Neurochem 75: 1103–1114.
23 Anderson K. D., Sengupta J., Morin M., Neve R. L., Valenzuela C. F. and Perrone-Bizzozero N. I. (2001) Overexpression of HuD accelerates neurite outgrowth and increases GAP-43 mRNA expression in cortical neurons and retinoic acid-induced embryonic stem cells in vitro. Exp Neurol 168: 250–258.
24 Kasashima K., Terashima K., Yamamoto K., Sakashita E. and Sakamoto H. (1999) Cytoplasmic localization is required for the mammalian ELAV-like protein HuD to induce neuronal differentiation. Genes Cells 4: 667–683.
25 Mobarak C. D., Anderson K. D., Morin M., Beckel-Mitchener A., Rogers S. L., Furneaux H. M., 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.
26 Akamatsu W., Fujihara H., Mitsuhashi T., Yano M., Shibata S., Hayakawa Y., Okano H. J., Sakakibara S., Takano H., Takano T., Takahashi T., Noda T. and Okano H. (2005) The RNA-binding protein HuD regulates neuronal cell identity and maturation. Proc Natl Acad Sci U S A 102: 4625–4630.
27 Quattrone A., Pascale A., Nogues X., Zhao W., Gusev P., Pacini A. and Alkon D. L. (2001) Posttranscriptional regulation of gene expression in learning by the neuronal ELAV-like mRNA-stabilizing proteins. Proc Natl Acad Sci U S A 98: 11668–11673.
28 Pascale A., Gusev P. A., Amadio M., Dottorini T., Govoni S., Alkon D. L. and Quattrone A. (2004) Increase of the RNA-binding protein HuD and posttranscriptional up-regulation of the GAP-43 gene during spatial memory. Proc Natl Acad Sci USA 101: 1217–1222.
29 Bolognani F., Qiu S., Tanner D. C., Paik J., Perrone-Bizzozero N. I. and Weeber E. J. (2007) Associative and spatial learning and memory deficits in transgenic mice overexpressing the RNA-binding protein HuD. Neurobiol Learn Mem 87: 635–643.
30 Gorospe M. (2003) HuR in the mammalian genotoxic response: post-transcriptional multitasking. Cell Cycle 2: 412–414.
31 Lal A., Kawai T., Yang X., Mazan-Mamczarz K. and Gorospe M. (2005) Antiapoptotic function of RNA-binding protein

HuR effected through prothymosin alpha. Embo J 24: 1852–1862.
32 Abdelmohsen K., Lal A., Kim H. H. and Gorospe M. (2007) Posttranscriptional orchestration of an anti-apoptotic program by HuR. Cell Cycle 6: 1288–1292.
33 Mazroui R., Di Marco S., Clair E., von Roretz C., Tenenbaum S. A., Keene J. D., Saleh M. and Gallouzi I. E. (2008) Caspase-mediated cleavage of HuR in the cytoplasm contributes to pp32/PHAP-I regulation of apoptosis. J Cell Biol 180: 113–127.
34 Lopez de Silanes I., Lal A. and Gorospe M. (2005) HuR: post-transcriptional paths to malignancy. RNA Biol 2: 11–13.
35 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.
36 Cherry J., Karschner V., Jones H. and Pekala P. H. (2006) HuR, an RNA-binding protein, involved in the control of cellular differentiation. In Vivo 20: 17–23.
37 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.
38 Yaman I., Fernandez J., Sarkar B., Schneider R. J., Snider M. D., Nagy L. E. and Hatzoglou M. (2002) Nutritional control of mRNA stability is mediated by a conserved AU-rich element that binds the cytoplasmic shuttling protein HuR. J Biol Chem 277: 41539–41546.
39 McMullen M. R., Cocuzzi E., Hatzoglou M. and Nagy L. E. (2003) Chronic ethanol exposure increases the binding of HuR to the TNFalpha 3'-untranslated region in macrophages. J Biol Chem 278: 38333–38341.
40 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.
41 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.
42 Myer V. E., Fan X. C. and Steitz J. A. (1997) Identification of HuR as a protein implicated in AUUUA-mediated mRNA decay. Embo J 16: 2130–2139.
43 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.
44 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.
45 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.
46 Okano H. J. and Darnell R. B. (1997) A hierarchy of Hu RNA binding proteins in developing and adult neurons. J Neurosci 17: 3024–3037.
47 Wang X. and Tanaka Hall T. M. (2001) Structural basis for recognition of AU-rich element RNA by the HuD protein. Nat Struct Biol 8: 141–145.
48 Inoue M., Muto Y., Sakamoto H. and Yokoyama S. (2000) NMR studies on functional structures of the AU-rich element-binding domains of Hu antigen C. Nucleic Acids Res 28: 1743–1750.
49 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.
50 Abe R., Yamamoto K. and Sakamoto H. (1996) Target specificity of neuronal RNA-binding protein, Mel-N1: direct binding to the 3' untranslated region of its own mRNA. Nucleic Acids Res 24: 2011–2016.
51 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.
52 Kasashima K., Sakashita E., Saito K. and Sakamoto H. (2002) Complex formation of the neuron-specific ELAV-like Hu RNA-binding proteins. Nucleic Acids Res 30: 4519–4526.
53 Fan X. C. and Steitz J. A. (1998) HNS, a nuclear-cytoplasmic shuttling sequence in HuR. Proc Natl Acad Sci U S A 95: 15293–15298.
54 Wilkinson M. F. and Shyu A. B. (2001) Multifunctional regulatory proteins that control gene expression in both the nucleus and the cytoplasm. Bioessays 23: 775–787.
55 Keene J. D. (2007) RNA regulons: coordination of post-transcriptional events. Nat Rev Genet 8: 533–543.
56 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.
57 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.
58 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.
59 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.
60 Levy N. S., Chung S., Furneaux H. and Levy A. P. (1998) Hypoxic stabilization of vascular endothelial growth factor mRNA by the RNA-binding protein HuR. J Biol Chem 273: 6417–6423.
61 Atasoy U., Curry S. L., Lopez de Silanes I., Shyu A. B., Casolaro V., Gorospe M. and Stellato C. (2003) Regulation of eotaxin gene expression by TNF-alpha and IL-4 through mRNA stabilization: involvement of the RNA-binding protein HuR. J Immunol 171: 4369–4378.
62 Bolognani F., Tanner D. C., Merhege M., Deschenes-Furry J., Jasmin B. and Perrone-Bizzozero N. I. (2006) In vivo post-transcriptional regulation of GAP-43 mRNA by overexpression of the RNA-binding protein HuD. J Neurochem 96: 790–801.
63 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.
64 Kullmann M., Gopfert U., Siewe B. and Hengst L. (2002) ELAV/Hu proteins inhibit p27 translation via an IRES element in the p27 5'UTR. Genes Dev 16: 3087–3099.
65 Zhu H., Hasman R. A., Barron V. A., Luo G. and Lou H. (2006) A nuclear function of Hu proteins as neuron-specific alternative RNA processing regulators. Mol Biol Cell 17: 5105–5114.
66 Zhu H., Hinman M. N., Hasman R. A., Mehta P. and Lou H. (2008) Regulation of neuron-specific alternative splicing of neurofibromatosis type 1 pre-mRNA. Mol Cell Biol 28: 1240–1251.
67 Zhu H., Zhou H. L., Hasman R. A. and Lou H. (2007) Hu proteins regulate polyadenylation by blocking sites containing U-rich sequences. J Biol Chem 282: 2203–2210.
68 Bellavia D., Mecarozzi M., Campese A. F., Grazioli P., Talora C., Frati L., Gullino A. and Screpanti I. (2007) Notch3 and the Notch3-upregulated RNA-binding protein HuD regulate Ikaros alternative splicing. EMBO J 26: 1670–1680.
69 Barreau C., Paillard L. and Osborne H. B. (2005) AU-rich elements and associated factors: are there unifying principles? Nucleic Acids Res 33: 7138–7150.
70 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.
71 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.
72 Chen C. Y. and Shyu A. B. (1995) AU-rich elements: characterization and importance in mRNA degradation. Trends Biochem Sci 20: 465–470.
73 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.
74 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.
75 Chen C. Y., Gherzi R., Ong S. E., Chan E. L., Raijmakers R., Pruijn G. L., 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.
76 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.
77 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.
78 Fialcowitz-White E. J., Brewer B. Y., Ballin J. D., Willis C. D., Toth E. A., Toth E. A. and Wilson G. M. (2007) Specific protein domains mediate cooperative assembly of HuR oligomers on AU-rich mRNA-destabilizing sequences. J Biol Chem 282: 20948–20959.
79 Toba G. and White K. (2008) The third RNA recognition motif of Drosophila ELAV protein has a role in multimerization. Nucleic Acids Res 36: 1390–1399.
80 David P. S., Tanveer R. and Port J. D. (2007) FRET-detectable interactions between the ARE binding proteins, HuR and p37AUF1. RNA 13: 1453–1468.
81 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.
82 Abdelmohsen K., Pullmann R., Jr., Lal A., Kim H. H., Galban S., Yang X., Blethrow J. D., Walker M., Shubert J., Gillespie D. A., Furneaux H. and Gorospe M. (2007) Phosphorylation of HuR by Chk2 regulates SIRT1 expression. Mol Cell 25: 543–557.
83 Li H., Park S., Kilburn B., Jelinek M. A., Henschen-Edman A., Aswad D. W., Stallcup M. R. and Laird-Offringa I. A. (2002) Lipopolysaccharide-induced methylation of HuR, an mRNA-stabilizing protein, by CARM1. Coactivator-associated arginine methyltransferase. J Biol Chem 277: 44623–44630.
84 Fujiwara T., Mori Y., Chu D. L., Koyama Y., Miyata S., Tanaka H., Yachi K., Kubo T., Yoshikawa H. and Tohyama M. (2006) CARM1 regulates proliferation of PC12 cells by methylating HuD. Mol Cell Biol 26: 2273–2285.
85 Kawai T., Lal A., Yang X., Galban S., Mazan-Mamczarz K. and Gorospe M. (2006) Translational control of cytochrome c by RNA-binding proteins TIA-1 and HuR. Mol Cell Biol 26: 3295–3307.
86 Galban S., Kuwano Y., Pullmann R., Jr., Martindale J. L., Kim H. H., Lal A., Abdelmohsen K., Yang X., Dang Y., Liu J. O., Lewis S. M., Holcik M. and Gorospe M. (2008) RNA-binding proteins HuR and PTB promote the translation of hypoxia-inducible factor 1alpha. Mol Cell Biol 28: 93–107.
87 Bhattacharyya S. N., Habermacher R., Martine U., Closs E. I. and Filipowicz W. (2006) Relief of microRNA-mediated translational repression in human cells subjected to stress. Cell 125: 1111–1124.
88 Shyu A. B., Wilkinson M. F. and van Hoof A. (2008) Messenger RNA regulation: to translate or to degrade. EMBO J 27: 471–481.
89 Filipowicz W., Bhattacharyya S. N. and Sonenberg N. (2008) Mechanisms of post-transcriptional regulation by microRNAs: are the answers in sight? Nat Rev Genet 9: 102–114.
90 Keene J. D. (1999) Why is Hu where? Shuttling of early-response-gene messenger RNA subsets. Proc Natl Acad Sci USA 96: 5–7.
91 Prechtel A. T., Chemnitz J., Schirmer S., Ehlers C., Langbein-Detsch I., Stulke J., Dabauvalle M. C., Kehlenbach R. H. and Hauber J. (2006) Expression of CD83 is regulated by HuR via a novel cis-active coding region RNA element. J Biol Chem 281: 10912–10925.
92 Gantt K., Cherry J., Tenney R., Karschner V. and Pekala P. H. (2005) An early event in adipogenesis, the nuclear selection of the CCAAT enhancer-binding protein {beta} (C/EBP{beta}) mRNA by HuR and its translocation to the cytosol. J Biol Chem 280: 24768–24774.
93 Jones H., Carver M. and Pekala P. H. (2007) HuR binds to a single site on the C/EBPbeta mRNA of 3T3-L1 adipocytes. Biochem Biophys Res Commun 355: 217–220.
94 Millard S. S., Vidal A., Markus M. and Koff A. (2000) A U-rich element in the 5' untranslated region is necessary for the translation of p27 mRNA. Mol Cell Biol 20: 5947–5959.
95 Meng Z., King P. H., Nabors L. B., Jackson N. L., Chen C. Y., Emanuel P. D. and Blume S. W. (2005) The ELAV RNA-stability factor HuR binds the 5'-untranslated region of the human IGF-IR transcript and differentially represses cap-dependent and IRES-mediated translation. Nucleic Acids Res 33: 2962–2979.
96 Leandersson K., Riesbeck K. and Andersson T. (2006) Wnt-5a mRNA translation is suppressed by the Elav-like protein HuR in human breast epithelial cells. Nucleic Acids Res 34: 3988–3999.
97 Ratti A., Fallini C., Colombrita C., Pascale A., Laforenza U., Quattrone A. and Silani V. (2008) Post-transcriptional Regulation of Neuro-oncological Ventral Antigen 1 by the Neuronal RNA-binding Proteins ELAV. J Biol Chem 283: 7531–7541.
98 Hatzoglou M., Fernandez J., Yaman I. and Closs E. (2004) Regulation of cationic amino acid transport: the story of the CAT-1 transporter. Annu Rev Nutr 24: 377–399.
99 Haeussler J., Haeusler J., Striebel A. M., Assum G., Vogel W., Furneaux H. and Krone W. (2000) Tumor antigen HuR binds specifically to one of five protein-binding segments in the 3'-untranslated region of the neurofibromin messenger RNA. Biochem Biophys Res Commun 267: 726–732.
100 Soller M. and White K. (2004) Elav. Curr Biol 14: R53.
101 Penalva L. O. and Sanchez L. (2003) RNA binding protein sex-lethal (Sxl) and control of Drosophila sex determination and dosage compensation. Microbiol Mol Biol Rev 67: 343–359, table of contents.
102 Rosenfeld M. G., Amara S. G. and Evans R. M. (1984) Alternative RNA processing: determining neuronal phenotype. Science 225: 1315–1320.
103 Lou H. and Gagel R. F. (1999) Mechanism of tissue-specific alternative RNA processing of the calcitonin CGRP gene. Front Horm Res 25: 18–33.
104 Crenshaw E. B., 3rd, Russo A. F., Swanson L. W. and Rosenfeld M. G. (1987) Neuron-specific alternative RNA processing in transgenic mice expressing a metallothionein-calcitonin fusion gene. Cell 49: 389–398.
105 Zhou H. L., Baraniak A. P. and Lou H. (2007) Role for Fox-1/Fox-2 in mediating the neuronal pathway of calcitonin/calcitonin gene-related peptide alternative RNA processing. Mol Cell Biol 27: 830–841.
106 Zhu H., Hasman R. A., Young K. M., Kedersha N. L. and Lou H. (2003) U1 snRNP-dependent function of TIAR in the regulation of alternative RNA processing of the human calcitonin/CGRP pre-mRNA. Mol Cell Biol 23: 5959–5971.

107 Cheng Y., Miura R. M. and Tian B. (2006) Prediction of mRNA polyadenylation sites by support vector machine. Bioinformatics 22: 2320–2325.
108 Liu D., Brockman J. M., Dass B., Hutchins L. N., Singh P., McCarrey J. R., MacDonald C. C. and Graber J. H. (2007) Systematic variation in mRNA 3'-processing signals during mouse spermatogenesis. Nucleic Acids Res 35: 234–246.
109 Andersen L. B., Ballester R., Marchuk D. A., Chang E., Gutmann D. H., Saulino A. M., Camonis J., Wigler M. and Collins F. S. (1993) A conserved alternative splice in the von Recklinghausen neurofibromatosis (NF1) gene produces two neurofibromin isoforms, both of which have GTPase-activating protein activity. Mol Cell Biol 13: 487–495.
110 Huynh D. P., Nechiporuk T. and Pulst S. M. (1994) Differential expression and tissue distribution of type I and type II neurofibromins during mouse fetal development. Dev Biol 161: 538–551.
111 Gutmann D. H., Zhang Y. and Hirbe A. (1999) Developmental regulation of a neuron-specific neurofibromatosis 1 isoform. Ann Neurol 46: 777–782.
112 Yunoue S., Tokuo H., Fukunaga K., Feng L., Ozawa T., Nishi T., Kikuchi A., Hattori S., Saya H. and Araki N. (2003) Neurofibromatosis type I tumor suppressor neurofibromin regulates neuronal differentiation via its GTPase-activating protein function toward Ras. J Biol Chem 278: 26958–26969.
113 Molnar A. and Georgopoulos K. (1994) The Ikaros gene encodes a family of functionally diverse zinc finger DNA-binding proteins. Mol Cell Biol 14: 8292–8303.
114 Hahm K., Ernst P., Lo K., Kim G. S., Turck C. and Smale S. T. (1994) The lymphoid transcription factor LyF-1 is encoded by specific, alternatively spliced mRNAs derived from the Ikaros gene. Mol Cell Biol 14: 7111–7123.
115 Beverly L. J. and Capobianco A. J. (2003) Perturbation of Ikaros isoform selection by MLV integration is a cooperative event in Notch(IC)-induced T cell leukemogenesis. Cancer Cell 3: 551–564.
116 Bellavia D., Mecarozzi M., Campese A. F., Grazioli P., Gulino A. and Screpanti I. (2007) Notch and Ikaros: not only converging players in T cell leukemia. Cell Cycle 6: 2730–2734.