Received: 22 December 2021 | Revised: 18 March 2022 | Accepted: 23 March 2022

DOI: 10.1002/wrna.1733

FOCUS ARTICLE

WIREs RNA WILEY

Regulation of neuronal RNA signatures by ELAV/Hu proteins

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Funding information
Valérie Hilgers is funded by the Max Planck Society, the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation; Project-ID 403222702) SFB 1381, the DFG under Germany's Excellence Strategy (CIBSS—EXC-2189—Project ID 390939984) and the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation program (Grant agreement no. ERC-2018-STG-803258).

Edited by: Carol Lutz, Associate Editor and Jeff Wilusz, Editor-in-Chief

Abstract

The RNA-binding proteins encoded by the highly conserved *elav/Hu* gene family, found in all metazoans, regulate the expression of a wide range of genes, at both the co-transcriptional and posttranscriptional level. Nervous-system-specific ELAV/Hu proteins are prominent for their essential role in neuron differentiation, and mutations have been associated with human neurodevelopmental and neurodegenerative diseases. Drosophila ELAV, the founding member of the protein family, mediates the synthesis of neuronal RNA signatures by promoting alternative splicing and alternative polyadenylation of hundreds of genes. The recent identification of ELAV's direct RNA targets revealed the protein's central role in shaping the neuronal transcriptome, and highlighted the importance of neuronal transcript signatures for neuron maintenance and organism survival. Animals have evolved multiple cellular mechanisms to ensure robustness of ELAV/Hu function. In Drosophila, *elav* autoregulates in a 3′UTR-dependent manner to maintain optimal protein levels. A complete absence of ELAV causes the activation and nuclear localization of the normally cytoplasmic paralogue FNE, in a process termed EXon-Activated functional Rescue (EXAR). Other species, including mammals, seem to utilize different strategies, such as protein redundancy, to maintain ELAV protein function and effectively safeguard the identity of the neuronal transcriptome.

This article is categorized under:

RNA Processing > 3′ End Processing
RNA in Disease and Development > RNA in Development
RNA Interactions with Proteins and Other Molecules > Protein-RNA Interactions: Functional Implications

KEYWORDS

alternative polyadenylation, ELAV proteins, neuron, RNA

1 | INTRODUCTION

The nervous system is composed of polarized and complex cells, in which it is particularly critical to coordinate gene expression in a robust but dynamic manner. One strategy used by neurons to achieve high functional versatility is the specific expression of RNAs found in no other cell type, including non-coding RNAs such as microRNAs, long-non-coding RNAs and circRNAs. In addition, in neurons, the generation of multiple mRNA isoforms through alternative processing of mRNA precursors greatly increases the regulatory potential of individual genes (Andreassi et al., 2018). Notably, a shift towards the selection of more distal polyadenylation (poly[A]) sites in hundreds of genes (Hilgers et al., 2011; Miura et al., 2013; Smibert et al., 2012; Ulitsky et al., 2012) generates exons and 3′untranslated regions (3′UTRs) referred to as "neuronal RNA signatures." The extraordinary diversity of the neuronal transcriptome has only started to be appreciated in recent years, with the emergence of new transcriptomics technologies. While not all neuronal RNA signatures are expected to perform a specific function, many studies point to the fact that they are collectively relevant for the development and maintenance of the nervous system. In this review, we focus on neuronal 3′UTRs and their impact on neuronal identity and function.

2 | TRANSCRIPTOME DIVERSIFICATION THROUGH ALTERNATIVE POLYADENYLATION

Nascent transcripts undergo cleavage and polyadenylation (CPA) to produce mature mRNAs. Upon recognition of a poly(A) site by the CPA machinery, cleavage of the precursor mRNA (pre-mRNA) is followed by polyadenylation of the newly created 3′end of the transcript (Shi & Manley, 2015). In many metazoan genes, the presence and use of several functional poly(A) sites generates mRNA isoforms with different 3′ends, in a process called alternative polyadenylation (APA; reviewed in Gruber & Zavolan, 2019). While APA isoforms can arise from imprecision in the selection of processing sites, causing gene expression noise (Raser & O'Shea, 2005; Xu & Zhang, 2020), specific APA patterns are likely to result from active regulation and to be of biological relevance. For example, coherent shifts towards the use of more proximal or distal 3′end sites occur in cancer (Mayr & Bartel, 2009), as a response to stimuli (Flavell et al., 2008) or stress (Hollerer et al., 2016; Zheng et al., 2018), and in a tissue-specific manner (Lianoglou et al., 2013; Sanfilippo et al., 2017; Zhang et al., 2005). When alternative 3′ends are located upstream of the stop codon, coding sequence APA (CDS-APA) gives rise to mRNAs that differ in their protein coding potential. More commonly however, transcripts with longer 3′UTRs are generated, in a process termed UTR-APA.

3′UTRs regulate mRNA fate through multiple mechanisms (Mayr, 2019). They constitute the main binding platform for microRNAs and RNA-binding proteins, and are enriched in mRNA posttranslational modifications such as N6-methyladenosine (m^6A; Meyer et al., 2012). Through these regulatory factors, 3′UTRs directly influence mRNA stability, translation, localization, and incorporation into RNP granules. 3′UTRs can even mediate the localization and function of the encoded protein by directing the co-translational recruitment of transport factors (Berkovits & Mayr, 2015) or complex partners (Lee & Mayr, 2019). UTR-APA can majorly affect transcriptome identity and function, and its disruption is associated with multiple diseases including cancer, and neurological and immunological pathologies (Gruber & Zavolan, 2019; Mohanan et al., 2021).

3 | THE ELAV/HU FAMILY OF RNA-BINDING PROTEINS

ELAV/Hu family proteins are highly conserved RNA-binding proteins (RBPs) named after their first described member, Drosophila Embryonic Lethal Abnormal Vision (ELAV; Campos et al., 1985; Campos et al., 1987; Homyk Jr. et al., 1985). Since in metazoans, most *elav*-related genes encode proteins expressed in all neurons throughout development, ELAV/Hu proteins represent well-established markers for neuronal identity across the animal kingdom (Pascale et al., 2008; Robinow & White, 1988; Yao et al., 1993).

3.1 | Expression and localization

Vertebrate and arthropod *elav/Hu* genes derive from one common ancestor and have independently undergone duplication and/or retrotransposition during evolution, leading to functional diversification of the encoded proteins

(Samson, 2008). Drosophila possesses three ELAV/Hu family proteins: the founding member ELAV, Found in NEurons (FNE), and RNA-Binding Protein 9 (RBP9). In neurons, ELAV is restricted to the nucleus, whereas FNE localizes to the cytoplasm (Samson & Chalvet, 2003); RBP9, expressed in later stages of development, has been found in both compartments (Kim & Baker, 1993; Park et al., 1998). In contrast to Drosophila, mammals possess a ubiquitous ELAV/Hu protein, HuR. The three other paralogues HuB, HuC, and HuD are expressed specifically in neurons. All four mammalian Hu proteins shuttle between the nucleus and the cytoplasm, and their distribution varies in a cell-type and context-dependent manner. Although HuR is best known for protecting mRNAs from degradation in the cytoplasm, in neurons the RBP is predominantly found in the nucleus, consistent with a role for ELAV/Hu proteins in the consecutive regulation of multiple steps of RNA metabolism (Antic & Keene, 1997; Hinman & Lou, 2008; Keene, 1999).

ELAV/Hu proteins have been shown to be components of multiple types of phase-separated ribonucleoprotein (RNP) granules: in the nucleus, ELAV associates with Cajal bodies (Yannoni & White, 1997). Cytoplasmic ELAV/Hu proteins were found in neuronal granules (Antic & Keene, 1998; Gao & Keene, 1996) and stress granules (Gallouzi et al., 2000; Markmiller et al., 2018). Moreover, pathogenic insoluble inclusions often contain ELAV/Hu proteins (Bowles et al., 2021; de Santis et al., 2019; Lu et al., 2009). Like many RBPS, ELAV/Hu proteins contain disordered regions and as such, are prone to biomolecular condensation. Whether this propensity to associate with condensates and aggregates is tied to their molecular function, is currently unclear.

3.2 | Structure and RNA-binding

The members of the ELAV/Hu protein family are structurally similar (Samson, 2008). They contain three characteristic, highly conserved RNA Recognition Motifs (RRMs). The third RRM is typically separated from the first two by a less conserved hinge region that in some cases contains signals that regulate protein distribution in the cell (Figure 1). The hinge region of HuR contains an amino acid segment termed HNS (HuR nucleocytoplasmic shuttling sequence) with both nuclear export and import elements (Fan & Steitz, 1998a); that of ELAV contains a signal for nuclear localization (Yannoni & White, 1999). ELAV/Hu proteins display a high degree of amino acid sequence similarity; individual family members can bind to, and regulate, each other's RNA target sequences in vitro or when ectopically expressed (Borgeson & Samson, 2005); rather than displaying individual target specificity, the localization and concentration of individual members of the protein family seem to be determining factors in dictating the selection of RNA targets (Zaharieva et al., 2015). Binding studies in human, rodent (Nicholson et al., 2017; Scheckel et al., 2016) and Drosophila (Carrasco et al., 2020) brain have revealed direct gene- and sequence-specific binding to functional RNA targets. Surprisingly, in flies and mammals, sites of ELAV/Hu crosslinking, rather than presenting a precise consensus motif, are enriched in a uridine-rich 6-mer commonly found in non-coding RNA regions, especially in 3′UTRs and around poly(A) sites. Thus, the binding specificity, rather than dictated by a consensus sequence, is likely regulated by additional mechanisms. For example, posttranslational modifications, and the modulation of ELAV/Hu multimerization, may affect target specificity (Brauer et al., 2014; Grammatikakis et al., 2017; Pabis et al., 2019; Soller & White, 2005; Toba & White, 2008); features such as RNA structure or RNA modifications may also influence binding. Moreover, ELAV recruitment occurs co-transcriptionally, and depends, at least partially, on promoter sequence and/or chromatin environment (Oktaba et al., 2015).

3.3 | Physiological functions

Both mammalian and Drosophila ELAV/Hu proteins play important roles in neuronal differentiation and during branching and establishment of neuronal connections (Akamatsu et al., 1999; Alizzi et al., 2021; Lim & Alkon, 2012; Tebaldi et al., 2018). In addition, ELAV/Hu proteins regulate synaptogenesis and synaptic plasticity (Dell'Orco

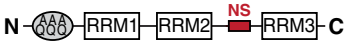

FIGURE 1 Structure of ELAV/Hu family proteins. The three RNA recognition motifs (RRMs) are common to all family members; some, for example ELAV, possess an unstructured N-terminal alanine-glutamine-rich (AQ-rich) region. The hinge region of ELAV also includes an amino acid sequence that confers nuclear localization (NS)

et al., 2020; Haussmann et al., 2008; Tiruchinapalli et al., 2008). Accordingly, mutations can severely affect motor functions, memory and behavior. In fly *fne* mutants, the morphology of the mushroom body, an important memory center, is disrupted, possibly leading to the observed impairment in courtship behavior (Zanini et al., 2012). *rbp9* mutants show altered numbers of synaptic boutons at neuromuscular junctions and develop age-dependent, progressive motor deficits. Interestingly, FNE and RBP9 play distinct roles during neuronal development and maintenance, but act redundantly in the regulation of synaptic plasticity (Zaharieva et al., 2015). A recent study in honey bees, which possess a single *elav/Hu* family gene, directly demonstrated a role for dynamic ELAVL2 expression in learning and memory (Ustaoglu et al., 2021). In mammals, ELAV/Hu proteins are up-regulated after memory formation; HuC and HuD knockdown impairs learning performance and causes motor/sensory defects in adult mice in several models (Akamatsu et al., 2005; Pascale et al., 2004; Quattrone et al., 2001). HuC is required to maintain neurotransmitter levels, and knockout mice display impaired synaptic signaling and disrupted axonal terminals, resulting in progressive motor deficits and epileptic seizures (Ince-Dunn et al., 2012; Ogawa et al., 2018). While the crucial role of ELAV/Hu proteins in neuron differentiation, maintenance, and function, has been extensively demonstrated, and their association with numerous neurological diseases has been known for a long time (Mirisis & Carew, 2019; Yano et al., 2016), it has been difficult to determine how their molecular function ties into the neurological phenotypes: rather than targeting specific pathways, ELAV/Hu proteins seem to act in a rather pleiotropic manner on multiple and diverse molecular targets.

4 | ELAV-MEDIATED ALTERNATIVE POLYADENYLATION

In the nucleus, ELAV/Hu proteins control neuron-specific alternative splicing and alternative polyadenylation in flies (Carrasco et al., 2020; Hilgers et al., 2012; Koushika et al., 1996; Lisbin et al., 2001; Soller & White, 2003; Wei et al., 2020) and mammals (Grassi et al., 2018; Mansfield & Keene, 2012; Zhu et al., 2006). In the cytoplasm, ELAV/Hu proteins regulate mRNA stability, localization, and/or translation (Ince-Dunn et al., 2012; Mukherjee et al., 2011; Pascale et al., 2005; Tebaldi et al., 2018; Tiruchinapalli et al., 2008; Yokoi et al., 2017). Recent studies have shown that Drosophila ELAV, the only predominantly nuclear ELAV/Hu family member, represents the central regulator of the neuronal RNA landscape in vivo. ELAV regulates every single site of neuron-specific APA by directly binding to nascent mRNAs during transcription, thereby inhibiting 3′end processing at proximal poly(A) sites (Carrasco et al., 2020; Figure 2). ELAV also directly mediates hundreds of neuron-specific splicing events (Lee et al., 2021; Box 1).

4.1 | In Drosophila

First accounts of ELAV-mediated APA emerged over two decades ago in the laboratory of Kalpana White. The lack of a neuron-specific splice isoform of a protein encoded by *neuroglian* in *elav* mutant flies, and the ectopic generation of neuronal Neuroglian upon ectopic expression of ELAV, showed that ELAV is necessary and sufficient for neuron-specific RNA processing (Koushika et al., 1996). Rather than, as initially proposed, acting on splice site usage, ELAV

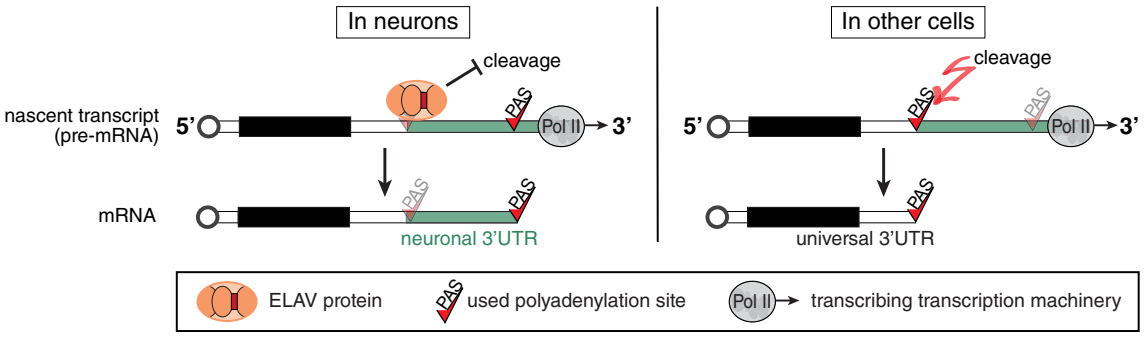

FIGURE 2 ELAV mediates alternative polyadenylation in neurons. The pan-neuronal RNA-binding protein ELAV directly binds in the vicinity of proximal poly(A) sites (PAS) of hundreds of genes and inhibits cleavage and polyadenylation. Transcription elongation beyond the proximal poly(A) site causes the synthesis of additional RNA sequences, usually an extended 3′UTR (neuronal 3′UTR). In cells in which ELAV is not expressed, cleavage and polyadenylation occur at the proximal poly(A) site

BOX 1 Coordination of ELAV-mediated APA and AS

ELAV regulates alternative splicing (AS) of hundreds of transcripts, thereby generating neuron-specific mRNA isoforms, often differing in their coding region. Hence, ELAV's influence on neuronal signatures affects not only mRNA regulation, but also the makeup of the proteome itself. Interestingly, a large fraction of ELAV's splicing targets also undergo neuronal APA, indicating that ELAV links AS and APA (Carrasco et al., 2020; Lee et al., 2021; Zhang et al., 2019).

inhibits a proximal poly(A) site, causing the transcription, and use of, a distal 3′ splice site (Lisbin et al., 2001). The neuron-enriched splicing pattern of another gene, *erect wing* (*ewg*), was also shown to be the product of ELAV-mediated CDS-APA: ELAV directly binds a U-rich region downstream of the proximal *ewg* 3′end processing site, causing transcriptional read-through and splicing of the last intron. Intriguingly, in vitro, ELAV does not compete with the CPA machinery for poly(A) site recognition, but directly inhibits *ewg* cleavage (Koushika et al., 2000; Soller & White, 2003, 2005).

The nervous system specific elongation of 3′UTRs during development was described first in Drosophila (Hilgers et al., 2011; Smibert et al., 2012), closely followed by vertebrate model systems (Miura et al., 2013; Ulitsky et al., 2012). In vivo studies identified ELAV as necessary and sufficient to promote this 3′UTR extension, and proposed that ELAV and its homologues broadly regulate neural APA (Hilgers et al., 2012). Two more recent studies formally demonstrated such a global role for ELAV/Hu proteins in Drosophila neurons (Carrasco et al., 2020; Wei et al., 2020). Transcriptomics analyses in sorted populations from Drosophila embryos (McCorkindale et al., 2019) made it possible to unambiguously identify neuron-specific 3′UTRs, and discriminate them from other tissue- or stage-specific APA events. Strikingly, ELAV was shown to directly bind transcripts of all genes that undergo neural-specific APA, typically around the proximal poly(A) site. Moreover, virtually all neuron-specific 3′UTRs were downregulated in *elav* mutant embryos (Carrasco et al., 2020). ELAV/Hu proteins were also shown to be sufficient to induce a global shift towards more distal 3′end site selection in non-neural cultured cells (Wei et al., 2020). These studies showed that ELAV represents the central regulator of neuronal APA.

4.2 | In other animals

In mammals, ELAV/Hu proteins are best known for their role in mRNA stabilization (Brennan & Steitz, 2001; Colombrita et al., 2013; Fan & Steitz, 1998b), and in miRNA-mediated regulation in the cytoplasm (Bhattacharyya et al., 2006; Lu et al., 2021; Vasudevan & Steitz, 2007). However, ELAV/Hu proteins were also shown to regulate neuronal AS and APA: in human neurons, all four Hu proteins bind to proximal poly(A) sites to promote 3′UTR extension of the transcript encoding HuR (Mansfield & Keene, 2012). Knock-down of *Elavl3* (also known as *HuC*) causes 3′UTR shortening in a subset of genes and impairs neuronal differentiation in a neural stem cell model (Grassi et al., 2018). Since ELAV/Hu proteins in mammals shuffle between the nucleus and the cytoplasm, disentangling their functions in co-transcriptional processing from posttranscriptional regulation has been challenging (Colombrita et al., 2013). Knock-downs and knockouts of individual Hu proteins have had little effect on the global neuronal transcriptome, indicating functional redundancy between the members of mammalian members of the ELAV/Hu protein family. It will be interesting to study the molecular effect of a global Hu protein loss in the mammalian brain; it is likely that this will cause the depletion of neuronal APA, as it does in Drosophila.

4.3 | Physiological consequences of loss of ELAV-mediated APA

In Drosophila, about 15–20% of genes that undergo neuronal APA are affected at the protein-coding level: ELAV-mediated CDS-APA is required for the expression of often essential neuron-specific protein isoforms, including those of Ewg, Neuroglian, Zelda, and giant Ankyrin. One recent study particularly highlighted ELAV's direct and indirect effects on the neuronal proteome: Disruption of the ELAV-dependent CDS-APA of the gene *Srrm234* impaired the expression

of the neuron-specific *enhancer of microexons* (eMIC) domain, and caused genome-wide microexon skipping and pervasive neurological defects in flies (Torres-Méndez et al., 2022).

The effects of a loss of neuronal 3′UTRs, on the other hand, are much more difficult to predict. Several studies have shown that individual neuron-specific 3′UTR isoforms play an important role in neurogenesis or neuronal function. For example, 3′UTR extension drives the upregulation of the conserved transcription factor Prospero in larval neurons (Samuels et al., 2020); ELAV-mediated AS and APA of the mRNA encoding the transmembrane receptor Dscam1 are required for axon outgrowth (Zhang et al., 2019). In mice, the specific depletion of the neuron-enriched, long 3′UTR isoform of *Calmodulin 1* caused dorsal root ganglion disorganization and impairment of hippocampal neuron activation (Bae et al., 2020). Notably, ELAV regulates Hox gene function and embryonic patterning through at least two independent mechanisms. First, ELAV-mediated AS and APA of *Ultrabithorax* (*Ubx*) pre-mRNAs is essential for proper Ubx protein expression, and ELAV removal causes aberrant reprogramming of Ubx-controlled neural differentiation routines (Rogulja-Ortmann et al., 2014). Second, the ELAV-mediated elongation of the non-coding RNA *iab-8* into the *abdominal-A* (*abd-A*) locus represses *abd-A* transcription, thereby restricting Abd-A protein to parasegments 7 through 12 and preventing aberrant and deleterious expression in the posterior CNS (Castro Alvarez et al., 2021).

5 | ROBUSTNESS OF ELAV/HU PROTEIN FUNCTION

In Drosophila, as a consequence of the spatial segregation of ELAV/Hu proteins into the nuclear and cytoplasmic compartments, ELAV constitutes the central, limiting mediator of neuronal APA and alternative splicing. Loss and misexpression of ELAV in genetically modified flies cause impaired and premature neural differentiation, respectively (Campos et al., 1985; Lai et al., 2012), suggesting that the proper deployment of the neuronal transcriptome is vulnerable to fluctuations in ELAV expression. In the endogenous context, several mechanisms act to preserve neuronal RNAs: ELAV expression is spatially regulated, and maintained to optimal levels, posttranscriptionally. Moreover, nuclear ELAV function can be rescued by FNE through a cellular strategy termed EXon-Activated functional Rescue (EXAR).

5.1 | Posttranscriptional repression of ELAV function outside of neurons

The gene *elav* is transcribed ubiquitously, albeit at low levels; the silencing of *elav* mRNAs in all cells except neurons is a strategy to avoid a potentially deleterious ELAV function from this background expression. The microRNAs *miR-279* and *miR-996*, as well as the protein Mei-P26, are expressed in a pattern complementary to ELAV, and their ectopic expression represses a GFP sensor carrying the *elav* 3′UTR. Moreover, endogenous ELAV is upregulated in *miR279/996* mutant clones. Whether *elav* transcripts are destabilized, or translationally inhibited, by these factors, remains to be uncovered. Either way, the appropriate cell-type-specific expression of ELAV in neurons is facilitated by the repression of mature *elav* transcripts in non-neuronal cells (Sanfilippo et al., 2016).

5.2 | ELAV autoregulation

Interestingly, the gene *elav* itself is subjected to 3′UTR extension. It was proposed over two decades ago that ELAV binds to its own mRNA to promote 3′UTR extension (Samson, 1998). Direct binding of ELAV to the proximal poly(A) site of the *elav* 3′UTR was later formally demonstrated in vitro (Borgeson & Samson, 2005) and in vivo (Carrasco et al., 2020; Hilgers et al., 2012); the functional dependence of *elav* 3′UTR extension on ELAV protein was shown in embryos specifically lacking the *elav* coding sequence (Figure 3).

In the endogenous, wild-type context, ELAV expression in neurons is subjected to tight control and remains at a defined level independently of *elav* gene dosage. In transgenes that lack the long 3′UTR, this regulation is lost, and ELAV expression becomes directly proportional to copy number; further, overexpression of short, but not long, *elav* transgenes caused lethality. These experiments showed that the long 3′UTR functions to avoid toxic ELAV overexpression in neurons (Samson, 1998). ELAV clearly mediates the differential expression of one APA isoform over the other; whether it also acts on *elav* mRNA remains to be seen. Since posttranscriptional regulation occurs

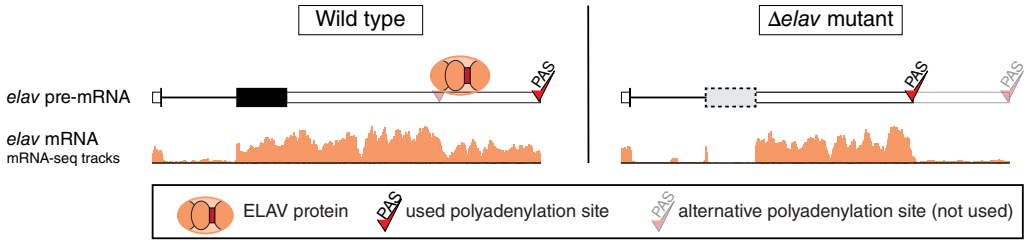

FIGURE 3 ELAV autoregulation through extension of its own 3′UTR. ELAV protein binds its own pre-mRNA to promote the expression of an extended *elav* transcript. In *Δelav* mutants, ELAV is not expressed, and the *elav* 3′UTR is significantly shortened. Note the lack of mRNA-seq signal for the *elav* coding sequence in *Δelav* embryos. mRNA-seq data is from 18 to 22 h Drosophila embryos (Carrasco et al., 2020); the gene model excludes 5′UTR isoforms for simplicity

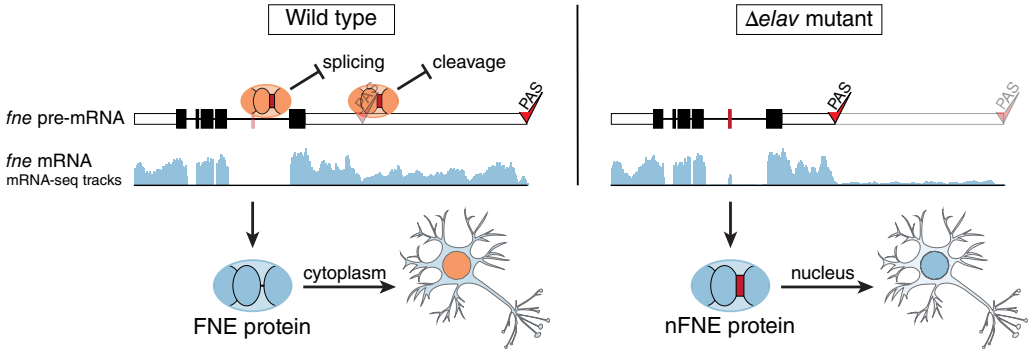

FIGURE 4 EXAR in Drosophila embryos. In the nucleus of wild-type neurons, ELAV directly binds to the *fne* pre-mRNA and inhibits mini-exon inclusion. The resulting FNE protein localizes to the cytoplasm. In *Δelav* mutant embryos, the mini-exon, which encodes amino acids required for nuclear localization, is derepressed and spliced in (red box). nFNE localizes to the nucleus and assumes nuclear ELAV functions. Note that *fne* harbors an ELAV-dependent neuronal 3′UTR. mRNA-seq data is from 18 to 22 h Drosophila embryos (Carrasco et al., 2020); the gene model excludes upstream 5′UTR exons for simplicity

in the cytoplasm, it is likely that other, yet to be discovered factors are involved in the modulation of the stability or translation of *elav* transcripts.

5.3 | EXon-activated functional rescue (EXAR) through FNE

The observation that virtually every neuron-specific 3′UTR was downregulated in *elav* mutant embryos prompted the conclusion that ELAV acts as the central, essential effector of neuronal APA (Carrasco et al., 2020). Surprisingly, a concomitant loss of ELAV and FNE—a cytoplasmic ELAV paralogue—caused complete eradication of all neuronal 3′UTRs, while deleting FNE alone did not affect APA at all. Therefore, the cooperative effect between ELAV and FNE could not be attributed to functional redundancy between the two proteins. Instead, Carrasco et al. demonstrated that FNE regulates neuronal APA only in the absence of ELAV, through a cellular strategy they termed EXon-Activated functional Rescue (EXAR). In *elav* null mutants, the *fne* RNA acquires a previously unannotated mini-exon that encodes, in the hinge region, a stretch of 15 amino acids homologous to the ELAV nuclear localization sequence (NS). As a result, the NS-containing FNE protein, nFNE, localizes to the nucleus, where it takes over ELAV functions such as AS and APA. Under wild-type conditions, ELAV directly binds to the mini-exon 5′splice site, inhibits mini-exon inclusion and nFNE expression, thereby restricting FNE to the cytoplasm (Carrasco et al., 2020; Figure 4).

nFNE activation through EXAR ensures that the molecular functions of ELAV can still be performed when ELAV is impaired. As opposed to functional redundancy, a clear hierarchy is at play in EXAR: FNE acts as a "second-in-command" when, and only when, the central effector ELAV is not functional. Notably, EXAR does not rescue the lethality of *elav* null mutants, raising questions about the physiological relevance of this mechanism. It is likely that

endogenously, EXAR works as a fail-safe strategy for situations of ELAV fluctuation and partial loss-of-function, but is not efficient enough to compensate for a complete loss of ELAV.

5.4 | Protein redundancy

Even though the *elav* gene structure, including the fne mini-exon, is conserved (Samson, 2008), functional rescue between ELAV/Hu family members is quite specific to Drosophila. Unlike *D. melanogaster*, in which mini-exon inclusion is undetectable in wild-type conditions, homologous hinge region sequences are expressed endogenously in more distantly related insects and in mammals (Wang et al., 2010). As a result, ELAV/Hu proteins are both nuclear and cytoplasmic and likely act on a largely common set of target transcripts, which can buffer the loss of individual paralogues. However, functional redundancy is not complete, since mutation of individual ELAV/Hu family members causes neurological phenotypes (see Section 3.3).

6 | CONCLUSION

The crucial importance of the ELAV/Hu family of proteins is highlighted by the profoundly deleterious effects caused by mutating one of its members, on neuronal differentiation and function. Elucidating the physiological and molecular function of ELAV/Hu proteins remains a challenge, since they act through multiple pathways, regulating RNA metabolism both co-transcriptionally and posttranscriptionally, through thousands of targets. Moreover, several cellular strategies that ensure robustness of ELAV/Hu protein function further complicate genetic and molecular studies. Much remains to be discovered; for example, thousands of direct RNA targets have been reported in flies and mammals, including noncoding RNAs, but a clear physiological function has been demonstrated for only a fraction of them. Members of the ELAV/Hu protein family display partially overlapping expression patterns, and can interchangeably bind to the same targets; their respective function mainly, but not exclusively, depends on cellular localization and concentration. It is therefore likely that ELAV/Hu proteins not only complement each other, but also perform distinct functions on the same targets. For example, I propose that in Drosophila brains, FNE and RBP9 posttranscriptionally regulate the neuronal signatures whose synthesis depends on ELAV co-transcriptional function.

ACKNOWLEDGMENTS

I apologize to my colleagues whose work I could not cite due to space restrictions. I thank Dominika Grzejda, Judit Carrasco, Carlos Alfonso-Gonzalez, Sakshi Gorey, Matthias Soller, and Marie-Laure Samson for stimulating discussions that helped shape this review. Open access funding enabled and organized by Projekt DEAL.

CONFLICT OF INTEREST

The author declares no conflict of interest.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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REFERENCES

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., & Okano, H. (2005). The RNA-binding protein HuD regulates neuronal cell identity and maturation. *Proceedings of the National Academy of Sciences of the United States of America*, *102*(12), 4625–4630. https://doi.org/10.1073/pnas.0407523102

Akamatsu, W., Okano, H. J., Osumi, N., Inoue, T., Nakamura, S., Sakakibara, S., Miura, M., Matsuo, N., Darnell, R. B., & Okano, H. (1999). Mammalian ELAV-like neuronal RNA-binding proteins HuB and HuC promote neuronal development in both the central and the peripheral nervous systems. *Proceedings of the National Academy of Sciences of the United States of America*, *96*(17), 9885–9890. https://doi.org/10.1073/pnas.96.17.9885

Alizzi, R. A., Xu, D., Tenenbaum, C. M., Wang, W., & Gavis, E. R. (2021). The ELAV/Hu protein found in neurons regulates cytoskeletal and ECM adhesion inputs for space-filling dendrite growth. *PLoS Genetics*, *16*(12), e1009235. https://doi.org/10.1371/journal.pgen.1009235

Andreassi, C., Crerar, H., & Riccio, A. (2018). Post-transcriptional processing of mRNA in neurons: The vestiges of the RNA world drive transcriptome diversity. *Frontiers in Molecular Neuroscience*, *11*, 304. https://doi.org/10.3389/fnmol.2018.00304

Antic, D., & Keene, J. D. (1997). Embryonic lethal abnormal visual RNA-binding proteins involved in growth, differentiation, and posttranscriptional gene expression. *American Journal of Human Genetics*, *61*(2), 273–278. https://doi.org/10.1086/514866

Antic, D., & Keene, J. D. (1998). Messenger ribonucleoprotein complexes containing human ELAV proteins: Interactions with cytoskeleton and translational apparatus. *Journal of Cell Science*, *111*(Pt 2), 183–197.

Bae, B., Gruner, H. N., Lynch, M., Feng, T., So, K., Oliver, D., Mastick, G. S., Yan, W., Pieraut, S., & Miura, P. (2020). Elimination of Calm1 long 3′-UTR mRNA isoform by CRISPR-Cas9 gene editing impairs dorsal root ganglion development and hippocampal neuron activation in mice. *RNA*, *26*(10), 1414–1430. https://doi.org/10.1261/rna.076430.120

Berkovits, B. D., & Mayr, C. (2015). Alternative 3′ UTRs act as scaffolds to regulate membrane protein localization. *Nature*, *522*(7556), 363–367. https://doi.org/10.1038/nature14321

Bhattacharyya, S. N., Habermacher, R., Martine, U., Closs, E. I., & Filipowicz, W. (2006). Relief of microRNA-mediated translational repression in human cells subjected to stress. *Cell*, *125*(6), 1111–1124. https://doi.org/10.1016/j.cell.2006.04.031

Borgeson, C. D., & Samson, M. L. (2005). Shared RNA-binding sites for interacting members of the Drosophila ELAV family of neuronal proteins. *Nucleic Acids Research*, *33*(19), 6372–6383. https://doi.org/10.1093/nar/gki942

Bowles, K. R., Silva, M. C., Whitney, K., Bertucci, T., Berlind, J. E., Lai, J. D., Garza, J. C., Boles, N. C., Mahali, S., Strang, K. H., Marsh, J. A., Chen, C., Pugh, D. A., Liu, Y., Gordon, R. E., Goderie, S. K., Chowdhury, R., Lotz, S., Lane, K., … Temple, S. (2021). ELAVL4, splicing, and glutamatergic dysfunction precede neuron loss in MAPT mutation cerebral organoids. *Cell*, *184*(17), 4547–4563.e4517. https://doi.org/10.1016/j.cell.2021.07.003

Brauer, U., Zaharieva, E., & Soller, M. (2014). Regulation of ELAV/Hu RNA-binding proteins by phosphorylation. *Biochemical Society Transactions*, *42*(4), 1147–1151. https://doi.org/10.1042/BST20140103

Brennan, C. M., & Steitz, J. A. (2001). HuR and mRNA stability. *Cellular and Molecular Life Sciences*, *58*(2), 266–277. https://doi.org/10.1007/pl00000854

Campos, A. R., Grossman, D., & White, K. (1985). Mutant alleles at the locus elav in Drosophila melanogaster lead to nervous system defects. A developmental-genetic analysis. *Journal of Neurogenetics*, *2*(3), 197–218. https://doi.org/10.3109/01677068509100150

Campos, A. R., Rosen, D. R., Robinow, S. N., & White, K. (1987). Molecular analysis of the locus elav in Drosophila melanogaster: A gene whose embryonic expression is neural specific. *The EMBO Journal*, *6*(2), 425–431. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC553413/pdf/emboj00242-0137.pdf

Carrasco, J., Rauer, M., Hummel, B., Grzejda, D., Alfonso-Gonzalez, C., Lee, Y., Wang, Q., Puchalska, M., Mittler, G., & Hilgers, V. (2020). ELAV and FNE determine neuronal transcript signatures through EXon-activated rescue. *Molecular Cell*, *80*(1), 156–163.e156. https://doi.org/10.1016/j.molcel.2020.09.011

Castro Alvarez, J. J., Revel, M., Carrasco, J., Cléard, F., Pauli, D., Hilgers, V., Karch, F., & Maeda, R. K. (2021). Repression of the Hox gene abd-A by ELAV-mediated transcriptional interference. *PLoS Genetics*, *17*(11), e1009843. https://doi.org/10.1371/journal.pgen.1009843

Colombrita, C., Silani, V., & Ratti, A. (2013). ELAV proteins along evolution: Back to the nucleus? *Molecular and Cellular Neurosciences*, *56*, 447–455. https://doi.org/10.1016/j.mcn.2013.02.003

de Santis, R., Alfano, V., de Turris, V., Colantoni, A., Santini, L., Garone, M. G., Antonacci, G., Peruzzi, G., Sudria-Lopez, E., Wyler, E., Anink, J. J., Aronica, E., Landthaler, M., Pasterkamp, R. J., Bozzoni, I., & Rosa, A. (2019). Mutant FUS and ELAVL4 (HuD) aberrant crosstalk in amyotrophic lateral sclerosis. *Cell Reports*, *27*(13), 3818–3831.e3815. https://doi.org/10.1016/j.celrep.2019.05.085

Dell'Orco, M., Oliver, R. J., & Perrone-Bizzozero, N. (2020). HuD binds to and regulates circular RNAs derived from neuronal development- and synaptic plasticity-associated genes. *Frontiers in Genetics*, *11*, 790. https://doi.org/10.3389/fgene.2020.00790

Fan, X. C., & Steitz, J. A. (1998a). HNS, a nuclear-cytoplasmic shuttling sequence in HuR. *Proceedings of the National Academy of Sciences of the United States of America*, *95*(26), 15293–15298. https://doi.org/10.1073/pnas.95.26.15293

Fan, X. C., & Steitz, J. A. (1998b). Overexpression of HuR, a nuclear-cytoplasmic shuttling protein, increases the in vivo stability of ARE-containing mRNAs. *The EMBO Journal*, *17*(12), 3448–3460. https://doi.org/10.1093/emboj/17.12.3448

Flavell, S. W., Kim, T. K., Gray, J. M., Harmin, D. A., Hemberg, M., Hong, E. J., Markenscoff-Papadimitriou, E., Bear, D. M., & Greenberg, M. E. (2008). Genome-wide analysis of MEF2 transcriptional program reveals synaptic target genes and neuronal activity-dependent polyadenylation site selection. *Neuron*, *60*(6), 1022–1038. https://doi.org/10.1016/j.neuron.2008.11.029

Gallouzi, I. E., Brennan, C. M., Stenberg, M. G., Swanson, M. S., Eversole, A., Maizels, N., & Steitz, J. A. (2000). HuR binding to cytoplasmic mRNA is perturbed by heat shock. *Proceedings of the National Academy of Sciences of the United States of America*, *97*(7), 3073–3078. https://doi.org/10.1073/pnas.97.7.3073

Gao, F. B., & Keene, J. D. (1996). Hel-N1/Hel-N2 proteins are bound to poly(A)+ mRNA in granular RNP structures and are implicated in neuronal differentiation. *Journal of Cell Science*, *109*(Pt 3), 579–589.

Grammatikakis, I., Abdelmohsen, K., & Gorospe, M. (2017). Posttranslational control of HuR function. *Wiley Interdisciplinary Reviews RNA*, *8*(1), e1372. https://doi.org/10.1002/wrna.1372

Grassi, E., Santoro, R., Umbach, A., Grosso, A., Oliviero, S., Neri, F., Conti, L., Ala, U., Provero, P., DiCunto, F., & Merlo, G. R. (2018). Choice of alternative polyadenylation sites, mediated by the RNA-binding protein Elavl3, plays a role in differentiation of inhibitory neuronal progenitors. *Frontiers in Cellular Neuroscience*, *12*, 518. https://doi.org/10.3389/fncel.2018.00518

Gruber, A. J., & Zavolan, M. (2019). Alternative cleavage and polyadenylation in health and disease. *Nature Reviews Genetics*, *20*(10), 599–614. https://doi.org/10.1038/s41576-019-0145-z

Haussmann, I. U., White, K., & Soller, M. (2008). Erect wing regulates synaptic growth in Drosophila by integration of multiple signaling pathways. *Genome Biology*, *9*(4), R73. https://doi.org/10.1186/gb-2008-9-4-r73

Hilgers, V., Lemke, S. B., & Levine, M. (2012). ELAV mediates 3′ UTR extension in the Drosophila nervous system. *Genes & Development*, *26*(20), 2259–2264. https://doi.org/10.1101/gad.199653.112

Hilgers, V., Perry, M. W., Hendrix, D., Stark, A., Levine, M., & Haley, B. (2011). Neural-specific elongation of 3′UTRs during Drosophila development. *Proceedings of the National Academy of Sciences of the United States of America*, *108*(38), 15864–15869. https://doi.org/10.1073/pnas.1112672108

Hinman, M. N., & Lou, H. (2008). Diverse molecular functions of Hu proteins. *Cellular and Molecular Life Sciences*, *65*(20), 3168–3181. https://doi.org/10.1007/s00018-008-8252-6

Hollerer, I., Curk, T., Haase, B., Benes, V., Hauer, C., Neu-Yilik, G., Bhuvanagiri, M., Hentze, M. W., & Kulozik, A. E. (2016). The differential expression of alternatively polyadenylated transcripts is a common stress-induced response mechanism that modulates mammalian mRNA expression in a quantitative and qualitative fashion. *RNA*, *22*(9), 1441–1453. https://doi.org/10.1261/rna.055657.115

Homyk, T., Jr., Isono, K., & Pak, W. L. (1985). Developmental and physiological analysis of a conditional mutation affecting photoreceptor and optic lobe development in *Drosophila melanogaster*. *Journal of Neurogenetics*, *2*(5), 309–324. https://doi.org/10.3109/01677068509102326

Ince-Dunn, G., Okano, H. J., Jensen, K. B., Park, W. Y., Zhong, R., Ule, J., Mele, A., Fak, J. J., Yang, C., Zhang, C., Yoo, J., Herre, M., Okano, H., Noebels, J. L., & Darnell, R. B. (2012). Neuronal Elav-like (Hu) proteins regulate RNA splicing and abundance to control glutamate levels and neuronal excitability. *Neuron*, *75*(6), 1067–1080. https://doi.org/10.1016/j.neuron.2012.07.009

Keene, J. D. (1999). Why is Hu where? Shuttling of early-response-gene messenger RNA subsets. *Proceedings of the National Academy of Sciences of the United States of America*, *96*(1), 5–7. https://doi.org/10.1073/pnas.96.1.5

Kim, Y. J., & Baker, B. S. (1993). The Drosophila gene rbp9 encodes a protein that is a member of a conserved group of putative RNA binding proteins that are nervous system-specific in both flies and humans. *The Journal of Neuroscience*, *13*(3), 1045–1056. https://doi.org/10.1523/jneurosci.13-03-01045.1993

Koushika, S. P., Lisbin, M. J., & White, K. (1996). ELAV, a Drosophila neuron-specific protein, mediates the generation of an alternatively spliced neural protein isoform. *Current Biology*, *6*(12), 1634–1641.

Koushika, S. P., Soller, M., & White, K. (2000). The neuron-enriched splicing pattern of Drosophila erect wing is dependent on the presence of ELAV protein. *Molecular and Cellular Biology*, *20*(5), 1836–1845. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC85364/pdf/mb001836.pdf

Lai, S. L., Miller, M. R., Robinson, K. J., & Doe, C. Q. (2012). The snail family member Worniu is continuously required in neuroblasts to prevent Elav-induced premature differentiation. *Developmental Cell*, *23*(4), 849–857. https://doi.org/10.1016/j.devcel.2012.09.007

Lee, S., Wei, L., Zhang, B., Goering, R., Majumdar, S., Wen, J., Taliaferro, J. M., & Lai, E. C. (2021). ELAV/Hu RNA binding proteins determine multiple programs of neural alternative splicing. *PLoS Genetics*, *17*(4), e1009439. https://doi.org/10.1371/journal.pgen.1009439

Lee, S. H., & Mayr, C. (2019). Gain of additional BIRC3 protein functions through 3′-UTR-mediated protein complex formation. *Molecular Cell*, *74*, 701–712.e9. https://doi.org/10.1016/j.molcel.2019.03.006

Lianoglou, S., Garg, V., Yang, J. L., Leslie, C. S., & Mayr, C. (2013). Ubiquitously transcribed genes use alternative polyadenylation to achieve tissue-specific expression. *Genes & Development*, *27*(21), 2380–2396. https://doi.org/10.1101/gad.229328.113

Lim, C. S., & Alkon, D. L. (2012). Protein kinase C stimulates HuD-mediated mRNA stability and protein expression of neurotrophic factors and enhances dendritic maturation of hippocampal neurons in culture. *Hippocampus*, *22*(12), 2303–2319. https://doi.org/10.1002/hipo.22048

Lisbin, M. J., Qiu, J., & White, K. (2001). The neuron-specific RNA-binding protein ELAV regulates neuroglian alternative splicing in neurons and binds directly to its pre-mRNA. *Genes & Development*, *15*(19), 2546–2561. https://doi.org/10.1101/gad.903101

Lu, L., Wang, S., Zheng, L., Li, X., Suswam, E. A., Zhang, X., Wheeler, C. G., Nabors, L. B., Filippova, N., & King, P. H. (2009). Amyotrophic lateral sclerosis-linked mutant SOD1 sequesters Hu antigen R (HuR) and TIA-1-related protein (TIAR): Implications for impaired post-transcriptional regulation of vascular endothelial growth factor. *The Journal of Biological Chemistry*, *284*(49), 33989–33998. https://doi.org/10.1074/jbc.M109.067918

Lu, Y. L., Liu, Y., McCoy, M. J., & Yoo, A. S. (2021). MiR-124 synergism with ELAVL3 enhances target gene expression to promote neuronal maturity. *Proceedings of the National Academy of Sciences of the United States of America*, *118*(22), e2015454118. https://doi.org/10.1073/pnas.2015454118

Mansfield, K. D., & Keene, J. D. (2012). Neuron-specific ELAV/Hu proteins suppress HuR mRNA during neuronal differentiation by alternative polyadenylation. *Nucleic Acids Research*, *40*(6), 2734–2746. https://doi.org/10.1093/nar/gkr1114

Markmiller, S., Soltanieh, S., Server, K. L., Mak, R., Jin, W., Fang, M. Y., Luo, E. C., Krach, F., Yang, D., Sen, A., Fulzele, A., Wozniak, J. M., Gonzalez, D. J., Kankel, M. W., Gao, F. B., Bennett, E. J., Lecuyer, E., & Yeo, G. W. (2018). Context-dependent and disease-specific diversity in protein interactions within stress granules. *Cell*, *172*(3), 590–604 e513. https://doi.org/10.1016/j.cell.2017.12.032

Mayr, C. (2019). What are 3′ UTRs doing? *Cold Spring Harbor Perspectives in Biology*, *11*(10), a034728. https://doi.org/10.1101/cshperspect.a034728

Mayr, C., & Bartel, D. P. (2009). Widespread shortening of 3′UTRs by alternative cleavage and polyadenylation activates oncogenes in cancer cells. *Cell*, *138*(4), 673–684. https://doi.org/10.1016/j.cell.2009.06.016

McCorkindale, A. L., Wahle, P., Werner, S., Jungreis, I., Menzel, P., Shukla, C. J., Abreu, R. L. P., Irizarry, R. A., Meyer, I. M., Kellis, M., & Zinzen, R. P. (2019). A gene expression atlas of embryonic neurogenesis in Drosophila reveals complex spatiotemporal regulation of lncRNAs. *Development*, *146*(6), dev175265. https://doi.org/10.1242/dev.175265

Meyer, K. D., Saletore, Y., Zumbo, P., Elemento, O., Mason, C. E., & Jaffrey, S. R. (2012). Comprehensive analysis of mRNA methylation reveals enrichment in 3′ UTRs and near stop codons. *Cell*, *149*(7), 1635–1646. https://doi.org/10.1016/j.cell.2012.05.003

Mirisis, A. A., & Carew, T. J. (2019). The ELAV family of RNA-binding proteins in synaptic plasticity and long-term memory. *Neurobiology of Learning and Memory*, *161*, 143–148. https://doi.org/10.1016/j.nlm.2019.04.007

Miura, P., Shenker, S., Andreu-Agullo, C., Westholm, J. O., & Lai, E. C. (2013). Widespread and extensive lengthening of 3′UTRs in the mammalian brain. *Genome Research*, *23*(5), 812–825. https://doi.org/10.1101/gr.146886.112

Mohanan, N. K., Shaji, F., Koshre, G. R., & Laishram, R. S. (2021). Alternative polyadenylation: An enigma of transcript length variation in health and disease. *Wiley Interdisciplinary Reviews RNA*, *13*(1), e1692. https://doi.org/10.1002/wrna.1692

Mukherjee, N., Corcoran, D. L., Nusbaum, J. D., Reid, D. W., Georgiev, S., Hafner, M., Ascano, M., Jr., Tuschl, T., Ohler, U., & Keene, J. D. (2011). Integrative regulatory mapping indicates that the RNA-binding protein HuR couples pre-mRNA processing and mRNA stability. *Molecular Cell*, *43*(3), 327–339. https://doi.org/10.1016/j.molcel.2011.06.007

Nicholson, C. O., Friedersdorf, M., & Keene, J. D. (2017). Quantifying RNA binding sites transcriptome-wide using DO-RIP-seq. *RNA*, *23*(1), 32–46. https://doi.org/10.1261/rna.058115.116

Ogawa, Y., Kakumoto, K., Yoshida, T., Kuwako, K. I., Miyazaki, T., Yamaguchi, J., Konno, A., Hata, J., Uchiyama, Y., Hirai, H., Watanabe, M., Darnell, R. B., Okano, H., & Okano, H. J. (2018). Elavl3 is essential for the maintenance of Purkinje neuron axons. *Scientific Reports*, *8*(1), 2722. https://doi.org/10.1038/s41598-018-21130-5

Oktaba, K., Zhang, W., Lotz, T. S., Jun, D. J., Lemke, S. B., Ng, S. P., Esposito, E., Levine, M., & Hilgers, V. (2015). ELAV links paused pol II to alternative polyadenylation in the Drosophila nervous system. *Molecular Cell*, *57*(2), 341–348. https://doi.org/10.1016/j.molcel.2014.11.024

Pabis, M., Popowicz, G. M., Stehle, R., Fernandez-Ramos, D., Asami, S., Warner, L., Garcia-Maurino, S. M., Schlundt, A., Martinez-Chantar, M. L., Diaz-Moreno, I., & Sattler, M. (2019). HuR biological function involves RRM3-mediated dimerization and RNA binding by all three RRMs. *Nucleic Acids Research*, *47*(2), 1011–1029. https://doi.org/10.1093/nar/gky1138

Park, S. J., Yang, E. S., Kim-Ha, J., & Kim, Y. J. (1998). Down regulation of extramacrochaetae mRNA by a Drosophila neural RNA binding protein Rbp9 which is homologous to human Hu proteins. *Nucleic Acids Research*, *26*(12), 2989–2994. https://doi.org/10.1093/nar/26.12.2989

Pascale, A., Amadio, M., & Quattrone, A. (2008). Defining a neuron: neuronal ELAV proteins. *Cellular and Molecular Life Sciences*, *65*(1), 128–140. https://doi.org/10.1007/s00018-007-7017-y

Pascale, A., Amadio, M., Scapagnini, G., Lanni, C., Racchi, M., Provenzani, A., Govoni, S., Alkon, D. L., & Quattrone, A. (2005). Neuronal ELAV proteins enhance mRNA stability by a PKCalpha-dependent pathway. *Proceedings of the National Academy of Sciences of the United States of America*, *102*(34), 12065–12070. https://doi.org/10.1073/pnas.0504702102

Pascale, A., Gusev, P. A., Amadio, M., Dottorini, T., Govoni, S., Alkon, D. L., & Quattrone, A. (2004). Increase of the RNA-binding protein HuD and posttranscriptional up-regulation of the GAP-43 gene during spatial memory. *Proceedings of the National Academy of Sciences of the United States of America*, *101*(5), 1217–1222. https://doi.org/10.1073/pnas.0307674100

Quattrone, A., Pascale, A., Nogues, X., Zhao, W., Gusev, P., Pacini, A., & Alkon, D. L. (2001). Posttranscriptional regulation of gene expression in learning by the neuronal ELAV-like mRNA-stabilizing proteins. *Proceedings of the National Academy of Sciences of the United States of America*, *98*(20), 11668–11673. https://doi.org/10.1073/pnas.191388398

Raser, J. M., & O'Shea, E. K. (2005). Noise in gene expression: origins, consequences, and control. *Science*, *309*(5743), 2010–2013. https://doi.org/10.1126/science.1105891

Robinow, S., & White, K. (1988). The locus elav of Drosophila melanogaster is expressed in neurons at all developmental stages. *Developmental Biology*, *126*(2), 294–303. https://doi.org/10.1016/0012-1606(88)90139-X

Rogulja-Ortmann, A., Picao-Osorio, J., Villava, C., Patraquim, P., Lafuente, E., Aspden, J., Thomsen, S., Technau, G. M., & Alonso, C. R. (2014). The RNA-binding protein ELAV regulates Hox RNA processing, expression and function within the Drosophila nervous system. *Development*, *141*(10), 2046–2056. https://doi.org/10.1242/dev.101519

Samson, M. L. (1998). Evidence for 3′ untranslated region-dependent autoregulation of the Drosophila gene encoding the neuronal nuclear RNA-binding protein ELAV. *Genetics*, *150*(2), 723–733. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1460370/pdf/9755203.pdf

Samson, M.-L. (2008). Rapid functional diversification in the structurally conserved ELAV family of neuronal RNA binding proteins. *BMC Genomics*, *9*(1), 392. https://doi.org/10.1186/1471-2164-9-392

Samson, M. L., & Chalvet, F. (2003). Found in neurons, a third member of the Drosophila elav gene family, encodes a neuronal protein and interacts with elav. *Mechanisms of Development*, *120*(3), 373–383. https://doi.org/10.1016/s0925-4773(02)00444-6

Samuels, T. J., Arava, Y., Järvelin, A. I., Robertson, F., Lee, J. Y., Yang, L., Yang, C.-P., Lee, T., Ish-Horowicz, D., & Davis, I. (2020). Neuronal upregulation of Prospero protein is driven by alternative mRNA polyadenylation and Syncrip-mediated mRNA stabilisation. *Biology Open*, *9*(5), bio049684. https://doi.org/10.1242/bio.049684

Sanfilippo, P., Smibert, P., Duan, H., & Lai, E. C. (2016). Neural specificity of the RNA-binding protein Elav is achieved by post-transcriptional repression in non-neural tissues. *Development*, *143*(23), 4474–4485. https://doi.org/10.1242/dev.141978

Sanfilippo, P., Wen, J., & Lai, E. C. (2017). Landscape and evolution of tissue-specific alternative polyadenylation across Drosophila species. *Genome Biology*, *18*, 229. https://doi.org/10.1186/s13059-017-1358-0

Scheckel, C., Drapeau, E., Frias, M. A., Park, C. Y., Fak, J., Zucker-Scharff, I., Kou, Y., Haroutunian, V., Ma'ayan, A., Buxbaum, J. D., & Darnell, R. B. (2016). Regulatory consequences of neuronal ELAV-like protein binding to coding and non-coding RNAs in human brain. *eLife*, *5*, e10421. https://doi.org/10.7554/eLife.10421

Shi, Y., & Manley, J. L. (2015). The end of the message: Multiple protein-RNA interactions define the mRNA polyadenylation site. *Genes & Development*, *29*(9), 889–897. https://doi.org/10.1101/gad.261974.115

Smibert, P., Miura, P., Westholm, J. O., Shenker, S., May, G., Duff, M. O., Zhang, D., Eads, B. D., Carlson, J., Brown, J. B., Eisman, R. C., Andrews, J., Kaufman, T., Cherbas, P., Celniker, S. E., Graveley, B. R., & Lai, E. C. (2012). Global patterns of tissue-specific alternative polyadenylation in Drosophila. *Cell Reports*, *1*(3), 277–289. https://doi.org/10.1016/j.celrep.2012.01.001

Soller, M., & White, K. (2003). ELAV inhibits 3′-end processing to promote neural splicing of ewg pre-mRNA. *Genes & Development*, *17*(20), 2526–2538. https://doi.org/10.1101/gad.1106703

Soller, M., & White, K. (2005). ELAV multimerizes on conserved AU4-6 motifs important for ewg splicing regulation. *Molecular and Cellular Biology*, *25*(17), 7580–7591. https://doi.org/10.1128/MCB.25.17.7580-7591.2005

Tebaldi, T., Zuccotti, P., Peroni, D., Kohn, M., Gasperini, L., Potrich, V., Bonazza, V., Dudnakova, T., Rossi, A., Sanguinetti, G., Conti, L., Macchi, P., D'Agostino, V., Viero, G., Tollervey, D., Huttelmaier, S., & Quattrone, A. (2018). HuD is a neural translation enhancer acting on mTORC1-responsive genes and counteracted by the Y3 small non-coding RNA. *Molecular Cell*, *71*(2), 256–270.e210. https://doi.org/10.1016/j.molcel.2018.06.032

Tiruchinapalli, D. M., Ehlers, M. D., & Keene, J. D. (2008). Activity-dependent expression of RNA binding protein HuD and its association with mRNAs in neurons. *RNA Biology*, *5*(3), 157–168. https://doi.org/10.4161/rna.5.3.6782

Toba, G., & White, K. (2008). The third RNA recognition motif of Drosophila ELAV protein has a role in multimerization. *Nucleic Acids Research*, *36*(4), 1390–1399. https://doi.org/10.1093/nar/gkm1168

Torres-Méndez, A., Pop, S., Bonnal, S., Almudi, I., Avola, A., Roberts, R. J. V., Paolantoni, C., Alcaina-Caro, A., Martín-Anduaga, A., Haussmann, I. U., Morin, V., Casares, F., Soller, M., Kadener, S., Roignant, J. Y., Prieto-Godino, L., & Irimia, M. (2022). Parallel evolution of a splicing program controlling neuronal excitability in flies and mammals. *Science Advances*, *8*(4), eabk0445. https://doi.org/10.1126/sciadv.abk0445

Ulitsky, I., Shkumatava, A., Jan, C. H., Subtelny, A. O., Koppstein, D., Bell, G. W., Sive, H., & Bartel, D. P. (2012). Extensive alternative polyadenylation during zebrafish development. *Genome Research*, *22*(10), 2054–2066. https://doi.org/10.1101/gr.139733.112

Ustaoglu, P., Gill, J. K., Doubovetzky, N., Haussmann, I. U., Dix, T. C., Arnold, R., Devaud, J. M., & Soller, M. (2021). Dynamically expressed single ELAV/Hu orthologue elavl2 of bees is required for learning and memory. *Communications Biology*, *4*(1), 1234. https://doi.org/10.1038/s42003-021-02763-1

Vasudevan, S., & Steitz, J. A. (2007). AU-rich-element-mediated upregulation of translation by FXR1 and Argonaute 2. *Cell*, *128*(6), 1105–1118. https://doi.org/10.1016/j.cell.2007.01.038

Wang, H., Molfenter, J., Zhu, H., & Lou, H. (2010). Promotion of exon 6 inclusion in HuD pre-mRNA by Hu protein family members. *Nucleic Acids Research*, *38*(11), 3760–3770. https://doi.org/10.1093/nar/gkq028

Wei, L., Lee, S., Majumdar, S., Zhang, B., Sanfilippo, P., Joseph, B., Miura, P., Soller, M., & Lai, E. C. (2020). Overlapping activities of ELAV/Hu family RNA binding proteins specify the extended neuronal 3′UTR landscape in Drosophila. *Molecular Cell*, *80*(1), 140–155.e146. https://doi.org/10.1016/j.molcel.2020.09.007

Xu, C., & Zhang, J. (2020). A different perspective on alternative cleavage and polyadenylation. *Nature Reviews Genetics*, *21*(1), 63–64. https://doi.org/10.1038/s41576-019-0198-z

Yannoni, Y. M., & White, K. (1997). Association of the neuron-specific RNA binding domain-containing protein ELAV with the coiled body in Drosophila neurons. *Chromosoma*, *105*(6), 332–341. https://doi.org/10.1007/bf02529748

Yannoni, Y. M., & White, K. (1999). Domain necessary for Drosophila ELAV nuclear localization: Function requires nuclear ELAV. *Journal of Cell Science*, *112* (Pt 24), 4501–4512. Retrieved from http://jcs.biologists.org/content/joces/112/24/4501.full.pdf

Yano, M., Hayakawa-Yano, Y., & Okano, H. (2016). RNA regulation went wrong in neurodevelopmental disorders: The example of Msi/Elavl RNA binding proteins. *International Journal of Developmental Neuroscience*, *55*, 124–130. https://doi.org/10.1016/j.ijdevneu.2016.01.002

Yao, K. M., Samson, M. L., Reeves, R., & 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. *Journal of Neurobiology*, *24*(6), 723–739. https://doi.org/10.1002/neu.480240604

Yokoi, S., Udagawa, T., Fujioka, Y., Honda, D., Okado, H., Watanabe, H., Katsuno, M., Ishigaki, S., & Sobue, G. (2017). 3′UTR length-dependent control of SynGAP isoform alpha2 mRNA by FUS and ELAV-like proteins promotes dendritic spine maturation and cognitive function. *Cell Reports*, *20*(13), 3071–3084. https://doi.org/10.1016/j.celrep.2017.08.100

Zaharieva, E., Haussmann, I. U., Brauer, U., & Soller, M. (2015). Concentration and localization of Coexpressed ELAV/Hu proteins control specificity of mRNA processing. *Molecular and Cellular Biology*, *35*(18), 3104–3115. https://doi.org/10.1128/MCB.00473-15

Zanini, D., Jallon, J. M., Rabinow, L., & Samson, M. L. (2012). Deletion of the Drosophila neuronal gene found in neurons disrupts brain anatomy and male courtship. *Genes, Brain and Behavior*, *11*(7), 819–827. https://doi.org/10.1111/j.1601-183X.2012.00817.x

Zhang, H., Lee, J. Y., & Tian, B. (2005). Biased alternative polyadenylation in human tissues. *Genome Biology*, *6*(12), R100. https://doi.org/10.1186/gb-2005-6-12-r100

Zhang, Z., So, K., Peterson, R., Bauer, M., Ng, H., Zhang, Y., Kim, J. H., Kidd, T., & Miura, P. (2019). Elav-mediated exon skipping and alternative polyadenylation of the Dscam1 gene are required for axon outgrowth. *Cell Reports*, *27*(13), 3808–3817.e3807. https://doi.org/10.1016/j.celrep.2019.05.083

Zheng, D., Wang, R., Ding, Q., Wang, T., Xie, B., Wei, L., Zhong, Z., & Tian, B. (2018). Cellular stress alters 3′UTR landscape through alternative polyadenylation and isoform-specific degradation. *Nature Communications*, *9*(1), 2268. https://doi.org/10.1038/s41467-018-04730-7

Zhu, H., Hasman, R. A., Barron, V. A., Luo, G., & Lou, H. (2006). A nuclear function of Hu proteins as neuron-specific alternative RNA processing regulators. *Molecular Biology of the Cell*, *17*(12), 5105–5114. https://doi.org/10.1091/mbc.e06-02-0099

How to cite this article: Hilgers, V. (2022). Regulation of neuronal RNA signatures by ELAV/Hu proteins. *WIREs RNA*, e1733. https://doi.org/10.1002/wrna.1733