Published in final edited form as:

Wiley Interdiscip Rev RNA. 2012 January ; 3(1): 104–121. doi:10.1002/wrna.107.

The importance of CELF control: molecular and biological roles of the CUG-BP, Elav-like family of RNA binding proteins

Twishasri Dasgupta and

Department of Cell Biology, Lerner Research Institute, Cleveland Clinic

Andrea N. Ladd

Department of Cell Biology, Lerner Research Institute, Cleveland Clinic

Abstract

RNA processing is important for generating protein diversity and modulating levels of protein expression. The CUG-BP, Elav-like family (CELF) of RNA binding proteins regulate several steps of RNA processing in the nucleus and cytoplasm, including pre-mRNA alternative splicing, C to U RNA editing, deadenylation, mRNA decay, and translation. *In vivo*, CELF proteins have been shown to play roles in gametogenesis and early embryonic development, heart and skeletal muscle function, and neurosynaptic transmission. Dysregulation of CELF-mediated programs has been implicated in the pathogenesis of human diseases affecting the heart, skeletal muscles, and nervous system.

Keywords

CELF; CUG-BP; ETR-3; Bruno-like; alternative splicing; RNA editing; deadenylation; mRNA decay; translation; myotonic dystrophy

Introduction

The completion of the Human Genome Project revealed that there are far fewer genes than predicted. Combined with the development of technologies for high throughput transcriptome and proteome analyses, it has become increasingly clear that one gene can produce several proteins. Both post-transcriptional regulation of RNAs and post-translational modification of proteins contribute to proteome diversity. The amount of gene expression can also be regulated at the RNA or protein level, by modulating production or stability.

Each step in the life of a transcript, including its production, modification, transport, translation, and decay, is tightly controlled. Many RNA binding protein families differentially interact with specific subsets of transcripts and regulate one or more of these steps. An example is the CUG-BP, Elav-like family (CELF). CELF proteins are found in the nucleus and cytoplasm. They regulate multiple facets of gene expression, including pre-mRNA alternative splicing, RNA editing, deadenylation, mRNA stability, and translation. We review both the molecular and biological roles of CELF proteins, and recap evidence that implicates members of this family in human diseases. Because different family members were identified at different times in different tissues and different species, they are found in the literature under a host of names. In this review, we summarize what is known about CELF proteins using a consistent nomenclature.

THE CELF FAMILY

Before being recognized as a family, individual CELF clones were isolated. CELF1 was first isolated from human cells as a nuclear protein that binds to a (CUG) RNA.¹⁸ *CELF2* was first cloned in a screen of genes expressed in fetal heart.² It arose again in screens for genes up-regulated during apoptosis in neuroblastoma cells³ and for genes related to CELF1 in mouse liver.⁴ A *celf3* clone was identified in *Xenopus* neural tissue.⁵

Two groups described the CELF proteins as a family. Good and colleagues called them the Bruno-like (Brunol) proteins after the homologous *Drosophila* protein Bruno.⁶ Ladd et al. dubbed them the CUG-BP and ETR-3-like factor (CELF) proteins after the first two family members identified in humans, CUG binding protein (CUG-BP, now called CELF1) and elav-type RNA binding protein 3 (ETR-3, now called CELF2).⁷ At that time, cDNA or EST sequences for five human CELF proteins, CELF1-5, were found in the databases, and a sixth was predicted from genomic sequence. Expression of CELF6 was validated a few years later.⁸ Family members have been called by a plethora of names (Table 1), until a recent consensus in the field agreed on a common nomenclature: CUGBP, Elav-like family member 1 (CELF1), CELF2, CELF3, CELF4, CELF5, and CELF6. It should be noted that in early reports, CUG-BP1 and CUG-BP2 referred to phosphoisoforms of CELF1^{1, 9}, whereas in later studies CUG-BP1 was used for all isoforms of CELF1 and CUG-BP2 referred to CELF2.

CELF protein subfamilies

CELF homologs are found in deuterostomes, protostomes, and plants, but not in bacteria, Archaea, or yeast.^{6, 10} Phylogenetic analysis of human CELF proteins divides them into two distinct subfamilies: CELF1-2, and CELF3-6.⁶⁻⁸ Each subfamily is represented by at least one member in plants and animals, suggesting the ancestral CELF gene underwent a duplication soon after it arose.¹⁰ Most invertebrates, including primitive chordates, have only two CELF proteins. *Drosophila*, however, has three paralogs, Bruno (Bru), Bru-2, and Bru-3.⁶ Bru and Bru-2 likely result from a lineage-specific tandem duplication and belong to the CELF1-2 subfamily. While phylogenetic analysis suggests that Bru-3 belongs to the CELF3-6 subfamily⁶, it has been suggested that Bru-3 is functionally more similar to CELF1 than Bru or Bru-2.¹¹ The number of CELF proteins expanded in vertebrates. Mice have six CELF proteins as in humans. Chicken has five due to partial deletion of the *CELF3* gene.¹⁰ Paralogs of *celf1-5*, but not *celf6*, have been identified in *Xenopus tropicalis* and *laevis*.¹² As a pseudotetraploid *X. laevis* has two of each gene, and cDNAs have been cloned for second paralogs of *celf1*, *celf2*, and *celf3*.¹² Only *celf1-5* are listed in the NCBI database for zebrafish, although ESTs and predicted protein sequences suggest there could be eight Celf proteins due to expansion of the CELF3-6 subfamily.¹⁰

The CELF1-2 subfamily is broadly expressed, while the CELF3-6 subfamily exhibits more restricted expression. CELF1 and CELF2 have overlapping yet distinct expression profiles, both being highest in heart, skeletal muscle, and brain.^{3, 6-8} CELF3 and CELF5 are detected primarily in the nervous system, while CELF6 is found in nervous system, kidney, and testis.^{6-8, 13} There are conflicting reports of CELF4 expression. In one study, *CELF4* transcripts were broadly detected in most human tissues⁸, but in several others *CELF4* mRNA was detected only in the nervous system.¹³⁻¹⁵ This discrepancy may result from using probes against different regions of the *CELF4* transcript. CELF1 and CELF2 are also broadly expressed in the developing embryo with high expression in myogenic tissues, while CELF3-6 are primarily found in embryonic neural tissues.^{5, 10, 12, 16-18} Differential expression of the subfamilies is conserved. In *C. elegans* ETR-1, the CELF1-2 homolog, is expressed in developing muscle¹⁹ while UNC-75, the CELF3-6 homolog, is expressed exclusively in the nervous system.¹³

CELF protein structure and function

CELF proteins share a common domain structure with the Elav family (which includes the Hu antigen proteins), but are evolutionarily distinct.⁶ Each CELF protein contains three RNA binding domains of the RNA recognition motif (RRM) variety, two at the N terminus and one at the C terminus (Figure 1). A unique linker region called the “divergent domain” separates RRM2 and RRM3. The divergent domain was so named because sequence similarities in this domain divide CELF proteins into the two distinct subfamilies.⁷

Interactions with RNA—Like many RNA binding proteins, CELF protein binding sites lack a tight consensus sequence. Rather, CELF proteins have been shown to interact with a number of sequence elements in different contexts. In early studies, CELF1 and CELF2 were both identified as proteins that bind to (CUG)₈ repeats^{1, 4}, but yeast three-hybrid analysis indicated CELF1 prefers UG dinucleotide repeats over CUG repeats.²⁰ U/G-rich sequences were also identified as preferred binding sites of recombinant CELF1 and CELF2 by systematic evolution of ligands by exponential enrichment (SELEX). CELF1 binds with high affinity to elements enriched with UGU trinucleotides²¹, whereas CELF2 preferentially binds to UG repeats and UGUU motifs.²² From these studies U/G-rich motifs emerge as a common theme, but CELF1 was also shown to bind to CCG repeats in the 5' region of *C/EBPβ* transcripts *in vitro*²³, to a G/C-rich region in the 5' UTR of *cyclin D1*²⁴, and to U(A/G) repeats in the 3' UTR of *Eg* and *c-mos* transcripts.²⁵ Surface plasmon resonance analysis suggested that recombinant CELF1 prefers “pure” UG dinucleotide repeats to other U/G-rich sequences such as (UAUG)₇ or (UUG)₁₀, but cannot rule out their use *in vivo*.²⁶ NMR chemical shift perturbation indicated that CELF1 RRM3 binds better to UG repeats than CUG and UA repeats in solution, although it will bind all three.²⁷ Binding preferences for CELF3-6 are less well characterized, but are theorized to be similar based on the high degree of conservation within CELF protein RRM3.

Several studies suggest that RRM1 and RRM2 are important for CELF protein:RNA interactions. Deletional analyses indicate that CELF1 binds to CUG sequences¹ and A/U-rich elements²⁸ through the first two RRM3s. Likewise, CELF2 binds to a Bruno response element through RRM1 and RRM2⁶, and CELF4 needs either RRM1 or RRM2 for binding a muscle-specific splicing enhancer (MSE).²⁹ In yeast three-hybrid experiments, however, CELF1 binding to UG repeats was not abrogated by loss of any single RRM, and RRM3 was sufficient to bind RNA when combined with the divergent domain.²⁶ Furthermore, a truncated recombinant CELF1 containing RRM3 bound to a G/C-rich riboprobe, whereas truncated proteins containing either RRM1 or RRM2 did not.²⁴ Together these studies suggest that different RRM3s may bind distinct substrates. Interestingly, non-overlapping segments of CELF2 containing either RRM1 and RRM2 with the first half of the divergent domain or RRM3 with the second half of the divergent domain bind specifically to MSE RNA *in vitro*, suggesting the RRM3s may be redundant.²⁹ Crystal and solution structures of CELF1 domains indicate that the manner in which RRM1 and RRM2 interact with RNA is similar, but differs from that of RRM3.^{27, 30}

Although not an RNA binding domain itself, the divergent domain likely plays a role in mediating the interaction with RNA. The largest effect on CELF1 binding in yeast three-hybrid experiments came from deletions of the divergent domain, not changes in the RRM3s.^{20, 26} Strikingly, experiments with chimeric proteins demonstrated that binding via the first two RRM3s of CELF4 is strongly influenced by the divergent domain sequence associated with them.³¹ It is not clear whether the divergent domain affects RNA binding by mediating protein:protein interactions, or by conveying an important conformational state.

Beyond RNA binding—The divergent domain has also been shown to contain sequences that are important for function separable from RNA binding. Deletion of a portion of the human CELF4 divergent domain immediately downstream of RRM2 resulted in loss of splicing activity, despite retaining RNA binding.²⁹ Additionally, deletion of RRM1 and part of RRM2 in a naturally occurring splice variant of CELF4 lacking part of RRM3 results in a protein that does not bind to RNA, but can inhibit the splicing activity of full length CELF proteins.^{29, 32} Deletion of the CELF2 divergent domain also ablates splicing activity but not RNA binding.³³ Distinct but overlapping regions within the divergent domains of human CELF2 and CELF4 activate splicing of different alternative exons.³¹ Regions required for repression of splicing overlap with those required for activation. Deletion and domain-swapping experiments demonstrated that its divergent domain bestows the ability to induce CFTR exon skipping on CELF2, but not CELF1.³³ Similarly, replacement of the *Xenopus* celf1 divergent domain with that of celf3 conferred a celf3-specific translational activity to the chimeric protein.³⁴ Together, these data suggest that different regulatory events have different requirements, perhaps due to the formation of target-specific complexes.

Several regions of CELF2 were identified that control its subcellular localization.³⁵ Two regions confer nuclear localization, the C-terminal 20 amino acids and the last quadrant of the divergent domain. The C-terminus contains arginine and lysine residues typical of conventional nuclear localization signals, whereas the divergent domain does not. The divergent domain also contains a region that confers cytoplasmic localization via the CRM1-dependent nuclear export pathway. Deletional analyses of CELF1 indicated the divergent domain is required for exclusion from the nucleolus, localization in the perinuclear compartment, and translocation to cytoplasmic stress granules.³⁶

Yeast two-hybrid assays suggested that CELF1 can interact with itself.²⁸ Ablation of CELF1 oligomerization by mutating or directing a peptide against a 27-amino acid region within the divergent domain abolished these homotypic interactions and CELF1-dependent deadenylation.³⁷ RNA binding was also lost, however, so it is not clear if CELF1:CELF1 interactions are required for deadenylation activity *per se*.

Regulatory effects of phosphorylation—Shortly after its discovery, CELF1 was shown to be a phosphoprotein.⁹ CELF1 is phosphorylated by multiple kinases, with varying effects. Hyper-phosphorylation by protein kinase C (PKC) increases its half-life.³⁸ Although the phosphorylated residue(s) has not been identified, CELF1 has multiple PKC consensus sites. Phosphorylation by cyclinD3/cdk4 at Ser302 in the divergent domain promotes association with the initiation factor eIF2, affecting translation of CELF1 targets.³⁹ Phosphorylation of Ser302 also increases RNA binding *in vitro*, as does phosphorylation of Ser28 in RRM1.²⁴ Thus differential phosphorylation of CELF1 may control specific protein:protein and protein:RNA interactions. This is important, as many of the studies investigating *in vitro* RNA binding used un-phosphorylated recombinant proteins. CELF2-6 also have several predicted phosphorylation sites.^{7, 8} It remains to be seen whether phosphorylation is a common mechanism for regulating CELF function.

NUCLEAR ROLES OF THE CELF PROTEINS

Celf1 has been shown to co-localize with nascent transcripts on lampbrush chromosomes in amphibian oocytes.⁴⁰ The loading of CELF1 onto specific transcripts as they are transcribed supports a regulatory role for CELF proteins in co-transcriptional or post-transcriptional RNA processing. Indeed, one of the earliest roles ascribed to CELF proteins is regulation of pre-mRNA alternative splicing.^{7, 41} Although alternative splicing regulation is generally considered the primary function of CELF proteins in the nucleus, CELF2 has also been implicated in nuclear C to U RNA editing in mammalian cells.^{42, 43}

Alternative splicing

CELF proteins have been shown to activate inclusion of some alternatively spliced regions, but silence others. They typically bind to intronic sequences in their pre-mRNA targets. While early studies relied on the use of *in vitro* assays and minigene reporters in cultured cells, later studies have expanded to include regulation of endogenous transcripts *in vivo*.

Exogenous minigenes—Studies performed in cultured cells with transiently transfected minigenes have identified a number of alternative regions regulated by CELF proteins (Table 2). The first targets of CELF-mediated splicing activity were identified based on the presence of CUG sequences in the introns adjacent to regulated exons (e.g.⁴¹). SELEX for CELF2 suggested several others.²² Interestingly, CELF proteins can have positive or negative effects on splicing of different pre-mRNAs.

Minigenes can be easily modified to identify transcript features that control splicing, *cis*-acting regulatory elements, and *trans*-acting factors that bind these elements.⁴⁴ Minigene data must be interpreted cautiously, however, as splicing elements may not behave the same in the context of a full-length pre-mRNA expressed under its own promoter. Likewise, regulatory factors identified *in vitro* or in over-expression studies may not be determinative for regulation *in vivo*. One example is cardiac troponin T (cTNT) exon 5. Although cTNT minigenes indicated that CELF proteins are important for activation of exon 5 inclusion^{7, 8, 32, 41, 45}, recent transgenic mice studies suggest that cTNT exon 5 alternative splicing is unresponsive *in vivo* to over-expression of CELF1⁴⁶ or repression of CELF activity (A. Ladd, unpublished data).

Endogenous targets—The investigation of alternative splicing regulation *in vivo* has been aided by the development of transgenic mouse models and the advent of splicing microarrays. Splicing microarrays have identified alternative splicing events that undergo fetal-to-adult transitions in the heart.⁴⁶ These changes correlate with a decline in CELF1 and CELF2 expression, and nearly half responded to over-expression of CELF1 in transgenic mice. These data support an earlier model in which it was proposed that down-regulation of CELF proteins in the heart drives developmental stage-specific alternative splicing transitions.⁴⁵ Additional bioinformatically-predicted CELF targets have been shown to respond to changes in CELF activity in cells and mice.^{47, 48} Endogenous transcripts that respond to changes in CELF activity are summarized in Table 3.

Biochemical identification of direct targets—An important caveat of *in vivo* studies is that responding to changes in CELF activity does not necessarily indicate that a transcript is a direct target, as it could lie downstream in a pathway regulated by a direct target. Crosslinking immunoprecipitation (CLIP) allows co-purification of a protein from cells with fragments of its target RNAs, called “CLIP tags”⁴⁹. This can be combined with high throughput sequencing (HITS-CLIP) to generate a comprehensive putative target set.⁵³ To date, CLIP has only been performed for one CELF protein, CELF1, from mouse hindbrain.⁵⁰ Tags representing 206 genes were identified, 64% of which were intronic, consistent with a role in alternative splicing regulation. The alternative splicing of only one, *Gabt4*, has been shown to respond to changes in CELF1 activity thus far.⁵⁰ Future studies are needed to integrate sets of targets identified by different methods, and to compare these between different tissues and family members.

Mechanisms of CELF-mediated alternative splicing—The mechanisms of CELF-mediated alternative splicing are not fully understood, but studies by several groups point to the U2 snRNP. CELF2 enhanced complex A formation on a cTNT exon 5 substrate, which was previously shown to be activated by binding of CELF2 to sites in the downstream

intron.⁵¹ CELF2 bound components of the U2 snRNP and promoted binding of U2 snRNA to the upstream intron. These data suggest a model in which CELF2 binds downstream and acts across the regulated exon to promote U2 assembly on the branch site.

The U2 snRNP is also implicated in CELF-mediated silencing. *In vitro* binding and splicing experiments suggested that CELF proteins inhibit inclusion of *NF1* exon 23a through interference with binding of U2AF⁶⁵, which interacts with the polypyrimidine tract of the 3' splice site and promotes assembly of the U2 snRNP at the branch site.⁵² Likewise, U2AF⁶⁵ was displaced from *CFTR* transcripts by CELF2, inhibiting exon inclusion.³³ Interestingly, CELF1 failed to displace U2AF⁶⁵ and had no effect on *CFTR* alternative splicing. Using the *NMDA R1* N1 cassette exon, Dembowski and colleagues developed a “branch site-perimeter binding” model, in which silencing depends on multiple CELF binding sites near the branch site.⁴⁷ They identified a set of exons that had a similar arrangement of U/G motifs closely positioned at the boundaries of predicted branch sites, which were inhibited by CELF2.

It remains to be determined whether interactions with U2 are an essential for CELF function on all splicing targets. The generation of genome-wide data sets allows the construction of RNA binding maps, in which known regulatory outcomes (e.g. from microarray studies) are integrated with positional information from HITS-CLIP data. In addition to being a powerful tool for predicting the functional consequences of binding, RNA binding maps can offer insight into mechanism.⁵³ The development of CELF protein RNA binding maps is a valuable objective for future investigations.

RNA editing

In mammals, the apolipoprotein B (*APOB*) mRNA undergoes C to U editing in the nucleus.⁵⁴ APOBEC-1, an RNA cytidine deaminase, and APOBEC-1 complementation factor (ACF) form the core of the C to U editing holoenzyme. Function of this holoenzyme is regulated in part by CELF2.⁴³ CELF2 was identified as an apobec-1 interacting protein in a yeast two-hybrid screen.⁴² Co-immunoprecipitation and co-localization studies in liver cells indicated that CELF2 forms a complex with APOBEC-1, ACF, and the *APOB* RNA.⁴² Addition of CELF2 to a cell-free system inhibits C to U editing, while antisense inhibition of CELF2 in hepatoma cells promoted editing.⁴² Likewise, knockdown of CELF2 in an intestinal cell line stimulated *APOB* editing.⁴³ CELF2 levels inversely correlate with levels of *APOB* editing in mouse tissues and cultured human cells.⁴³ Other transcripts undergo nuclear C to U editing in mammalian cells⁵⁵, but a role for CELF proteins in these events has not yet been reported.

CYTOPLASMIC ROLES OF THE CELF PROTEINS

Just as CELF proteins bind to introns in pre-mRNAs to mediate alternative splicing in the nucleus, they have also been shown to bind to 5' and 3' UTRs in mature mRNAs to regulate deadenylation, mRNA stability, and translation in the cytoplasm.

Deadenylation

In *Xenopus*, celf1 was first identified by its ability to bind a U(A/G)-containing embryo deadenylation element (EDEN) and promote rapid EDEN-mediated deadenylation and silencing of maternal transcripts.²⁵ When RNAs were co-immunoprecipitated with celf1 complexes from egg extracts and hybridized on microarrays over 150 putative targets were identified.⁵⁶ More than 90% of these contained EDEN motifs in the 3' UTR, and included transcripts encoding proteins involved in cell cycle control and oocyte maturation. Human CELF1 also binds EDEN motifs, and can functionally replace the deadenylation activity of *Xenopus* celf1 in immunodepleted egg extracts.^{57, 58}

Deadenylation is not only important for silencing of maternal transcripts, but is also a rate-limiting step in the turnover of most eukaryotic mRNAs. CELF1 was shown to bind to *C-FOS* and *TNF α* transcripts and promote deadenylation in HeLa cell extracts.⁵⁹ CELF1 also interacts with poly(A)-specific ribonuclease (PARN), suggesting CELF1 may facilitate deadenylation by recruiting or stabilizing the deadenylase on its substrates.⁵⁹

While it is clear that CELF1 can promote mRNA deadenylation, it might also induce polyadenylation. Some putative targets of *celf1* (e.g. *frl1*) are polyadenylated in the zygote.⁶⁰ PARN has been shown to be part of the cytoplasmic polyadenylation element binding protein (cpeb) complex in *Xenopus* oocytes, where it deadenylates transcripts as the poly(A) tail is added.⁶⁰ Dissociation of PARN occurs following phosphorylation of cpeb, allowing the poly(A) tail to lengthen. Recruitment of transcripts into this complex by *celf1* could theoretically promote deadenylation or polyadenylation, depending on whether PARN is present. It remains to be determined whether *celf1* stimulates the polyadenylation of any targets.

mRNA stability

CELF1 binds to a conserved GU-rich element (GRE) that is enriched in the 3' UTRs of short-lived transcripts in human primary T cells, and promotes GRE-dependent mRNA decay.⁶¹ Interestingly, a CELF2 antibody failed to supershift GRE-containing complexes, suggesting this activity is specific to CELF1. Transcripts that co-purify with CELF1 in HeLa and C2C12 cytoplasmic extracts were significantly enriched for GRE and GU-repeats in the 3' UTRs.^{62, 63} Many of these encode proteins involved in cell cycle, growth, and apoptosis. CELF1-associated transcripts in myoblasts have shorter half lives, and are stabilized by siRNA-mediated knockdown of CELF1.^{62, 64} As mRNA decay often follows poly(A) tail shortening, it remains to be determined whether CELF1-mediated degradation is a consequence of its deadenylation activity.

While CELF1 promotes mRNA decay, CELF2 binds to A/U-rich sequences in the 3' UTR of *cyclooxygenase-2* (*COX2*) transcripts and stabilizes them, while simultaneously inhibiting their translation.⁶⁵ COX-2 is an enzyme that synthesizes prostaglandins, which provide a protective effect against ionizing radiation. CELF2 is induced following irradiation, whereas knockdown of CELF2 up-regulates COX-2 expression and decreases radiation-induced apoptosis.^{65, 66} Prostaglandins inhibit CELF2 expression in intestinal cells, indicating COX-2 may regulate CELF2 in a negative feedback loop.⁶⁷ CELF2 similarly stabilizes and inhibits translation of *MCL1*, an anti-apoptotic factor in the BCL2 family, via binding to its 3' UTR.⁶⁸

Translation

CELF proteins have been shown to regulate translation through multiple mechanisms (Figure 2). In *Drosophila*, Bru was first described as a repressor of *oskar* (*osk*) translation via binding to its 3' UTR.⁶⁹ Bru interacts with Cup, which associates with the 5'-cap binding initiation factor eIF4E, suggesting translational repression occurs via a 5'/3' interaction mediated by an eIF4E-Cup-Bru complex.⁷⁰ Bru also binds to *gurken* and *Cyclin A* 3' UTRs and inhibits translation.^{71, 72} As described above, CELF2 has likewise been shown to inhibit the translation of *COX2* and *MCL1* mRNAs.^{65, 68} The mechanism is not well understood, but CELF2 has been shown to compete with HuR, an activator of translation, for binding to A/U-rich elements in the *COX2* 3' UTR.⁷³ Under conditions of environmental stress, CELF1 is routed to stress granules in HeLa cells, suggesting it may also play a role in translational silencing.³⁶

Like Bru, *Xenopus* celf3 was shown to bind to the 3' UTR of *cyclin A* transcripts, but unlike Bru, celf3 binding stimulated translation.³⁴ Mammalian CELF1 induces translation of *p21*, a cell cycle inhibitor, by binding to a G/C-rich sequence in its 5' UTR.^{74, 75} CELF1 also binds to sequences in the 5' region of *C/EBP β* , where it promotes the use of a downstream AUG translation initiation codon. This gives rise to a truncated form of the C/EBP β protein, liver inhibitory protein (LIP), in a variety of contexts.^{23, 39, 76-78} Phosphorylation of CELF1 activates an interaction with subunits of the initiation factor eIF2, leading to recruitment of ribosomes to the downstream AUG start site.^{39, 79} A CELF1-eIF2 complex also binds to the 5' UTR of *HDAC1* and increases HDAC1 protein levels in aging liver.⁸⁰

Binding of CELF1 to the 3' UTR of *SHMT*, a folate-dependent enzyme, has been implicated in internal ribosome entry site (IRES)-mediated translation.^{81, 82} A mechanism whereby CELF1-hnRNP H complexes promote circularization of the transcript by mediating 5'/3' interactions has been proposed.⁸¹ Whether CELF1 recruits the translation machinery to the *SHMT* 5' UTR via its interaction with eIF2 or another initiation factor remains to be determined.

Integration of cytoplasmic functions

Polyadenylation status, transcript stability, and translation are intimately related. For example, deadenylated transcripts are often degraded or translationally silent. How cytoplasmic CELF functions are mechanistically coupled is unclear. In addition, opposing effects may be observed in different contexts. Tethering CELF1 to the 3' UTR of a reporter reduced RNA levels while maintaining protein production.⁸³ This suggests a decrease in mRNA stability and increase in translation, the opposite of the effects of CELF2 on *COX2* RNA. As with alternative splicing, the construction of RNA binding maps combining 5' and 3' UTR binding sites with regulatory information may help elucidate the mechanisms by which the functions of CELF proteins are integrated in the cytoplasm.

CELF PROTEIN PHENOTYPES

Much of what is known about the molecular roles of CELF proteins has been investigated *in vitro* or in cultured cells. Animal models shed light on the biological roles of CELF proteins *in vivo*. CELF proteins have been knocked down, knocked out, or inhibited in a variety of species. Consistent with their expression patterns, members of the CELF1-2 subfamily are important for striated muscle function, while the entire family is implicated in nervous system function. Both subfamilies likely participate in gametogenesis and zygotic development.

Mutations in invertebrate CELF proteins

Three mutant alleles in the *arrest* (*aret*) locus have been shown to disrupt the coding region of the *Drosophila* Bru protein.⁸⁴ The *aret* mutants have gametogenesis defects in both males and females.^{85, 86} Bru translationally represses *osk*, which is important for germ cell formation and posterior body patterning.⁶⁹ Bru also represses *gurken*, which is essential for axis formation, and *Cyclin A*, which drives the release of meiotic arrest and mitotic reentry.^{71, 72} Celf1 is maternally expressed in zygotes and egg extracts in zebrafish and frogs^{12, 87}, and a cluster of mRNAs involved in the meiotic process of oocyte maturation were identified as potential targets of celf1 deadenylation.⁵⁶ These data suggest that CELF1 may play a conserved role in the switch from meiosis to mitosis and oocyte to zygote.

In *C. elegans*, inactivation of ETR-1 by RNAi caused elongation defects and embryonic lethality.¹⁹ Worms that survived to hatching had paralysis and defects in muscle attachment. Mutations in *C. elegans* *unc-75* caused defects in motor neuron axon sprouting and synaptic function, but not neuron differentiation.¹³ Mosaic analysis indicated that UNC-75 affects

cholinergic and GABAergic synaptic transmission between motor neurons and target muscles. *Unc-75* mutants also had behavioral defects in egg laying and feeding that are characteristic of cholinergic transmission defects.

CELF proteins in the frog embryo

While initially uniform in early *Xenopus* embryos, *celf1* becomes enriched in dorsal mesoderm at neurula and tailbud stages, particularly presomitic mesoderm.⁸⁸ Somites are transient structures that give rise to multiple tissues including body muscles. Inhibiting *celf1* function by injecting antisense oligonucleotides or neutralizing antibodies leads to severe defects in somite segmentation, but not differentiation.⁸⁸ This phenotype has been attributed to de-repression of *Su(H)*, a component of the notch signaling pathway.⁸⁹ CELF1 is also expressed in the somites in chick and mouse embryos, perhaps indicating a conserved role in segmentation.^{10, 18}

Although initially isolated as a neural-enriched gene⁵, knockdown of *Xenopus celf3* in embryos inhibited proliferation and subsequent differentiation in the endoderm.³⁴ Conversely, over-expression of *celf3* stimulated proliferation.

Knockout mice

Similar to *Bru* mutants in flies, *Celf1* and *Celf3* knockout mice both indicate a role in gametogenesis. Mice lacking CELF3 are fertile with normal testicular morphology.⁹⁰ A significant reduction in the total sperm count was observed, however, suggesting that CELF3 deficiency affects the progression of spermatogenesis. Since fertility was not adversely affected, CELF3 is not essential for spermatogenesis, but compensation by another CELF protein is possible. In contrast to *Celf3*-null mice, disruption of *Celf1* resulted in severely impaired fertility in both males and females.¹⁸ Homozygous males are hypofertile, with a blockage in spermiogenesis prior to spermatid elongation, increased apoptosis, and down-regulation of germ cell markers. Histological analysis of the ovaries revealed no obvious changes in the oocytes or follicles, so the basis for reduced fertility in females remains unknown. *Celf1* knockout mice also exhibit a high rate of perinatal mortality and growth retardation, perhaps due to impaired placental function. Their failure to thrive and fertility deficits may make future characterization of the effects of loss of CELF1 on other organ systems such as heart, muscle, and brain more difficult in this model.

Consistent with neurotransmission defects in *unc-75* mutants, CELF4 deficiency is associated with a seizure disorder in the Frequent flier (*Ff*) mouse model of epilepsy, so named for its phenotype of convulsions followed by a running-bouncing phase.¹⁵ The *Ff* mutation arises from a transgenic insertion in the *Celf4* gene, which results in loss of function. A significantly reduced seizure threshold was observed in heterozygotes with progression of recurrent limbic and tonic clonic seizures. Homozygotes did not usually survive in the original C57BL/6J background, but those that survived in F₂ hybrid backgrounds displayed spike-wave discharges, the hallmark of absence epilepsy. This suggests that although all six CELF proteins are expressed in brain, CELF4 plays a non-redundant role in neural function.

Transgenic dominant negative CELF protein models

Two mouse models have been generated that use tissue-specific expression of a nuclear dominant negative CELF protein, NLSCELFΔ, to investigate CELF-mediated alternative splicing programs *in vivo*. The dominant negative approach offers several advantages. First, all CELF proteins present in a tissue (e.g. CELF1 and CELF2 in striated muscle) are simultaneously inhibited. NLSCELFΔ has been shown to inhibit the splicing activity of all six family members.³² Second, because NLSCELFΔ is restricted to the nucleus, it

presumably blocks only the nuclear function of CELF proteins, leaving cytoplasmic functions intact. Third, tissue-specific repression is easily achieved through use of tissue-specific promoters. Disadvantages are that individual functions of family members cannot be separated, and repression may not be complete.

MHC-*CELFA* mice express *NLSCELFA* under a cardiac muscle-specific promoter.⁴⁸ MHC-*CELFA* mice develop defects in CELF-mediated alternative splicing, dilated cardiomyopathy, and severe cardiac dysfunction. These changes were attributed to loss of CELF activity because over-expression of human *CELF1* in bitransgenic mice partially rescued both splicing defects and overt heart disease. Myo-*CELFA* mice express *NLSCELFA* under a skeletal muscle-specific promoter.⁹¹ Unlike the MHC-*CELFA* mice, these mice display small changes in alternative splicing and have a mild phenotype. Myo-*CELFA* mice exhibit reduced muscle interstitia, increased variability in fiber size, and a slight increase in slow twitch fibers. These modest changes may indicate that CELF proteins are more determinative for alternative splicing in heart than in skeletal muscle. Alternatively, *NLSCELFA* may more effectively inhibit CELF activity in MHC-*CELFA* hearts than in Myo-*CELFA* muscles.

CELF PROTEINS IN DISEASE

Consistent with their expression patterns and animal model phenotypes, CELF proteins have been implicated in pathogenesis in the heart, skeletal muscle, and nervous system.

CELF proteins in cardiac pathogenesis

The human *CELF2* gene is located at chromosome 10p13-14. *CELF2* has been proposed as a candidate gene for two genetic disorders affecting the heart that arise from mutations in this vicinity. Arrhythmogenic right ventricular dysplasia (ARVD) is characterized by progressive myocardial cell loss and fibrosis.⁹² A familial form of ARVD, designated ARVD6, maps to the locus 10p12-14, which contains *CELF2*. ARVD6 has high penetrance, early onset, and a high incidence of sudden death. Although no mutation was found in ARVD6 family members that would be predicted to change the *CELF2* protein sequence, variations were detected that cosegregate with the disease.⁹² Partial monosomy 10p is a rare chromosomal aberration associated with symptoms of the DiGeorge syndrome spectrum. Haploinsufficiency within the proximal region of 10p, *DGCR2*, has been linked to heart and thymus defects. *CELF2* was the only known gene found within a critical 300 kb region of *DGCR2* identified by deletion mapping of partial monosomy 10p patients.⁹³ Although loss of *CELF2* was not proven to cause heart defects in either ARVD6 or partial monosomy 10p, it is a promising candidate for future clinical and functional studies.

CELF proteins in muscle diseases

The best-studied example of involvement of a CELF protein in disease is that of *CELF1* in myotonic dystrophy (DM). DM is a form of muscular dystrophy that affects several systems, including skeletal muscle and heart. DM results from expression of RNAs containing expanded CUG or CCUG repeats that form double-stranded hairpins, accumulate in nuclear foci, and disrupt the processing of other RNAs.⁹⁴ *CELF1* was first identified as a CUG-repeat binding protein¹, leading to the proposal that the mutant RNAs sequester *CELF1*. Later studies indicated that another splicing factor, muscleblind-like 1 (*MBNL1*), is sequestered.^{95, 96} *CELF1*, however, does not interact with long CUG repeats or co-localize with mutant RNAs in cells.^{97, 98} Rather, *CELF1* is up-regulated in DM heart and skeletal muscle.^{99, 100} PKC-mediated phosphorylation of *CELF1* stabilizes the protein without a change in *CELF1* transcript levels.^{38, 101} Increased *CELF1* activity disrupts alternative splicing and translation events that are directly linked to disease symptoms.^{75, 99, 102} DM

pathogenesis is now believed to result from a combination of effects including CELF1 over-expression and MBNL1 sequestration.

Transgenic mouse models that over-express CELF1 in striated muscle tissues support its contribution to DM pathogenesis. CELF1 is constitutively over-expressed in skeletal muscle in two models.^{103, 104} Both exhibited defects in muscle histology and physiology that recapitulate features of DM. Timchenko et al. showed that CELF1 over-expression enhanced translation of p21 and MEF2A¹⁰⁴, while Ho et al. demonstrated changes in alternative splicing of muscle transcripts.¹⁰³ Because embryonic muscle development is impaired in these mice, the effects of CELF1 over-expression in adult muscle are difficult to assess. Bitransgenic mice that inducibly over-express CELF1 demonstrated that elevated CELF1 in adult skeletal muscle is sufficient to induce impaired muscle performance, muscle wasting, and alternative splicing changes seen in DM.¹⁰⁵ Inducible over-expression of CELF1 in heart likewise exhibits echocardiographic, electrocardiographic, and histological abnormalities seen in DM patients.¹⁰⁶

While the dysregulation of CELF1 in DM is thought to be a primary pathogenic event, a recent study found that changes in alternative splicing and increases in CELF1 and CELF2 are also seen in other types of muscular dystrophy and muscle injury.¹⁰⁷ Elevated levels of CELF2 have also been reported in Duchenne and Becker muscular dystrophies.^{108, 109} These changes may reflect the reiteration of early developmental programs during muscle regeneration.

CELF proteins in nervous system disorders

Although DM is primarily considered a striated muscle disease, patients also have symptoms affecting the nervous system, including cognitive impairment.⁹⁴ Several exons of *tau*, which is involved in forming protein aggregates in Alzheimer's disease and other dementias, are regulated by CELF proteins and are dysregulated in DM.¹¹⁰⁻¹¹⁴ Interestingly, skipping of *tau* exon 10, which encodes a microtubule binding domain, is induced by over-expression of CELF2, but not over-expression of CELF1 or MBNL1 knockdown.¹¹⁵ CELF1 and CELF2 are both up-regulated in DM brains, and increased exon 10 skipping correlates with elevated CELF protein levels.¹¹⁵ Polymorphisms in *CELF2* were also identified in a genome-wide association study of late-onset Alzheimer's disease that are significantly associated with high risk alleles of *APOE*.¹¹⁶

Expression of an expanded CUG repeat-containing RNA in the neurodegenerative disorder spinocerebellar ataxia type 8 (SCA8) may follow a similar mechanism to DM, as MBNL1 is sequestered and CELF/MBNL alternative splicing is disrupted in neurons.⁵⁰ Fragile X syndrome, a form of hereditary mental retardation, is caused by a CGG repeat expansion in the 5' UTR of the *FMR1* gene that leads to silencing and loss of the FMRP protein.¹¹⁷ In a transgenic fly that expresses 90 CGG repeats over-expression of CELF1 suppresses the neurodegenerative phenotype through interaction with the CGG-binding protein hnRNP A2/B1.¹¹⁸

The mechanistic similarities between CELF-mediated pathogenesis in the nervous system and striated muscle suggest that although CELF family members are differentially expressed throughout the body, they fulfill similar functional niches in different cell types. As additional CELF targets are identified, it will be interesting to determine whether common pathways are involved in pathogenesis in different cell types.

Conclusion

CELF proteins have been shown to regulate RNA processing at several important steps in the nucleus and cytoplasm, but little is known about whether or how these disparate

activities are coordinated. Dysregulation of CELF1-mediated alternative splicing and translation have been shown to contribute to pathogenesis in DM.^{103, 104} It is easy to imagine that shuttling of CELF1 between subcellular compartments serves to coordinate these processes in healthy tissues.

Many tissues express multiple CELF proteins, but the extent of functional redundancy and individual contributions of specific family members have not been determined in most cases. Many studies have focused on only a single CELF protein, and CELF1 and CELF2 have received greater attention than CELF3-6. The distinct phenotypes of CELF knockout mice despite overlapping expression patterns suggest that different family members do indeed play different roles *in vivo*. The elucidation and comparison of targets regulated by each family member will help to delineate specific CELF regulons.

Acknowledgments

We apologize to anyone whose work was not cited due to lack of space. Research in the Ladd laboratory is supported by the National Institutes of Health (1R01HL089376).

References

1. Timchenko L, Miller J, Timchenko N, Devore D, Datar K, Lin L, Roberts R, Caskey C, Swanson M. Identification of a (CUG)_n triplet repeat RNA-binding protein and its expression in myotonic dystrophy. *Nuc Acids Res.* 1996; 24:4407–4414.
2. Hwang D, Hwang W, Liew C. Single pass sequencing of a unidirectional human fetal heart cDNA library to discover novel genes of the cardiovascular system. *J Molec Cell Cardiol.* 1994; 26:1329–1333. [PubMed: 7869393]
3. Choi D, Ito T, Mitsui Y, Sakaki Y. Fluorescent differential display analysis of gene expression in apoptotic neuroblastoma cells. *Gene.* 1998; 223:21–31. [PubMed: 9858671]
4. Lu X, Timchenko N, Timchenko L. Cardiac elav-type RNA-binding protein (ETR-3) binds to RNA CUG repeats expanded in myotonic dystrophy. *Hum Mol Gen.* 1999; 8:53–60. [PubMed: 9887331]
5. Knecht A, Good P, Dawid I, Harland R. Dorsal-ventral patterning and differentiation of noggin-induced neural tissue in the absence of mesoderm. *Development.* 1995; 121:1927–1936. [PubMed: 7601005]
6. Good P, Chen Q, Warner S, Herring D. A family of human RNA-binding proteins related to the *Drosophila* Bruno translational regulator. *J Biol Chem.* 2000; 275:28583–28592. [PubMed: 10893231]
7. Ladd A, Charlet-B N, Cooper T. The CELF family of RNA binding proteins is implicated in cell-specific and developmentally regulated alternative splicing. *Mol Cell Biol.* 2001; 21:1285–1296. [PubMed: 11158314]
8. Ladd A, Nguyen N, Malhotra K, Cooper T. CELF6, a member of the CELF family of RNA binding proteins, regulates muscle-specific splicing enhancer-dependent alternative splicing. *J Biol Chem.* 2004; 279:17756–17764. [PubMed: 14761971]
9. Roberts R, Timchenko N, Miller J, Reddy S, Caskey C, Swanson M, Timchenko L. Altered phosphorylation and intracellular distribution of a (CUG)_n triplet repeat RNA-binding protein in patients with myotonic dystrophy and in myotonin protein kinase knockout mice. *Proc Natl Acad Sci.* 1997; 94:13221–13226. [PubMed: 9371827]
10. Brimacombe KR, Ladd AN. Cloning and embryonic expression patterns of the chicken CELF family. *Dev Dyn.* 2007; 236:2216–2224. [PubMed: 17584860]
11. Delaunay J, Le Mee G, Ezzeddine N, Labesse G, Terzian C, Capri M, Ait-Ahmed O. The *Drosophila* Bruno paralogue Bru-3 specifically binds the EDEN translational repression element. *Nucleic Acids Res.* 2004; 32:3070–3082. [PubMed: 15181172]
12. Wu J, Li C, Zhao S, Mao B. Differential expression of the Brunol/CELF family genes during *Xenopus laevis* early development. *Int J Dev Biol.* 2010; 54:209–214. [PubMed: 19757395]

13. Loria P, Duke A, Rand J, Hobert O. Two neuronal, nuclear-localized RNA binding proteins involved in synaptic transmission. *Curr Biol*. 2003; 13:1317–1323. [PubMed: 12906792]
14. Meins M, Schlickum S, Wilhelm C, Missbach J, Yadav S, Glaser B, Grzmil M, Burfeind P, Laccone F. Identification and characterization of murine Brunol4, a new member of the elav/bruno family. *Cytogenet Genome Res*. 2002; 97:254–260. [PubMed: 12438720]
15. Yang Y, Mahaffey CL, Berube N, Maddatu TP, Cox GA, Frankel WN. Complex seizure disorder caused by Brunol4 deficiency in mice. *PLoS Genet*. 2007; 3:e124. [PubMed: 17677002]
16. Choi D, Ito T, Tsukahara F, Hirai M, Sakaki Y. Developmentally regulated expression of mNapor encoding an apoptosis-induced ELAV-type RNA binding protein. *Gene*. 1999; 237:135–142. [PubMed: 10524244]
17. Choi DK, Yoo KW, Hong SK, Rhee M, Sakaki Y, Kim CH. Isolation and expression of Napor/CUG-BP2 in embryo development. *Biochem Biophys Res Commun*. 2003; 305:448–454. [PubMed: 12763013]
18. Kress C, Gautier-Courteille C, Osborne HB, Babinet C, Paillard L. Inactivation of CUG-BP1/CELF1 causes growth, viability, and spermatogenesis defects in mice. *Mol Cell Biol*. 2007; 27:1146–1157. [PubMed: 17130239]
19. Milne C, Hodgkin J. ETR-1, a homologue of a protein linked to myotonic dystrophy, is essential for muscle development in *Caenorhabditis elegans*. *Curr Biol*. 1999; 9:1243–1246. [PubMed: 10556089]
20. Takahashi N, Sasagawa N, Suzuki K, Ishiura S. The CUG-binding protein binds specifically to UG dinucleotide repeats in a yeast three-hybrid system. *Biochem Biophys Res Commun*. 2000; 277:518–523. [PubMed: 11032753]
21. Marquis J, Paillard L, Audic Y, Cosson B, Danos O, Le Bec C, Osborne HB. CUG-BP1/CELF1 requires UGU-rich sequences for high-affinity binding. *Biochem J*. 2006; 400:291–301. [PubMed: 16938098]
22. Faustino N, Cooper T. Identification of putative new splicing targets for ETR-3 using its SELEX sequences. *Mol Cell Biol*. 2005; 25:879–887. [PubMed: 15657417]
23. Timchenko NA, Welm AL, Lu X, Timchenko LT. CUG repeat binding protein (CUGBP1) interacts with the 5' region of C/EBPbeta mRNA and regulates translation of C/EBPbeta isoforms. *Nuc Acids Res*. 1999; 27:4517–4525.
24. Salisbury E, Sakai K, Schoser B, Huichalaf C, Schneider-Gold C, Nguyen H, Wang GL, Albrecht JH, Timchenko LT. Ectopic expression of cyclin D3 corrects differentiation of DM1 myoblasts through activation of RNA CUG-binding protein, CUGBP1. *Exp Cell Res*. 2008; 314:2266–2278. [PubMed: 18570922]
25. Paillard L, Omilli F, Legagneux V, Bassez T, Maniey D, Osborne H. EDEN and EDEN-BP, a *cis* element and an associated factor that mediate sequence-specific mRNA deadenylation in *Xenopus* embryos. *EMBO J*. 1998; 17:278–287. [PubMed: 9427761]
26. Mori D, Sasagawa N, Kino Y, Ishiura S. Quantitative analysis of CUG-BP1 binding to RNA repeats. *J Biochem*. 2008; 143:377–383. [PubMed: 18039683]
27. Tsuda K, Kuwasako K, Takahashi M, Someya T, Inoue M, Terada T, Kobayashi N, Shirouzu M, Kigawa T, Tanaka A, et al. Structural basis for the sequence-specific RNA-recognition mechanism of human CUG-BP1 RRM3. *Nuc Acids Res*. 2009; 37:5151–5166.
28. Bonnet-Corven S, Audic Y, Omilli F, Osborne HB. An analysis of the sequence requirements of EDEN-BP for specific RNA binding. *Nuc Acids Res*. 2002; 30:4667–4674.
29. Singh G, Charlet BN, Han J, Cooper TA. ETR-3 and CELF4 protein domains required for RNA binding and splicing activity in vivo. *Nuc Acids Res*. 2004; 32:1232–1241.
30. Teplova M, Song J, Gaw HY, Teplov A, Patel DJ. Structural insights into RNA recognition by the alternate-splicing regulator CUG-binding protein 1. *Structure*. 2010; 18:1364–1377. [PubMed: 20947024]
31. Han J, Cooper TA. Identification of CELF splicing activation and repression domains in vivo. *Nuc Acids Res*. 2005; 33:2769–2780.
32. Charlet-B N, Logan P, Singh G, Cooper T. Dynamic antagonism between ETR-3 and PTB regulates cell type-specific alternative splicing. *Mol Cell*. 2002; 9:649–658. [PubMed: 11931771]

33. Dujardin G, Buratti E, Charlet-Berguerand N, Martins de Araujo M, Mbopda A, Le Jossic-Corcos C, Pagani F, Ferec C, Corcos L. CELF proteins regulate CFTR pre-mRNA splicing: essential role of the divergent domain of ETR-3. *Nuc Acids Res.* 2010; 38:7273–7285.
34. Horb LD, Horb ME. BrunoL1 regulates endoderm proliferation through translational enhancement of cyclin A2 mRNA. *Dev Biol.* 2010; 345:156–169. [PubMed: 20633547]
35. Ladd A, Cooper T. Multiple domains control the subcellular localization and activity of ETR-3, a regulator of nuclear and cytoplasmic RNA processing events. *J Cell Sci.* 2004; 117:3519–3529. [PubMed: 15226369]
36. Fujimura K, Kano F, Murata M. Dual localization of the RNA binding protein CUGBP-1 to stress granule and perinucleolar compartment. *Exp Cell Res.* 2008; 314:543–553. [PubMed: 18164289]
37. Cosson B, Gautier-Courteille C, Maniey D, Ait-Ahmed O, Lesimple M, Osborne HB, Paillard L. Oligomerization of EDEN-BP is required for specific mRNA deadenylation and binding. *Biol Cell.* 2006; 98:653–665. [PubMed: 16836486]
38. Kuyumcu-Martinez NM, Wang GS, Cooper TA. Increased steady-state levels of CUGBP1 in myotonic dystrophy 1 are due to PKC-mediated hyperphosphorylation. *Mol Cell.* 2007; 28:68–78. [PubMed: 17936705]
39. Timchenko LT, Salisbury E, Wang GL, Nguyen H, Albrecht JH, Hershey JW, Timchenko NA. Age-specific CUGBP1-eIF2 complex increases translation of CCAAT/enhancer-binding protein beta in old liver. *J Biol Chem.* 2006; 281:32806–32819. [PubMed: 16931514]
40. Morgan GT. Localized co-transcriptional recruitment of the multifunctional RNA-binding protein CELF1 by lampbrush chromosome transcription units. *Chromosome Res.* 2007; 15:985–1000. [PubMed: 18095176]
41. Philips A, Timchenko L, Cooper T. Disruption of splicing regulated by a CUG-binding protein in myotonic dystrophy. *Science.* 1998; 280:737–741. [PubMed: 9563950]
42. Anant S, Henderson J, Mukhopadhyay D, Navaratnam N, Kennedy S, Min J, Davidson N. Novel role for RNA-binding protein CUGBP2 in mammalian RNA editing. *J Biol Chem.* 2001; 276:47338–47351. [PubMed: 11577082]
43. Chen Z, Eggerman TL, Patterson AP. ApoB mRNA editing is mediated by a coordinated modulation of multiple apoB mRNA editing enzyme components. *Am J Physiol Gastrointest Liver Physiol.* 2007; 292:G53–65. [PubMed: 16920700]
44. Cooper TA. Use of minigene systems to dissect alternative splicing elements. *Methods.* 2005; 37:331–340. [PubMed: 16314262]
45. Ladd A, Stenberg M, Swanson M, Cooper T. A dynamic balance between activation and repression regulates pre-mRNA alternative splicing during heart development. *Dev. Dyn.* 2005; 233:783–793. [PubMed: 15830352]
46. Kalsotra A, Xiao X, Ward AJ, Castle JC, Johnson JM, Burge CB, Cooper TA. A postnatal switch of CELF and MBNL proteins reprograms alternative splicing in the developing heart. *Proc Natl Acad Sci U S A.* 2008; 105:20333–20338. [PubMed: 19075228]
47. Dembowski JA, Grabowski PJ. The CUGBP2 splicing factor regulates an ensemble of branchpoints from perimeter binding sites with implications for autoregulation. *PLoS Genet.* 2009; 5:e1000595. [PubMed: 19680430]
48. Ladd A, Taffet G, Hartley C, Kearney D, Cooper T. Cardiac-specific repression of CELF activity disrupts alternative splicing and causes cardiomyopathy. *Mol Cell Biol.* 2005; 25:6267–6278. [PubMed: 15988035]
49. Ule J, Jensen K, Mele A, Darnell RB. CLIP: a method for identifying protein-RNA interaction sites in living cells. *Methods.* 2005; 37:376–386. [PubMed: 16314267]
50. Daughters RS, Tuttle DL, Gao W, Ikeda Y, Moseley ML, Ebner TJ, Swanson MS, Ranum LP. RNA gain-of-function in spinocerebellar ataxia type 8. *PLoS Genet.* 2009; 5:e1000600. [PubMed: 19680539]
51. Goo YH, Cooper TA. CUGBP2 directly interacts with U2 17S snRNP components and promotes U2 snRNA binding to cardiac troponin T pre-mRNA. *Nuc Acids Res.* 2009; 37:4275–4286.
52. Barron VA, Zhu H, Hinman MN, Ladd AN, Lou H. The neurofibromatosis type I pre-mRNA is a novel target of CELF protein-mediated splicing regulation. *Nuc Acids Res.* 2010; 38:253–264.

53. Darnell RB. HITS-CLIP: panoramic views of protein-RNA regulation in living cells. *WIREs RNA*. 2010; 1:266–286. [PubMed: 21935890]
54. Blanc V, Davidson NO. APOBEC-1-mediated RNA editing. *Wiley Interdiscip Rev Syst Biol Med*. 2010; 2:594–602. [PubMed: 20836050]
55. Blanc V, Davidson NO. C-to-U RNA editing: mechanisms leading to genetic diversity. *J Biol Chem*. 2003; 278:1395–1398. [PubMed: 12446660]
56. Graindorge A, Le Tonqueze O, Thuret R, Pollet N, Osborne HB, Audic Y. Identification of CUG-BP1/EDEN-BP target mRNAs in *Xenopus tropicalis*. *Nuc Acids Res*. 2008; 36:1861–1870.
57. Paillard L, Legagneux V, Maniey D, Osborne H. c-Jun ARE targets mRNA deadenylation by an EDEN-BP (embryo deadenylation element-binding protein)-dependent pathway. *J Biol Chem*. 2002; 277:3232–3235. [PubMed: 11707455]
58. Paillard L, Legagneux V, Osborne H. A functional deadenylation assay identifies human CUG-BP as a deadenylation factor. *Biol of the Cell*. 2003; 95:107–113.
59. Moraes KC, Wilusz CJ, Wilusz J. CUG-BP binds to RNA substrates and recruits PARN deadenylase. *RNA*. 2006; 12:1084–1091. [PubMed: 16601207]
60. Kim JH, Richter JD. Opposing polymerase-deadenylase activities regulate cytoplasmic polyadenylation. *Mol Cell*. 2006; 24:173–183. [PubMed: 17052452]
61. Vlasova IA, Bohjanen PR. Posttranscriptional regulation of gene networks by GU-rich elements and CELF proteins. *RNA Biol*. 2008; 5:201–207. [PubMed: 18971639]
62. Lee JE, Lee JY, Wilusz J, Tian B, Wilusz CJ. Systematic analysis of cis-elements in unstable mRNAs demonstrates that CUGBP1 is a key regulator of mRNA decay in muscle cells. *PLoS One*. 2010; 5:e11201. [PubMed: 20574513]
63. Rattenbacher B, Beisang D, Wiesner DL, Jeschke JC, von Hohenberg M, St Louis-Vlasova IA, Bohjanen PR. Analysis of CUGBP1 targets identifies GU-repeat sequences that mediate rapid mRNA decay. *Mol Cell Biol*. 2010; 30:3970–3980. [PubMed: 20547756]
64. Zhang L, Lee JE, Wilusz J, Wilusz CJ. The RNA-binding protein CUGBP1 regulates stability of tumor necrosis factor mRNA in muscle cells: implications for myotonic dystrophy. *J Biol Chem*. 2008; 283:22457–22463. [PubMed: 18559347]
65. Mukhopadhyay D, Houchen C, Kennedy S, Dieckgraefe B, Anant S. Coupled mRNA stabilization and translational silencing of cyclooxygenase-2 by a novel RNA binding protein, CUGBP2. *Mol Cell*. 2003; 11:113–126. [PubMed: 12535526]
66. Mukhopadhyay D, Jung J, Murmu N, Houchen CW, Dieckgraefe BK, Anant S. CUGBP2 plays a critical role in apoptosis of breast cancer cells in response to genotoxic injury. *Ann N Y Acad Sci*. 2003; 1010:504–509. [PubMed: 15033780]
67. Murmu N, Jung J, Mukhopadhyay D, Houchen CW, Riehl TE, Stenson WF, Morrison AR, Arumugam T, Dieckgraefe BK, Anant S. Dynamic antagonism between RNA-binding protein CUGBP2 and cyclooxygenase-2-mediated prostaglandin E2 in radiation damage. *Proc Natl Acad Sci U S A*. 2004; 101:13873–13878. [PubMed: 15358864]
68. Subramaniam D, Natarajan G, Ramalingam S, Ramachandran I, May R, Queimado L, Houchen CW, Anant S. Translation inhibition during cell cycle arrest and apoptosis: Mcl-1 is a novel target for RNA binding protein CUGBP2. *Am J Physiol Gastrointest Liver Physiol*. 2008; 294:G1025–1032. [PubMed: 18292181]
69. Kim-Ha J, Kerr K, Macdonald P. Translational regulation of *oskar* mRNA by bruno, an ovarian RNA-binding protein, is essential. *Cell*. 1995; 81:403–412. [PubMed: 7736592]
70. Nakamura A, Sato K, Hanyu-Nakamura K. Drosophila cup is an eIF4E binding protein that associates with Bruno and regulates oskar mRNA translation in oogenesis. *Dev Cell*. 2004; 6:69–78. [PubMed: 14723848]
71. Filardo P, Ephrussi A. Bruno regulates gurken during Drosophila oogenesis. *Mech Dev*. 2003; 120:289–297. [PubMed: 12591598]
72. Sugimura I, Lilly MA. Bruno inhibits the expression of mitotic cyclins during the prophase I meiotic arrest of Drosophila oocytes. *Dev Cell*. 2006; 10:127–135. [PubMed: 16399084]
73. Sureban SM, Murmu N, Rodriguez P, May R, Maheshwari R, Dieckgraefe BK, Houchen CW, Anant S. Functional antagonism between RNA binding proteins HuR and CUGBP2 determines the fate of COX-2 mRNA translation. *Gastroenterology*. 2007; 132:1055–1065. [PubMed: 17383427]

74. Iakova P, Wang GL, Timchenko L, Michalak M, Pereira-Smith OM, Smith JR, Timchenko NA. Competition of CUGBP1 and calreticulin for the regulation of p21 translation determines cell fate. *Embo J*. 2004; 23:406–417. [PubMed: 14726956]
75. Timchenko N, Iakova P, Cai Z-J, Smith J, Timchenko L. Molecular basis for impaired muscle differentiation in myotonic dystrophy. *Mol Cell Biol*. 2001; 21:6927–6938. [PubMed: 11564876]
76. Baldwin BR, Timchenko NA, Zahnow CA. Epidermal growth factor receptor stimulation activates the RNA binding protein CUG-BP1 and increases expression of C/EBPbeta-LIP in mammary epithelial cells. *Mol Cell Biol*. 2004; 24:3682–3691. [PubMed: 15082764]
77. Wang GL, Salisbury E, Shi X, Timchenko L, Medrano EE, Timchenko NA. HDAC1 promotes liver proliferation in young mice via interactions with C/EBPbeta. *J Biol Chem*. 2008; 283:26179–26187. [PubMed: 18622014]
78. Welm AL, Mackey SL, Timchenko LT, Darlington GJ, Timchenko NA. Translational induction of liver-enriched transcriptional inhibitory protein during acute phase response leads to repression of CCAAT/enhancer binding protein alpha mRNA. *J Biol Chem*. 2000; 275:27406–27413. [PubMed: 10854425]
79. Timchenko NA, Wang GL, Timchenko LT. RNA CUG-binding protein 1 increases translation of 20-kDa isoform of CCAAT/enhancer-binding protein beta by interacting with the alpha and beta subunits of eukaryotic initiation translation factor 2. *J Biol Chem*. 2005; 280:20549–20557. [PubMed: 15788409]
80. Wang GL, Salisbury E, Shi X, Timchenko L, Medrano EE, Timchenko NA. HDAC1 cooperates with C/EBPalpha in the inhibition of liver proliferation in old mice. *J Biol Chem*. 2008; 283:26169–26178. [PubMed: 18622015]
81. Fox JT, Stover PJ. Mechanism of the internal ribosome entry site-mediated translation of serine hydroxymethyltransferase 1. *J Biol Chem*. 2009; 284:31085–31096. [PubMed: 19734143]
82. Woeller CF, Fox JT, Perry C, Stover PJ. A ferritin-responsive internal ribosome entry site regulates folate metabolism. *J Biol Chem*. 2007; 282:29927–29935. [PubMed: 17702748]
83. Barreau C, Watrin T, Beverley Osborne H, Paillard L. Protein expression is increased by a class III AU-rich element and tethered CUG-BP1. *Biochem Biophys Res Commun*. 2006; 347:723–730.
84. Webster PJ, Liang L, Berg CA, Lasko P, Macdonald PM. Translational repressor bruno plays multiple roles in development and is widely conserved. *Genes Dev*. 1997; 11:2510–2521. [PubMed: 9334316]
85. Castrillon DH, Gonczy P, Alexander S, Rawson R, Eberhart CG, Viswanathan S, DiNardo S, Wasserman SA. Toward a molecular genetic analysis of spermatogenesis in *Drosophila melanogaster*: characterization of male-sterile mutants generated by single P element mutagenesis. *Genetics*. 1993; 135:489–505. [PubMed: 8244010]
86. Schupbach T, Wieschaus E. Female sterile mutations on the second chromosome of *Drosophila melanogaster*. II. Mutations blocking oogenesis or altering egg morphology. *Genetics*. 1991; 129:1119–1136. [PubMed: 1783295]
87. Suzuki H, Maegawa S, Nishibu T, Sugiyama T, Yasuda K, Inoue K. Vegetal localization of the maternal mRNA encoding an EDEN-BP/Bruno-like protein in zebrafish. *Mech Dev*. 2000; 93:205–209. [PubMed: 10781958]
88. Gautier-Courteille C, Le Clainche C, Barreau C, Audic Y, Graindorge A, Maniey D, Osborne HB, Paillard L. EDEN-BP-dependent post-transcriptional regulation of gene expression in *Xenopus* somitic segmentation. *Development*. 2004; 131:6107–6117. [PubMed: 15548579]
89. Cibois M, Gautier-Courteille C, Vallee A, Paillard L. A strategy to analyze the phenotypic consequences of inhibiting the association of an RNA-binding protein with a specific RNA. *RNA*. 2010; 16:10–15. [PubMed: 19933768]
90. Dev A, Nayernia K, Meins M, Adham I, Lacone F, Engel W. Mice deficient for RNA-binding protein *brunol1* show reduction of spermatogenesis but are fertile. *Mol Reprod Dev*. 2007
91. Berger DS, Moyer M, Kliment GM, van Lunteren E, Ladd AN. Expression of a dominant negative CELF protein in vivo leads to altered muscle organization, fiber size, and subtype. *PLoS One*. 2011; 6(4):e19274. [PubMed: 21541285]

92. Li D, Bachinski L, Roberts R. Genomic organization and isoform-specific tissue expression of human NAPOR (CUGBP2) as a candidate gene for familial arrhythmogenic right ventricular dysplasia. *Genomics*. 2001; 74:396–401. [PubMed: 11414768]
93. Lichtner P, Attié-Bitach T, Schuffenhauer S, Henwood J, Bouvagnet P, Scambler P, Meitinger T, Vekemans M. Expression and mutation analysis of *Brunol3*, a candidate gene for heart and thymus developmental defects associated with partial monosomy 10p. *J Mol Med*. 2002; 80:431–442. [PubMed: 12110949]
94. Schoser B, Timchenko L. Myotonic dystrophies 1 and 2: complex diseases with complex mechanisms. *Curr Genomics*. 2010; 11:77–90. [PubMed: 20885816]
95. Fardaei M, Rogers M, Thorpe H, Larkin K, Hamshire M, Harper P, Brook J. Three proteins, MBNL, MBLL and MBXL, co-localize *in vivo* with nuclear foci of expanded-repeat transcripts in DM1 and DM2 cells. *Hum Mol Gen*. 2002; 11:805–814. [PubMed: 11929853]
96. Mankodi A, Urbinati C, Yuan Q-P, Moxley R, Sansone V, Krym M, Henderson D, Schalling M, Swanson M, Thornton C. Muscleblind localizes to nuclear foci of aberrant RNA in myotonic dystrophy types 1 and 2. *Hum Mol Gen*. 2001; 10:2165–2170. [PubMed: 11590133]
97. Fardaei M, Larkin K, Brook J, Hamshire M. *In vivo* co-localization of MBNL protein with *DMPK* expanded-repeat transcripts. *Nuc Acids Res*. 2001; 29:2766–2771.
98. Michalowski S, Miller J, Urbinati C, Paliouras M, Swanson M, Griffith J. Visualization of double-stranded RNAs from the myotonic dystrophy protein kinase gene and interactions with CUG-binding protein. *Nuc Acids Res*. 1999; 27:3534–3542.
99. Savkur R, Phillips A, Cooper T. Aberrant regulation of insulin receptor alternative splicing is associated with insulin resistance in myotonic dystrophy. *Nat Genet*. 2001; 29:40–47. [PubMed: 11528389]
100. Timchenko N, Cai Z-J, Welm A, Reddy S, Ashizawa T, Timchenko L. RNA CUG repeats sequester CUGBP1 and alter protein levels and activity of CUGBP1. *J Biol Chem*. 2001; 276:7820–7826. [PubMed: 11124939]
101. Nezu Y, Kino Y, Sasagawa N, Nishino I, Ishiura S. Expression of MBNL and CELF mRNA transcripts in muscles with myotonic dystrophy. *Neuromuscul Disord*. 2007; 17:306–312. [PubMed: 17331722]
102. Charlet-B N, Savkur R, Singh G, Philips A, Grice E, Cooper T. Loss of the muscle-specific chloride channel in type I myotonic dystrophy lead to misregulated alternative splicing. *Mol Cell*. 2002; 10:45–53. [PubMed: 12150906]
103. Ho TH, Bundman D, Armstrong DL, Cooper TA. Transgenic mice expressing CUG-BP1 reproduce splicing mis-regulation observed in myotonic dystrophy. *Hum Mol Genet*. 2005; 14:1539–1547. [PubMed: 15843400]
104. Timchenko NA, Patel R, Iakova P, Cai ZJ, Quan L, Timchenko LT. Overexpression of CUG triplet repeat-binding protein, CUGBP1, in mice inhibits myogenesis. *J Biol Chem*. 2004; 279:13129–13139. [PubMed: 14722059]
105. Ward AJ, Rimer M, Killian JM, Dowling JJ, Cooper TA. CUGBP1 overexpression in mouse skeletal muscle reproduces features of myotonic dystrophy type 1. *Hum Mol Genet*. 2010; 19:3614–3622. [PubMed: 20603324]
106. Koshelev M, Sarma S, Price RE, Wehrens XH, Cooper TA. Heart-specific overexpression of CUGBP1 reproduces functional and molecular abnormalities of myotonic dystrophy type 1. *Hum Mol Genet*. 2010; 19:1066–1075. [PubMed: 20051426]
107. Orengo JP, Ward AJ, Cooper TA. Alternative splicing dysregulation secondary to skeletal muscle regeneration. *Ann Neurol*. 2010
108. Bakay M, Zhao P, Chen J, Hoffman E. A web-accessible complete transcriptome of normal human and DMD muscle. *Neuromusc Disorders*. 2002; 12:S125–S141. [PubMed: 12206807]
109. Sironi M, Cagliani R, Pozzoli U, Bardoni A, Comi G, Giorda R, Bresolin N. The dystrophin gene is alternatively spliced throughout its coding sequence. *FEBS Lett*. 2002; 517:163–166. [PubMed: 12062429]
110. Leroy O, Dhaenens CM, Schraen-Maschke S, Belarbi K, Delacourte A, Andreadis A, Sablonniere B, Buee L, Sergeant N, Caillet-Boudin ML. ETR-3 represses Tau exons 2/3 inclusion, a splicing

- event abnormally enhanced in myotonic dystrophy type I. *J Neurosci Res.* 2006; 84:852–859. [PubMed: 16862542]
111. Leroy O, Wang J, Maurage CA, Parent M, Cooper T, Buee L, Sergeant N, Andreadis A, Caillet-Boudin ML. Brain-specific change in alternative splicing of Tau exon 6 in myotonic dystrophy type 1. *Biochim Biophys Acta.* 2006; 1762:460–467. [PubMed: 16487687]
 112. Sergeant N, Sablonniere B, Schraen-Maschke S, Ghestem A, Maurage CA, Wattez A, Vermersch P, Delacourte A. Dysregulation of human brain microtubule-associated tau mRNA maturation in myotonic dystrophy type 1. *Hum Mol Genet.* 2001; 10:2143–2155. [PubMed: 11590131]
 113. Vermesch P, Sergeant N, Ruchoux M, Hofmann-Radvanyi H, Wattez A, Petit H, Dwailly P, Delacourte A. Specific tau variants in the brains of patients with myotonic dystrophy. *Neurology.* 1996; 47:711–717. [PubMed: 8797469]
 114. Wang J, Gao QS, Wang Y, Lafyatis R, Stamm S, Andreadis A. Tau exon 10, whose missplicing causes frontotemporal dementia, is regulated by an intricate interplay of cis elements and trans factors. *J Neurochem.* 2004; 88:1078–1090. [PubMed: 15009664]
 115. Dhaenens CM, Tran H, Frandemich ML, Carpentier C, Schraen-Maschke S, Sistiaga A, Goicoechea M, Eddarkaoui S, Van Brussels E, Obriot H, et al. Mis-splicing of Tau exon 10 in myotonic dystrophy type 1 is reproduced by overexpression of CELF2 but not by MBNL1 silencing. *Biochim Biophys Acta.* 2011; 1812:732–742. [PubMed: 21439371]
 116. Wijsman EM, Pankratz ND, Choi Y, Rothstein JH, Faber KM, Cheng R, Lee JH, Bird TD, Bennett DA, Diaz-Arrastia R, et al. Genome-wide association of familial late-onset Alzheimer's disease replicates BIN1 and CLU and nominates CUGBP2 in interaction with APOE. *PLoS Genet.* 2011; 7:e1001308. [PubMed: 21379329]
 117. Ranum LP, Cooper TA. RNA-mediated neuromuscular disorders. *Annu Rev Neurosci.* 2006; 29:259–277. [PubMed: 16776586]
 118. Sofola OA, Jin P, Qin Y, Duan R, Liu H, de Haro M, Nelson DL, Botas J. RNA-binding proteins hnRNP A2/B1 and CUGBP1 suppress fragile X CCG premutation repeat-induced neurodegeneration in a *Drosophila* model of FXTAS. *Neuron.* 2007; 55:565–571. [PubMed: 17698010]
 119. Wollerton MC, Gooding C, Wagner EJ, Garcia-Blanco MA, Smith CW. Autoregulation of polypyrimidine tract binding protein by alternative splicing leading to nonsense-mediated decay. *Mol Cell.* 2004; 13:91–100. [PubMed: 14731397]
 120. Philips A, Cooper T. RNA processing and human disease. *Cell Molec Life Sci.* 1999 In press.
 121. Poleev A, Hartmann A, Stamm S. A trans-acting factor, isolated by the three-hybrid system, that influences alternative splicing of the amyloid precursor protein minigene. *Eur J Biochem.* 2000; 267:4002–4010. [PubMed: 10866799]
 122. Jiang H, Mankodi A, Swanson MS, Moxley RT, Thornton CA. Myotonic dystrophy type 1 is associated with nuclear foci of mutant RNA, sequestration of muscleblind proteins and deregulated alternative splicing in neurons. *Hum Mol Genet.* 2004; 13:3079–3088. [PubMed: 15496431]
 123. Zhang L, Liu W, Grabowski P. Coordinate repression of a trio of neuron-specific splicing events by the splicing regulator PTB. *RNA.* 1999; 5:117–130. [PubMed: 9917071]
 124. Zhang W, Haiying L, Kyounga H, Grabowski P. Region-specific alternative splicing in the nervous system: Implications for regulation by the RNA-binding protein NAPOR. *RNA.* 2002; 8:671–685. [PubMed: 12022233]
 125. Gromak N, Matlin A, Cooper T, Smith C. Antagonistic regulation of a-actinin alternative splicing by CELF proteins and polypyrimidine tract binding protein. *RNA.* 2003; 9:443–456. [PubMed: 12649496]
 126. Suzuki H, Jin Y, Otani H, Yasuda K, Inoue K. Regulation of alternative splicing of a-actinin transcript by Bruno-like proteins. *Genes to Cells.* 2002; 7:133–141. [PubMed: 11895477]
 127. Sureau A, Sauliere J, Expert-Bezancon A, Marie J. CELF and PTB proteins modulate the inclusion of the beta-tropomyosin exon 6B during myogenic differentiation. *Exp Cell Res.* 2011; 317:94–106. [PubMed: 20875406]

128. Chapple JP, Anthony K, Martin TR, Dev A, Cooper TA, Gallo JM. Expression, localization and tau exon 10 splicing activity of the brain RNA-binding protein TNRC4. *Hum Mol Genet.* 2007; 16:2760–2769. [PubMed: 17725984]
129. Li K, Arikan MC, Andreadis A. Modulation of the membrane-binding domain of tau protein: splicing regulation of exon 2. *Brain Res Mol Brain Res.* 2003; 116:94–105. [PubMed: 12941465]
130. Terenzi F, Brimacombe KR, Penn MS, Ladd AN. CELF-mediated alternative splicing is required for cardiac function during early, but not later, postnatal life. *J Mol Cell Cardiol.* 2009; 46:395–404. [PubMed: 19073192]

FIGURE 1.

All six human CELF proteins share a common domain structure, with three RRM RNA binding domains and a divergent domain between the second and third RRM.⁸ Each family member exists in multiple isoforms due to alternative splicing and/or the generation of alternative 5' ends by differential promoter usage (not shown).

FIGURE 2.

CELF proteins regulate translation through multiple mechanisms. **A.** The *Drosophila* CELF protein, Bru, forms a complex with Cup and the 5' cap binding initiation factor, eIF4E, leading to circularization and translational repression of the mRNA. **B.** CELF2 inhibits translation by binding to A/U-rich elements in the 3' UTR and competing with HuR, an activator of translation. **C.** Binding of CELF1 to sequences in the short open reading frame (sORF) in the 5' region of *C/EPBβ* promotes usage of a downstream start codon, giving rise to the truncated LIP protein. Phosphorylated CELF1 interacts with the initiation factor, eIF2, which is thought to promote use of the downstream AUG by increased ribosome scanning. **D.** Interaction of CELF1 and hnRNP H at the two ends of the transcript aids circularization. This has been proposed to facilitate translation from an internal ribosome entry site by increasing ribosome recycling.

Table 1
Nomenclature and phenotypes of CELF family orthologs

Species	Family Member(s)	Gene	Alternative Protein Names	Loss of Function Phenotype
<u>Vertebrates</u>				
<i>Homo sapiens</i> (human)	CELF1	<i>CELF1</i>	CUG-BP, CUG-BP1, BRUNOL2, NAB50	
	CELF2	<i>CELF2</i>	ETR-3, CUG-BP2, NAPOR, BRUNOL3	
	CELF3	<i>CELF3</i>	TNRC4, BRUNOL1, CAGH4	
	CELF4	<i>CELF4</i>	BRUNOL4	
	CELF5	<i>CELF5</i>	BRUNOL5	
	CELF6	<i>CELF6</i>	BRUNOL6	
<i>Mus musculus</i> (mouse)	CELF1	<i>Celf1</i>	CUG-BP, CUG-BP1, BRUNOL2	Spermatogenesis defects
	CELF2	<i>Celf2</i>	ETR-3, CUG-BP2, NAPOR, BRUNOL3	
	CELF3	<i>Celf3</i>	TNRC4, BRUNOL1, CAGH4	Reduced sperm count
	CELF4	<i>Celf4</i>	BRUNOL4	Seizure Disorder
	CELF5	<i>Celf5</i>	BRUNOL5	
	CELF6	<i>Celf6</i>	BRUNOL6	
<i>Gallus gallus</i> (chicken)	CELF1	<i>CELF1</i>	CUG-BP, CUG-BP1	
	CELF2	<i>CELF2</i>	ETR-3, CUG-BP2	
	CELF3	Deleted		
	CELF4	<i>CELF4</i>	BRUNOL4	
	CELF5	<i>CELF5</i>	BRUNOL5	
	CELF6	<i>CELF6</i>	BRUNOL6	
<i>Xenopus laevis</i> (frog)	CELF1	<i>celf1a</i>	brunol1a, EDEN-BP	Somite segmentation defects
		<i>celf1b</i>	brunol1b	
	CELF2	<i>celf2a</i>	brunol2a	Endoderm proliferation and differentiation defects
		<i>celf2b</i>	brunol2b	
	CELF3	<i>celf3a</i>	brunol3a, etr1	
		<i>celf3b</i>	brunol3b	
	CELF4	<i>celf4</i>	brunol4	
	CELF5	<i>celf5</i>	brunol5	
	CELF6	Missing		
	CELF1	<i>celf1</i>	cugbp1	
<i>Xenopus tropicalis</i> (frog)	CELF2	<i>celf2</i>	etr3	
	CELF3	<i>celf3</i>	tnrc4	
	CELF4	<i>celf4</i>	brunol4	
	CELF5	<i>celf5</i>	brunol5	
	CELF6	Missing		

Species	Family Member(s)	Gene	Alternative Protein Names	Loss of Function Phenotype
<i>Danio rerio</i> (Zebrafish)	CELF1	<i>celf1</i>	Cugbp1, Brul	
	CELF2	<i>celf2</i>	Etr-3, Cugbp2	
	CELF3	<i>celf3</i>	Etr-1	
	CELF4	<i>celf4</i>		
	CELF5	<i>celf5</i>		
	CELF6	Missing		
<u>Invertebrates</u>				
<i>Caenorhabditis elegans</i> (worm)	CELF1-2	<i>etr-1</i>		Elongation and muscle attachment defects
	CELF3-6	<i>unc-75</i>		Synaptic transmission defects
<i>Drosophila melanogaster</i> (fruit fly)	CELF1-2	<i>bru</i>		Gametogenesis defects
		<i>bru-2</i>		
	CELF3-6	<i>bru-3</i>		

Table 2
CELF-mediated alternative splicing targets identified using minigenes

Target	Splicing event	Minigene species	References
PTB	Exon 11 Inclusion	Human	31, 119
cTNT	Exon 5 Inclusion	Human, Chicken	7, 8, 22, 32, 120
MTMR1	Exon 2.1 Inclusion	Human	22
Nf1	Exon 23a Skipping	Human	52
CIC1	Intron 2 Retention	Human	102
APP	Exon 8 Skipping	Human	121
GABT4	Exon 7 Inclusion	Human	50
NMDAR1	Exon 5 Skipping	Human, Rat	31, 122-124
	Exon 21 Inclusion		
Insulin Receptor	Exon 11 Skipping	Human	8, 31, 99
	Exon SM Inclusion	Rat	125, 126
α -Actinin	Exon NM Skipping		
β -Tropomyosin	Exon 6B Inclusion	Chicken	127
CFTR	Exon 9 Inclusion	Human	22, 33
	Exon 2 Skipping	Human	110-112, 114, 122, 128, 129
Tau	Exon 3 Skipping	Human	
	Exon 6 Skipping	Human	
	Exon 10 Inclusion*	Human	

* CELF4 has been shown to induce exon 10 inclusion in a *tau* minigene¹²⁸, but CELF2 has been implicated in *tau* exon 10 skipping in DM¹¹⁵

Table 3
Endogenous CELF-mediated alternative splicing targets

Target	Splicing event	Tissues or cell types used	Reference
Sorbs1	Exon 6 Skipping	cardiac tissues	46
C10orf97	Exon 5 Inclusion	cardiac and skeletal muscle tissues	46, 91
Atp2b1	Exon 21 Skipping	cardiac tissues	46
Mfn2	Exon 3 Skipping	cardiac tissues	46
	Exon 14-3'Spice site Skipping		
Ank2	Exon 21 Skipping	cardiac tissues	46
Capzb	Exon 8 Skipping	cardiac and skeletal muscle tissues	46, 91
Tmem 134	Exon 5 Inclusion	cardiac tissues	46
Mtmr3	Exon 16 Skipping	cardiac tissues	46
Ppfibp	Exon 4 Skipping	cardiac tissues	46
Fox-2	Exon 13 Skipping	cardiac tissues	46
2900002H16Rik	Exon 4 Skipping	cardiac tissues	46
Ablim1	Exon 9 Skipping	cardiac tissues	46
Mbnl2	Exon 8 Inclusion	cardiac tissues	46
	ME Exon 6 Inclusion	cardiac and skeletal muscle tissues	46, 91
H2afy	ME Exon 7 Skipping		
Actn4	ME exon 5 Skipping	cardiac tissues	46
	ME exon 6 Inclusion		
Xpo7	Exon 4-5' Splice Site Skipping	cardiac tissues	46
FXR1h	Exon 15 Skipping	cardiac tissues	46
Mtmr1	Exon 2.1 Inclusion Exon 2.2*	cardiac tissues	48, 130
Bin1	Exon10 Skipping	cardiac tissues	48, 130
Itgb1	Exon D Inclusion	cardiac tissues	48, 130
Mef2A	Exon 16 Inclusion	cardiac tissues	48, 130
Nrap	Exon 12 Skipping	skeletal muscle tissues	91
MAP4	Exon 15 Skipping	HEK293T cells	47
SORBS1	Exon 5 Skipping	HEK293T cells	47
PPF1BP1	Exon 19 Skipping	HEK293T cells	47
SMARCE1	Exon 4 Skipping	HEK293T cells	47
Fox2	Exon 11 Skipping	HEK293T cells	47
CELF2	Exon 16 Skipping	HEK293T cells	47
NFAT	Exon 2 Skipping	HEK293T cells	47
CTBP1	Exon 2 Skipping	HEK293T cells	47
PTER	Exon 3 Skipping	HEK293T cells	47

Target	Splicing event	Tissues or cell types used	Reference
MLLT10	Exon 13 Skipping	HEK293T cells	⁴⁷

* Same splicing pattern in response to over-expression of CELF1 and repression of CELF activity in the heart