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Roles for RNA-binding proteins in development and disease

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Abstract

RNA-binding protein activities are highly regulated through protein levels, intracellular localization, and post-translation modifications. During development, mRNA processing of specific gene sets is regulated through manipulation of functional RNA-binding protein activities. The impact of altered RNA-binding protein activities also affects human diseases in which there are either a gain-of-function or loss-of-function causes pathogenesis. We will discuss RNA-binding proteins and their normal developmental RNA metabolism and contrast how their function is disrupted in disease.

Keywords

RNA-binding protein; RNA processing; Development; Neurodegenerative disease; Protein aggregate; Protein mis-regulation

1. Introduction

RNA-binding proteins (RBP) regulate RNA processing at multiple levels including alternative splicing, mRNA stability, mRNA localization and translation efficiency. As proteins highly involved in regulation, the expression of RBPs tends to be highly regulated. One mechanism of regulating the activities of RBPs in nuclear and cytoplasmic compartments is through changes in cellular localization. In the nucleus, some RBPs function as alternative splicing regulators often during development and/or in a tissue specific manner (Blencowe, 2006; Grabowski, 2011; Johnson, 2003; Wang et al., 2008). There are a growing number of examples in which alternative splicing changes have functional consequences (Giudice et al., 2014; Grabowski, 2011). In the cytoplasm, RBPs can function in the regulation of mRNA localization, mRNA stability or regulate the efficiency with which specific mRNAs are translated. As with the regulation of alternative splicing, regulation of cytoplasmic RNA processing events commonly occur during

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development (Jambor et al., 2015; Pilaz and Silver, 2015). The functions of RBPs can be disrupted in disease as either a primary cause of disease or a consequence. Understanding the normal roles of RBPs during periods of physiological change, such as during development, can reveal important aspects of function that are directly relevant to the pathogenic mechanisms and consequences in disease.

In this minireview, we discuss two sets of RBPs that control developmental transitions that are mis-regulated in disease: CUG-BP, Elav-like family (CELF) and Muscleblind-like (MBNL) proteins that have pathogenic roles in myotonic dystrophy (DM) and *fused* in sarcoma (FUS) and TAR DNA-binding protein (TDP-43) proteins that have pathogenic roles in amyotrophic lateral sclerosis (ALS). These RBPs are sequestered, hyperactive, or aggregated in the disease states. In the case of DM, the activities of both CELF and MBNL proteins revert to fetal patterns thereby promoting fetal mRNA processing of their targets in adult tissue. In neurons affected in ALS, FUS and TDP-43 are depleted from the nucleus and aggregate in the cytoplasm. Mutations in the *FUS* or *TARDBP* genes have a direct role in ALS pathogenesis; evidence also indicates that aggregation of FUS and TDP-43 causes both a loss-of-function and a gain-of-function. Currently, it is unknown if TDP-43 and FUS mRNA processing targets revert back to embryonic patterns; although it is clear there is mis-regulation of mRNA processing in ALS directly due to TDP-43 and FUS aggregation.

2. CELF and MBNL in DM

The CELF family contains six paralogs in humans and mice (CELF1-6), which can be subdivided into two groups containing CELF1-2 and CELF3-6 based on phylogenetic analysis (Dasgupta and Ladd, 2012). Structurally, CELF proteins contain three RNA recognition motifs (RRMs) in which RRM1 and 2 are adjacent near the N-terminus, RRM3 is near the C-terminus and a unique domain of ~200 residues separates RRM2 and RRM3 (Ladd et al., 2004). CELF proteins bind preferentially to G/U-rich RNA sequence motifs (Faustino and Cooper, 2005; Marquis et al., 2006) and have well-established nuclear and cytoplasmic functions. In the nucleus, CELF proteins regulate alternative splicing to promote exon exclusion of some targets or inclusion of others (Kalsotra et al., 2008). Minigene and CLIP-seq studies have demonstrated that CELF proteins directly bind to pre-mRNAs, typically within introns, to promote the regulated splicing pattern (Daughters et al., 2009; Ladd et al., 2001; Wang et al., 2015). In the cytoplasm, CELF proteins affect mRNA translation or mRNA stability by binding the 3'UTR of the regulated mRNA (Blanc and Davidson, 2003; Subramaniam et al., 2008; Vlasova and Bohjanen, 2008; Zhang et al., 2008).

The MBNL family consists of three paralogs: MBNL1-3 (Kanadia et al., 2003a). MBNL proteins bind RNA through two pairs of zinc finger domains, which consist of CCCH amino acid residues (Begemann et al., 1997). MBNL proteins bind to YGCY motifs, preferring UGCU (Du et al., 2010; Goers et al., 2010). As regulators of alternative splicing both CELF and MBNL proteins typically bind to the introns downstream or upstream of an alternative exon to promote inclusion or exclusion, respectively (Kalsotra et al., 2008; Wang et al., 2012). In the cytoplasm, MBNL affects mRNA localization or stability by binding the 3' UTR of target mRNAs (Llamusi et al., 2013; Masuda et al., 2012).

CELF and MBNL have overlapping targets for alternative splicing and mRNA stability, and are most often antagonistic regulators of these common targets (Masuda et al., 2012; Wang et al., 2015). As splicing regulators, CELF and MBNL proteins promote the opposite effects on splice site or exon usage. Global analysis of CLIP-seq data shows that CELF and MBNL proteins overlap with thousands of 3' UTR targets and affect mRNA stability (Wang et al., 2015). CELF-bound mRNA targets are degraded while MBNL1 binding leads to mRNA localization to the membrane for translation (Wang et al., 2015).

2.1 Development

During development, changes in the abundance and localization of CELF and MBNL proteins have been shown to promote global splicing transitions (Kalsotra et al., 2008). CELF and cytoplasmic MBNL protein levels are high in embryonic mouse skeletal muscle and CELF protein levels decrease while MBNL localizes to the nucleus during postnatal development (Kalsotra et al., 2008; Lin et al., 2006). CELF protein levels also drop during mouse heart postnatal development while MBNL protein levels increase (Kalsotra et al., 2008). Therefore in both striated muscles, the nuclear activities of CELF and MBNL proteins change in opposite directions. Studies of the developmental effects of CELF and MBNL proteins have focused on alternative splicing transitions in striated muscle. There are also likely to be important cytoplasmic effects during development that remain to be identified. The genes that undergo the developmental splicing transition are enriched for vesicular trafficking, among other functional categories, and induced CELF1 expression in transgenic mice reverts a subset of vesicular trafficking genes to the fetal splicing pattern (Giudice et al., 2014). MBNL proteins have been shown to maintain the differentiated state in cells relevant to brain and muscle, by repressing embryonic stem cell alternative splicing pattern (Han et al., 2013).

Interestingly, the dramatic drop in CELF protein levels during heart development is not due to a corresponding drop in mRNA levels (Kalsotra et al., 2008). CELF protein down regulation is regulated by at least two mechanisms. First, CELF translation is repressed postnatally due to up-regulation of microRNAs (miRNAs) including miR-23a/b. Second CELF1 protein is dephosphorylated during postnatal development resulting in protein destabilization (Kalsotra et al., 2010; Kuyumcu-Martinez et al., 2007).

2.2 Disease - DM

DM is an autosomal dominant disease that affects multiple organ systems including skeletal muscle, heart and brain. DM is the most common form of adult-onset muscular dystrophy and the second most common form of muscular dystrophy overall. Mutations in two different genes cause different forms of DM: DM type 1 (DM1) and DM type 2 (DM2). DM1 is caused by an expansion of a CTG repeat in the 3' UTR in the *DMPK* gene, and DM2 is caused by a CCTG repeat in intron 1 of the *CNBP* gene (Brook et al., 1992; Ranum et al., 1998). Unaffected individuals contain fewer than 34 repeats in the *DMPK* gene while affected individuals have from 80 to thousands of repeats. In both DM1 and DM2, the expanded allele is transcribed and the expanded repeat within the RNA forms a hairpin structure and aggregates into nuclear foci (Napierała and Krzyzosiak, 1997). MBNL proteins are sequestered on the RNA foci substantially reducing the pool of functional protein and

interfering with normal MBNL function (Kanadia et al., 2003b; Kanadia et al., 2006; Miller et al., 2000). In addition, PKC is activated by an unknown mechanism which phosphorylates and stabilizes CELF1 proteins and results in increased CELF1 function (Kuyumcu-Martinez et al., 2007). Increased CELF and decreased MBNL function revert the splicing patterns back to the splicing pattern seen in fetal tissue (Charlet-B et al., 2002; Philips et al., 1998; Savkur et al., 2001).

Mis-regulated mRNA processing by CELF and MBNL proteins has been linked to predominant symptoms in DM and DM1 in particular. The best characterized is aberrant splicing of the muscle-specific chloride channel (CLCN1) which has been demonstrated to cause the myotonia for which the disease is named (Charlet-B et al., 2002; Mankodi et al., 2002). MBNL1 knockout mice and compound loss of the two MBNL paralogs expressed in striated muscle (*Mbnl1*^{-/-}, *Mbnl2*^{+/-}) produce phenotypes consistent with DM, including dramatic tissue pathology and mis-regulated alternative splicing including *Clcn1*, supporting the role of MBNL loss of function in disease pathogenesis (Kanadia et al., 2003b; Lee et al., 2013). The mis-regulation of insulin receptor alternative splicing correlates with insulin resistance seen in patients (Savkur et al., 2001; Sen et al., 2010). Muscle weakness has been correlated with numerous splicing changes in DM1 (Nakamori et al., 2013). One particular example, *CACNA1S* (CaV1.1) exon 29 is antagonistically regulated by MBNL1 and CELF1 and associated with muscle weakness (Tang et al., 2012). Muscle pathology is also linked to mis-regulation of CELF1 mRNA translation targets (Jones et al., 2012). Another clinical feature of DM1 is altered cognition. Mis-splicing of MBNL targets have been identified in brains of *Mbnl2* knockout mice (Charizanis et al., 2012; Goodwin et al., 2015). The clinical features associated with expression of fetal isoforms and reversion of cytoplasmic functions to fetal patterns has demonstrated the necessity of the developmental transitions for normal function in adult tissues.

3. FUS and TDP-43 in ALS

Familial forms of ALS are caused by mutations in a large and growing number of genes. Here we highlight TDP-43 and FUS proteins as they are linked to ALS pathogenesis independent of mutations present in the *TARDBP* or *FUS* genes, respectively (Giordana et al., 2010; Neumann et al., 2006; Neumann et al., 2009).

3.1 Normal roles of TDP-43 and FUS

TDP-43 is a DNA- and RNA-binding protein with both cytoplasmic and nuclear functions. TDP-43 contains two RRM, a nuclear localization signal (NLS), nuclear export signal (NES), and a glycine-rich domain (GRD) (Fiesel and Kahle, 2011). TDP-43 is predominantly but not exclusively localized to the nucleus and depending upon its localization has functions in mRNA metabolism including alternative splicing, mRNA stability, and miRNA biogenesis (Buratti et al., 2010; Polymenidou et al., 2011; Volkening et al., 2009). Homozygous knockout of TDP-43 (*Tardbp*^{-/-}) is embryonic lethal at E3.5 to E8.5 demonstrating a requirement for early embryonic development (Kraemer et al., 2010; Sephton et al., 2010). Similar to expression of CELF1 during heart development, TDP-43 protein levels decrease during mouse brain postnatal development while TDP-43 mRNA

levels remain constant (Huang et al., 2010; Sephton et al., 2010). The mechanism of TDP-43 down regulation during development is unknown. The developmental targets of TDP-43 is also currently unknown.

FUS was identified as an oncogenic fusion protein that along with EWSR1 and TAF15 make up the FET family of proteins (Croizat et al., 1993)(Wu et al., 2009). Similar to TDP-43, FUS contains a NES, NLS, GRD, and a single RRM (Fiesel and Kahle, 2011). FUS is a DNA- and RNA-binding protein with evidence for functions in DNA repair, transcriptional regulation, alternative splicing regulation, RNA localization, and association with stress granules (Sama et al., 2014). FUS has also been shown to couple transcription to alternative splicing by interacting with both RNA polymerase II and U1 snRNP (Yu and Reed, 2015). Homozygous knockout of *Fus* produces perinatal lethality in inbred mouse lines (Hicks et al., 2000). However, *Fus* knockout animals survived in an outbred strain, but developed neurodegenerative pathology (Kino et al., 2015). During postnatal development, FUS protein levels decrease in brain, skeletal muscle, heart, and liver tissue while mRNA levels remain constant (Huang et al., 2010). Similar to the current state of knowledge for TDP-43, the developmental targets of FUS and how FUS-dependent mRNA regulation affects development remain to be determined.

3.2 Disease – ALS

ALS is a neurodegenerative disease that affects motor neurons resulting in rapidly progressive loss of muscle function. Ninety percent of ALS cases are sporadic; however the familial forms of ALS in which the disease is dominantly inherited provides insight into mechanisms of pathogenesis.

Involvement of TDP-43 and FUS in ALS pathogenesis has been discussed in several excellent reviews (Fiesel and Kahle, 2011; Lourenco et al., 2015; Orozco and Edbauer, 2013; Polymenidou et al., 2012). A critical pathological effect in ALS (and other neurodegenerative diseases) is aggregate formation of TDP-43 and FUS in the cytoplasm and depletion of these proteins from the nucleus (Giordana et al., 2010; Kwiatkowski et al., 2009; Neumann et al., 2009). A recent and important proposal is that the protein aggregates found in ALS have prion-like qualities and reflect an anomaly of the normal protein-protein interactions required for the dynamic nature of RNA metabolism. TDP-43 and FUS, as well as other RBPs shown to have pathogenic effects in ALS, contain prion-like domains (Gitler and Shorter, 2011; King et al., 2012). Furthermore, mutations in these genes that cause ALS accelerate the prion-like domain's ability to self-seed (Kim et al., 2013; Shorter and Taylor, 2013).

Recent work suggests that both loss and gain of TDP-43 and FUS functions contribute to ALS pathogenesis, however, the specific mechanisms and functional consequences are as yet unclear. Loss of TDP-43 causes inclusion of non-conserved, cryptic exons leading to non-sense mediated decay of TDP-43 splicing targets (Ling et al., 2015). In addition, cytoplasmic aggregation of TDP-43 is likely to affect its function in transporting mRNA as mRNP granules (Alami et al., 2014). Coexpression of TDP-43 with FMRP, with which TDP-43 forms a complex involved in transport of mRNPs, prevents TDP-43 aggregation and restores appropriate translation (Coyne et al., 2015). Loss of TDP-43 in mice causes age-

dependent progressive motor neuron degeneration indicating that loss of TDP-43 activity is consistent with features of ALS (Wu et al., 2012; Yang et al., 2014)'. Mutated FUS seen in ALS patients causes a loss of interaction between FUS and U1 snRNP (Sun et al., 2015) suggesting the potential for pathogenic effects via disrupted co-transcriptional RNA processing. Although FUS loss does not cause lethality, it causes a unique neurodegenerative phenotype distinctive from ALS, which indicates that FUS loss-of-function is disruptive to normal neural function but may not be the driving force of ALS pathogenesis (Kino et al., 2015). FUS gains function through mutations that have a greater association with SMN protein in the cytoplasm (Sun et al., 2015). In addition, SOD1, another ALS-associated protein, is misfolded in familial ALS patients with mutations in FUS or TDP-43 and can be misfolded just by the overexpression of wildtype FUS or TDP-43 (Pokrishevsky et al., 2012).

4. Conclusion

CELF and MBNL proteins illustrate that RBPs are highly regulated during development to perform both nuclear and cytoplasmic functions and that both functions are disrupted in diseases that affect the activities of the proteins. TDP43 and FUS illustrate that RBP functions require dynamic interactions between proteins and RNA within transient complexes and that disruption of proper association-dissociation leads to pathogenesis (Fig. 1). It is well established that CELF1 and MBNL1 activities revert to the fetal pattern and shift a regulatory program such that functions required in adult tissues are lost. In the examples of MBNL1, TDP-43, and FUS, the level of protein is not strongly altered but rather the functionality of the protein is affected. This highlights that different effects on protein function including localization, temporal expression, or amount of protein can result in pathogenicity.

Further investigation is required to fully understand the normal balance of RNA-binding protein function and the disruption of the function that leads to disease. Some functions of CELF and MBNL proteins during development are well established, but because studies have primarily focused on splicing regulation during development and its disruption in disease, there is likely to be important roles for these proteins in other aspects of RNA metabolism. Less is known about the functions of FUS and TDP-43 during development. An understanding of the functions of these RNA binding proteins in development will provide insight into their roles during disease pathogenesis.

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Highlights

- RBPs can be developmentally regulated for normal RNA metabolism.
- RBPs are mis-regulated in certain diseases (e.g. DM and ALS).
- This minireview stresses the importance of proper RBP level and localization.

Fig. 1. Protein levels and subcellular localization of RBPs during development and disease. (A) CELF1 (blue) is regulated by phosphorylation and microRNAs while MBNL1 (red) localization changes during development. In DM, the presence of DMPK expanded CUG-repeat (green) leads to hyper-phosphorylation and stability of CELF1 to increase CELF1 activity, and MBNL1 activity is lost by sequestration by the RNA foci. (B) Normally, TDP-43 (purple) and FUS (orange) are predominantly nuclear proteins that have reduced protein levels during development. Due to mutations (*) in TDP-43 and FUS, TDP-43 and

FUS proteins aggregate in the cytoplasm causing a loss of function of TDP-43 and gain of function of FUS.

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