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• REVIEW •

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RNA-binding proteins in neurological diseases

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Emerging studies support that RNA-binding proteins (RBPs) play critical roles in human biology and pathogenesis. RBPs are essential players in RNA processing and metabolism, including pre-mRNA splicing, polyadenylation, transport, surveillance, mRNA localization, mRNA stability control, translational control and editing of various types of RNAs. Aberrant expression of and mutations in RBP genes affect various steps of RNA processing, altering target gene function. RBPs have been associated with various diseases, including neurological diseases. Here, we mainly focus on selected RNA-binding proteins including Nova-1/Nova-2, HuR/HuB/HuC/HuD, TDP-43, Fus, Rbfox1/Rbfox2, QKI and FMRP, discussing their function and roles in human diseases.

RNA-binding proteins, post-transcriptional regulation, neurological diseases

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RNA-binding proteins (RBPs) play key roles in post-transcriptional processing of RNAs, which can occur at every aspect of the life of mRNAs, from pre-mRNA splicing to mRNA localization, turnover, polyadenylation, translational control, nuclear export, and editing [1,2]. Post-transcriptional regulation adds substantial complexity to the control of gene expression and allows the cell to fine-tune its protein composition in order to respond to developmental or other stimuli, so it plays important roles in diverse cellular processes [3]. RBPs interact with specific *cis*-regulatory mRNA elements to form ribonucleoprotein (RNP) complexes, thereby regulating expression/function of their target RNAs [4]. There are more than 800 RBPs encoded by the human genome with approximately 40 different types of domain motifs, including RNA recognition motifs (RRM), K-homology (KH) domains, RGG (Arginine-Glycine-Glycine) boxes, double-stranded RNA binding motifs (dsRBM), DEAD/DEAH boxes and Piwi/Argonaute/Zwille (PAZ) domains [5]. Although many RNA binding proteins are expressed ubiquitously in a wide range of tissues/cell types, numerous examples have been reported in which specific RBPs are expressed in cell- or tissue-specific manners. On the other hand, many macromolecular complexes contain more than one RNA-binding protein, and the specific combinations of different RBPs may be cell- or tissue type-specific. Such cell/tissue-specific RBP expression patterns increase genetic diversity and also contribute to the cell/tissue-unique manifestations when a specific RBP is mutated or defective [6]. Many RBPs have multiple target genes and modulate expression/function of an array of target RNAs. Consequently, disrupting the function of a single RBP often affects post-transcriptional regulation of many RNA transcripts, a phenomenon increasingly recognized in

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human diseases associated with RBPs. Although the precise mechanisms by which RBPs achieve cell-type specific post-transcriptional control of their target genes are poorly understood, genetic studies have identified a large number of mutations in RBP genes in a wide range of human diseases, suggesting the critical importance of RBPs in human biology and pathogenesis [7].

1 RBPs in post-transcriptional gene regulation

The life of an mRNA begins in the nucleus, when a gene is transcribed by RNA polymerase II to produce its pre-mRNA transcript(s) [8]. In many cases, the 5′-end capping and 3′-end polyadenylation of the pre-mRNA occur co-transcriptionally. While the RNA is being transcribed by the RNA polymerase complex, the intervening sequences (introns) are removed from the pre-mRNA transcript, a process known as "pre-mRNA splicing", by the multi-component splicing machinery called the spliceosome. Pre-mRNA splicing is under complex regulation by splicing enhancers or splicing silencers that interact with exonic or intronic *cis*-acting elements. Best known examples of splicing regulators include the serine-arginine rich protein family (SR proteins) and heterogeneous ribonucleoproteins (hnRNPs) [9]. The coordinated 5′-capping, pre-mRNA splicing and 3′-polyadenylation processes act to produce a mature mRNA ready for transport to the cytoplasm for translation. Once in the cytoplasm, the mRNA is associated with the protein synthesis machinery, which may be localized in the endoplasmic reticulum (ER) [10] or at sites far away from the nucleus. For example, in neurons, mRNAs may be transported to axons and dendrites for local protein synthesis [11]. During mRNA transport, translation is repressed and the mRNA is protected from degradation [12]. The mRNAs are not distributed homogenously throughout the cytoplasm. Imaging studies have revealed two types of cytoplasmic RNA granules, processing (P)-bodies and stress granules, the RNA-protein complexes in which mRNAs could accumulate. P-bodies contain components for RNA decay, whereas stress granules frequently contain translation initiation factors [13–15]. As shown in Figure 1, there is significant overlap between the components of P-bodies and stress granules. Under conditions of cellular stresses, the mRNAs can be sequestered into RNA granules, an RBP-dependent process that is often reversible [16]. Throughout its life, the mRNA is associated with a host of RBPs, many of which act in more than one aspect of post-transcriptional gene regulation. Such intimate association between mRNAs and RBPs contributes to the complex temporal and spatial regulation of eukaryotic gene expression. The detailed coverage of specific functional activities of each RBP is not the goal of this review. Here, we will use a few examples of neuronally expressed RBPs to illustrate the importance of RBPs in human diseases.

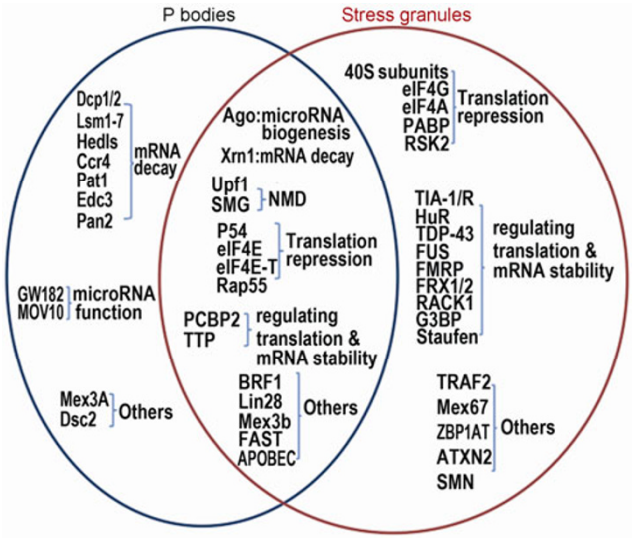

Figure 1 Known components of P bodies and stress granules. A diagram showing the distinct and shared RBPs detected in P bodies and stress granules. For details about these proteins, the readers are referred to several expert reviews [13–15].

2 Post-transcriptional regulation in the nervous system

Increasing evidence supports that post-transcriptional regulation plays an important role in the nervous system [17]. First, a number of RBPs, including the well-known Rbfox3, HuB/HuC/HuD and Nova, are specifically expressed or enriched in neurons [18]. Second, alternative splicing seems to be particularly active in the nervous system, as compared with other tissues [19]. Alternative splicing regulates expression and function of almost each category of genes critical for neuronal function, ranging from nerve growth factors, membrane proteins such as receptors to intracellular signaling molecules, thereby influencing neurophysiology [20–24]. Third, local protein synthesis guided by mRNAs localized in axons is required for neuron function, including learning and memory [25–27]. Consequently, the dysfunction of or defects in RNA-binding proteins lead to defective post-transcriptional gene regulation, resulting in neurological diseases [7].

3 Distinct families of RBPs and their involvement in neurological diseases

Genetic studies have revealed mutations in or dysregulation of RBPs in a wide range of human diseases. Here we focus on those in the nervous system. These RBPs share certain structural features, as shown in Figure 2. Two types of genetic mutations are best characterized, mutations affecting the protein coding regions of RBP genes and mutations in

the regulatory regions of RBP genes [28]. In addition, multiple RBPs are found in aggregates in neurodegenerative disorders, although it remains to be determined whether such aggregate formation is a direct cause of disease or a secondary event. In this review, we will describe several examples to illustrate the roles of RBPs in neurological diseases and discuss underlying pathogenetic mechanisms (Table 1). The readers are directed to a number of excellent reviews on the roles of RNA binding proteins in human diseases in general [28–32].

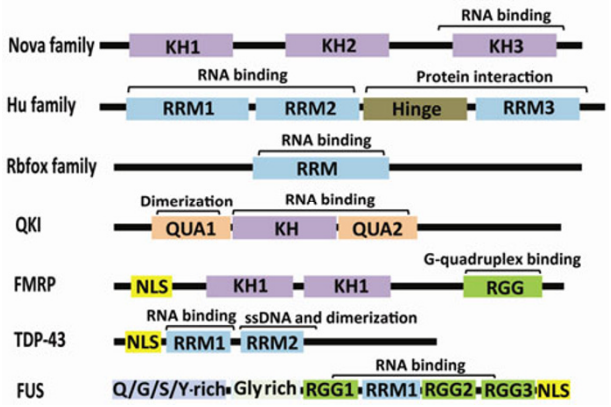

Figure 2 A diagrammatic illustration of domain structures present in the selected RBPs described in this article. KH, K-homology domain; RRM, RNA recognition motif; NLS, nuclear localization sequence; QUA, quaking domain; RGG, Arg-Gly-Gly motifs. The drawing is not to the scale.

3.1 The Nova family

The proteins of the Nova (neuro-oncological ventral antigen) family, including Nova-1 and Nova-2, are neuron-specific RNA-binding proteins. Nova-1 is expressed primarily in the hindbrain and ventral spinal cord, whereas the expression of Nova-2 is restricted to the neocortex [33,34]. Nova-1 protein was first identified as a target antigen in an autoimmune syndrome known as paraneoplastic opsoclonus myoclonus ataxia (POMA), a form of paraneoplastic neurodegeneration (PND) [33,35]. Tumors outside of the nervous system can sometimes trigger ectopic expression of RBPs that are normally restricted to the central nervous system. The immune system recognizes the ectopically expressed RBPs as non-self antigens and initiates autoimmune responses against these antigens, resulting in PND. POMA is caused by auto-antibodies against Nova-1/Nova-2 triggered by ectopic expression of these proteins in breast cancers. Nova-1 knockout mice die postnatally from a motor deficit associated with apoptotic death of brainstem and spinal neurons [36]. The hallmark pathological defect in POMA patients is neuronal cell death in the regions of the brainstem and spinal cord where Nova-1 is normally expressed. Thus, Nova-1 plays an important role in neuronal cell survival in the postnatal stage [36].

Nova-1 and Nova-2 contain three KH domains, connected by flexible linker regions (Figure 1). The third KH domain of Nova (KH3) is necessary and sufficient for specific RNA binding. The X-ray crystallography structure reveals

Table 1 Selected RBPs associated with neurological diseases[a)]

RBPs	RBD	RNA motif	Enrichment of mRNA targets	Posttranscriptional regulation	Neurological disease	Pathological alteration	Ref.
Nova	KH	Cluster of YCAY	Synaptic proteins; Neuronal inhibition	Alternative splicing; Polyadenylation	Paraneoplastic opsoclonus myoclonus ataxia (POMA)	Ectopic expression; Autoantibody production	[33,37,38,41]
Hu	RRM	AU-rich	Amino acid biosynthesis; Synaptic cytoskeletal dynamics	Stability; Translation; Alternative splicing; Polyadenylation	Paraneoplastic subacute sensory neuropathy syndrome	Ectopic expression; Autoantibody production	[46,52,55,57,62]
Rbfox	RRM	(U)GCAUG	Transmission; Membrane excitability	Alternative splicing; Polyadenylation	Mental retardation; Epilepsy; Autism	Mutation	[68,72,73,77]
QKI	KH Qua2	A(C/U)UAA(C/U)	Myelination in CNS; Vascular development	Stability; Translation; Alternative splicing; Localization	Psychiatric diseases; Schizophrenia; Ataxia	Reduction of expression	[80,82,86,91]
FMRP	RGG	G-quadruplex	Presynaptic proteins; Postsynaptic proteins	Translation; Transport; Stability	Fragile X syndrome	Repeat expansion mutation	[29,97,105]
TDP-43	RRM	$(UG)_n$ repeat	Neuronal development; Neuron survival; Synaptic transmission	Alternative splicing; MicroRNA biogenesis; Stability; Transport	Amyotrophic lateral sclerosis; Frontotemporal lobar dementia	Mutation; Cytoplasmic aggregate	[117,126,128,129]
Fus	RRM GRR	GGUG	Vesicle transport; Neuronal impulse; Neuronal projection	Transcription; Alternative splicing; Transport; Gene silencing	Amyotrophic lateral sclerosis; Frontotemporal lobar dementia	Mutation; Cytoplasmic aggregate	[126,149,150]

a) Y is a pyrimidine residue.

that the Nova protein holds the RNA in its grip by forming a molecular vice with the invariant Gly-X-X-Gly motif on one side and the variable loop of KH3 domain on the other. *In vitro* binding experiments have established that Nova proteins interact with sequence-specific clusters of YCAY elements (where Y is a pyrimidine) on pre-mRNA and can regulate alternative splicing [37,38]. A large number of candidate RNA targets regulated by Nova proteins have been identified based on the prediction of clusters of YCAY elements. Among these, the α2 subunit of the glycine (GlyRα2) receptor and the γ2 subunit of the GABAA (GABAA γ2) receptor are two targets that have been confirmed using cell biological and *in vitro* splicing assays [34,36,39,40].

To demonstrate that Nova is a key player in PND pathogenesis, Darnell and colleagues systematically searched for Nova target RNA genes using genome-wide methods. First, an ultraviolet cross-linking coupled immunoprecipitation (CLIP) approach was developed to analyze Nova-RNA complexes. Three-quarters of the Nova-interacting RNA transcripts identified encode proteins involved in the neuronal synaptic function, and one-third of these play a role in inhibitory transmission. This study provided compelling evidence that Nova-1 could regulate a subset of RNA transcripts encoding multiple components of the inhibitory synapses [41]. Second, exon-junction splicing microarrays were used to assess RNA splicing changes in Nova1-null mice. Fifty Nova1-dependent targets were identified. Interestingly, nearly all 50 targets contained clusters of YCAY elements and encoded synaptic proteins [42]. Third, high-throughput sequencing was performed using RNAs isolated by crosslinking-coupled immunoprecipitation (HITS-CLIP), leading to the identification of Nova-2 CLIP tags and construction of genome-wide footprints. Both CLIP and HITS-CLIP have been used to study direct protein-RNA interactions in tissue and living cells. Two methods use ultraviolet irradiation to induce covalent crosslinks between protein-RNA complexes *in situ*, allowing rigorous purification of RBPs along with small fragments of RNA. RNAs purified by CLIP are amplified and sequenced, however, HITS-CLIP applies high-throughput sequencing methods to RNAs purified by CLIP and provides a genome-wide biochemical approach. Their study demonstrated that the position of Nova binding determines the outcome of alternative splicing. Interestingly, many Nova-2 binding sites are localized to 3′ untranslated regions (3′ UTRs), suggesting that Nova regulates alternative polyadenylation in the brain [43]. Finally, they combined CLIP, exon-junction splicing microarrays, HITS-CLIP, and more sophisticated bioinformatics identified a set of 700 Nova-dependent alternatively spliced exons and a smaller number of alternative 3′ UTRs in the mouse brain [44]. The identification of these Nova target genes predicts a role of Nova in synaptic function, especially in mediating inhibitory responses and suggests potential association of Nova with other neurological diseases [43,44].

3.2 Hu/ELAVL protein family

The mammalian Hu/embryonic lethal abnormal vision-like (ELAVL) family of RNA-binding proteins was another family of RBPs originally identified as antigens associated with paraneoplastic neurological syndrome [45]. Paraneoplastic subacute sensory neuropathy/encephalomyelopathy syndrome, the neurologic disorder associated with the Hu antigen, is caused by the production of auto-antibodies against the Hu family proteins triggered by their ectopic expression in small cell lung cancers [46]. In mammals, the Hu protein family includes four members, HuR, HuB, HuC and HuD. Of the four Hu family members, HuR is widely expressed in many cell types, whereas HuB, HuC and HuD are expressed specifically in central and peripheral neurons [46,47]. Neuronal Hu proteins play an important role in neuronal development, neuronal plasticity and memory [48–50]. Homozygous HuD knockout mice exhibited transient impairment of embryonic cranial nerve development, with reduced neurosphere formation, suggesting a role of HuD in neural stem cells [48]. Over-expression of HuD in forebrain neurons of HuD transgenic mice resulted in impaired acquisition and retention of memories [51]. Furthermore, antisense-mediated knockdown of HuC in mice resulted in impaired spatial learning [52]. These studies suggest the involvement of HuC and HuD in learning and memory.

Hu proteins contain three RNA recognition motifs (RRMs), referred to as RRM1, RRM2 and RRM3. There is a basic hinge region between RRM2 and RRM3. RRM1 and RRM2 cooperate to recognize and bind to AU-rich sequences on mRNA targets, thereby achieving their post-transcriptional regulation [51,53]. Hu proteins are great multi-tasking proteins regulating various aspects of RNA metabolism in both cytoplasmic and nuclear compartments. In the cytoplasm, the most well-known molecular function of Hu proteins is to regulate mRNA stability. Hu proteins bind to AU-rich elements (AREs) located at the 3′ UTRs of GAP-43, VEGF, GLUT1, eotaxin and c-fos, thereby enhancing their mRNA stability [54–58]. Approximately 5%–8% of human protein-coding genes contain AREs, suggesting the overall importance of Hu proteins in regulating mRNA stability [54,59]. In addition to mRNA stability, Hu proteins also regulate translation by acting as either enhancers or repressors of translation of their target genes, including neurofilament M, hypoxia inducible factor 1a and CD83 [56,60,61]. In the nucleus, Hu proteins serve as regulators of polyadenylation and alternative splicing [62]. To date, at least four splicing targets of Hu proteins have been characterized, including Calcitonin/CGRP, NF1, Fas and HuD [63–67]. Hu proteins bind to AREs and interact with spliceosomal factors to regulate exon inclusion or

exclusion [67].

Extensive HuC-RNA interactions in the mouse brain have been revealed by using combined HITS-CLIP, splicing-sensitive microarrays and bioinformatic analyses to examine HuC knockout mice. In neurons, 699 robust intronic and 3′ UTR HuC binding sites were identified. HuC preferentially binds to conserved U-rich sequences interspersed with G residues at exon-intron junctions to either repress or enhance the inclusion of alternative exons. Gene ontology (GO) analysis demonstrated prominent roles of HuC proteins in mRNA stability, regulation of genes involved in amino acid biosynthesis and alternative splicing regulation of transcripts involved in synaptic cytoskeletal dynamics. A subset of these HuC binding sites are mapped to the 3′ UTRs of genes involved in pathways regulating glutamate, a major excitatory neurotransmitter in the brain. Indeed, glutamate levels were reduced in the HuC knockout mouse brains, and both homozygous and heterozygous knockout mice showed spontaneous epilepsy. This phenotype confirmed the notion that neuron-specific HuC controlled the excitation-inhibition balance in the brain, as suggested by the genome-wide analysis of HuC targets [52].

3.3 Rbfox family

The members of the Rbfox family of RNA-binding proteins, including Rbfox1, Rbfox2 and Rbfox3, are evolutionarily conserved regulators of tissue-specific alternative splicing in metazoans. Rbfox1 and Rbfox2 proteins are expressed in muscle, heart, and brain tissues [68]. In the brain, these proteins are exclusively expressed in neurons [69]. Rbfox3 is neuron-specific [70]. Rbfox1 was originally identified in a yeast two-hybrid screen as a protein that interacts with the spinocerebellar ataxia type-2 gene (SCA2) [71]. Subsequent studies demonstrated that mutations in the Rbfox1 gene were associated with mental retardation, epilepsy and autism [72–74]. The CNS-specific conditional Rbfox1 knockout mice exhibited spontaneous seizures [75]. In contrast, the CNS-specific deletion of Rbfox2 disrupts cerebellar development [76].

Rbfox1, 2 and 3 share a common protein domain structure and contain a single RNA-binding domain. The Rbfox family specifically recognizes the (U)GCAUG element in regulated exons or in flanking introns, either promoting or repressing alternative splicing of a number of target exons [68]. A recent study using HITS-CLIP demonstrated that Rbfox2 interacted with GCAUG elements in embryonic stem (ES) cells, consistent with biochemical studies [77]. The position-dependent splicing map based on the position of GCAUG elements around alternative exons is consistent with earlier bioinformatics predictions made by analyzing UGCAUG binding sites and support a role for Rbfox2 as a splicing factor in neurons. Interestingly, many of the predicted Rbfox2 alternatively spliced target transcripts themselves encoded splicing factors, suggesting the possibility of a higher-order regulatory network. Gene ontology (GO) analysis of the Rbfox2 splicing regulatory network demonstrated enrichment of a biologically coherent set of transcripts encoding proteins mediating synaptic transmission and membrane excitability [77,78]. Genome-wide analysis of brain RNA of Rbfox2 knock-out mice identified numerous splicing changes altering proteins important both for brain development and mature neuronal function.

3.4 QUAKING

QUAKING (QKI) belongs to the STAR (signal transducer and activator of RNA) family of RNA-binding proteins. It regulates differentiation of myelin-forming oligodendrocyte and Schwann cells [79]. In the adult mouse CNS, QKI is highly expressed in glial cells, including astrocytes and oligodendrocytes [80]. Expression of QKI mRNA is reduced in disease-related regions from postmortem brain tissue samples from patients, and multiple studies suggested that QKI is a strong candidate gene for several psychiatric diseases including schizophrenia and ataxia [81,82]. The role of QKI in myelination was further supported by the quaking-viable mutation in mice (qkv mice) [83]. The qkv mice showed severe CNS dysmyelination because the myelin fails to undergo compaction in the CNS due to a large deletion upstream of the quaking locus [83].

QKI proteins consist of a central KH domain flanked by two conserved subdomains, referred to as Qua1 and Qua2. QKI proteins bind the consensus RNA element A(C/U)UAA(C/U) via the KH and Qua2 regions, while the Qua1 domain is essential for homodimerization [84–86]. Multiple studies demonstrated that QKI played key roles in oligodendroglia differentiation and maturation by controlling post-transcriptional gene expression including mRNA stabilization, translation, subcellular localization and alternative splicing [79,81,87–90]. By PAR-CLIP (photoactivatable ribonucleoside-enhanced CLIP) and bioinformatics prediction, over a thousand mRNA species are targeted by QKI in mice. In HITS-CLIP, living cells or tissue samples are irradiated with UV light at a wavelength of 254 nm, however in PAR-CLIP, cells are fed with 4-thiouridine, which becomes incorporated into newly transcribed RNA, allowing crosslinking with UV light at 365 nm and can be used to pinpoint the crosslinked nucleotide. Although many proteins encoded by target mRNAs are important for myelination in CNS, targets of QKI are not restricted to the control of myelin-related gene expression and are implicated in multiple biological processes such as smooth muscle cell differentiation, vascular development and heart development [91,92].

3.5 Fragile X mental retardation protein

Fragile X mental retardation protein (FMRP), the product of the FMR1 gene, encodes an RNA-binding protein associated with fragile X mental retardation. A repeat expansion

mutation in the 5′ UTR of FMR1 causes hypermethylation of the genes' promoter, leading to transcriptional silencing. This mutation causes fragile-X syndrome (FXS) the most common genetic cause of mental retardation, with an incidence of approximately 1:4000 males and 1:8000 females [93,94]. Affected individuals display a variety of intellectual deficits, ranging from mild learning impairments to abnormal social behaviors and autism. Expansion of the CGG repeats >200 copies leads to transcriptional silencing of FMR1 and a loss of the FMRP expression [95–97]. Interestingly, individual males with an intermediate number of CGG repeats in the FMR1 gene (50–200), referred to as premutation alleles, do not develop FXS, but develop fragile X tremor ataxia syndrome (FXTAS), a late-onset disorder characterized by dementia, gait abnormality, and tremor [98]. In individuals with FXTAS, the FMR1 gene is not completely silenced [99]. In the case of FXTAS, the expanded CGG repeat is transcribed as part of the FMR1 mRNA. Several RBPs that interact with the CCG repeat-containing RNAs, including hnRNPA2/B1 and MBNL, are sequestered away from their normal locations and prevented from executing their normal function. The loss of function of these RBPs leads to development of FXTAS, as demonstrated by the observation that overexpression of these RBPs rescues the disease phenotypes in FXTAS animal models [100–102].

FMRP is expressed in all cell types of the nervous system except mature glia. In neurons, FMRP is enriched in the cell body, although it is also detected in granules in dendrites and axons during development [103]. FMRP is critical for the formation of synapses and neural circuits [104]. FMRP contains two KH domains, an RGG box, a nuclear localization and export signals (NLS and NES). FMRP binds to G-quadruplex structures, possibly via its RGG box, to participate in different steps of post-transcriptional gene regulation [29,105,106]. The RNA G-quadruplex is formed by stacks of planar layers of guanine tetrad units and regulates different steps of RNA processing [107], including protein translation and stability of a subset of mRNAs in neurons [108]. First, FMRP is a polyribosome-associated RNA-binding protein in dendrites that regulates translation of mRNAs required for synaptic plasticity by stalling ribosomal translocation on target mRNAs [109–111]. Second, FMRP may play a role in the transport of RNAs to the synapse in a microtubule-dependent manner [112]. Finally, FMRP is involved in mRNA stability control of PSD95, a key regulator of synaptic plasticity [113]. In knock-out mice, the loss of FMRP leads to reduced glutamate-induced transport of mRNA to the synapse, and decreased distribution of FMRP target RNAs and their proteins in the synapses [114,115]. Recently, HITS-CLIP experiments uncovered >800 candidate FMRP target mRNAs in the mouse brain. Such FMRP-targets are markedly enriched in both presynaptic and postsynaptic regions, consistent with the synaptic function of FMRP [29]. Together these studies suggest that the loss of FMRP function in regulating transport, translation and stability of target RNAs result in the deficits seen in FXS patients and animal models.

3.6 RRM-RGG families: TDP-43 and FUS

The DNA/RNA-binding protein TDP-43 (Tar-DNA binding protein 43) was initially identified as a transcriptional repressor of HIV. TDP-43 is a member of hnRNP family, with structural similarity to hnRNPs A1 and A2 [116]. Since 2006, TDP-43 has attracted more attention following the landmark discovery that TDP-43 was a major component of intraneuronal insoluble aggregates detected in degenerating neurons in patients affected by amyotrophic lateral sclerosis (ALS) or fronto-temporal lobar degeneration (FTLD) [117]. More than 30 missense TDP-43 mutations have been identified among ALS patients, however, the majority FTLD-TDP43 patients have no detectable TDP-43 mutations, although they show TDP-43 positive pathological lesions [118–120]. It remains unclear how mutations in TDP-43 lead to ALS, although they appear to enhance its cytoplasmic aggregate formation, suggesting a key role of a toxic aggregation of TDP-43 in these neurological diseases [118,120]. Mice over-expressing wild-type human TDP-43 or mouse TDP-43 developed ALS/FTLD-like neurodegeneration with cytoplasmic ubiquitin-positive TDP-43 aggregation, indicating that too much TDP-43 may be just as detrimental as mutant TDP-43 [121–123]. ALS is considered the most common adult-onset motor neuron disease and results from loss of motor neurons in the brain and spinal cord. ALS is a fatal disease and most patients die of paralysis of the respiratory muscles [124]. ALS is frequently diagnosed post mortem, by the loss of motor neurons and presence of intra-neuronal inclusion bodies [125]. Dominant mutations in the TDP-43 gene are implicated in ~ 4% of familial ALS cases [126]. FTLD is the second most common form of early-onset dementia. A pathological hallmark for FTLD-TDP43 is the presence of ubiquitinylated intra-neuronal TDP-43 positive protein aggregates in the affected brain regions [127]. Recently TDP-43 proteinopathy has been associated with mutations in other ALS and FTLD related genes such as GRP or VCP, which may suggest a new pathological mechanism for TDP-43 proteinopathy.

TDP-43 contains a nuclear localization signal (NLS), a nuclear export signal (NES), two RRMs and a glycine-rich domain at carboxyl terminus. The NLS and NES allow TDP-43 to be continuously shuttled between the nucleus and the cytoplasm. TDP-43 recognizes $(UG)_n$ repeat elements in target mRNAs by its RRM1 [128,129]. The glycine-rich domain mediates the interactions between TDP-43 and other proteins such as hnRNPs [130]. TDP-43 also contains a Q/N-rich prion-like element that mediates its interaction with polyQ aggregates. The biological function of TDP-43 is multi-faceted, including transcriptional regu-

lation and post-transcriptional regulation at the different stages such as pre-mRNA splicing, microRNA biogenesis, RNA stability and RNA transport [131]. Here, we briefly discuss the role of TDP-43 in pre-mRNA splicing and mRNA stability control. By binding to the UG repeats of the target pre-mRNA transcripts, TDP-43 modulates alternative splicing of many genes, such as the cystic fibrosis transmembrane conductance regulator (CFTR) gene, SMN2 and apolipoprotein A-II [132−134]. CFTR was the first identified target gene whose pre-mRNA splicing is regulated by TDP-43. CFTR contains an alternative exon 9, and the exclusion of exon 9 results in a nonfunctional CFTR protein [133]. TDP-43 inhibits exon 9 inclusion by binding specifically to an UG containing splicing silencing element located at the 3′-splice site within intron 8 of CFTR. Second, TDP-43 regulates mRNA stability by binding to the 3′-UTR of the target mRNAs including the low molecular weight neurofilament (NFL), cyclin-dependent kinase 6 (CDK6), and histone deacetylase 6 (HDAC6) genes [135,136]. HDAC6 is well characterized as a target of TDP-43. TDP-43 promotes HDAC6 mRNA stability through binding to UG repeat elements in the 3′-UTR. Interestingly, HDAC6 has been implicated in suppression of neurodegeneration by autophagic degradation of aggregated proteins in Alzheimer's disease and Parkinson's disease [137]. Recent genomic studies using HITS-CLIP identified about 7000 protein-coding RNA substrates for TDP-43 in brain and cell lines. Consistent with multiple roles of TDP-43 in post-transcriptional regulation, TDP-43 binding is enriched in long intronic regions, the 3′-UTR of mRNA, and nuclear ncRNAs [138−142]. Many targets are involved in neuronal development, neuron survival, and synaptic transmission, suggesting a critical role of TDP-43 in neurons.

Homozygous disruption of TDP-43 in mice is embryonically lethal, whereas disrupting one copy of the gene leads to more subtle motor disturbances and muscle weakness, which is similar to transgenic animals [143,144]. Therefore, TDP-43 may be linked to disease via loss-of-function in which pathological TDP-43 fails to exercise its function in post-transcriptional gene expression. On the other hand, simple overexpression of the wild-type TDP-43 in animal models recapitulates many pathological and clinical features of TDP-43 proteinopathy patients [123,145−147]. Analyses of FTLD patients with TDP-43 immunoreactive protein aggregates show that the TDP-43 protein level is frequently increased [121,148]. It remains to be determined whether gain-of-function toxicity or loss-of-function haplo- insufficiency is the major pathogenic mechanism underlying various TDP-43 proteinopathies in humans.

4 Other ALS-associated RBPs

Similar to TDP-43, several other RNA-binding proteins have been linked to ALS. First, the RNA-binding protein FUS (fused in sarcoma, also known as translocated in liposarcoma, TLS) is associated with ALS and FTLD. FUS-immunoreactive aggregates are pathological hallmarks for ALS-FUS and FTLD-FUS. A large number of FUS mutations have been identified in ALS-FUS since 2009 [149]. Approximately 4% of familial ALS cases contain mutations in the FUS gene [126]. FUS and TDP-43 share similarities in protein structure and functional activities. FUS contains a single RRM domain, a Glycine-rich domain, a lysine-rich region, a glutamine-glycine-serine-tyrosine (QGSY)-rich region, two arginine-glycine rich regions, a zinc finger motif and a C-terminal nuclear localization signal (Figure 1). FUS recognizes GGUG motifs on RNAs via its RNA binding domain [150]. FUS is predominantly localized to the nucleus and plays a role in transcription, mRNA splicing transport, and gene silencing. Hippocampal neurons from homozygous FUS knock-out mice displayed reduced spine numbers and abnormal dendritic spines as long thin filopodia-like structures. This may be associated with the FUS function in regulating transport of mRNAs encoding actin-stabilizing proteins to maintain the normal dendritic spine morphology [151,152].

Discoveries of mutations in TDP-43 and *FUS* in ALS suggest that other RBPs may be involved in pathogenesis of neurodegenerative diseases. A screen in yeast identified 133 human RBPs, that were cytotoxic and formed cytoplasmic aggregates, similar to TDP-43 and FUS [153]. Some of these RBPs contain prion-like sequences. Mutations in ALS patients were found in *TAF15*, which were also independently found in another study [154]. Subsequently mutations were also identified in the *EWSR1* gene in ALS patients [155]. Discovery of mutations in the hnRNPA1 gene suggest involvement of hnRNAPA1 in ALS and multisystem proteinopathy [156]. These mutations were found to affect the prion-like domains of the RBPs and accelerated hnRNP fibrillization and, similar to mutations in TDP-43 that increase amyloid fibril formation [156].

An hexanucleotide repeat expansion in the *C9orf72* gene has been identified as a common genetic change associated with both familial and sporadic ALS [157,158]. Similar to FXTAS, intranuclear RNA foci have been observed in ALS patients carrying the *C9orf72* repeat expansion [157]. A model of RNA toxicity has been postulated for ALS associated with *C9orf72* repeat expansion. Several RBPs have been identified that interact with the expanded repeat, including Pur-α [159], hnRNPA3 [160] and ADARB2 [161]. ADARB2 is a double-stranded RNA adenosine deaminase, a family of RBPs that edit pre-mRNAs by altering adenosines to inosines. Inosines in RNA are recognized as guanosines by the splicing and translational machinery. Thus, editing can create new splice sites in pre-mRNAs or result in translated proteins with altered amino acid sequences [162,163]. ADARB2 was shown to co-localize with RNA foci in both induced pluripotent stem cells (iPSCs) and post mortem tissue from repeat expansion carriers. Gluta-

mate-mediated excitotoxicity, RNA foci and sequestering of ADARB2 to foci, were all reduced when antisense oligonucleotides targeting the toxic repeat containing *C9orf72* RNA were introduced into the iPSCs. Along with ADARB2, 18 additional proteins were reported to associate with the hexanucleotide repeat [161]. Together, these studies suggest that RBPs may play important roles in ALS pathogenesis.

Mutations in the senataxin gene, encoding a DNA/RNA helicase, lead to a rare form of ALS with typically juvenile onset (ALS4) and the inherited ataxia, AOA2 (ataxia oculomotor apraxia 2) [164,165]. Recent studies indicate that senataxin plays a role in DNA repair, suggesting the involvement of DNA repair in pathophysiology of ALS4 [166].

Interestingly, intermediate lengths of expanded polyglutamine tracts (polyQ) in the RNA-binding protein ATXN2 were found to increase risk for ALS [167]. CAG-expansions in the ATXN2 gene cause spinocerebellar ataxia-type II (SCA2), an autosomal-dominant neurodegenerative disorder [168]. In SCA2, the polyQ tract in ATXN2 is expanded from the normal 22Q to 34Q or more. PolyQ-expanded ATXN2 forms cytosolic aggregates, where it may acquire a toxic gain of function [169]. The normal cellular function of ATXN2 is largely unknown. However, the presence of a like-Sm (LSM)-type RNA-binding domain and its association with other RBPs suggest potential involvement of ATXN2 in RNA metabolism. Many unanswered questions remain as to how polyQ expansion of ATXN2 ultimately causes neurodegeneration.

5 Concluding remarks

It has become increasingly clear that RBPs are important players in neurodegenerative disorders and other human diseases. The advent of high-throughput technologies, bioinformatics analysis, genetic mouse models and biochemical approaches significantly advanced our understanding of the involvement of RBPs in human diseases. However, the molecular mechanisms by which genetic mutations or aberrant expression of RBPs lead to impairment of neuronal function remain to be elucidated.

Most RBPs are involved in multiple steps of post-transcriptional regulation and interact with a large number of RNA targets. Recent studies show that different genetic mutations or defects in different genes may lead to the same pathological lesions and clinical manifestations. For example, genetic alterations in different genes could lead to TDP-43 proteinopathy. Both Rbfox and QKI are implicated in ataxia [170]. On the other hand, the same mutation may have distinct clinical manifestations in different individuals. It is interesting to note that many of these RBPs play multiple roles in different steps of RNA processing. For example, both FUS and TDP-43 likely regulate pre-mRNA splicing of multiple genes. It remains to be determined whether splicing defects in a subset of substrate genes important for neuronal survival and function contribute to neurodegeneration in FUS or TDP-43 proteinopathies. Therefore, identification of a specific set of disease-causing defects in RNA processing is highly demanding. It is even more challenging to elucidate the mechanisms by which the cell integrates information from myriad of different RBPs to coordinate the RNA metabolism and to determine cellular heath and function.

Although significant efforts have been invested into understanding biological function of RBPs and pathogenic mechanisms underlying the human diseases associated with RBPs, a number of questions remain to be addressed. For example, why do mutations in ubiquitously expressed RBP genes (such as TDP-43 or FUS) cause ALS that predominantly affects motor neurons? What factors prevent such genetic mutations from causing neuronal damage early on in life? How do such mutations cause neuronal death? How do RNA processing defects contribute to neuronal loss?

Disease-causing mutations in RBP genes may not result in a loss of gene expression, therefore, gene knockout models may not necessarily be disease-relevant (e.g., embryonic lethality in TDP-43-deficient mice). On the other hand, transgenic animals overexpressing either wild-type or mutant RBPs may result in neurodegenerative pathology and functional impairment. It is likely that both loss of the normal function and gain-of-function neurotoxicity contribute to the pathogenesis of these diseases. Animal models in combination with molecular and cellular studies have begun to address pathogenetic mechanisms, however, much more work is necessary to identify the early events and biomarkers for neural damage and for developing effective therapeutic approaches to these devastating diseases.

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