

Disruption of RNA Metabolism in Neurological Diseases and Emerging Therapeutic Interventions

Julia K. Nussbacher,¹ Ricardos Tabet,^{2,3} Gene W. Yeo,^{1,*} and Clotilde Lagier-Tourenne^{2,3,*}

¹Department of Cellular and Molecular Medicine, Institute for Genomic Medicine, UCSD Stem Cell Program, University of California, San Diego, La Jolla, CA, USA

²Department of Neurology, The Sean M. Healey and AMG Center for ALS at Mass General, Massachusetts General Hospital, Harvard Medical School, Charlestown, MA 02129, USA

³Broad Institute of Harvard University and MIT, Cambridge, MA 02142, USA

*Correspondence: geneyeo@ucsd.edu (G.W.Y.), clagier-tourenne@mgh.harvard.edu (C.L.-T.)

<https://doi.org/10.1016/j.neuron.2019.03.014>

RNA binding proteins are critical to the maintenance of the transcriptome via controlled regulation of RNA processing and transport. Alterations of these proteins impact multiple steps of the RNA life cycle resulting in various molecular phenotypes such as aberrant RNA splicing, transport, and stability. Disruption of RNA binding proteins and widespread RNA processing defects are increasingly recognized as critical determinants of neurological diseases. Here, we describe distinct mechanisms by which the homeostasis of RNA binding proteins is compromised in neurological disorders through their reduced expression level, increased propensity to aggregate or sequestration by abnormal RNAs. These mechanisms all converge toward altered neuronal function highlighting the susceptibility of neurons to deleterious changes in RNA expression and the central role of RNA binding proteins in preserving neuronal integrity. Emerging therapeutic approaches to mitigate or reverse alterations of RNA binding proteins in neurological diseases are discussed.

During their life cycle, messenger RNAs (mRNAs) undergo extensive processing steps including splicing, polyadenylation, editing, transport, translation, and turnover. This elaborate process is highly dynamic and requires a complex interplay among RNA binding proteins (RBPs) to finely modulate co- and post-transcriptional processing of transcripts. RBPs bind RNA molecules at specific sequences or secondary structures in order to facilitate several steps of RNA processing, both in the nucleus and cytoplasm. Genome-wide approaches have provided major insights into the multiple ways RBPs influence the fate of their targets (Nussbacher et al., 2015). Indeed, RBPs are increasingly recognized as multifunctional proteins since a specific RBP may associate with different protein complexes to influence several processing steps of its RNA targets. It is therefore not surprising that altered interaction between an RBP and its targets can lead to severe pathological phenotypes. In fact, the weight of evidence supports that defects in RNA regulation play a crucial role in neurodevelopmental dysfunction and neurodegenerative diseases. The mechanisms by which RBPs are altered in neurological disorders are diverse, and in several cases the molecular details remain elusive. However, a few themes have emerged, including impaired RBP expression, cellular mislocalization and aggregation of RBPs, and sequestration of RBPs by transcripts and/or abnormal proteins with pathological repeat expansions (Figure 1; Table 1). Even small changes in RBP expression or activity may be amplified due to their broad impacts on expression, splicing, and translation of hundreds of RNA substrates, and their functional disruption can have global and significantly deleterious outcomes on the health of neurons. Here, we discuss the multiple ways by which perturbations of RNA homeostasis are associated with neurological diseases. The groundbreaking recognition of RBPs as major contributors to neurodegenerative

diseases is illustrated by specific examples and descriptions of genome-wide studies that documented the impact of widespread RNA processing misregulation in disease. Methods to therapeutically restore the functions of RBPs are emerging and current strategies to combat RBP homeostasis perturbations are also discussed.

Reduced Expression of RBPs in Neurological Disorders

Neurological disorders may be induced by mutations reducing or silencing the expression level of RBPs (Figure 1A) as demonstrated for SMN1 in spinal muscular atrophy and FMRP in fragile X syndrome (FXS).

Altered SMN1 Expression in Spinal Muscular Atrophy

Spinal muscular atrophy (SMA), characterized by degeneration of lower motor neurons and severe muscular atrophy, has an incidence rate of ~1 in 6,000–10,000. This autosomal recessive disease is caused by loss-of-function mutations, homozygous deletions, and less frequently point mutations, in the survival of motor neuron 1 (SMN1) gene (Lefebvre et al., 1995). The closely related SMN2 gene generates only low levels of functional SMN protein. Indeed, a C > T difference in SMN2 results in the exclusion of exon 7 in 80%–90% of transcripts and the production of a truncated protein unable to efficiently compensate for loss of SMN1 (Kashima and Manley, 2003; Lorson et al., 1999). Notably, inefficient splicing of SMN2 exon 7 is markedly exacerbated in motor neurons, contributing at least partially to the vulnerability of motor neurons in SMA patients (Ruggiu et al., 2012). SMN is essential for the assembly of small nuclear ribonucleoproteins (snRNPs), achieved through self-oligomerization followed by interaction with small nuclear RNAs (snRNAs), gemins, and Sm proteins in the cytoplasm (Li et al., 2014; Liu et al., 1997; Pellizzoni et al., 1998; So et al., 2016). This complex is imported to



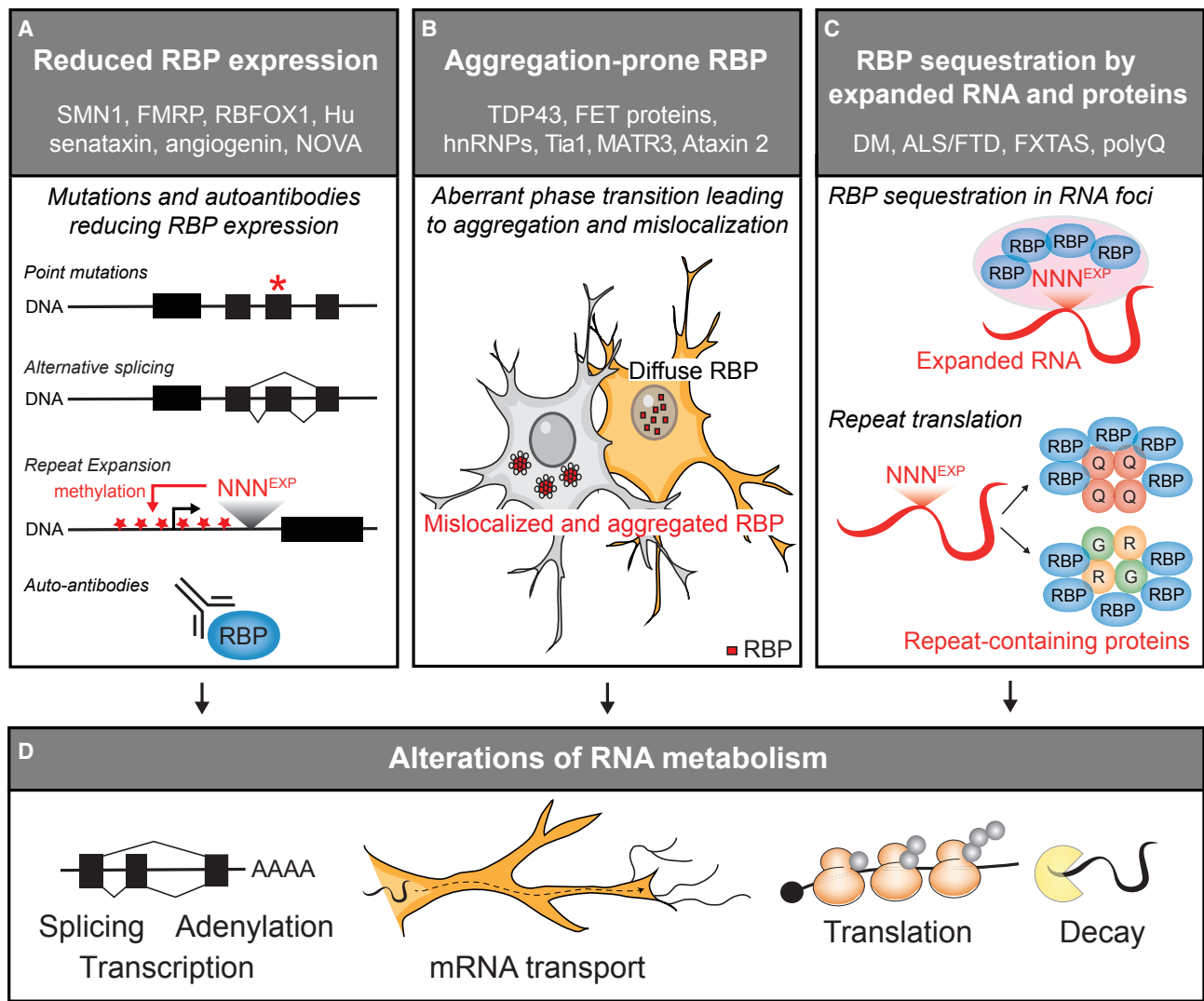


Figure 1. Mechanisms Leading to Disruption of RBPs in Neurodegenerative Diseases

(A) Expression of RBPs may be reduced by point mutations, abnormal splicing, aberrant repeat expansions that repress the RBP transcription, and neutralization by autoantibodies in paraneoplastic neurological syndromes.
 (B) Abnormal phase transition of RBPs with low complexity domains leads to their aggregation and mislocalization with loss of function and/or gain of novel toxic properties.
 (C) Expanded repeats in microsatellite diseases may lead to sequestration of RBPs into RNA foci and/or abnormal interaction with repeat-containing proteins translated from the expansion.
 (D) Disruption of RBPs has a widespread effect on the metabolism of their RNA targets including abnormal splicing, polyadenylation, transport, translation, and decay with downstream effects on cellular morphology and function.

the nucleus and the final assembly of the snRNPs takes place in Cajal bodies. In SMA, abnormal snRNP assembly is either due to deletion or to missense mutations that disrupt the ability of SMN protein to oligomerize and/or to interact with Sm proteins (Lorson et al., 1998; Pellizzoni et al., 1999; Seng et al., 2015; Wan et al., 2005). Loss of SMN1 function causes tissue- and snRNP-specific changes in the snRNP repertoire that result in widespread splicing defects (Gabanella et al., 2007; Huo et al., 2014; Imlach et al., 2012; Lotti et al., 2012; Zhang et al., 2008). Indeed, loss of SMN protein preferentially reduces the accumulation of minor (U12-dependent) spliceosome (Gabanella et al.,

2007; Zhang et al., 2008) and mainly affects the expression of U12-targeted introns, including in the gene Stasimon that encodes a protein required for motor circuit function (Imlach et al., 2012; Lotti et al., 2012). While widespread splicing changes were observed in tissues of SMA mice (Zhang et al., 2008), they are more apparent in late-symptomatic animals; hence, it is difficult to discriminate between direct effects of SMN deficiency and consequences of neurodegenerative processes (Bäumer et al., 2009). Notably, independent from its role on splicing, U1 snRNP protects pre-mRNAs from premature cleavage and polyadenylation (Kaida et al., 2010) and promotes

Table 1. Summary of RBPs Involved in Neurological Diseases

RBP	Disease	Mechanism
Reduced RBP Expression		
Survival of motor neuron 1 and 2 (SMN1 and SMN2)	spinal muscular atrophy (SMA)	loss-of-function mutations, homozygous deletions
Fragile X mental retardation protein (FMRP)	fragile X syndrome (FXS)	CGG > 200 repeat expansion in the 5' UTR of <i>FMR1</i>
Fox-1 homolog (RBFOX1)/ataxin 2-binding protein 1 (A2BP1)	autism spectrum disorder (ASD), intellectual disability, epilepsy	chromosomal translocations, copy number variations, mutations
Senataxin (SETX)	ataxia with oculomotor apraxia type 2 (AOA2), amyotrophic lateral sclerosis 4 (ALS4)	recessive mutations (AO2), dominant mutations (ALS4)
Angiogenin (ANG)	amyotrophic lateral sclerosis (ALS)	mutations
Neuro-oncological ventral antigen 1/2 (NOVA1/2)	paraneoplastic opsoclonus-myoclonus ataxia (POMA)	autoantibodies
Hu proteins (HuB, HuC, HuD)	paraneoplastic sensory neuronopathy and/or paraneoplastic encephalomyelitis (PSN-PEM)	autoantibodies
Aggregation-Prone RBPs		
TAR DNA binding protein 43 (TDP-43)	ALS, frontotemporal dementia (FTD)	mutations, mislocalization
FET proteins (fused in sarcoma/translocated in liposarcoma, FUS/TLS; Ewing's sarcoma, EWS; TATA-binding protein-associated factor 15, TAF-15)	ALS, FTD	mutations, mislocalization, aberrant methylation
Heterogeneous ribonucleoprotein particles (hnRNPA2B1, hnRNPA1)	ALS, FTD	mutations
T cell-restricted intracellular antigen-1 (TIA1)	ALS, FTD	mutations
Matrin 3 (MATR3)	ALS	mutations
Ataxin 2 (ATXN2)	spinocerebellar ataxia type 2 (SCA2), ALS	CAG ₂₇₋₃₃ (ALS) or CAG > 33 (SCA2) repeat expansion in the coding sequence of <i>ATXN2</i>
RBP Sequestration by Expanded RNA and Proteins		
DM1 protein kinase (DMPK)	myotonic dystrophy type 1 (DM1)	CTG _{50-3,500} repeat expansion in the 3' UTR of <i>DMPK</i> sequesters MBNL
Zinc finger 9 (ZFN9)	myotonic dystrophy type 2 (DM2)	CCTG _{75-11,000} repeat expansion in intron 1 of <i>ZFN9</i> sequesters MBNL
<i>C9ORF72</i>	ALS, FTD	(G ₄ C ₂) > 30 repeat expansion in intron 1 of <i>C9ORF72</i> . Both expanded transcripts and dipeptide repeat proteins interact with RBPs
FMRP	fragile x-associated tremor/ataxia syndrome (FXTAS)	CGG ₅₅₋₂₀₀ repeat expansion in the 5' UTR of <i>FMR1</i> recruits Pur- α and hnRNPA2/B1

transcription elongation in the sense-coding direction, highlighting how loss of SMN and alteration of snRNP assembly may impact diverse steps of RNA processing (Almada et al., 2013). Several lines of evidence also support a general role of SMN as a chaperone that promotes the assembly of various RNP complexes and influences several aspects of RNA metabolism including the 3' processing of histone mRNAs (Tisdale et al., 2013) and mRNA decay and transport (Li et al., 2014). In neurons, SMN and a subset of its gemin partners localize to dendrites and axons (Carrel et al., 2006; Pagliardini et al., 2000; Todd et al., 2010; Zhang et al., 2006), contributing to neuronal mRNA trafficking and local translation of specific transcripts including β -actin mRNA (Jablonka et al., 2007; Kye et al., 2014; Rathod et al., 2012; Rossoll et al., 2002; Zhang et al., 2006). Consistently, SMN-deficient motor neurons exhibit reduced neurite length and

altered growth cones (Akten et al., 2011; Fallini et al., 2011; Rossoll et al., 2003).

A striking convergence between SMA and amyotrophic lateral sclerosis (ALS), another motor neuron disease, has been uncovered. Indeed, SMN-Gemins complexes cluster into membraneless nuclear structures called gems (gemini of Cajal bodies) (Liu and Dreyfuss, 1996) that are lost in cells of SMA patients. Surprisingly, the number of gems is also reduced in cells of ALS patients (Sun et al., 2015; Yamashita et al., 2013; Yamazaki et al., 2012; Yu et al., 2015) and various ALS mouse models expressing mutant SOD1, FUS, or TDP-43 (Gertz et al., 2012; Ishihara et al., 2013; Kariya et al., 2012; Shan et al., 2010; Sun et al., 2015). Notably, increasing neuronal SMN levels extended lifespan and attenuated neurodegeneration in mutant TDP-43 mice (Perera et al., 2016) and improved survival of induced

pluripotent stem cell (iPSC)-derived motor neurons with TDP-43 and SOD1 mutations (Rodriguez-Muela et al., 2017). In addition, SMN and specific proteins from the U1 snRNP complexes were shown to directly interact with FUS, an RBP associated with ALS and frontotemporal dementia (FTD) (Gerbino et al., 2013; Groen et al., 2013; Sun et al., 2015; Yamazaki et al., 2012; Yu et al., 2015). The U1 snRNA is directly bound by FUS (Lagier-Tourenne et al., 2012) and is redistributed to the cytoplasm (Gerbino et al., 2013; Yu et al., 2015) along with the SmB/B' proteins (Yu et al., 2015) in the presence of ALS-associated FUS mutations. The mechanisms leading to a reduced number of nuclear gems in different forms of ALS are not elucidated but could result from the abnormal cytoplasmic interactions of SMN with ALS-related aggregation-prone proteins. The association of SMN and ALS is further supported by human genetic studies; specifically, aberrant SMN1 copy number appears to be associated with an increased risk of ALS (Corcia et al., 2009). While SMN dysfunction emerges as a common feature in SMA and ALS, the exact mechanisms leading to motor neuron loss remain elusive. Indeed, the splicing, stability, and transport of hundreds of mRNAs are predicted to be altered by SMN dysfunction; however, the relative contribution of these events to motor neuron death is not yet established.

Reduced Expression of FMRP and RBFOX1 in Autism Spectrum Disorders

Certain autism spectrum disorders (ASDs) can be attributed to the reduced expression of RBPs including fragile X mental retardation protein (FMRP) and fox-1 homolog (RBFOX1, also known as ataxin 2-binding protein 1, A2BP1). FXS is the most common inherited form of neurodevelopmental disorder with autistic symptoms. It occurs in ~1 in 4,000 males and 1 in 8,000 females and is characterized by behavioral disorders, learning disabilities, and distinctive physical features including macroorchidism. FXS is caused by a CGG > 200 microsatellite repeat expansion in the 5' UTR of the *FMR1* gene, which encodes FMRP (Kremer et al., 1991; Oberlé et al., 1991; Verkerk et al., 1991; Yu et al., 1991). This large CGG expansion induces the silencing of the *FMR1* gene via DNA hypermethylation, as well as histone hypoacetylation and hypermethylation (Coffee et al., 1999; Kumari et al., 2012; Oberlé et al., 1991; Sutcliffe et al., 1992; Verkerk et al., 1991), leading to the absence of FMRP in FXS patient neurons (Devys et al., 1993; Verheij et al., 1993). FMRP is necessary for the maintenance and stability of neural synapses (Coffee et al., 1999; Kumari et al., 2012; Weiler and Greenough, 1999). Indeed, neuronal dendrites from FXS patients and mouse models have abnormally long, thin, and dense spines, which correlate with immature-like morphology, supporting a role for FMRP in synaptic pruning and maturation (He and Portera-Cailliau, 2013; Irwin et al., 2001). It is noteworthy that a tight regulation of mRNA localization and spatial activation of translation is crucial to maintain neuronal integrity and synaptic function. This is in part facilitated by the formation of RNA granules composed of RNA molecules, interacting RBPs, motor proteins, and adaptor complexes that function to coordinate the transport of RNAs to specific subcellular locations (Anderson and Kedersha, 2009; Chyung et al., 2018). While in transit, translation of mRNAs is often repressed in order to ensure protein production only upon the receipt of proper signals at the appropriate loca-

tion (Anderson and Kedersha, 2009). FMRP plays a critical role in synaptic plasticity and metabotropic glutamate receptor (mGluR)-dependent long-term depression by controlling the transport and local translation of specific mRNA targets in synapses (Hagerman et al., 2017; Santoro et al., 2012). In neurons FMRP interacts with kinesins including KIF3C and KIF5 to control the transport of its mRNA targets (Davidovic et al., 2007; Dichtenberg et al., 2008) and localizes to the postsynaptic spaces of dendritic spines where it may function as a translational repressor influencing local protein synthesis (Feng et al., 1997; Schütt et al., 2009; Weiler et al., 1997; Zalfa et al., 2003). FMRP is present in different RNA granules, including stress granules, which form when translation initiation is impaired (Anderson and Kedersha, 2006; Antar et al., 2004; Bagni and Oostra, 2013; Dichtenberg et al., 2008; Kanai et al., 2004; Kao et al., 2010; Zalfa et al., 2006). FMRP also associates with polyribosomes (Feng et al., 1997; Khandjian et al., 1996; Weiler et al., 1997), and genome-wide analyses support that FMRP binds to coding sequences of mRNAs associated with stalled ribosomes, including transcripts implicated in other ASDs (Ascano et al., 2012; Darnell and Klann, 2013; Darnell et al., 2011). However, there is also evidence that FMRP binds to non-coding regions of mRNAs and several FMRP RNA binding sites have been proposed, including G quadruplex, Kissing complex, SoSlip structure, and ACUG UGGA motifs (Ascano et al., 2012; Bechara et al., 2009; Brown et al., 2001; Darnell et al., 2001, 2005; Edbauer et al., 2010; Gessert et al., 2010; Miyashiro et al., 2003; Muddashetty and Bassell, 2009; Schaeffer et al., 2001; Suhl et al., 2014; Tian et al., 2013; Vasilyev et al., 2015). Several models for translational control by FMRP have been proposed. FMRP can prevent the tethering of the ribosome at the 5' end of the mRNA by recruiting cytoplasmic FMRP-interacting protein 1 (CYFIP1), which prevents interaction between translation initiation factors (Napoli et al., 2008). FMRP also directly binds to the L5 protein on the 80S ribosome (Chen et al., 2014), and an alternate model is that FMRP causes ribosomes to stall during the elongation phase of translation (Ceman et al., 2003; Darnell et al., 2011; Stefani et al., 2004). However, it should be noted that direct interaction of FMRP with ribosomal subunits (Chen et al., 2014) was observed with a N-terminally truncated form of the protein in *Drosophila*, a species with only one *Fmr1* gene, while mammals also express somewhat functionally redundant paralogs *FXR1P* and *FXR2P*. In addition, FMRP was shown to recruit RNA-induced silencing complex (RISC) to mRNA targets, repressing translation through the RNA interference pathway (Caudy et al., 2002; Edbauer et al., 2010; Ishizuka et al., 2002; Jin et al., 2004; Muddashetty et al., 2011; Plante et al., 2006). Finally, an indirect mechanism was recently proposed with the identification of the diacylglycerol kinase kappa ($Dgk\kappa$) mRNA as a major target of FMRP in mouse cortical neurons (Tabet et al., 2016). Loss of FMRP is associated with decreased expression of the DGKK protein, a master regulator of lipid signaling that converts diacylglycerol (DAG) to phosphatidic acid (PA). Since downstream effectors of DAG and PA lipids influence protein translation, it was proposed that FMRP can regulate protein synthesis by an indirect (DAG-mediated) rather than a direct mechanism (Hagerman et al., 2017; Tabet et al., 2016).

In addition to mRNA transport and translation regulation, FMRP plays a role in other steps of RNA metabolism including mRNA stability (Zalfa et al., 2007), RNA editing (Shamay-Ramot et al., 2015), and the splicing of its own mRNA (Didiot et al., 2008). Although FMRP is predominantly cytoplasmic, it shuttles between the cytoplasm and the nucleus (Feng et al., 1997; Willemssen et al., 1996). Nuclear FMRP was found to associate with Cajal bodies (Dury et al., 2013) and was identified as a chromatin-binding protein that participates in the DNA damage response (Alpatov et al., 2014). In addition, FMRP interacts with other RBPs involved in neurological diseases including SMN (Piazzon et al., 2008) and TDP-43 (Majumder et al., 2016; Wang et al., 2008; Yu et al., 2012). Overall, FMRP loss may affect multiple steps of RNA processing leading to synaptic dysfunction associated with autism in FXS patients. An additional mechanism of FMRP-mediated neurodegenerative pathology involves intermediate repeat expansion in *FMR1*, which is linked to increased mRNA expression but reduced FMRP protein expression; this mechanism is discussed in detail in a subsequent section.

Reduced expression of another RBP, RBFOX1, has been associated with autism, intellectual disability, and epilepsy (Bhalla et al., 2004; Bill et al., 2013; Martin et al., 2007; Sebat et al., 2007; Voineagu et al., 2011). The predominant nuclear isoform of RBFOX1 is necessary for appropriate neuronal excitation through control of the alternative splicing of numerous transcripts, including genes associated with increased susceptibility to autism (Weyn-Vanhentenryck et al., 2014). Downregulation of RBFOX1 and aberrant splicing of putative RBFOX1 targets was identified by RNA sequencing (RNA-seq) in the cortex of autistic patients (Voineagu et al., 2011). A neural-specific knockout mouse model of RBFOX1 revealed aberrant splicing of mRNA targets involved in neuronal excitation and synaptic function (Gehman et al., 2011). Furthermore, these mice present with epilepsy, a frequent comorbidity in autistic-like traits. Besides splicing regulation in the nucleus, a cytoplasmic RBFOX1 isoform lacking the nuclear localization signal is produced from alternatively spliced transcripts. Cytoplasmic RBFOX1 mostly binds 3' UTRs and regulates mRNA stability and translation of cortical development and autism-related genes (Lee et al., 2016a). Notably, another RBP, ELAVL2/HuB, was shown to affect the splicing and transcription of transcripts also targeted by FMRP and RBFOX1. Common targets include several synaptic proteins and ASD-associated genes (Berto et al., 2016), suggesting coordinated regulation and providing an additional candidate for ASD targeted therapeutics. ELAVL/Hu proteins are also involved in neurological diseases related to the production of autoantibodies and their roles are discussed in greater detail in the corresponding section.

Senataxin: An RNA/DNA Helicase Involved in Neurodegeneration

A severe recessive disorder called ataxia with oculomotor apraxia type 2 (AOA2) (Moreira et al., 2004) and a dominant juvenile form of ALS (ALS4) (Chen et al., 2004) are caused by mutations in the Senataxin gene (*SETX*), an RNA/DNA helicase. AOA2 is an autosomal recessive genetic disorder characterized by cerebellar atrophy, oculomotor apraxia, and axonal sensorimotor neuropathy, while ALS4 is a dominantly inherited form of

juvenile ALS. Most Senataxin mutations cause premature protein termination, interfere with the function of the helicase, or affect N-terminal protein interaction domains (Chen et al., 2004; Criscuolo et al., 2006; Duquette et al., 2005; Fogel and Perlman, 2006; Moreira et al., 2004). Senataxin regulates gene expression by inducing transcriptional termination (Skourti-Stathaki et al., 2011). An RNA/DNA duplex is formed with the nascent mRNA, behind polymerase II (Pol II) and after 3' cleavage of poly(A) sites. Senataxin acts to resolve these duplex structures, allowing access for the 5'–3' exonuclease Xrn2 at 3' cleavage poly(A) sites and promoting Rad51 foci formation to facilitate double-strand break repair at active genes (Cohen et al., 2018). This facilitates 3' transcript degradation and consequent Pol II termination (Alzu et al., 2012; Skourti-Stathaki et al., 2011; Yüce and West, 2013). Another study reveals that it is the dimethylation of arginine residues in the C-terminal domain of Pol II that recruits SMN, which can then interact with senataxin to facilitate the resolution of R-loops and affect transcription termination (Zhao et al., 2016).

Angiogenin Loss of Function: A Role for tRNA Maturation in Neurodegeneration

Heterozygous missense mutations in the coding region of hypoxia-inducible factor angiogenin (*ANG*) segregate with familial and sporadic ALS, resulting in ANG loss of function (Fernández-Santiago et al., 2009; Greenway et al., 2006; Paubel et al., 2008; Wu et al., 2007; Zou et al., 2012). ANG, a member of the pancreatic ribonuclease A (RNase A) superfamily (Fett et al., 1985), is a key factor in the control of motor neuron survival by protecting against excitotoxic injury (Kieran et al., 2008). Several aspects of ANG are necessary to induce angiogenesis, the process of new blood-vessel growth, including ribonuclease activity, basement membrane degradation, signaling transduction, and nuclear translocation (Gao and Xu, 2008). In cancer cells, ANG can move into the nucleus and is able to bind to DNA and stimulate ribosomal RNA transcription (Tsuji et al., 2005). ANG is also an important component of stress-induced translational repression by inducing cleavage of tRNAs and accumulation of tRNA-derived, stress-induced small RNAs (tiRNAs) (Fu et al., 2009; Yamasaki et al., 2009). ANG mutants associated with ALS are unable to induce angiogenesis because of a deficiency in ribonuclease activity, nuclear import, and nuclear localization (Crabtree et al., 2007; Greenway et al., 2006; Wu et al., 2007).

Autoantibodies against RBPs Cause Loss-of-Function Phenotypes Associated with Neurological Disorders

Paraneoplastic opsoclonus-myoclonus ataxia (POMA) is a neurological syndrome caused by the secretion of auto-antibodies against neuro-oncological ventral antigen (Nova) 1 and 2 expressed by systemic tumors (Buckanovich et al., 1993), with an estimated incidence of 1 in 5,000,000. Nova-1 and Nova-2 are neuron-specific nuclear RBPs that are involved in the regulation of splicing (Ule et al., 2003, 2005b, 2006) and alternative polyadenylation (Licatalosi et al., 2008). Deficit of Nova proteins alters the processing of genes involved in inhibitory synaptic transmission (Dredge et al., 2005), as well as synaptogenesis (Ruggiu et al., 2009) and neuronal migration (Yano et al., 2010).

Anti-neuronal Nuclear (Hu) antibody associated paraneoplastic sensory neuronopathy and/or paraneoplastic encephalomyelitis (PSN-PEM) is an autoimmune disorder in which

patients develop symptoms of CNS dysfunction and/or sensory neuropathy not caused by metastases or other disorders (Lukacs et al., 2012; Senties-Madrid and Vega-Boada, 2001). ELAV/Hu proteins (HuB, HuC, and HuD) are a family of RNA-binding proteins implicated in neuronal differentiation and maintenance (Akamatsu et al., 1999; Anderson et al., 2000; Wakamatsu and Weston, 1997; Zhu et al., 2006). Hu proteins affect many post-transcriptional aspects of RNA metabolism, from splicing to translation. They regulate mRNA stability by interacting with AU-rich elements (AREs) in 3' UTRs (Anderson et al., 2000; Darnell et al., 2011; Deschênes-Furry et al., 2006; Fan and Steitz, 1998; Hinman and Lou, 2008; Myer et al., 1997) and were also shown to modulate translation of mRNA targets through diverse mechanisms. Most frequently, Hu proteins were found to increase translation initiation, either by binding to the 3' UTR and promoting stability (Antic et al., 1999; Kawai et al., 2006; Mazan-Mamczarz et al., 2003) or by binding to the 5' UTR (Galbán et al., 2008). In the nucleus, Hu proteins interact with U-rich sequences to promote RNA stability, polyadenylation, splicing, and translation (Bellavia et al., 2007; Zhu et al., 2006, 2007, 2008).

Aggregation-Prone RBPs at the Core of Neurodegenerative Diseases

Multiple RBPs Containing Low Complexity Domains Are Mutated in ALS and FTD

Another emerging mechanism relies on the intrinsic aggregation-prone properties of several RBPs that form abnormal inclusion bodies in pathological conditions (Figure 1B). This process may result in either a gain of toxic properties, a loss of function, or both, via the RBP's cellular mislocalization and sequestration into inclusions. Disruption of RBP homeostasis has emerged as a major disease mechanism in ALS and FTD, two neurodegenerative conditions with clinical, genetic, and pathological overlap. ALS, which has an incidence of 1–2 cases per 100,000 each year, is an adult onset motor neuron disease characterized by degeneration of motor neurons in the brain and spinal cord leading to progressive muscle weakness and fatal paralysis. FTD has a prevalence of approximately 20 in 100,000 and is characterized by alterations in behavior, personality, and language associated with atrophy of the frontal and temporal lobes. Motor neuron disease and cognitive deficits of variable severity can be concomitant in patients or within families indicating a spectrum of clinical phenotypes that relate to common neuropathologic lesions in ALS and FTD.

Aggregation of different RBPs, including TAR DNA binding protein 43 (TDP-43) (Arai et al., 2006; Neumann et al., 2006), the FET proteins (Fused in sarcoma/Translocated in liposarcoma, FUS/TLS; Ewing's sarcoma, EWS; and TATA-binding protein-associated factor 15, TAF-15) (Couthouis et al., 2011, 2012; Kwiatkowski et al., 2009; Vance et al., 2009), a subset of heterogeneous ribonucleoprotein proteins (hnRNPA2B1 and hnRNPA1) (Kim et al., 2013), the Matrin 3 (MATR3) (Johnson et al., 2014), and the T cell-restricted intracellular antigen-1 (TIA1) (Mackenzie et al., 2017) proteins, have been implicated in a spectrum of neurodegenerative diseases including ALS and FTD.

Initial evidence pointing to a role for RNA processing in ALS and FTD has been the groundbreaking discovery of TDP-43 as a component of cytoplasmic and ubiquitinated inclusions in neurons of patients with sporadic ALS and FTD (Arai et al., 2006; Neumann et al., 2006). This finding was rapidly followed by the identification of TDP-43 mutations as genetic causes of ALS and FTD (Lagier-Tourenne et al., 2010). TDP-43 is primarily nuclear but shuttles between the nucleus and cytoplasm and has been implicated in several steps of RNA metabolism including splicing, transport, RNA stability, and translational repression within stress granules (Alami et al., 2014; Costessi et al., 2014; Fiesel et al., 2012; Li et al., 2013; Polymenidou et al., 2011; Tollervy et al., 2011). Most TDP-43 mutations causing familial ALS cluster within the C-terminal domain, which corresponds to a glycine-rich region essential for interactions with other proteins (Lagier-Tourenne et al., 2010). The N-terminal part of TDP-43 was recently shown to mediate physiological TDP-43 oligomerization and to antagonize the formation of pathologic aggregates (Afroz et al., 2017). Notably, TDP-43 pathology is also characterized by a striking loss of nuclear staining in neurons with cytoplasmic aggregation (Neumann et al., 2006). Determining the effect of this nuclear loss on the processing of TDP-43 mRNA targets in affected neurons represents a crucial step in elucidating disease mechanisms in TDP-43 proteinopathies.

In addition to TDP-43, mutations and mislocalization of the FET family proteins (FUS/TLS, EWS and TAF15) are also associated with ALS and/or FTD. FUS/TLS is a primarily nuclear RBP involved in various aspects of the RNA life cycle, including transcription, splicing and mRNA transport (Kanai et al., 2004; Lagier-Tourenne et al., 2010), that was identified in cytoplasmic inclusions of ALS and/or FTD patient neurons distinct from TDP-43 positive inclusions (Kwiatkowski et al., 2009; Neumann et al., 2009; Vance et al., 2009). FUS/TLS harbors a N-terminal domain enriched in glutamine, glycine, serine, and tyrosine residues that contains disease-causing mutations (Lagier-Tourenne et al., 2010). However, the majority of ALS and FTD-associated mutations are found in a C-terminal, non-canonical nuclear localization signal (NLS). Mutations in the NLS impair appropriate nuclear targeting (Dormann et al., 2010), likely contributing to subsequent cytoplasmic aggregation. Nuclear import of FUS is also influenced by abnormal arginine methylation of the protein in ALS and FTD (Dormann et al., 2010; Suárez-Calvet et al., 2016; Tradewell et al., 2012). Similarly, dominant mutations in both TAF15 and EWS have been identified in ALS and/or FTD patients (Couthouis et al., 2011, 2012), and both proteins were found in cytoplasmic aggregates in sporadic ALS (Couthouis et al., 2011, 2012) and FUS-positive sporadic FTD patients (Neumann et al., 2011).

Two members of the family of hnRNPs, hnRNPA2/B1, and hnRNPA1 were associated with a multisystemic disorder that includes ALS and FTD (Kim et al., 2013). Mutations in the C-terminal glycine-rich region of the proteins were identified in families with ALS or with a condition called multisystem proteinopathy (MSP) that includes inclusion body myopathy, Paget's disease of the bone, FTD, and ALS (Kim et al., 2013). Finally, ALS-causing mutations were identified in the gene encoding MATR3 (Johnson et al., 2014), an RBP interacting with TDP-43 (Ling et al., 2010), and involved in RNA splicing (Coelho et al., 2015), export

(Boehringer et al., 2017), and stability (Salton et al., 2011), and in the gene encoding Tia1 (Mackenzie et al., 2017), a major component of stress granules (Gilks et al., 2004; Kedersha et al., 2000).

A unifying feature shared by RBPs associated with neurodegenerative diseases is the presence of prion-like domains (PrLDs) that promote the formation of self-nucleating aggregates (March et al., 2016). PrLDs are characterized by low complexity sequences rather than a specific primary sequence and are enriched in polar, uncharged amino acids such as glutamine, asparagine, tyrosine, serine, and glycine (Alberti et al., 2009; Ross et al., 2005; Toombs et al., 2010; Wang et al., 2018). Such low complexity domains (LCDs) composed of only a few amino acids were predicted in more than 200 proteins in the human genome (Lancaster et al., 2014) with a clear enrichment for RNA and DNA binding proteins. These domains appear to be crucial for the dynamic assembly and disassembly of ribonucleoprotein granules (RNPs) (Gitler and Shorter, 2011; Kim et al., 2013; King et al., 2012; Li et al., 2013), as well as to confer the intrinsically aggregation-prone property of several RBPs associated with neurodegenerative diseases.

Indeed, low-complexity domains were recently shown to enable liquid-liquid phase separation, a process crucial for the dynamic formation of supramolecular assemblies constituting membraneless cellular compartments (Lin et al., 2015; Molliex et al., 2015; Nott et al., 2015; Patel et al., 2015). The ability of RBPs to undergo phase separation is intimately linked to their dynamic association with various nuclear and cytoplasmic membraneless organelles, including the nucleolus, nuclear speckles, Cajal bodies, nuclear pores, cytoplasmic stress granules, and P-bodies (Brangwynne et al., 2009; Frey et al., 2006; Hyman et al., 2014; Jain et al., 2016; Li et al., 2012). Failure to maintain liquid phase homeostasis has been shown to trigger fibrillization of RBPs and is proposed to be at the root of abnormal inclusions observed in neurodegenerative diseases including ALS, FTD, and Alzheimer's disease (March et al., 2016; Boeynaems et al., 2018; Burke et al., 2015; Ambadipudi et al., 2017; Conicella et al., 2016; Han et al., 2012; Hernández-Vega et al., 2017; Kato et al., 2012; Mackenzie et al., 2017; Mateju et al., 2017; Molliex et al., 2015; Monahan et al., 2017; Murakami et al., 2015; Murray et al., 2017; Patel et al., 2015; Xiang et al., 2015). Phase transition behavior from solute to liquid-like, gel-like, and solid states is influenced by various factors including temperature, local protein and RNA concentrations, or the amino acid composition and post-translational modifications of the LCDs (Boeynaems et al., 2018; Maharana et al., 2018; Wang et al., 2018). Mutations associated with neurodegeneration largely cluster within low complexity domains in the N-terminal domain of FUS, TAF15, and EWS, and in the C-terminal of TDP-43, hnRNP2/B1, hnRNP1, and Tia1. Disease-associated mutations in the LCDs alter the physicochemical properties of RBPs with respect to phase separation leading to a faster and sometimes irreversible transition of RBPs from liquid droplets into less dynamic structures such as hydrogels and eventually fibrillar solids reminiscent of the aggregations observed in post-mortem human CNS tissues (Kim et al., 2013). Notably, increasing the cytoplasmic concentration of RBPs results in liquid-liquid phase separation and increased assembly of stress granules (Guo et al., 2018; Kim et al., 2013; Lin et al., 2015; Mackenzie et al.,

2017; Molliex et al., 2015; Murakami et al., 2015; Patel et al., 2015). Hence, while ALS and/or FTD mutations in the NLS of FUS do not directly alter the propensity of FUS to aggregate (Sun et al., 2011), they impair nuclear import of FUS leading to higher cytoplasmic concentration and phase separation of the protein into FUS droplets that mature to more solid aggregates.

While it appears that RBPs are prone to aggregation and may play a role in disease propagation due to PrLDs (Polymenidou and Cleveland, 2011), a great deal remains unknown about the interplay between the proteins, the mechanistic role of the various mutations in each of the proteins, and the potential for aggregate gain of function.

Genome-wide Studies Reveal Complex Networks between Disease-Associated RBPs and Their RNA Targets

Notably, RBP levels are tightly regulated and genome-wide analyses of binding sites by cross-linking immunoprecipitation (CLIP) approaches (Licatalosi et al., 2008; Ule et al., 2005a) have uncovered complex regulatory mechanisms between different RBPs (Huelga et al., 2012; Lagier-Tourenne et al., 2012; Martinez et al., 2016; Polymenidou et al., 2011; Rogelj et al., 2012; Sanford et al., 2009; Tollervey et al., 2011; Yeo et al., 2009). Indeed, RBPs often bind their own RNAs, as well as RNAs encoding other RBPs, generating feedback loops crucial for RNA homeostasis. For example, both TDP-43 and FUS/TLS bind their own mRNAs and induce dose-dependent splicing alterations that produce transcripts subjected to nonsense-mediated decay as part of complex auto-regulatory mechanisms (Avendaño-Vázquez et al., 2012; Ayala et al., 2011; Bembich et al., 2014; D'Alton et al., 2015; Lagier-Tourenne et al., 2012; Polymenidou et al., 2011; Zhou et al., 2013). ALS-associated mutations in FUS/TLS were shown to compromise this auto-regulatory loop and may participate in a feedforward mechanism enhancing FUS/TLS aggregation in affected neurons (Zhou et al., 2013). Notably, increasing the amount of human up-frameshift protein 1 (hUPF1), an ATP-dependent RNA helicase and central nonsense-mediated decay factor, was shown to rescue toxicity associated with both FUS and TDP-43 overexpression (Barmada et al., 2015; Ju et al., 2011). In addition, intertwined relationships between different aggregation-prone RBPs have been observed, including decreased levels of FUS transcript upon TDP-43 knockdown (Polymenidou et al., 2011), or elevated TAF15 levels upon FUS reduction (Lagier-Tourenne et al., 2012). Consequently, disruption of one RBP may induce widespread alterations of RNA processing engaging direct RNA targets as well as transcripts not bound by the primary altered RBP.

Systematic analyses of the RNA targets of aggregation-prone RBPs, in particular, comparisons of the binding patterns between TDP-43 and FUS/TLS, the members of the FET family (FUS/TLS-EWSR1-TAF15), Tia1, or the hnRNP family have revealed points of convergence but also striking differences in the role of each RBP (Alarcón et al., 2015; Blechingberg et al., 2012; Colombrita et al., 2012; Goodarzi et al., 2012; Hoell et al., 2011; Huelga et al., 2012; Ibrahim et al., 2013; Ishigaki et al., 2012; Kapeli et al., 2016; Lagier-Tourenne et al., 2012; Martinez et al., 2016; Meyer et al., 2018; Nakaya et al., 2013; Polymenidou et al., 2011; Rogelj et al., 2012; Sephton et al., 2011;

Tollervey et al., 2011; Vogler et al., 2018; Xiao et al., 2011). Although genome-wide analyses established that TDP-43 and FUS/TLS have mostly distinct functions in regulating the processing of their targets, an unexpected role was uncovered in sustaining the levels of mRNAs transcribed from genes with exceptionally long introns that encode proteins essential for neuronal function (Lagier-Tourenne et al., 2012; Polymenidou et al., 2011). A subset of these proteins was also found to be reduced in TDP-43 aggregate-containing motor neurons in sporadic ALS, supporting a common loss-of-function pathway as one component underlying motor neuron death in TDP-43 and FUS/TLS proteinopathies (Lagier-Tourenne et al., 2012). In addition, several hundred splicing alterations were identified upon reduction of aggregation-prone RBPs (Ibrahim et al., 2013; Ishigaki et al., 2012; Lagier-Tourenne et al., 2012; Martinez et al., 2016; Paronetto et al., 2011; Polymenidou et al., 2011; Rogelj et al., 2012; Tollervey et al., 2011). Only a subset of TDP-43-regulated exons identified by RNA-seq had previous expressed sequence tag (EST)/mRNA evidence for alternative splicing, demonstrating a role for TDP-43 to regulate previously unknown alternative splicing events (Polymenidou et al., 2011). Among non-annotated aberrant splicing events, approximately 50 exons were found to be included upon TDP-43 depletion, consistent with a normal role for TDP-43 in repressing the inclusion of cryptic exons (Ling et al., 2015; Tan et al., 2016). Abnormal inclusion of cryptic exons (Ling et al., 2015) along with aberrant splicing of the *SORTILIN 1* and *POLDIP3* transcripts (Prudencio et al., 2012; Shiga et al., 2012) observed in brain tissues of ALS and/or FTD patients with TDP-43 pathology strongly support a loss-of-function mechanism in TDP-43 proteinopathies. Among thousands of TDP-43-mediated RNA alterations, human stathmin-2 was recently shown to be the most affected transcript upon TDP-43 loss (Kim et al., 2019; Melamed et al., 2019). Aberrant splicing and premature polyadenylation lead to accumulation of a truncated transcript in affected tissues from sporadic and C9ORF72-related ALS patients and restoration of stathmin-2 levels was shown to be crucial for axonal regeneration upon TDP-43 loss. In addition, FUS was shown to mediate the interaction between RNA polymerase II and U1 snRNP, hence coupling transcription to splicing (Sun et al., 2015; Yu et al., 2015). Besides alternative splicing, both TDP-43 and FUS/TLS were proposed to influence the use of alternative polyadenylation sites (Masuda et al., 2015; Rot et al., 2017). Genomic approaches also provided insights on the RNA binding sites and roles on alternative splicing and polyadenylation of hnRNPA2B1 (Alarcón et al., 2015; Goodarzi et al., 2012; Martinez et al., 2016) and Tia1 (Meyer et al., 2018). Notably, hnRNPA2B1 was found to bind m⁶A-bearing RNAs and elicit similar alternative splicing effects as the m⁶A writer METTL3 (Alarcón et al., 2015). Finally, TDP-43, FUS and hnRNPA2B1 were shown to bind microRNAs and modulate microRNA biogenesis with broad implications on RNA biology (Alarcón et al., 2015; Eitan and Hornstein, 2016). Overall, genomic approaches have uncovered widespread RNA processing alterations in aggregation-prone neurodegenerative diseases. Identification of splicing and/or expression alterations that directly contribute to neuronal dysfunction may have major implications for therapeutic development in ALS and FTD.

The Cytoplasmic Roles of RBPs Are Also Altered in ALS and FTD

It is noteworthy that alterations of an RBP's cytoplasmic functions may play a role in neurodegeneration along with loss of nuclear functions in affected neurons. Indeed, aggregation-prone RBPs involved in ALS and/or FTD such as TDP-43, FUS/TLS, TAF-15, EWSR1, hnRNPA1, and hnRNPA2/B1 have been implicated in RNA transport and local translation of their RNA targets (Alami et al., 2014; Andersson et al., 2008; Jean-Philippe et al., 2013; Lagier-Tourenne et al., 2010). Notably, the cytoplasmic function of TDP-43 in delivering mRNA targets to distal neuronal compartments is impaired in the presence of ALS-related mutations (Alami et al., 2014). Comparative CLIP analysis of wild-type FET proteins (FUS, EWSR1, and TAF15) and ALS mutant FUS/TLS has also revealed that mutant FUS/TLS binds more cytoplasmic targets, as demonstrated by increased fraction of binding on mature RNAs (UTRs and coding regions), consistent with the cytoplasmic mislocalization of mutant FUS/TLS (Hoell et al., 2011). A role of the FET proteins in mRNA transport has been proposed (Andersson et al., 2008), but it is still unknown whether ALS-linked mutations disrupt the transport of specific mRNA targets. However, deficient axonal transport of mitochondria and lysosomes was recently identified in iPSC-derived motor neurons with FUS mutations (Guo et al., 2017; Naumann et al., 2018). Expression of human mutant FUS in transgenic mice was also shown to activate an integrated stress response and to inhibit intra-axonal protein synthesis in hippocampal neurons and sciatic nerves (Lopez-Erauskin et al., 2018).

Finally, aggregation-prone RBPs involved in neurodegeneration often co-localize with markers of stress granules. Stress granules are dynamic, self-assembling, membraneless structures that contain translationally arrested mRNA-protein complexes, and usually form in the presence of cellular stress. They are believed to function as a triage unit to determine whether mRNAs should be degraded, released for translation, or remain sequestered and translationally repressed (Protter and Parker, 2016). As discussed above, unbalanced assembly and disassembly of stress granules through altered phase separation homeostasis is likely to play a role in the emergence of cytoplasmic inclusions observed in patients (Bosco et al., 2010; Li et al., 2013; Mackenzie et al., 2017; Mitchell and Parker, 2014; Murakami et al., 2015; Patel et al., 2015; Ramaswami et al., 2013; Wolozin, 2012). The implementation of enzymatic-based biotin proximity labeling identified hundreds of known and previously unknown stress granule-associated proteins, and it was shown *in vivo* that a subset of these stress granule-associated RBPs is capable of modulating the toxicity driven by mutant ALS-associated RBPs FUS and TDP-43 (Markmiller et al., 2018). The RBP TIA1 was also shown to interact with tau to promote tau misfolding, leading to protein aggregation and formation of stress granules both *in vitro* (Vanderweyde et al., 2016) and *in vivo* (Apicco et al., 2018). Finally, besides the cytoplasmic role of aggregation-prone RBPs in stress granules, TDP-43 was recently shown to play a functional role in muscle formation and regeneration through the constitution of amyloid-like oligomeric assemblies called “myo-granules” which are cleared as myofibers mature (Vogler et al., 2018).

Aggregation of RBPs in Polyglutamine Expansion Diseases

CAG trinucleotide repeat expansions encoding for long polyglutamine (polyQ) stretches are associated with several neurodegenerative diseases including Huntington's disease (a progressive disorder resulting in impaired cognition, psychiatric manifestations and abnormal movements called chorea) and several spinocerebellar ataxias (SCAs). It is noteworthy that polyQ-containing proteins were shown to sequester different RBPs containing LCDs (Figure 1C). In particular, FUS was identified by a proteomic approach as a major component of the intranuclear polyQ aggregates in cellular models of Huntington's disease and SCA3 (Doi et al., 2008) and found to colocalize with aggregates in brains from mice and patients (Doi et al., 2008, 2010). Sequestration of TDP-43 dependent on its C-terminal LCD was also observed in cellular models overexpressing polyQ (Fuentealba et al., 2010). However, it is still not established whether altered processing of TDP-43 and FUS RNA targets contribute to the pathogenesis of polyQ neurodegenerative diseases.

Besides the indirect effect of polyQ stretches in sequestering RBPs, trinucleotide (CAG) repeats within two RBPs, ataxin 1 and ataxin 2, cause SCA types 1 and 2 (SCA1 and SCA2). SCA2 is triggered by an expansion of more than 33 repeats and is characterized by a progressive cerebellar ataxia leading to abnormal balance, eye movements, and speech, often associated with dystonia, chorea, or dementia. In addition to SCA2, intermediate-length polyQ expansions (27–33 glutamines) in the *ATXN2* gene are associated with an increased risk of ALS (Elden et al., 2010). Ataxin 2 interacts with the poly(A)-binding protein (PABP) and DEAD/H-box RNA helicase (DDX6) (Ciosk et al., 2004; Hua and Zhou, 2004; Nonhoff et al., 2007; Ralser et al., 2005), two components of stress granules and P-bodies, which are stress-induced cytoplasmic foci that have a role in RNA degradation and may dock with stress granules to receive RNAs for turnover (Kedersha et al., 2005). Alteration of ataxin 2 levels interferes with the assembly of P-bodies and stress granules, and ataxin 2 is required for miRNA-mediated repression of several translational reporters *in vivo* (McCann et al., 2011; Nonhoff et al., 2007). Direct binding of ataxin 2 to RNAs was recently demonstrated using a CLIP approach (Yokoshi et al., 2014). Ataxin 2 binds a uridine-rich element within the 3' UTR of its targets in a PABP1-independent manner and likely plays a role in the regulation of polyadenylation and stabilization of its mRNA targets (Ostrowski et al., 2017; Yokoshi et al., 2014). In addition, ataxin 2 was shown to assemble with polyribosomes (Satterfield and Pallanck, 2006) and global reduction of protein synthesis in *Atxn2* knockout mice suggest that ataxin 2 promotes general translation (Fittschen et al., 2015). Finally, ataxin 2 was shown to preserve genome integrity by repressing the deleterious accumulation of RNA-DNA R-loop structures within the nucleus (Salvi et al., 2014). The exact mechanisms leading to either SCA2 or ALS are not well understood; however, ataxin 2 protein was identified as a major modulator of TDP-43 toxicity. Indeed, ataxin 2 directly interacts with TDP-43 and its reduction suppresses TDP-43 toxicity in various models by affecting its recruitment to stress granules and aggregation propensity (Becker et al., 2017; Elden et al., 2010; Hart and Gitler, 2012).

Similarly, ataxin 2 interacts with FUS and was identified as a modifier of FUS pathology (Farg et al., 2013; Nihei et al., 2012). The intertwined relationship between ataxin 2 and other ALS-associated RBPs, including the striking repression of TDP-43 toxicity when lowering ataxin 2 levels in various animal models, designates ataxin 2 as a promising therapeutic target in ALS (Becker et al., 2017; Elden et al., 2010).

RBP Sequestration by Expanded RNAs and Proteins RNA-Mediated Toxicity: An Alternative Path to Disrupt RBPs

Advances in our understanding of several neurodegenerative disorders have brought RBPs into the spotlight as having key roles in the development of pathology. The examples described above involve mutations within genes encoding RBPs; however, an alternative mechanism leading to the functional disruption of RBPs has emerged in diseases linked to microsatellite repeat expansions (Figure 1C). This mechanism, called RNA-mediated toxicity, involves sequestration of RBPs through binding to transcripts that contain hundreds or thousands of repeats, rendering the RBP unavailable for other substrates. Various RBPs have been implicated as essential contributors to such expansion-mediated pathology. Myotonic dystrophy (dystrophia myotonica, DM), ALS, FTD, and fragile X tremor and ataxia syndrome (FXTAS) are all characterized by genomic repeat expansions that, when transcribed into RNA, can sequester RBPs. This may occur in a stoichiometric fashion in which expansion of the repeat creates an excess of RBP binding sites. Alternatively, an RBP may have a greater affinity for either the primary or secondary structure of the repeat expansion. In both cases, the RBP is prevented from interacting with its endogenous targets, causing a multitude of biological effects that ultimately contribute to pathology.

RNA Toxicity in Myotonic Dystrophies

Myotonic dystrophies types 1 and 2 (DM1 and DM2) are inherited multisystemic disorders affecting ~1 in 8,000 individuals. Adult forms are characterized by muscular weakness and delayed relaxation of skeletal muscles (myotonia) and may include cataracts, learning disability, hypersomnia, insulin resistance, and heart conduction defects. Congenital forms of DM1 result in severe neonatal hypotonia and breathing defects associated with cognitive disabilities. In both DM1 and DM2 a genomic repeat expansion results in the accumulation of repeat-containing transcripts that aberrantly sequester RBPs. DM1 is caused by a (CTG)_{50 > 3,500} repeat expansion in the 3' UTR of the *DMPK* gene (Brook et al., 1992), while a repeat expansion of (CCTG)_{75 > 11,000} located in intron 1 of the *ZNF9* gene causes DM2 (Liquori et al., 2001). Transcripts containing expanded (CTG) or (CCTG) repeats sequester and compromise the functions of RBPs from the muscle blind like protein family (MBNL1, 2, and 3) (Mankodi et al., 2000; Wang et al., 2012a). MBNL1 is a splicing regulator that co-localizes to CUG expansion-containing nuclear RNA foci (Jiang et al., 2004), and *in vivo* overexpression of MBNL1 efficiently reverses the DM1 myotonia phenotype (Kanadia et al., 2006). More specifically, it has been shown that sequestration of MBNL1 results in the aberrant splicing of several genes, a subset of which is directly involved in muscle function and the DM1 phenotype (Freyermuth et al.,

2016; Goodwin et al., 2015; Lee and Cooper, 2009). In addition to MBNL1 disruption, steady-state levels of CUGBP1, another splicing regulator, increase due to protein kinase C-mediated hyperphosphorylation in response to the expression of repeat expansions (Kuyumcu-Martinez et al., 2007). Notably, functional disruption of another member of the muscle blind like family, MBNL2, was shown to account for the neurological features (learning difficulties, daytime sleepiness) observed in DM1 patients (Charizanis et al., 2012). Depletion of MBNL3 was also shown to cause defects in muscle regeneration and a spectrum of DM1 age-associated pathologies including abnormal glucose metabolism in knockout mice (Choi et al., 2016; Poulos et al., 2013). Disruption of MBNL proteins in DM1 tissues leads to the re-emergence of developmentally immature alternative splicing. In addition to their role in alternative splicing, MBNL proteins have also been shown to bind the 3' UTRs of their targets, affecting polyadenylation, RNA localization and turnover (Goodwin et al., 2015; Masuda et al., 2012; Wang et al., 2012a). Depletion of MBNL proteins led to misregulated polyadenylation, which was also observed in mouse models expressing CUG repeats and in muscles and brains from DM1 patients (Batra et al., 2014; Goodwin et al., 2015).

RNA Toxicity in ALS and FTD Linked to C9orf72

A hexanucleotide (G₄C₂) repeat expansion in the *C9ORF72* gene represents the most frequent genetic cause of ALS and FTD (DeJesus-Hernandez et al., 2011; Renton et al., 2011), with patients exhibiting hundreds to thousands of repeats compared to less than 30 in healthy individuals. At present, it is unresolved as to whether neurodegeneration is due to a loss of C9ORF72 function, a gain of toxic function or a combination of the two. However, accumulating evidence supports an RNA toxicity mechanism as a key mediator of pathogenesis. Indeed, transcripts containing the G₄C₂ repeat expansion have been shown to accumulate into nuclear RNA foci similar to those observed in myotonic dystrophy (DeJesus-Hernandez et al., 2011; Gendron et al., 2014). As in other microsatellite diseases, foci containing C₄G₂ expanded RNAs transcribed from the antisense strand of the *C9ORF72* locus have also been identified in cells and tissues from C9ORF72 ALS and/or FTD patients (Gendron et al., 2013; Lagier-Tourenne et al., 2013; Mizielinska et al., 2013; Mori et al., 2013a; Zu et al., 2013). A flurry of reports have proposed several candidate RBPs that may interact with *C9ORF72* hexanucleotide repeats *in vitro* (Cooper-Knock et al., 2014; Mori et al., 2013b; Reddy et al., 2013; Xu et al., 2013), including hnRNP H1/F (Conlon et al., 2016), ALYREF, SRSF2 (Cooper-Knock et al., 2014; Lee et al., 2013), hnRNPA1 (Sareen et al., 2013), hnRNP A3 (Mori et al., 2013b), ADARB2 (Donnelly et al., 2013), Pur- α (Sareen et al., 2013), and Nucleolin (Haeusler et al., 2014), that were found to partially co-localize with sense strand RNA foci. Transcriptome profiles in cells and brains from C9ORF72 ALS patients were shown to be distinct from sporadic ALS (Conlon et al., 2016; Cooper-Knock et al., 2015; Donnelly et al., 2013; Lagier-Tourenne et al., 2013; Prudencio et al., 2015; Sareen et al., 2013); however, it has not yet been established which RBP(s) is/are functionally disrupted by sequestration in RNA foci. Only hnRNP H-dependent splicing alterations have been reported in cells and tissues expressing G₄C₂ expansions (Con-

lon et al., 2016; Lee et al., 2013). Importantly, the capacity for the antisense RNA C₄G₂ repeat expansion to sequester RBPs requires further investigation and there is no consensus yet whether one or several RBPs are functionally disrupted in C9ORF72 disease leading to misregulation of their RNA targets. While interaction between RBPs and the expanded repeat RNA has been proposed, the structural and biochemical details remain relatively unknown. It has been proposed that the guanine-rich repeats form a G-quadruplex that preferentially binds a subset of RBPs (Fratta et al., 2012; Haeusler et al., 2014; Reddy et al., 2013; Zamiri et al., 2014). It will be interesting to more thoroughly explore structural dependencies of RNA-RBP interactions on a broader scale. An additional mechanism of disrupting RNA metabolism in C9ORF72 disease and other repeat expansion disorders is called repeat-associated non-ATG-dependent (RAN) translation and is discussed in detail below.

RNA Toxicity in FXTAS

Fragile X-associated tremor/ataxia syndrome (FXTAS) is yet another neurodegenerative repeat expansion disease where an RNA-mediated toxicity has been proposed as a mechanism underlying disease pathology. FXTAS presents with movement abnormalities including intention tremor, cerebellar ataxia, or parkinsonism and, in some instances, cognitive impairment. FXTAS is characterized by a CGG₅₅ > 200 expansion in the 5' UTR of the *FMR1* gene (Hagerman and Hagerman, 2007; Hagerman et al., 2001; Jacquemont et al., 2003). Although larger repeat expansions cause FXS as discussed above, intermediate repeat expansions of 55–200 repeats lead to a clinically distinct neurodegenerative disorder with late-onset ataxia and tremor. Contrary to FXS, the intermediate repeat expansion in *FMR1* is associated with increased mRNA expression but reduced expression of the FMRP protein proportional to repeat length (Kenneson et al., 2001). The mechanism of pathogenesis is still debated, but there is some evidence that the repeat expansion in RNA acts to sequester RBPs. For example, both Pur- α and hnRNPA2/B1 have been reported to bind the repeat expansion in a fly model (Jin et al., 2007; Sofola et al., 2007). These RBPs typically function in mRNA localization and transport in neurons, and loss of function by sequestration could lead to a severe phenotype. Perhaps most compellingly, overexpression of Pur- α and hnRNPA2/B1 RBPs in the fly model alleviated the neurodegenerative phenotype. In addition to these RBPs, significant work demonstrated sequestration of DGCR8 and DROSHA in both human and mouse FXTAS tissues (Sellier et al., 2013). DGCR8 and DROSHA co-localize with CGG repeats, and the processing of miRNAs was found to be reduced in patient tissues, further supporting a sequestration model. In addition, while dendritic complexity and neuronal cell viability was reduced in primary cultures of mouse cortical neurons expressing CGG repeats, overexpression of DGCR8 in these cells rescued both dendritic morphology and neuronal cell death. Surprisingly, Sellier et al. identified Pur- α and hnRNPA2/B1 as binding CGG repeats, but preferentially to non-pathogenic short repeats. In addition to DGCR8 and DROSHA, sequential sequestration of Sam 68, hnRNPG, and MBNL1 by CGG repeat expansions was identified and confirmed by fluorescence in situ hybridization (FISH)/immunofluorescence (IF) (Sellier et al., 2010). Loss

of Sam68, an RBP involved in regulation of alternative splicing, causes defects in motor coordination (Lukong and Richard, 2008) supporting that sequestration of Sam68 may play a significant role in the FXTAS pathology, although further transcriptome-wide studies identifying the splicing defects in FXTAS patients must be undertaken.

Sequestration of RBPs by Abnormal Proteins Translated from Expanded Repeats

As discussed above, long polyQ stretches translated from repeat expansions in coding regions were shown to directly or indirectly alter RBP homeostasis. In addition, transcripts with repeat expansions in coding and non-coding regions were shown to undergo RAN translation, producing abnormal mono or dipeptide repeat proteins (Zu et al., 2011). RAN translation occurs in the absence of an AUG start codon, in multiple reading frames of the same transcript, and within coding as well non-coding regions. This mechanism has now been described in several expansion diseases including SCA8 (Zu et al., 2011) and SCA31 (Ishiguro et al., 2017), DM1 and DM2 (Zu et al., 2017; Zu et al., 2011), Huntington's disease (Bañez-Coronel et al., 2015), FXTAS (Todd et al., 2013), and C9ORF72 ALS and/or FTD (Ash et al., 2013; Gendron et al., 2013; Mori et al., 2013a, 2013b, 2013c; Zu et al., 2013). In C9ORF72 disease, 5 different dipeptide repeat (DPR) proteins translated through RAN translation from both G₄C₂ and C₄G₂ transcripts accumulate in patients. Increasing evidence supports that DPR proteins, in particular, arginine-rich poly-GR and poly-PR proteins, are toxic and play a central role in neurodegeneration due to C9ORF72 expansions (Kwon et al., 2014; May et al., 2014; Mizielinska et al., 2014; Sabeti et al., 2018; Tao et al., 2015; Wen et al., 2014; Yamakawa et al., 2015; Zhang et al., 2014). Most importantly, arginine-containing DPRs were recently shown to interact mainly with RBPs and proteins with LCDs including TDP-43, hnRNPA1, and FUS/TLS (Kanekura et al., 2016; Lee et al., 2016b; Lin et al., 2016; Lopez-Gonzalez et al., 2016). Poly-GR and poly-PR were shown to perturb the phase separation of proteins with LCDs and disrupt the assembly and function of membrane-less organelles such as stress granules, nucleoli and the nuclear pore complex (Boeynaems et al., 2017; Lee et al., 2016b; Lin et al., 2016; Shi et al., 2017). In particular, accumulation of DPRs (Boeynaems et al., 2016; Freibaum et al., 2015; Jovičić et al., 2015; Rossi et al., 2015; Zhang et al., 2015), as well as other aggregation-prone proteins such as TDP-43 (Chou et al., 2018), Huntingtin (Gasset-Rosa et al., 2017; Grima et al., 2017), and Tau (Eftekharzadeh et al., 2018), disrupts the integrity of the nuclear pores, alters nuclear-cytoplasmic transport and induces nuclear retention of RNAs. In addition, poly-GR and poly-PR may trigger splicing alterations through aberrant association with U2 snRNP (Yin et al., 2017) and impair protein translation through interaction with ribosomal proteins (Kanekura et al., 2016; Lopez-Gonzalez et al., 2016; Zhang et al., 2018).

Therapeutic Avenues for the Treatment of RBP-Mediated Neurodegeneration

Application of powerful high-throughput technologies has revealed an RBP-mediated component to several neurological diseases, and with the discovery of novel players comes the potential for new therapeutic targets.

Therapeutics that Restore RBP Expression or Function

Several therapeutic strategies have been developed to restore RBP expression. In SMA, antisense oligonucleotides (ASOs) have been designed to bind an intronic silencer and enhance inclusion of exon 7 of *SMN2* (Figure 2Ai). It was indeed shown that ASO terminal modifications can produce differential RBP recruitment to either enhance or repress exon inclusion (Rigo et al., 2012a). This approach increased *SMN2* levels and improved survival and neuromuscular function in SMA mice (Hua et al., 2008, 2010, 2011; Passini et al., 2011; Rigo et al., 2012b). Most importantly, intrathecal administration of an ASO modulating *SMN2* splicing (Nursinen) was shown to be beneficial in SMA patients (Chiriboga et al., 2016; Finkel et al., 2016, 2017; Mercuri et al., 2018) and represents the first FDA approved ASO-mediated treatment in neurodegenerative diseases. *SMN* depletion was also successfully achieved in patients using an adeno-associated virus (AAV)-mediated gene therapy approach (Figure 2Aiv) (Mendell et al., 2017), altogether demonstrating that restoration of *SMN* protein level is therapeutic (Groen et al., 2018). Other approaches have been investigated in preclinical studies to increase functional *SMN*. For example, modified ASOs were used to increase *SMN2* expression in primary neurons by inhibiting the interaction between a long noncoding RNA (*SMN*-antisense 1) and the polycomb repressive complex 2 (PRC2), which normally repress *SMN2* expression (Woo et al., 2017). In addition, splicing regulation of *SMN2* exon 7 was achieved using small molecules that successfully restored full-length *SMN2* protein in two mouse models through recruitment of the U1 snRNP (Figure 2Aii) (Naryshkin et al., 2014; Pinard et al., 2017; Sivaramakrishnan et al., 2017), which normally has a weak interaction with the 5' splice site of exon 7 (Roca and Krainer, 2009). By enhancing promoter activation, histone deacetylase (HDAC) inhibitors were also shown to alleviate symptoms and increase mean survival in SMA mouse models by increasing *SMN2* levels (Figure 2Aiii) (Avila et al., 2007; Lai et al., 2017; Mutsaers et al., 2011); however, survival benefit was modest when compared to ASO therapy (38 days versus 243 days). Finally, it was recently shown that administration of flunarizine in patient fibroblasts and a mouse model of SMA can facilitate localization of functional *SMN* into nuclear cajal bodies (Sapaly et al., 2018), facilitating proper association of RNPs and thus proper splicing. Though the exact role of *SMN* in cajal bodies is unknown, it is proposed that *SMN* acts as a chaperone for snRNPs thereby affecting the splicing repertoire.

Innovative therapeutic strategies based on the CRISPR technology are emerging, including the development of methods to increase the expression of specific proteins. In the case of FXS, dCas9-Tet1 recruitment to the repeat expansion in the 5' UTR of *FMR1* was shown to cause targeted demethylation of the repeat expansion resulting in reactivation of *FMR1* expression (Figure 2Av) (Liu et al., 2018). Removal of the repeat expansion by CRISPR/Cas9 has also been shown to restore *FMR1* expression in cultured cells (Xie et al., 2016). Furthermore, small molecules that recapitulate the role of RBPs whose function is lost or aberrant in the disease state show great potential as targeted therapeutics. The loss of FMRP and its role in stalling ribosomal translocation has been phenotypically reversed (Figure 2Avi), as shown by dendritic spine maturation

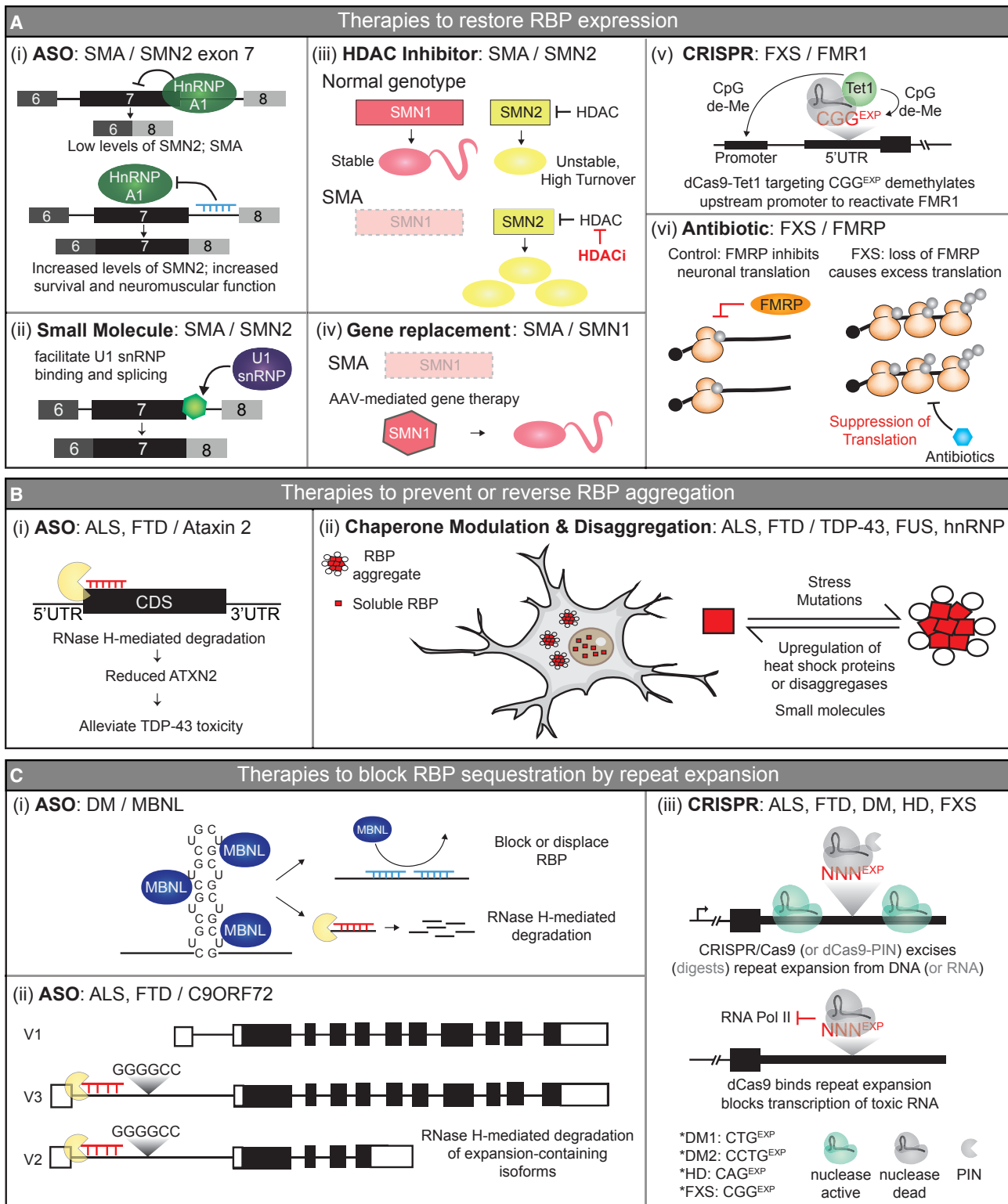


Figure 2. Therapeutic Strategies to Restore the Level and Function of RBPs in Neurodegenerative Diseases

(A) Strategies to restore RBP expression level. (i) Antisense oligonucleotides (ASOs) enhance inclusion of SMN2 exon 7 resulting in increased levels of SMN2 protein. (ii) Exon 7 inclusion in SMN2 achieved via small-molecule recruiting U1 snRNP. (iii) Restoration of SMN2 proteins levels through inhibition of Histone deacetylases (HDACs). (iv) Restoration of SMN1 protein level by AAV-mediated gene therapy. (v) Promotion of FMR1 expression using deactivated Cas9 (dCas9)

(legend continued on next page)

and restored synaptic structure, by the tetracycline analog minocycline in flies (Siller and Broadie, 2011) and mice (Bilousova et al., 2009). The antibiotic functions by occupying the A-site of the ribosome to stall translation and has been specifically shown to inhibit the overexpression of the matrix metalloproteinase-9 that occurs in *Fmr1* knockout mice (Bilousova et al., 2009). There remains some contention as to how exactly minocycline works to alleviate FXS pathology, either through global translational suppression or the suppression of specific targets; however, improvements in adolescent patient anxiety and behavior during a 3-month clinical trial (Paribello et al., 2010), as well as behavioral and cognitive improvements in adults and children (Leigh et al., 2013; Utari et al., 2010), support the need for additional characterization of the mechanism.

Therapeutics that Reverse RBP Aggregation

As discussed herein, aggregation-prone RBPs are tightly regulated and influence the processing of numerous RNA targets, precluding therapeutic strategies that reduce the overall level of the protein including ASO-mediated gene silencing. While infusion of ASOs that have specific chemical modifications leading to RNase-H mediated degradation of RNA targets holds great promise in several dominantly inherited neurodegenerative diseases (Miller et al., 2013; Schoch and Miller, 2017; Smith et al., 2006), ASO-mediated reduction of TDP-43, FUS, TAF15, or hnRNPA2B1 in the CNS of wild-type mice triggered hundreds of RNA expression and splicing alterations (Kapeli et al., 2016; Lagier-Tourenne et al., 2012; Martinez et al., 2016; Polymenidou et al., 2011). Yet, RNase-H-dependent ASOs were successfully used to target ataxin-2, a major modulator of RBP toxicity. Indeed, ASOs targeting ataxin-2 administered to the CNS of TDP-43 transgenic mice extended their survival and improved motor function, in addition to reducing aggregation of TDP-43 protein (Becker et al., 2017) (Figure 2Bi). Importantly, the approach may apply to the vast majority of sporadic ALS patients and other neurodegenerative diseases with TDP-43 proteinopathy.

In addition, efforts to modulate the misfolding and aggregation properties of RBPs have been undertaken in various models via the modulation of chaperone proteins (Figure 2Bii) (Carlomagno et al., 2014; Chen et al., 2016; Crippa et al., 2016; Gregory et al., 2012; Jackrel et al., 2014; Li et al., 2016; Park et al., 2018; Wang et al., 2017). Pharmacological upregulation of heat shock response pathways or genetic upregulation of a single heat shock protein were shown to inhibit TDP-43 aggregation. Notably, re-engineering of the yeast heat shock protein 104 (hsp104) enhanced its recognition of TDP-43 and FUS/TLS leading to significant RBP disaggregation (Jackrel et al., 2014). Most recently, nuclear import receptors were also shown to act as chaperones and potent disaggregases beyond their role in nuclear transport of RBPs (Guo et al., 2018; Hofweber et al.,

2018; Qamar et al., 2018; Yoshizawa et al., 2018). Indeed, Transportin 1 (also called Karyopherin- β 2) was shown to inhibit phase separation, assembly in stress granules and fibrillization of FUS/TLS (Guo et al., 2018; Hofweber et al., 2018; Qamar et al., 2018; Yoshizawa et al., 2018), TAF15, EWSR1, hnRNPA1, and hnRNPA2 (Guo et al., 2018), while Importin- α and Karyopherin- β 1 prevented TDP-43 fibrillization (Guo et al., 2018). While these approaches based on modulating the level and activity of chaperones are challenging to implement in human patients, the drastic effect on aberrant phase separation merits further investigation to determine the therapeutic potential of disaggregases in neurodegenerative diseases.

Finally, small molecules can aid in the clearance of toxic accumulations of TDP-43 observed in ALS and/or FTD patients through the stimulation of autophagy (Barmada et al., 2014; Wang et al., 2012b). Inducing autophagy via small molecules improved TDP-43 clearance and mitigated toxicity in cultured neurons and astrocytes with mutant TDP-43 (Barmada et al., 2014) and improved behavior and pathology in a mouse model overexpressing TDP-43 (Wang et al., 2012b). More recently, binding of TDP-43 to poly(ADP-ribose) (PAR) was shown to promote the liquid-liquid phase transition of TDP-43, and small-molecule inhibition of the PAR polymerase Tankyrase-1/2 was found to antagonize TDP-43-associated pathology (McGurk et al., 2018). Yet another application of small-molecule therapies involves targeting the spliceosome. Though this strategy has yet to be utilized in the treatment of splicing-related neurodegenerative diseases, it has been demonstrated that the spliceosome is subject to significant modulation by small molecules, particularly via targeting of the SF3b1 subunit (Webb et al., 2013). Alternative splicing can also be modulated by small molecules targeting serine-arginine-rich proteins that bind to specific sequences called exonic splicing enhancer (ESE) and influence the choice of splice sites (Soret et al., 2005).

Therapeutics that Prevent RBP Sequestration by Repeat Expansion

ASO based therapies are now being brought to bear in several neurodegenerative disorders linked to repeat expansions either located in non-coding regions, as in myotonic dystrophy (Mulderson et al., 2009; Wheeler et al., 2009, 2012; Wu et al., 2004) and C9ORF72 ALS and/or FTD (Lagier-Tourenne et al., 2013; Donnelly et al., 2013; Sareen et al., 2013; Jiang et al., 2016), or encoding for polyQ tracts as in Huntington's disease (Kordasiewicz et al., 2012) and SCAs (Moore et al., 2017; Scoles et al., 2017; Toonen et al., 2017). Depending on chemical modifications, ASOs can either bind to the repeats, alter secondary structure formation and attenuate RBP binding as shown for MBNL1 in DM1 (Wheeler et al., 2009) (Figure 2Ci), or trigger RNase H-mediated degradation of their target RNAs as demonstrated in DM1

fused to Tet1 that when targeted to CGG repeat expansion leads to demethylation of the upstream promoter and gene re-activation. (vi) The tetracycline analog minocycline blocks excessive translation caused by loss of FMRP.

(B) Strategies to prevent or reverse RBP aggregation. (i) RNase-H-dependent ASOs reducing the levels of ataxin 2 alleviate TDP-43 aggregation and toxicity. (ii) Modulation of chaperones such as heat shock proteins (hsp) and disaggregases such as nuclear import factors reverse abnormal phase transition and aggregation of RBPs.

(C) Strategies to block RBP sequestration by repeat-expanded RNAs and proteins. ASOs have been applied to alleviate RBP sequestration by repeat expanded RNAs both by blocking and displacing muscleblind proteins or by degrading the toxic RNA in myotonic dystrophy (i), ASOs inducing isoform-specific degradation of expansion-containing transcripts alleviated pathology without reducing overall levels of C9ORF72 in ALS and/or FTD (ii). CRISPR technology was applied to repeat expansion diseases to either excise the repeat expansion or to block RNA Pol II transcription in the absence of nuclease activity (iii).

(Wheeler et al., 2012) (Figure 2Ci) and C9ORF72-mediated ALS and/or FTD (Donnelly et al., 2013; Jiang et al., 2016; Lagier-Touranne et al., 2013; Sareen et al., 2013) (Figure 2Cii). The latter is most frequently used and currently under testing in several clinical trials (Schoch and Miller, 2017). Notably, repeat expansions are often transcribed from both DNA strands and ASOs have so far mostly been developed to target expanded transcripts from the sense strand. An alternative approach targeting the transcription elongation factor Spt4/SUPT4H1 was shown to decrease production of both sense and antisense expanded transcripts in yeast, flies and C9ORF72 patient fibroblasts, resulting in a reduction of foci and DPR proteins from both strands (Kramer et al., 2016).

Beyond ASOs, aberrant interaction between RBPs and expanded RNAs can be modulated by small molecules. Modified RNA aptamer-based screens and computer-aided RNA-structure docking strategies have identified lead compounds that bind to RNA in a structure-specific manner. Compounds that bind to expanded CUG repeats were reported to block RBP binding and relieve alternative splicing defects by increasing MBNL availability in *in vitro* models of DM (Childs-Disney et al., 2013, 2014). Other small molecules function by disrupting structural motifs of mRNA necessary for RBP binding (Coonrod et al., 2013; Simone et al., 2018; Su et al., 2014). Recently, daunorubicin hydrochloride was shown to bind CUG repeats and alleviate MBNL titration in cardiomyocytes in *Drosophila* DM1 models, rescuing molecular and cardiac function associated with DM1 (Chakraborty et al., 2018).

Last, the potential therapeutic applications for CRISPR/Cas9 are rapidly increasing, and recent work suggests that this system may provide a means to specifically target toxic RNA. Batra et al. utilized an RNA-targeting Cas9 system to degrade neurodegeneration (DM, C9-ALS, and HD)-associated microsatellite repeat expansions (Batra et al., 2017). In both exogenously expressed repeat expansions as well as patient cells, targeting repeat-containing transcripts by an RNA-specific Cas9 system resulted in a reversal of several molecular phenotypes, including degradation of the RNA, foci elimination, restoration of RBP localization, reduction in toxic protein production, and correction of aberrant splicing patterns (Batra et al., 2017). Expanded DNA can also be targeted by Cas9 either to remove the expanded repeat through DNA excision (van Agtmaal et al., 2017), or by using a non-cleaving dCas9/gRNA complex that coats the repeat DNA and blocks transcription of the toxic RNA (Figure 2Ciii) (Pinto et al., 2017). While CRISPR is a highly promising targeted therapeutic, there are several issues to consider. The first is off-target effects. While specificity at the RNA-level by RNA-targeting CRISPR approaches can be measured by RNA-seq, off-target DNA modifications at the DNA-level is difficult to detect and quantify. The second issue is delivery; while AAV is a commonly utilized delivery vehicle for human therapy, its carrying capacity is limited and Cas9 is a relatively large protein. Attempts to shrink Cas9 (Batra et al., 2017), to split Cas9 (Chew et al., 2016), and to identify Cas proteins that are smaller for RNA (such as Cas13) (Cox et al., 2017; Konermann et al., 2018; Yan et al., 2018) and DNA (Harrington et al., 2018) targeting will ultimately address this issue. Finally, there is evidence suggesting that high-levels of Cas9 elicits an immune response (Chew et al., 2016), which is prob-

lematic but could potentially be circumvented by immunosuppression or possibly by manipulation to “humanize” CRISPR/Cas proteins.

Perspectives and Summary

Mutations in RBPs and RNAs have been revealed as central to the disease pathology of many neurological disorders. Pathology may arise from reduced RBP expression, aggregation of RBPs with LCDs, or sequestration of RBPs by expanded RNAs and proteins in microsatellite diseases. Separately from the mechanism, disruption of RBPs lead to major alterations in RNA homeostasis including splicing, polyadenylation, transport, translation, and decay (Figure 1). Major advances in sequencing-based technology have provided the opportunity to determine the influence of multifunctional RBPs on their RNA targets (Nussbacher et al., 2015). These studies have demonstrated that widespread alteration of multiple steps involved in RNA homeostasis is a key contributor to the molecular basis leading to neuronal dysfunction. Most importantly, multiple therapeutic approaches that restore the level and/or function of RBPs are showing great potential for clinical applications and, in some instances, are already improving disease progression in patients. However, we still lack a clear understanding of how these aberrantly expressed RBPs and RNAs contribute to selective vulnerability of neuronal cells. Additionally, the direct versus indirect changes that lead to differences in pathology remain undistinguished. Nevertheless, a great deal has been done to elucidate commonalities among phenotypes and to clarify general mechanistic observations among these diseases. Ultimately, it may be the case that despite shared global characteristics of RBP loss, aggregation, or sequestration, the underlying molecular mechanisms are distinct. This emphasizes the need for disease-specific therapeutic approaches that exploit the pioneering efforts of other fields such as AAV-mediated gene therapy or CRISPR.

ACKNOWLEDGMENTS

This work was supported by grants from the NIH (NS103172, MH107367, EY029166, HG009889, HG004659 to G.W.Y. and NS087227 to C.L.-T.), from Target ALS (to C.L.-T. and G.W.Y.), the ALS Association (to C.L.-T. and G.W.Y.), the Pape Adams Foundation, the Association Française contre les Myopathies, and ALS Finding a Cure (to C.L.-T.). R.T. was supported by a grant from the Philippe Foundation. J.K.N. is an Achievement Rewards for College Scientists Foundation Fellow and was partially supported by the Genentech Foundation fellowship and the University of California, San Diego, Cancer Training Program through an institutional training grant from the National Institute of General Medical Sciences, T32CA067754.

DECLARATION OF INTERESTS

G.W.Y. is co-founder, member of the Board of Directors, equity holder, and paid consultant for Locana and Eclipse Biolnnovations. G.W.Y. is co-founder of Enzerna and ProteoNA. G.W.Y. is a paid consultant for Aquinnah Pharmaceuticals and Ionis Pharmaceuticals and scientific advisory board member to Ribometrix and the Allen Institute of Immunology. The terms of this arrangement have been reviewed and approved by the University of California, San Diego in accordance with its conflict of interest policies. The authors declare no other competing financial interests.

REFERENCES

Afroz, T., Hock, E.M., Ernst, P., Foglieni, C., Jambau, M., Gilhespy, L.A.B., Laferriere, F., Maniecka, Z., Plückthun, A., Mittl, P., et al. (2017). Functional and

- dynamic polymerization of the ALS-linked protein TDP-43 antagonizes its pathologic aggregation. *Nat. Commun.* 8, 45.
- Akamatsu, W., Okano, H.J., Osumi, N., Inoue, T., Nakamura, S., Sakakibara, S., Miura, M., Matsuo, N., Darnell, R.B., and Okano, H. (1999). Mammalian ELAV-like neuronal RNA-binding proteins HuB and HuC promote neuronal development in both the central and the peripheral nervous systems. *Proc. Natl. Acad. Sci. USA* 96, 9885–9890.
- Akten, B., Kye, M.J., Hao le, T., Wertz, M.H., Singh, S., Nie, D., Huang, J., Merianda, T.T., Twiss, J.L., Beattie, C.E., et al. (2011). Interaction of survival of motor neuron (SMN) and HuD proteins with mRNA cpG15 rescues motor neuron axonal deficits. *Proc. Natl. Acad. Sci. USA* 108, 10337–10342.
- Alami, N.H., Smith, R.B., Carrasco, M.A., Williams, L.A., Winborn, C.S., Han, S.S.W., Kiskinis, E., Winborn, B., Freibaum, B.D., Kanagaraj, A., et al. (2014). Axonal transport of TDP-43 mRNA granules is impaired by ALS-causing mutations. *Neuron* 81, 536–543.
- Alarcón, C.R., Goodarzi, H., Lee, H., Liu, X., Tavazoie, S., and Tavazoie, S.F. (2015). HNRNPA2B1 is a mediator of m(6)A-dependent nuclear RNA processing events. *Cell* 162, 1299–1308.
- Alberti, S., Halfmann, R., King, O., Kapila, A., and Lindquist, S. (2009). A systematic survey identifies prions and illuminates sequence features of prionogenic proteins. *Cell* 137, 146–158.
- Almada, A.E., Wu, X., Kriz, A.J., Burge, C.B., and Sharp, P.A. (2013). Promoter directionality is controlled by U1 snRNP and polyadenylation signals. *Nature* 499, 360–363.
- Alpatov, R., Lesch, B.J., Nakamoto-Kinoshita, M., Blanco, A., Chen, S., Stützer, A., Armache, K.J., Simon, M.D., Xu, C., Ali, M., et al. (2014). A chromatin-dependent role of the fragile X mental retardation protein FMRP in the DNA damage response. *Cell* 157, 869–881.
- Alzu, A., Bermejo, R., Begnis, M., Lucca, C., Piccini, D., Carotenuto, W., Saponaro, M., Brambati, A., Cocito, A., Foiani, M., and Liberi, G. (2012). Senataxin associates with replication forks to protect fork integrity across RNA-polymerase-II-transcribed genes. *Cell* 151, 835–846.
- Ambadipudi, S., Biernat, J., Riedel, D., Mandelkow, E., and Zweckstetter, M. (2017). Liquid-liquid phase separation of the microtubule-binding repeats of the Alzheimer-related protein Tau. *Nat. Commun.* 8, 275.
- Anderson, P., and Kedersha, N. (2006). RNA granules. *J. Cell Biol.* 172, 803–808.
- Anderson, P., and Kedersha, N. (2009). RNA granules: post-transcriptional and epigenetic modulators of gene expression. *Nat. Rev. Mol. Cell Biol.* 10, 430–436.
- Anderson, K.D., Morin, M.A., Beckel-Mitchener, A., Mobarak, C.D., Neve, R.L., Furneaux, H.M., Burry, R., and Perrone-Bizzozero, N.I. (2000). Overexpression of HuD, but not of its truncated form HuD I+II, promotes GAP-43 gene expression and neurite outgrowth in PC12 cells in the absence of nerve growth factor. *J. Neurochem.* 75, 1103–1114.
- Andersson, M.K., Ståhlberg, A., Arvidsson, Y., Olofsson, A., Semb, H., Stenman, G., Nilsson, O., and Aman, P. (2008). The multifunctional FUS, EWS and TAF15 proto-oncoproteins show cell type-specific expression patterns and involvement in cell spreading and stress response. *BMC Cell Biol.* 9, 37.
- Antar, L.N., Afroz, R., Dichtenberg, J.B., Carroll, R.C., and Bassell, G.J. (2004). Metabotropic glutamate receptor activation regulates fragile x mental retardation protein and FMR1 mRNA localization differentially in dendrites and at synapses. *J. Neurosci.* 24, 2648–2655.
- Antic, D., Lu, N., and Keene, J.D. (1999). ELAV tumor antigen, Hel-N1, increases translation of neurofilament M mRNA and induces formation of neurites in human teratocarcinoma cells. *Genes Dev.* 13, 449–461.
- Apicco, D.J., Ash, P.E.A., Maziuk, B., LeBlang, C., Medalla, M., Al Abdullatif, A., Ferragud, A., Botelho, E., Ballance, H.I., Dhawan, U., et al. (2018). Reducing the RNA binding protein TIA1 protects against tau-mediated neurodegeneration in vivo. *Nat. Neurosci.* 21, 72–80.
- Arai, T., Hasegawa, M., Akiyama, H., Ikeda, K., Nonaka, T., Mori, H., Mann, D., Tsuchiya, K., Yoshida, M., Hashizume, Y., and Oda, T. (2006). TDP-43 is a component of ubiquitin-positive tau-negative inclusions in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. *Biochem. Biophys. Res. Commun.* 351, 602–611.
- Ascano, M., Jr., Mukherjee, N., Bandaru, P., Miller, J.B., Nusbaum, J.D., Corcoran, D.L., Langlois, C., Munschauer, M., Dewell, S., Hafner, M., et al. (2012). FMRP targets distinct mRNA sequence elements to regulate protein expression. *Nature* 492, 382–386.
- Ash, P.E., Bieniek, K.F., Gendron, T.F., Caulfield, T., Lin, W.L., DeJesus-Hernandez, M., van Blitterswijk, M.M., Jansen-West, K., Paul, J.W., 3rd, Rademakers, R., et al. (2013). Unconventional translation of C9ORF72 GGGGCC expansion generates insoluble polypeptides specific to c9FTD/ALS. *Neuron* 77, 639–646.
- Avendaño-Vázquez, S.E., Dhir, A., Bembich, S., Buratti, E., Proudfoot, N., and Baralle, F.E. (2012). Autoregulation of TDP-43 mRNA levels involves interplay between transcription, splicing, and alternative polyA site selection. *Genes Dev.* 26, 1679–1684.
- Avila, A.M., Burnett, B.G., Taye, A.A., Gabanella, F., Knight, M.A., Hartenstein, P., Cizman, Z., Di Prospero, N.A., Pellizzoni, L., Fischbeck, K.H., and Sumner, C.J. (2007). Trichostatin A increases SMN expression and survival in a mouse model of spinal muscular atrophy. *J. Clin. Invest.* 117, 659–671.
- Ayala, V., Granado-Serrano, A.B., Cacabelos, D., Naudí, A., Ilieva, E.V., Boada, J., Caraballo-Mirallas, V., Lladó, J., Ferrer, I., Pamplona, R., and Portero-Otin, M. (2011). Cell stress induces TDP-43 pathological changes associated with ERK1/2 dysfunction: implications in ALS. *Acta Neuropathol.* 122, 259–270.
- Bagni, C., and Oostra, B.A. (2013). Fragile X syndrome: from protein function to therapy. *Am. J. Med. Genet. A.* 161A, 2809–2821.
- Bañez-Coronel, M., Ayhan, F., Tarabochia, A.D., Zu, T., Perez, B.A., Tusi, S.K., Pletnikova, O., Borchelt, D.R., Ross, C.A., Margolis, R.L., et al. (2015). RAN translation in Huntington disease. *Neuron* 88, 667–677.
- Barmada, S.J., Serio, A., Arjun, A., Bilican, B., Daub, A., Ando, D.M., Tsvetkov, A., Pleiss, M., Li, X., Peisach, D., et al. (2014). Autophagy induction enhances TDP43 turnover and survival in neuronal ALS models. *Nat. Chem. Biol.* 10, 677–685.
- Barmada, S.J., Ju, S., Arjun, A., Batarse, A., Archbold, H.C., Peisach, D., Li, X., Zhang, Y., Tank, E.M., Qiu, H., et al. (2015). Amelioration of toxicity in neuronal models of amyotrophic lateral sclerosis by hUPF1. *Proc. Natl. Acad. Sci. USA* 112, 7821–7826.
- Batra, R., Charizanis, K., Manchanda, M., Mohan, A., Li, M., Finn, D.J., Goodwin, M., Zhang, C., Sobczak, K., Thornton, C.A., and Swanson, M.S. (2014). Loss of MBNL leads to disruption of developmentally regulated alternative polyadenylation in RNA-mediated disease. *Mol. Cell* 56, 311–322.
- Batra, R., Nelles, D.A., Pirie, E., Blue, S.M., Marina, R.J., Wang, H., Chaim, I.A., Thomas, J.D., Zhang, N., Nguyen, V., et al. (2017). Elimination of toxic microsatellite repeat expansion RNA by RNA-targeting Cas9. *Cell* 170, 899–912.
- Bäumer, D., Lee, S., Nicholson, G., Davies, J.L., Parkinson, N.J., Murray, L.M., Gillingwater, T.H., Ansorge, O., Davies, K.E., and Talbot, K. (2009). Alternative splicing events are a late feature of pathology in a mouse model of spinal muscular atrophy. *PLoS Genet.* 5, e1000773.
- Bechara, E.G., Didiot, M.C., Melko, M., Davidovic, L., Bensaid, M., Martin, P., Castets, M., Pogoniec, P., Khandjian, E.W., Moine, H., and Bardoni, B. (2009). A novel function for fragile X mental retardation protein in translational activation. *PLoS Biol.* 7, e16.
- Becker, L.A., Huang, B., Bieri, G., Ma, R., Knowles, D.A., Jafar-Nejad, P., Messing, J., Kim, H.J., Soriano, A., Auburger, G., et al. (2017). Therapeutic reduction of ataxin-2 extends lifespan and reduces pathology in TDP-43 mice. *Nature* 544, 367–371.
- Bellavia, D., Mecarozzi, M., Campese, A.F., Grazioli, P., Talora, C., Frati, L., Gulino, A., and Screpanti, I. (2007). Notch3 and the Notch3-upregulated RNA-binding protein HuD regulate Ikaros alternative splicing. *EMBO J.* 26, 1670–1680.
- Bembich, S., Herzog, J.S., De Conti, L., Stuani, C., Avendaño-Vázquez, S.E., Buratti, E., Baralle, M., and Baralle, F.E. (2014). Predominance of spliceosomal complex formation over polyadenylation site selection in TDP-43 autoregulation. *Nucleic Acids Res.* 42, 3362–3371.

Berto, S., Usui, N., Konopka, G., and Fogel, B.L. (2016). ELAVL2-regulated transcriptional and splicing networks in human neurons link neurodevelopment and autism. *Hum. Mol. Genet* 15, 2451–2464.

Bhalla, K., Phillips, H.A., Crawford, J., McKenzie, O.L., Mulley, J.C., Eyre, H., Gardner, A.E., Kremmidiotis, G., and Callen, D.F. (2004). The de novo chromosome 16 translocations of two patients with abnormal phenotypes (mental retardation and epilepsy) disrupt the A2BP1 gene. *J. Hum. Genet.* 49, 308–311.

Bill, B.R., Lowe, J.K., Dybuncio, C.T., and Fogel, B.L. (2013). Orchestration of neurodevelopmental programs by RBFOX1: implications for autism spectrum disorder. *Int. Rev. Neurobiol.* 113, 251–267.

Bilousova, T.V., Dansie, N., Ngo, M., Aye, J., Charles, J.R., Ethell, D.W., and Ethell, I.M. (2009). Minocycline promotes dendritic spine maturation and improves behavioural performance in the fragile X mouse model. *J. Med. Genet.* 46, 94–102.

Blechingberg, J., Luo, Y., Bolund, L., Damgaard, C.K., and Nielsen, A.L. (2012). Gene expression responses to FUS, EWS, and TAF15 reduction and stress granule sequestration analyses identifies FET-protein non-redundant functions. *PLoS ONE* 7, e46251.

Boehringer, A., Garcia-Mansfield, K., Singh, G., Bakkar, N., Pirrotte, P., and Bowser, R. (2017). ALS associated mutations in Matrin 3 alter protein-protein interactions and impede mRNA nuclear export. *Sci. Rep.* 7, 14529.

Boeynaems, S., Bogaert, E., Michiels, E., Gijssels, I., Sieben, A., Jovičić, A., De Baets, G., Scheveneels, W., Steyaert, J., Cuijt, I., et al. (2016). Drosophila screen connects nuclear transport genes to DPR pathology in c9ALS/FTD. *Sci. Rep.* 6, 20877.

Boeynaems, S., Bogaert, E., Kovacs, D., Konijnenberg, A., Timmerman, E., Volkov, A., Guharoy, M., De Decker, M., Jaspers, T., Ryan, V.H., et al. (2017). Phase separation of C9orf72 dipeptide repeats perturbs stress granule dynamics. *Mol. Cell* 65, 1044–1055.

Boeynaems, S., Alberti, S., Fawzi, N.L., Mittag, T., Polymenidou, M., Rousseau, F., Schymkowitz, J., Shorter, J., Wolozin, B., Van Den Bosch, L., et al. (2018). Protein phase separation: a new phase in cell biology. *Trends Cell Biol.* 28, 420–435.

Bosco, D.A., Lemay, N., Ko, H.K., Zhou, H., Burke, C., Kwiatkowski, T.J., Jr., Sapp, P., McKenna-Yasek, D., Brown, R.H., Jr., and Hayward, L.J. (2010). Mutant FUS proteins that cause amyotrophic lateral sclerosis incorporate into stress granules. *Hum. Mol. Genet.* 19, 4160–4175.

Brangwynne, C.P., Eckmann, C.R., Courson, D.S., Rybarska, A., Hoeghe, C., Gharakhani, J., Jülicher, F., and Hyman, A.A. (2009). Germline P granules are liquid droplets that localize by controlled dissolution/condensation. *Science* 324, 1729–1732.

Brook, J.D., McCurrach, M.E., Harley, H.G., Buckler, A.J., Church, D., Aburatani, H., Hunter, K., Stanton, V.P., Thirion, J.P., Hudson, T., et al. (1992). Molecular basis of myotonic dystrophy: expansion of a trinucleotide (CTG) repeat at the 3' end of a transcript encoding a protein kinase family member. *Cell* 68, 799–808.

Brown, V., Jin, P., Ceman, S., Darnell, J.C., O'Donnell, W.T., Tenenbaum, S.A., Jin, X., Feng, Y., Wilkinson, K.D., Keene, J.D., et al. (2001). Microarray identification of FMRP-associated brain mRNAs and altered mRNA translational profiles in fragile X syndrome. *Cell* 107, 477–487.

Buckanovich, R.J., Posner, J.B., and Darnell, R.B. (1993). Nova, the paraneoplastic Ri antigen, is homologous to an RNA-binding protein and is specifically expressed in the developing motor system. *Neuron* 11, 657–672.

Burke, K.A., Janke, A.M., Rhine, C.L., and Fawzi, N.L. (2015). Residue-by-residue view of in vitro FUS granules that bind the C-terminal domain of RNA polymerase II. *Mol. Cell* 60, 231–241.

Carlomagno, Y., Zhang, Y., Davis, M., Lin, W.L., Cook, C., Dunmore, J., Tay, W., Menkosky, K., Cao, X., Petrucelli, L., and Deture, M. (2014). Casein kinase II induced polymerization of soluble TDP-43 into filaments is inhibited by heat shock proteins. *PLoS ONE* 9, e90452.

Carrel, T.L., McWhorter, M.L., Workman, E., Zhang, H., Wolstencroft, E.C., Lorson, C., Bassell, G.J., Burghes, A.H., and Beattie, C.E. (2006). Survival motor neuron function in motor axons is independent of functions required for small nuclear ribonucleoprotein biogenesis. *J. Neurosci.* 26, 11014–11022.

Caudy, A.A., Myers, M., Hannon, G.J., and Hammond, S.M. (2002). Fragile X-related protein and VIG associate with the RNA interference machinery. *Genes Dev.* 16, 2491–2496.

Ceman, S., O'Donnell, W.T., Reed, M., Patton, S., Pohl, J., and Warren, S.T. (2003). Phosphorylation influences the translation state of FMRP-associated polyribosomes. *Hum. Mol. Genet.* 12, 3295–3305.

Chakraborty, M., Sellier, C., Ney, M., Villa, P., Charlet-Berguerand, N., Artero, R., and Llamusi, B. (2018). Daunorubicin reduces MBNL1 titration by expanded CUG repeat RNA and rescues cardiac dysfunctions in a Drosophila model of myotonic dystrophy. *Dis. Model. Mech.*

Charizanis, K., Lee, K.Y., Batra, R., Goodwin, M., Zhang, C., Yuan, Y., Shiue, L., Cline, M., Scotti, M.M., Xia, G., et al. (2012). Muscleblind-like 2-mediated alternative splicing in the developing brain and dysregulation in myotonic dystrophy. *Neuron* 75, 437–450.

Chen, Y.Z., Bennett, C.L., Huynh, H.M., Blair, I.P., Puls, I., Irobi, J., Dierick, I., Abel, A., Kennerson, M.L., Rabin, B.A., et al. (2004). DNA/RNA helicase gene mutations in a form of juvenile amyotrophic lateral sclerosis (ALS4). *Am. J. Hum. Genet.* 74, 1128–1135.

Chen, E., Sharma, M.R., Shi, X., Agrawal, R.K., and Joseph, S. (2014). Fragile X mental retardation protein regulates translation by binding directly to the ribosome. *Mol. Cell* 54, 407–417.

Chen, H.J., Mitchell, J.C., Novoselov, S., Miller, J., Nishimura, A.L., Scotter, E.L., Vance, C.A., Cheetham, M.E., and Shaw, C.E. (2016). The heat shock response plays an important role in TDP-43 clearance: evidence for dysfunction in amyotrophic lateral sclerosis. *Brain* 139, 1417–1432.

Chew, W.L., Tabebordbar, M., Cheng, J.K., Mali, P., Wu, E.Y., Ng, A.H., Zhu, K., Wagers, A.J., and Church, G.M. (2016). A multifunctional AAV-CRISPR-Cas9 and its host response. *Nat. Methods* 13, 868–874.

Childs-Disney, J.L., Stepniak-Konieczna, E., Tran, T., Yildirim, I., Park, H., Chen, C.Z., Hoskins, J., Southall, N., Marugan, J.J., Patnaik, S., et al. (2013). Induction and reversal of myotonic dystrophy type 1 pre-mRNA splicing defects by small molecules. *Nat. Commun.* 4, 2044.

Childs-Disney, J.L., Yildirim, I., Park, H., Lohman, J.R., Guan, L., Tran, T., Sarkar, P., Schatz, G.C., and Disney, M.D. (2014). Structure of the myotonic dystrophy type 2 RNA and designed small molecules that reduce toxicity. *ACS Chem. Biol.* 9, 538–550.

Chiriboga, C.A., Swoboda, K.J., Darras, B.T., Iannaccone, S.T., Montes, J., De Vivo, D.C., Norris, D.A., Bennett, C.F., and Bishop, K.M. (2016). Results from a phase 1 study of nusinersen (ISIS-SMN(Rx)) in children with spinal muscular atrophy. *Neurology* 86, 890–897.

Choi, J., Dixon, D.M., Dansithong, W., Abdallah, W.F., Roos, K.P., Jordan, M.C., Trac, B., Lee, H.S., Comai, L., and Reddy, S. (2016). Muscleblind-like 3 deficit results in a spectrum of age-associated pathologies observed in myotonic dystrophy. *Sci. Rep.* 6, 30999.

Chou, C.C., Zhang, Y., Umoh, M.E., Vaughan, S.W., Lorenzini, I., Liu, F., Sayegh, M., Donlin-Asp, P.G., Chen, Y.H., Duong, D.M., et al. (2018). TDP-43 pathology disrupts nuclear pore complexes and nucleocytoplasmic transport in ALS/FTD. *Nat. Neurosci.* 21, 228–239.

Chyung, E., LeBlanc, H.F., Fallon, J.R., and Akins, M.R. (2018). Fragile X granules are a family of axonal ribonucleoprotein particles with circuit-dependent protein composition and mRNA cargos. *J. Comp. Neurol.* 526, 96–108.

Ciosk, R., DePalma, M., and Priess, J.R. (2004). ATX-2, the *C. elegans* ortholog of ataxin 2, functions in translational regulation in the germline. *Development* 131, 4831–4841.

Coelho, M.B., Attig, J., Bellora, N., König, J., Hallegger, M., Kayikci, M., Eyraes, E., Ule, J., and Smith, C.W. (2015). Nuclear matrix protein Matrin3 regulates alternative splicing and forms overlapping regulatory networks with PTB. *EMBO J.* 34, 653–668.

Coffee, B., Zhang, F., Warren, S.T., and Reines, D. (1999). Acetylated histones are associated with FMR1 in normal but not fragile X-syndrome cells. *Nat. Genet.* 22, 98–101.

Cohen, S., Puget, N., Lin, Y.L., Clouaire, T., Aguirrebengoa, M., Rocher, V., Pasero, P., Canitrot, Y., and Legube, G. (2018). Senataxin resolves RNA:DNA

- hybrids forming at DNA double-strand breaks to prevent translocations. *Nat. Commun.* **9**, 533.
- Colombrita, C., Onesto, E., Megiorni, F., Pizzuti, A., Baralle, F.E., Buratti, E., Silani, V., and Ratti, A. (2012). TDP-43 and FUS RNA-binding proteins bind distinct sets of cytoplasmic messenger RNAs and differently regulate their post-transcriptional fate in motoneuron-like cells. *J. Biol. Chem.* **287**, 15635–15647.
- Conicella, A.E., Zerze, G.H., Mittal, J., and Fawzi, N.L. (2016). ALS mutations disrupt phase separation mediated by α -helical structure in the TDP-43 low-complexity C-terminal domain. *Structure* **24**, 1537–1549.
- Conlon, E.G., Lu, L., Sharma, A., Yamazaki, T., Tang, T., Shneider, N.A., and Manley, J.L. (2016). The C9ORF72 GGGGCC expansion forms RNA G-quadruplex inclusions and sequesters hnRNP H to disrupt splicing in ALS brains. *eLife* **5**. Published online September 13, 2016. <https://doi.org/10.7554/eLife.17820>.
- Coonrod, L.A., Nakamori, M., Wang, W., Carrell, S., Hilton, C.L., Bodner, M.J., Siboni, R.B., Docter, A.G., Haley, M.M., Thornton, C.A., and Berglund, J.A. (2013). Reducing levels of toxic RNA with small molecules. *ACS Chem. Biol.* **8**, 2528–2537.
- Cooper-Knock, J., Walsh, M.J., Higginbottom, A., Robin Highley, J., Dickman, M.J., Edbauer, D., Ince, P.G., Wharton, S.B., Wilson, S.A., Kirby, J., et al. (2014). Sequestration of multiple RNA recognition motif-containing proteins by C9orf72 repeat expansions. *Brain* **137**, 2040–2051.
- Cooper-Knock, J., Bury, J.J., Heath, P.R., Wyles, M., Higginbottom, A., Gels thorpe, C., Highley, J.R., Hautbergue, G., Rattray, M., Kirby, J., and Shaw, P.J. (2015). C9ORF72 GGGGCC expanded repeats produce splicing dysregulation which correlates with disease severity in amyotrophic lateral sclerosis. *PLoS ONE* **10**, e0127376.
- Corcia, P., Camu, W., Praline, J., Gordon, P.H., Vourch, P., and Andres, C. (2009). The importance of the SMN genes in the genetics of sporadic ALS. *Amyotroph. Lateral Scler.* **10**, 436–440.
- Costessi, L., Porro, F., Iaconcig, A., and Muro, A.F. (2014). TDP-43 regulates β -adducin (Add2) transcript stability. *RNA Biol.* **11**, 1280–1290.
- Couthouis, J., Hart, M.P., Shorter, J., DeJesus-Hernandez, M., Erion, R., Oristano, R., Liu, A.X., Ramos, D., Jethava, N., Hosangadi, D., et al. (2011). A yeast functional screen predicts new candidate ALS disease genes. *Proc. Natl. Acad. Sci. USA* **108**, 20881–20890.
- Couthouis, J., Hart, M.P., Erion, R., King, O.D., Diaz, Z., Nakaya, T., Ibrahim, F., Kim, H.J., Mojsilovic-Petrovic, J., Panossian, S., et al. (2012). Evaluating the role of the FUS/TLS-related gene EWSR1 in amyotrophic lateral sclerosis. *Hum. Mol. Genet.* **21**, 2899–2911.
- Cox, D.B.T., Gootenberg, J.S., Abudayyeh, O.O., Franklin, B., Kellner, M.J., Jung, J., and Zhang, F. (2017). RNA editing with CRISPR-Cas13. *Science* **358**, 1019–1027.
- Crabtree, B., Thiyagarajan, N., Prior, S.H., Wilson, P., Iyer, S., Ferns, T., Shapiro, R., Brew, K., Subramanian, V., and Acharya, K.R. (2007). Characterization of human angiogenin variants implicated in amyotrophic lateral sclerosis. *Biochemistry* **46**, 11810–11818.
- Crippa, V., Cicardi, M.E., Ramesh, N., Seguin, S.J., Ganassi, M., Bigi, I., Diacci, C., Zelotti, E., Baratashvili, M., Gregory, J.M., et al. (2016). The chaperone HSPB8 reduces the accumulation of truncated TDP-43 species in cells and protects against TDP-43-mediated toxicity. *Hum. Mol. Genet.* **25**, 3908–3924.
- Criscuolo, C., Chessa, L., Di Giandomenico, S., Mancini, P., Saccà, F., Grieco, G.S., Piane, M., Barbieri, F., De Michele, G., Banfi, S., et al. (2006). Ataxia with oculomotor apraxia type 2: a clinical, pathologic, and genetic study. *Neurology* **66**, 1207–1210.
- D'Alton, S., Altshuler, M., and Lewis, J. (2015). Studies of alternative isoforms provide insight into TDP-43 autoregulation and pathogenesis. *RNA* **21**, 1419–1432.
- Darnell, J.C., and Klann, E. (2013). The translation of translational control by FMRP: therapeutic targets for FXS. *Nat. Neurosci.* **16**, 1530–1536.
- Darnell, J.C., Jensen, K.B., Jin, P., Brown, V., Warren, S.T., and Darnell, R.B. (2001). Fragile X mental retardation protein targets G quartet mRNAs important for neuronal function. *Cell* **107**, 489–499.
- Darnell, J.C., Fraser, C.E., Mostovetsky, O., Stefani, G., Jones, T.A., Eddy, S.R., and Darnell, R.B. (2005). Kissing complex RNAs mediate interaction between the fragile-X mental retardation protein KH2 domain and brain polyribosomes. *Genes Dev.* **19**, 903–918.
- Darnell, J.C., Van Driesche, S.J., Zhang, C., Hung, K.Y., Mele, A., Fraser, C.E., Stone, E.F., Chen, C., Fak, J.J., Chi, S.W., et al. (2011). FMRP stalls ribosomal translocation on mRNAs linked to synaptic function and autism. *Cell* **146**, 247–261.
- Davidovic, L., Jaglin, X.H., Lepagnol-Bestel, A.M., Tremblay, S., Simonneau, M., Bardoni, B., and Khandjian, E.W. (2007). The fragile X mental retardation protein is a molecular adaptor between the neurospecific KIF3C kinesin and dendritic RNA granules. *Hum. Mol. Genet.* **16**, 3047–3058.
- DeJesus-Hernandez, M., Mackenzie, I.R., Boeve, B.F., Boxer, A.L., Baker, M., Rutherford, N.J., Nicholson, A.M., Finch, N.A., Flynn, H., Adamson, J., et al. (2011). Expanded GGGGCC hexanucleotide repeat in noncoding region of C9ORF72 causes chromosome 9p-linked FTD and ALS. *Neuron* **72**, 245–256.
- Deschênes-Furry, J., Perrone-Bizzozero, N., and Jasmin, B.J. (2006). The RNA-binding protein HuD: a regulator of neuronal differentiation, maintenance and plasticity. *BioEssays* **28**, 822–833.
- Devys, D., Lutz, Y., Rouyer, N., Bellocq, J.P., and Mandel, J.L. (1993). The FMR-1 protein is cytoplasmic, most abundant in neurons and appears normal in carriers of a fragile X premutation. *Nat. Genet.* **4**, 335–340.
- Dicthenberg, J.B., Swanger, S.A., Antar, L.N., Singer, R.H., and Bassell, G.J. (2008). A direct role for FMRP in activity-dependent dendritic mRNA transport links filopodial-spine morphogenesis to fragile X syndrome. *Dev. Cell* **14**, 926–939.
- Didiot, M.C., Tian, Z., Schaeffer, C., Subramanian, M., Mandel, J.L., and Moine, H. (2008). The G-quartet containing FMRP binding site in FMR1 mRNA is a potent exonic splicing enhancer. *Nucleic Acids Res.* **36**, 4902–4912.
- Doi, H., Okamura, K., Bauer, P.O., Furukawa, Y., Shimizu, H., Kurosawa, M., Machida, Y., Miyazaki, H., Mitsui, K., Kuroiwa, Y., and Nukina, N. (2008). RNA-binding protein TLS is a major nuclear aggregate-interacting protein in huntingtin exon 1 with expanded polyglutamine-expressing cells. *J. Biol. Chem.* **283**, 6489–6500.
- Doi, H., Koyano, S., Suzuki, Y., Nukina, N., and Kuroiwa, Y. (2010). The RNA-binding protein FUS/TLS is a common aggregate-interacting protein in polyglutamine diseases. *Neurosci. Res.* **66**, 131–133.
- Donnelly, C.J., Zhang, P.W., Pham, J.T., Haeusler, A.R., Mistry, N.A., Viden-sky, S., Daley, E.L., Poth, E.M., Hoover, B., Fines, D.M., et al. (2013). RNA toxicity from the ALS/FTD C9ORF72 expansion is mitigated by antisense intervention. *Neuron* **80**, 415–428.
- Dormann, D., Rodde, R., Edbauer, D., Bentmann, E., Fischer, I., Hruscha, A., Than, M.E., Mackenzie, I.R., Capell, A., Schmid, B., et al. (2010). ALS-associated fused in sarcoma (FUS) mutations disrupt Transportin-mediated nuclear import. *EMBO J.* **29**, 2841–2857.
- Dredge, B.K., Stefani, G., Engelhard, C.C., and Darnell, R.B. (2005). Nova autoregulation reveals dual functions in neuronal splicing. *EMBO J.* **24**, 1608–1620.
- Duquette, A., Roddier, K., McNabb-Baltar, J., Gosselin, I., St-Denis, A., Dicaire, M.J., Loisel, L., Labuda, D., Marchand, L., Mathieu, J., et al. (2005). Mutations in senataxin responsible for Quebec cluster of ataxia with neuropathy. *Ann. Neurol.* **57**, 408–414.
- Dury, A.Y., El Fatimy, R., Tremblay, S., Rose, T.M., Côté, J., De Koninck, P., and Khandjian, E.W. (2013). Nuclear fragile X mental retardation protein is localized to Cajal bodies. *PLoS Genet.* **9**, e1003890.
- Edbauer, D., Neilson, J.R., Foster, K.A., Wang, C.F., Seeburg, D.P., Batterton, M.N., Tada, T., Dolan, B.M., Sharp, P.A., and Sheng, M. (2010). Regulation of synaptic structure and function by FMRP-associated microRNAs miR-125b and miR-132. *Neuron* **65**, 373–384.
- Eftekhazadeh, B., Daigle, J.G., Kapinos, L.E., Coyne, A., Schiantarelli, J., Car-lomagno, Y., Cook, C., Miller, S.J., Dujardin, S., Amaral, A.S., et al. (2018). Tau protein disrupts microcytoplasmic transport in Alzheimer's disease. *Neuron* **99**, 925–940.

- Eitan, C., and Hornstein, E. (2016). Vulnerability of microRNA biogenesis in FTD-ALS. *Brain Res.* 1647, 105–111.
- Elden, A.C., Kim, H.J., Hart, M.P., Chen-Plotkin, A.S., Johnson, B.S., Fang, X., Armakola, M., Geser, F., Greene, R., Lu, M.M., et al. (2010). Ataxin-2 intermediate-length polyglutamine expansions are associated with increased risk for ALS. *Nature* 466, 1069–1075.
- Fallini, C., Zhang, H., Su, Y., Silani, V., Singer, R.H., Rossoll, W., and Bassell, G.J. (2011). The survival of motor neuron (SMN) protein interacts with the mRNA-binding protein HuD and regulates localization of poly(A) mRNA in primary motor neuron axons. *J. Neurosci.* 31, 3914–3925.
- Fan, X.C., and Steitz, J.A. (1998). Overexpression of HuR, a nuclear-cytoplasmic shuttling protein, increases the in vivo stability of ARE-containing mRNAs. *EMBO J.* 17, 3448–3460.
- Farg, M.A., Soo, K.Y., Warraich, S.T., Sundaramoorthy, V., Blair, I.P., and Atkin, J.D. (2013). Ataxin-2 interacts with FUS and intermediate-length polyglutamine expansions enhance FUS-related pathology in amyotrophic lateral sclerosis. *Hum. Mol. Genet.* 22, 717–728.
- Feng, Y., Gutekunst, C.A., Eberhart, D.E., Yi, H., Warren, S.T., and Hersch, S.M. (1997). Fragile X mental retardation protein: nucleocytoplasmic shuttling and association with somatodendritic ribosomes. *J. Neurosci.* 17, 1539–1547.
- Fernández-Santiago, R., Hoenig, S., Lichtner, P., Sperfeld, A.D., Sharma, M., Berg, D., Weichenrieder, O., Illig, T., Eger, K., Meyer, T., et al. (2009). Identification of novel Angiogenin (ANG) gene missense variants in German patients with amyotrophic lateral sclerosis. *J. Neurol.* 256, 1337–1342.
- Fett, J.W., Strydom, D.J., Lobb, R.R., Alderman, E.M., Bethune, J.L., Riordan, J.F., and Vallee, B.L. (1985). Isolation and characterization of angiogenin, an angiogenic protein from human carcinoma cells. *Biochemistry* 24, 5480–5486.
- Fiesel, F.C., Weber, S.S., Supper, J., Zell, A., and Kahle, P.J. (2012). TDP-43 regulates global translational yield by splicing of exon junction complex component SKAR. *Nucleic Acids Res.* 40, 2668–2682.
- Finkel, R.S., Chiriboga, C.A., Vajsar, J., Day, J.W., Montes, J., De Vivo, D.C., Yamashita, M., Rigo, F., Hung, G., Schneider, E., et al. (2016). Treatment of infantile-onset spinal muscular atrophy with nusinersen: a phase 2, open-label, dose-escalation study. *Lancet* 388, 3017–3026.
- Finkel, R.S., Mercuri, E., Darras, B.T., Connolly, A.M., Kuntz, N.L., Kirschner, J., Chiriboga, C.A., Saito, K., Servais, L., Tizzano, E., et al.; ENDEAR Study Group (2017). Nusinersen versus sham control in infantile-onset spinal muscular atrophy. *N. Engl. J. Med.* 377, 1723–1732.
- Fittschen, M., Lastres-Becker, I., Halbach, M.V., Damrath, E., Gispert, S., Azizov, M., Walter, M., Müller, S., and Auburger, G. (2015). Genetic ablation of ataxin-2 increases several global translation factors in their transcript abundance but decreases translation rate. *Neurogenetics* 16, 181–192.
- Fogel, B.L., and Perlman, S. (2006). Novel mutations in the senataxin DNA/RNA helicase domain in ataxia with oculomotor apraxia 2. *Neurology* 67, 2083–2084.
- Fratta, P., Mizielinska, S., Nicoll, A.J., Zloh, M., Fisher, E.M., Parkinson, G., and Isaacs, A.M. (2012). C9orf72 hexanucleotide repeat associated with amyotrophic lateral sclerosis and frontotemporal dementia forms RNA G-quadruplexes. *Sci. Rep.* 2, 1016.
- Freibaum, B.D., Lu, Y., Lopez-Gonzalez, R., Kim, N.C., Almeida, S., Lee, K.H., Badders, N., Valentine, M., Miller, B.L., Wong, P.C., et al. (2015). GGGGCC repeat expansion in C9orf72 compromises nucleocytoplasmic transport. *Nature* 525, 129–133.
- Frey, S., Richter, R.P., and Görlich, D. (2006). FG-rich repeats of nuclear pore proteins form a three-dimensional meshwork with hydrogel-like properties. *Science* 314, 815–817.
- Freyermuth, F., Rau, F., Kokunai, Y., Linke, T., Sellier, C., Nakamori, M., Kino, Y., Arandel, L., Jollet, A., Thibault, C., et al. (2016). Splicing misregulation of SCN5A contributes to cardiac-conduction delay and heart arrhythmia in myotonic dystrophy. *Nat. Commun.* 7, 11067.
- Fu, H., Feng, J., Liu, Q., Sun, F., Tie, Y., Zhu, J., Xing, R., Sun, Z., and Zheng, X. (2009). Stress induces tRNA cleavage by angiogenin in mammalian cells. *FEBS Lett.* 583, 437–442.
- Fuentealba, R.A., Udan, M., Bell, S., Wegorzewska, I., Shao, J., Diamond, M.I., Weihl, C.C., and Baloh, R.H. (2010). Interaction with polyglutamine aggregates reveals a Q/N-rich domain in TDP-43. *J. Biol. Chem.* 285, 26304–26314.
- Gabanella, F., Butchbach, M.E., Saieva, L., Carissimi, C., Burghes, A.H., and Pellizzoni, L. (2007). Ribonucleoprotein assembly defects correlate with spinal muscular atrophy severity and preferentially affect a subset of spliceosomal snRNPs. *PLoS ONE* 2, e921.
- Galbán, S., Kuwano, Y., Pullmann, R., Jr., Martindale, J.L., Kim, H.H., Lal, A., Abdelmohsen, K., Yang, X., Dang, Y., Liu, J.O., et al. (2008). RNA-binding proteins HuR and PTB promote the translation of hypoxia-inducible factor 1alpha. *Mol. Cell. Biol.* 28, 93–107.
- Gao, X., and Xu, Z. (2008). Mechanisms of action of angiogenin. *Acta Biochim. Biophys. Sin. (Shanghai)* 40, 619–624.
- Gasset-Rosa, F., Chillon-Marin, C., Goginashvili, A., Atwal, R.S., Artates, J.W., Tabet, R., Wheeler, V.C., Bang, A.G., Cleveland, D.W., and Lagier-Tourange, C. (2017). Polyglutamine-expanded Huntingtin exacerbates age-related disruption of nuclear integrity and nucleocytoplasmic transport. *Neuron* 94, 48–57.
- Gehman, L.T., Stoilov, P., Maguire, J., Damianov, A., Lin, C.H., Shiue, L., Ares, M., Jr., Mody, I., and Black, D.L. (2011). The splicing regulator Rbfox1 (A2BP1) controls neuronal excitation in the mammalian brain. *Nat. Genet.* 43, 706–711.
- Gendron, T.F., Bieniek, K.F., Zhang, Y.J., Jansen-West, K., Ash, P.E., Caulfield, T., Daugherty, L., Dunmore, J.H., Castanedes-Casey, M., Chew, J., et al. (2013). Antisense transcripts of the expanded C9orf72 hexanucleotide repeat form nuclear RNA foci and undergo repeat-associated non-ATG translation in c9FTD/ALS. *Acta Neuropathol.* 126, 829–844.
- Gendron, T.F., Belzil, V.V., Zhang, Y.J., and Petrucelli, L. (2014). Mechanisms of toxicity in C9FTLD/ALS. *Acta Neuropathol.* 127, 359–376.
- Gerbino, V., Carri, M.T., Cozzolino, M., and Achsel, T. (2013). Mislocalised FUS mutants stall spliceosomal snRNPs in the cytoplasm. *Neurobiol. Dis.* 55, 120–128.
- Gertz, B., Wong, M., and Martin, L.J. (2012). Nuclear localization of human SOD1 and mutant SOD1-specific disruption of survival motor neuron protein complex in transgenic amyotrophic lateral sclerosis mice. *J. Neuropathol. Exp. Neurol.* 71, 162–177.
- Gessert, S., Bugner, V., Tecza, A., Pinker, M., and Kühl, M. (2010). FMR1/FXR1 and the miRNA pathway are required for eye and neural crest development. *Dev. Biol.* 341, 222–235.
- Gilks, N., Kedersha, N., Ayodele, M., Shen, L., Stoecklin, G., Dember, L.M., and Anderson, P. (2004). Stress granule assembly is mediated by prion-like aggregation of TIA-1. *Mol. Biol. Cell* 15, 5383–5398.
- Gitler, A.D., and Shorter, J. (2011). RNA-binding proteins with prion-like domains in ALS and FTLU. *Prion* 5, 179–187.
- Goodarzi, H., Najafabadi, H.S., Oikonomou, P., Greco, T.M., Fish, L., Salavati, R., Cristea, I.M., and Tavazoie, S. (2012). Systematic discovery of structural elements governing stability of mammalian messenger RNAs. *Nature* 485, 264–268.
- Goodwin, M., Mohan, A., Batra, R., Lee, K.Y., Charizanis, K., Fernández Gómez, F.J., Eddarkaoui, S., Sergeant, N., Buée, L., Kimura, T., et al. (2015). MBNL sequestration by toxic RNAs and RNA misprocessing in the myotonic dystrophy brain. *Cell Rep.* 12, 1159–1168.
- Greenway, M.J., Andersen, P.M., Russ, C., Ennis, S., Cashman, S., Donaghy, C., Patterson, V., Swingler, R., Kieran, D., Prehn, J., et al. (2006). ANG mutations segregate with familial and ‘sporadic’ amyotrophic lateral sclerosis. *Nat. Genet.* 38, 411–413.
- Gregory, J.M., Barros, T.P., Meehan, S., Dobson, C.M., and Luheshi, L.M. (2012). The aggregation and neurotoxicity of TDP-43 and its ALS-associated 25 kDa fragment are differentially affected by molecular chaperones in *Drosophila*. *PLoS ONE* 7, e31899.
- Grima, J.C., Daigle, J.G., Arbez, N., Cunningham, K.C., Zhang, K., Ochaba, J., Geater, C., Morozko, E., Stocksdale, J., Glatzer, J.C., et al. (2017). Mutant Huntingtin disrupts the nuclear pore complex. *Neuron* 94, 93–107.

- Groen, E.J., Fumoto, K., Blokhuis, A.M., Engelen-Lee, J., Zhou, Y., van den Heuvel, D.M., Koppers, M., van Diggelen, F., van Heest, J., Demmers, J.A., et al. (2013). ALS-associated mutations in FUS disrupt the axonal distribution and function of SMN. *Hum. Mol. Genet.* **22**, 3690–3704.
- Groen, E.J.N., Talbot, K., and Gillingwater, T.H. (2018). Advances in therapy for spinal muscular atrophy: promises and challenges. *Nat. Rev. Neurol.* **14**, 214–224.
- Guo, W., Naujock, M., Fumagalli, L., Vandoorne, T., Baatsen, P., Boon, R., Ordoñas, L., Patel, A., Welters, M., Vanwelden, T., et al. (2017). HDAC6 inhibition reverses axonal transport defects in motor neurons derived from FUS-ALS patients. *Nat. Commun.* **8**, 861.
- Guo, L., Kim, H.J., Wang, H., Monaghan, J., Freyermuth, F., Sung, J.C., O'Donovan, K., Fare, C.M., Diaz, Z., Singh, N., et al. (2018). Nuclear-import receptors reverse aberrant phase transitions of RNA-binding proteins with prion-like domains. *Cell* **173**, 677–692.
- Haeusler, A.R., Donnelly, C.J., Periz, G., Simko, E.A., Shaw, P.G., Kim, M.S., Maragakis, N.J., Troncoso, J.C., Pandey, A., Sattler, R., et al. (2014). C9orf72 nucleotide repeat structures initiate molecular cascades of disease. *Nature* **507**, 195–200.
- Hagerman, P.J., and Hagerman, R.J. (2007). Fragile X-associated tremor/ataxia syndrome—an older face of the fragile X gene. *Nat. Clin. Pract. Neurol.* **3**, 107–112.
- Hagerman, R.J., Leehey, M., Heinrichs, W., Tassone, F., Wilson, R., Hills, J., Grigsby, J., Gage, B., and Hagerman, P.J. (2001). Intention tremor, parkinsonism, and generalized brain atrophy in male carriers of fragile X. *Neurology* **57**, 127–130.
- Hagerman, R.J., Berry-Kravis, E., Hazlett, H.C., Bailey, D.B., Jr., Moine, H., Kooy, R.F., Tassone, F., Gantois, I., Sonenberg, N., Mandel, J.L., and Hagerman, P.J. (2017). Fragile X syndrome. *Nat. Rev. Dis. Primers* **3**, 17065.
- Han, T.W., Kato, M., Xie, S., Wu, L.C., Mirzaei, H., Pei, J., Chen, M., Xie, Y., Allen, J., Xiao, G., and McKnight, S.L. (2012). Cell-free formation of RNA granules: bound RNAs identify features and components of cellular assemblies. *Cell* **149**, 768–779.
- Harrington, L.B., Burstein, D., Chen, J.S., Paez-Espino, D., Ma, E., Witte, I.P., Cofsky, J.C., Kyrpides, N.C., Banfield, J.F., and Doudna, J.A. (2018). Programmed DNA destruction by miniature CRISPR-Cas14 enzymes. *Science* **362**, 839–842.
- Hart, M.P., and Gitler, A.D. (2012). ALS-associated ataxin 2 polyQ expansions enhance stress-induced caspase 3 activation and increase TDP-43 pathological modifications. *J. Neurosci.* **32**, 9133–9142.
- He, C.X., and Portera-Cailliau, C. (2013). The trouble with spines in fragile X syndrome: density, maturity and plasticity. *Neuroscience* **251**, 120–128.
- Hernández-Vega, A., Braun, M., Scharrel, L., Jahnel, M., Wegmann, S., Hyman, B.T., Alberti, S., Diez, S., and Hyman, A.A. (2017). Local nucleation of microtubule bundles through tubulin concentration into a condensed Tau phase. *Cell Rep.* **20**, 2304–2312.
- Hinman, M.N., and Lou, H. (2008). Diverse molecular functions of Hu proteins. *Cell. Mol. Life Sci.* **65**, 3168–3181.
- Hoell, J.I., Larsson, E., Runge, S., Nusbaum, J.D., Duggimpudi, S., Farazi, T.A., Hafner, M., Borkhardt, A., Sander, C., and Tuschl, T. (2011). RNA targets of wild-type and mutant FET family proteins. *Nat. Struct. Mol. Biol.* **18**, 1428–1431.
- Hofweber, M., Hutten, S., Bourgeois, B., Spreitzer, E., Niedner-Boblentz, A., Schifferer, M., Ruepp, M.D., Simons, M., Niessing, D., Madl, T., et al. (2018). Phase separation of FUS is suppressed by its nuclear import receptor and arginine methylation. *Cell* **173**, 706–719.
- Hua, Y., and Zhou, J. (2004). Survival motor neuron protein facilitates assembly of stress granules. *FEBS Lett.* **572**, 69–74.
- Hua, Y., Vickers, T.A., Okunola, H.L., Bennett, C.F., and Krainer, A.R. (2008). Antisense masking of an hnRNP A1/A2 intronic splicing silencer corrects SMN2 splicing in transgenic mice. *Am. J. Hum. Genet.* **82**, 834–848.
- Hua, Y., Sahashi, K., Hung, G., Rigo, F., Passini, M.A., Bennett, C.F., and Krainer, A.R. (2010). Antisense correction of SMN2 splicing in the CNS rescues necrosis in a type III SMA mouse model. *Genes Dev.* **24**, 1634–1644.
- Hua, Y., Sahashi, K., Rigo, F., Hung, G., Horev, G., Bennett, C.F., and Krainer, A.R. (2011). Peripheral SMN restoration is essential for long-term rescue of a severe spinal muscular atrophy mouse model. *Nature* **478**, 123–126.
- Huelga, S.C., Vu, A.Q., Arnold, J.D., Liang, T.Y., Liu, P.P., Yan, B.Y., Donohue, J.P., Shiue, L., Hoon, S., Brenner, S., et al. (2012). Integrative genome-wide analysis reveals cooperative regulation of alternative splicing by hnRNP proteins. *Cell Rep.* **7**, 167–178.
- Huo, Q., Kayikci, M., Odermatt, P., Meyer, K., Michels, O., Saxena, S., Ule, J., and Schümperli, D. (2014). Splicing changes in SMA mouse motoneurons and SMN-depleted neuroblastoma cells: evidence for involvement of splicing regulatory proteins. *RNA Biol.* **11**, 1430–1446.
- Hyman, A.A., Weber, C.A., and Jülicher, F. (2014). Liquid-liquid phase separation in biology. *Annu. Rev. Cell Dev. Biol.* **30**, 39–58.
- Ibrahim, F., Maragkakis, M., Alexiou, P., Maronski, M.A., Dichter, M.A., and Mourelatos, Z. (2013). Identification of in vivo, conserved, TAF15 RNA binding sites reveals the impact of TAF15 on the neuronal transcriptome. *Cell Rep.* **3**, 301–308.
- Imlach, W.L., Beck, E.S., Choi, B.J., Lotti, F., Pellizzoni, L., and McCabe, B.D. (2012). SMN is required for sensory-motor circuit function in *Drosophila*. *Cell* **151**, 427–439.
- Irwin, S.A., Patel, B., Idupulapati, M., Harris, J.B., Crisostomo, R.A., Larsen, B.P., Kooy, F., Willems, P.J., Cras, P., Kozlowski, P.B., et al. (2001). Abnormal dendritic spine characteristics in the temporal and visual cortices of patients with fragile-X syndrome: a quantitative examination. *Am. J. Med. Genet.* **98**, 161–167.
- Ishigaki, S., Masuda, A., Fujioka, Y., Iguchi, Y., Katsuno, M., Shibata, A., Urano, F., Sobue, G., and Ohno, K. (2012). Position-dependent FUS-RNA interactions regulate alternative splicing events and transcriptions. *Sci. Rep.* **2**, 529.
- Ishiguro, T., Sato, N., Ueyama, M., Fujikake, N., Sellier, C., Kanegami, A., Tokuda, E., Zamiri, B., Gall-Duncan, T., Mirceta, M., et al. (2017). Regulatory role of RNA chaperone TDP-43 for RNA misfolding and repeat-associated translation in SCA31. *Neuron* **94**, 108–124.e7.
- Ishihara, T., Arizumi, Y., Shiga, A., Kato, T., Tan, C.F., Sato, T., Miki, Y., Yokoo, M., Fujino, T., Koyama, A., et al. (2013). Decreased number of Gemini of coiled bodies and U12 snRNA level in amyotrophic lateral sclerosis. *Hum. Mol. Genet.* **22**, 4136–4147.
- Ishizuka, A., Siomi, M.C., and Siomi, H. (2002). A *Drosophila* fragile X protein interacts with components of RNAi and ribosomal proteins. *Genes Dev.* **16**, 2497–2508.
- Jablonka, S., Beck, M., Lechner, B.D., Mayer, C., and Sendtner, M. (2007). Defective Ca²⁺ channel clustering in axon terminals disturbs excitability in motoneurons in spinal muscular atrophy. *J. Cell Biol.* **179**, 139–149.
- Jackrel, M.E., DeSantis, M.E., Martinez, B.A., Castellano, L.M., Stewart, R.M., Caldwell, K.A., Caldwell, G.A., and Shorter, J. (2014). Potentiated Hsp104 variants antagonize diverse proteotoxic misfolding events. *Cell* **156**, 170–182.
- Jacquemont, S., Hagerman, R.J., Leehey, M., Grigsby, J., Zhang, L., Brunberg, J.A., Greco, C., Des Portes, V., Jardini, T., Levine, R., et al. (2003). Fragile X premutation tremor/ataxia syndrome: molecular, clinical, and neuroimaging correlates. *Am. J. Hum. Genet.* **72**, 869–878.
- Jain, S., Wheeler, J.R., Walters, R.W., Agrawal, A., Barsic, A., and Parker, R. (2016). ATPase-modulated stress granules contain a diverse proteome and substructure. *Cell* **164**, 487–498.
- Jean-Philippe, J., Paz, S., and Caputi, M. (2013). hnRNP A1: the Swiss army knife of gene expression. *Int. J. Mol. Sci.* **14**, 18999–19024.
- Jiang, H., Mankodi, A., Swanson, M.S., Moxley, R.T., and Thornton, C.A. (2004). 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.* **13**, 3079–3088.

- Jiang, J., Zhu, Q., Gendron, T.F., Saberi, S., McAlonis-Downes, M., Seelman, A., Stauffer, J.E., Jafar-Nejad, P., Drenner, K., Schulte, D., et al. (2016). Gain of toxicity from ALS/FTD-linked repeat expansions in C9ORF72 is alleviated by antisense oligonucleotides targeting GGGGCC-containing RNAs. *Neuron* 90, 535–550.
- Jin, P., Zarnescu, D.C., Ceman, S., Nakamoto, M., Mowrey, J., Jongens, T.A., Nelson, D.L., Moses, K., and Warren, S.T. (2004). Biochemical and genetic interaction between the fragile X mental retardation protein and the microRNA pathway. *Nat. Neurosci.* 7, 113–117.
- Jin, P., Duan, R., Qurashi, A., Qin, Y., Tian, D., Rosser, T.C., Liu, H., Feng, Y., and Warren, S.T. (2007). Pur alpha binds to rCGG repeats and modulates repeat-mediated neurodegeneration in a Drosophila model of fragile X tremor/ataxia syndrome. *Neuron* 55, 556–564.
- Johnson, J.O., Piro, E.P., Boehringer, A., Chia, R., Feit, H., Renton, A.E., Pflner, H.A., Abramzon, Y., Marangi, G., Winborn, B.J., et al.; ITALS GEN (2014). Mutations in the Matrin 3 gene cause familial amyotrophic lateral sclerosis. *Nat. Neurosci.* 17, 664–666.
- Jovičić, A., Mertens, J., Boeynaems, S., Bogaert, E., Chai, N., Yamada, S.B., Paul, J.W., 3rd, Sun, S., Herdy, J.R., Bieri, G., et al. (2015). Modifiers of C9orf72 dipeptide repeat toxicity connect neurocytoplasmic transport defects to FTD/ALS. *Nat. Neurosci.* 18, 1226–1229.
- Ju, S., Tardiff, D.F., Han, H., Divya, K., Zhong, Q., Maquat, L.E., Bosco, D.A., Hayward, L.J., Brown, R.H., Jr., Lindquist, S., et al. (2011). A yeast model of FUS/TLS-dependent cytotoxicity. *PLoS Biol.* 9, e1001052.
- Kaida, D., Berg, M.G., Younis, I., Kasim, M., Singh, L.N., Wan, L., and Dreyfuss, G. (2010). U1 snRNP protects pre-mRNAs from premature cleavage and polyadenylation. *Nature* 468, 664–668.
- Kanadia, R.N., Shin, J., Yuan, Y., Beattie, S.G., Wheeler, T.M., Thornton, C.A., and Swanson, M.S. (2006). Reversal of RNA missplicing and myotonia after muscleblind overexpression in a mouse poly(CUG) model for myotonic dystrophy. *Proc. Natl. Acad. Sci. USA* 103, 11748–11753.
- Kanai, Y., Dohmae, N., and Hirokawa, N. (2004). Kinesin transports RNA: isolation and characterization of an RNA-transporting granule. *Neuron* 43, 513–525.
- Kanekura, K., Yagi, T., Cammack, A.J., Mahadevan, J., Kuroda, M., Harms, M.B., Miller, T.M., and Urano, F. (2016). Poly-dipeptides encoded by the C9ORF72 repeats block global protein translation. *Hum. Mol. Genet.* 25, 1803–1813.
- Kao, D.I., Aldridge, G.M., Weiler, I.J., and Greenough, W.T. (2010). Altered mRNA transport, docking, and protein translation in neurons lacking fragile X mental retardation protein. *Proc. Natl. Acad. Sci. USA* 107, 15601–15606.
- Kapeli, K., Pratt, G.A., Vu, A.Q., Hutt, K.R., Martinez, F.J., Sundaraman, B., Batra, R., Freese, P., Lambert, N.J., Huelga, S.C., et al. (2016). Distinct and shared functions of ALS-associated proteins TDP-43, FUS and TAF15 revealed by multisystem analyses. *Nat. Commun.* 7, 12143.
- Kariya, S., Re, D.B., Jacquier, A., Nelson, K., Przedborski, S., and Monani, U.R. (2012). Mutant superoxide dismutase 1 (SOD1), a cause of amyotrophic lateral sclerosis, disrupts the recruitment of SMN, the spinal muscular atrophy protein to nuclear Cajal bodies. *Hum. Mol. Genet.* 21, 3421–3434.
- Kashima, T., and Manley, J.L. (2003). A negative element in SMN2 exon 7 inhibits splicing in spinal muscular atrophy. *Nat. Genet.* 34, 460–463.
- Kato, M., Han, T.W., Xie, S., Shi, K., Du, X., Wu, L.C., Mirzaei, H., Goldsmith, E.J., Longgood, J., Pei, J., et al. (2012). Cell-free formation of RNA granules: low complexity sequence domains form dynamic fibers within hydrogels. *Cell* 149, 753–767.
- Kawai, T., Lal, A., Yang, X., Galban, S., Mazan-Mamczarz, K., and Gorospe, M. (2006). Translational control of cytochrome c by RNA-binding proteins TIA-1 and HuR. *Mol. Cell. Biol.* 26, 3295–3307.
- Kedersha, N., Cho, M.R., Li, W., Yacono, P.W., Chen, S., Gilks, N., Golan, D.E., and Anderson, P. (2000). Dynamic shuttling of TIA-1 accompanies the recruitment of mRNA to mammalian stress granules. *J. Cell Biol.* 151, 1257–1268.
- Kedersha, N., Stoecklin, G., Ayodele, M., Yacono, P., Lykke-Andersen, J., Fritzler, M.J., Scheuner, D., Kaufman, R.J., Golan, D.E., and Anderson, P. (2005). Stress granules and processing bodies are dynamically linked sites of mRNP remodeling. *J. Cell Biol.* 169, 871–884.
- Kenneson, A., Zhang, F., Hagedorn, C.H., and Warren, S.T. (2001). Reduced FMRP and increased FMR1 transcription is proportionally associated with CGG repeat number in intermediate-length and premutation carriers. *Hum. Mol. Genet.* 10, 1449–1454.
- Khandjian, E.W., Corbin, F., Woerly, S., and Rousseau, F. (1996). The fragile X mental retardation protein is associated with ribosomes. *Nat. Genet.* 12, 91–93.
- Kieran, D., Sebastia, J., Greenway, M.J., King, M.A., Connaughton, D., Concannon, C.G., Fenner, B., Hardiman, O., and Prehn, J.H. (2008). Control of motoneuron survival by angiogenin. *J. Neurosci.* 28, 14056–14061.
- Kim, H.J., Kim, N.C., Wang, Y.D., Scarborough, E.A., Moore, J., Diaz, Z., MacLea, K.S., Freibaum, B., Li, S., Mollieux, A., et al. (2013). Mutations in prion-like domains in hnRNPA2B1 and hnRNPA1 cause multisystem proteinopathy and ALS. *Nature* 495, 467–473.
- King, O.D., Gitler, A.D., and Shorter, J. (2012). The tip of the iceberg: RNA-binding proteins with prion-like domains in neurodegenerative disease. *Brain Res.* 1462, 61–80.
- Klim, J.R., Williams, L.A., Limone, F., Guerra San Juan, I., Davis-Dusenbery, B.N., Mordes, D.A., Burberry, A., Steinbaugh, M.J., Gamage, K.K., Kirchner, R., et al. (2019). ALS-implicated protein TDP-43 sustains levels of STMN2, a mediator of motor neuron growth and repair. *Nat. Neurosci.* 22, 167–179.
- Konermann, S., Lotfy, P., Brideau, N.J., Oki, J., Shokhiev, M.N., and Hsu, P.D. (2018). Transcriptome engineering with RNA-targeting type VI-D CRISPR effectors. *Cell* 173, 665–676.
- Kordasiewicz, H.B., Stanek, L.M., Wancewicz, E.V., Mazur, C., McAlonis, M.M., Pytel, K.A., Artates, J.W., Weiss, A., Cheng, S.H., Shihabuddin, L.S., et al. (2012). Sustained therapeutic reversal of Huntington's disease by transient repression of huntingtin synthesis. *Neuron* 74, 1031–1044.
- Kramer, N.J., Carlomagno, Y., Zhang, Y.J., Almeida, S., Cook, C.N., Gendron, T.F., Prudencio, M., Van Blitterswijk, M., Belzil, V., Couthouis, J., et al. (2016). Spt4 selectively regulates the expression of C9orf72 sense and antisense mutant transcripts. *Science* 353, 708–712.
- Kremer, E.J., Pritchard, M., Lynch, M., Yu, S., Holman, K., Baker, E., Warren, S.T., Schlessinger, D., Sutherland, G.R., and Richards, R.I. (1991). Mapping of DNA instability at the fragile X to a trinucleotide repeat sequence p(CCG)n. *Science* 252, 1711–1714.
- Kumari, D., Lokanga, R., Yudkin, D., Zhao, X.N., and Usdin, K. (2012). Chromatin changes in the development and pathology of the fragile X-associated disorders and Friedreich ataxia. *Biochim. Biophys. Acta* 1819, 802–810.
- Kuyumcu-Martinez, N.M., Wang, G.S., and Cooper, T.A. (2007). Increased steady-state levels of CUGBP1 in myotonic dystrophy 1 are due to PKC-mediated hyperphosphorylation. *Mol. Cell* 28, 68–78.
- Kwiatkowski, T.J., Jr., Bosco, D.A., Leclerc, A.L., Tamrazian, E., Vandenberg, C.R., Russ, C., Davis, A., Gilchrist, J., Kasarskis, E.J., Munsat, T., et al. (2009). Mutations in the FUS/TLS gene on chromosome 16 cause familial amyotrophic lateral sclerosis. *Science* 323, 1205–1208.
- Kwon, I., Xiang, S., Kato, M., Wu, L., Theodoropoulos, P., Wang, T., Kim, J., Yun, J., Xie, Y., and McKnight, S.L. (2014). Poly-dipeptides encoded by the C9orf72 repeats bind nucleoli, impede RNA biogenesis, and kill cells. *Science* 345, 1139–1145.
- Kye, M.J., Niederst, E.D., Wertz, M.H., Gonçalves, Ido.C., Akten, B., Dover, K.Z., Peters, M., Riessland, M., Neveu, P., Wirth, B., et al. (2014). SMN regulates axonal local translation via miR-183/mTOR pathway. *Hum. Mol. Genet.* 23, 6318–6331.
- Lagier-Tourenne, C., Polymenidou, M., and Cleveland, D.W. (2010). TDP-43 and FUS/TLS: emerging roles in RNA processing and neurodegeneration. *Hum. Mol. Genet.* 19 (R1), R46–R64.
- Lagier-Tourenne, C., Polymenidou, M., Hutt, K.R., Vu, A.Q., Baughn, M., Huelga, S.C., Clutario, K.M., Ling, S.C., Liang, T.Y., Mazur, C., et al. (2012). Divergent roles of ALS-linked proteins FUS/TLS and TDP-43 intersect in processing long pre-mRNAs. *Nat. Neurosci.* 15, 1488–1497.
- Lagier-Tourenne, C., Baughn, M., Rigo, F., Sun, S., Liu, P., Li, H.R., Jiang, J., Watt, A.T., Chun, S., Katz, M., et al. (2013). Targeted degradation of sense and

- antisense C9orf72 RNA foci as therapy for ALS and frontotemporal degeneration. *Proc. Natl. Acad. Sci. USA* **110**, E4530–E4539.
- Lai, J.I., Leman, L.J., Ku, S., Vickers, C.J., Olsen, C.A., Montero, A., Ghadiri, M.R., and Gottesfeld, J.M. (2017). Cyclic tetrapeptide HDAC inhibitors as potential therapeutics for spinal muscular atrophy: Screening with iPSC-derived neuronal cells. *Bioorg. Med. Chem. Lett.* **27**, 3289–3293.
- Lancaster, A.K., Nutter-Upham, A., Lindquist, S., and King, O.D. (2014). PLAAC: a web and command-line application to identify proteins with prion-like amino acid composition. *Bioinformatics* **30**, 2501–2502.
- Lee, J.E., and Cooper, T.A. (2009). Pathogenic mechanisms of myotonic dystrophy. *Biochem. Soc. Trans.* **37**, 1281–1286.
- Lee, Y.B., Chen, H.J., Peres, J.N., Gomez-Deza, J., Attig, J., Stalekar, M., Troakes, C., Nishimura, A.L., Scotter, E.L., Vance, C., et al. (2013). Hexanucleotide repeats in ALS/FTD form length-dependent RNA foci, sequester RNA binding proteins, and are neurotoxic. *Cell Rep.* **5**, 1178–1186.
- Lee, J.A., Damianov, A., Lin, C.H., Fontes, M., Parikshak, N.N., Anderson, E.S., Geschwind, D.H., Black, D.L., and Martin, K.C. (2016a). Cytoplasmic Rbfox1 regulates the expression of synaptic and autism-related genes. *Neuron* **89**, 113–128.
- Lee, K.H., Zhang, P., Kim, H.J., Mitrea, D.M., Sarkar, M., Freibaum, B.D., Cika, J., Coughlin, M., Messing, J., Molliex, A., et al. (2016b). C9orf72 dipeptide repeats impair the assembly, dynamics, and function of membrane-less organelles. *Cell* **167**, 774–788.
- Lefebvre, S., Bürglen, L., Reboullet, S., Clermont, O., Burlet, P., Viollet, L., Benichou, B., Cruaud, C., Millasseau, P., Zeviani, M., et al. (1995). Identification and characterization of a spinal muscular atrophy-determining gene. *Cell* **80**, 155–165.
- Leigh, M.J., Nguyen, D.V., Mu, Y., Winarni, T.I., Schneider, A., Chechi, T., Polussa, J., Doucet, P., Tassone, F., Rivera, S.M., et al. (2013). A randomized double-blind, placebo-controlled trial of minocycline in children and adolescents with fragile x syndrome. *J. Dev. Behav. Pediatr.* **34**, 147–155.
- Li, P., Banjade, S., Cheng, H.C., Kim, S., Chen, B., Guo, L., Llaguno, M., Hollingsworth, J.V., King, D.S., Banani, S.F., et al. (2012). Phase transitions in the assembly of multivalent signalling proteins. *Nature* **483**, 336–340.
- Li, Y.R., King, O.D., Shorter, J., and Gitler, A.D. (2013). Stress granules as crucibles of ALS pathogenesis. *J. Cell Biol.* **201**, 361–372.
- Li, D.K., Tisdale, S., Lotti, F., and Pellizzoni, L. (2014). SMN control of RNP assembly: from post-transcriptional gene regulation to motor neuron disease. *Semin. Cell Dev. Biol.* **32**, 22–29.
- Li, S., Zhang, P., Freibaum, B.D., Kim, N.C., Kolaitis, R.M., Molliex, A., Kanagaraj, A.P., Yabe, I., Tanino, M., Tanaka, S., et al. (2016). Genetic interaction of hnRNP A2B1 and DNAJB6 in a Drosophila model of multisystem proteinopathy. *Hum. Mol. Genet.* **25**, 936–950.
- Licatalosi, D.D., Mele, A., Fak, J.J., Ule, J., Kayikci, M., Chi, S.W., Clark, T.A., Schweitzer, A.C., Blume, J.E., Wang, X., et al. (2008). HITS-CLIP yields genome-wide insights into brain alternative RNA processing. *Nature* **456**, 464–469.
- Lin, Y., Protter, D.S., Rosen, M.K., and Parker, R. (2015). Formation and maturation of phase-separated liquid droplets by RNA-binding proteins. *Mol. Cell* **60**, 208–219.
- Lin, Y., Mori, E., Kato, M., Xiang, S., Wu, L., Kwon, I., and McKnight, S.L. (2016). Toxic PR poly-dipeptides encoded by the C9orf72 repeat expansion target LC domain polymers. *Cell* **167**, 789–802.
- Ling, S.C., Albuquerque, C.P., Han, J.S., Lagier-Tourenne, C., Tokunaga, S., Zhou, H., and Cleveland, D.W. (2010). ALS-associated mutations in TDP-43 increase its stability and promote TDP-43 complexes with FUS/TLS. *Proc. Natl. Acad. Sci. USA* **107**, 13318–13323.
- Ling, J.P., Pletnikova, O., Troncoso, J.C., and Wong, P.C. (2015). TDP-43 repression of nonconserved cryptic exons is compromised in ALS-FTD. *Science* **349**, 650–655.
- Liquori, C.L., Ricker, K., Moseley, M.L., Jacobsen, J.F., Kress, W., Naylor, S.L., Day, J.W., and Ranum, L.P. (2001). Myotonic dystrophy type 2 caused by a CCTG expansion in intron 1 of ZNF9. *Science* **293**, 864–867.
- Liu, Q., and Dreyfuss, G. (1996). A novel nuclear structure containing the survival of motor neurons protein. *EMBO J.* **15**, 3555–3565.
- Liu, Q., Fischer, U., Wang, F., and Dreyfuss, G. (1997). The spinal muscular atrophy disease gene product, SMN, and its associated protein SIP1 are in a complex with spliceosomal snRNP proteins. *Cell* **90**, 1013–1021.
- Liu, X.S., Wu, H., Krzisch, M., Wu, X., Graef, J., Muffat, J., Hnisz, D., Li, C.H., Yuan, B., Xu, C., et al. (2018). Rescue of Fragile X Syndrome Neurons by DNA methylation editing of the FMR1 gene. *Cell* **172**, 979–992.
- Lopez-Erauskin, J., Tadokoro, T., Baughn, M.W., Myers, B., McAlonis-Downes, M., Chillon-Marinan, C., Asiaban, J.N., Artates, J., Bui, A.T., Vetto, A.P., et al. (2018). ALS/FTD-linked mutation in FUS suppresses intra-axonal protein synthesis and drives disease without nuclear loss of function of FUS. *Neuron* **100**, 816–830.
- Lopez-Gonzalez, R., Lu, Y., Gendron, T.F., Karydas, A., Tran, H., Yang, D., Petrucelli, L., Miller, B.L., Almeida, S., and Gao, F.B. (2016). Poly(GR) in C9ORF72-related ALS/FTD compromises mitochondrial function and increases oxidative stress and DNA damage in iPSC-derived motor neurons. *Neuron* **92**, 383–391.
- Lorson, C.L., Strasswimmer, J., Yao, J.M., Baleja, J.D., Hahnen, E., Wirth, B., Le, T., Burghes, A.H., and Androphy, E.J. (1998). SMN oligomerization defect correlates with spinal muscular atrophy severity. *Nat. Genet.* **19**, 63–66.
- Lorson, C.L., Hahnen, E., Androphy, E.J., and Wirth, B. (1999). A single nucleotide in the SMN gene regulates splicing and is responsible for spinal muscular atrophy. *Proc. Natl. Acad. Sci. USA* **96**, 6307–6311.
- Lotti, F., Imlach, W.L., Saieva, L., Beck, E.S., Hao, T., Li, D.K., Jiao, W., Mentis, G.Z., Beattie, C.E., McCabe, B.D., and Pellizzoni, L. (2012). An SMN-dependent U12 splicing event essential for motor circuit function. *Cell* **151**, 440–454.
- Lukacs, S., Szabo, N., and Woodhams, S. (2012). Rare association of anti-hu antibody positive paraneoplastic neurological syndrome and transitional cell bladder carcinoma. *Case Rep. Urol.* **2012**, 724940.
- Lukong, K.E., and Richard, S. (2008). Motor coordination defects in mice deficient for the Sam68 RNA-binding protein. *Behav. Brain Res.* **189**, 357–363.
- Mackenzie, I.R., Nicholson, A.M., Sarkar, M., Messing, J., Purice, M.D., Pottier, C., Annu, K., Baker, M., Perkerson, R.B., Kurti, A., et al. (2017). TIA1 mutations in amyotrophic lateral sclerosis and frontotemporal dementia promote phase separation and alter stress granule dynamics. *Neuron* **95**, 808–816.
- Maharana, S., Wang, J., Papadopoulos, D.K., Richter, D., Pozniakovskiy, A., Poser, I., Bickle, M., Rizk, S., Guillén-Boixet, J., Franzmann, T.M., et al. (2018). RNA buffers the phase separation behavior of prion-like RNA binding proteins. *Science* **360**, 918–921.
- Majumder, P., Chu, J.F., Chatterjee, B., Swamy, K.B., and Shen, C.J. (2016). Co-regulation of mRNA translation by TDP-43 and fragile X syndrome protein FMRP. *Acta Neuropathol.* **132**, 721–738.
- Mankodi, A., Logigian, E., Callahan, L., McClain, C., White, R., Henderson, D., Krym, M., and Thornton, C.A. (2000). Myotonic dystrophy in transgenic mice expressing an expanded CUG repeat. *Science* **289**, 1769–1773.
- March, Z.M., King, O.D., and Shorter, J. (2016). Prion-like domains as epigenetic regulators, scaffolds for subcellular organization, and drivers of neurodegenerative disease. *Brain Res.* **1647**, 9–18.
- Markmiller, S., Soltanieh, S., Server, K.L., Mak, R., Jin, W., Fang, M.Y., Luo, E.C., Krach, F., Yang, D., Sen, A., et al. (2018). Context-dependent and disease-specific diversity in protein interactions within stress granules. *Cell* **172**, 590–604.
- Martin, C.L., Duvall, J.A., Ilkin, Y., Simon, J.S., Arreaza, M.G., Wilkes, K., Alvarez-Retuerto, A., Whichello, A., Powell, C.M., Rao, K., et al. (2007). Cytogenetic and molecular characterization of A2BP1/FOX1 as a candidate gene for autism. *Am. J. Med. Genet. B. Neuropsychiatr. Genet.* **144B**, 869–876.
- Martinez, F.J., Pratt, G.A., Van Nostrand, E.L., Batra, R., Huelga, S.C., Kapeli, K., Freese, P., Chun, S.J., Ling, K., Gelboin-Burkhart, C., et al. (2016). Protein-RNA networks regulated by normal and ALS-associated mutant HNRNPA2B1 in the nervous system. *Neuron* **92**, 780–795.

- Masuda, A., Andersen, H.S., Doktor, T.K., Okamoto, T., Ito, M., Andresen, B.S., and Ohno, K. (2012). CUGBP1 and MBNL1 preferentially bind to 3' UTRs and facilitate mRNA decay. *Sci. Rep.* 2, 209.
- Masuda, A., Takeda, J., Okuno, T., Okamoto, T., Ohkawara, B., Ito, M., Ishigaki, S., Sobue, G., and Ohno, K. (2015). Position-specific binding of FUS to nascent RNA regulates mRNA length. *Genes Dev.* 29, 1045–1057.
- Mateju, D., Franzmann, T.M., Patel, A., Kopach, A., Boczek, E.E., Maharana, S., Lee, H.O., Carra, S., Hyman, A.A., and Alberti, S. (2017). An aberrant phase transition of stress granules triggered by misfolded protein and prevented by chaperone function. *EMBO J.* 36, 1669–1687.
- May, S., Hornburg, D., Schludi, M.H., Arzberger, T., Rentzsch, K., Schwenk, B.M., Grässer, F.A., Mori, K., Kremmer, E., Banzhaf-Strathmann, J., et al. (2014). C9orf72 FTL/ALS-associated Gly-Ala dipeptide repeat proteins cause neuronal toxicity and Unc119 sequestration. *Acta Neuropathol.* 128, 485–503.
- Mazan-Mamczarz, K., Galbán, S., López de Silanes, I., Martindale, J.L., Atsroy, U., Keene, J.D., and Gorospe, M. (2003). RNA-binding protein HuR enhances p53 translation in response to ultraviolet light irradiation. *Proc. Natl. Acad. Sci. USA* 100, 8354–8359.
- McCann, C., Holohan, E.E., Das, S., Dervan, A., Larkin, A., Lee, J.A., Rodrigues, V., Parker, R., and Ramaswami, M. (2011). The Ataxin-2 protein is required for microRNA function and synapse-specific long-term olfactory habituation. *Proc. Natl. Acad. Sci. USA* 108, E655–E662.
- McGurk, L., Gomes, E., Guo, L., Mojsilovic-Petrovic, J., Tran, V., Kalb, R.G., Shorter, J., and Bonini, N.M. (2018). Poly(ADP-ribose) prevents pathological phase separation of TDP-43 by promoting liquid demixing and stress granule localization. *Mol. Cell* 71, 703–717.
- Melamed, Z., Lopez-Erauskin, J., Baughn, M.W., Zhang, O., Drenner, K., Sun, Y., Freyermuth, F., McMahon, M.A., Beccari, M.S., Artates, J.W., et al. (2019). Premature polyadenylation-mediated loss of stathmin-2 is a hallmark of TDP-43-dependent neurodegeneration. *Nat. Neurosci.* 22, 180–190.
- Mendell, J.R., Al-Zaidy, S., Shell, R., Arnold, W.D., Rodino-Klapac, L.R., Prior, T.W., Lowes, L., Alfano, L., Berry, K., Church, K., et al. (2017). Single-dose gene-replacement therapy for spinal muscular atrophy. *N. Engl. J. Med.* 377, 1713–1722.
- Mercuri, E., Darras, B.T., Chiriboga, C.A., Day, J.W., Campbell, C., Connolly, A.M., Iannaccone, S.T., Kirschner, J., Kuntz, N.L., Saito, K., et al.; CHERISH Study Group (2018). Nusinersen versus sham control in later-onset spinal muscular atrophy. *N. Engl. J. Med.* 378, 625–635.
- Meyer, C., Garzia, A., Mazzola, M., Gerstberger, S., Molina, H., and Tuschl, T. (2018). The TIA1 RNA-binding protein family regulates EIF2AK2-mediated stress response and cell cycle progression. *Mol. Cell* 69, 622–635.
- Miller, T.M., Pestronk, A., David, W., Rothstein, J., Simpson, E., Appel, S.H., Andres, P.L., Mahoney, K., Allred, P., Alexander, K., et al. (2013). An antisense oligonucleotide against SOD1 delivered intrathecally for patients with SOD1 familial amyotrophic lateral sclerosis: a phase 1, randomised, first-in-man study. *Lancet Neurol.* 12, 435–442.
- Mitchell, S.F., and Parker, R. (2014). Principles and properties of eukaryotic mRNPs. *Mol. Cell* 54, 547–558.
- Miyashiro, K.Y., Beckel-Mitchener, A., Purk, T.P., Becker, K.G., Barret, T., Liu, L., Carbonetto, S., Weiler, I.J., Greenough, W.T., and Eberwine, J. (2003). RNA cargoes associating with FMRP reveal deficits in cellular functioning in Fmr1 null mice. *Neuron* 37, 417–431.
- Mizielinska, S., Lashley, T., Norona, F.E., Clayton, E.L., Ridler, C.E., Fratta, P., and Isaacs, A.M. (2013). C9orf72 frontotemporal lobar degeneration is characterised by frequent neuronal sense and antisense RNA foci. *Acta Neuropathol.* 126, 845–857.
- Mizielinska, S., Grönke, S., Niccoli, T., Ridler, C.E., Clayton, E.L., Devoy, A., Moens, T., Norona, F.E., Woollacott, I.O.C., Pietrzyk, J., et al. (2014). C9orf72 repeat expansions cause neurodegeneration in Drosophila through arginine-rich proteins. *Science* 345, 1192–1194.
- Molliex, A., Temirov, J., Lee, J., Coughlin, M., Kanagaraj, A.P., Kim, H.J., Mittag, T., and Taylor, J.P. (2015). Phase separation by low complexity domains promotes stress granule assembly and drives pathological fibrillization. *Cell* 163, 123–133.
- Monahan, Z., Ryan, V.H., Janke, A.M., Burke, K.A., Rhoads, S.N., Zerze, G.H., O'Meally, R., Dignon, G.L., Conicella, A.E., Zheng, W., et al. (2017). Phosphorylation of the FUS low-complexity domain disrupts phase separation, aggregation, and toxicity. *EMBO J.* 36, 2951–2967.
- Moore, L.R., Rajpal, G., Dillingham, I.T., Qutob, M., Blumenstein, K.G., Gattis, D., Hung, G., Kordasiewicz, H.B., Paulson, H.L., and McLoughlin, H.S. (2017). Evaluation of antisense oligonucleotides targeting ATXN3 in SCA3 mouse models. *Mol. Ther. Nucleic Acids* 7, 200–210.
- Moreira, M.C., Klur, S., Watanabe, M., Németh, A.H., Le Ber, I., Moniz, J.C., Tranchant, C., Aubourg, P., Tazir, M., Schöls, L., et al. (2004). Senataxin, the ortholog of a yeast RNA helicase, is mutant in ataxia-ocular apraxia 2. *Nat. Genet.* 36, 225–227.
- Mori, K., Arzberger, T., Grässer, F.A., Gijssels, I., May, S., Rentzsch, K., Weng, S.M., Schludi, M.H., van der Zee, J., Cruts, M., et al. (2013a). Bidirectional transcripts of the expanded C9orf72 hexanucleotide repeat are translated into aggregating dipeptide repeat proteins. *Acta Neuropathol.* 126, 881–893.
- Mori, K., Lammich, S., Mackenzie, I.R., Forné, I., Zilow, S., Kretzschmar, H., Edbauer, D., Janssens, J., Kleinberger, G., Cruts, M., et al. (2013b). hnRNP A3 binds to GGGGCC repeats and is a constituent of p62-positive/TDP43-negative inclusions in the hippocampus of patients with C9orf72 mutations. *Acta Neuropathol.* 125, 413–423.
- Mori, K., Weng, S.M., Arzberger, T., May, S., Rentzsch, K., Kremmer, E., Schmid, B., Kretzschmar, H.A., Cruts, M., Van Broeckhoven, C., et al. (2013c). The C9orf72 GGGGCC repeat is translated into aggregating dipeptide-repeat proteins in FTL/ALS. *Science* 339, 1335–1338.
- Muddashetty, R., and Bassell, G.J. (2009). A boost in microRNAs shapes up the neuron. *EMBO J.* 28, 617–618.
- Muddashetty, R.S., Nalavadi, V.C., Gross, C., Yao, X., Xing, L., Laur, O., Warren, S.T., and Bassell, G.J. (2011). Reversible inhibition of PSD-95 mRNA translation by miR-125a, FMRP phosphorylation, and mGluR signaling. *Mol. Cell* 42, 673–688.
- Mulders, S.A., van den Broek, W.J., Wheeler, T.M., Croes, H.J., van Kuik-Romeijn, P., de Kimpe, S.J., Furling, D., Platenburg, G.J., Gourdon, G., Thornton, C.A., et al. (2009). Triplet-repeat oligonucleotide-mediated reversal of RNA toxicity in myotonic dystrophy. *Proc. Natl. Acad. Sci. USA* 106, 13915–13920.
- Murakami, T., Qamar, S., Lin, J.Q., Schierle, G.S., Rees, E., Miyashita, A., Costa, A.R., Dodd, R.B., Chan, F.T., Michel, C.H., et al. (2015). ALS/FTD mutation-induced phase transition of FUS liquid droplets and reversible hydrogels into irreversible hydrogels impairs RNP granule function. *Neuron* 88, 678–690.
- Murray, D.T., Kato, M., Lin, Y., Thurber, K.R., Hung, I., McKnight, S.L., and Tycko, R. (2017). Structure of FUS protein fibrils and its relevance to self-assembly and phase separation of low-complexity domains. *Cell* 171, 615–627.
- Mutsaers, C.A., Wishart, T.M., Lamont, D.J., Riessland, M., Schreml, J., Comley, L.H., Murray, L.M., Parson, S.H., Lochmüller, H., Wirth, B., et al. (2011). Reversible molecular pathology of skeletal muscle in spinal muscular atrophy. *Hum. Mol. Genet.* 20, 4334–4344.
- Myer, V.E., Fan, X.C., and Steitz, J.A. (1997). Identification of HuR as a protein implicated in AUUUA-mediated mRNA decay. *EMBO J.* 16, 2130–2139.
- Nakaya, T., Alexiou, P., Maragkakis, M., Chang, A., and Mourelatos, Z. (2013). FUS regulates genes coding for RNA-binding proteins in neurons by binding to their highly conserved introns. *RNA* 19, 498–509.
- Napoli, I., Mercaldo, V., Boyle, P.P., Eleuteri, B., Zalfa, F., De Rubeis, S., Di Marino, D., Mohr, E., Massimi, M., Falconi, M., et al. (2008). The fragile X syndrome protein represses activity-dependent translation through CYFIP1, a new 4E-BP. *Cell* 134, 1042–1054.
- Naryshkin, N.A., Weetall, M., Dakka, A., Narasimhan, J., Zhao, X., Feng, Z., Ling, K.K., Karp, G.M., Qi, H., Woll, M.G., et al. (2014). Motor neuron disease. SMN2 splicing modifiers improve motor function and longevity in mice with spinal muscular atrophy. *Science* 345, 688–693.
- Naumann, M., Pal, A., Goswami, A., Lojewski, X., Japtok, J., Vehlow, A., Naujock, M., Günther, R., Jin, M., Stanslowsky, N., et al. (2018). Impaired DNA damage response signaling by FUS-NLS mutations leads to neurodegeneration and FUS aggregate formation. *Nat. Commun.* 9, 335.

- Neumann, M., Sampathu, D.M., Kwong, L.K., Truax, A.C., Micsenyi, M.C., Chou, T.T., Bruce, J., Schuck, T., Grossman, M., Clark, C.M., et al. (2006). Ubiquitinated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. *Science* **314**, 130–133.
- Neumann, M., Rademakers, R., Roeber, S., Baker, M., Kretschmar, H.A., and Mackenzie, I.R. (2009). A new subtype of frontotemporal lobar degeneration with FUS pathology. *Brain* **132**, 2922–2931.
- Neumann, M., Bentmann, E., Dormann, D., Jawaid, A., DeJesus-Hernandez, M., Ansorge, O., Roeber, S., Kretschmar, H.A., Munoz, D.G., Kusaka, H., et al. (2011). FET proteins TAF15 and EWS are selective markers that distinguish FTLD with FUS pathology from amyotrophic lateral sclerosis with FUS mutations. *Brain* **134**, 2595–2609.
- Nihei, Y., Ito, D., and Suzuki, N. (2012). Roles of ataxin-2 in pathological cascades mediated by TAR DNA-binding protein 43 (TDP-43) and Fused in Sarcoma (FUS). *J. Biol. Chem.* **287**, 41310–41323.
- Nonhoff, U., Ralsler, M., Welzel, F., Piccini, I., Balzereit, D., Yaspo, M.L., Lehrach, H., and Krobitsch, S. (2007). Ataxin-2 interacts with the DEAD/H-box RNA helicase DDX6 and interferes with P-bodies and stress granules. *Mol. Biol. Cell* **18**, 1385–1396.
- Nott, T.J., Petsalaki, E., Farber, P., Jervis, D., Fussner, E., Plochowitz, A., Craggs, T.D., Bazett-Jones, D.P., Pawson, T., Forman-Kay, J.D., and Baldwin, A.J. (2015). Phase transition of a disordered nuage protein generates environmentally responsive membraneless organelles. *Mol. Cell* **57**, 936–947.
- Nussbacher, J.K., Batra, R., Lagier-Tourenne, C., and Yeo, G.W. (2015). RNA-binding proteins in neurodegeneration: Seq and you shall receive. *Trends Neurosci.* **38**, 226–236.
- Oberlé, I., Rousseau, F., Heitz, D., Kretz, C., Devys, D., Hanauer, A., Boué, J., Bertheas, M.F., and Mandel, J.L. (1991). Instability of a 550-base pair DNA segment and abnormal methylation in fragile X syndrome. *Science* **252**, 1097–1102.
- Ostrowski, L.A., Hall, A.C., and Mekhail, K. (2017). Ataxin-2: from RNA control to human health and disease. *Genes (Basel)*. Published online June 5, 2017. <https://doi.org/10.3390/genes8060157>.
- Pagliardini, S., Giavazzi, A., Setola, V., Lizier, C., Di Luca, M., DeBiasi, S., and Battaglia, G. (2000). Subcellular localization and axonal transport of the survival motor neuron (SMN) protein in the developing rat spinal cord. *Hum. Mol. Genet.* **9**, 47–56.
- Paribello, C., Tao, L., Folino, A., Berry-Kravis, E., Tranfaglia, M., Ethell, I.M., and Ethell, D.W. (2010). Open-label add-on treatment trial of minocycline in fragile X syndrome. *BMC Neurol.* **10**, 91.
- Park, S.K., Arslan, F., Kanneganti, V., Barmada, S.J., Purushothaman, P., Verma, S.C., and Liebman, S.W. (2018). Overexpression of a conserved HSP40 chaperone reduces toxicity of several neurodegenerative disease proteins. *Prion* **12**, 16–22.
- Paronetto, M.P., Miñana, B., and Valcárcel, J. (2011). The Ewing sarcoma protein regulates DNA damage-induced alternative splicing. *Mol. Cell* **43**, 353–368.
- Passini, M.A., Bu, J., Richards, A.M., Kinnecom, C., Sardi, S.P., Stanek, L.M., Hua, Y., Rigo, F., Matson, J., Hung, G., et al. (2011). Antisense oligonucleotides delivered to the mouse CNS ameliorate symptoms of severe spinal muscular atrophy. *Sci. Transl. Med.* **3**, 72ra18.
- Patel, A., Lee, H.O., Jawerth, L., Maharana, S., Jahnel, M., Hein, M.Y., Stoyanov, S., Mahamid, J., Saha, S., Franzmann, T.M., et al. (2015). A liquid-to-solid phase transition of the ALS protein FUS accelerated by disease mutation. *Cell* **162**, 1066–1077.
- Paubel, A., Violette, J., Amy, M., Praline, J., Meininger, V., Camu, W., Corcia, P., Andres, C.R., and Vourc'h, P.; French Amyotrophic Lateral Sclerosis (ALS) Study Group (2008). Mutations of the ANG gene in French patients with sporadic amyotrophic lateral sclerosis. *Arch. Neurol.* **65**, 1333–1336.
- Pellizzoni, L., Kataoka, N., Charroux, B., and Dreyfuss, G. (1998). A novel function for SMN, the spinal muscular atrophy disease gene product, in pre-mRNA splicing. *Cell* **95**, 615–624.
- Pellizzoni, L., Charroux, B., and Dreyfuss, G. (1999). SMN mutants of spinal muscular atrophy patients are defective in binding to snRNP proteins. *Proc. Natl. Acad. Sci. USA* **96**, 11167–11172.
- Perera, N.D., Sheehan, R.K., Crouch, P.J., White, A.R., Horne, M.K., and Turner, B.J. (2016). Enhancing survival motor neuron expression extends lifespan and attenuates neurodegeneration in mutant TDP-43 mice. *Hum. Mol. Genet.* **25**, 4080–4093.
- Piazzon, N., Rage, F., Schlotter, F., Moine, H., Branlant, C., and Massenet, S. (2008). In vitro and in cellulo evidences for association of the survival of motor neuron complex with the fragile X mental retardation protein. *J. Biol. Chem.* **283**, 5598–5610.
- Pinard, E., Green, L., Reutlinger, M., Weetall, M., Naryshkin, N.A., Baird, J., Chen, K.S., Paushkin, S.V., Metzger, F., and Ratni, H. (2017). Discovery of a novel class of survival motor neuron 2 splicing modifiers for the treatment of spinal muscular atrophy. *J. Med. Chem.* **60**, 4444–4457.
- Pinto, B.S., Saxena, T., Oliveira, R., Mendez-Gomez, H.R., Cleary, J.D., Denes, L.T., McConnell, O., Arboleda, J., Xia, G., Swanson, M.S., et al. (2017). Impeding transcription of expanded microsatellite repeats by deactivated Cas9. *Mol. Cell* **68**, 479–490.
- Plante, I., Davidovic, L., Ouellet, D.L., Gobeil, L.A., Tremblay, S., Khandjian, E.W., and Provost, P. (2006). Dicer-derived microRNAs are utilized by the fragile X mental retardation protein for assembly on target RNAs. *J. Biomed. Biotechnol.* **2006**, 64347.
- Polymenidou, M., and Cleveland, D.W. (2011). The seeds of neurodegeneration: prion-like spreading in ALS. *Cell* **147**, 498–508.
- Polymenidou, M., Lagier-Tourenne, C., Hutt, K.R., Huelga, S.C., Moran, J., Liang, T.Y., Ling, S.C., Sun, E., Wancewicz, E., Mazur, C., et al. (2011). Long pre-mRNA depletion and RNA missplicing contribute to neuronal vulnerability from loss of TDP-43. *Nat. Neurosci.* **14**, 459–468.
- Poulos, M.G., Batra, R., Li, M., Yuan, Y., Zhang, C., Darnell, R.B., and Swanson, M.S. (2013). Progressive impairment of muscle regeneration in muscle-blind-like 3 isoform knockout mice. *Hum. Mol. Genet.* **22**, 3547–3558.
- Protter, D.S.W., and Parker, R. (2016). Principles and properties of stress granules. *Trends Cell Biol.* **26**, 668–679.
- Prudencio, M., Jansen-West, K.R., Lee, W.C., Gendron, T.F., Zhang, Y.J., Xu, Y.F., Gass, J., Stuan, C., Stetler, C., Rademakers, R., et al. (2012). Misregulation of human sortilin splicing leads to the generation of a nonfunctional progranulin receptor. *Proc. Natl. Acad. Sci. USA* **109**, 21510–21515.
- Prudencio, M., Belzil, V.V., Batra, R., Ross, C.A., Gendron, T.F., Pregent, L.J., Murray, M.E., Overstreet, K.K., Piazza-Johnston, A.E., Desaro, P., et al. (2015). Distinct brain transcriptome profiles in C9orf72-associated and sporadic ALS. *Nat. Neurosci.* **18**, 1175–1182.
- Qamar, S., Wang, G., Randle, S.J., Ruggeri, F.S., Varela, J.A., Lin, J.Q., Phillips, E.C., Miyashita, A., Williams, D., Strohl, F., et al. (2018). FUS phase separation is modulated by a molecular chaperone and methylation of arginine cation- π interactions. *Cell* **173**, 720–734.
- Ralsler, M., Albrecht, M., Nonhoff, U., Lengauer, T., Lehrach, H., and Krobitsch, S. (2005). An integrative approach to gain insights into the cellular function of human ataxin-2. *J. Mol. Biol.* **346**, 203–214.
- Ramaswami, M., Taylor, J.P., and Parker, R. (2013). Altered ribostasis: RNA-protein granules in degenerative disorders. *Cell* **154**, 727–736.
- Rathod, R., Havlicek, S., Frank, N., Blum, R., and Sendtner, M. (2012). Laminin induced local axonal translation of β -actin mRNA is impaired in SMN-deficient motoneurons. *Histochem. Cell Biol.* **138**, 737–748.
- Reddy, K., Zamiri, B., Stanley, S.Y., Macgregor, R.B., Jr., and Pearson, C.E. (2013). The disease-associated r(GGGGCC) $_n$ repeat from the C9orf72 gene forms tract length-dependent uni- and multimolecular RNA G-quadruplex structures. *J. Biol. Chem.* **288**, 9860–9866.
- Renton, A.E., Majounie, E., Waite, A., Simón-Sánchez, J., Rollinson, S., Gibbs, J.R., Schymick, J.C., Laaksovirta, H., van Swieten, J.C., Myllykangas, L., et al.; ITALSGEN Consortium (2011). A hexanucleotide repeat expansion in C9ORF72 is the cause of chromosome 9p21-linked ALS-FTD. *Neuron* **72**, 257–268.

- Rigo, F., Hua, Y., Chun, S.J., Prakash, T.P., Krainer, A.R., and Bennett, C.F. (2012a). Synthetic oligonucleotides recruit ILF2/3 to RNA transcripts to modulate splicing. *Nat. Chem. Biol.* **8**, 555–561.
- Rigo, F., Hua, Y., Krainer, A.R., and Bennett, C.F. (2012b). Antisense-based therapy for the treatment of spinal muscular atrophy. *J. Cell Biol.* **199**, 21–25.
- Roca, X., and Krainer, A.R. (2009). Recognition of atypical 5' splice sites by shifted base-pairing to U1 snRNA. *Nat. Struct. Mol. Biol.* **16**, 176–182.
- Rodriguez-Muela, N., Litterman, N.K., Norabuena, E.M., Mull, J.L., Galazo, M.J., Sun, C., Ng, S.Y., Makhortova, N.R., White, A., Lynes, M.M., et al. (2017). Single-cell analysis of SMN reveals its broader role in neuromuscular disease. *Cell Rep.* **18**, 1484–1498.
- Rogelj, B., Easton, L.E., Bogu, G.K., Stanton, L.W., Rot, G., Curk, T., Zupan, B., Sugimoto, Y., Modic, M., Haberman, N., et al. (2012). Widespread binding of FUS along nascent RNA regulates alternative splicing in the brain. *Sci. Rep.* **2**, 603.
- Ross, E.D., Edskes, H.K., Terry, M.J., and Wickner, R.B. (2005). Primary sequence independence for prion formation. *Proc. Natl. Acad. Sci. USA* **102**, 12825–12830.
- Rossi, S., Serrano, A., Gerbino, V., Giorgi, A., Di Francesco, L., Nencini, M., Bozzo, F., Schininà, M.E., Bagni, C., Cestra, G., et al. (2015). Nuclear accumulation of mRNAs underlies G4C2-repeat-induced translational repression in a cellular model of C9orf72 ALS. *J. Cell Sci.* **128**, 1787–1799.
- Rossoll, W., Kröning, A.K., Ohndorf, U.M., Steegborn, C., Jablonka, S., and Sendtner, M. (2002). Specific interaction of Smn, the spinal muscular atrophy determining gene product, with hnRNP-R and gry-rbp/hnRNP-Q: a role for Smn in RNA processing in motor axons? *Hum. Mol. Genet.* **11**, 93–105.
- Rossoll, W., Jablonka, S., Andreassi, C., Kröning, A.K., Karle, K., Monani, U.R., and Sendtner, M. (2003). Smn, the spinal muscular atrophy-determining gene product, modulates axon growth and localization of beta-actin mRNA in growth cones of motoneurons. *J. Cell Biol.* **163**, 801–812.
- Rot, G., Wang, Z., Huppertz, I., Modic, M., Lenče, T., Hallegger, M., Haberman, N., Curk, T., von Mering, C., and Ule, J. (2017). High-resolution RNA maps suggest common principles of splicing and polyadenylation regulation by TDP-43. *Cell Rep.* **19**, 1056–1067.
- Ruggiu, M., Herbst, R., Kim, N., Jevsek, M., Fak, J.J., Mann, M.A., Fischbach, G., Burden, S.J., and Darnell, R.B. (2009). Rescuing Z⁺ agrin splicing in Nova null mice restores synapse formation and unmasks a physiologic defect in motor neuron firing. *Proc. Natl. Acad. Sci. USA* **106**, 3513–3518.
- Ruggiu, M., McGovern, V.L., Lotti, F., Saieva, L., Li, D.K., Kariya, S., Monani, U.R., Burghes, A.H., and Pellizzoni, L. (2012). A role for SMN exon 7 splicing in the selective vulnerability of motor neurons in spinal muscular atrophy. *Mol. Cell Biol.* **32**, 126–138.
- Saber, S., Stauffer, J.E., Jiang, J., Garcia, S.D., Taylor, A.E., Schulte, D., Ohkubo, T., Schloffman, C.L., Maldonado, M., Baughn, M., et al. (2018). Sense-encoded poly-GR dipeptide repeat proteins correlate to neurodegeneration and uniquely co-localize with TDP-43 in dendrites of repeat-expanded C9orf72 amyotrophic lateral sclerosis. *Acta Neuropathol.* **135**, 459–474.
- Salton, M., Elkon, R., Borodina, T., Davydov, A., Yaspo, M.L., Halperin, E., and Shiloh, Y. (2011). Matr3 binds and stabilizes mRNA. *PLoS ONE* **6**, e23882.
- Salvi, J.S., Chan, J.N., Szafranski, K., Liu, T.T., Wu, J.D., Olsen, J.B., Khanam, N., Poon, B.P., Emili, A., and Mekhalif, K. (2014). Roles for Pbp1 and caloric restriction in genome and lifespan maintenance via suppression of RNA-DNA hybrids. *Dev. Cell* **30**, 177–191.
- Sanford, J.R., Wang, X., Mort, M., Vanduy, N., Cooper, D.N., Mooney, S.D., Edenberg, H.J., and Liu, Y. (2009). Splicing factor SFRS1 recognizes a functionally diverse landscape of RNA transcripts. *Genome Res.* **19**, 381–394.
- Santoro, M.R., Bray, S.M., and Warren, S.T. (2012). Molecular mechanisms of fragile X syndrome: a twenty-year perspective. *Annu. Rev. Pathol.* **7**, 219–245.
- Sapaly, D., Dos Santos, M., Delers, P., Biondi, O., Quérol, G., Houdebine, L., Khoobary, K., Girardet, F., Bulet, P., Armand, A.S., et al. (2018). Small-molecule flunarizine increases SMN protein in nuclear Cajal bodies and motor function in a mouse model of spinal muscular atrophy. *Sci. Rep.* **8**, 2075.
- Sareen, D., O'Rourke, J.G., Meera, P., Muhammad, A.K., Grant, S., Simpkinson, M., Bell, S., Carmona, S., Ornelas, L., Sahabian, A., et al. (2013). Targeting RNA foci in iPSC-derived motor neurons from ALS patients with a C9orf72 repeat expansion. *Sci. Transl. Med.* **5**, 208ra149.
- Satterfield, T.F., and Pallanck, L.J. (2006). Ataxin-2 and its Drosophila homolog, ATX2, physically assemble with polyribosomes. *Hum. Mol. Genet.* **15**, 2523–2532.
- Schaeffer, C., Bardoni, B., Mandel, J.L., Ehresmann, B., Ehresmann, C., and Moine, H. (2001). The fragile X mental retardation protein binds specifically to its mRNA via a purine quartet motif. *EMBO J.* **20**, 4803–4813.
- Schoch, K.M., and Miller, T.M. (2017). Antisense oligonucleotides: translation from mouse models to human neurodegenerative diseases. *Neuron* **94**, 1056–1070.
- Schütt, J., Falley, K., Richter, D., Kreienkamp, H.J., and Kindler, S. (2009). Fragile X mental retardation protein regulates the levels of scaffold proteins and glutamate receptors in postsynaptic densities. *J. Biol. Chem.* **284**, 25479–25487.
- Scoles, D.R., Meera, P., Schneider, M.D., Paul, S., Dansithong, W., Figueroa, K.P., Hung, G., Rigo, F., Bennett, C.F., Otis, T.S., and Pulst, S.M. (2017). Antisense oligonucleotide therapy for spinocerebellar ataxia type 2. *Nature* **544**, 362–366.
- Sebat, J., Lakshmi, B., Malhotra, D., Troge, J., Lese-Martin, C., Walsh, T., Yamrom, B., Yoon, S., Krasnitz, A., Kendall, J., et al. (2007). Strong association of de novo copy number mutations with autism. *Science* **316**, 445–449.
- Sellier, C., Rau, F., Liu, Y., Tassone, F., Hukema, R.K., Gattoni, R., Schneider, A., Richard, S., Willemsen, R., Elliott, D.J., et al. (2010). Sam68 sequestration and partial loss of function are associated with splicing alterations in FXTAS patients. *EMBO J.* **29**, 1248–1261.
- Sellier, C., Freyermuth, F., Tabet, R., Tran, T., He, F., Ruffenach, F., Alunni, V., Moine, H., Thibault, C., Page, A., et al. (2013). Sequestration of DROSHA and DGCR8 by expanded CGG RNA repeats alters microRNA processing in fragile X-associated tremor/ataxia syndrome. *Cell Rep.* **3**, 869–880.
- Seng, C.O., Magee, C., Young, P.J., Lorson, C.L., and Allen, J.P. (2015). The SMN structure reveals its crucial role in snRNP assembly. *Hum. Mol. Genet.* **24**, 2138–2146.
- Senties-Madrid, H., and Vega-Boada, F. (2001). Paraneoplastic syndromes associated with anti-Hu antibodies. *Isr. Med. Assoc. J.* **3**, 94–103.
- Sephton, C.F., Cenik, C., Kucukural, A., Dammer, E.B., Cenik, B., Han, Y., Dewey, C.M., Roth, F.P., Herz, J., Peng, J., et al. (2011). Identification of neuronal RNA targets of TDP-43-containing ribonucleoprotein complexes. *J. Biol. Chem.* **286**, 1204–1215.
- Shamay-Ramot, A., Khmermesh, K., Porath, H.T., Barak, M., Pinto, Y., Wachtel, C., Zilberberg, A., Lerer-Goldshtein, T., Efroni, S., Levanon, E.Y., and Appelbaum, L. (2015). Fmrp interacts with Adar and regulates RNA editing, synaptic density and locomotor activity in zebrafish. *PLoS Genet.* **11**, e1005702.
- Shan, X., Chiang, P.M., Price, D.L., and Wong, P.C. (2010). Altered distributions of Gemini of coiled bodies and mitochondria in motor neurons of TDP-43 transgenic mice. *Proc. Natl. Acad. Sci. USA* **107**, 16325–16330.
- Shi, K.Y., Mori, E., Nizami, Z.F., Lin, Y., Kato, M., Xiang, S., Wu, L.C., Ding, M., Yu, Y., Gall, J.G., and McKnight, S.L. (2017). Toxic PR_n poly-dipeptides encoded by the C9orf72 repeat expansion block nuclear import and export. *Proc. Natl. Acad. Sci. USA* **114**, E1111–E1117.
- Shiga, A., Ishihara, T., Miyashita, A., Kuwabara, M., Kato, T., Watanabe, N., Yamahira, A., Kondo, C., Yokoseki, A., Takahashi, M., et al. (2012). Alteration of POLDIP3 splicing associated with loss of function of TDP-43 in tissues affected with ALS. *PLoS ONE* **7**, e43120.
- Siller, S.S., and Broadie, K. (2011). Neural circuit architecture defects in a Drosophila model of Fragile X syndrome are alleviated by minocycline treatment and genetic removal of matrix metalloproteinase. *Dis. Model. Mech.* **4**, 673–685.
- Simone, R., Balendra, R., Moens, T.G., Preza, E., Wilson, K.M., Heslegrave, A., Woodling, N.S., Niccoli, T., Gilbert-Jaramillo, J., Abdelkarim, S., et al. (2018). G-quadruplex-binding small molecules ameliorate C9orf72 FTD/ALS pathology *in vitro* and *in vivo*. *EMBO Mol. Med.* **10**, 22–31.

- Sivaramakrishnan, M., McCarthy, K.D., Campagne, S., Huber, S., Meier, S., Augustin, A., Heckel, T., Meistermann, H., Hug, M.N., Birrer, P., et al. (2017). Binding to SMN2 pre-mRNA-protein complex elicits specificity for small molecule splicing modifiers. *Nat. Commun.* **8**, 1476.
- Skourti-Stathaki, K., Proudfoot, N.J., and Gromak, N. (2011). Human senataxin resolves RNA/DNA hybrids formed at transcriptional pause sites to promote Xrn2-dependent termination. *Mol. Cell* **42**, 794–805.
- Smith, R.A., Miller, T.M., Yamanaka, K., Monia, B.P., Condon, T.P., Hung, G., Lobsiger, C.S., Ward, C.M., McAlonis-Downes, M., Wei, H., et al. (2006). Antisense oligonucleotide therapy for neurodegenerative disease. *J. Clin. Invest.* **116**, 2290–2296.
- So, B.R., Wan, L., Zhang, Z., Li, P., Babiash, E., Duan, J., Younis, I., and Dreyfuss, G. (2016). A U1 snRNP-specific assembly pathway reveals the SMN complex as a versatile hub for RNP exchange. *Nat. Struct. Mol. Biol.* **23**, 225–230.
- Sofola, O.A., Jin, P., Qin, Y., Duan, R., Liu, H., de Haro, M., Nelson, D.L., and Botas, J. (2007). RNA-binding proteins hnRNP A2/B1 and CUGBP1 suppress fragile X CGG premutation repeat-induced neurodegeneration in a Drosophila model of FXTAS. *Neuron* **55**, 565–571.
- Soret, J., Bakkour, N., Maire, S., Durand, S., Zekri, L., Gabut, M., Fic, W., Divita, G., Rivalle, C., Dauzonne, D., et al. (2005). Selective modification of alternative splicing by indole derivatives that target serine-arginine-rich protein splicing factors. *Proc. Natl. Acad. Sci. USA* **102**, 8764–8769.
- Stefani, G., Fraser, C.E., Darnell, J.C., and Darnell, R.B. (2004). Fragile X mental retardation protein is associated with translating polyribosomes in neuronal cells. *J. Neurosci.* **24**, 7272–7276.
- Su, Z., Zhang, Y., Gendron, T.F., Bauer, P.O., Chew, J., Yang, W.Y., Fostvedt, E., Jansen-West, K., Belzil, V.V., Desaro, P., et al. (2014). Discovery of a biomarker and lead small molecules to target r(GGGGCC)-associated defects in c9FTD/ALS. *Neuron* **84**, 239.
- Suárez-Calvet, M., Neumann, M., Arzberger, T., Abou-Ajram, C., Funk, E., Hartmann, H., Edbauer, D., Kremmer, E., Göbl, C., Resch, M., et al. (2016). Monomethylated and unmethylated FUS exhibit increased binding to Transportin and distinguish FTLD-FUS from ALS-FUS. *Acta Neuropathol.* **131**, 587–604.
- Suhl, J.A., Chopra, P., Anderson, B.R., Bassell, G.J., and Warren, S.T. (2014). Analysis of FMRP mRNA target datasets reveals highly associated mRNAs mediated by G-quadruplex structures formed via clustered WGGA sequences. *Hum. Mol. Genet.* **23**, 5479–5491.
- Sun, Z., Diaz, Z., Fang, X., Hart, M.P., Chesi, A., Shorter, J., and Gitler, A.D. (2011). Molecular determinants and genetic modifiers of aggregation and toxicity for the ALS disease protein FUS/TLS. *PLoS Biol.* **9**, e1000614.
- Sun, S., Ling, S.C., Qiu, J., Albuquerque, C.P., Zhou, Y., Tokunaga, S., Li, H., Qiu, H., Bui, A., Yeo, G.W., et al. (2015). ALS-causative mutations in FUS/TLS confer gain and loss of function by altered association with SMN and U1-snRNP. *Nat. Commun.* **6**, 6171.
- Sutcliffe, J.S., Nelson, D.L., Zhang, F., Pieretti, M., Caskey, C.T., Saxe, D., and Warren, S.T. (1992). DNA methylation represses FMR-1 transcription in fragile X syndrome. *Hum. Mol. Genet.* **1**, 397–400.
- Tabet, R., Moutin, E., Becker, J.A., Heintz, D., Fouillen, L., Flatter, E., Krężel, W., Alunni, V., Koebel, P., Dembélé, D., et al. (2016). Fragile X mental retardation protein (FMRP) controls diacylglycerol kinase activity in neurons. *Proc. Natl. Acad. Sci. USA* **113**, E3619–E3628.
- Tan, Q., Yalamanchili, H.K., Park, J., De Maio, A., Lu, H.C., Wan, Y.W., White, J.J., Bondar, V.V., Sayegh, L.S., Liu, X., et al. (2016). Extensive cryptic splicing upon loss of RBM17 and TDP43 in neurodegeneration models. *Hum. Mol. Genet.* **25**, 5083–5093.
- Tao, Z., Wang, H., Xia, Q., Li, K., Li, K., Jiang, X., Xu, G., Wang, G., and Ying, Z. (2015). Nucleolar stress and impaired stress granule formation contribute to C9orf72 RAN translation-induced cytotoxicity. *Hum. Mol. Genet.* **24**, 2426–2441.
- Tian, H., Cao, Y.X., Zhang, X.S., Liao, W.P., Yi, Y.H., Lian, J., Liu, L., Huang, H.L., Liu, W.J., Yin, M.M., et al. (2013). The targeting and functions of miRNA-383 are mediated by FMRP during spermatogenesis. *Cell Death Dis.* **4**, e617.
- Tisdale, S., Lotti, F., Saieva, L., Van Meerbeke, J.P., Crawford, T.O., Sumner, C.J., Mentis, G.Z., and Pellizzoni, L. (2013). SMN is essential for the biogenesis of U7 small nuclear ribonucleoprotein and 3'-end formation of histone mRNAs. *Cell Rep.* **5**, 1187–1195.
- Todd, A.G., Shaw, D.J., Morse, R., Stebbings, H., and Young, P.J. (2010). SMN and the Gemin proteins form sub-complexes that localise to both stationary and dynamic neurite granules. *Biochem. Biophys. Res. Commun.* **394**, 211–216.
- Todd, P.K., Oh, S.Y., Krans, A., He, F., Sellier, C., Frazer, M., Renoux, A.J., Chen, K.C., Scaglione, K.M., Basur, V., et al. (2013). CGG repeat-associated translation mediates neurodegeneration in fragile X tremor ataxia syndrome. *Neuron* **78**, 440–455.
- Tollervy, J.R., Curk, T., Rogelj, B., Briese, M., Cereda, M., Kayikci, M., König, J., Hortobágyi, T., Nishimura, A.L., Zupunski, V., et al. (2011). Characterizing the RNA targets and position-dependent splicing regulation by TDP-43. *Nat. Neurosci.* **14**, 452–458.
- Toombs, J.A., McCarty, B.R., and Ross, E.D. (2010). Compositional determinants of prion formation in yeast. *Mol. Cell. Biol.* **30**, 319–332.
- Toonen, L.J.A., Rigo, F., van Attikum, H., and van Roon-Mom, W.M.C. (2017). Antisense oligonucleotide-mediated removal of the polyglutamine repeat in spinocerebellar ataxia type 3 mice. *Mol. Ther. Nucleic Acids* **8**, 232–242.
- Tradewell, M.L., Yu, Z., Tibshirani, M., Boulanger, M.C., Durham, H.D., and Richard, S. (2012). Arginine methylation by PRMT1 regulates nuclear-cytoplasmic localization and toxicity of FUS/TLS harbouring ALS-linked mutations. *Hum. Mol. Genet.* **21**, 136–149.
- Tsuji, T., Sun, Y., Kishimoto, K., Olson, K.A., Liu, S., Hirukawa, S., and Hu, G.F. (2005). Angiogenin is translocated to the nucleus of HeLa cells and is involved in ribosomal RNA transcription and cell proliferation. *Cancer Res.* **65**, 1352–1360.
- Ule, J., Jensen, K.B., Ruggiu, M., Mele, A., Ule, A., and Darnell, R.B. (2003). CLIP identifies Nova-regulated RNA networks in the brain. *Science* **302**, 1212–1215.
- Ule, J., Jensen, K., Mele, A., and Darnell, R.B. (2005a). CLIP: a method for identifying protein-RNA interaction sites in living cells. *Methods* **37**, 376–386.
- Ule, J., Ule, A., Spencer, J., Williams, A., Hu, J.S., Cline, M., Wang, H., Clark, T., Fraser, C., Ruggiu, M., et al. (2005b). Nova regulates brain-specific splicing to shape the synapse. *Nat. Genet.* **37**, 844–852.
- Ule, J., Stefani, G., Mele, A., Ruggiu, M., Wang, X., Taneri, B., Gaasterland, T., Blencowe, B.J., and Darnell, R.B. (2006). An RNA map predicting Nova-dependent splicing regulation. *Nature* **444**, 580–586.
- Utari, A., Chonchaiya, W., Rivera, S.M., Schneider, A., Hagerman, R.J., Faradz, S.M., Ethell, I.M., and Nguyen, D.V. (2010). Side effects of minocycline treatment in patients with fragile X syndrome and exploration of outcome measures. *Am. J. Intellect. Dev. Disabil.* **115**, 433–443.
- van Agtmaal, E.L., André, L.M., Willemsse, M., Cumming, S.A., van Kessel, I.D.G., van den Broek, W.J.A.A., Gourdon, G., Furling, D., Mouly, V., Monckton, D.G., et al. (2017). CRISPR/Cas9-induced (CTG·CAG)_n repeat instability in the myotonic dystrophy type 1 locus: implications for therapeutic genome editing. *Mol. Ther.* **25**, 24–43.
- Vance, C., Rogelj, B., Hortobágyi, T., De Vos, K.J., Nishimura, A.L., Sreedharan, J., Hu, X., Smith, B., Ruddy, D., Wright, P., et al. (2009). Mutations in FUS, an RNA processing protein, cause familial amyotrophic lateral sclerosis type 6. *Science* **323**, 1208–1211.
- Vanderweyde, T., Apicco, D.J., Youmans-Kidder, K., Ash, P.E.A., Cook, C., Lummertz da Rocha, E., Jansen-West, K., Frame, A.A., Citro, A., Leszyk, J.D., et al. (2016). Interaction of tau with the RNA-binding protein TIA1 regulates tau pathophysiology and toxicity. *Cell Rep.* **15**, 1455–1466.
- Vasilyev, N., Polonskaia, A., Darnell, J.C., Darnell, R.B., Patel, D.J., and Serganov, A. (2015). Crystal structure reveals specific recognition of a G-quadruplex RNA by a β -turn in the RGG motif of FMRP. *Proc. Natl. Acad. Sci. USA* **112**, E5391–E5400.
- Verheij, C., Bakker, C.E., de Graaff, E., Keulemans, J., Willemsen, R., Verkerk, A.J., Galjaard, H., Reuser, A.J., Hoogeveen, A.T., and Oostra, B.A. (1993).

Characterization and localization of the FMR-1 gene product associated with fragile X syndrome. *Nature* 363, 722–724.

Verkerk, A.J., Pieretti, M., Sutcliffe, J.S., Fu, Y.H., Kuhl, D.P., Pizzuti, A., Reiner, O., Richards, S., Victoria, M.F., Zhang, F.P., et al. (1991). Identification of a gene (FMR-1) containing a CGG repeat coincident with a breakpoint cluster region exhibiting length variation in fragile X syndrome. *Cell* 65, 905–914.

Vogler, T.O., Wheeler, J.R., Nguyen, E.D., Hughes, M.P., Britson, K.A., Lester, E., Rao, B., Betta, N.D., Whitney, O.N., Ewachiw, T.E., et al. (2018). TDP-43 and RNA form amyloid-like myo-granules in regenerating muscle. *Nature* 563, 508–513.

Voineagu, I., Wang, X., Johnston, P., Lowe, J.K., Tian, Y., Horvath, S., Mill, J., Cantor, R.M., Blencowe, B.J., and Geschwind, D.H. (2011). Transcriptomic analysis of autistic brain reveals convergent molecular pathology. *Nature* 474, 380–384.

Wakamatsu, Y., and Weston, J.A. (1997). Sequential expression and role of Hu RNA-binding proteins during neurogenesis. *Development* 124, 3449–3460.

Wan, L., Battle, D.J., Yong, J., Gubitza, A.K., Kolb, S.J., Wang, J., and Dreyfuss, G. (2005). The survival of motor neurons protein determines the capacity for snRNP assembly: biochemical deficiency in spinal muscular atrophy. *Mol. Cell. Biol.* 25, 5543–5551.

Wang, I.F., Wu, L.S., Chang, H.Y., and Shen, C.K. (2008). TDP-43, the signature protein of FTL-D-U, is a neuronal activity-responsive factor. *J. Neurochem.* 105, 797–806.

Wang, E.T., Cody, N.A., Jog, S., Biancolella, M., Wang, T.T., Treacy, D.J., Luo, S., Schroth, G.P., Housman, D.E., Reddy, S., et al. (2012a). Transcriptome-wide regulation of pre-mRNA splicing and mRNA localization by muscleblind proteins. *Cell* 150, 710–724.

Wang, I.F., Guo, B.S., Liu, Y.C., Wu, C.C., Yang, C.H., Tsai, K.J., and Shen, C.K. (2012b). Autophagy activators rescue and alleviate pathogenesis of a mouse model with proteinopathies of the TAR DNA-binding protein 43. *Proc. Natl. Acad. Sci. USA* 109, 15024–15029.

Wang, P., Wander, C.M., Yuan, C.X., Bereman, M.S., and Cohen, T.J. (2017). Acetylation-induced TDP-43 pathology is suppressed by an HSF1-dependent chaperone program. *Nat. Commun.* 8, 82.

Wang, J., Choi, J.M., Holehouse, A.S., Lee, H.O., Zhang, X., Jahnel, M., Maharana, S., Lemaître, R., Pozniakovskiy, A., Drechsel, D., et al. (2018). A molecular grammar governing the driving forces for phase separation of prion-like RNA binding proteins. *Cell* 174, 688–699.

Webb, T.R., Joyner, A.S., and Potter, P.M. (2013). The development and application of small molecule modulators of SF3b as therapeutic agents for cancer. *Drug Discov. Today* 18, 43–49.

Weiler, I.J., and Greenough, W.T. (1999). Synaptic synthesis of the fragile X protein: possible involvement in synapse maturation and elimination. *Am. J. Med. Genet.* 83, 248–252.

Weiler, I.J., Irwin, S.A., Klintsova, A.Y., Spencer, C.M., Brazelton, A.D., Miyashiro, K., Comery, T.A., Patel, B., Eberwine, J., and Greenough, W.T. (1997). Fragile X mental retardation protein is translated near synapses in response to neurotransmitter activation. *Proc. Natl. Acad. Sci. USA* 94, 5395–5400.

Wen, X., Tan, W., Westergard, T., Krishnamurthy, K., Markandaiah, S.S., Shi, Y., Lin, S., Schneider, N.A., Monaghan, J., Pandey, U.B., et al. (2014). Antisense proline-arginine RAN dipeptides linked to C9ORF72-ALS/FTD form toxic nuclear aggregates that initiate in vitro and in vivo neuronal death. *Neuron* 84, 1213–1225.

Weyn-Vanhenyck, S.M., Mele, A., Yan, Q., Sun, S., Farny, N., Zhang, Z., Xue, C., Herre, M., Silver, P.A., Zhang, M.Q., et al. (2014). HITS-CLIP and integrative modeling define the Rbfox splicing-regulatory network linked to brain development and autism. *Cell Rep.* 6, 1139–1152.

Wheeler, T.M., Sobczak, K., Lueck, J.D., Osborne, R.J., Lin, X., Dirksen, R.T., and Thornton, C.A. (2009). Reversal of RNA dominance by displacement of protein sequestered on triplet repeat RNA. *Science* 325, 336–339.

Wheeler, T.M., Leger, A.J., Pandey, S.K., MacLeod, A.R., Nakamori, M., Cheng, S.H., Wentworth, B.M., Bennett, C.F., and Thornton, C.A. (2012). Targeting nuclear RNA for in vivo correction of myotonic dystrophy. *Nature* 488, 111–115.

Willemsen, R., Bontekoe, C., Tamanini, F., Galjaard, H., Hoogeveen, A., and Oostra, B. (1996). Association of FMRP with ribosomal precursor particles in the nucleolus. *Biochem. Biophys. Res. Commun.* 225, 27–33.

Wolozin, B. (2012). Regulated protein aggregation: stress granules and neurodegeneration. *Mol. Neurodegener.* 7, 56.

Woo, C.J., Maier, V.K., Davey, R., Brennan, J., Li, G., Brothers, J., 2nd, Schwartz, B., Gordo, S., Kasper, A., Okamoto, T.R., et al. (2017). Gene activation of SMN by selective disruption of lncRNA-mediated recruitment of PRC2 for the treatment of spinal muscular atrophy. *Proc. Natl. Acad. Sci. USA* 114, E1509–E1518.

Wu, H., Lima, W.F., Zhang, H., Fan, A., Sun, H., and Crooke, S.T. (2004). Determination of the role of the human RNase H1 in the pharmacology of DNA-like antisense drugs. *J. Biol. Chem.* 279, 17181–17189.

Wu, D., Yu, W., Kishikawa, H., Folkert, R.D., Iafra, A.J., Shen, Y., Xin, W., Sims, K., and Hu, G.F. (2007). Angiogenin loss-of-function mutations in amyotrophic lateral sclerosis. *Ann. Neurol.* 62, 609–617.

Xiang, S., Kato, M., Wu, L.C., Lin, Y., Ding, M., Zhang, Y., Yu, Y., and McKnight, S.L. (2015). The LC domain of hnRNPA2 adopts similar conformations in hydrogel polymers, liquid-like droplets, and nuclei. *Cell* 163, 829–839.

Xiao, S., Sanelli, T., Dib, S., Sheps, D., Findlater, J., Bilbao, J., Keith, J., Zinman, L., Rogaeva, E., and Robertson, J. (2011). RNA targets of TDP-43 identified by UV-CLIP are deregulated in ALS. *Mol. Cell. Neurosci.* 47, 167–180.

Xie, N., Gong, H., Suhl, J.A., Chopra, P., Wang, T., and Warren, S.T. (2016). Re-activation of FMR1 by CRISPR/Cas9-mediated deletion of the expanded CGG-repeat of the fragile X chromosome. *PLoS ONE* 11, e0165499.

Xu, Z., Poidevin, M., Li, X., Li, Y., Shu, L., Nelson, D.L., Li, H., Hales, C.M., Gearing, M., Wingo, T.S., and Jin, P. (2013). Expanded GGGGCC repeat RNA associated with amyotrophic lateral sclerosis and frontotemporal dementia causes neurodegeneration. *Proc. Natl. Acad. Sci. USA* 110, 7778–7783.

Yamakawa, M., Ito, D., Honda, T., Kubo, K., Noda, M., Nakajima, K., and Suzuki, N. (2015). Characterization of the dipeptide repeat protein in the molecular pathogenesis of c9FTD/ALS. *Hum. Mol. Genet.* 24, 1630–1645.

Yamasaki, S., Ivanov, P., Hu, G.F., and Anderson, P. (2009). Angiogenin cleaves tRNA and promotes stress-induced translational repression. *J. Cell Biol.* 185, 35–42.

Yamashita, T., Chai, H.L., Teramoto, S., Tsuji, S., Shimazaki, K., Muramatsu, S., and Kwak, S. (2013). Rescue of amyotrophic lateral sclerosis phenotype in a mouse model by intravenous AAV9-ADAR2 delivery to motor neurons. *EMBO Mol. Med.* 5, 1710–1719.

Yamazaki, T., Chen, S., Yu, Y., Yan, B., Haertlein, T.C., Carrasco, M.A., Tapia, J.C., Zhai, B., Das, R., Lalancette-Hebert, M., et al. (2012). FUS-SMN protein interactions link the motor neuron diseases ALS and SMA. *Cell Rep.* 2, 799–806.

Yan, W.X., Chong, S., Zhang, H., Makarova, K.S., Koonin, E.V., Cheng, F.H., and Scott, D.A. (2018). Cas13d is a compact RNA-targeting type VI CRISPR effector positively modulated by a WYL-domain-containing accessory protein. *Mol. Cell* 70, 327–339.

Yano, M., Hayakawa-Yano, Y., Mele, A., and Darnell, R.B. (2010). Nova2 regulates neuronal migration through an RNA switch in disabled-1 signaling. *Neuron* 66, 848–858.

Yeo, G.W., Coufal, N.G., Liang, T.Y., Peng, G.E., Fu, X.D., and Gage, F.H. (2009). An RNA code for the FOX2 splicing regulator revealed by mapping RNA-protein interactions in stem cells. *Nat. Struct. Mol. Biol.* 16, 130–137.

Yin, S., Lopez-Gonzalez, R., Kunz, R.C., Gangopadhyay, J., Borufka, C., Gygi, S.P., Gao, F.B., and Reed, R. (2017). Evidence that C9ORF72 dipeptide repeat proteins associate with U2 snRNP to cause mis-splicing in ALS/FTD patients. *Cell Rep.* 19, 2244–2256.

Yokoshi, M., Li, Q., Yamamoto, M., Okada, H., Suzuki, Y., and Kawahara, Y. (2014). Direct binding of Ataxin-2 to distinct elements in 3' UTRs promotes mRNA stability and protein expression. *Mol. Cell* 55, 186–198.

Yoshizawa, T., Ali, R., Jiou, J., Fung, H.Y.J., Burke, K.A., Kim, S.J., Lin, Y., Peebles, W.B., Saltzberg, D., Soniat, M., et al. (2018). Nuclear import receptor

- inhibits phase separation of FUS through binding to multiple sites. *Cell* 173, 693–705.
- Yu, S., Pritchard, M., Kremer, E., Lynch, M., Nancarrow, J., Baker, E., Holman, K., Mulley, J.C., Warren, S.T., Schlessinger, D., et al. (1991). Fragile X genotype characterized by an unstable region of DNA. *Science* 252, 1179–1181.
- Yu, Z., Fan, D., Gui, B., Shi, L., Xuan, C., Shan, L., Wang, Q., Shang, Y., and Wang, Y. (2012). Neurodegeneration-associated TDP-43 interacts with fragile X mental retardation protein (FMRP)/Staufen (STAU1) and regulates SIRT1 expression in neuronal cells. *J. Biol. Chem.* 287, 22560–22572.
- Yu, Y., Chi, B., Xia, W., Gangopadhyay, J., Yamazaki, T., Winkelbauer-Hurt, M.E., Yin, S., Eliasse, Y., Adams, E., Shaw, C.E., and Reed, R. (2015). U1 snRNP is mislocalized in ALS patient fibroblasts bearing NLS mutations in FUS and is required for motor neuron outgrowth in zebrafish. *Nucleic Acids Res.* 43, 3208–3218.
- Yüce, Ö., and West, S.C. (2013). Senataxin, defective in the neurodegenerative disorder ataxia with oculomotor apraxia 2, lies at the interface of transcription and the DNA damage response. *Mol. Cell. Biol.* 33, 406–417.
- Zalfa, F., Giorgi, M., Primerano, B., Moro, A., Di Penta, A., Reis, S., Oostra, B., and Bagni, C. (2003). The fragile X syndrome protein FMRP associates with BC1 RNA and regulates the translation of specific mRNAs at synapses. *Cell* 112, 317–327.
- Zalfa, F., Achsel, T., and Bagni, C. (2006). mRNPs, polysomes or granules: FMRP in neuronal protein synthesis. *Curr. Opin. Neurobiol.* 16, 265–269.
- Zalfa, F., Eleuteri, B., Dickson, K.S., Mercaldo, V., De Rubeis, S., di Penta, A., Tabolacci, E., Chiurazzi, P., Neri, G., Grant, S.G., and Bagni, C. (2007). A new function for the fragile X mental retardation protein in regulation of PSD-95 mRNA stability. *Nat. Neurosci.* 10, 578–587.
- Zamiri, B., Reddy, K., Macgregor, R.B., Jr., and Pearson, C.E. (2014). TMPyP4 porphyrin distorts RNA G-quadruplex structures of the disease-associated r(GGGGCC)_n repeat of the C9orf72 gene and blocks interaction of RNA-binding proteins. *J. Biol. Chem.* 289, 4653–4659.
- Zhang, H., Xing, L., Rossoll, W., Wichterle, H., Singer, R.H., and Bassell, G.J. (2006). Multiprotein complexes of the survival of motor neuron protein SMN with Gemins traffic to neuronal processes and growth cones of motor neurons. *J. Neurosci.* 26, 8622–8632.
- Zhang, Z., Lotti, F., Dittmar, K., Younis, I., Wan, L., Kasim, M., and Dreyfuss, G. (2008). SMN deficiency causes tissue-specific perturbations in the repertoire of snRNAs and widespread defects in splicing. *Cell* 133, 585–600.
- Zhang, Y.J., Jansen-West, K., Xu, Y.F., Gendron, T.F., Bieniek, K.F., Lin, W.L., Sasaguri, H., Caulfield, T., Hubbard, J., Daugherty, L., et al. (2014). Aggregation-prone c9FTD/ALS poly(GA) RAN-translated proteins cause neurotoxicity by inducing ER stress. *Acta Neuropathol.* 128, 505–524.
- Zhang, K., Donnelly, C.J., Haeusler, A.R., Grima, J.C., Machamer, J.B., Steinwald, P., Daley, E.L., Miller, S.J., Cunningham, K.M., Vidensky, S., et al. (2015). The C9orf72 repeat expansion disrupts nucleocytoplasmic transport. *Nature* 525, 56–61.
- Zhang, Y.J., Gendron, T.F., Ebbert, M.T.W., O'Raw, A.D., Yue, M., Jansen-West, K., Zhang, X., Prudencio, M., Chew, J., Cook, C.N., et al. (2018). Poly(GR) impairs protein translation and stress granule dynamics in C9orf72-associated frontotemporal dementia and amyotrophic lateral sclerosis. *Nat. Med.* 24, 1136–1142.
- Zhao, D.Y., Gish, G., Braunschweig, U., Li, Y., Ni, Z., Schmitges, F.W., Zhong, G., Liu, K., Li, W., Moffat, J., et al. (2016). SMN and symmetric arginine dimethylation of RNA polymerase II C-terminal domain control termination. *Nature* 529, 48–53.
- Zhou, Y., Liu, S., Liu, G., Oztürk, A., and Hicks, G.G. (2013). ALS-associated FUS mutations result in compromised FUS alternative splicing and autoregulation. *PLoS Genet.* 9, e1003895.
- Zhu, H., Hasman, R.A., Barron, V.A., Luo, G., and Lou, H. (2006). A nuclear function of Hu proteins as neuron-specific alternative RNA processing regulators. *Mol. Biol. Cell* 17, 5105–5114.
- Zhu, H., Zhou, H.L., Hasman, R.A., and Lou, H. (2007). Hu proteins regulate polyadenylation by blocking sites containing U-rich sequences. *J. Biol. Chem.* 282, 2203–2210.
- Zhu, H., Hinman, M.N., Hasman, R.A., Mehta, P., and Lou, H. (2008). Regulation of neuron-specific alternative splicing of neurofibromatosis type 1 pre-mRNA. *Mol. Cell. Biol.* 28, 1240–1251.
- Zou, Z.Y., Wang, X.N., Liu, M.S., Sun, Q., Li, X.G., Cui, L.Y., and Kong, J. (2012). Identification of a novel missense mutation in angiogenin in a Chinese amyotrophic lateral sclerosis cohort. *Amyotroph. Lateral Scler.* 13, 270–275.
- Zu, T., Gibbens, B., Doty, N.S., Gomes-Pereira, M., Huguet, A., Stone, M.D., Margolis, J., Peterson, M., Markowski, T.W., Ingram, M.A., et al. (2011). Non-ATG-initiated translation directed by microsatellite expansions. *Proc. Natl. Acad. Sci. USA* 108, 260–265.
- Zu, T., Liu, Y., Bañez-Coronel, M., Reid, T., Pletnikova, O., Lewis, J., Miller, T.M., Harms, M.B., Falchook, A.E., Subramony, S.H., et al. (2013). RAN proteins and RNA foci from antisense transcripts in C9ORF72 ALS and frontotemporal dementia. *Proc. Natl. Acad. Sci. USA* 110, E4968–E4977.
- Zu, T., Cleary, J.D., Liu, Y., Banez-Coronel, M., Bubenik, J.L., Ayhan, F., Ashizawa, T., Xia, G., Clark, H.B., Yachnis, A.T., et al. (2017). RAN translation regulated by muscleblind proteins in myotonic dystrophy type 2. *Neuron* 95, 1292–1305.