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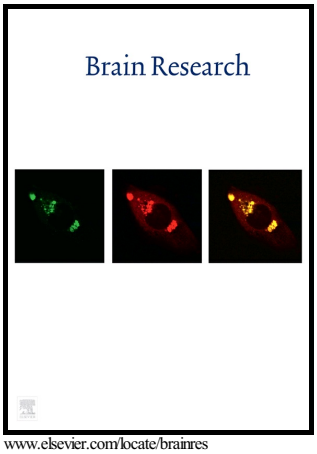

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Mechanisms of FUS Mutations in Familial Amyotrophic Lateral Sclerosis

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Running Head: Mechanisms of FUS in Neurodegeneration

ABSTRACT

Recent advances in the genetics of amyotrophic lateral sclerosis (ALS) have provided key mechanistic insights to the pathogenesis of this devastating neurodegenerative disease. Among many etiologies for ALS, the identification of mutations and proteinopathies in two RNA binding proteins, TDP-43 (TARDBP or TAR DNA binding protein 43) and its closely related RNA/DNA binding protein FUS (fused in sarcoma), raises the intriguing possibility that perturbations to the RNA homeostasis and metabolism in neurons may contribute to the pathogenesis of these diseases. Although the similarities between TDP-43 and FUS suggest that mutations and proteinopathy involving these two proteins may converge on the same mechanisms leading to neurodegeneration, there is increasing evidence that FUS mutations target distinct mechanisms to cause early disease onset and aggressive progression of disease. This review focuses on the recent advances on the molecular, cellular and genetic approaches to uncover the mechanisms of wild type and mutant FUS proteins during development and in neurodegeneration. These findings provide important insights to understand how FUS mutations may perturb the maintenance of dendrites through fundamental processes in RNA splicing, RNA transport and DNA damage response/repair. These results contribute to the understanding of phenotypic manifestations in neurodegeneration related to FUS mutations, and to identify important directions for future investigations.

Keywords: RNA Binding Protein, Fused in sarcoma (FUS), Prion-like Property, Low Complexity Domain, DNA Damage Repair, RNA Splicing, Amyotrophic Lateral Sclerosis (ALS), Frontotemporal Dementia (FTD)

1. Introduction

1.1. The Expanding Genetic Landscape of Amyotrophic Lateral Sclerosis

Amyotrophic lateral sclerosis (ALS) is an adult-onset neurodegenerative disease that affects upper and lower motor neurons. As initially described by Jean-Martin Charcot more than 140 years ago, the key clinical features in ALS patients include muscle wasting, and progressive loss of spinal motor neurons and upper motor neurons and their axons in the lateral columns of the spinal cord. Recent advances in human genetics have identified many genetic loci that are mutated in patients with famililal ALS (FALS). Among a growing number of genes involved in FALS, mutations in four genes account for the majority of cases. These mutations include missense mutations in superoxide dismutase 1 (*SOD1*), two genes encoding RNA/DNA binding proteins, *TDP-43* (*TARDBP* or TAR-DNA-binding protein-43) and *FUS/TLS* (*fused in sarcoma/translocation in liposarcoma* or *FUS*), and the GGGGCC hexanucleotide expansions in *C9ORF72* gene (Cirulli et al., 2015; Lee et al., 2012; Ling et al., 2013). The discovery of TDP-43 as a major component in the ubiquitin-positive, tau-negative insoluble protein aggregates in neurons and glia represents a major breakthrough in FTD (frontotemporal dementia) and ALS research (Arai et al., 2006; Neumann et al., 2006). Moreover, the impact of this discovery goes beyond the identification of a single disease gene and essentially ushers in a new era of research that focuses on the potential contributions of transcription, RNA splicing and RNA metabolism on neurodegenerative diseases.

TDP-43 is originally identified to bind to the TAR DNA sequence in HIV-1 genome to regulate viral gene expression (Ou et al., 1995). Under physiological conditions, TDP-43

is a ubiquitous nuclear protein, however, in FTD patients, TDP-43 aggregates are present predominantly in neuronal cytoplasm and dystrophic neuronal processes (Arai et al., 2006; Neumann et al., 2006). This distinct feature, defined as TDP-43 proteinopathy, constitutes a major neuropathological diagnosis entity in sporadic ALS (ALS-TDP). Several subsequent studies show that dominant mutations in the *TARDBP* gene can also be identified in FALS patients (Lattante et al., 2013). The identification of autosomal dominant mutations in the *FUS* gene in a large kindred of familial ALS (FALS) further expanded the genetic and neuropathological landscape of ALS (Kwiatkowski et al., 2009; Vance et al., 2009). Similar to TDP-43, FUS proteins reside primarily in the neuronal nuclei, but in ALS-FUS patients FUS proteins form large aggregates in the cytoplasm. The morphology of FUS proteinopathy in FALS ranges from diffuse and dense cytoplasmic aggregate present in late onset cases, to basophilic inclusions commonly found in juvenile FALS with FUS-P525L mutation. Finally, in 2011 two groups independently reported the GGGGCC hexanucleotide repeat expansions in the noncoding region of the *C9ORF72* gene as causal links to ALS and FTD (DeJesus-Hernandez et al., 2011; Renton et al., 2011). Although TDP-43 proteinopathy can be detected in FTD and ALS patients with *C9ORF72* mutations, the neuropathological features in these cases are quite heterogeneous and also include prominent ubiquitin and p62 positive, but TDP-43 negative intracytoplasmic and intranuclear inclusions (Bigio, 2012; Mackenzie et al., 2014).

1.2. Early Disease Onset in FALS Caused by FUS Mutations

It is estimated that mutations in *TARDBP* and *FUS* each account for ~5% of FALS, whereas the GGGGCC expansion mutations in *C9ORF72* account for 20-40% of ALS and FTD-ALS cases, depending on the population studied (Cirulli et al., 2015). One important feature noted in a recent study indicates that the age of disease onset for FALS caused by *FUS*, *TARDBP* and *C9ORF72* mutations differ quite drastically. Mutations in *FUS* account for ~35% of FALS in patients younger than 40 years old, whereas mutations in *C9ORF72* are much more common in patients older than 50 years of age (Millecamps et al., 2012). Indeed, meta-analyses of 154 ALS cases with *FUS* mutations (including FALS and SALS with *de novo FUS* mutations) show an average disease onset of 43.8 ± 17.4 years (**Figure 1**)(Deng et al., 2014a; Lattante et al., 2013). More than 60% of cases with *FUS* mutations show disease onset before 45 years of age, with many juvenile ALS cases presenting with disease onset in late teens and early 20's (**Figure 1**)(Baumer et al., 2010; Huang et al., 2010). These findings are similar to those from another study using smaller sample size, and show that the average disease onset for *FUS*, *SOD1* or *TARDBP* mutations is 43.6 ± 15.8, 47.7 ± 13.0 and 54.7 ± 15.3, respectively (Yan et al., 2010). Kaplan-Meier survival analysis shows statistically significant differences in the age of onset among these three mutations. This distinctive feature of *FUS* mutations raises the intriguing hypothesis that mutations in FUS may target divergent mechanisms that perturb the development, maintenance and homeostasis of the nervous system in early postnatal life and in the aging process.

This review focuses on the recent progress on the molecular, cellular and genetic approaches to uncover the mechanisms of wild type and mutant FUS proteins. These

findings provide important insights to understand how FUS mutations may perturb the fundamental processes in DNA damage response/repair, RNA splicing, and RNA transport, to interpret the phenotypic manifestations in neurodegeneration related to FUS mutations, and to identify important directions for future investigations.

2. Physical Properties of FUS and Their Implications in RNA Metabolism

2.1. RNA Binding Properties of FUS

FUS is identified as an oncogene that undergoes chromosomal translocation in myxoid liposarcoma, in which the N-terminal transcriptional activation domain of FUS is fused to CHOP (CAAT enhancer-binding homologous protein), a growth arrest and DNA-damage inducible member of the C/EBP family of transcription factors (Crozat et al., 1993; Rabbitts et al., 1993). Subsequent studies further reveal that chromosomal translocations involving FUS can be identified in several other human cancers, including acute myeloid leukemia, where the N-terminus of *FUS* gene is translocated to the *ERG* gene, a member of the ETS transcription factor family (Ichikawa et al., 1994; Prasad et al., 1994). Structurally, FUS belongs to a family of FET RNA binding proteins, including FUS, Ewing's sarcoma RNA binding protein 1 (EWSR1) and Tata-binding protein-associated factor 2N (TAF-15), that are known to interact with the C-terminal domain of RNA polymerase II (RNAP II) and general transcription factor TFIID (Das et al., 2007; Kwon et al., 2013; Schwartz et al., 2012; Tan and Manley, 2009). Full length human FUS protein contains 526 amino acids that can be divided into the N-terminal "prion-like" or low complexity (LC) Q/G/S/Y domain (amino acids 1-165) and Gly-rich region (amino acids 166-267)(**Figure 2**). The C-terminal half of FUS protein contains an RNA

recognition motif (RRM)(amino acids 285-371), two Arg-Gly-Gly (RGG)-repeat regions (amino acids 371-422 and 453-501), interrupted by a Cys_2-Cys_2 zinc-finger motif (ZNF)(amino acids 422-453), and a non-conventional nuclear localization signal (NLS)(amino acids 510-526), which interacts with the nuclear transport receptor Transportin 1 (**Figure 2**)(Dormann et al., 2012; Dormann et al., 2010; Iko et al., 2004). Most of the FALS-associated FUS mutations cluster in the N-terminal LC domain, the second RGG domain and NLS in the C-terminal (**Figure 2**).

Following its discovery as an oncogene involved in chromosomal translocation in malignant tumors, several studies have elucidated the biochemical properties of FUS as an RNA binding protein that regulates splicing. First, by UV cross-linking, it has been shown that FUS can bind to RNA. The binding seems not to depend on the RRM in the C-terminus, but rather on the zinc finger (ZnF) motif (Iko et al., 2004; Zinszner et al., 1997). Second, FUS is an abundant nuclear protein that can form stable complex with many members of the heterogeneous ribonuclear protein (hnRNP) family and can be co-purified from nuclear extracts by single-stranded DNA affinity chromatography (Calvio et al., 1995; Zinszner et al., 1994; Zinszner et al., 1997). One study suggests that the stability of the FUS-hnRNP complex is dependent on the integrity of its constituent RNA (Zinszner et al., 1994). While these results do not prove that FUS can directly interact with RNA, they suggest that at least some component(s) of the FUS-hnRNP complex has RNA-binding activity.

To further determine if FUS directly binds to specific RNA sequence, Lerga and colleagues use an *in vitro* selection assay and identify a common GGUG motif in RNA oligoribonucleotides that bind to recombinant FUS protein (Lerga et al., 2001). These

results are verified using UV cross-linking combined with competition and immunoprecipitation in nuclear extracts. The ability of FUS to directly interact with RNA has been further examined using in FUS antibody immunoprecipitates from mouse and human brain tissues, followed by CLIP (cross-linking immunoprecipitation)-RNA sequencing (CLIP-Seq)(Lagier-Tourenne et al., 2012). This approach shows that both mouse and human FUS proteins bind to RNAs that contain an enriched GUGGU motif, different from the GU-rich binding sequence reported for TDP-43 (Polymenidou et al., 2011; Tollervey et al., 2011). However, several studies using similar CLIP-seq technology do not find similar consensus RNA binding sequences for FUS (Colombrita et al., 2012; Hoell et al., 2011; Ishigaki et al., 2012; Rogelj et al., 2012). Instead, the results from these studies support the idea that FUS binding sites in RNA tend to form stable secondary structures, such as the stem-and-loop structure (Hoell et al., 2011; Ishigaki et al., 2012). Similarly, FUS has also been shown to interact with the short RNA repeats r(UUAGGG) in the G-quadruplex telomeric repeat-containing RNA (TERRA) by forming unique secondary and tertiary structures (Takahama et al., 2013). In a recent study, Wang and colleagues examine the specificity of the putative FUS-binding RNA motifs using electrophoretic mobility shift assays (EMSA), and show that FUS binds to all the repeats with K_d values within a 10-fold range (Wang et al., 2015). Surprisingly, RNAs without any of the reported binding motifs also bind to FUS with similar affinity. Together, these results support that the nucleic acid binding property in FUS can be rather generic or “promiscuous”, and is dictated by the secondary or tertiary structure of RNA.

2.2. Roles of FUS in RNA Splicing

Given the nature of FUS-RNA interactions, what would be the physiological role of FUS in RNA metabolism? Previous CLIP-seq studies show that most RNAs that bind to FUS contain intronic sequences. Perhaps the most unique feature is that in genes with long intron, FUS-RNA binding exhibits a distinct “sawtooth” CLIP pattern (Lagier-Tourenne et al., 2012; Rogelj et al., 2012), with substantially higher FUS cluster density at the beginning of introns and a gradual decrease toward the 3’ sequence. These results suggest that FUS is co-transcriptionally deposited onto the nascent RNA transcripts. In addition, FUS binding has been identified around the alternatively spliced exons and in the promoter antisense strands in several genes implicated in neurodevelopmental and neurodegenerative diseases (Ishigaki et al., 2012), suggesting that FUS may involve in alternative splicing and transcription. The support for FUS in RNA splicing is further underscored by the identification of FUS as an direct interacting partner with splicing factors, SC35 and SRSF10 (serine/arginine-rich splicing factor 10), and as one of the ~50 non-snRNP proteins in the pre-spliceosome (Behzadnia et al., 2007; Wahl et al., 2009; Yang et al., 1998). While these results provide physical evidence of FUS in RNA splicing, it remains unclear how FUS regulates the recognition of the 5’ splice junction, the formation and stability of Complex A, and the efficiency of splicing. Finally, two studies use epitope-tagged FUS to identify FUS interactome in nuclear extracts from HeLa cells and show that FUS can also interact with SMN and U1 snRNP (Sun et al., 2015; Yamazaki et al., 2012). The results that FUS can interact with SMN is intriguing because SMN is implicated in fatal childhood motor neuron disease spinal muscular atrophy (SMA) and the organization of Gemini of Cajal bodies (Gems).

Consistent with these findings, knocking down FUS or expression of FALS-associated FUS mutant proteins severely compromise the formation of Gems in HeLa cells and fibroblasts from FALS patients, respectively. In primary neurons, FALS-associated FUS mutant proteins promote SMN protein aggregation in the cytoplasm and axons of primary neurons (Groen et al., 2013; Yamazaki et al., 2012). In a recent study, Sun and colleagues further show that the RGG domain in FUS and the Tudor domain in SMN are required for direct interaction. Surprisingly, FALS-associated FUS mutations enhance the interaction between FUS and SMN, and thereby affecting the normal functions of SMN by reducing Gems bodies and changing the state steady level of snRNA in transgenic mouse tissues and in fibroblasts from patients expressing mutant FUS proteins (Sun et al., 2015). Global analysis of RNA splicing reveals that mutant FUS-dependent splicing changes mimic partial FUS loss of activity, independent of cytosolic mislocalization. These results provide evidence for both gain and loss of function caused by ALS-linked mutations in FUS and the potential convergence in pathological pathways of ALS and SMA.

2.3. Prion-like Property of FUS and Its Implication in Liquid-to-Solid Phase Transition

The presence of TDP-43 and FUS proteinopathy in ALS raises the intriguing possibility that TDP-43 and FUS may have a high propensity to form protein aggregates. Indeed, using a bioinformatics algorithm designed to identify proteins with “prion-like” or low complexity (LC) domain (Alberti et al., 2009), it has been shown that the N-terminal Q/G/S/Y domain and part of the Gly-rich region in FUS (amino acids 1-239) and the C-terminal Gly-rich region of TDP-43 (amino acids 277-414) respectively

rank 15th and 69th among 27,879 proteins in the human proteome for their prion-like property (Cushman et al., 2010; Gitler and Shorter, 2011)(**Figure 2**). Consistent with this idea, expression of TDP-43 and FUS in the baker's yeast *Saccharomyces cerevisiae* shows that both proteins are more prone to form protein aggregates that resemble proteinopathy in human diseases and that the protein aggregate formation is dependent on the prion-like domains (Fushimi et al., 2011; Sun et al., 2011). These findings underscore the value of yeasts as a model organism that can be used for genetic screens to identify modifiers that can alleviate FUS proteinopathy. Indeed, such screens reveal many potential candidates that are implicated in RNA metabolic process, ribosome biogenesis, response to cellular stress, etc (Sun et al., 2011). Similar approach has also been exploited as an effective screen to identify the causal links between two other FET family members, EWSR1 and TAF15, to the pathogenesis of ALS (Couthouis et al., 2012; Couthouis et al., 2011). Several other RNA-binding proteins with similar prion-like properties have been implicated in the organization of stress granules and perturbations to this process may also contribute to ALS and other neurodegenerative diseases (Li et al., 2013).

One major advance in understanding the biophysical property of FUS and its role in the formation of RNA granules comes from the observation of a small molecule chemical 5-aryl-isoxazole-3-carboxyamide, which when biotinylated acquires the unique property to aggregate and disaggregate RNA granules in a soluble, cell-free system (Han et al., 2012; Kato et al., 2012). Using biotinylated isoxazole (b-isox), McKnight and colleagues use an elegant and highly efficient hydrogel formation assay and identify that several RNA binding proteins, including FUS, TDP-43, and hnRNPA1, have a high

propensity to co-precipitate with b-isox using their LC domain. Many of these proteins have been implicated in the formation of stress granules. Consistent with this idea, mutations that alter the highly conserved [G/S]Y[G/S] motif within the LC domain of FUS completely abolish its ability to form stress granules in cells. The authors use transmission electron microscopy (TEM) and X-ray diffraction analyses to show that FUS form amyloid-like filamentous protein aggregates with prominent filamentous cross-β structure that resembles amyloid proteins. Indeed, both biophysical measurements and ultrastructural analyses show that the fibrillary FUS proteins in the hydrogel resemble FUS aggregates identified in the cytoplasm of spinal motor neurons in a patient with FUS-P525L mutation using immunogold EM (Huang et al., 2010; Kato et al., 2012).

Knowing the unique property of FUS in hydrogel formation, one critical and intuitive question is how FALS-associated mutations in FUS might alter the biophysical properties of FUS and thereby affects the function of FUS in RNA-protein complex formation. To investigate this, Alberti and colleagues show that when cells experience DNA damage or heat stress, FUS rapidly accumulates in distinct compartments in the nucleus and cytoplasm, respectively. They then use an imaging technique, known as “half-bleach” (Brangwynne et al., 2009), to show that FUS redistributes rapidly within stress granules in the cytoplasm and in nuclear FUS assemblies. Their results show that FUS granules undergo frequent fusion, and as soon as they interact, these granules undergo rapid relaxation into a spherical shape (Patel et al., 2015). These results indicate that the FUS-containing compartments, which exist in liquid droplets and hydrogel states, are reversible and extremely dynamic. Interestingly, FUS mutations

associated with FALS promote a conversion of FUS-containing liquid droplets to fiber state, which results in impaired protein synthesis in axons and leads to neurotoxicity (Murakami et al., 2015; Patel et al., 2015).

2.4. LC Domain of FUS in High-Order Assembly of Protein-RNA Complex

The ability of FUS to form RNA-protein complexes is further revealed using a 48 nucleotide (nt)-long RNA (prD RNA) from the promoter region of DNMT3b gene (Schwartz et al., 2012; Schwartz et al., 2013). It is important to note that this prD RNA does not contain any of the previously identified FUS binding motifs (Lagier-Tourenne et al., 2012; Lerga et al., 2001), yet exhibit robust binding to recombinant FUS proteins in electrophoretic mobility shift assays (Schwartz et al., 2013). This provides a convenient tool to characterize the essential role of the LC domain in FUS and its mutual interactions with RNA to form high-order protein-RNA complexes. Similar high-order assemblies have been reported using recombinant FUS proteins and synthetic RNA from the intron-exon boundary and 3'UTR of the *bdnf* gene (Qiu et al., 2014). Many of these *bdnf* RNA probes do not contain the reported FUS binding motif, again supporting the notion that the RNA structure is perhaps more important for FUS interaction. Interestingly, RNA-FUS assemblies appear to be critical for its interaction with the CTD of RNA polymerase II (Schwartz et al., 2013).

Using the FUS-*bdnf* RNA interactions, Qiu and colleagues show that mutant FUS-R521C proteins form more stable and higher order protein-RNA assemblies, which are more difficult to dissociate in competition assays (Qiu et al., 2014). Interestingly, in transgenic mice expressing FUS-R521C, the majority of mutant FUS proteins are in the

nuclei of spinal motor neurons, suggesting that the presence of high-order mutant FUS-RNA assemblies may interfere with the transcription and/or RNA splicing. Consistent with these results, expression of mutant FUS proteins or siRNA knockdown of FUS in fibroblasts alters the distribution of RNA polymerase II within the nuclei. These results are further confirmed using fibroblasts derived from FALS patients with FUS mutations (Nomura et al., 2014; Schwartz et al., 2014).

3. FUS Mutations and Neurodegeneration in Model Organisms

3.1. Dendrite and Synaptic Defects in Rodent Models of FUS Mutations

To characterize the consequences of expressing mutant FUS proteins in the nervous system, several groups have used a number of transgenic strategies in mice or rats to model disease conditions caused by FUS mutations. Results from these studies show that expressing mutant FUS proteins causes consistent neurodegenerative phenotypes. For instance, both the transgenic mouse and rat models expressing mutant FUS-R521C proteins develop early onset ALS-like symptoms, including hindlimb paralysis, muscle wasting, and reduced innervation at the neuromuscular junction (NMJ)(Huang et al., 2011; Qiu et al., 2014; Sharma et al., 2016). The cardinal phenotypes include age-dependent reductions in dendritic arborization and synaptic density in the spinal motor neurons and cortical neurons in the sensorimotor cortex of the FUS-R521C transgenic mice (**Figure 3**)(Qiu et al., 2014). Similar dendritic arborization defects have also been reported in neurons in the entorhinal cortex of *Camk2a-tTA* transgenic rats (Huang et al., 2012), and in the spinal motor neurons and cortical neurons of Cre-inducible transgenic mouse lines that express FUS-R521G in

the nervous system (Sephton et al., 2014). The dendritic phenotype caused by FUS-R521C can be recapitulated in cultured cortical neurons, and can be partially rescued by exogenous BDNF (Qiu et al., 2014). In side-by-side comparisons, FUS-R521C and FUS-P525L cause more severe dendritic growth defects compared to wild type FUS. These results support the notion that wild type and mutant FUS affect dendritic growth in gene dosage-dependent manner. In light of these findings, it is interesting to note that transgenic mice expressing higher level of wild type FUS also show early onset motor neuron degeneration in a dosage-dependent manner (Mitchell et al., 2013). Consistent with these results, mutations in the 3' UTR of the FUS gene have been identified in several FALS patients. These mutations drastically increase the FUS protein expression in the patients' fibroblasts, at levels higher than that in FUS-R521C fibroblasts (Sabatelli et al., 2013), supporting the notion that wild type FUS expressed at exceedingly high levels can be pathogenic.

Unlike the severe loss of spinal motor neurons in the *SOD1*^{G93A} mice, the neuron loss phenotype in different FUS transgenic models appears to be more modest. At end stage, FUS-R521C transgenic mice and rats, and transgenic mice expressing FUS-R521G or a truncated FUS mutant protein (amino acids 1-359), show greater than 50% preservation of spinal motor neurons (**Table 1**)(Huang et al., 2011; Qiu et al., 2014; Sephton et al., 2014; Sharma et al., 2016; Shelkovnikova et al., 2013). The majority of mutant FUS-R521C proteins are located within the nuclei of spinal motor neurons in these transgenic animals, with few neurons showing evidence of FUS-R521C protein aggregates in the cytoplasm. The lack of prominent cytoplasmic FUS inclusion in the FUS-R521C transgenic models is quite different from the pathology observed in patients

with FALS caused by FUS mutations (Huang et al., 2010; Kwiatkowski et al., 2009; Vance et al., 2009). Another interesting observation is that few neurons in FUS-R521C transgenic rats and wild type FUS transgenic mice show accumulation of ubiquitin-positive inclusions in the cytoplasm. Curiously, however, most of the ubiquitin-positive cytoplasmic inclusions do not contain mutant or wild type FUS proteins (Huang et al., 2011; Mitchell et al., 2013).

The discrepancy of neuropathology in the rodent models and human patients raise several intriguing questions regarding the cause and significance of FUS-positive cytoplasmic inclusions in FALS. One possible explanation for the lack of FUS+ cytoplasmic inclusions in transgenic rodent models is that the cytoplasmic aggregation of wild type or mutant FUS proteins may be age- and dosage-dependent. Depending on the efficiency of nucleus-to-cytoplasm translocation for wild type and mutant FUS proteins, the early postnatal lethality in most of the transgenic mice or rats may not have given FUS proteins sufficient time to accumulate in the neuronal cytoplasm. Alternatively, it is possible that mouse spinal motor neurons may develop inherent homeostatic mechanisms to maintain the certain level FUS expression (Dini Modigliani et al., 2014). In this regard, only when expressed at exceedingly high level using viral vectors, such as AAV1, will the FUS proteins begin to accumulate in the neuronal cytoplasm (Verbeeck et al., 2012). Regardless of the mechanism, the observations that transgenic mice and rats develop severe neurodegenerative phenotypes even in the absence of prominent FUS proteinopathy in neuronal cytoplasm suggest that increase of wild type FUS proteins or the presence of mutant FUS proteins in nucleus is sufficient to cause disease, most likely through the perturbations of DNA damage repair/response

and RNA splicing machinery (Qiu et al., 2014; Wang et al., 2013). Interestingly, FALS-related mutation FUS-R521G exhibits a drastic shift in binding preference from the intronic sequences to sequences in the 3'UTR (Hoell et al., 2011).

While the results from the murine models suggest that mutant FUS proteins may acquire gain-of-function properties to interact with wild type FUS proteins and new RNA targets, we are still at the very early stage of uncovering the mechanism(s) of FUS mutations that contribute to impairments in neuronal survival and defects in dendritic growth and synaptic connectivity. In Sections 4 and 5, we summarize the recent findings on the effects of FUS mutations in DNA damage repair and RNA splicing, which are likely to have synergistic contributions to the dendritic and synaptic defects. These results not only provide important insights on the potential target genes, which might be preferentially affected by the DNA damage repair and RNA splicing defects caused by FUS mutations, they also provide future directions to establish both *in vitro* and *in vivo* approaches to characterize how accumulation of mutant FUS proteins in neuronal cytoplasm affects ribosomal functions and RNA transport in dendrites and axons.

3.2. FUS-mediated Neurodegeneration in Other Model Organisms

In addition to the rodent models of FUS mutations, results from other model organisms, including yeasts, *Xenopus*, and rodents, have revealed a wealth of information regarding the *in vivo* functions of FUS during organismal development, and how FUS mutations may disrupt these functions and contribute to the neurodegenerative process. There are several additional studies performed in other model organisms, including *Drosophila melanogaster*, *C. elegans* and zebrafish (*Danio*

rerio), which provide novel insights on the genetic interactions between FUS and other FALS-related genes, such as *TARDBP* and *SOD1*.

Drosophila has a single homolog of FUS, encoded by the *cabeza* (*caz*) gene on X chromosome, that shares 53% amino acid identity to its mammalian counterpart (Stolow and Haynes, 1995). The *Drosophila* Caz protein contains 399 amino acids and is expressed in neurons, glia and muscle cells. Loss-of-function analyses show that only 14% of male *caz* mutant larvae successfully undergo pupation and eclose to become adults (Wang et al., 2011). The *caz* mutants that do survive into adulthood exhibit severe locomotion phenotype and a markedly reduced survival in postnatal life. The eclosion phenotype in *caz* mutants can be rescued by neuron-specific transgenic expression of Caz, wild type human FUS ($hFUS^{WT}$), $hFUS^{R522H}$ or $hFUS^{P525L}$ at the same expression level, suggesting that $hFUS^{WT}$, $hFUS^{R522H}$ and $hFUS^{P525L}$ can functionally restore the role of Caz during eclosion. However, neuron-specific transgenic expression of Caz or $hFUS^{WT}$ only partially restores the locomotion and longevity phenotype, whereas neither $hFUS^{R522H}$ nor $hFUS^{P525L}$ is capable of restoring the locomotion or longevity phenotype. Interestingly, Drosophila *tbph* mutants lacking TDP-43 homolog TBPH also show similar phenotype in eclosion, locomotion and longevity. Whereas expression of TBPH in *caz* mutants cannot rescue loss of Caz phenotype, overexpression of Caz in *tbph* mutants restores eclosion, locomotion and longevity. These results support the model that *caz* and *tbph* genetically interact in Drosophila to regulate neuronal development and longevity (Wang et al., 2011). Several other studies using *Drosophila* as a model system also show that expressing mutant human FUS proteins, $hFUS^{R518K}$, $hFUS^{R521H}$ or $hFUS^{R521C}$, in the eye, motor neurons or the nervous

system leads to eye degeneration, defects in locomotion and increase in mortality (Lanson et al., 2011). Detailed analyses of the locomotion defects indicate that mutant FUS proteins cause synaptic defects before the degeneration of motor neurons. The synaptic defects include disorganization of the presynaptic active zone protein brunchpilot, reduced quantal contents and miniature presynaptic currents, and reduced synaptic currents in the postsynaptic muscle cells (Shahidullah et al., 2013).

The effects of FUS in synaptic functions have also been investigated using the zebrafish larvae as a model system. Antisense morpholino (AMO) knockdown of FUS in zebrafish causes motor behavioral defects reflected as reduced touch-evoked escape response (TEER) and marked reductions in the branching and length of motor axons (Kabashi et al., 2011). Similar to the observations in Drosophila, AMO knockdown of FUS and expressing mutant human FUS proteins in zebrafish also cause defects in synaptic transmission at NMJ by reducing the presynaptic quantal contents (Armstrong and Drapeau, 2013).

4. Mechanism of FUS Mutations in Neurodegeneration: DNA Damage Defects

4.1. DNA Damage & Neurodegeneration

Both prokaryotic and eukaryotic organisms use highly evolutionarily conserved mechanisms to repair DNA damages caused by radiation from the environment or by endogenous sources, such as free radicals produced within the cells. Mutations in DNA repair machinery have been linked to hereditary neurodegenerative diseases (Jackson and Bartek, 2009; McKinnon, 2009; Rass et al., 2007). For instance, ataxia telangiectasia (AT) is an autosomal recessive, early onset neurodegenerative disease

caused by mutations in the *ATM* gene, which encodes a protein kinase that regulates the cellular response to DNA double strand breaks (DSBs). Patients with mutations in the components of the DNA damage sensor complex MRN (MRE11-RAD50-NBS1) also develop severe neurological symptoms, with hypersensitivity to ionizing radiation and genome instability. Another DNA damage repair machinery involves base excision repair (BER), which is the primary mechanism that handles spontaneous DNA damage caused by free radicals and reactive oxygen species (ROS). Patients with mutations in critical components of the BER machinery, including CSA (also known as excision repair cross-complementing rodent repair deficiency, complementation group 6 or ERCC6), CSB (ERCC8), XPD (ERCC2) and XPG (ERCC5), develop Cockyane syndrome, characterized by retinal degeneration, microcephaly, deafness and loss of Purkinje cells in the cerebellum (Cleaver et al., 2009). In addition to the inherited forms of neurodegenerative diseases, DNA damage and genomic instability have also been linked to late-onset neurodegenerative diseases, such as Alzheimer's disease and Parkinson's disease (Anderson et al., 1996; Bender et al., 2006; Lu et al., 2004).

4.2. Roles of FUS in DNA Damage Response and Repair

Several studies have implicated FUS in the DNA damage response and repair machinery during development and in postnatal life. For instance, two groups have independently generated mice lacking FUS (*FUS*$^{-/-}$ mutants) and show that FUS deficiency consistently leads to a marked increase in DNA damage that affect a wide range of cell types during perinatal development and in postnatal life (**Table 1**)(Hicks et al., 2000; Kuroda et al., 2000). When maintained in the C57Bl6 background, *FUS*$^{-/-}$

mice are perinatal lethal and exhibit severe deficiency in B lymphocyte development. In contrast, *FUS*$^{-/-}$ mice maintained in the mixed 129svev;CD1 genetic background survive into adulthood and show male sterility. Regardless of the genetic background, however, one consistent phenotype in both independent *FUS*$^{-/-}$ mouse lines are the presence of genomic instability and increased vulnerability to ionizing irradiation. Consequently, *FUS*$^{-/-}$ mice present with increased numbers of unpaired and mispaired chromosomal axes in pre-meiotic spermatocytes and in B lymphocytes (Hicks et al., 2000; Kuroda et al., 2000).

The robust genomic instability and increased DNA damage phenotype in *FUS*$^{-/-}$ mice raise the intriguing possibility that FUS might be involved in DNA damage response and repair machinery. Indeed, FUS has been suggested to be involved in the formation of D-loops, an essential step in homologous recombination, and normally presents in chromosome pairing, DNA repair and telomeres (Baechtold et al., 1999; Bertrand et al., 1999). Interestingly, wild type FUS, but not FUS-CHOP fusion protein can be phosphorylated by ATM (ataxia-telangiectasia mutated) in response to DNA double strand breaks (DSBs)(Gardiner et al., 2008). In addition, FUS has been shown to promote homologous DNA pairing, a key step in homologous recombination (HR), whereas the oncogenic fusion protein FUS-CHOP, in which the C-terminal domain of FUS was replaced by the DNA-binding and leucine zipper dimerization domain of CHOP (Crozat et al., 1993), is unable to promote DNA pairing. Since the Gly-rich domain is retained in the FUS-CHOP protein, these data suggest that the N-terminal domain of FUS may be involved to DNA repair through interaction with other proteins in the DNA repair machinery. Consistent with this notion, FUS has been shown to directly

interact with CBP/p300, an acetyltransferase, through its N-terminal domain, and leads to the inhibition of *CCND1* transcription following DNA damage (Wang et al., 2008), suggesting that FUS may play multiple roles in response to DNA damage. Surprisingly, the ability of FUS to respond to DNA damage depends on the allosteric interaction with single-stranded, low copy number long noncoding RNA transcripts (Wang et al., 2008). Together, these results underscore the unique feature of FUS as RNA and DNA binding protein in regulating the DNA damage response and repair process.

To determine how FUS affects DSB repair, Wang and colleagues use siRNA to knockdown FUS and show that loss of FUS affects homologous recombination and non-homologous end joint (NHEJ)-mediated DSB repairs in both U2OS cells and primary neurons (Wang et al., 2013). Using γH2AX immunoreactivity as an early marker of DNA damage and a prerequisite marker for DSB repair (Fillingham et al., 2006; Pilch et al., 2003), they further demonstrate that FUS is rapidly recruited to DNA damage sites, which precedes the accumulation of γH2AX. These results, independently confirmed by other groups (Mastrocola et al., 2013; Rulten et al., 2014), suggest that recruitment of FUS to damaged chromatin is required to elicit an effective DDR. The study by Rulten and colleagues further show that FUS recruitment to DNA damage foci is dependent on poly (ADP-ribose) polymerase 1 (PARP1). However, it is unclear if these results indicate the presence of hierarchy of protein complex formation involving PARP1 and FUS in the assembly of DNA response/repair machinery, or the requirement of PARP1 is a cell type-specific phenomenon (Rulten et al., 2014; Wang et al., 2013). Finally, DSB can induce nucleus-to-cytoplasm translocation of FUS and causes phosphorylation of FUS in the C-terminus by DNA-dependent protein kinase (DNA-PK)(Deng et al., 2014b).

These studies provide the first molecular clue for the previous observations that *FUS*$^{-/-}$ mice exhibit enhanced radiation sensitivity, growth retardation, immunodeficiency, and increased genomic instability (Hicks et al., 2000; Kuroda et al., 2000).

4.3. FUS Mutations & HDAC-dependent DNA Damage Response/Repair

Given the critical role of FUS in DNA damage response/repair, it is interesting to note that FALS-associated mutations in FUS do not affect the recruitment of mutant FUS proteins to DNA damage foci. Rather, the mechanism by which FUS regulates DNA damage repair machinery depends on its ability to directly interact with chromatin remodeling factor histone deacetylase 1 (HDAC1), which plays fundamental role in DNA repair and the maintenance of genomic stability (**Figure 4A**). Deficiency in HDAC1 and the closely related HDAC2 causes severe hypersensitivity to DNA damaging agents and persistent phenotypes related to DNA repair defects, including dysregulation of histone acetylation, abnormalities in heterochromatin formation, and aberrant expansion and re-condensing of the chromatin structure in DNA repair process (Dinant et al., 2008; Lukas et al., 2011; Miller et al., 2010). Interestingly, loss of HDAC1 has been reported to sensitize neurons to DNA damage and induce aberrant cell cycle re-entry, while the overexpression of HDAC1 protects neurons from genotoxic agents (Dobbin et al., 2013; Kim et al., 2008). Indeed, both neurons with FUS deficiency and *HDAC1*$^{-/-}$ neurons exhibit increased DNA damage following etoposide treatment (Dobbin et al., 2013; Wang et al., 2013), supporting the notion that the interaction between FUS and HDAC1 plays an important role in maintaining genome stability and integrity in neurons.

The fact that FALS mutations are transmitted in an autosomal dominant manner and FALS mutations do not affect FUS recruitment to DNA damage foci lead to the hypothesis that mutant FUS proteins may have dominant-negative effect that interferes with its interaction with HDAC1 and the subsequent assembly of DNA repair machinery (**Figure 4A**). Indeed, structure-function analyses show that the Glycine-rich domain (amino acids 156-262) and C-terminal domain (amino acids 450-526) of FUS are required for FUS-HDAC1 interaction (Wang et al., 2013). Remarkably, these two domains in FUS harbor most of the FALS mutations, and FUS mutations in these two domains, including FUS-R244C, FUS-R514S and FUS-R521C, show impaired interaction with HDAC1 and reduced DSB repair efficiency when expressed in cells. Consistent with these results, wild type FUS can be detected in a protein complex with HDAC1 in the control spinal cord tissues. In contrast, protein extracts from FUS-R521C transgenic mice show no detectable complex formation between FUS-R521C and HDAC1 (Qiu et al., 2014). Interestingly, the presence of FUS-R521C almost completely abolishes the protein-protein interaction between wild type FUS and HDAC1 in FUS-R521C transgenic mice. This dominant effect of FUS-R521C is due to the abnormal gain-of-function property of the mutant FUS-R521C protein in forming more stable complex with wild type FUS protein in both HEK293T cells and in FUS-R521C transgenic mice. Consistent with these results, spinal motor neurons and cortical neurons in FUS-R521C transgenic mice and in patients with FUS-R521C or FUS-P525L mutation show a robust increase of γH2AX staining (Qiu et al., 2014; Wang et al., 2013).

The demonstration that DNA damage repair defects contribute to the pathogenesis of neurodegeneration caused by FUS mutations further underscores the critical role of

DNA damage repair in neurodegenerative conditions. Indeed, several previous studies have shown that increased levels of 8-hydroxydeoxyguanosine (8-OHdG) residues, a marker of oxidative DNA damage, can be identified in the spinal cord of both sporadic and familial ALS patients (Ferrante et al., 1997). Age-related motor neuron degeneration has been observed in mice lacking the DNA repair protein ERCC1, suggesting that the accumulation of DNA damage contributes to the motor neuron vulnerability (de Waard et al., 2010). To determine whether FUS mutations cause widespread or selective target genes, Qiu and colleagues performed a quantitative PCR-based formamidopyrimidine-DNA glycosylase (FPG), a based excision repair enzyme, assay to identify oxidized purine residues in a highly selected group of neural genes (Qiu et al., 2014). Their results show that signatures of DNA damage can be detected in the 5' and 3' UTR of genes that involve in synaptic transmission (*NR2A* and *GluR2*) and dendritic growth (*Bdnf*). One interesting caveat is that the DNA damage in these genes appears to be more prominent in the cortex than in the spinal cord. Hence, future experiments are needed to reveal additional targets at the genome-wide level. While these results suggest that perturbations to multiple signaling pathways may converge on the DNA damage repair defects leading to neurodegeneration, it is important to note that DNA damage due to defects in oxidative stress and nucleotide excision repair is quite different from that caused by double stranded DNA breaks or defects in the ATM pathways. Finally, it is unclear why motor neurons are more susceptible to FUS mutations despite the fact that almost all neurons express mutant FUS proteins and the evidence of DNA damage can also be detected in cortical neurons of FUS-R521C transgenic mice and human disease tissues (Qiu et al., 2014;

Wang et al., 2013). Such “selective vulnerability” is a common theme in neurodegenerative diseases. One potential mechanism is that motor neurons may produce excessively higher amount of mitochondrial reactive oxygen species, which may create a vicious cycle that further promotes the accumulation of DNA damage repair defects (Cleaver et al., 2013; Rulten et al., 2014).

5. Mechanism of FUS Mutations in Neurodegeneration: RNA Splicing Defects

5.1. Effects of FUS Mutations in RNA Transcription/Splicing Defects

The causal link between FUS mutations and DNA damage defects provides critical mechanistic insights to neurodegeneration because the process to repair DNA damage is tightly coupled to transcription through regulating the activity of RNA polymerase II and the subsequent RNA processing, including RNA splicing and transport (Cleaver et al., 2009; Kornblihtt et al., 2004; Munoz et al., 2009). Furthermore, a plethora of evidence support that RNA transcription in the eukaryotic cells is a highly dynamic and tightly regulated process that involves multiple intricately connected steps, including splicing of pre-mRNA and transport of mature mRNA to its final destinations (Moore and Proudfoot, 2009; Reed and Hurt, 2002). In the nervous system, these regulatory mechanisms are known to generate a vast diversity of RNA transcripts that control cell fate determination, axon guidance, dendritic growth and synaptic functions (Li et al., 2007; Martin and Ephrussi, 2009). Perturbations to these critical mechanisms have been implicated in neuromuscular diseases, neurodevelopmental disorders and neurodegenerative diseases (Cooper et al., 2009).

Consistent with these findings, two recent studies show that both human patients with FUS-R521C or FUS-P525L mutation, and FUS-R521C transgenic mice exhibited evidence of DNA damage in cortical neurons and spinal motor neurons (Qiu et al., 2014; Wang et al., 2013). These results indicate that the FUS-R521C transgenic mice provide an invaluable system to identify neural genes implicated in DNA damage during neurodegeneration (Graff et al., 2012; Lu et al., 2004). Indeed, a PCR-based screening approach shows that the 5' non-coding exons of the mouse *Bdnf* gene, which contain transcriptional start sites and are required splicing of long intronic sequences to generate mature *Bdnf* mRNA, consistently exhibit evidence of DNA damage. These results lead to the identification of retentions of 5' splice junctions in the *Bdnf* mRNA and defects in transporting *Bdnf* mRNA to distal dendrites. Using electrophoretic mobility shift assays (EMSA), it is further demonstrated that, compared to wild type FUS, mutant FUS-R521C proteins form more stable protein-RNA complex to 5' splice junction and the 3'UTR sequences of *Bdnf* pre-mRNA (Qiu et al., 2014)(**Figure 4B**). These results support the idea that FALS-associated FUS mutation FUS-R521C exhibits aberrant gain-of-function properties, including forming more stable protein-protein interactions with the endogenous wild type FUS and more stable protein-RNA complex, which most likely alter the ability of FUS to recruit DNA damage repair machinery and the equilibrium of the interactions between FUS and RNA in the splicing machinery, respectively (**Figure 4**)(Qiu et al., 2014; Wang et al., 2013). Similar phenotypes of FUS mutations in DNA damage repair and RNA splicing machinery have been reported in several other studies using biochemical, cell biology and bioinformatics analyses (Hoell et al., 2011; Mastrocola et al., 2013; Zhou et al., 2014).

Given the highly efficient process in RNA splicing, the observations that FUS-R521C can form more stable protein-RNA complex raise the intriguing question as to whether this gain-of-function property may have more widespread intron retention effects on the transcriptomes or only affect a selective subset of target genes. To distinguish these two possibilities, Qiu and colleagues perform genome-wide survey in the transcriptomes of FUS-R521C spinal cord using RNA-seq and show two primary defects involving the transcription and RNA splicing in selective genes that are critical for dendritic growth and synaptic functions (Qiu et al., 2014). For instance, RNA-seq results in the spinal cord of FUS-R521C mutants show perturbations in the expression or splicing of genes involved in the organization of extracellular matrix, including members of the *collagen* and *cadherin* gene families that regulate the specificity of axonal projection and target innervation (Robles and Baier, 2012; Sanes and Yamagata, 2009). Interestingly, similar targets have also been identified in the RNA-seq analyses of FUS MO-treated *Xenopus* morphants (Dichmann and Harland, 2012), suggesting that FUS-R521C phenotype may recapitulate certain transcriptional and RNA splicing defects in FUS loss-of-function mutants. Another intriguing feature of the RNA-seq results in FUS-R521C spinal cord is that many target genes in the extracellular matrix assembly GO categories (GO:0005581, GO:0005201, GO:0005578 and GO:0031012) have also been shown to be transcriptional targets of DNA damage response gene Cockyane syndrome B (CSB) and HDAC1 (Newman et al., 2006), and are frequently misregulated and misspliced in the motor neurons of SALS patients (Rabin et al., 2010). While these results are correlative, they raise the interesting possibility that the recruitment of FUS, HDAC1 and

CSB may constitute a critical step in the repair of damaged DNA in FALS caused by FUS mutations and in SALS.

Finally, one remarkable feature in the spinal cord of FUS-R521C transgenic mice is the up-regulation of genes that are functionally related to immune response, complement activation and chemotaxis (Qiu et al., 2014). Consistent with these findings, the FUS-R521C spinal cord show pronounced microgliosis. Since neither wild type FUS or FUS-R521C proteins can be detected in the microglia, these results support the idea that non-cell autonomous mechanisms, triggered by damaged neurons or reactive astroglia, may activate microglia and contribute to the neurodegeneration in ALS. Interestingly, similar non-cell autonomous mechanisms have been reported in the mutant SOD1 models (Boillee et al., 2006a; Boillee et al., 2006b). Alternatively, the defects in DNA damage repair and RNA splicing caused by mutant FUS-R521C may occur in glia cells, which promotes astroglial activation and/or degeneration of oligodendroglia, further contributing to the dendritic loss and synaptic degeneration of spinal motor neurons in FUS-R521C mice.

5.2. Mechanisms of FUS Mutations in RNA Spicing Machinery

Several studies indicate that FUS can physically interact with SMN and U1 snRNP, and that loss of FUS or expressing FALS-associated FUS mutations disrupts the organization of Gemini of Cajal bodies (Gems), where the presence of TDP-43, FUS and another fatal motor neuron disease gene product SMN are required to regulate the assembly of the Gems in several different cell types (Battle et al., 2006; Sun et al., 2015; Yamazaki et al., 2012). Consistent with these findings, *FUS*$^{-/-}$ hippocampal

neurons show a near complete loss of Gems. Interestingly, the integrity of Gems and splicesome is severely perturbed in the spinal motor neurons of patients with sporadic ALS, which most likely is due to the loss of nuclear TDP-43 and prominent up-regulations of U snRNAs and snRNPs (Tsuiji et al., 2013). In addition to its roles in the organization of splicesome, FUS is implicated in the integrity of paraspeckles, which are subnuclear structures that regulate gene expression by nuclear retention of RNA (Bond and Fox, 2009). The core paraspeckle proteins include DBHS (*Drosophila melanogaster* behavior, human splicing) proteins, PSF/SFPQ, P45NRB/NONO, and PSPC1. In addition, a long noncoding RNA (lncRNA) *NEAT1* is also required to maintain the integrity of paraspeckles. In a recent study, FUS and TDP-43 are shown to interact with *NEAT1* (Nishimoto et al., 2013), raising the possibility that perturbations to both proteins may disrupt the integrity or maintenance of paraspeckles. Consistent with this idea, spinal motor neurons in transgenic mice expressing truncated FUS mutant protein (amino acids 1-359) show cytoplasmic aggregates of P45NRB/NONO. Although confocal images from these transgenic neurons indicate that P45NRB/NONO proteins and mutant FUS proteins are in close proximity, it is unclear whether the presumed complex between P45NRB/NONO and FUS are disrupted by the presence of mutant FUS proteins (Shelkovnikova et al., 2013). It is also unclear if the number and distribution of paraspeckles are disrupted in the spinal motor neurons of these transgenic mice and in patients with FALS or SALS. Another alternative mechanism for mutant FUS proteins to interfere with the integrity of paraspeckles is by altering the expression of lncRNA *NEAT1*. Indeed, RNA-seq analyses of spinal cord from FUS-

R521C transgenic mice show that the *NEAT1* levels are 2-3 folds higher than that in the age-matched controls (Qiu et al., 2014).

6. Future Directions

6.1. Hierarchy of FUS Mutations and Proteinopathy

The discovery of dominant FUS mutations as one of the major causal links for FALS has opened up new windows to understanding the pathogenesis of ALS. Judging from the biophysical properties of FUS, it is tempting to propose that the FALS-associated FUS mutations alter the liquid-solid phase transition in FUS and thereby dominantly interfere with the ability of wild type FUS in DNA damage repair and RNA splicing, leading to both structural and functional defects in dendritic/axonal growth and synaptic transmission. The fundamental effects of mutant FUS proteins can be attributed to its aberrant gain-of-function properties that alter the homeostasis of the interactions of wild type FUS and its the interacting partners, including proteins, pre-mRNAs and lncRNAs, in the DNA damage response/repair and RNA splicing machinery. In many aspects, these gain-of-function properties truly reflect broad and essential roles of wild type FUS in the embryonic and postnatal development, and in the maintenance of the organisms and the nervous system.

Notwithstanding these new insights into FUS mutations, it remains a challenge to determine how these new mechanisms help in understanding the pathogenesis of FALS and SALS. With the availability of transgenic models for FUS and TDP-43, we may begin to determine whether mutations in FUS or TDP-43 target similar or divergent mechanisms that eventually lead to neurodegeneration. This is especially important

given that TDP-43 proteinopathy is a major neuropathological feature not only in FALS, but also in SALS. Several directions for future research include (1) whether FUS and TDP-43 proteins use similar or different intracellular trafficking mechanisms for their transport in and out of the neuronal nucleus, (2) how FUS and TDP-43 proteinopathies promote degeneration in spinal motor neurons and other types of neurons, (3) how FUS and TDP-43 mutations might cause non-cell autonomous mechanisms to promote glial pathology, and (4) whether patient-specific induced pluripotent stem cell (iPSC)-derived neurons can provide a new model system more closely related to human disease. Indeed, the combined strengths of using iPSC-derived neurons and model organism *Drosophila* have led to the identification of novel functions of TDP-43 in mRNA transport in axons (Alami et al., 2014). Future studies using similar approaches can also provide more insights to unravel the mechanisms and their hierarchical interactions in the pathogenesis of human diseases. The iPSC approach will also provide important tools to identify potential therapeutic targets that are specific for different mutations in FUS-mediated FALS.

6.2. The Expanding Repertoire of RNA Machinery in Neurodegeneration

The identification of mutations in *FUS*, *TARDBP* and *C9ORF72* in ALS and FTD-ALS has expanded the landscape of neurodegenerative diseases caused by defects in RNA metabolism machinery. These studies raise the intriguing questions that *FUS*, *TARDBP* and *C9ORF72* may be just tips of an iceberg, and that dysregulations in RNA machinery may have broader roles in other neurodegenerative diseases? Indeed, results from several recent studies indicate that the answers to both questions are

positive. For instance, mutations in the prion-like domains of hnRNPA2B1 and hnRNAPA1 accelerate filamentous protein aggregate formation, increase the propensity of stress granule formation in neuronal cytoplasm and can contribute to the pathology in patients with multisystem proteinopathy and ALS (Kim et al., 2013; Molliex et al., 2015). Furthermore, chemical mutagenesis screens for recessive mutations (Neuroscience Mutagenesis Facility) have reported two mutations, *nmf291* and *nmf205*, that result in neurological phenotypes. The *nmf291* allele is caused by a 5-nucleotide deletion in the U2 snRNA, which leads to profound dysregulation in RNA splicing and an age-dependent, progressive degeneration of the cerebellum (An et al., 2008). In contrast, the *nmf205* mutation results in loss of GTPBP2 due to a point mutation in the consensus splice donor site of intron 6 of *Gtpbp2*, leading to missplicing of *Gtpbp2* mRNA and a premature stop codon. Since GTPBP2 is an essential binding partner for the ribosome recycling protein pelota, loss of GTPBP2 results in widespread ribosomal stalling and profound age-dependent neurodegeneration in the cerebellum of *nmf205* mutants (Ishimura et al., 2014). Finally, two recent studies show that mutations in the human *cleavage and polyadenylation factor subunit 1* (*CLP1*) gene, which encodes a multifunctional kinase implicated in the maturation of tRNA, mRNA and siRNA, cause severe neurodegeneration in the cerebellum (Karaca et al., 2014; Schaffer et al., 2014). Consistent with these findings, mouse mutants that express kinase-dead mutant CLP1 show neurodegeneration, characterized by a progressive loss of spinal motor neurons, axonal degeneration in the peripheral nerves, and denervation of neuromuscular junctions, leading to impaired motor function, muscle weakness, paralysis and fatal respiratory failure (Hanada et al., 2013). Together, these findings further expand and

reinforce the critical role of RNA metabolism in maintaining the normal functions of the nervous system.

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References

Alami, N.H., Smith, R.B., Carrasco, M.A., Williams, L.A., Winborn, C.S., Han, S.S., 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.

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.

An, J.J., Gharami, K., Liao, G.Y., Woo, N.H., Lau, A.G., Vanevski, F., Torre, E.R., Jones, K.R., Feng, Y., Lu, B.*, et al.* (2008). Distinct role of long 3' UTR BDNF mRNA in spine morphology and synaptic plasticity in hippocampal neurons. Cell *134*, 175-187.

Anderson, A.J., Su, J.H., and Cotman, C.W. (1996). DNA damage and apoptosis in Alzheimer's disease: colocalization with c-Jun immunoreactivity, relationship to brain area, and effect of postmortem delay. J Neurosci *16*, 1710-1719.

Arai, T., Hasegawa, M., Akiyama, H., Ikeda, K., Nonaka, T., Mori, H., Mann, D., Tsuchiya, K., Yoshida, M., Hashizume, Y.*, et al.* (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.

Armstrong, G.A., and Drapeau, P. (2013). Loss and gain of FUS function impair neuromuscular synaptic transmission in a genetic model of ALS. Hum Mol Genet *22*, 4282-4292.

Baechtold, H., Kuroda, M., Sok, J., Ron, D., Lopez, B.S., and Akhmedov, A.T. (1999). Human 75-kDa DNA-pairing protein is identical to the pro-oncoprotein TLS/FUS and is able to promote D-loop formation. J Biol Chem *274*, 34337-34342.

Battle, D.J., Kasim, M., Yong, J., Lotti, F., Lau, C.K., Mouaikel, J., Zhang, Z., Han, K., Wan, L., and Dreyfuss, G. (2006). The SMN complex: an assembly machine for RNPs. Cold Spring Harb Symp Quant Biol *71*, 313-320.

Baumer, D., Hilton, D., Paine, S.M., Turner, M.R., Lowe, J., Talbot, K., and Ansorge, O. (2010). Juvenile ALS with basophilic inclusions is a FUS proteinopathy with FUS mutations. Neurology *75*, 611-618.

Behzadnia, N., Golas, M.M., Hartmuth, K., Sander, B., Kastner, B., Deckert, J., Dube, P., Will, C.L., Urlaub, H., Stark, H.*, et al.* (2007). Composition and three-dimensional EM structure of double affinity-purified, human prespliceosomal A complexes. Embo J *26*, 1737-1748.

Bender, A., Krishnan, K.J., Morris, C.M., Taylor, G.A., Reeve, A.K., Perry, R.H., Jaros, E., Hersheson, J.S., Betts, J., Klopstock, T.*, et al.* (2006). High levels of mitochondrial DNA deletions in substantia nigra neurons in aging and Parkinson disease. Nat Genet *38*, 515-517.

Bertrand, P., Akhmedov, A.T., Delacote, F., Durrbach, A., and Lopez, B.S. (1999). Human POMp75 is identified as the pro-oncoprotein TLS/FUS: both POMp75 and POMp100 DNA homologous pairing activities are associated to cell proliferation. Oncogene *18*, 4515-4521.

Betley, J.N., Wright, C.V., Kawaguchi, Y., Erdelyi, F., Szabo, G., Jessell, T.M., and Kaltschmidt, J.A. (2009). Stringent specificity in the construction of a GABAergic presynaptic inhibitory circuit. Cell *139*, 161-174.

Bigio, E.H. (2012). Motor neuron disease: the C9orf72 hexanucleotide repeat expansion in FTD and ALS. Nature reviews Neurology *8*, 249-250.

Boillee, S., Vande Velde, C., and Cleveland, D.W. (2006a). ALS: a disease of motor neurons and their nonneuronal neighbors. Neuron *52*, 39-59.

Boillee, S., Yamanaka, K., Lobsiger, C.S., Copeland, N.G., Jenkins, N.A., Kassiotis, G., Kollias, G., and Cleveland, D.W. (2006b). Onset and progression in inherited ALS determined by motor neurons and microglia. Science *312*, 1389-1392.

Bond, C.S., and Fox, A.H. (2009). Paraspeckles: nuclear bodies built on long noncoding RNA. J Cell Biol *186*, 637-644.

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

Calvio, C., Neubauer, G., Mann, M., and Lamond, A.I. (1995). Identification of hnRNP P2 as TLS/FUS using electrospray mass spectrometry. Rna *1*, 724-733.

Cirulli, E.T., Lasseigne, B.N., Petrovski, S., Sapp, P.C., Dion, P.A., Leblond, C.S., Couthouis, J., Lu, Y.F., Wang, Q., Krueger, B.J.*, et al.* (2015). Exome sequencing in amyotrophic lateral sclerosis identifies risk genes and pathways. Science *347*, 1436-1441.

Cleaver, J.E., Bezrookove, V., Revet, I., and Huang, E.J. (2013). Conceptual developments in the causes of Cockayne syndrome. Mech Ageing Dev *134*, 284-290.

Cleaver, J.E., Lam, E.T., and Revet, I. (2009). Disorders of nucleotide excision repair: the genetic and molecular basis of heterogeneity. Nat Rev Genet *10*, 756-768.

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.

Cooper, T.A., Wan, L., and Dreyfuss, G. (2009). RNA and disease. Cell *136*, 777-793.

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.

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 U S A *108*, 20881-20890.

Crozat, A., Aman, P., Mandahl, N., and Ron, D. (1993). Fusion of CHOP to a novel RNA-binding protein in human myxoid liposarcoma. Nature *363*, 640-644.

Cushman, M., Johnson, B.S., King, O.D., Gitler, A.D., and Shorter, J. (2010). Prion-like disorders: blurring the divide between transmissibility and infectivity. J Cell Sci *123*, 1191-1201.

Das, R., Yu, J., Zhang, Z., Gygi, M.P., Krainer, A.R., Gygi, S.P., and Reed, R. (2007). SR proteins function in coupling RNAP II transcription to pre-mRNA splicing. Mol Cell *26*, 867-881.

de Waard, M.C., van der Pluijm, I., Zuiderveen Borgesius, N., Comley, L.H., Haasdijk, E.D., Rijksen, Y., Ridwan, Y., Zondag, G., Hoeijmakers, J.H., Elgersma, Y.*, et al.* (2010). Age-related motor neuron degeneration in DNA repair-deficient Ercc1 mice. Acta Neuropathol *120*, 461-475.

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.

Deng, H., Gao, K., and Jankovic, J. (2014a). The role of FUS gene variants in neurodegenerative diseases. Nature reviews Neurology *10*, 337-348.

Deng, Q., Holler, C.J., Taylor, G., Hudson, K.F., Watkins, W., Gearing, M., Ito, D., Murray, M.E., Dickson, D.W., Seyfried, N.T.*, et al.* (2014b). FUS is phosphorylated by DNA-PK and accumulates in the cytoplasm after DNA damage. J Neurosci *34*, 7802-7813.

Dichmann, D.S., and Harland, R.M. (2012). fus/TLS orchestrates splicing of developmental regulators during gastrulation. Genes Dev *26*, 1351-1363.

Dinant, C., Houtsmuller, A.B., and Vermeulen, W. (2008). Chromatin structure and DNA damage repair. Epigenetics & chromatin *1*, 9.

Dini Modigliani, S., Morlando, M., Errichelli, L., Sabatelli, M., and Bozzoni, I. (2014). An ALS-associated mutation in the FUS 3'-UTR disrupts a microRNA-FUS regulatory circuitry. Nature communications *5*, 4335.

Dobbin, M.M., Madabhushi, R., Pan, L., Chen, Y., Kim, D., Gao, J., Ahanonu, B., Pao, P.C., Qiu, Y., Zhao, Y.*, et al.* (2013). SIRT1 collaborates with ATM and HDAC1 to maintain genomic stability in neurons. Nat Neurosci *16*, 1008-1015.

Dormann, D., Madl, T., Valori, C.F., Bentmann, E., Tahirovic, S., Abou-Ajram, C., Kremmer, E., Ansorge, O., Mackenzie, I.R., Neumann, M.*, et al.* (2012). Arginine methylation next to the PY-NLS modulates Transportin binding and nuclear import of FUS. Embo J *31*, 4258-4275.

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.

Ferrante, R.J., Browne, S.E., Shinobu, L.A., Bowling, A.C., Baik, M.J., MacGarvey, U., Kowall, N.W., Brown, R.H., Jr., and Beal, M.F. (1997). Evidence of increased oxidative damage in both sporadic and familial amyotrophic lateral sclerosis. J Neurochem *69*, 2064-2074.

Fillingham, J., Keogh, M.C., and Krogan, N.J. (2006). GammaH2AX and its role in DNA double-strand break repair. Biochemistry and cell biology = Biochimie et biologie cellulaire *84*, 568-577.

Fushimi, K., Long, C., Jayaram, N., Chen, X., Li, L., and Wu, J.Y. (2011). Expression of human FUS/TLS in yeast leads to protein aggregation and cytotoxicity, recapitulating key features of FUS proteinopathy. Protein & cell *2*, 141-149.

Gardiner, M., Toth, R., Vandermoere, F., Morrice, N.A., and Rouse, J. (2008). Identification and characterization of FUS/TLS as a new target of ATM. The Biochemical journal *415*, 297-307.

Gitler, A.D., and Shorter, J. (2011). RNA-binding proteins with prion-like domains in ALS and FTLD-U. Prion *5*, 179-187.

Graff, J., Rei, D., Guan, J.S., Wang, W.Y., Seo, J., Hennig, K.M., Nieland, T.J., Fass, D.M., Kao, P.F., Kahn, M.*, et al.* (2012). An epigenetic blockade of cognitive functions in the neurodegenerating brain. Nature *483*, 222-226.

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.

Han, T.W., Kato, M., Xie, S., Wu, L.C., Mirzaei, H., Pei, J., Chen, M., Xie, Y., Allen, J., Xiao, G.*, et al.* (2012). Cell-free formation of RNA granules: bound RNAs identify features and components of cellular assemblies. Cell *149*, 768-779.

Hanada, T., Weitzer, S., Mair, B., Bernreuther, C., Wainger, B.J., Ichida, J., Hanada, R., Orthofer, M., Cronin, S.J., Komnenovic, V.*, et al.* (2013). CLP1 links tRNA metabolism to progressive motor-neuron loss. Nature *495*, 474-480.

Hicks, G.G., Singh, N., Nashabi, A., Mai, S., Bozek, G., Klewes, L., Arapovic, D., White, E.K., Koury, M.J., Oltz, E.M.*, et al.* (2000). Fus deficiency in mice results in defective B-lymphocyte development and activation, high levels of chromosomal instability and perinatal death. Nat Genet *24*, 175-179.

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.

Huang, C., Tong, J., Bi, F., Wu, Q., Huang, B., Zhou, H., and Xia, X.G. (2012). Entorhinal cortical neurons are the primary targets of FUS mislocalization and ubiquitin aggregation in FUS transgenic rats. Hum Mol Genet *21*, 4602-4614.

Huang, C., Zhou, H., Tong, J., Chen, H., Liu, Y.J., Wang, D., Wei, X., and Xia, X.G. (2011). FUS transgenic rats develop the phenotypes of amyotrophic lateral sclerosis and frontotemporal lobar degeneration. PLoS Genet *7*, e1002011.

Huang, E.J., Zhang, J., Geser, F., Trojanowski, J.Q., Strober, J.B., Dickson, D.W., Brown, R.H., Jr., Shapiro, B.E., and Lomen-Hoerth, C. (2010). Extensive FUS-immunoreactive pathology in juvenile amyotrophic lateral sclerosis with basophilic inclusions. Brain Pathol *20*, 1069-1076.

Ichikawa, H., Shimizu, K., Hayashi, Y., and Ohki, M. (1994). An RNA-binding protein gene, TLS/FUS, is fused to ERG in human myeloid leukemia with t(16;21) chromosomal translocation. Cancer Res *54*, 2865-2868.

Iko, Y., Kodama, T.S., Kasai, N., Oyama, T., Morita, E.H., Muto, T., Okumura, M., Fujii, R., Takumi, T., Tate, S.*, et al.* (2004). Domain architectures and characterization of an RNA-binding protein, TLS. J Biol Chem *279*, 44834-44840.

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.

Ishimura, R., Nagy, G., Dotu, I., Zhou, H., Yang, X.L., Schimmel, P., Senju, S., Nishimura, Y., Chuang, J.H., and Ackerman, S.L. (2014). RNA function. Ribosome

stalling induced by mutation of a CNS-specific tRNA causes neurodegeneration. Science *345*, 455-459.

Jackson, S.P., and Bartek, J. (2009). The DNA-damage response in human biology and disease. Nature *461*, 1071-1078.

Kabashi, E., Bercier, V., Lissouba, A., Liao, M., Brustein, E., Rouleau, G.A., and Drapeau, P. (2011). FUS and TARDBP but not SOD1 interact in genetic models of amyotrophic lateral sclerosis. PLoS Genet *7*, e1002214.

Karaca, E., Weitzer, S., Pehlivan, D., Shiraishi, H., Gogakos, T., Hanada, T., Jhangiani, S.N., Wiszniewski, W., Withers, M., Campbell, I.M.*, et al.* (2014). Human CLP1 mutations alter tRNA biogenesis, affecting both peripheral and central nervous system function. Cell *157*, 636-650.

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.

Kim, D., Frank, C.L., Dobbin, M.M., Tsunemoto, R.K., Tu, W., Peng, P.L., Guan, J.S., Lee, B.H., Moy, L.Y., Giusti, P.*, et al.* (2008). Deregulation of HDAC1 by p25/Cdk5 in neurotoxicity. Neuron *60*, 803-817.

Kim, H.J., Kim, N.C., Wang, Y.D., Scarborough, E.A., Moore, J., Diaz, Z., MacLea, K.S., Freibaum, B., Li, S., Molliex, A.*, et al.* (2013). Mutations in prion-like domains in hnRNPA2B1 and hnRNPA1 cause multisystem proteinopathy and ALS. Nature *495*, 467-473.

Kornblihtt, A.R., de la Mata, M., Fededa, J.P., Munoz, M.J., and Nogues, G. (2004). Multiple links between transcription and splicing. Rna *10*, 1489-1498.

Kuroda, M., Sok, J., Webb, L., Baechtold, H., Urano, F., Yin, Y., Chung, P., de Rooij, D.G., Akhmedov, A., Ashley, T.*, et al.* (2000). Male sterility and enhanced radiation sensitivity in TLS(-/-) mice. Embo J *19*, 453-462.

Kwiatkowski, T.J., Jr., Bosco, D.A., Leclerc, A.L., Tamrazian, E., Vanderburg, 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., Kato, M., Xiang, S., Wu, L., Theodoropoulos, P., Mirzaei, H., Han, T., Xie, S., Corden, J.L., and McKnight, S.L. (2013). Phosphorylation-regulated binding of RNA polymerase II to fibrous polymers of low-complexity domains. Cell *155*, 1049-1060.

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.

Lanson, N.A., Jr., Maltare, A., King, H., Smith, R., Kim, J.H., Taylor, J.P., Lloyd, T.E., and Pandey, U.B. (2011). A Drosophila model of FUS-related neurodegeneration reveals genetic interaction between FUS and TDP-43. Hum Mol Genet *20*, 2510-2523.

Lattante, S., Rouleau, G.A., and Kabashi, E. (2013). TARDBP and FUS mutations associated with amyotrophic lateral sclerosis: summary and update. Hum Mutat *34*, 812-826.

Lee, E.B., Lee, V.M., and Trojanowski, J.Q. (2012). Gains or losses: molecular mechanisms of TDP43-mediated neurodegeneration. Nat Rev Neurosci *13*, 38-50.

Lerga, A., Hallier, M., Delva, L., Orvain, C., Gallais, I., Marie, J., and Moreau-Gachelin, F. (2001). Identification of an RNA binding specificity for the potential splicing factor TLS. J Biol Chem *276*, 6807-6816.

Li, Q., Lee, J.A., and Black, D.L. (2007). Neuronal regulation of alternative pre-mRNA splicing. Nat Rev Neurosci *8*, 819-831.

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.

Ling, S.C., Polymenidou, M., and Cleveland, D.W. (2013). Converging mechanisms in ALS and FTD: disrupted RNA and protein homeostasis. Neuron *79*, 416-438.

Lu, T., Pan, Y., Kao, S.Y., Li, C., Kohane, I., Chan, J., and Yankner, B.A. (2004). Gene regulation and DNA damage in the ageing human brain. Nature *429*, 883-891.

Lukas, J., Lukas, C., and Bartek, J. (2011). More than just a focus: The chromatin response to DNA damage and its role in genome integrity maintenance. Nature cell biology *13*, 1161-1169.

Mackenzie, I.R., Frick, P., and Neumann, M. (2014). The neuropathology associated with repeat expansions in the C9ORF72 gene. Acta Neuropathol *127*, 347-357.

Martin, K.C., and Ephrussi, A. (2009). mRNA localization: gene expression in the spatial dimension. Cell *136*, 719-730.

Mastrocola, A.S., Kim, S.H., Trinh, A.T., Rodenkirch, L.A., and Tibbetts, R.S. (2013). The RNA-binding protein fused in sarcoma (FUS) functions downstream of poly(ADP-ribose) polymerase (PARP) in response to DNA damage. J Biol Chem *288*, 24731-24741.

McKinnon, P.J. (2009). DNA repair deficiency and neurological disease. Nat Rev Neurosci *10*, 100-112.

Millecamps, S., Boillee, S., Le Ber, I., Seilhean, D., Teyssou, E., Giraudeau, M., Moigneu, C., Vandenberghe, N., Danel-Brunaud, V., Corcia, P.*, et al.* (2012).

Phenotype difference between ALS patients with expanded repeats in C9ORF72 and patients with mutations in other ALS-related genes. J Med Genet *49*, 258-263.

Miller, K.M., Tjeertes, J.V., Coates, J., Legube, G., Polo, S.E., Britton, S., and Jackson, S.P. (2010). Human HDAC1 and HDAC2 function in the DNA-damage response to promote DNA nonhomologous end-joining. Nat Struct Mol Biol *17*, 1144-1151.

Mitchell, J.C., McGoldrick, P., Vance, C., Hortobagyi, T., Sreedharan, J., Rogelj, B., Tudor, E.L., Smith, B.N., Klasen, C., Miller, C.C.*, et al.* (2013). Overexpression of human wild-type FUS causes progressive motor neuron degeneration in an age- and dose-dependent fashion. Acta neuropathologica *125*, 273-288.

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.

Moore, M.J., and Proudfoot, N.J. (2009). Pre-mRNA processing reaches back to transcription and ahead to translation. Cell *136*, 688-700.

Munoz, M.J., Perez Santangelo, M.S., Paronetto, M.P., de la Mata, M., Pelisch, F., Boireau, S., Glover-Cutter, K., Ben-Dov, C., Blaustein, M., Lozano, J.J.*, et al.* (2009). DNA damage regulates alternative splicing through inhibition of RNA polymerase II elongation. Cell *137*, 708-720.

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.

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.

Newman, J.C., Bailey, A.D., and Weiner, A.M. (2006). Cockayne syndrome group B protein (CSB) plays a general role in chromatin maintenance and remodeling. Proc Natl Acad Sci U S A *103*, 9613-9618.

Nishimoto, Y., Nakagawa, S., Hirose, T., Okano, H.J., Takao, M., Shibata, S., Suyama, S., Kuwako, K., Imai, T., Murayama, S.*, et al.* (2013). The long non-coding RNA nuclear-enriched abundant transcript 1_2 induces paraspeckle formation in the motor neuron during the early phase of amyotrophic lateral sclerosis. Molecular brain *6*, 31.

Nomura, T., Watanabe, S., Kaneko, K., Yamanaka, K., Nukina, N., and Furukawa, Y. (2014). Intranuclear aggregation of mutant FUS/TLS as a molecular pathomechanism of amyotrophic lateral sclerosis. J Biol Chem *289*, 1192-1202.

Ou, S.H., Wu, F., Harrich, D., Garcia-Martinez, L.F., and Gaynor, R.B. (1995). Cloning and characterization of a novel cellular protein, TDP-43, that binds to human immunodeficiency virus type 1 TAR DNA sequence motifs. Journal of virology *69*, 3584-3596.

Patel, A., Lee, H.O., Jawerth, L., Maharana, S., Jahnel, M., Hein, M.Y., Stoynov, 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.

Pilch, D.R., Sedelnikova, O.A., Redon, C., Celeste, A., Nussenzweig, A., and Bonner, W.M. (2003). Characteristics of gamma-H2AX foci at DNA double-strand breaks sites. Biochemistry and cell biology = Biochimie et biologie cellulaire *81*, 123-129.

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.

Prasad, D.D., Ouchida, M., Lee, L., Rao, V.N., and Reddy, E.S. (1994). TLS/FUS fusion domain of TLS/FUS-erg chimeric protein resulting from the t(16;21) chromosomal translocation in human myeloid leukemia functions as a transcriptional activation domain. Oncogene *9*, 3717-3729.

Qiu, H., Lee, S., Shang, Y., Wang, W.Y., Au, K.F., Kamiya, S., Barmada, S., Finkbeiner, S., Lui, H., Carlton, C.E.*, et al.* (2014). ALS-associated mutation FUS-R521C causes DNA damage and RNA splicing defects. J Clin Invest *124*.

Rabbitts, T.H., Forster, A., Larson, R., and Nathan, P. (1993). Fusion of the dominant negative transcription regulator CHOP with a novel gene FUS by translocation t(12;16) in malignant liposarcoma. Nat Genet *4*, 175-180.

Rabin, S.J., Kim, J.M., Baughn, M., Libby, R.T., Kim, Y.J., Fan, Y., La Spada, A., Stone, B., and Ravits, J. (2010). Sporadic ALS has compartment-specific aberrant exon splicing and altered cell-matrix adhesion biology. Hum Mol Genet *19*, 313-328.

Rass, U., Ahel, I., and West, S.C. (2007). Defective DNA repair and neurodegenerative disease. Cell *130*, 991-1004.

Reed, R., and Hurt, E. (2002). A conserved mRNA export machinery coupled to pre-mRNA splicing. Cell *108*, 523-531.

Renton, A.E., Majounie, E., Waite, A., Simon-Sanchez, J., Rollinson, S., Gibbs, J.R., Schymick, J.C., Laaksovirta, H., van Swieten, J.C., Myllykangas, L.*, et al.* (2011). A hexanucleotide repeat expansion in C9ORF72 is the cause of chromosome 9p21-linked ALS-FTD. Neuron *72*, 257-268.

Robles, E., and Baier, H. (2012). Assembly of synaptic laminae by axon guidance molecules. Curr Opin Neurobiol.

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.

Rulten, S.L., Rotheray, A., Green, R.L., Grundy, G.J., Moore, D.A., Gomez-Herreros, F., Hafezparast, M., and Caldecott, K.W. (2014). PARP-1 dependent recruitment of the amyotrophic lateral sclerosis-associated protein FUS/TLS to sites of oxidative DNA damage. Nucleic Acids Res *42*, 307-314.

Sabatelli, M., Moncada, A., Conte, A., Lattante, S., Marangi, G., Luigetti, M., Lucchini, M., Mirabella, M., Romano, A., Del Grande, A.*, et al.* (2013). Mutations in the 3' untranslated region of FUS causing FUS overexpression are associated with amyotrophic lateral sclerosis. Hum Mol Genet *22*, 4748-4755.

Sanes, J.R., and Yamagata, M. (2009). Many paths to synaptic specificity. Annu Rev Cell Dev Biol *25*, 161-195.

Schaffer, A.E., Eggens, V.R., Caglayan, A.O., Reuter, M.S., Scott, E., Coufal, N.G., Silhavy, J.L., Xue, Y., Kayserili, H., Yasuno, K.*, et al.* (2014). CLP1 founder mutation links tRNA splicing and maturation to cerebellar development and neurodegeneration. Cell *157*, 651-663.

Schwartz, J.C., Ebmeier, C.C., Podell, E.R., Heimiller, J., Taatjes, D.J., and Cech, T.R. (2012). FUS binds the CTD of RNA polymerase II and regulates its phosphorylation at Ser2. Genes Dev *26*, 2690-2695.

Schwartz, J.C., Podell, E.R., Han, S.S., Berry, J.D., Eggan, K.C., and Cech, T.R. (2014). FUS is sequestered in nuclear aggregates in ALS patient fibroblasts. Mol Biol Cell *25*, 2571-2578.

Schwartz, J.C., Wang, X., Podell, E.R., and Cech, T.R. (2013). RNA seeds higher-order assembly of FUS protein. Cell reports *5*, 918-925.

Sephton, C.F., Tang, A.A., Kulkarni, A., West, J., Brooks, M., Stubblefield, J.J., Liu, Y., Zhang, M.Q., Green, C.B., Huber, K.M.*, et al.* (2014). Activity-dependent FUS dysregulation disrupts synaptic homeostasis. Proc Natl Acad Sci U S A *111*, E4769-4778.

Shahidullah, M., Le Marchand, S.J., Fei, H., Zhang, J., Pandey, U.B., Dalva, M.B., Pasinelli, P., and Levitan, I.B. (2013). Defects in synapse structure and function precede motor neuron degeneration in Drosophila models of FUS-related ALS. J Neurosci *33*, 19590-19598.

Sharma, A., Lyashchenko, A.K., Lu, L., Nasrabady, S.E., Elmaleh, M., Mendelsohn, M., Nemes, A., Tapia, J.C., Mentis, G.Z., and Shneider, N.A. (2016). ALS-associated

mutant FUS induces selective motor neuron degeneration through toxic gain of function. Nature communications *7*, 10465.

Shelkovnikova, T.A., Peters, O.M., Deykin, A.V., Connor-Robson, N., Robinson, H., Ustyugov, A.A., Bachurin, S.O., Ermolkevich, T.G., Goldman, I.L., Sadchikova, E.R.*, et al.* (2013). Fused in sarcoma (FUS) protein lacking nuclear localization signal (NLS) and major RNA binding motifs triggers proteinopathy and severe motor phenotype in transgenic mice. J Biol Chem *288*, 25266-25274.

Stolow, D.T., and Haynes, S.R. (1995). Cabeza, a Drosophila gene encoding a novel RNA binding protein, shares homology with EWS and TLS, two genes involved in human sarcoma formation. Nucleic Acids Res *23*, 835-843.

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. Nature communications *6*, 6171.

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.

Takahama, K., Takada, A., Tada, S., Shimizu, M., Sayama, K., Kurokawa, R., and Oyoshi, T. (2013). Regulation of telomere length by G-quadruplex telomere DNA- and TERRA-binding protein TLS/FUS. Chem Biol *20*, 341-350.

Tan, A.Y., and Manley, J.L. (2009). The TET family of proteins: functions and roles in disease. Journal of molecular cell biology *1*, 82-92.

Testa, D., Lovati, R., Ferrarini, M., Salmoiraghi, F., and Filippini, G. (2004). Survival of 793 patients with amyotrophic lateral sclerosis diagnosed over a 28-year period. Amyotrophic lateral sclerosis and other motor neuron disorders : official publication of the World Federation of Neurology, Research Group on Motor Neuron Diseases *5*, 208-212.

Tollervey, J.R., Curk, T., Rogelj, B., Briese, M., Cereda, M., Kayikci, M., Konig, J., Hortobagyi, 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.

Tsuiji, H., Iguchi, Y., Furuya, A., Kataoka, A., Hatsuta, H., Atsuta, N., Tanaka, F., Hashizume, Y., Akatsu, H., Murayama, S.*, et al.* (2013). Spliceosome integrity is defective in the motor neuron diseases ALS and SMA. EMBO molecular medicine *5*, 221-234.

Vance, C., Rogelj, B., Hortobagyi, 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.

Verbeeck, C., Deng, Q., Dejesus-Hernandez, M., Taylor, G., Ceballos-Diaz, C., Kocerha, J., Golde, T., Das, P., Rademakers, R., Dickson, D.W.*, et al.* (2012). Expression of Fused in sarcoma mutations in mice recapitulates the neuropathology of FUS proteinopathies and provides insight into disease pathogenesis. Mol Neurodegener *7*, 53.

Wahl, M.C., Will, C.L., and Luhrmann, R. (2009). The spliceosome: design principles of a dynamic RNP machine. Cell *136*, 701-718.

Wang, J.W., Brent, J.R., Tomlinson, A., Shneider, N.A., and McCabe, B.D. (2011). The ALS-associated proteins FUS and TDP-43 function together to affect Drosophila locomotion and life span. J Clin Invest *121*, 4118-4126.

Wang, W.Y., Pan, L., Su, S.C., Quinn, E.J., Sasaki, M., Jimenez, J.C., Mackenzie, I.R., Huang, E.J., and Tsai, L.H. (2013). Interaction of FUS and HDAC1 regulates DNA damage response and repair in neurons. Nat Neurosci *16*, 1383-1391.

Wang, X., Arai, S., Song, X., Reichart, D., Du, K., Pascual, G., Tempst, P., Rosenfeld, M.G., Glass, C.K., and Kurokawa, R. (2008). Induced ncRNAs allosterically modify RNA-binding proteins in cis to inhibit transcription. Nature *454*, 126-130.

Wang, X., Schwartz, J.C., and Cech, T.R. (2015). Nucleic acid-binding specificity of human FUS protein. Nucleic Acids Res *43*, 7535-7543.

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 reports *2*, 799-806.

Yan, J., Deng, H.X., Siddique, N., Fecto, F., Chen, W., Yang, Y., Liu, E., Donkervoort, S., Zheng, J.G., Shi, Y.*, et al.* (2010). Frameshift and novel mutations in FUS in familial amyotrophic lateral sclerosis and ALS/dementia. Neurology *75*, 807-814.

Yang, L., Embree, L.J., Tsai, S., and Hickstein, D.D. (1998). Oncoprotein TLS interacts with serine-arginine proteins involved in RNA splicing. J Biol Chem *273*, 27761-27764.

Zhou, Y., Liu, S., Ozturk, A., and Hicks, G.G. (2014). FUS-regulated RNA metabolism and DNA damage repair: Implications for amyotrophic lateral sclerosis and frontotemporal dementia pathogenesis. Rare diseases *2*, e29515.

Zinszner, H., Albalat, R., and Ron, D. (1994). A novel effector domain from the RNA-binding protein TLS or EWS is required for oncogenic transformation by CHOP. Genes Dev *8*, 2513-2526.

Zinszner, H., Sok, J., Immanuel, D., Yin, Y., and Ron, D. (1997). TLS (FUS) binds RNA in vivo and engages in nucleo-cytoplasmic shuttling. J Cell Sci *110 (Pt 15)*, 1741-1750.

Figure Legends

Figure 1. The Age of Disease Onset in FALS Patients with FUS Mutations. (A) Meta-analyses of 154 FALS patients (either familial ALS inherited mutations or sporadic ALS with *de novo* mutations) show a predilection early disease onset. Compared to all FUS mutations and the most common mutations that occur in amino acid 521 (FUS-R521C), FUS-P525L mutation tends to occur in late teens and early 20's and represents a much more aggressive form of disease. (B) For sporadic ALS (SALS), about 35.9% and 34.9% of patients show disease onset in the range of 41-55 and 56-65 years old. In contrast, more than 60% of ALS patients with FUS mutations show disease onset before 40 years old. *Statistics for SALS have been adapted from the study by Testa and colleagues (Testa et al., 2004).

Figure 2. Schematic Diagrams of Genomic Organization of the Human *FUS* Gene, *FUS* Mutations Identified in ALS, and Functional Domains in FUS Proteins.

The human FUS gene consists of 15 exons, spanning ~14.9 Kb, and is located on chromosome 16p11.2. The *FUS* mRNA transcripts are predicted to contain a 3,433 bp 3'UTR, which has been recently shown to contain 4 disease-related variants. The full length human FUS protein contains 526 amino acids that can be further divided into several functional domains, including the "prion-like" or low complexity (LC) domain that contains the Q/G/S/Y-rich region (amino acids 1-165) and the G-rich region (amino acids 165-267), the Arginine-rich motif (RRM, amino acids 285-371), two Arg-Gly-Gly (RGG)-repeat regions (amino acids 371-422 and 453-501), interrupted by a Cys_2-Cys_2 zinc-finger motif (ZNF)(amino acids 422-453), and a non-conventional nuclear

localization signal (NLS)(amino acids 510-526). The majority of FALS-related mutations are more commonly found in (1) the G-rich region, (2) the 2nd RGG region and (3) the NLS. Additional structural and functional domains in FUS include the prion-like domains and the HDAC1-interacting domains.

Figure 3. Dendritic and Synaptic Phenotypes Caused by FUS Mutations.

Neurolucida tracing shows that the dendritic arbors in control motor neurons, highlighted by Golgi staining techniques, had 6 to 8 intersections per radial distance within 100 μm from the cell body, followed by a gradual reduction in the number of dendritic arbors from 100 to 250 μm. Compared to the control, the number of dendritic arbors in the FUS-R521C motor neurons shows no change within the first 50 μm from the cell body, but a significant reduction is noted from 50 to 250 μm, resulting in reduced cumulative dendritic area. To determine if the dendritic phenotype in FUS-R521C spinal motor neurons affected synaptic connectivity, we use ChAT (green) and FUS immunostaining to characterize the density of synapses surrounding motor neurons (Betley et al., 2009). Our results show that FUS proteins are present primarily in neuronal nuclei, but also show extensive colocalization with ChAT+ boutons and synaptophysin-immunoreactive presynaptic terminals. Remarkably, the density of ChAT+ boutons and SIPT showed significant reductions in the anterior horn of FUS-R521C spinal cord. To further characterize the synaptic defects, we use electron microscopy (EM) to ascertain the morphology and density of synapses within 100 μm radius of the cell body of spinal motor neurons, and show that the cell bodies of control motor neurons are surrounded by synaptic terminals arranged as rosette-like structures

(Betley et al., 2009). In contrast, the size of post-synaptic density and the number of synapse per unit area are reduced in FUS-R521C motor neurons. Similar dendrite and synaptic defects are also noted in the apical and basal dendrites of the pyramidal neurons in layer IV-V of the sensorimotor cortex (Qiu et al., 2014), and neurons in the entorhinal cortex (Huang et al., 2012).

Figure 4. Mechanisms of Wild Type and Mutant FUS in DNA Damage Repair/Response and RNA Splicing.

(A) Wild type FUS is rapidly recruited to DNA damage foci caused by double-stranded breaks, where it interacts with chromatin remodeling factor HDAC1. Although FUS-R521C can still be recruited to DNA damage foci, it fails to interact with HDAC1 and PARP1. Due to the defects in DNA repair/response machinery, neurons in FUS-R521C transgenic mice show increased DNA damage (indicated by blue asterisks and the presence of double-stranded breaks). (B) In addition to its role in DNA damage repair, several lines of evidence indicate that FUS can also regulate pre-mRNA splicing. Results from CLIP-RT-PCR and protein-RNA interactions in EMSA assays show that both wild type FUS and FUS-R521C can interact with selective oligoribonucleotides from *Bdnf* exon-intron boundaries. Whereas the equilibrium of wild type FUS-RNA interactions appears to be more dynamic, FUS-R521C tends to form more stable protein-RNA complexes that are more difficult to dissociate. (Figure adapted from Qiu et al., 2014, with permissions from the *Journal of Clinical Investigation*.)

Table 1. Summary of FUS loss-of-function and ALS-associated FUS mutations in model organisms.

Mutation	Species	Targeting methods	Phenotypes	References

LOF 2000	Mouse	Knockout	- Male sterility	Kuroda et al.,
			-↑ Unpaired & mispaired chromosomal	
axes in spermatocytes				
		Knockout	- Perinatal lethal	Hicks et al.,
2000				
			- Defects in B lymphocytes	
			-↑ Genomic instability	
		Conditional knockout	- No phenotype in adult neurons	Sharma et al.,
2016				
	Drosophila	Deletion in *caz* gene	- Pupation and eclosion defects	Wang et al.,
2011				
			- Adult *caz* mutants show reduced	
survival and locomotor defects				
- TBPH cannot rescue *caz* phenotype				
	Xenopus	Antisense MO	- Severe gastrulation defects	Dichmann &
		knock-down	- ***RNA-seq*:** splicing defects in genes in	Hartland, 2012
FGF, cell adhesion, and other major				
signaling pathways				
LOF/GOF 2011	Zebrafish	Antisense MO	- Axon & behavior defects	Kabashi et al.,
		knock-down	- Rescued by WT FUS, but not	
FUS-R521C or FUS-R521H				
			- Toxic GOF phenotype in FUS-R521H	
		Antisense MO	- Impaired motor activity	Armstrong &
			- Reduced NMJ synaptic transmission	Drapeau, 2013
			- FUS-R521H reduces synaptic fidelity	
GOF 2010	Zebrafish	mRNA injection	- Cytoplasmic mislocalization	Bosco et al.,
		WT FUS, FUS-H517Q	- Stress granule formation	
		-R521G, -R495X		
		or -G515X		
GOF 2011	Rat	Tet-inducible system	- ALS-like phenotype	Huang et al.,
		WT FUS and	- Early postnatal mortality	
		FUS-R521C		
		Camk2a-tTA inducible	- Dendrite defects in neurons in the	Huang et al.,
2012				
		WT FUS and	entorhinal cortex	
		FUS-R521C	- Golgi & mitochondria defects	
			- Protein ubiquitination defects	
GOF al., 2012	*C. elegans*	Pan-neuronal promoter	- Motor defects, shortened lifespan	Murakami et
		FUS-R514G, -R521G	- Neuronal dysfunctions	
		-R522G, R524S, P525L		
GOF 2013	Mouse	Mouse PrP promoter	- ALS-like phenotypes in homozygotes	Mitchell et al.,
		WT human FUS	- FUS+/ubiquitin– cytoplasmic inclusions	

GOF	Mouse	Thy1 promoter	- Cytoplasmic FUS aggregates	Shelkovnikova et al.,
		FUS 1-359	- Motor neuron loss	2013 & 2014
			- Disruption of paraspeckle	
GOF	Mouse	FLAG-FUS-R521C	- ALS-like phenotypes w/ moderate	Qiu et al., 2014
		transgenic expression	loss of spinal motor neurons	
		by Syrian hamster	- Severe dendrite/synapse defects	
		PrP promoter	- Neuroinflammation	
			- ***RNA-seq*****:** transcription & splicing	
			defects in genes for neural development	
GOF	Mouse	FUS-WT or FUS-R521G	- ALS-like phenotypes in FUS-R521G Tg	Sephton et al., 2014
		transgenic expression	- Modest loss of spinal motor neurons	
		by ubiquitous Cre	- Severe dendritic defects	
		promoter	- Neuroinflammation	
			- Dysregulation of mGluR & synaptic proteins	
			- No neurological phenotype in FUS-WT	
Tg mice				
GOF	Mouse	FUS-WT, FUS-R521C and	- Phenotype FUS-P525L > FUS-R521C	Sharma et al., 2016
		FUS-P525L transgenic	- Modest loss of spinal motor neurons	
		expression from *MAPT*	- Loss of NMJ synapse	
		locus	- Astrogliosis and microgliosis	
			- No phenotype in FUS-WT Tg mice	
GOF	*Drosophila*	Global or neural	- Severe eye degeneration	Lanson et al., 2011
		expression of	- Pupal mortality & locomotor defects	Daigle et al., 2013
		human FUS	- Cytoplasmic mislocalization	Shahidullah et al.,
			- Stress granule formation	2013
			- Synapse defects precede neuro-	
degeneration				

*Abbreviations: LOF: loss of function; GOF: gain of function; MO: morpholino; PrP: prion.

HIGHLIGHTS

- RNA/DNA binding protein FUS (fused in sarcoma) regulates RNA homeostasis and DNA damage repair
- Prion-like or low complexity domain in FUS regulates liquid to solid phase transition.
- FALS-related mutations in FUS cause dendritic and synaptic defects in model organisms

- FUS mutations affect RNA splicing and DNA damage response/repair via a dominant negative mechanism.

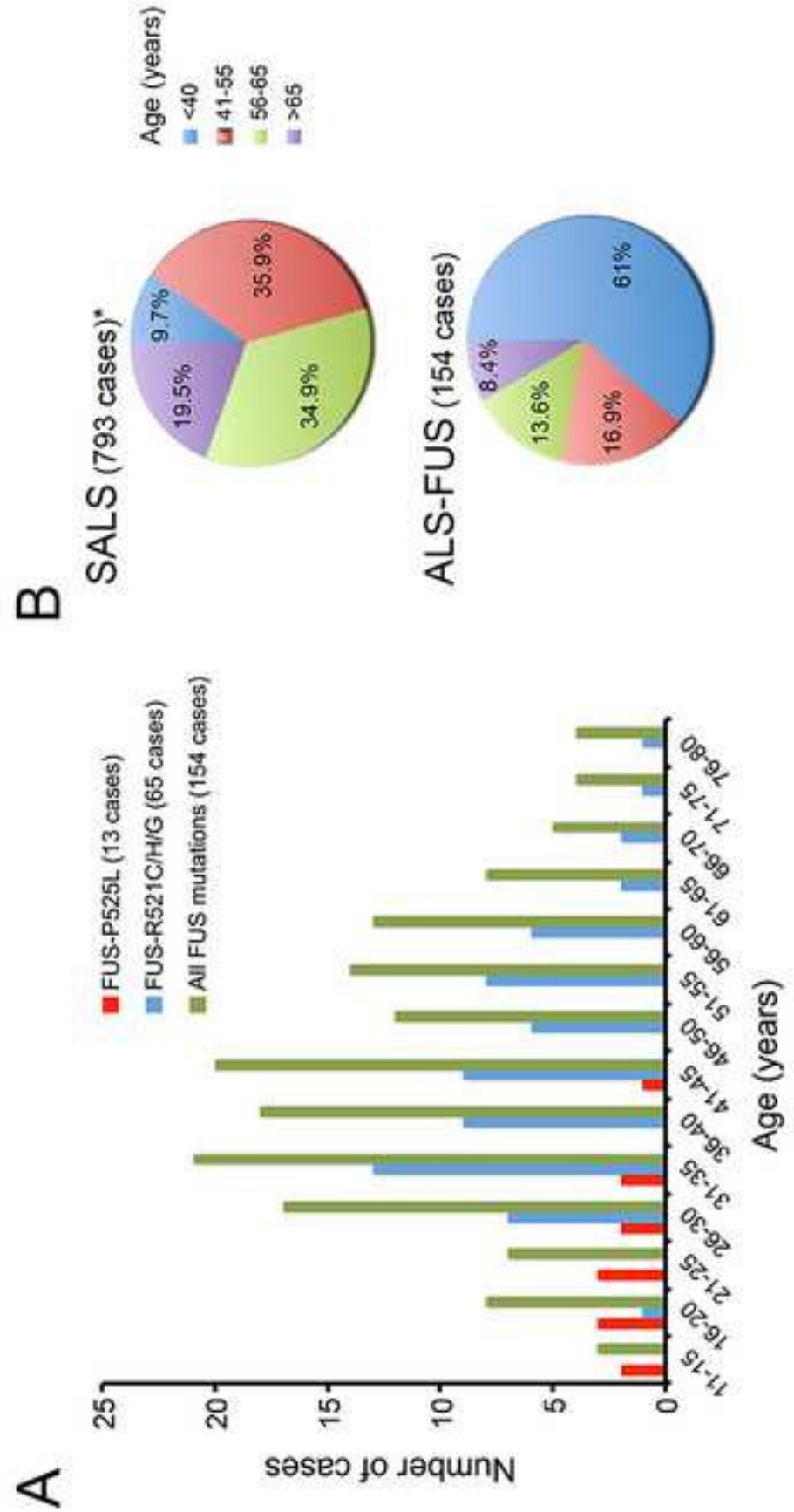

Figure 1

Figure 2

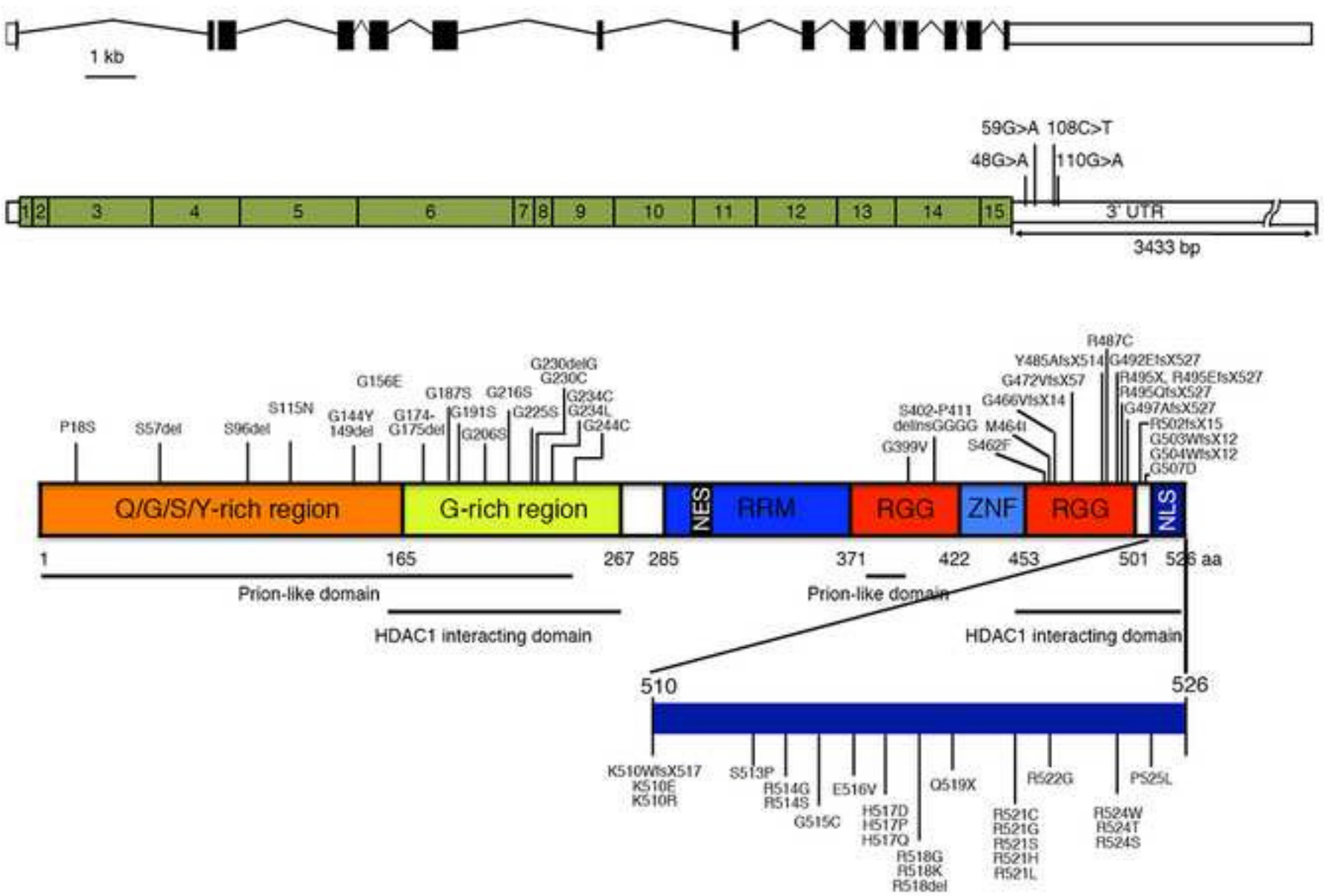

Figure 3

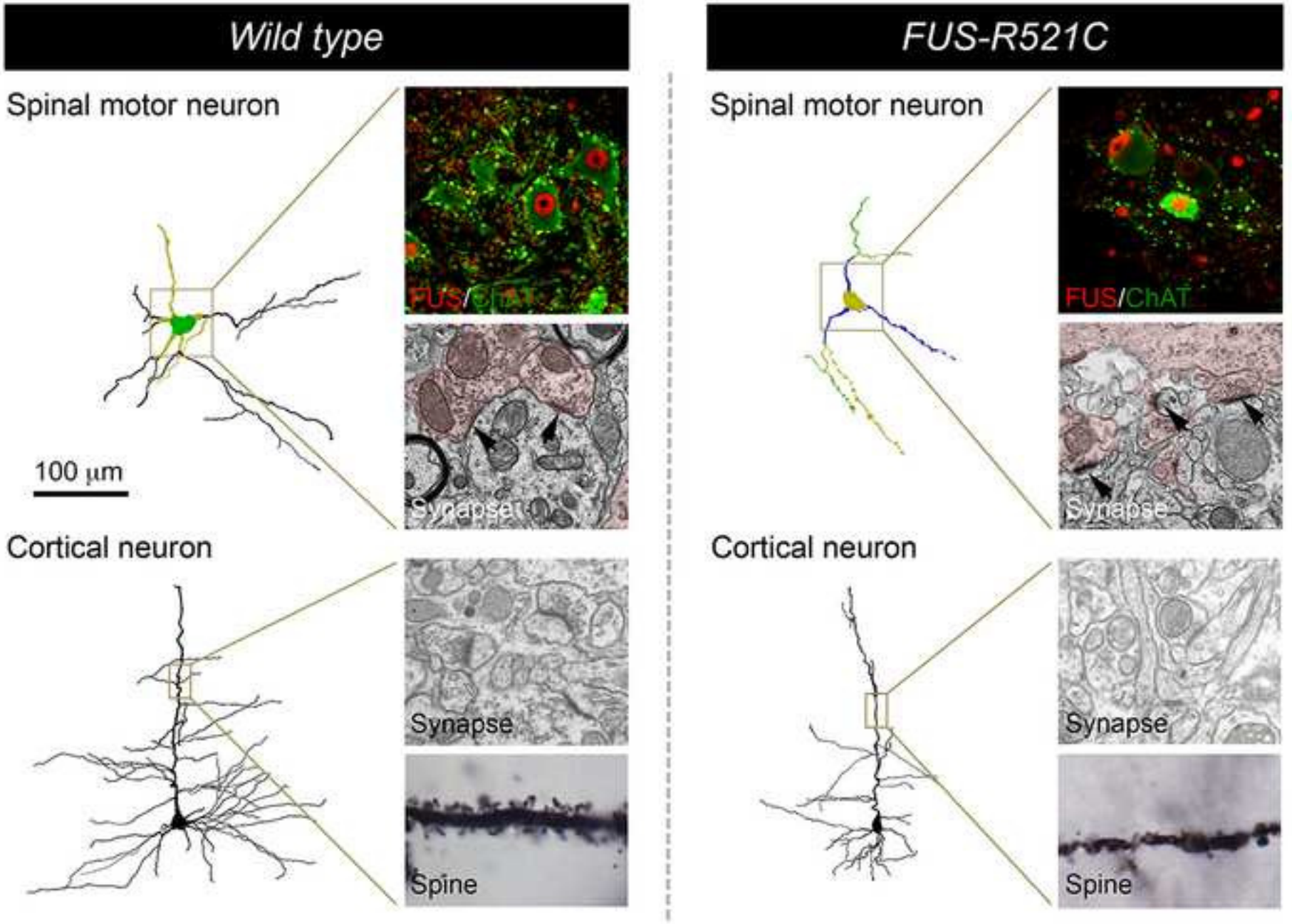

Figure 4