Taylor & Francis
Taylor & Francis Group

REVIEW

Role of FET proteins in neurodegenerative disorders

Francesca Svetoni[a,b], Paola Frisone[b], and Maria Paola Paronetto[a,b]

[a]University of Rome "Foro Italico," Rome, Italy; [b]Laboratory of Cellular and Molecular Neurobiology, Fondazione Santa Lucia, Rome, Italy

ABSTRACT

Neurodegenerative disorders such as Alzheimer disease (AD), frontotemporal dementia (FTD), amyotrophic lateral sclerosis (ALS), Parkinson disease (PD), Huntington's disease (HD), and multiple sclerosis (MS) affect different neuronal cells, and have a variable age of onset, clinical symptoms, and pathological features. Despite the great progress in understanding the etiology of these disorders, the underlying mechanisms remain largely unclear. Among the processes affected in neurodegenerative diseases, alteration in RNA metabolism is emerging as a crucial player. RNA-binding proteins (RBPs) are involved at all stages of RNA metabolism and display a broad range of functions, including modulation of mRNA transcription, splicing, editing, export, stability, translation and localization and miRNA biogenesis, thus enormously impacting regulation of gene expression. On the other hand, aberrant regulation of RBP expression or activity can contribute to disease onset and progression. Recent reports identified mutations causative of neurological disorders in the genes encoding a family of RBPs named FET (FUS/TLS, EWS and TAF15). This review summarizes recent works documenting the involvement of FET proteins in the pathology of ALS, FTLD, essential tremor (ET) and other neurodegenerative diseases. Moreover, clinical implications of recent advances in FET research are critically discussed.

Introduction

FUS (*Fused in liposarcoma*), EWS (*Ewing Sarcoma*) and TAF15 (*TATA binding associated factor 15*) belong to the FET family of DNA and RNA binding proteins, together with the *Drosophila* ortholog *Cabeza*. FET proteins are highly conserved and ubiquitously expressed. They contain several conserved domains: a serine-tyrosine-glycine-glutamine (SYGQ) domain embedded in the DNA activation domain (AD), 3 glycine-arginine (RGG) rich regions that affect RNA binding, one conserved RNA-binding domain (RBD, formed by a RNA-recognition motif, RRM), and a zinc finger domain that is also involved in nucleic acid binding (Fig. 1).[1,2]

FET proteins display several physiological roles within the cell. They are involved in multiple steps of DNA/RNA metabolism and in the maintenance of genomic stability.[3,4,5] Moreover, all members of the FET family interact with the transcription pre-initiation complex, formed by the RNA Polymerase II (RNAPII) and the TFIID complex, and with several transcription factors.[1,2,6,7] In addition to transcription, FET proteins affect also alternative splicing by recruiting the spliceosome machinery and splicing factors, such as the heterogeneous nuclear ribonucleoproteins (hnRNPs) and the SR (Serine-Arginine) proteins, to nascent pre-mRNAs.[8-13] Importantly, FET proteins are physiologically subjected to different environmental signals that induce post-translational modifications in their RBD and in the RGG domains, thus modulating their activity.[14] In this way, FET proteins can couple signal transduction network with modulation of transcription and RNA metabolism, thus globally impacting on gene expression programs.

FET proteins are involved in sarcoma translocations that give rise to in frame fusion proteins comprising the N-terminal part of a FET protein and a transcription factor belonging to the ETS family at the carboxy-terminus.[15,16] These aberrant chimeras are potent transcription factors that guide oncogenic transformation.

FET proteins are expressed in most human tissues, where they mainly localize into the cell nucleus.[17] Interestingly, FUS protein is also localized in dendritic granules and spines in neurons where it plays a role in mRNA transport into dendrites, which represents an essential process for local protein synthesis and synaptic plasticity.[18,19] Recently, it has been suggested the involvement of FET proteins in neurological diseases, such as frontotemporal lobar degeneration (FTLD) and amyotrophic lateral sclerosis (ALS), where they have been found in cytoplasmic aggregates.[20-24] For instance, it was shown that TAF15 and EWS co-accumulate with FUS in neuronal and glial cytoplasmic inclusions in FTLD patients.[20] Remarkably, the cells bearing cytoplasmic inclusions frequently show reduced nuclear staining of all FET proteins.[20] Although it has been recognized as a pathological hallmark of these diseases, the mechanism leading to cytoplasmic mislocalization of FET proteins in neurodegenerative disease is not well understood yet. In particular, it is still unresolved whether neurodegeneration is caused by the loss of an essential function displayed by FET proteins, and/or other RNA binding proteins (RBPs) ("loss-of-function"), or by a gain of toxic properties of these protein aggregates ("gain-of-function"), or by a combination of both. In this regard, recent data appear
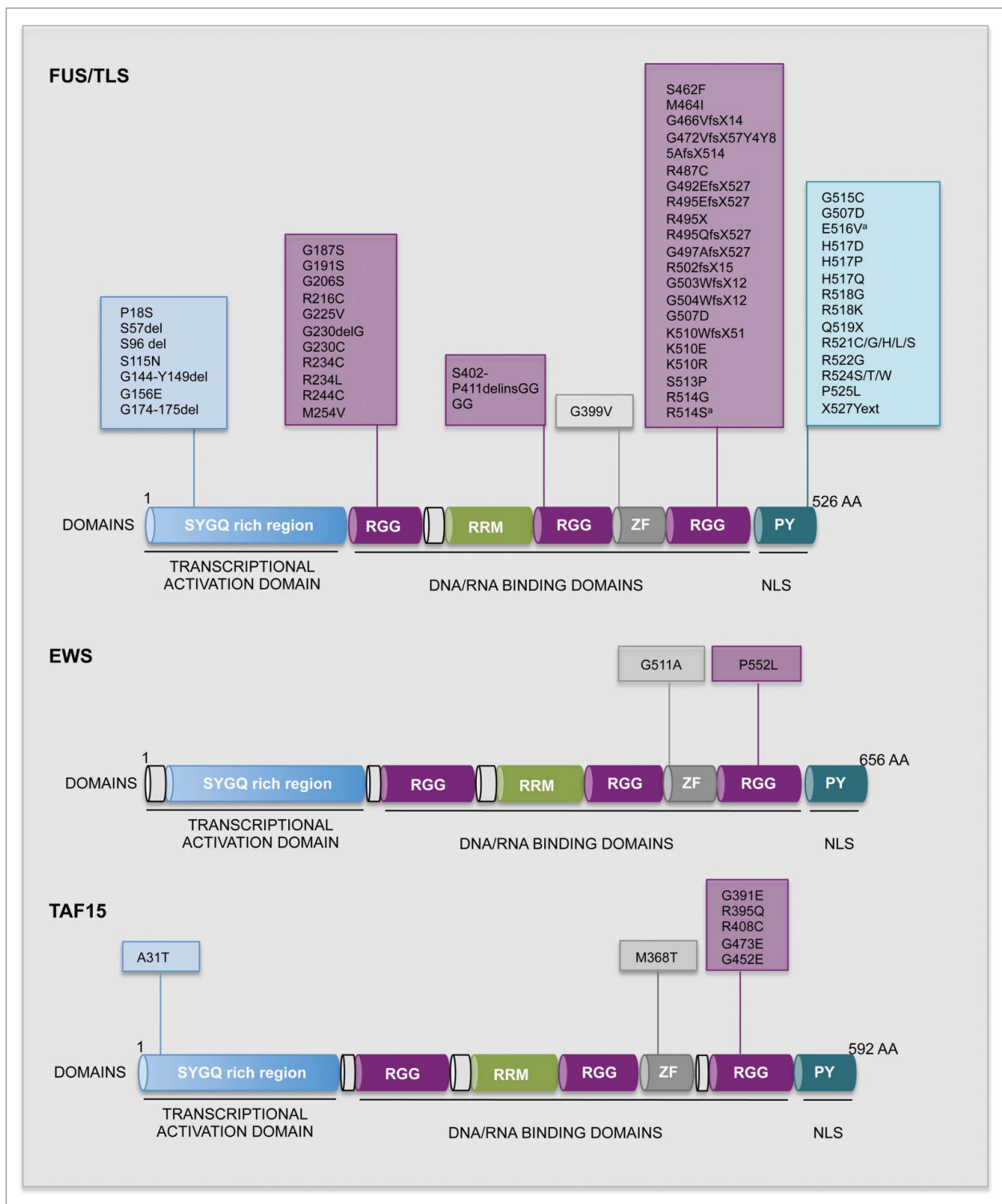
Figure 1. Schematic representation of the 3 members of the FET family (FUS, EWS and TAF15). FET proteins share the same domain structure. SYGQ = serine, tyrosine, glycine and glutamine; RGG = represents a region enriched in arginine-glycine-glycine motifs; RRM = RNA-binding domain; ZF = Cys2/Cys2-type zinc finger motif; PY = proline-tyrosine nuclear localization signal (NLS). In the figure, a schematic overview of FUS, EWS and TAF15 mutations identified in ALS patients is represented. del = deletion; ins = insertion; fs = frameshift; X = stop.

to support a disease model in which motor neuron degeneration occurs through a toxic gain of function mechanism that does not involve the normal activity of these RBPs.[25,26] In this manuscript we will review and discuss recent papers highlighting the critical role played by FET proteins in neurological disorders.

Genetic models of FET ablation in mice

The physiological functions of FUS and EWS have been recently unveiled by the analyses of mouse knockout models of the *Fus/Tls* and *Ewsr1* (encoding EWS) genes, whereas *Taf15* mouse knockout models have not been developed yet. FET mutant

mice display very similar phenotypes, such as perinatal lethality, sterility, enhanced radiation sensitivity and defects in B cell development.[4,27,28]

Mouse models of Ewsr1 gene ablation

Ewsr1 knockout mice are sterile, due to meiotic arrest and apoptosis of pachytene spermatocytes.[4] Notably, EWS protein is critically important for the completion of meiosis in both males and females. After the formation of bivalents, synapsed chromosomes initiate homologous recombination, which is essential for proper segregation of chromosomes. *Ewsr1*-null spermatocytes display a reduction in the number of MLH1 foci, which mark the sites of recombination. As a consequence, *Ewsr1*-null spermatocytes undergo massive apoptosis and gamete maturation is completely arrested.[4] *Ewsr1*−/− mice display also aging-like characteristics, such as kyphosis, reduced bone density and loss of subcutaneous fat.[4] EWS protein is also required for the completion of B cell development and *Ewsr1*-null mice display a severe lymphopenia, whereas erythropoiesis is normal.[4] Furthermore, *Ewsr1*-null mice display smaller thymus and spleen compared with their littermates, reflected in a markedly reduced cellularity of these organs, with a slight decrease in the proportion of T cells and a marked reduction in both immature and mature B cell populations.[4] Further studies indicated that *Ewsr1*$^{−/−}$ mice exhibit a progressive and severe postnatal atrophy of haematopoietic organs due to pronounced reduction in the number of lymphoid progenitor stem cells compared with wild type mice, while the myeloid progenitors are not affected, suggesting that the drop in the lymphoid population might result from the skewing of stem progenitor cells toward the myeloid lineage.[29] Notably, mice with deficiency in DNA repair genes display a similar pattern of haematopoietic lineage skewing as that described for *Ewsr1*$^{−/−}$ mice.[30] Related to this similar phenotype, *Ewsr1*$^{−/−}$ mice are highly susceptible to ionizing radiation, and 2 genetic screens identified *EWSR1* gene as required for resistance to ionizing radiations and to the treatment with the topoisomerase I inhibitor camptothecin.[4,31,32] Since in human cells EWS protein was shown to regulate the alternative splicing of genes involved in the DNA damage response (DDR), like *ABL1* and *CHEK2*, it is possible that EWS plays a direct role in the DDR by modulating the expression of genes involved in this biological response.[5]

Lastly, *Ewsr1* deficient mice display drastically reduced interscapular brown adipose tissue (BAT) compared to their wild type littermates.[33] *Ewsr1* mutant brown preadipocytes fail to differentiate due to the loss of *Bmp7* (bone morphogenic protein), expression, which is a critical early factor for brown adipogenesis.[33] Moreover, mouse embryonic fibroblasts (MEFs) lacking *Ewsr1* fail to undergo adipogenesis, due to a significant reduction in the expression of early adipogenic regulators such as Bmp2, Bmp4 Cebpβ, and Cebpδ (CCAAT/enhancer binding protein β and δ).[34]

Mouse models of Fus/Tls gene ablation

Similar to deletion of the *Ewsr1* gene, inactivation of *Fus/Tls* in mice leads to defects in B cell development and fertility defects, but the molecular mechanisms affected by EWS and FUS deficiencies are different.[27,28] Mating of *Fus/Tls*$^{−/−}$ animals with wild-type counterparts revealed complete male sterility and reduced fertility of females.[27] Interestingly, FUS protein is completely excluded from the synapsed X-Y chromosomes, a chromatin region transcriptionally inactive also called sex body.[27,35] In *Fus/Tls*$^{−/−}$ testes, most homologous chromosomes do synapse; however, the few that fail to execute this essential step properly are sufficient to interfere with meiosis accomplishment, thus causing male sterility.[27] *Fus/Tls*$^{−/−}$ haematopoietic stem cells (HSCs) are highly susceptible to radiation both *in vitro* and *in vivo* and show delayed repair of radiation-induced DNA damage.[36] The proliferation and differentiation of *Fus/Tls*$^{−/−}$ haematopoietic progenitors appear normal *in vitro*. However, the number of colony-forming cells present in long-term cultures is significantly reduced. *Fus/Tls*$^{−/−}$ HSCs have impaired long-term repopulating capacity and fail to repopulate in recipient mice.[36] Furthermore, FUS protein displays an intrinsic role in the proliferative response of B cells to specific mitogenic stimuli and it is required for the maintenance of genomic stability.[36]

These observations demonstrate that EWS and FUS display similar but non-redundant functions *in vivo*.

Neuronal functions of FET proteins

No major neuronal defects have been reported in *Fus/Tls* or *Ewsr1* knockout mice, highlighting the possibility that EWS and FUS functions are not essential for normal neuronal development and/or that they play redundant roles in neurons, carried out by the other member of the family on demand. Although *Fus/Tls*$^{−/−}$ mice do not manifest ALS- or ET-like phenotypes until nearly 2 years, they show distinct histological and behavioral alterations upon aging, including vacuolation in hippocampus, hyperactivity, and reduction in anxiety-like behavior.[37] Knockout mice show changes in the expression of genes related to neurological diseases, including upregulation of *Taf15* and *Hnrnpa1*, while they have normal morphology of RNA-related granules.[37] Moreover, FUS has been shown to transport the *Nd1-L* mRNA, encoding an actin-stabilizing protein, to neuronal dendrites.[38] Since *Fus/Tls*$^{−/−}$ hippocampal neurons display abnormal spine morphology, this defect could be attributed to actin destabilization by the improper supply of *Nd1-L* mRNA to the dendrites. Nevertheless, exogenous expression of FUS in *Fus/Tls*$^{−/−}$ neurons did not rescue the abnormal spine phenotypes. Thus, FUS nucleo-cytoplasmic shuttling during neuronal maturation may be critical for the recruitment of *Nd1-L* mRNA into the pool of ribonucleoproteins near the spines. This function might be relevant for the acquisition of neuronal activity at early developmental stages and not recoverable by exogenous expression of the protein in mature neurons.[38] Thus, although FUS depletion causes phenotypes possibly related to neuropsychiatric and neurodegenerative conditions, they appear distinct from ALS and ET.

Notably, postnatal elimination of FUS in mice has no effect on motor neuron survival, providing unequivocal evidence that FUS loss-of-function alone does not underlie motor neuron degeneration.[25] Furthermore, postnatal elimination of the endogenous FUS in a *hFUSP525L* mutant mouse model has no effect on the initiation and early progression of motor neuron loss, indicating that mutant FUS toxicity does not involve an

excess of FUS activity.[25] Recently a novel mouse model lacking the last exon of *Fus/Tls* gene has been generated, resulting in the production of a truncated FUS protein without the nuclear localization signal (NLS) that localizes almost exclusively to the cytoplasm.[26] Remarkably, this *Fus/Tls* ΔNLS knockin mouse model displays expression and splicing alterations consistent with loss of FUS nuclear function. However, unlike *Fus/Tls*⁻/⁻ mice, they exhibit perinatal motor neuron loss, rescued by motor neuron expression of wild−type FUS.[26] These findings indicate that cytoplasmic FUS mislocalization exerts not only a nuclear loss of function effect, but also a toxic gain of function within the cytoplasm of motor neurons required to trigger neuronal death.

The presence of only one member of the FET family in fruit-flies renders the ablation phenotype more severe in locomotion, longevity, and neuromuscular junction (NMJ) expansion[39]. In fact, ablation of the *Drosophila* FET hortologue *cabeza* led to decreased adult viability, locomotor disabilities at both larval and adult stage, and shorter life span.[39,40] All these phenotypes were rescued by transgenic wild-type *Drosophila Caz (cabeza)* or human *FUS*, revealing a remarkable conservation of protein function during evolution.[38] Remarkably, selective inactivation of *cabeza* in adult neurons did not affect motor performance and life span, documenting the requirement of neuronal *Caz* during development, but not for the maintenance of adult neuronal function.[41] Thus, loss of neuronal FUS/Caz function is not sufficient to cause adult motor neuron degeneration in fruitflies.

Genetic ablation of *FUS* in zebrafish led to abnormal motor behavior measured as a deficient touch-evoked escape response and reduced outgrowth of hyperbranched axons from motor neurons.[42] Moreover, *Fus* depleted zebrafish displayed aberrant structure of the neuromuscular junctions (NMJs) with reduced success of synaptic transmission.[42] *Ewsr1* knockdown in zebrafish leads to defects in the brain and spinal cord. In particular, upon depletion of *Ewsr1* gene cells of the central nervous system (CNS) were more prone to apoptosis. This was probably due to the fact that EWS protein interacts with a neuronal specific factor that regulates neuronal outgrowth and prevents apoptotic cell death by binding the promoter of target genes.[43]

Collectively from these reports we can conclude that in lower organisms the phenotype associated with genetic ablation models becomes more severe. Thus, a partial redundancy in FET function could be responsible of the milder neuronal phenotype associated with FET deficiency in mammals.

FET proteins in neurodegenerative diseases

Amyotrophic lateral sclerosis

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease that primarily targets motor neurons. The average age at onset is 60 years, and annual incidence is 1 to 2 per 100,000 individuals. ALS leads to a progressive muscular weakness caused by the degeneration of motor neurons in the brain and spinal cord, thus resulting in paralysis and death due to respiratory failure within a few years from onset.[44] Approximately 10% of ALS is dominantly inherited, while the remaining 90% of patients (referred to as sporadic) do not have familial history.[44] Autosomal dominant familial ALS (FALS) is clinically and pathologically indistinguishable from the sporadic disease (SALS). Few genes have been associated with familial ALS, including *SOD1*, the gene encoding superoxide dismutase 1; *ANG*, the gene encoding angiogenin; expanded repeats in the *C9ORF72*, the chromosome 9 open reading frame 72 and *TARDP*, encoding TAR DNA binding protein TDP-43.[45-49] The discovery of mutations in another RNA/DNA binding protein, FUS, rapidly followed the identification of TDP-43 mutations in ALS. *FUS/TLS* mutations account for about 5% of cases of FALS.[23,24] Notably, mutant FUS proteins share common features with mutant TDP-43. In fact, in ALS, mutated TDP-43 is partially excluded from the nuclei of neurons, while it accumulates in cytoplasmic aggregates,[50,51] and most patients with *FUS/TLS*-associated ALS disease display FUS-immunoreactive cytoplasmic inclusions.[23,24]

Since 2009, when the first *FUS/TLS* mutation was associated to ALS, more then 50 mutations have been identified in this locus (Fig. 1).[52] Most of them are missense mutations, with few exceptions, and they mainly cluster in 2 regions. Approximately two-thirds of mutations are located in exons 12–15. These exons encode the zinc-finger motif domain, the second and third RGG domain, and the nuclear localization signal. The remaining mutations are located in exons 3–6, which encode the QGSY-rich and the first RGG domains.[23,24,52] Importantly, none of the ALS-FUS cases investigated showed alteration in the subcellular distribution of TAF15 or EWS and there is not evidence of co-accumulation of these proteins in the FUS-positive pathological inclusions.[53] Thus, cytoplasmic accumulation of FUS *per se* does not trigger alterations in the subcellular distribution of its homologues and does not lead to sequestration of TAF15 and EWS into FUS inclusions.

Several ALS-associated FUS mutations occur within the nuclear localization signal (NLS) and impair nuclear import to a degree that correlates with the age of disease onset. Nuclear import of FUS depends on transportin, and interference with this transport pathway is sufficient to cause cytoplasmic redistribution and recruitment of FUS into stress granules.[54] Other *FUS/TLS* mutations affect the splice-acceptor site of intron 13 and induce skipping of exon 14, leading to the C-terminal truncation of FUS protein (p.G466VfsX14, Fig. 1), with a dramatic increase in the cytoplasmic localization of truncated FUS protein compared to wild-type FUS.[55] These observations suggest that forced accumulation of FUS in the cytoplasm leads to aggregates that might exert toxic effects and trigger neuron degeneration.

Development of animal models of the disease greatly helped the understanding of the molecular mechanisms underlying the involvement of FET proteins in ALS. Overexpression of mutant (R521C substitution), but not normal, human FUS in rats induced progressive paralysis and displayed a substantial loss of neurons in the cortex and hippocampus, accompanied by ubiquitin aggregation.[56,57] Interestingly, although FUS transgenic rats that overexpressed the wild-type human FUS were asymptomatic at young ages, at advanced ages they showed a significant loss of cortical and hippocampal neurons, demonstrating that increased expression of normal FUS is sufficient to induce neuron death accompanied by ubiquitin aggregation, thus recapitulating some features of ALS and FTLD.[57]

Similarly, FUS overexpression in mice showed significant loss of motor neurons, coupled with pathological phenotype recapitulating many aspects of FUS-ALS. The phenotype strongly depends on the expression level of the protein, and is associated with the translocation of FUS protein to the cytoplasm.[58]

Overexpression of the ALS-linked mutant of *FUS* R521H in zebrafish caused motor deficiency.[42] Moreover, the generation of transgenic *C. elegans* worms expressing mutant or wild-type (WT) human FUS, showed that only the mutant proteins mislocalize in the cytoplasm and cause progressive motor dysfunction, with phenotype severity that was directly correlated with the severity of the illness caused by the same mutation in humans.[58] Fly models of FUS also recapitulate several features of the human disease.[59,40] Targeted expression of FUS in the fly brain and motor neurons led to the pupal lethality and larval locomotor defects.[60] Moreover, conditional expression of mutant FUS in neurons drastically reduce the life span and climbing abilities compared to FUS wild type flies.[61,62] From these reports we can argue that these models closely replicate key features of the human illness.

Recently, genetic variants in *TAF15* and *EWSR1* genes have been reported in a small number of ALS patients, supporting a more general role for FET proteins in neurodegeneration.[63-65] TAF15 and EWS were identified as candidate RBPs in a yeast functional screen with the aim of identifying human proteins containing RNA recognition motifs (RRM) with properties similar to FUS and TDP-43.[64] Genomic sequencing of exons 13–16 of *TAF15* in 735 individuals diagnosed with ALS, allowed the identification of 3 patient-specific nonsynonymous missense variants (G391E, R408C, and G473E; Fig. 1). These variants were all located in highly conserved regions of TAF15 and correspond to the analogous regions where many FUS mutations are located, comprising the RGG domain and the PY-motif in the C-terminal domain, which plays a role in nuclear localization.[65,66] Importantly, these ALS-related variants of TAF15 showed an accumulation in cytoplasmic foci of dendrites and axons.[64] Analogously, the exons (15–18) of *EWSR1* gene encoding the same conserved region of the protein were sequenced in 817 individuals diagnosed with ALS and in 1082 healthy individuals.[65] This approach identified 2 missense mutations in *EWSR1* gene in 2 unrelated ALS patients with sporadic disease.[65] These mutations are located in the first (G511A) and second (P552L) RGG of EWS (Fig. 1) and neither one was present in 1082 sequenced controls, strongly supporting their clinical significance.[65] Remarkably, transfection of wild type and mutated *EWSR1* into primary motor neurons cultured from rat embryos revealed that wild type EWS primarily localized to the nucleus, whereas both ALS-specific variants showed increased cytoplasmic and neuritic accumulation. Even more importantly, mutated TAF15 and EWS cause neurodegeneration when expressed in *Drosophila*.[64,65]

All mutant forms of FET protein have increased aggregation tendency and altered subcellular localization and they have been proposed to disrupt RNA and protein homeostasis. Nevertheless, the pathogenetic mechanism by which mutated FET proteins causes neurodegeneration associated with ALS is still unknown. Several factors could be involved in FET-mediated toxicity, including stress granule formation, the presence of prion-like domain, arginine methylation, and/or mysregulation

of their RNA targets (Fig. 2).[67-71] These issues will be critically discussed below.

Frontotemporal dementia

Frontotemporal dementia (FTD) is a clinical syndrome characterized by progressive deterioration in behavior, personality and/or language skills, with relative preservation of memory.[72] FTD is the second most common form of dementia after Alzheimer disease and results from degeneration of the cortex of the frontal and temporal lobes, often in conjunction with the degeneration of subcortical regions.[73] This pattern of degeneration gives rise to a spectrum of behavioral, language, and movement disorders. The molecular bases of FTD are heterogeneous, leading to phenotype variability. As mentioned, a common feature of FTD is the selective degeneration of the frontal and temporal lobes, and the term "frontotemporal lobar degeneration" (FTLD) is often used.[72]

Although most cases are considered sporadic, FTLD displays a strong genetic component, with a family history occurrence in approximately 40% of cases, and an autosomal dominant pattern of inheritance observed in 25–50% of these patients.[73,74] Mutations in the microtubule associated protein tau gene (*MAPT*) and in progranulin gene (*PGRN*), both located on chromosome 17, were identified in families with FTLD.[75,76] Recently, the presence of GGGGCC hexanucleotide repeat expansions in the first intron of the *c9orf72* gene were identified as the most common genetic cause of familial FTLD, accounting for approximately 20% of all familial cases.[22,57,77] Moreover, mutations in 2 other genes, *VCP*, encoding the vasolin-containing protein, and *CHMP2B*, encoding the chromatin-modifying protein 2B, are responsible for a minority of familial FTLD cases.[78]

Most cases of FTLD presented abnormal intracellular accumulation of disease-specific proteins. Initially, only the FTLD subgroup characterized by the aggregation of hyper-phosphorylated tau protein in neurons and glia, classified as FTLD-tau, was described.[79] However, most cases of FTLD are not associated with tau pathology, but are instead characterized by ubiquitin positive neuronal inclusions, then termed FTLD-U.[80] TDP-43 was also reported as a ubiquitinated pathological protein in several cases of FTLD-U (subsequently renamed FTLD-TDP).[48] Recently, aberrant localization of FUS protein has been described as a component of the inclusions in several subtypes of FTLD, now included in the FTLD-FUS group. FTLD-FUS includes cases with ubiquitin-positive inclusions (FTLD-U), neuronal intermediate filament inclusion disease (NIFID) and basophilic inclusion body disease (BIBD).[21,80] Notably, only few FUS mutations have been reported in patients with an FTLD or FTLD-ALS phenotype.[81,82] However, further studies in large FTLD cohorts observed no mutations, suggesting that FUS has only a limited role in the genetic etiology of FTLD.[21,82,83] Moreover, abnormal co-accumulation of all 3 FET proteins into pathological inclusions has been described in all subtypes of FTLD-FUS.[84,85] Biochemical analysis of proteins extracted from FTLD-FUS brains revealed increased insolubility of all FET proteins, without other obvious disease-associated changes, such as truncation or abnormal protein phosphorylation.[21]
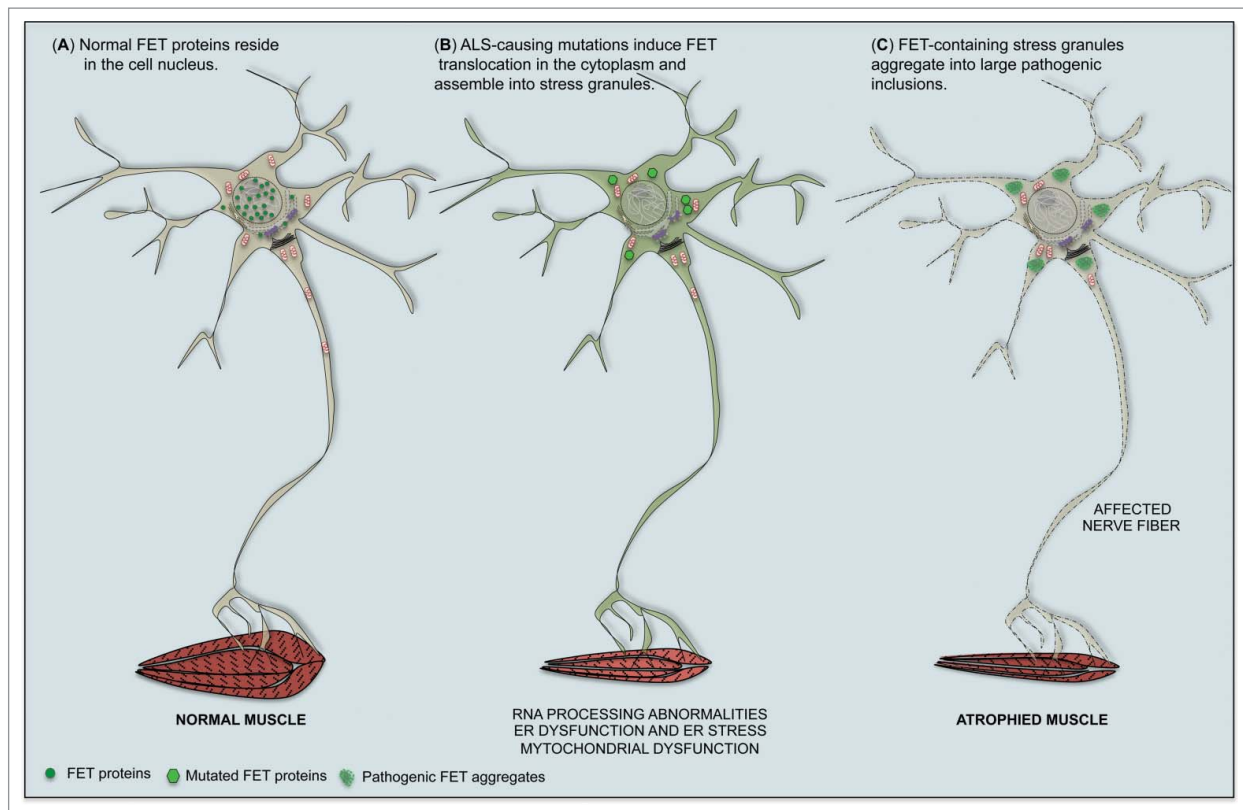
Figure 2. Impairment of nucleo-cytoplasmic shuttling of FET proteins contributes to neurodegenerative disease and muscular atrophy. In normal conditions, FET proteins display several physiological functions, including transcription, pre-mRNA splicing, mRNA biogenesis, stress granules formation and mRNA stability, by binding target RNAs both in the nucleus and in the cytoplasm and are engaged in nucleo-cytoplasmic shuttling (a). Mutations within the NLS or NES of FET proteins and factors arising during aging, like reduced expression of nuclear transport factors or oxidative damage, as well as post-translational modifications, such as phosphorylation, arginine methylation and proteolytic cleavage events that remove the NLS, can cause cytoplasmic accumulation of FET proteins within stress granules (b). Such stressors may also include environmental toxins or mutations in neuro-protective/stress protective genes, such as *GRN* or *VCP*. Since the formation of stress granules is a reversible process, this step can potentially be reversed upon release of stress or by upregulation of protective factors, like chaperones or neuronal growth factors, such as Progranulin. However, persistent cellular stress or genetic risk factors leads to the conversion of FET-containing stress granules into large pathological inclusions. This in turn can cause degeneration of motoneurons and muscular atrophy (c).

Since only rarely mutations in the *FUS* gene are causal for FTLD, it is still debated what causes FET protein translocation. In the case of FUS, it is known that, under normal physiological conditions, this protein shuttles between the nucleus and cytoplasm through the nuclear pore and its nuclear import is assisted by transportin1 (TRN1), which is responsible of most of the nucleocytoplasmic transport traffic within the cell.[86] Binding to TRN1 is dependent on the PY motif located at the carboxy-terminus of FUS.[87] Interference with this transport pathway leads to cytoplasmic redistribution and recruitment of FUS into stress granules. Thus, FUS protein mislocalization in cases of FTLD-FUS might be due to a general defect of transportin-mediated nuclear import.[55] Indeed, inhibition of transportin-mediated nuclear import resulted in recruitment and co-localization of all FET proteins into stress granules, while alterations in the subcellular distribution of other transportin cargos such as hnRNPA1 and SAM68 was not affected.[86,87] This observation suggests that FET proteins are particularly sensitive to alteration of this pathway. Another possibility is that the normal nuclear import of FET proteins is affected by abnormal post-translational modifications occurring in the FTLD condition. In this regard, it has been shown that arginine methylation and phosphorylation modulate FET proteins nucleo-cytoplasmic transport, protein–protein interactions and

stability. Arginine methylation of a site adjacent to the C-terminal PY motif impairs TRN1-mediated nuclear import of FUS.[88] In addition, the N terminus of FUS contains a low-complexity domain that can aggregate into fibrils mimicking features of RNA granules; aggregation of the FUS low-complexity domain can be disrupted by DNA-PK-mediated phosphorylation.[88,89] On the other hand, FUS phosphorylation on tyrosine 526 by Src kinases reduces its interaction with TRN1 and might consequentially affect the transport of FUS into the nucleus.[90] Thus, abnormal localization of FUS protein could also result from post-translational modifications triggered at the onset of FTLD.

TAF15 co-localization with aberrant FUS was reported in all cases analyzed, while subtle disease-specific differences were observed for EWS. Interestingly, TAF15 localization was differentially regulated in HeLa and neuronal HT22 cell lines. Although predominantly nuclear in both cell lines, in HT22 cells TAF15 co-localized in the cytoplasm with a subset of RNA granules, thus supporting the hypothesis of a role for TAF15 in RNA transport and/or local RNA translation in neurons.[91]

Hence, co-accumulation of all FET proteins in the characteristic pathologic inclusions seems a specific feature of FTLD-FUS, but not of ALS-FUS. Moreover, in all ALS cases presenting FUS inclusions, mutations in the *FUS/TLS* gene caused the

disease, whereas only a few FTLD cases with FUS inclusions harbor *FUS/TLS* mutations.

Essential tremor disease

Essential tremor (ET) is a neurodegenerative disorder characterized by postural and/or kinetic tremor. It is one of the most common adult-onset movement disorders. The main feature is postural tremor of the arms, but head, legs, trunk, voice, jaw, and facial muscles may also be involved. Notably, in most families affected by the disease, autosomal dominant inheritance can be demonstrated.[92]

Recent exome sequencing of a large pedigree of individuals within a French-Canadian family affected by an autosomal dominant form of familial ET revealed a nonsense mutation in *FUS/TLS* gene (c.868C>T) as pathogenic.[93] This ET variant in exon 9 of *FUS/TLS* corresponds to a stop mutation (p.Q290X). The nonsense mutation is located in the predicted nuclear exporting signal (NES) of FUS protein (amino acids 289–298), which is a short amino acid sequence that targets the protein for export from the nucleus to the cytoplasm through the nuclear pore complex. The corresponding transcribed mRNA is mainly degraded by nonsense mediated decay (NMD), suggesting a loss-of-function disease mechanism.[93] Moreover, a novel risk variant c.1176G>A (p.M392I) was identified as potential ET susceptibility factor.[94] This variant resided in exon 12 of the *FUS/TLS* gene, causing the substitution of Met392 with Ile.[94] Exome sequencing analysis has also recently identified a non-sense mutation in FUS, p.R377W, in one patient with family history of disease, with one brother diagnosed with parkinsonism and one sister and one uncle with possible ET. This mutation was located in the second RGG domain of FUS protein, in which mutations for ALS and FTD have not been described.[95] Furthermore, sequencing of the *EWSR1* gene in ET patients identified a rare p.R471C substitution in a single subject with familial ET. The pathogenicity of this substitution remains equivocal, as DNA samples from relatives were not available and the genotyping of 404 additional ET subjects did not reveal any further carriers.[96] Collectively, these studies suggest that mutations in FET proteins may underlie the pathology of ET and further studies are needed to elucidate the mechanisms of such pathological effects.

Parkinson disease

Parkinson disease (PD) is a progressive disorder of the nervous system that affects several regions of the brain devoted to the control of balance and movement. In addition, PD can affect emotions and thinking ability (cognition).[97] Generally, PD onset occurs after age 50 (late-onset disease), although early-onset symptoms before this age and even before age 20 have been described (referred to as juvenile-onset PD).[97] Despite familial history had been recognized as a common feature in PD, only in the last 15 years the contribution of genetics has been deeply explored. These studies have conducted to the identification of few genes that are clearly responsible for mendelian forms of the disease, either with autosomal dominant (*SNCA, LRRK2*) or recessive (*PARK2/Parkin, PINK1, DJ-1, ATP13A2*) inheritance.[98] Given the involvement of *FUS*

mutations in ET ethiopathology, the role of FUS variants was also investigated in PD but it did not identify any novel non-synonymous variant affecting the subjects' susceptibility to PD.[99]

Alzheimer disease

Alzheimer disease (AD) is the most common form of dementia. Genetic factors are involved in 25–40% of AD patients and, in some cases, AD segregates as an autosomal dominant trait in families.[100] To date, 3 genes have been identified that, when mutated, cause AD: the Aβ amyloid precursor protein gene (*APP*), the presenilin 1 (*PSEN1*) and the presenilin 2 (*PSEN2*) genes. Together, these mutations are responsible for 30–50% of autosomal dominant AD cases, and about 0.5% of all AD cases.[100] No mutations in FET genes have been reported in AD patients.

FET proteins and RNA processing: New potential targets in neurodegenerative disease

As discussed above, dysregulation of RBPs is emerging as a prominent pathogenic mechanism underlying ALS. Most ALS-related FUS mutants display disrupted nucleo-cytoplasmic shuttling, leading to increased cytoplasmic levels of the protein and formation of aggregates. Cytoplasmic accumulation and aggregation of FUS, TAF15, and EWS was also found in a subset of FTLD cases, further supporting the hypothesis of a dysregulation of FET-dependent RNA metabolism in the disease.[54] Thus, identification of common RNA targets driving or contributing to pathogenesis is an intriguing open challenge.

FUS and EWS preferentially bind polyU and polyG sequences.[101,102] RNA binding is mediated by the RBD, while the 3 RGG motifs cooperate with the RBD to increase the affinity for RNA.[9,13,101-104] The RGG motifs are sites of post-translational modifications that modulate RNA binding affinity and affect protein-protein interactions.[9,14] The carboxy-terminus of TAF15 contains 20 copies of Gly-Gly-Tyr-Gly-Gly-Asp-Arg repeats; this region is likely to have a role in RNA binding.[6,14,105] Importantly, FUS was initially identified in purified human spliceosomes assembled *in vitro*, while *in vitro* splicing assays demonstrated its implication in the regulation of alternative splicing.[9,106,107] FUS protein crosslinks the pre-mRNA 3′ splice site during the second step of splicing.[108] On the other hand, EWS was initially shown to interact with the branch-point recognizing protein BBP/SF1 and with U1C, one of the protein components of U1 snRNP, suggesting a role in modulating 3′ and/ or 5′ splice-site recognition.[109,110]

Identification of the RNA targets bound by FET proteins *in vivo* is an essential step toward elucidation of their functions. FET RNA targets were reported for FLAG-tagged proteins expressed in 293T cells, revealing prominent binding of FUS to introns, while EWS and TAF15 preferentially bind coding sequences and 3′UTRs in target RNAs.[111] Notably, FUS and TAF15 crosslink sequences nearby the 3′ splice site, while EWS preferentially binds near the 5′ splice sites of target pre-mRNAs.[111,13] UV crosslinking and immunoprecipitation sequencing (CLIP-seq) experiments highlighted an EWS signature in exonic regions nearby the 5′ splice sites. Mechanistically,

EWS recruitment of U1snRNP and U2AF to the flanking splice sites of the target exons, favoring their definition and, consequently, their inclusion.[13] RNA targets for endogenous FUS in mouse brains and human autopsy brains document that nascent RNAs constitute the major class of substrates for FUS.[112-114] Interestingly, no significant overlap between the binding sites or splicing changes is seen between FUS and TDP-43, although both proteins are directly involved in ALS and they both bind and regulate genes with long introns and important for neuronal development, highlighting the possibility that binding of TDP-43 or FUS to long introns may facilitate transcription elongation and ensure normal and correct splicing of long introns, preventing unproductive splicing events.[115,116] Nevertheless, a subset of common targets was identified, opening the possibility of RNA regulation as a key mechanism underlying pathophysiological features of ALS and FTD diseases.[113-115]

FUS regulates splicing of genes coding for other RBPs by binding to their highly conserved introns.[115,116] These results have important implications for understanding the impact of FUS in neurodegeneration, as they suggest that perturbations of FUS may have widespread effects in the neuronal transcriptome via dysregulation of the processing of various RBP transcripts.[116] Among them, FUS regulates its own transcript by repression of exon 7 splicing, thus leading to a transcript subjected to NMD.[117] FUS protein harboring R521G, R522G or ΔExon15 ALS-related mutations show deficiencies in both exon 7 repression and autoregulation of FUS protein levels. These observations suggest that compromised autoregulation of FUS can directly exacerbate the pathogenic accumulation of the protein in the cytoplasm in neurons of ALS patients.[117] Importantly, FUS, EWS and TAF15 target pre-mRNAs largely overlap in Hek293 cells, while mutated FUS proteins (FUS-R521G or FUS-R521H) display an elevated fraction of unique crosslinks, pointing toward an altered, rather than disrupted, binding profile.[111] Gene categories related to unfolded protein response (UPR) and endoplasmic reticulum (ER), are overrepresented among transcripts uniquely bound by cytoplasmic FUS mutants,[111] suggesting that their dysregulation may contribute to the formation of cytoplasmic aggregates. In mouse and human brain tissues, FUS affects the expression of genes important for neuronal function, including synaptic genes.[113] FUS mRNA targets of relevance for neurodegenerative disorders include *SOD1*, the genes encoding medium and heavy chains of neurofilament (*NEFL, NEFM, NEFH*) and the glutamate transporter (*EAAT2*), ubiquilin 1 and 2 (*UBQLN1* and *UBQLN2*) and MAPT.[113] Comparison of FUS mRNA targets in mouse versus human brain revealed a relatively high degree (69%) of overlap, indicating that FUS-RNA interactome is evolutionary conserved.[113]

More recently, UV crosslinking and immunoprecipitation sequencing (CLIP-seq) experiments highlighted an EWS signature in exonic regions nearby the 5′ splice sites. Mechanistically, EWS recruitment of U1snRNP and U2AF to the flanking splice sites of the target exons, favoring their definition and, consequently, their inclusion.[13]

FET proteins bind to both coding and noncoding RNAs. FUS, EWS and TAF15 associate with Drosha and with the microprocessor complex.[118] Moreover, EWS crosslinks different classes of noncoding RNAs while FUS binds directly and contributes to the biogenesis of a subset of miRNAs with roles in neuronal function, differentiation and synaptogenesis.[13,119,120] Notably, global loss of miRNAs through Dicer knockdown results in neurodegeneration, suggesting that misregulation of miRNA biogenesis may contribute to neurodegeneration through chronic changes in synaptic functions.[121-124] Thus, it is possible that transcriptome alterations detected in FTLD and ALS patients derive, at least in part, from disruption of miRNA networks, potentially linked to improper FET proteins function. Indeed, recent studies using miRNA microarrays or RNA sequencing to assess alterations in the miRNA landscape in FTD-ALS revealed extensive changes in the expression of these small noncoding RNAs.[125-127]

As mentioned above, FET proteins display multiple roles in the RNA metabolism, from transcription to pre-mRNA splicing, miRNA processing, lncRNAs, mRNA stability, transport and control of mRNA translation, and they are even involved in the formation of stress granules (for a review see Ref [128]). Although their regulatory activities are similar, they regulate distinct RNA targets and show different and not-redundant functions both in normal and in pathogenetic conditions.

Now that genome-wide analyses have provided thousands of coding and noncoding RNAs either bound or processed by FET proteins, the next goal will be to determine whether these genes are dysregulated in neurodegenerative diseases bearing mutations in FET genes. Moreover, if a causative link is established, it will be crucial to assess whether disease-related phenotypes can be rescued by restoring the regulation of the disease-causing targets.

Prion-like domain in FET proteins

Increasing evidence supports a common mechanism driving neurodegeneration in clinically different conditions. Most neurodegenerative diseases, in fact, share the abnormal accumulation of misfolded insoluble proteins that aggregate in the neurons of affected individuals.[129] In prion disease, the infectious agent is the prion, a misfolded pathogenic protein with the ability to self-propagate and to transmit its conformation to other proteins, thus driving encephalopathy.[130] In neurodegenerative disease, instead, the misfolded aberrant proteins act as a seed for aggregation, thus sequestering their native isoforms and converting them into pathological aggregates, which gradually enlarge by recruitment and conversion of further native proteins, ultimately leading to the disease phenotype.[131] Notably, bioinformatics analysis revealed that many human RBPs, including TDP-43 and FET proteins, contain predicted "prion-like" domains, enriched in uncharged polar amino acids (such as asparagine, glutamine and tyrosine) and in glycine residues.[63,132,133,134] This finding raises the intriguing possibility that mutations in these aggregation-prone RBPs can trigger neurodegenerative diseases by acting as seeds in a prion-like fashion. Chronic cellular stress conditions or mutations in the amino acid sequence may convert the RBPs that are highly concentrated in the stress granules into irreversible protein aggregates. In turn, these protein aggregates can critically perturb protein homeostasis thus driving neurodegeneration.

The prion-like domain of TDP-43 is located at the carboxy-terminus of the protein (residues 277–414), while that of FUS is embedded in the N-terminal region (residues 1–239), but this RBP contains an additional potential prion-region in the first RGG domain (residues 391–405).[132] Importantly, the prion-like domain of TDP-43 plays a critical role in protein aggregation, since its deletion prevents aberrant TDP-43 misfolding.[132,135] Conversely, elevated expression of the C-terminal portion of TDP-43 containing the prion-like domain elicits toxicity and cytoplasmic TDP-43 aggregation in yeast and worm models.[136,137] Unlike TDP-43, FUS fragments that harbor the prion-like domain (amino acids 1–238) do not aggregate, unless they also contain a C-terminal RGG domain (amino acids 374–422). Thus, communication between different domains of the protein is necessary to promote FUS aggregation.[138] Importantly, both TDP-43 and FUS were found in stress granules of ALS patients.[139] Association of TDP43 with stress granules strongly depends on the presence of the prion-like domain, as deletion of this region inhibited the process.[140] In the case of FUS, the RGG-zinc finger domain is the most important domain for stress granules recruitment, although the glycine-rich domain and the RRM domain also contribute to FUS relocalization, whereas the prion-like glutamine-rich domain is dispensable.[140] Notably, mutations within this domain resulted in enhanced kinetics of stress granule formation and increased size of the granules.[139-141]

Depending on the type of stress, FUS rapidly shuttles from the nuclear liquid compartments to the cytoplasm; then, a population of FUS converts from a liquid state to an aggregated state, which resembles the pathological state of ALS patients harboring mutations in the FUS protein.[142,143] This conversion from liquid to solid is accelerated either by mutations in the prion-like domain that induce the early onset of ALS or by raising the protein concentration.[142,143]

Similarly to FUS, both TAF15 and EWS harbor a N-terminal prion-like domain (amino acids 1–149 and 1–280, respectively).[64,134] Moreover, TAF15 and EWS proteins were shown to form cytoplasmic aggregates in yeast and to display toxic features.[64]

As mentioned above, the accumulation of misfolded protein structures is a recurring and unifying facet of different neurodegenerative diseases, able to propagate disease from single or multiple sites of origin. Interestingly, many of the misfolded proteins found in these pathological inclusions are expressed in almost all cells, but they only seem to misfold and confer toxicity in specific neurons (i.e. motor neurons in ALS, dopaminergic neurons in PD). Agents able to revert protein misfolding and restore proteins to native form would likely have high therapeutic value. Indeed, a protein chaperone system able to rescue previously aggregated proteins has been described in yeast, but it has no metazoan orthologs.[144,145] This system is formed by the protein disaggregase Hsp104 and the 2 chaperones Hsp70 and Hsp40.[146] Remarkably, modifications of Hsp104 are able to disaggregate and rescue deleterious TDP-43, FUS, and TAF15 misfolding, whereas misfolded EWS was not buffered by Hsp104 variants.[146] Thus, rescue of the disease-associated proteins to their non-pathogenic states through the development of an engineered chaperon system might provide a highly promising strategy for halting or even reversing the progression of devastating neurodegenerative diseases. Further understanding of the mechanisms regulating protein processing and underlying protein aggregation, as well as of the toxic effects of misfolded proteins in the pathogenesis of neurodegeneration, will help in the development of rationally designed therapies to treat or prevent these disorders.

Concluding remarks

FET proteins play key roles in the regulation of gene expression, thus displaying similar but not redundant functions within the cell. The recent discovery of the implication of FET proteins in neurodegenerative diseases renewed the interest in elucidating their physiological functions. To date, it is still not clear which, if any, endogenous function of FET proteins is involved in the pathogenesis of these diseases. Many ongoing studies suggest a prion-like mechanism underlying the pathogenesis of ALS, as well as of other neurodegenerative disorders. The prion-like seeding and spreading of the proteins implicated in the disorder could trigger, from a single protein-misfolding event, an autocatalytic protein-protein pathological cascade, thus causing neurodegeneration. In this scenario, impairment of FUS autoregulation of its own RNA might contribute to create a feed-forward positive loop of protein production and aggregation, resulting in sequestration of newly produced FUS, together with its RNA targets, into the cytoplasmic aggregates. Importantly, further understanding of the role of FET proteins in neurodegenerative diseases might lead to the development of new tools and techniques for early diagnosis and identification of therapeutic targets for the treatment and prevention of these disorders.

The discovery of the involvement of FUS/TLS, EWS and TAF15 in ALS and in other neurodegenerative diseases reinforces the role of altered RNA processing as a driving mechanism in neurodegeneration. Well known examples include errors in RNA metabolism from loss of survival of motor neurons (SMN) in spinal muscular atrophy (SMA) and FMRP in fragile-X mental retardation, and many others (for a review see Refs.[147,148]).

Overall the emerging roles of FET proteins discussed in this review add considerable support to the proposal that defects in RNA processing play a central role in neurodegeneration.

References

1. Tan AY, Manley JL. The TET family of proteins: functions and roles in disease. J Mol Cell Biol 2009; 1:82-92; PMID:19783543; http://dx.doi.org/10.1093/jmcb/mjp025

2. Paronetto MP. Ewing sarcoma protein: a key player in human cancer. Int J Cell Biol 2013; 2013:642853; PMID: 24082883; http://dx.doi.org/10.1155/2013/642853

3. Baechtold H, Kuroda M, Sok J, Ron D, Lopez BS, Akhmedov AT. Human 75-kDa DNA-pairing protein is identical to the pro-onco-protein TLS/FUS and is able to promote D-loop formation. J Biol Chem 1999; 274:34337-42; PMID:10567410; http://dx.doi.org/10.1074/jbc.274.48.34337

4. Li H, Watford W, Li C, Parmelee A, Bryant MA, Deng C, O'Shea J, Lee SB. Ewing sarcoma gene EWS is essential for meiosis and B lymphocyte development. J Clin Invest 2007; 117:1314-23; PMID:17415412; http://dx.doi.org/10.1172/JCI31222

5. Paronetto MP, Miñana B, Valcárcel J. The Ewing sarcoma protein regulates DNA damage-induced alternative splicing. Mol Cell 2011; 43:353-68; PMID:21816343; http://dx.doi.org/10.1016/j.molcel.2011.05.035

6. Bertolotti A, Lutz Y, Heard DJ, Chambon P, Tora L. hTAF(II)68, a novel RNA/ssDNA-binding protein with homology to the pro-onco-proteins TLS/FUS and EWS is associated with both TFIID and RNA polymerase II. EMBO J 1996; 15:5022-31; PMID:8890175

7. Bertolotti A, Melot T, Acker J, Vigneron M, Delattre O, Tora L. EWS, but not EWS-FLI-1, is associated with both TFIID and RNA polymerase II: interactions between two members of the TET family, EWS and hTAFII68, and subunits of TFIID and RNA polymerase II complexes. Mol Cell Biol 1998; 18:1489-97; PMID:9488465; http://dx.doi.org/10.1128/MCB.18.3.1489

8. Yang L, Embree LJ, Tsai S, Hickstein DD. Oncoprotein TLS interacts with serine-arginine proteins involved in RNA splicing. J Biol Chem 1998; 273:27761-4; PMID:9774382; http://dx.doi.org/10.1074/jbc.273.43.27761

9. Lerga A, Hallier M, Delva L, Orvain C, Gallais I, Marie J, Moreau-Gachelin F. Identification of an RNA binding specificity for the potential splicing factor TLS. J Biol Chem 2001; 276:6807-16; PMID:11098054; http://dx.doi.org/10.1074/jbc.M008304200

10. Chansky HA, Hu M, Hickstein DD, Yang L. Oncogenic TLS/ERG and EWS/Fli-1 fusion proteins inhibit RNA splicing mediated by YB-1 protein. Cancer Res 2001; 61:3586-90; PMID:11325824

11. Meissner M, Lopato S, Gotzmann J, Sauermann G, Barta A. Proto-oncoprotein TLS/FUS is associated to the nuclear matrix and complexed with splicing factors PTB, SRm160, and SR proteins. Exp Cell Res 2003; 283:184-95; PMID:12581738; http://dx.doi.org/10.1016/S0014-4827(02)00046-0

12. Dutertre M, Sanchez G, De Cian MC, Barbier J, Dardenne E, Gratadou L, Dujardin G, Le Jossic-Corcos C, Corcos L, Auboeuf D. Cotranscriptional exon skipping in the genotoxic stress response. Nat Struct Mol Biol 2010; 17:1358-66; PMID: 20972445; http://dx.doi.org/10.1038/nsmb.1912

13. Paronetto MP, Bernardis I, Volpe E, Bechara E, Sebestyén E, Eyras E, Valcárcel J. Regulation of FAS exon definition and apoptosis by the Ewing sarcoma protein. Cell Rep 2014; 7:1211-26; PMID:24813895; http://dx.doi.org/10.1016/j.celrep.2014.03.077

14. Burd CG, Dreyfuss G. Conserved structures and diversity of functions of RNA-binding proteins. Science 1994; 265:615-21; PMID:8036511; http://dx.doi.org/10.1126/science.8036511

15. Crozat A, Aman P, Mandahl N, Ron D. Fusion of CHOP to a novel RNA-binding protein in human myxoid liposarcoma. Nature 1993; 363:640-4; PMID:8510758; http://dx.doi.org/10.1038/363640a0

16. Delattre O, Zucman J, Plougastel B, Desmaze C, Melot T, Peter M, Kovar H, Joubert I, de Jong P, Rouleau G, et al. Gene fusion with an ETS DNA-binding domain caused by chromosome translocation in human tumours. Nature 1992; 359:162-5; PMID: 1522903; http://dx.doi.org/10.1038/359162a0

17. Andersson MK, Ståhlberg A, Arvidsson Y, Olofsson A, Semb H, Stenman G, Nilsson O, Aman P. The multifunctional FUS, EWS and TAF15 proto-oncoproteins show cell type-specific expression patterns and involvement in cell spreading and stress response. BMC Cell Biol 2008; 9:37; PMID:18620564; http://dx.doi.org/10.1186/1471-2121-9-37

18. Aoki N, Higashi S, Kawakami I, Kobayashi Z, Hosokawa M, Katsuse O, Togo T, Hirayasu Y, Akiyama H. Localization of fused in sarcoma (FUS) protein to the post-synaptic density in the brain. Acta Neuropathol 2012; 124:383-94; PMID:22526020; http://dx.doi.org/10.1007/s00401-012-0984-6

19. Tolino M, Köhrmann M, Kiebler MA. RNA-binding proteins involved in RNA localization and their implications in neuronal diseases. Eur J Neurosci 2012; 35:1818-36; PMID:22708593; http://dx.doi.org/10.1111/j.1460-9568.2012.08160.x

20. Mackenzie IR, Neumann M. FET proteins in frontotemporal dementia and amyotrophic lateral sclerosis. Brain Res 2012; 1462:40-3; PMID: 22261247; http://dx.doi.org/10.1016/j.brainres.2011.12.010

21. Neumann M, Rademakers R, Roeber S, Baker M, Kretzschmar HA, Mackenzie IR. A new subtype of frontotemporal lobar degeneration with FUS pathology. Brain 2009; 132(Pt 11):2922-31; PMID:19674978; http://dx.doi.org/10.1093/brain/awp214

22. Rademakers R, Neumann M, Mackenzie IR. Advances in understanding the molecular basis of frontotemporal dementia. Nat Rev Neurol 2012; 8:423-34; PMID: 22732773; http://dx.doi.org/10.1038/nrneurol.2012.117

23. Kwiatkowski TJ, Jr, Bosco DA, Leclerc AL, Tamrazian E, Vanderburg CR, Russ C, Davis A, Gilchrist J, Kasarskis EJ, Munsat T, et al. Mutations in the FUS/TLS gene on chromosome 16 cause familial amyotrophic lateral sclerosis. Science 2009; 323:1205-8; PMID:19251627; http://dx.doi.org/10.1126/science.1166066

24. Vance C, Rogelj B, Hortobágyi T, De Vos KJ, Nishimura AL, Sreedharan J, Hu X, Smith B, Ruddy D, Wright P, et al. Mutations in FUS, an RNA processing protein, cause familial amyotrophic lateral sclerosis type 6. Science 2009; 323:1208-11; PMID:19251628; http://dx.doi.org/10.1126/science.1165942

25. Sharma A, Lyashchenko AK, Lu L, Nasrabady SE, Elmaleh M, Mendelsohn M, Nemes A, Tapia JC, Mentis GZ, Shneider NA. ALS-associated mutant FUS induces selective motor neuron degeneration through toxic gain of function. Nat Commun 2016; 7:10465; PMID:26842965; http://dx.doi.org/10.1038/ncomms10465

26. Scekic-Zahirovic J, Sendscheid O, El Oussini H, Jambeau M, Sun Y, Mersmann S, Wagner M, Dieterlé S, Sinniger J, Dirrig-Grosch S, et al. Toxic gain of function from mutant FUS protein is crucial to trigger cell autonomous motor neuron loss. EMBO J 2016; 35:1077-97; PMID:26951610; http://dx.doi.org/10.15252/embj.201592559

27. Kuroda M, Sok J, Webb L, Baechtold H, Urano F, Yin Y, Chung P, de Rooij DG, Akhmedov A, Ashley T, et al. Male sterility and enhanced radiation sensitivity in TLS(−/−) mice. EMBO J 2000; 19:453-62; PMID:10654943; http://dx.doi.org/10.1093/emboj/19.3.453

28. Hicks GG, Singh N, Nashabi A, Mai S, Bozek G, Klewes L, Arapovic D, White EK, Koury MJ, Oltz EM, et al. Fus deficiency in mice results in defective B-lymphocyte development and activation, high levels of chromosomal instability and perinatal death. Nat Genet 2000; 24:175-9; PMID:10655065; http://dx.doi.org/10.1038/72842

29. Cho J, Shen H, Yu H, Li H, Cheng T, Lee SB, Lee BC. Ewing sarcoma gene Ews regulates hematopoietic stem cell senescence. Blood 2011; 117:1156-66; PMID:21030557; http://dx.doi.org/10.1182/blood-2010-04-279349

30. Rossi DJ, Bryder D, Seita J, Nussenzweig A, Hoeijmakers J, Weissman IL. Deficiencies in DNA damage repair limit the function of haematopoietic stem cells with age. Nature 2007; 447:725-9; PMID:17554309; http://dx.doi.org/10.1038/nature05862

31. Hurov KE, Cotta-Ramusino C, Elledge SJ. A genetic screen identifies the Triple T complex required for DNA damage signaling and ATM and ATR stability. Genes Dev 2010; 24:1939-50; PMID:20810650; http://dx.doi.org/10.1101/gad.1934210

32. O'Connell BC, Adamson B, Lydeard JR, Sowa ME, Ciccia A, Bredemeyer AL, Schlabach M, Gygi SP, Elledge SJ, Harper JW. A genome-wide camptothecin sensitivity screen identifies a mammalian MMS22L-NFKBIL2 complex required for genomic stability. Mol Cell 2010; 40:645-57; PMID:21055985; http://dx.doi.org/10.1016/j.molcel.2010.10.022

33. Park JH, Kang HJ, Kang SI, Lee JE, Hur J, Ge K, Mueller E, Li H, Lee BC, Lee SB. A multifunctional protein, EWS, is essential for early brown fat lineage determination. Dev Cell 2013; 26:393-404; PMID:23987512; http://dx.doi.org/10.1016/j.devcel.2013.07.002

34. Park JH, Lee SB. An essential role for Ewing sarcoma gene (EWS) in early white adipogenesis. Obesity (Silver Spring) 2015; 23:138-44; PMID:25407167; http://dx.doi.org/10.1002/oby.20934

35. Handel MA. The XY body: a specialized meiotic chromatin domain. Exp Cell Res 2004; 296:57-63; PMID:15120994; http://dx.doi.org/10.1016/j.yexcr.2004.03.008

36. Sugawara T, Oguro H, Negishi M, Morita Y, Ichikawa H, Iseki T, Yokosuka O, Nakauchi H, Iwama A. FET family proto-oncogene Fus contributes to self-renewal of hematopoietic stem cells. Exp Hematol 2010; 38:696-706; PMID: 20412831; http://dx.doi.org/10.1016/j.exphem.2010.04.006

37. Kino Y, Washizu C, Kurosawa M, Yamada M, Miyazaki H, Akagi T, Hashikawa T, Doi H, Takumi T, Hicks GG, et al. FUS/TLS deficiency causes behavioral and pathological abnormalities distinct from amyotrophic lateral sclerosis. Acta Neuropathol Commun 2015; 3:24; PMID:25907258; http://dx.doi.org/10.1186/s40478-015-0202-6

38. Fujii R, Grossenbacher-Zinchuk O, Jamari I, Wang Y, Zinchuk V, Takumi T. TLS-GFP cannot rescue mRNP formation near spines and spine phenotype in TLS-KO. Neuroreport 2009; 20:57-61; PMID:18989236; http://dx.doi.org/10.1097/WNR.0b013e32831bedb0

39. Frickenhaus M, Wagner M, Mallik M, Catinozzi M, Storkebaum E. Highly efficient cell-type-specific gene inactivation reveals a key function for the Drosophila FUS homolog cabeza in neurons. Sci Rep 2015; 5:9107; PMID: 25772687; http://dx.doi.org/10.1038/srep09107

40. Stolow DT, Haynes SR. 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. 1995; 23:835-43; PMID:7708500; http://dx.doi.org/10.1093/nar/23.5.835

41. Chen Y, Yang M, Deng J, Chen X, Ye Y, Zhu L, Liu J, Ye H, Shen Y, Li Y, et al. Expression of human FUS protein in Drosophila leads to progressive neurodegeneration. Protein Cell 2011 Jun; 2(6):477-86; PMID:21748598; http://dx.doi.org/10.1007/s13238-011-1065-7

42. Armstrong GA, Drapeau P. Loss and gain of FUS function impair neuromuscular synaptic transmission in a genetic model of ALS. Hum Mol Genet 2013 Nov 1; 22(21):4282-92; PMID:23771027; http://dx.doi.org/10.1093/hmg/ddt278

43. Azuma M, Embree LJ, Sabaawy H, Hickstein DD. Ewing sarcoma protein ewsr1 maintains mitotic integrity and proneural cell survival in the zebrafish embryo. PLoS One 2007; 2:e979; PMID: 17912356; http://dx.doi.org/10.1371/journal.pone.0000979

44. Shaw CE, Al-Chalabi A, Leigh N. Progress in the pathogenesis of amyotrophic lateral sclerosis. Curr Neurol Neurosci Rep 2001; 1:69-76; PMID:11898502; http://dx.doi.org/10.1007/s11910-001-0078-7

45. Rosen DR, Siddique T, Patterson D, Figlewicz DA, Sapp P, Hentati A, Donaldson D, Goto J, O'Regan JP, Deng HX, et al. Mutations in Cu/Zn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis. Nature 1993; 362:59-62; PMID:8446170; http://dx.doi.org/10.1038/362059a0

46. Greenway MJ, Andersen PM, Russ C, Ennis S, Cashman S, Donaghy C, Patterson V, Swingler R, Kieran D, Prehn J, et al. ANG mutations segregate with familial and 'sporadic' amyotrophic lateral sclerosis. Nat Genet 2006; 38:411-3; http://dx.doi.org/10.1038/ng1742

47. DeJesus-Hernandez M, Mackenzie IR, Boeve BF, Boxer AL, Baker M, Rutherford NJ, Nicholson AM, Finch NA, Flynn H, Adamson J, et al. Expanded GGGGCC hexanucleotide repeat in noncoding region of C9ORF72 causes chromosome 9p-linked FTD and ALS. Neuron 2011; 72:245-56; PMID:21944778; http://dx.doi.org/10.1016/j.neuron.2011.09.011

48. Kabashi E, Valdmanis PN, Dion P, Spiegelman D, McConkey BJ, Vande Velde C, Bouchard JP, Lacomblez L, Pochigaeva K, Salachas F, et al. TARDBP mutations in individuals with sporadic and familial amyotrophic lateral sclerosis. Nat Genet 2008; 40:572-4; PMID:18372902; http://dx.doi.org/10.1038/ng.132

49. Sreedharan J, Blair IP, Tripathi VB, Hu X, Vance C, Rogelj B, Ackerley S, Durnall JC, Williams KL, Buratti E, et al. TDP-43 mutations in familial and sporadic amyotrophic lateral sclerosis. Science 2008; 319:1668-72; PMID:18309045; http://dx.doi.org/10.1126/science.1154584

50. Arai T, Hasegawa M, Akiyama H, Ikeda K, Nonaka T, Mori H, Mann D, Tsuchiya K, Yoshida M, Hashizume Y, et al. TDP-43 is a component of ubiquitin-positive tau-negative inclusions in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. Biochem Biophys Res Commun 2006; 351:602-11; PMID: 17084815; http://dx.doi.org/10.1016/j.bbrc.2006.10.093

51. Neumann M, Sampathu DM, Kwong LK, Truax AC, Micsenyi MC, Chou TT, Bruce J, Schuck T, Grossman M, Clark CM, et al. Ubiquitinated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. Science 2006; 314:130-3; PMID:17023659; http://dx.doi.org/10.1126/science.1134108

52. Deng H, Gao K, Jankovic J. The role of FUS gene variants in neurodegenerative diseases. Nat Rev Neurol 2014; 10:337-48; PMID:24840975; http://dx.doi.org/10.1038/nrneurol.2014.78

53. Neumann M, Bentmann E, Dormann D, Jawaid A, DeJesus-Hernandez M, Ansorge O, Roeber S, Kretzschmar HA, Munoz DG, Kusaka H, et al. FET proteins TAF15 and EWS are selective markers that distinguish FTLD with FUS pathology from amyotrophic lateral sclerosis with FUS mutations. Brain 2011; 134(Pt 9):2595-609; PMID:21856723; http://dx.doi.org/10.1093/brain/awr201

54. Dormann D, Rodde R, Edbauer D, Bentmann E, Fischer I, Hruscha A, Than ME, Mackenzie IR, Capell A, Schmid B, et al. ALS-associated fused in sarcoma (FUS) mutations disrupt Transportin-mediated nuclear import. EMBO J 2010; 29:2841-57; PMID:20606625; http://dx.doi.org/10.1038/emboj.2010.143

55. DeJesus-Hernandez M, Kocerha J, Finch N, Crook R, Baker M, Desaro P, Johnston A, Rutherford N, Wojtas A, Kennelly K, et al. De novo truncating FUS gene mutation as a cause of sporadic amyotrophic lateral sclerosis. Hum Mutat 2010; 31:E1377-89; PMID: 20232451; http://dx.doi.org/10.1002/humu.21241

56. Mitchell JC, McGoldrick P, Vance C, Hortobagyi T, Sreedharan J, Rogelj B, Tudor EL, Smith BN, Klasen C, Miller CC, et al. Overexpression of human wild-type FUS causes progressive motor neuron degeneration in an age- and dose-dependent fashion. Acta Neuropathol 2013; 125:273-88; PMID:22961620; http://dx.doi.org/10.1007/s00401-012-1043-z

57. Huang C, Zhou H, Tong J, Chen H, Liu YJ, Wang D, Wei X, Xia XG. FUS transgenic rats develop the phenotypes of amyotrophic lateral sclerosis and frontotemporal lobar degeneration. PLoS Genet 2011; 7:e1002011; PMID:21408206; http://dx.doi.org/10.1371/journal.pgen.1002011

58. Kabashi E, Bercier V, Lissouba A, Liao M, Brustein E, Rouleau GA, Drapeau P. FUS and TARDBP but not SOD1 interact in genetic models of amyotrophic lateral sclerosis. PLoS Genet 2011; 7(8):e1002214; PMID:21829392; http://dx.doi.org/10.1371/journal.pgen.1002214

59. Murakami T, Yang SP, Xie L, Kawano T, Fu D, Mukai A, Bohm C, Chen F, Robertson J, Suzuki H, et al. ALS mutations in FUS cause neuronal dysfunction and death in Caenorhabditis elegans by a dominant gain-of-function mechanism. Hum Mol Genet 2012; 21:1-9; PMID:21949354; http://dx.doi.org/10.1093/hmg/ddr417

60. Wang JW, Brent JR, Tomlinson A, Shneider NA, McCabe BD. The ALS-associated proteins FUS and TDP-43 function together to affect Drosophila locomotion and life span. J Clin Invest 2011; 121:4118-26; PMID: 21881207; http://dx.doi.org/10.1172/JCI57883

61. Lanson NA, Jr, Maltare A, King H, Smith R, Kim JH, Taylor JP, Lloyd TE, Pandey UB. A Drosophila model of FUS-related neurodegeneration reveals genetic interaction between FUS and TDP-43. Hum Mol Genet 2011; 20:2510-23; PMID:21487023; http://dx.doi.org/10.1093/hmg/ddr150

62. Shahidullah M, Le Marchand SJ, Fei H, Zhang J, Pandey UB, Dalva MB, Pasinelli P, Levitan IB. Defects in synapse structure and function precede motor neuron degeneration in Drosophila models of FUS-related ALS. J Neurosci 2013; 33(50):19590-8; PMID:24336723; http://dx.doi.org/10.1523/JNEUROSCI.3396-13.2013

63. Ticozzi N, Vance C, Leclerc AL, Keagle P, Glass JD, McKenna-Yasek D, Sapp PC, Silani V, Bosco DA, Shaw CE, et al. Mutational analysis reveals the FUS homolog TAF15 as a candidate gene for familial amyotrophic lateral sclerosis. Am J Med Genet B Neuropsychiatr Genet 2011; 156B(3):285-90; PMID:21438137; http://dx.doi.org/10.1002/ajmg.b.31158

64. Couthouis J, Hart MP, Shorter J, DeJesus-Hernandez M, Erion R, Oristano R, Liu AX, Ramos D, Jethava N, Hosangadi D, et al. A yeast functional screen predicts new candidate ALS disease genes. Proc Natl Acad Sci U S A 2011; 108:20881-90; PMID:22065782; http://dx.doi.org/10.1073/pnas.1109434108

65. Couthouis J, Hart MP, Erion R, King OD, Diaz Z, Nakaya T, Ibrahim F, Kim HJ, Mojsilovic-Petrovic J, Panossian S, et al. Evaluating the role of the FUS/TLS-related gene EWSR1 in amyotrophic lateral sclerosis. Hum Mol Genet 2012; 21:2899-911; PMID:22454397; http://dx.doi.org/10.1093/hmg/dds116

66. Gal J, Zhang J, Kwinter DM, Zhai J, Jia H, Jia J, Zhu H. Nuclear localization sequence of FUS and induction of stress granules by ALS mutants. Neurobiol Aging 2011; 32:2323.e27-40; PMID:20674093; http://dx.doi.org/10.1016/j.neurobiolaging.2010.06.010

67. Jobert L, Argentini M, Tora L. PRMT1 mediated methylation of TAF15 is required for its positive gene regulatory function. Exp Cell Res 2009 Apr 15; 315(7):1273-86; PMID:19124016; http://dx.doi.org/10.1016/j.yexcr.2008.12.008

68. Ling SC, Polymenidou M, Cleveland DW. Converging mechanisms in ALS and FTD: disrupted RNA and protein homeostasis. Neuron 2013; 79:416-38; PMID:23931993; http://dx.doi.org/10.1016/j.neuron.2013.07.033

69. Daigle JG, Lanson NA, Jr, Smith RB, Casci I, Maltare A, Monaghan J, Nichols CD, Kryndushkin D, Shewmaker F, Pandey UB. RNA-binding ability of FUS regulates neurodegeneration, cytoplasmic mislocalization and incorporation into stress granules associated with FUS carrying ALS-linked mutations. Hum Mol Genet 2013; 22:1193-205; PMID:23257289; http://dx.doi.org/10.1093/hmg/dds526

70. King OD, Gitler AD, Shorter J. The tip of the iceberg: RNA-binding proteins with prion-like domains in neurodegenerative disease. Brain Res 2012; 1462:61-80; PMID:22445064; http://dx.doi.org/10.1016/j.brainres.2012.01.016

71. Tradewell ML, Yu Z, Tibshirani M, Boulanger MC, Durham HD, Richard S. Arginine methylation by PRMT1 regulates nuclear-cytoplasmic localization and toxicity of FUS/TLS harbouring ALS-linked mutations. Hum Mol Genet 2012; 21:136-49; PMID:21965298; http://dx.doi.org/10.1093/hmg/ddr448

72. McKhann GM, Albert MS, Grossman M, Miller B, Dickson D, Trojanowski JQ. Work Group on Frontotemporal Dementia and Pick's Disease. Clinical and pathological diagnosis of frontotemporal dementia: report of the work group on frontotemporal dementia and pick's disease. Arch Neurol 2001; 58:1803-9; PMID:11708987; http://dx.doi.org/10.1001/archneur.58.11.1803

73. Seltman RE, Matthews BR. Frontotemporal lobar degeneration: epidemiology, pathology, diagnosis and management. CNS Drugs 2012; 26:841-70; PMID:22950490; http://dx.doi.org/10.2165/11640070-000000000-00000

74. Seelaar H, Kamphorst W, Rosso SM, Azmani A, Masdjedi R, de Koning I, Maat-Kievit JA, Anar B, Donker Kaat L, Breedveld GJ, et al. Distinct genetic forms of frontotemporal dementia. Neurology 2008; 71:1220-6; PMID:18703462; http://dx.doi.org/10.1212/01.wnl.0000319702.37497.72

75. Hutton M, Lendon CL, Rizzu P, Baker M, Froelich S, Houlden H, Pickering-Brown S, Chakraverty S, Isaacs A, Grover A, et al. Association of missense and 5'-splice-site mutations in tau with the inherited dementia FTDP-17. Nature 1998; 393:702-5; PMID:9641683; http://dx.doi.org/10.1038/31508

76. Baker M, Mackenzie IR, Pickering-Brown SM, Gass J, Rademakers R, Lindholm C, Snowden J, Adamson J, Sadovnick AD, Rollinson S, et al. Mutations in progranulin cause tau-negative frontotemporal dementia linked to chromosome 17. Nature 2006; 442:916-9; PMID:16862116; http://dx.doi.org/10.1038/nature05016

77. Renton AE, Majounie E, Waite A, Simón-Sánchez J, Rollinson S, Gibbs JR, Schymick JC, Laaksovirta H, van Swieten JC, Myllykangas L, et al. A hexanucleotide repeat expansion in C9ORF72 is the cause of chromosome 9p21-linked ALS-FTD. Neuron 2011; 72:257-68; PMID:21944779; http://dx.doi.org/10.1016/j.neuron.2011.09.010

78. Lipton AM, White CL, 3rd, Bigio EH. Frontotemporal lobar degeneration with motor neuron disease-type inclusions predominates in 76 cases of frontotemporal degeneration. Acta Neuropathol 2004; 108:379-85; PMID:15351890; http://dx.doi.org/10.1007/s00401-004-0900-9

79. Mackenzie IR, Neumann M, Bigio EH, Cairns NJ, Alafuzoff I, Kril J, Kovacs GG, Ghetti B, Halliday G, Holm IE, et al. Nomenclature for neuropathologic subtypes of frontotemporal lobar degeneration: consensus recommendations. Acta Neuropathol 2009; 117:15-8; PMID:19015862; http://dx.doi.org/10.1007/s00401-008-0460-5

80. Munoz DG, Neumann M, Kusaka H, Yokota O, Ishihara K, Terada S, Kuroda S, Mackenzie IR. FUS pathology in basophilic inclusion body disease. Acta Neuropathol 2009; 118:617-27; PMID:19830439; http://dx.doi.org/10.1007/s00401-009-0598-9

81. Van Langenhove T, van der Zee J, Sleegers K, Engelborghs S, Vandenberghe R, Gijselinck I, Van den Broeck M, Mattheijssens M, Peeters K, De Deyn PP, et al. Genetic contribution of FUS to frontotemporal lobar degeneration. Neurology 2010; 74:366-71; PMID:20124201; http://dx.doi.org/10.1212/WNL.0b013e3181ccc732

82. Broustal O, Camuzat A, Guillot-Noël L, Guy N, Millecamps S, Deffond D, Lacomblez L, Golfier V, Hannequin D, Salachas F, et al. French clinical and genetic research network on FTD/FTD-MND. FUS mutations in frontotemporal lobar degeneration with amyotrophic lateral sclerosis. J Alzheimers Dis 2010; 22:765-9; PMID:21158017

83. Snowden JS, Hu Q, Rollinson S, Halliwell N, Robinson A, Davidson YS, Momeni P, Baborie A, Griffiths TD, Jaros E, et al. The most common type of FTLD-FUS (aFTLD-U) is associated with a distinct clinical form of frontotemporal dementia but is not related to mutations in the FUS gene. Acta Neuropathol 2011; 122:99-110; PMID:21424531; http://dx.doi.org/10.1007/s00401-011-0816-0

84. Urwin H, Josephs KA, Rohrer JD, Mackenzie IR, Neumann M, Authier A, Seelaar H, Van Swieten JC, Brown JM, Johannsen P, et al. FUS pathology defines the majority of tau- and TDP-43-negative frontotemporal lobar degeneration. Acta Neuropathol 2010 Jul; 120(1):33-41; PMID:20490813; http://dx.doi.org/10.1007/s00401-010-0698-6

85. Davidson YS, Robinson AC, Hu Q, Mishra M, Baborie A, Jaros E, Perry RH, Cairns NJ, Richardson A, Gerhard A, et al. Nuclear carrier and RNA-binding proteins in frontotemporal lobar degeneration associated with fused in sarcoma (FUS) pathological changes. Neuropathol Appl Neurobiol 2013 Feb; 39(2):157-65; PMID:22497712; http://dx.doi.org/10.1111/j.1365-2990.2012.01274.x

86. Zinszner H, Sok J, Immanuel D, Yin Y, Ron D. TLS (FUS) binds RNA in vivo and engages in nucleo-cytoplasmic shuttling. J Cell Sci 1997; 110 (Pt 15):1741-50; PMID:9264461

87. Neumann M, Valori CF, Ansorge O, Kretzschmar HA, Munoz DG, Kusaka H, Yokota O, Ishihara K, Ang LC, Bilbao JM, et al. Transportin 1 accumulates specifically with FET proteins but no other transportin cargos in FTLD-FUS and is absent in FUS inclusions in ALS with FUS mutations. Acta Neuropathol 2012; 124(5):705-16; PMID:22842875; http://dx.doi.org/10.1007/s00401-012-1020-6

88. Dormann D, Madl T, Valori CF, Bentmann E, Tahirovic S, Abou-Ajram C, Kremmer E, Ansorge O, Mackenzie IR, Neumann M, et al. Arginine methylation next to the PY-NLS modulates Transportin binding and nuclear import of FUS. EMBO J 2012; 31(22):4258-75; PMID:22968170; http://dx.doi.org/10.1038/emboj.2012.261

89. Han TW, Kato M, Xie S, Wu LC, Mirzaei H, Pei J, Chen M, Xie Y, Allen J, Xiao G, et al. Cell-free formation of RNA granules: bound RNAs identify features and components of cellular assemblies. Cell 2012; 149(4):768-79; PMID:22579282; http://dx.doi.org/10.1016/j.cell.2012.04.016

90. Darovic S, PrparMihevc S, Župunski V, Gunčar G, Štalekar M, Lee YB, Shaw CE, Rogelj B. Phosphorylation of C-terminal tyrosine residue 526 in FUS impairs its nuclear import. J Cell Sci 2015 Nov 15; 128(22):4151-9; PMID:26403203; http://dx.doi.org/10.1242/jcs.176602

91. Marko M, Vlassis A, Guialis A, Leichter M. Domains involved in TAF15 subcellular localisation: dependence on cell type and ongoing transcription. Gene 2012; 506(2):331-8; PMID:22771914; http://dx.doi.org/10.1016/j.gene.2012.06.088

92. Louis ED. Essential tremor. Lancet Neurol 2005; 4(2):100-10; PMID:15664542; http://dx.doi.org/10.1016/S1474-4422(05)00991-9

93. Merner ND, Girard SL, Catoire H, Bourassa CV, Belzil VV, Rivière JB, Hince P, Levert A, Dionne-Laporte A, Spiegelman D, et al. Exome sequencing identifies FUS mutations as a cause of essential tremor. Am J Hum Genet 2012; 91:313-9; PMID:22863194; http://dx.doi.org/10.1016/j.ajhg.2012.07.002

94. Zheng W, Deng X, Liang H, Song Z, Gao K, Yang Y, Deng H. Genetic analysis of the fused in sarcoma gene in Chinese Han patients with essential tremor. Neurobiol Aging 2013; 34:2078.e3-4; PMID:23582660; http://dx.doi.org/10.1016/j.neurobiolaging.2013.03.001

95. Rajput A, Rajput AH, Rajput ML, Encarnacion M, Bernales CQ, Ross JP, Farrer MJ, Vilariño-Güell C. Identification of FUS p.R377W in essential tremor. Eur J Neurol 2014; 21:361-3; PMID:23834483; http://dx.doi.org/10.1111/ene.12231

96. Lorenzo-Betancor O, Ogaki K, Soto-Ortolaza A, Labbé C, Vilariño-Güell C, Rajput A, Rajput AH, Pastor P, Ortega S, Lorenzo E, et al. Analysis of nuclear export sequence regions of FUS-Related RNA-binding proteins in essential tremor. PLoS One 2014; 9:e111989; PMID:25375143; http://dx.doi.org/10.1371/journal.pone.0111989

97. Rodriguez-Oroz MC, Jahanshahi M, Krack P, Litvan I, Macias R, Bezard E, Obeso JA. Initial clinical manifestations of Parkinson disease: features and pathophysiological mechanisms. Lancet Neurol 2009; 8:1128-39; PMID:19909911; http://dx.doi.org/10.1016/S1474-4422(09)70293-5

98. De Rosa P, Marini ES, Gelmetti V, Valente EM. Candidate genes for Parkinson disease: lessons from pathogenesis. Clin Chim Acta 2015; 449:68-76; PMID:26048192; http://dx.doi.org/10.1016/j.cca.2015.04.042

99. Labbé C, Rayaprolu S, Soto-Ortolaza A, Ogaki K, Uitti RJ, Wszolek ZK, Ross OA. Investigating FUS variation in Parkinson disease. Parkinsonism Relat Disord 2014; 20 Suppl 1:S147-9; PMID:http://dx.doi.org/10.1016/S1353-8020(13)70035-X

100. Chouraki V, Seshadri S. Genetics of Alzheimer disease. Adv Genet 2014; 87:245-94; PMID:25311924; http://dx.doi.org/10.1016/B978-0-12-800149-3.00005-6

101. Ohno T, Ouchida M, Lee L, Gatalica Z, Rao VN, Reddy ES. The EWS gene, involved in Ewing family of tumors, malignant melanoma of soft parts and desmoplastic small round cell tumors, codes for an RNA binding protein with novel regulatory domains. Oncogene 1994; 9:3087-97; PMID:8084618

102. Tuerk C, Gold L. Systematic evolution of ligands by exponential enrichment: RNA ligands to bacteriophage T4 DNA polymerase. Science 1990; 249:505-10; PMID:2200121; http://dx.doi.org/10.1126/science.2200121

103. Iko Y, Kodama TS, Kasai N, Oyama T, Morita EH, Muto T, Okumura M, Fujii R, Takumi T, Tate S, et al. Domain architectures and characterization of an RNA-binding protein, TLS. J Biol Chem 2004; 279:44834-40; PMID:15299008; http://dx.doi.org/10.1074/jbc.M408552200

104. Liu X, Niu C, Ren J, Zhang J, Xie X, Zhu H, Feng W, Gong W. The RRM domain of human fused in sarcoma protein reveals a non-canonical nucleic acid binding site. Biochim Biophys Acta 2013; 1832:375-85; PMID:23200923; http://dx.doi.org/10.1016/j.bbadis.2012.11.012

105. Morohoshi F, Arai K, Takahashi EI, Tanigami A, Ohki M. Cloning and mapping of a human RBP56 gene encoding a putative RNA binding protein similar to FUS/TLS and EWS proteins. Genomics 1996; 38:51-7; PMID:8954779; http://dx.doi.org/10.1006/geno.1996.0591

106. Rappsilber J, Ryder U, Lamond AI, Mann M. Large-scale proteomic analysis of the human spliceosome. Genome Res 2002; 12:1231-45; PMID:12176931; http://dx.doi.org/10.1101/gr.473902

107. Zhou Z, Licklider LJ, Gygi SP, Reed R. Comprehensive proteomic analysis of the human spliceosome. Nature 2002; 419:182-5; PMID:12226669; http://dx.doi.org/10.1038/nature01031

108. Wu S, Green MR. Identification of a human protein that recognizes the 3' splice site during the second step of pre-mRNA splicing. EMBO J 1997; 16:4421-32; PMID:9250686; http://dx.doi.org/10.1093/emboj/16.14.4421

109. Zhang D, Paley AJ, Childs G. The transcriptional repressor ZFM1 interacts with and modulates the ability of EWS to activate transcription. J Biol Chem 1998; 273:18086-91; PMID:9660765; http://dx.doi.org/10.1074/jbc.273.29.18086

110. Knoop LL, Baker SJ. The splicing factor U1C represses EWS/FLI-mediated transactivation. J Biol Chem 2000; 275:24865-71; PMID:10827180; http://dx.doi.org/10.1074/jbc.M001661200

111. Hoell JI, Larsson E, Runge S, Nusbaum JD, Duggimpudi S, Farazi TA, Hafner M, Borkhardt A, Sander C, Tuschl T. RNA targets of wild-type and mutant FET family proteins. Nat Struct Mol Biol 2011; 18:1428-31; http://dx.doi.org/10.1038/nsmb.2163

112. Ishigaki S, Masuda A, Fujioka Y, Iguchi Y, Katsuno M, Shibata A, Urano F, Sobue G, Ohno K. Position-dependent FUS-RNA interactions regulate alternative splicing events and transcriptions. Sci Rep 2012; 2:529; PMID:22829983; http://dx.doi.org/10.1038/srep00529

113. Lagier-Tourenne C, Polymenidou M, Hutt KR, Vu AQ, Baughn M, Huelga SC, Clutario KM, Ling SC, Liang TY, Mazur C, et al. Divergent roles of ALS-linked proteins FUS/TLS and TDP-43 intersect in processing long pre-mRNAs. Nat Neurosci 2012; 15:1488-97; PMID:23023293; http://dx.doi.org/10.1038/nn.3230

114. Rogelj B, Easton LE, Bogu GK, Stanton LW, Rot G, Curk T, Zupan B, Sugimoto Y, Modic M, Haberman N, et al. Widespread binding of FUS along nascent RNA regulates alternative splicing in the brain. Sci Rep 2012; 2:603; PMID:22934129; http://dx.doi.org/10.1038/srep00603

115. Colombrita C, Onesto E, Buratti E, de la Grange P, Gumina V, Baralle FE, Silani V, Ratti A. From transcriptomic to protein level changes in TDP-43 and FUS loss-of-function cell models. Biochim Biophys Acta 2015; 1849:1398-410; PMID:26514432; http://dx.doi.org/10.1016/j.bbagrm.2015.10.015

116. Nakaya T, Alexiou P, Maragkakis M, Chang A, Mourelatos Z. FUS regulates genes coding for RNA-binding proteins in neurons by binding to their highly conserved introns. RNA 2013; 19:498-509; PMID:23389473; http://dx.doi.org/10.1261/rna.037804.112

117. Zhou Y, Liu S, Liu G, Oztürk A, Hicks GG. ALS-associated FUS mutations result in compromised FUS alternative splicing and autoregulation. PLoS Genet 2013; 9:e1003895; PMID:24204307; http://dx.doi.org/10.1371/journal.pgen.1003895

118. Gregory RI, Yan KP, Amuthan G, Chendrimada T, Doratotaj B, Cooch N, Shiekhattar R. The Microprocessor complex mediates the genesis of microRNAs. Nature 2004; 432(7014):235-40; PMID:15531877; http://dx.doi.org/10.1038/nature03120

119. Morlando M, Dini Modigliani S, Torrelli G, Rosa A, Di Carlo V, Caffarelli E, Bozzoni I. FUS stimulates microRNA biogenesis by facilitating co-transcriptional Drosha recruitment. EMBO J 2012; 31:4502-10; http://dx.doi.org/10.1038/emboj.2012.319

120. Dini Modigliani S, Morlando M, Errichelli L, Sabatelli M, Bozzoni I. An ALS-associated mutation in the FUS 3'-UTR disrupts a micro-RNA-FUS regulatory circuitry. Nat Commun 2014; 5:4335; PMID:25004804; http://dx.doi.org/10.1038/ncomms5335

121. Cuellar TL, Davis TH, Nelson PT, Loeb GB, Harfe BD, Ullian E, McManus MT. Dicer loss in striatal neurons produces behavioral and neuroanatomical phenotypes in the absence of neurodegeneration. Proc Natl Acad Sci U S A 2008; 105:5614-9; PMID:18385371; http://dx.doi.org/10.1073/pnas.0801689105

122. Davis TH, Cuellar TL, Koch SM, Barker AJ, Harfe BD, McManus MT, Ullian EM. Conditional loss of Dicer disrupts cellular and tissue morphogenesis in the cortex and hippocampus. J Neurosci 2008; 28:4322-30; PMID:18434510; http://dx.doi.org/10.1523/JNEUROSCI.4815-07.2008

123. Haramati S, Chapnik E, Sztainberg Y, Eilam R, Zwang R, Gershoni N, McGlinn E, Heiser PW, Wills AM, Wirguin I, et al. miRNA malfunction causes spinal motor neuron disease. Proc Natl Acad Sci U S A 2010; 107:13111-6; PMID:20616011; http://dx.doi.org/10.1073/pnas.1006151107

124. Schaefer A, O'Carroll D, Tan CL, Hillman D, Sugimori M, Llinas R, Greengard P. Cerebellar neurodegeneration in the absence of micro-RNAs. J Exp Med 2007; 204:1553-8; PMID:17606634; http://dx.doi.org/10.1084/jem.20070823

125. Campos-Melo D, Droppelmann CA, He Z, Volkening K, Strong MJ. Altered microRNA expression profile in Amyotrophic Lateral Sclerosis: a role in the regulation of NFL mRNA levels. Mol Brain 2013; 24; 6:26; PMID:23705811; http://dx.doi.org/10.1186/1756-6606-6-26

126. Chen-Plotkin AS, Unger TL, Gallagher MD, Bill E, Kwong LK, Volpicelli-Daley L, Busch JI, Akle S, Grossman M, Van Deerlin V, et al. TMEM106B, the risk gene for frontotemporal dementia, is regulated by the microRNA-132/212 cluster and affects progranulin pathways. J Neurosci 2012; 32:11213-27; PMID:22895706; http://dx.doi.org/10.1523/JNEUROSCI.0521-12.2012

127. Gascon E, Gao FB. The emerging roles of microRNAs in the pathogenesis of frontotemporal dementia-amyotrophic lateral sclerosis (FTD-ALS) spectrum disorders. J Neurogenet 2014; 28:30-40; PMID:24506814; http://dx.doi.org/10.3109/01677063.2013.876021

128. Ratti A, Buratti E. Physiological Functions and Pathobiology of TDP-43 and FUS/TLS proteins. J Neurochem 2016; PMID:27015757; http://dx.doi.org/10.1111/jnc.13625

129. Forman MS, Trojanowski JQ, Lee VM. Neurodegenerative diseases: a decade of discoveries paves the way for therapeutic breakthroughs. Nat Med 2004; 10:1055-63; PMID:15459709; http://dx.doi.org/10.1038/nm1113

130. Prusiner SB. Novel proteinaceous infectious particles cause scrapie. Science 1982; 216:136-44; PMID:6801762; http://dx.doi.org/10.1126/science.6801762

131. Maniecka Z, Polymenidou M. From nucleation to widespread propagation: A prion-like concept for ALS. Virus Res 2015; 207:94-105; PMID:25656065; http://dx.doi.org/10.1016/j.virusres.2014.12.032

132. Cushman M, Johnson BS, King OD, Gitler AD, Shorter J. Prion-like disorders: blurring the divide between transmissibility and infectivity. J Cell Sci 2010; 123(Pt 8):1191-201; PMID:20356930; http://dx.doi.org/10.1242/jcs.051672

133. Udan M, Baloh RH. Implications of the prion-related Q/N domains in TDP-43 and FUS. Prion 2011; 5:1-5; PMID:21135580; http://dx.doi.org/10.4161/pri.5.1.14265

134. Alberti S, Halfmann R, King O, Kapila A, Lindquist S. A systematic survey identifies prions and illuminates sequence features of prionogenic proteins. Cell 2009; 137:146-58; PMID:19345193; http://dx.doi.org/10.1016/j.cell.2009.02.044

135. Johnson BS, Snead D, Lee JJ, McCaffery JM, Shorter J, Gitler AD. TDP-43 is intrinsically aggregation-prone, and amyotrophic lateral sclerosis-linked mutations accelerate aggregation and increase toxicity. J Biol Chem 2009; 284:20329-39; PMID:19465477; http://dx.doi.org/10.1074/jbc.M109.010264

136. Johnson BS, McCaffery JM, Lindquist S, Gitler AD. A yeast TDP-43 proteinopathy model: Exploring the molecular determinants of TDP-43 aggregation and cellular toxicity. Proc Natl Acad Sci U S A 2008; 105:6439-44; PMID:18434538; http://dx.doi.org/10.1073/pnas.0802082105

137. Ash PE, Zhang YJ, Roberts CM, Saldi T, Hutter H, Buratti E, Petrucelli L, Link CD. Neurotoxic effects of TDP-43 overexpression in C. elegans. Hum Mol Genet 2010 Aug 15; 19(16):3206-18; PMID:20530643; http://dx.doi.org/10.1093/hmg/ddq230

138. Sun Z, Diaz Z, Fang X, Hart MP, Chesi A, Shorter J, Gitler AD. Molecular determinants and genetic modifiers of aggregation and toxicity for the ALS disease protein FUS/TLS. PLoS Biol 2011; 9: e1000614; http://dx.doi.org/10.1371/journal.pbio.1000614

139. Liu-Yesucevitz L, Bilgutay A, Zhang YJ, Vanderweyde T, Citro A, Mehta T, Zaarur N, McKee A, Bowser R, Sherman M, et al. Tar DNA binding protein-43 (TDP-43) associates with stress granules: analysis of cultured cells and pathological brain tissue. PLoS One 2010; 5(10):e13250; PMID:20948999; http://dx.doi.org/10.1371/journal.pone.0013250

140. Bentmann E, Neumann M, Tahirovic S, Rodde R, Dormann D, Haass C. Requirements for stress granule recruitment of fused in sarcoma (FUS) and TAR DNA-binding protein of 43 kDa (TDP-43). J Biol Chem 2012; 287:23079-94; PMID:22563080; http://dx.doi.org/10.1074/jbc.M111.328757

141. Ito D, Seki M, Tsunoda Y, Uchiyama H, Suzuki N. Nuclear transport impairment of amyotrophic lateral sclerosis-linked mutations in FUS/TLS. Ann Neurol 2011; 69:152-62; PMID:21280085; http://dx.doi.org/10.1002/ana.22246

142. Kato M, Han TW, Xie S, Shi K, Du X, Wu LC, Mirzaei H, Goldsmith EJ, Longgood J, Pei J, et al. Cell-free formation of RNA granules: low complexity sequence domains form dynamic fibers within hydrogels. Cell 2012; 149:753-67; PMID:22579281; http://dx.doi.org/10.1016/j.cell.2012.04.017

143. Patel A, Lee HO, Jawerth L, Maharana S, Jahnel M, Hein MY, Stoynov S, Mahamid J, Saha S, Franzmann TM, et al. A liquid-to-solid phase transition of the ALS Protein FUS accelerated by disease mutation. Cell 2015; 162:1066-77; PMID:26317470; http://dx.doi.org/10.1016/j.cell.2015.07.047

144. Glover JR, Lindquist S. Hsp104, Hsp70, and Hsp40: a novel chaperone system that rescues previously aggregated proteins. Cell 1998; 94:73-82; PMID:9674429; http://dx.doi.org/10.1016/S0092-8674(00)81223-4

145. Parsell DA, Kowal AS, Singer MA, Lindquist S. Protein disaggregation mediated by heat-shock protein Hsp104. Nature 1994; 372:475-8; PMID:7984243; http://dx.doi.org/10.1038/372475a0

146. Jackrel ME, Shorter J. Engineering enhanced protein disaggregases for neurodegenerative disease. Prion 2015; 9:90-109; PMID:25738979; http://dx.doi.org/10.1080/19336896.2015.1020277

147. Monani UR. Spinal muscular atrophy: a deficiency in a ubiquitous protein; a motor neuron-specific disease. Neuron 2005; 48:885-96; PMID:16364894; http://dx.doi.org/10.1016/j.neuron.2005.12.001

148. Darnell JC, Klann E. The translation of translational control by FMRP: therapeutic targets for FXS. Nat Neurosci 2013; 16:1530-6; PMID:23584741; http://dx.doi.org/10.1038/nn.3379