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FUS-mediated regulation of alternative RNA processing in neurons: insights from global transcriptome analysis

Authors

Akio Masuda,[1*] Jun-ichi Takeda,[1] and Kinji Ohno[1]

[1]Division of Neurogenetics, Center for Neurological Diseases and Cancer, Nagoya University Graduate School of Medicine, Nagoya, Japan

65 Tsurumai, Showa-ku, Nagoya 466-8550, Japan

*Corresponding author

Akio Masuda, MD, PhD

Division of Neurogenetics, Center for Neurological Diseases and Cancer,

Nagoya University Graduate School of Medicine,

65 Tsurumai, Showa-ku, Nagoya 466-8550, Japan

Phone: +81-52-744-2447, Fax: +81-52-744-2449, e-mail: amasuda@med.nagoya-u.ac.jp

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Abstract

Fused in sarcoma (FUS) is an RNA-binding protein that is causally associated with oncogenesis and neurodegeneration. Recently, the role of FUS in neurodegeneration has been extensively studied, because mutations in *FUS* are associated with amyotrophic lateral sclerosis (ALS), and the FUS protein has been identified as a major component of intracellular inclusions in neurodegenerative disorders including ALS and frontotemporal lobar degeneration. FUS is a key molecule in transcriptional regulation and RNA processing including processes such as pre-mRNA splicing and polyadenylation. Interaction of FUS with various components of the transcription machinery, spliceosome, and the 3′ end processing machinery has been identified. Furthermore, recent advances in high-throughput transcriptomic profiling approaches have enabled us to determine the mechanisms of FUS-dependent RNA processing networks at a cellular level. These analyses have revealed that depletion of FUS in neuronal cells affects alternative splicing and alternative polyadenylation of thousands of mRNAs. Gene Ontology analysis has suggested that FUS-modulated genes are implicated in neuronal functions and development. CLIP-seq of FUS has shown that FUS is frequently clustered around these alternative sites of nascent RNA. ChIP-seq of RNA polymerase II (RNAP II) has demonstrated that an interaction between FUS and nascent RNA downregulates local transcriptional activity of RNAP II, which is critically involved in RNA processing. Both alternative splicing and alternative polyadenylation are fundamental processes by which cells expand their transcriptomic diversity, and are particularly essential in the nervous system. Dependence of transcriptomic diversity on FUS makes the nervous system vulnerable to neurodegeneration, when FUS is functionally compromised.

INTRODUCTION

Fused in sarcoma (FUS), also known as translocated in sarcoma (TLS), belongs to the FET protein family, which includes EWS/EWSR1 (Ewing sarcoma breakpoint region 1) and TAF15/TAFII68 (TATA-binding protein-associated factor II, 68 kDa). The FET family proteins are ubiquitously expressed RNA binding proteins (RBPs), and genomic rearrangements in *FET* have been identified in human sarcomas and acute leukemias[1]. Mutations in these genes are associated with amyotrophic lateral sclerosis (ALS)[2-5], which is a neurodegenerative disease with muscle wasting due to progressive loss of upper and lower motor neurons in the brain and spinal cord. Mutations in *FUS* account for about 5% of the cases of familial ALS[2], and neuronal cytoplasmic inclusions positive for FUS and ubiquitin are commonly observed in these patients[2, 3]. ALS-causing mutations cluster in the nuclear localization sequence of FUS, which is essential for the nuclear import of FUS[6], and facilitates the formation of cytoplasmic inclusions. FUS-positive inclusions are also found in other neurodegenerative diseases. Frontotemporal lobar degeneration (FTLD) is a region-specific neurodegenerative disease with loss of neurons in the frontotemporal cortex, and it affects higher cognitive functions, such as behavior, language, memory, and personality. Although mutations in *FUS* have been rarely identified in cases of FTLD, FUS is present in the pathological inclusions in the majority of patients with FTLD who have no Tau- or TDP-43-containing inclusions[7, 8]. Furthermore, FUS has been identified in neuronal intranuclear inclusions in Huntington's disease, spinocerebellar ataxias 1, 2, 3, and dentatorubral-pallidoluysian atrophy[9-11]. Transgenic mice overexpressing FUS recapitulate some ALS-like phenotypes[12], and *Fus*-null hippocampal neurons exhibit abnormal morphology in dendritic spines and a low dendritic spine density[13].

FUS is predominantly localized in the nucleus[14]. FUS is involved in multiple steps of RNA metabolism such as transcription[15-17], alternative splicing[18, 19], and mRNA transport[20, 21], which are pivotal to various biological processes including neurogenesis. Recent advances in high-throughput sequencing technology have enabled us to determine the global mechanisms of regulatory networks in RNA processing (Figure 1). The challenge now is to completely understand the biological significance of FUS-regulated RNA processing and the underlying mechanisms of how dysregulation of these processes causes neurodegenerative disorders. In this review, we focus on the role of FUS in the regulation of RNA processing, which has been clarified by global transcriptomic analysis.

RECOGNITION OF SPECIFIC RNA BY FUS

Recognition of specific RNA by RBP is a key step in the regulation of RNA processing. Most RBPs directly interact with RNA through their RNA binding domains, which recognize specific structural features of target RNAs, such as a short sequence motifs and the secondary structure. Currently, high-throughput sequencing of a cDNA library generated by ultraviolet cross-linking and immunoprecipitation (CLIP-seq; Figure 1) is the most powerful tool to study protein-RNA interactions *in vivo*.

To globally identify the RNA binding sites of FUS, CLIP-seq analyses have been performed using various tissues and cells including mouse and human brain tissues[18, 19, 22], neuronal cells[16, 23], as well as other cell lines[24, 25]. In all these analyses, numerous FUS binding sites have been identified, and 60–80% of these binding sites were distributed in the intronic regions. The FUS-CLIP tag density is highest at the 5′ end and decreases toward the 3′ end, especially in long introns[16, 19, 22]. This binding pattern corresponds to the abundance of the nascent RNA, suggesting that FUS interacts with nascent RNA soon after it is generated[19, 22]. Gene Ontology analysis has shown that genes highly covered with FUS-CLIP tags are involved in neuron-specific functions, such as synaptic control, cell adhesion, and neuronal projection and recognition processes[23].

Analyses of CLIP-seq has revealed that FUS recognizes GU-rich motifs *in vivo*[16, 19, 22], which is similar to the GGUG motif identified using *in vitro* SELEX analysis[26]. In addition, NMR analysis showed that the zinc finger domain of FUS directly binds to GGUG-containing RNA[27]. Nevertheless, the enrichment of these GU-rich motifs is less represented in FUS-CLIP tags compared to the enrichment of motifs observed in CLIP-seq analyses of other RBPs[19, 22], suggesting that FUS binds to RNA with limited sequence specificity. Consistently, recent *in vitro* binding analysis has shown that FUS can bind to a wide range of nucleic acids and tends to bind to RNA in a length-dependent manner[28]. Additionally, FUS has been proposed to recognize a short-stem loop in target RNA[18, 24], and is also capable of binding to single strand DNA (ssDNA)[29] with less affinity than RNA[28]. Further analysis is required to understand how FUS achieves binding specificity.

High-throughput sequencing is now becoming a mainstream technology for the analysis of transcriptomes (Figure 1). RNA-seq has the potential to determine the number, structure, and abundance of transcripts by sequencing randomly fragmented RNA or cDNA[30, 31]. Additionally, Cap analysis gene expression (CAGE)-seq is specialized in identifying and quantifying the 5′ ends of transcripts based on cap-trapping[32, 33]. Conversely, PolyA-seq can specifically detect the 3′ ends of mRNAs by capturing transcript sequences immediately upstream of a polyA stretch[34]. RNAP II ChIP-seq is widely used for the analysis of RNAP II-dependent transcription status. In addition, Nascent-seq[35], which is an RNA-seq of nascent RNA localized in the chromatin fraction of cells, is also applicable.

We performed these high-throughput sequencing analyses using a mouse neuroblastoma N2A cells, after *Fus* was knocked down[16]. We detected 36,353 transcription start sites (TSSs), 3,386 alternative splice sites, and 46,081 polyA sites in these cells, and identified that FUS-CLIP clusters were enriched around all three sites. Enrichment of FUS-binding around alternative RNA processing sites has been detected also in other studies[18, 19, 22], indicating the importance of FUS-RNA interaction in the regulation of alternative RNA processing events. We describe the current understanding of how the interplay between FUS and RNA modulates the transcriptional activity and regulates RNA processing in a large subset of genes.

THE ROLE OF FUS IN TRANSCRIPTIONAL REGULATION

Initiation of transcription

The first step in mRNA biogenesis is transcription initiation, which involves the assembly of a large protein complex containing RNAP II and multiple general transcription factors (GTFs) on a promoter[36]. FUS directly interacts with one of the GTFs, TFIID[37]. In addition, ChIP on chip analysis of HeLa cells demonstrates binding of FUS to ssDNA in promoter regions[38], and our FUS CLIP-seq analysis

of the mouse brain shows the binding of FUS to antisense noncoding RNAs in the promoter regions in a wide array of genes[18]. Additionally, FUS ChIP-seq of HEK cells reveals that FUS migrates around the TSSs of most actively transcribed genes[39]. FUS downregulates transcriptional activity by impeding binding of Spi-1/ PU.1 to the promoter region[40]. FUS also downregulates transcription of the cyclin D1 (*CCND1*) gene in HeLa cells through the interaction with noncoding RNA generated from its promoter region and subsequent inhibition of the histone acetyltransferase (HAT) activity of CREB-binding protein (CBP) and p300[15]. Although the underlying global regulatory mechanism is still largely unknown, these results indicate that FUS associates with a promoter region and plays a pivotal role in the regulation of transcriptional activities.

Relationship between RNAP II and FUS

The transition from initiation to elongation is accompanied by a tightly controlled exchange of factors, which is orchestrated in part through the phosphorylation of RNAP II within the C-terminal domain (CTD) of its largest subunit[41]. CTD is mostly unphosphorylated during initial promoter binding, and RNAP II is then phosphorylated at serine-5, which is thought to destabilize the interactions between RNAP II and promoter-bound factors to facilitate promoter escape. Then, the transition into productive elongation is triggered by recruitment of the P-TEFb kinase, which phosphorylates serine-2 residues on CTD. As RNAP II elongates toward the 3′ end, serine-5 phosphorylation decreases, and serine-2 phosphorylation increases. The increase in serine-2 phosphorylation toward the 3′ end of the transcription unit occurs in parallel with the recruitment of factors and complexes involved in transcription elongation and mRNA processing.

FUS has been known to interact with RNAP II[39, 42, 43]. Recent reports show that FUS forms fibrous assemblies in a DNA/RNA-dependent manner, which can interact with CTD and modulate the transcriptional activity of RNAP II[44-46]. An *in vitro* kinase assay shows that FUS suppresses serine-2 phosphorylation on CTD, and RNAP II ChIP-seq analysis shows that *Fus*-knockdown HEK cells, as well as ALS patient-derived fibroblasts, have higher levels of serine-2 phosphorylation near the TSS compared to its downstream regions in a cell-type specific manner[39, 47]. A similar shift in RNAP II distribution toward TSS by *Fus*-knockdown was also observed in our RNAP II ChIP-seq analysis of N2A cells[16]. These findings indicate that FUS regulates transcriptional activity of RNAP II through direct interactions with CTD. Furthermore, our comprehensive analysis of CLIP-seq, RNAP II ChIP-seq, and Nascent-seq revealed that FUS-RNA interaction fine-tunes local transcription via RNAP II[16]. Our FUS CLIP-seq analysis detected 37,119 FUS-binding clusters in N2A cells, and FUS-dependent accumulation of RNAP II ChIP-tags and a concomitant decrease of Nascent-seq tags have been observed around these FUS-clustered regions, suggesting that binding of FUS to nascent RNA locally induces stalling of RNAP II and suppresses nascent transcription. Taken together, our current hypothesis is as follows (Figure 2): Nascent RNA stimulates a fibrous assembly of FUS, which in turn attenuates serine-2 phosphorylation on CTD through direct interaction between the fibrous FUS assembly and CTD. In this way, FUS precisely controls RNAP II-dependent transcription, which has a global impact on the transcriptional activity of RNAP II.

THE ROLE OF FUS IN RNA PROCESSING

During and immediately after the primary transcription by RNAP II, almost all mRNAs are extensively processed. Pre-mRNA-processing steps include an addition of a 7-methyl guanosine cap at the 5′ end (5′ capping), splicing of exons, and formation of the 3′ end by cleavage and polyadenylation. All these steps are closely associated with RNAP II-dependent transcription.

5′ capping

5′ capping occurs very early in transcription, and serine-5 phosphorylation on CTD has a crucial role in the 5′ capping of mRNA by directly interacting with both guanine-7-methyltransferase and the mRNA-capping enzyme[41]. Although the role of FUS in 5′ capping has not been well characterized, our CAGE-seq analysis of N2A cells shows that knockdown of *Fus* affects the usage of alternative TSSs. Furthermore, FUS-RNA interactions are enriched around these sites[16]. These results may be the

consequence of FUS-dependent activation/suppression of an alternative promoter; however, FUS may be involved in 5′ capping of a target RNA. Gene ontology analysis has revealed that genes whose TSSs are affected by *Fus*-knockdown are associated with transcription and nucleic acid metabolism[16].

Alternative splicing

During and immediately after transcription, almost all mRNAs are spliced. Alternative splicing through selection of pairs of splice sites in the pre-mRNA sequence is an important mechanism for the generation of diverse mRNA and protein variants. In humans, about 95% of the protein-coding multi-exon genes undergo alternative splicing, which is particularly prominent in neurons[48-50]. The splicing process is performed by the spliceosome, which comprises U snRNPs (U1, U2, U4, U5, and U6) and proteins recruited to the pre-mRNA. Alternative splicing is basically regulated by *cis*-acting elements such as exonic/intronic splicing enhancers or silencers in combination with *trans*-acting splicing factors, which recognize the *cis*-elements.

Various lines of evidences indicate that FUS regulates alternative splicing by interacting directly with the spliceosome and target RNAs. FUS is detectable in the spliceosome[51, 52] and directly binds to the splicing factors, including SR proteins[53, 54], polypyrimidine tract-binding protein (PTB)[54], and U1 snRNP[19, 55]. Recently, ALS-causing mutations in *FUS* have been shown to abnormally enhance the interaction of FUS with SMN and reduce the association with U1 snRNP[56]. Furthermore, U1 snRNP is mislocalized in ALS patient fibroblasts bearing mutations in *FUS*[57].

We and other researchers have reported based on data from microarray analysis and RNA-seq analysis, that hundreds of alternative splicing events are FUS-dependent in neuronal cells[18, 19, 22]. Knockdown of *Fus* induces skipping of 437 exons and inclusion of 631 exons in mouse ES cell-derived neurons[23], and skipping of 44 exons and inclusion of 55 exons in primary cortical neurons[18]. Similarly skipping of 21 exons and inclusion of 47 exons have been observed in *Fus*-knockout mice[22]. A recent analysis of ALS patient fibroblasts with *FUS* mutations identified aberrant skipping of 53 exons and inclusion of 57 exons[56]. These results suggest that FUS can promote both inclusion and skipping of an exon. Interestingly, we observed by exon array analysis of various primary cultured cells from the central nervous system that FUS regulates alternative splicing, rather than gene expression, in a region- and cell type-specific manner [58]. We have shown that alternative splicing events in the spinal motor and cortical neurons are very different from those in glial cells and cerebellar neurons, while the gene expression profiles of these cells show similar tendencies[58]. Importantly, FUS regulates alternative splicing of genes associated with neurodegenerative disorders, such as *Mapt*, *Camk2a*, *Fmr1*, and *Ndrg2* in primary cortical neurons and mouse brain[18, 19, 22]. Gene Ontology analysis has revealed that genes that undergo FUS-dependent alternative splicing in neuronal cells are implicated in neuronal functions including synaptogenesis, axonogenesis, and neuronal development[18, 22]. In contrast, Gene Ontology analysis in HeLa cells has shown a significant enrichment in genes regulating transcription[25]. These findings suggest that FUS-dependent alternative splicing is uniquely fine-tuned in a cell-specific manner. Dysregulation of this mechanism may thus cause synaptic dysfunction, axon withdrawal, and denervation, which potentially leads to neurodegeneration observed in ALS and FTLD (Figure 3).

In CLIP-seq analyses, a significant enrichment of FUS binding was observed around FUS-dependent alternative exons; however, no definite positional effects, which are often observed in other splicing factors[59], are recognized for FUS-dependent splicing regulation[18, 19, 22, 23, 25]. It is well established that splicing can happen co-transcriptionally and that splicing efficiency is sensitive to RNAP II elongation rate[60]. Aberration of alternative splicing events in FUS-depleted cells may be the result, in part, of FUS-dependent regulation of RNAP II activity[16]. Recent *in vitro* analysis using the HeLa cell nuclear extracts has revealed that FUS couples transcription with splicing by mediating an association between RNAP II and U1 snRNP[61]. FUS may be an intermediate factor that links transcription and splicing (Figure 3).

Alternative cleavage and polyadenylation

Almost all mRNAs undergo polyadenylation at their 3′ ends[62]. At the end of transcription, the 3′ terminal region of the newly made RNA is cleaved off at a polyA site by a set of RNA regulatory proteins

(the 3′ end processing machinery), which is followed by the synthesis of a polyA tail by the addition of adenine residues. Around 90 protein factors regulate this process, with cleavage and polyadenylation specificity factor (CPSF), cleavage simulator factor (CstF), cleavage factor I (CFI), cleavage factor II (CFII), polyA polymerase (PAP), and polyA binding protein (PABII) playing a crucial role. Similar to alternative splicing, RBPs, including splicing factors, are implicated in the regulation of alternative cleavage and polyadenylation (APA)[62]. APA often occurs in a tissue- or developmental stage-specific manner. Intriguingly, extensive usage of distal APA is prevalent in the mouse and human brain[63]. APA isoforms arising from the same gene should contain different 3′ untranslated regions (UTRs) and may encode different proteins. Accordingly, APA can significantly affect protein products, stability of the transcripts, its localization, and its translatability.

Recent advances in deep-sequencing techniques, which specifically detect polyadenylation sites of mRNAs, such as PolyA-seq, have revealed that ~70% of eukaryotic genes contain more than one polyA site[34, 62], thereby indicating that APA is widespread. We performed PolyA-seq analysis using N2A cells, and detected 46,081 polyA sites. Our analysis reveals that *Fus*-knockdown up- and down-regulates 1,033 and 1,977 APA sites, respectively, by more than 4-fold[16], suggesting that FUS can both activate and suppress APA in a wide array of genes. Consistently, 32 upregulated and 31 downregulated APA sites are detected in microarray analysis of the *Fus*-deficient mouse brain[16, 19], although the analysis is not specific for the detection of APA sites.

FUS interacts with components in the 3′ end processing machinery. Affinity purification combined with quantitative mass spectrometry analysis detected FUS-interaction with the 3′ end processing factors such as CPSF160 and PABPC1 (cytoplasmic polyA-binding protein 1)[56]. Our co-immunoprecipitation analysis using N2A cell extracts also detected interactions of FUS with CPSF160 as well as WDR33 and CstF64[16]. CPSF160 is a core component of the CPSF complex and is indispensable for polyadenylation signal (PAS)-dependent polyadenylation[64]. Since an RNA immunoprecipitation assay and an *in vitro* binding assay have demonstrated that FUS promotes binding of CPSF160 to PAS-containing RNA[16], the interaction between FUS and CPSF160 may be essential for FUS-dependent regulation of APA. Recent co-immunoprecipitation analysis using HeLa cell extracts showed that FUS, U1 snRNP, and CPSF160 form a large complex (~1.8 MDa) including SMN[56]. Interestingly, U1 snRNP globally controls APA in addition to its fundamental role in pre-mRNA splicing. It has been reported that knockdown of U1 snRNP causes frequent premature cleavage and polyadenylation in introns near TSS[65, 66]. These findings suggest that FUS may coordinately regulate APA through the interaction between FUS, U1 snRNP, and CPSF.

In addition to the direct interaction between FUS and the 3′ end processing factors, cleavage and polyadenylation of nascent transcripts is functionally coupled with transcriptional activity of RNAP II[67]. Simultaneous interaction of the CPSF complex with PAS and the body of RNAP II results in the stalling of RNAP II[67], which is thought to be an intermediate step leading to transcription termination. Conversely, pausing of RNAP II facilitates polyadenylation, presumably by promoting the assembly of the 3′ end processing machinery during slow transcription[68]. Our RNAP II ChIP-seq analysis of N2A cells revealed that FUS induces pausing of RNAP II upstream of APA sites that are upregulated by *Fus*-knockdown, as well as downstream of APA sites that are downregulated by *Fus*-knockdown[16]. As expected, our CLIP-seq analysis showed enrichment of FUS-RNA interaction at these sites where the FUS-dependent RNAP II pausing is prominent. Further analysis of the underlying mechanism of FUS-dependent regulation of APA revealed that when FUS binds upstream of the APA site, FUS simply induces stalling of RNAP II, resulting in the suppression of gene expression[16]. In contrast, when FUS binds immediately downstream of PAS, FUS promotes binding of CPSF160 to PAS-containing RNA, which leads to cleavage and polyadenylation of the bound RNA. These findings indicate that FUS regulates APA in a position-specific manner through the regulation of RNAP II transcriptional activity in combination with the 3′ end processing factors (Figure 4). Thus, RNAP II pausing needs to be induced in the right place at the right time to promote cleavage and polyadenylation of a nascent transcript.

Gene Ontology analysis of our PolyA-seq using N2A cells revealed that genes whose polyadenylation sites are affected by *Fus*-knockdown are associated with neuronal activities and synaptic transmission[16]. Furthermore, FUS regulates APA of *Ablim1* (actin binding LIM protein 1), which is involved in axon guidance, in the mouse brain[22]. FUS is also involved in polyadenylation of *GluA1* mRNA,

which regulates function of α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptor and FTLD/ALS-associated behavior in mice[69]. These results suggest that dysregulation of FUS-dependent APA may cause neurodegeneration in ALS and FTLD, along with dysregulated alternative splicing.

mRNA transport and local translation

Soon after the termination of transcription, mRNAs, which are capped, spliced, and polyadenylated, are transported to the cytoplasm. mRNAs are localized to specific regions of the cytoplasm, thereby allowing the local production of proteins.

Involvement of FUS in the RNA transport machinery has been identified in several molecules and genes, although a global role of FUS in this regulation is still under investigation. FUS has been detected in a large RNA-transporting granule, which associates with the microtubule-dependent kinesin motor protein KIF5B[70]. FUS also interacts with actin-based motors, Myo5A[71] and Myo6[72]. Furthermore, FUS is a component of adenomatous polyposis coli (APC)-containing ribonucleoprotein complexes in cell protrusions and is required for efficient translation of the *Kank2* mRNA (KN motif and ankyrin repeat domains 2)[73].

REGULATION OF mRNA ISOFORM-SPECIFIC EXPRESSION

FUS coordinates multistep RNA metabolic pathways, especially transcription and RNA processing, which may affect the mRNA expression level. Depletion of FUS-expression in neuronal cells, however, alter expressions of only a limited number of genes[16, 18, 19, 22, 23, 58]. For example, only 4 and 61 genes are significantly up- and down-regulated, respectively, by more than 2-folds by *Fus* knockdown in the mouse striatum[19]. Similarly, only tens of genes show more than a 2-fold change in the transcript level in the brain of *Fus*-knockout mice[22]. These results suggest that regulation of gene expression is probably not a principal function of FUS in neuronal cells.

Regulation of alternative transcription initiation, splicing, and polyadenylation is a prevalent mechanism for generating isoform diversity. As stated above, FUS is implicated in all three regulation steps, and substantially affects alternative splicing and polyadenylation of thousands of genes in neuronal cells. Our CAGE-seq and PolyA-seq analysis showed that more than 70% of genes expressed in N2A cells harbor two or more TSSs and two or more polyA sites[16]. FUS regulates usage of these alternative sites in as many as two-thirds of all expressed genes in N2A cells[16]. As expected, the affected transcripts are covered by more FUS-CLIP tags than unaffected transcripts. Taken together, these results strongly suggest that FUS is an essential regulator to achieve mRNA isoform diversity.

Conclusion

In summary, FUS is a multifunctional RNA binding protein that is implicated in a diverse array of RNA metabolism processes. FUS directly interacts with various components of the transcription machinery, spliceosome, and the 3′ end processing machinery. Furthermore, recently developed high throughput sequencing technologies have uncovered the FUS-dependent RNA processing networks in various cell types, especially in neuronal cells. In transcription, FUS associates with RNAP II in an RNA-dependent manner and suppresses its transcriptional activity. FUS forms a fibrous assembly in an RNA-dependent manner, which directly binds to CTD of RNAP II. Probably through the regulation of CTD phosphorylation, FUS precisely controls local transcriptional activity of RNAP II. In RNA processing, FUS is particularly involved in splicing and polyadenylation. Depletion of FUS induces aberrant regulation of these steps in a large number of genes. Among FUS-interacting partners, U1 snRNP and CPSF160 appear to have important roles in these regulations. U1 snRNP has been known to have a fundamental role in both splicing and premature cleavage and polyadenylation, and CPSF160 is an essential factor for polyadenylation. FUS, U1 snRNP and CPSF160 form a large complex in cells. Furthermore, FUS mediates an association between RNAP II and U1 snRNP, which is required for transcription-coupled splicing. FUS also promotes binding of CPSF160 to PAS-containing RNA. Interestingly, FUS regulates APA in a

position-specific manner through the regulation of RNAP II transcriptional activity in combination with CPSF160. When FUS binds upstream of a PAS of an APA site or a PAS is not present, FUS simply stalls RNAP II resulting in the suppression of gene expression. In contrast, when FUS binds immediately downstream of the PAS, FUS promotes binding of CPSF160 to PAS-containing RNA, which leads to cleavage and polyadenylation of the bound RNA. These findings suggest that a complex interplay between FUS, RNAP II, and associated RNA binding proteins determines the consequence of alternative RNA processing. FUS may be an intermediate factor that links transcription and RNA processing.

In neuronal cells, aberration of alternative splicing events and polyadenylation events by *Fus*-depletion and by *FUS*-mutations in ALS may compromise neuronal functions such as synaptogenesis, axonogenesis, and neuronal development. In conclusion, FUS-dependent RNA processing is uniquely fine-tuned in a cell-specific manner, dysregulation of which leads to neurodegeneration in ALS and FTLD.

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Figure legends

FIGURE 1. Transcriptome analysis by high throughput sequencing

(a) CLIP-seq produces a transcriptome-wide map of *in vivo* RNA-binding sites of a specific RNA-binding protein.

RNA-seq is a high-throughput sequencing method employed for genome-wide transcriptome profiling, which has the potential to elucidate the number, structure, and abundance of transcript by sequencing randomly fragmented RNA or cDNA.

CAGE-seq is a high-throughput, tag-based sequencing method designed to scrutinize the 5′ end of capped cDNAs.

PolyA-seq is a high-throughput sequencing method for the detection and quantification of the 3′ ends of polyadenylated transcripts.

(b) Nascent-Seq (genome-wide sequencing of nascent RNA) is a high-throughput sequencing method to identify chromatin-bound nascent RNA obtained from the lysis of cells and washing of cell nuclei with NUN buffer consisting of high concentrations of NaCl, urea, and NP-40. This sequencing method detects nascent RNA molecules attached to elongating RNAP II.

RNAP II ChIP-seq shows genome-wide distribution of RNAP II, which reflects the transcriptional status of RNAP II. Accumulation of RNAP II ChIP-tags is observed at the sites where RNAP II pauses.

FIGURE 2. FUS-dependent regulation of transcriptional activity of RNAP II

FUS interacts with the nascent RNA soon after its transcription, which stimulates formation of a fibrous assembly. Then, the FUS assembly binds to CTD, attenuates its serine-2 phosphorylation, and induces local stalling of RNAP II.

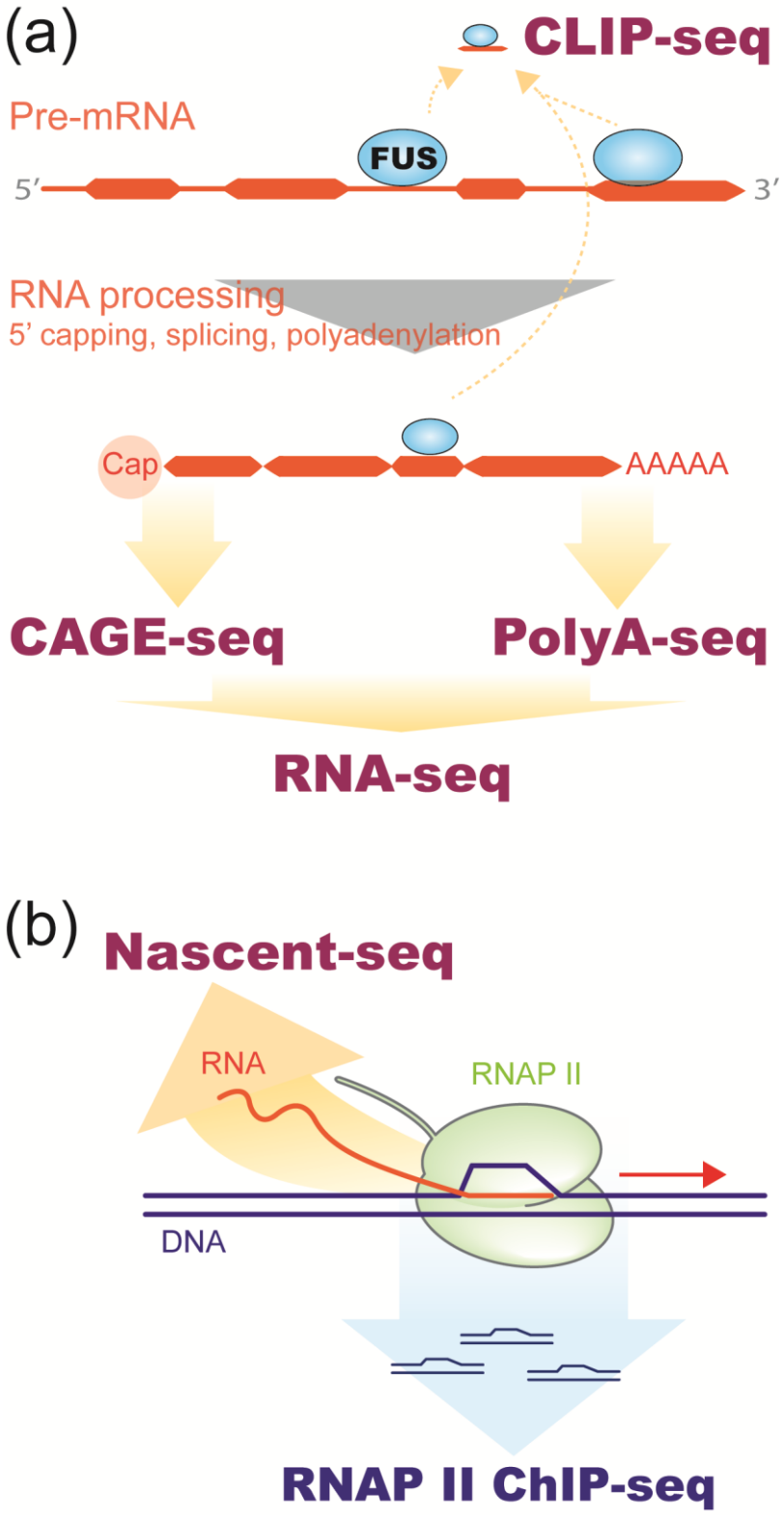
FIGURE 3. FUS-dependent regulation of alternative splicing

FUS regulates alternative splicing through direct interaction with spliceosome components, such as U1 snRNP, as well as through suppression of local transcriptional activity of RNAP II. FUS-dependent alternative splicing events are implicated in neuronal functions including synaptogenesis, axonogenesis, and neuronal development.

FIGURE 4. FUS-dependent regulation of alternative cleavage and polyadenylation

FUS is recruited to RNA immediately after its transcription and locally inhibits RNAP II transcription. When FUS binds immediately downstream of PAS, FUS promotes binding of CPSF160 to PAS-containing RNA, which leads to cleavage and polyadenylation of the bound RNA. When FUS binds upstream of the PAS of an APA site or a PAS is not present, only the transcription suppressive effect of FUS is observed and the FUS-bound transcript is downregulated. Through such regulation, FUS globally controls APA events, which are implicated in neuronal activities and synaptic transmission in neurons.

Figure 1


(a)
CLIP-seq
Pre-mRNA
FUS
5′
3′
RNA processing
5’ capping, splicing, polyadenylation
Cap
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PolyA-seq
RNA-seq
(b)
Nascent-seq
RNA
RNAP II
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RNAP II ChIP-seq

Figure 2

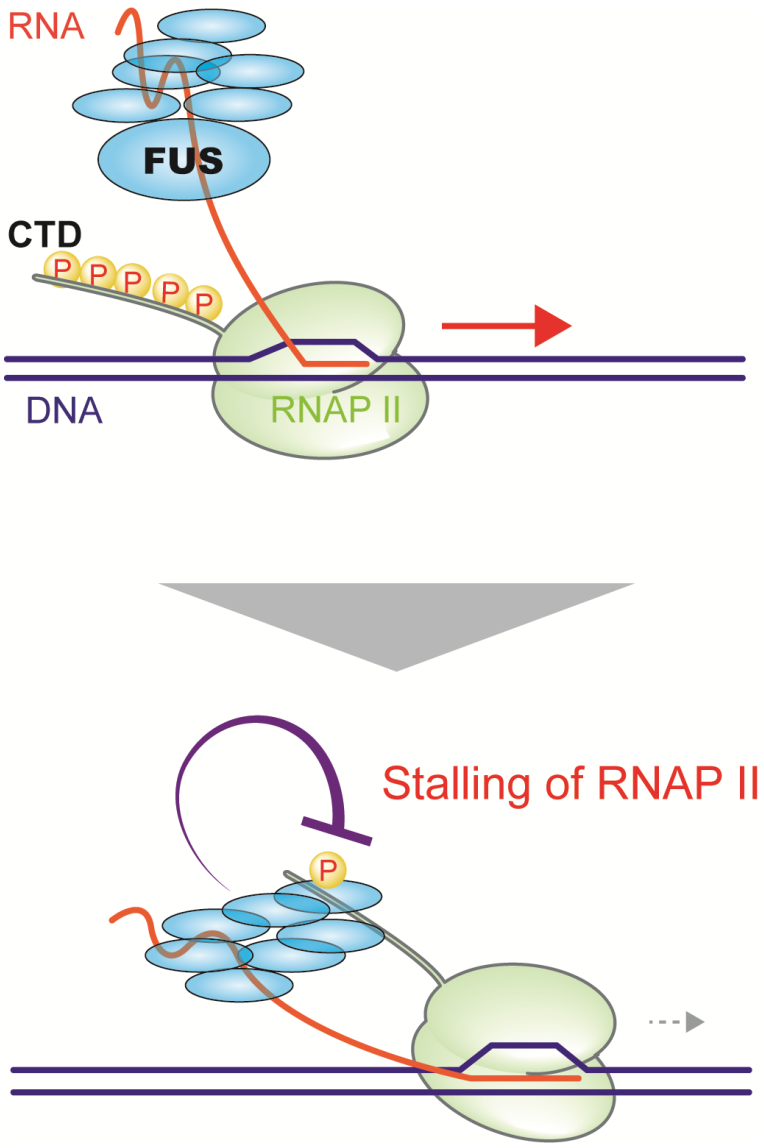
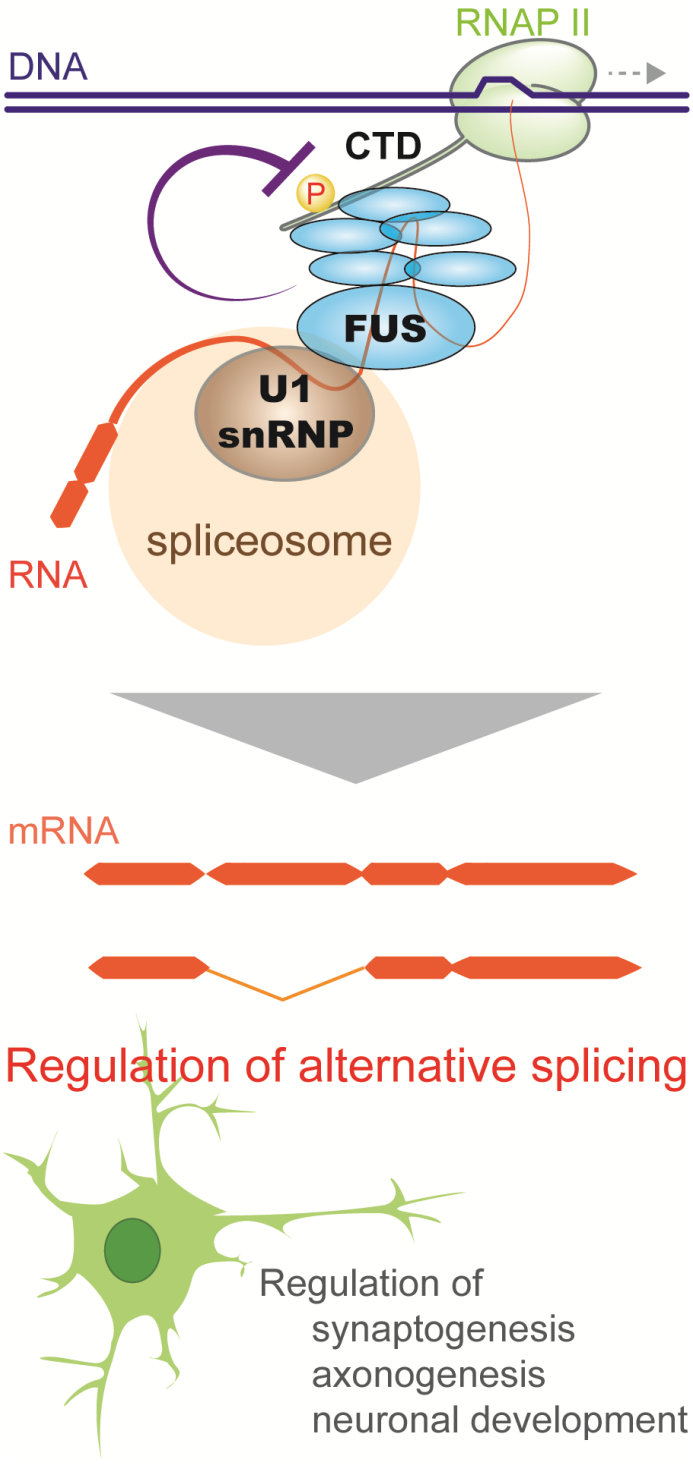


Figure 3


RNAP II
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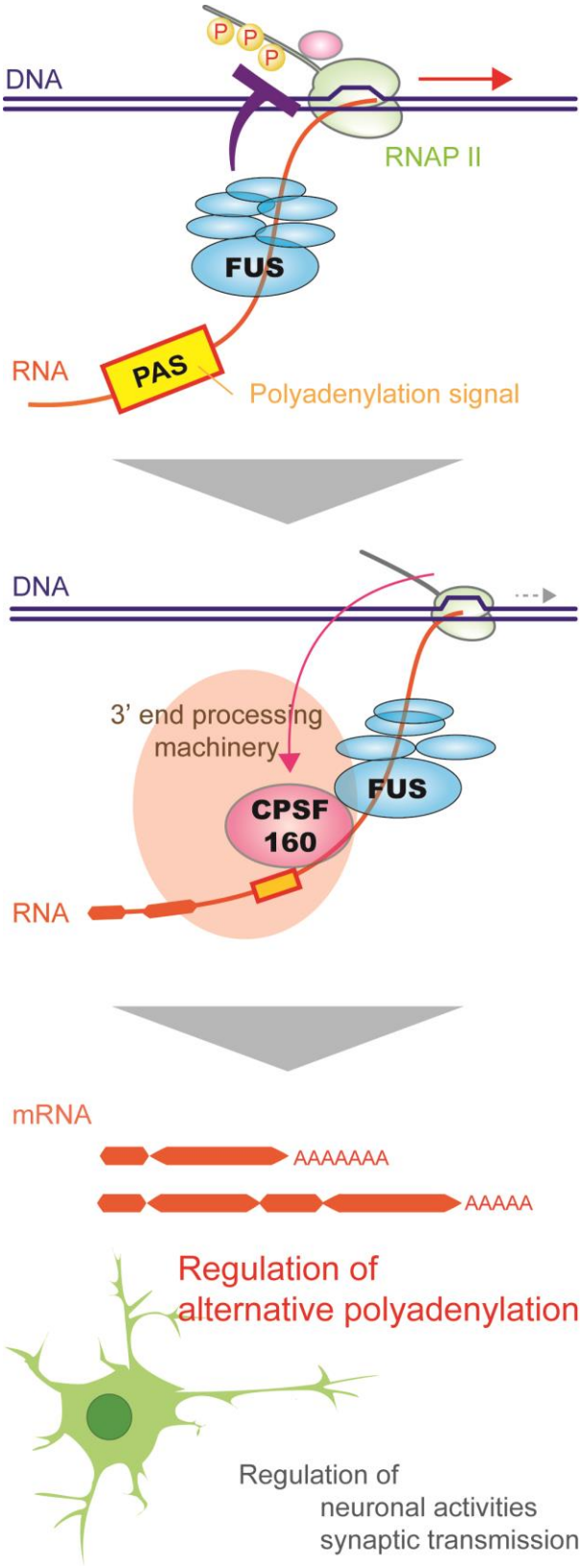
Regulation of alternative splicing
Regulation of
synaptogenesis
axonogenesis
neuronal development

Figure 4


P
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P
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RNAP II
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3' end processing
machinery
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RNA
mRNA
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Regulation of
alternative polyadenylation
Regulation of
neuronal activities
synaptic transmission