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Biochemical Properties and Biological Functions of FET Proteins

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Abstract

Members of the FET protein family, consisting of FUS, EWSR1, and TAF15, bind to RNA and contribute to the control of transcription, RNA processing, and the cytoplasmic fates of messenger RNAs in metazoa. FET proteins can also bind DNA, which may be important in transcription and DNA damage responses. FET proteins are of medical interest because chromosomal rearrangements of their genes promote various sarcomas and because point mutations in FUS or TAF15 can cause neurodegenerative diseases such as amyotrophic lateral sclerosis and frontotemporal lobar dementia. Recent results suggest that both the normal and pathological effects of FET proteins are modulated by low-complexity or prion-like domains, which can form higher-order assemblies with novel interaction properties. Herein, we review FET proteins with an emphasis on how the biochemical properties of FET proteins may relate to their biological functions and to pathogenesis.

Keywords

FUS; EWSR1; TAF15; low-complexity; RNA-binding; neurodegeneration

INTRODUCTION

The proper control of messenger RNA (mRNA) production and function is a key aspect of gene expression in mammalian cells. Important steps in the mRNA life cycle include transcription, splicing and polyadenylation, base methylation, nuclear–cytoplasmic transport, translation, and degradation.

Three insights have changed how we view mRNA biogenesis and function over the past few decades. First, multiple steps in mRNA biogenesis and function are mechanistically coupled such that RNA-binding proteins and RNA-processing machines can affect numerous steps in mRNA function (1–3). Second, the composition of any messenger ribonucleoprotein (mRNP) can dictate both the RNA processing of the transcript (e.g., alternative splicing) and the rates of mRNA translation and degradation (4, 5). Third, the composition of an

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mRNP can be influenced by (*a*) transcription-coupled processes that promote loading of proteins on nascent transcripts, (*b*) the competition for binding sites between different individual proteins, and (*c*) the competition for a given protein between different sites in the transcriptome (6, 7). The biogenesis of an mRNP has to be considered a process that is initiated and influenced by the transcription machinery with downstream consequences for mRNA function.

Key aspects of assembling a nascent mRNP are the mRNA–protein interactions that occur during transcription (1, 2). Such cotranscriptional assembly is dominated by the heterogeneous nuclear ribonucleoprotein particle (hnRNP) proteins, which tend to be highly abundant and ubiquitous RNA-binding proteins. hnRNP proteins are generally present in the nucleus, but many such proteins shuttle between the nucleus and cytosol (8, 9). Given their abundance and role in nascent precursor mRNA (pre-mRNA) packaging, hnRNP proteins have a significant impact on mRNA biogenesis and function (10). Moreover, some hnRNP proteins can also bind DNA as well as interact with the transcription machinery (11, 12). For these reasons, understanding the roles of hnRNP proteins in mRNA biogenesis and function is important.

A noteworthy family of hnRNP proteins is the FET proteins, which in vertebrates are FUS, EWSR1, and TAF15 (13, 14). These are abundant RNA- and DNA-binding proteins that interact with thousands of transcripts and affect multiple steps in mRNA biogenesis. FET proteins are interesting for three additional reasons. First, each FET protein contains an N-terminal domain (NTD) of low sequence complexity, which can form intermolecular assemblies with unique biochemical properties (15–18). Thus, understanding FET protein function may reveal new aspects of protein design and function. Second, translocation of a FET protein's low-complexity (LC) domain onto various DNA-binding proteins is a genetic abnormality that contributes to the formation of several different cancers (13, 14, 19). Finally, point mutations in either FUS or TAF15, some of which affect their nuclear–cytoplasmic shuttling, can cause neurodegenerative diseases such as amyotrophic lateral sclerosis (ALS) and frontotemporal lobar dementia (FTLD) (20, 21). Thus, understanding both normal and pathological FET protein function may further our understanding of these human diseases. In this light, we review the literature on FET proteins.

DOMAINS OF FET FAMILY MEMBERS

FET family members are found in multicellular organisms including vertebrates, plants, nematodes, and insects. Given the role of FET proteins in transcription and RNA processing (see the next two sections), one speculation is that the FET protein family evolved to facilitate the more complex coupling of transcription with RNA processing that occurs in multicellular organisms. Invertebrates and plants contain a single FET protein, which is called cabeza in *Drosophila melanogaster* (Figure 1). The family of three FET proteins arose in vertebrates, and each member is highly conserved from fish to mammals. The conservation of the three FET proteins in vertebrates suggests that they have specialized functions.

FET proteins have a common domain organization, including an N-terminal LC domain, RGG domains, a zinc finger (ZnF) domain, and an RNA recognition motif (RRM) (Figure 1). RRMs directly bind RNA in many RNA-binding proteins and are distinguished by their β1α1β2β3α2β54 secondary structure. FET RRMs are distinguished from other hnRNP RRMs because of the extended “KK-loop” between α1 and β2, and because of the conspicuous lack of two aromatic amino acids on β3 (22, 23). The lysines in the KK-loop of FUS are important for RNA binding (23). For other hnRNP proteins, the two aromatic residues on β3, which are not found in FET proteins, make important stacking interactions with nucleotides and can contribute to sequence-specific RNA recognition by the domain (24, 25). NMR chemical-shift analyses suggest that RNA binds the traditional β-sheet face of the FET protein RRM. However, the absence of these key aromatic residues on β3 suggests that the specific contacts between the RRM and RNA may differ from the canonical RRM–RNA interaction (23).

The RGG and ZnF domains also contribute to the binding of FUS to RNA. All FET proteins (Figure 2) share a ZnF domain with four cysteines coordinating the zinc ion (26). The isolated ZnF domain, along with its flanking RGG motifs, has at least as much affinity for RNA as that of the RRM (see the section titled Biochemical Properties of FET Proteins, below) (18). RGG domains in other hnRNP proteins mediate non-sequence-specific recognition of RNA (24, 27). Thus, both RNA-binding domains are likely to contribute to the RNA-binding properties of FET proteins (5), potentially allowing FET proteins to bind a greater diversity of RNA targets than a single domain.

The LC and RGG domains of FET proteins promote protein self-assembly into higher-order structures. These self-assembly domains are of interest for several reasons. First, many RNA-binding proteins contain either prion domains or “prion-like” LC and RGG domains, which form higher-order self-assemblies (15–18, 28, 29). In these cases, prion or prion-like domains are defined as regions of amino acid sequences with a computationally predicted tendency to form stacked β-amyloid assemblies. In some cases, such domains are important for the assembly of intracellular RNP granules, including P-bodies and stress granules (28–30). This finding suggests that cells use such prion-like domains as reversible assembly modules for large RNA–protein complexes.

In mammals, the LC domain of FET proteins possesses a repeated prion-like [S/G]Y[S/G] motif (Figure 1) (17, 18). This degenerate motif is weakly conserved in FET proteins throughout multicellular organisms, although at times the motif places an asparagine (N) adjacent to the tyrosine (Y). NY-rich motifs also have prion-like properties (31). By promoting the higher-order assembly of FET proteins, these extensive self-assembly domains appear to play important roles in modulating FET protein biological and pathological functions (see the sections titled Biochemical Properties of FET Proteins and Role of FET Proteins in Transcription, below).

BIOCHEMICAL PROPERTIES OF FET PROTEINS

FET Proteins Bind DNA

FET proteins bind single-stranded DNA (ssDNA) and double-stranded DNA (dsDNA). As with other hnRNP proteins, FET proteins were initially purified from mammalian cells and identified using immobilized ssDNA (32). Recombinant FET proteins shift ssDNA by electrophoretic mobility shift assays (EMSAs), and the binding can be competed with RNA, suggesting that both nucleic acids bind to the same site(s) or overlapping site(s) (22, 33, 34). Recombinant FET proteins also bind ssDNA and dsDNA in pull-down assays (35), but at least for FUS, this protein binds dsDNA in EMSAs with a much weaker dissociation constant (K_d) than that of ssDNA (X. Wang, J.C. Schwartz & T.R. Cech, unpublished data). Thus, a key question is whether weak DNA binding is simply a consequence of general and promiscuous nucleic acid binding (e.g., polyanion binding), or whether it is biologically meaningful.

The extent to which FET proteins directly bind DNA in vivo remains to be determined. FUS does precipitate from cells with chromatin and is released only upon DNase treatment (36). Moreover, FUS–DNA interactions recovered following formaldehyde cross-linking have been used for chromatin immunoprecipitation sequencing (ChIP-seq) or ChIP-array experiments. However, formaldehyde cross-linking also recovers tertiary interactions such as FUS interactions with other DNA-binding proteins (34, 37). Additional uncertainty comes from the observation that a UV cross-linking assay in cells failed to recover DNA bound to the FUS protein (38). FUS binds ssDNA in cell lysates but only when the FUS is phosphorylated in cells expressing the fusion protein BCR/ABL (39). Finally, FET proteins pull down from lysates with DNA substrates designed to model dsDNA breaks and Holliday junctions, although these interactions may be indirect (40).

Each FET protein can promote ssDNA invasion of dsDNA. This activity was discovered for FET proteins by use of an in vitro pairing on membrane (POM) assay, in which proteins immobilized on a nitrocellulose membrane show the ability to anneal DNA strands (41–43). This assay requires the protein to maintain activity after denaturing sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and immobilization on a nitrocellulose membrane. Because the RGG domains are predicted to be unstructured and therefore less affected by SDS denaturation, much of the POM activity (i.e., annealing activity) is presumably mediated through the RGG domains. One model of how RGG domains contribute to POM activity provides that nonspecific association of RGG repeats along the phosphodiester backbone may neutralize charge repulsion and thereby promote strand annealing. Promotion of the formation of duplex DNA is proposed to be a mechanism by which FET proteins can contribute to DNA break repair (see the section titled FET Proteins and DNA Damage Repair, below) (13, 41). The ability of FET proteins to promote ssDNA invasion of dsDNA raises the possibility that FET proteins can also promote ssRNA invasion of dsDNA or even dsRNA and, as such, may play unanticipated roles in higher-order nucleic acid interactions.

FET Proteins Bind RNA

Many observations clearly demonstrate that FET proteins directly bind RNA both in vitro and in vivo. This ability is consistent with the presence of domains shared with other RNA-binding proteins—the RRM and RGG–ZnF–RGG domains (22, 44–47). FET proteins can be purified by immobilized RNA in a manner similar to that using ssDNA (32). All three recombinant FET proteins either pull down with RNA or shift RNA by EMSA (18, 22, 26, 33–35, 38, 45, 46, 48–52). Moreover, FET proteins can be UV cross-linked to RNA targets in cells (37, 38, 51, 53–55), and the recognition of RNA by FUS promotes the latter's ability to form self-assemblies and to bind other proteins, including RNA polymerase II (RNA Pol II) and CBP/p300 (18, 37, 53).

FET proteins bind nucleic acids through two domains, the RRM and the RGG–ZnF–RGG motifs. For FUS and EWSR1, the RGG–ZnF–RGG domain possesses significant affinity for RNA and ssDNA (18, 48). Surface plasmon resonance (SPR) and NMR experiments confirm that the RRM of FUS binds RNA (23). However, by itself the RRM alone binds nucleic acids very weakly, and chemical shifts for the RRM are very similar whether bound to RNA, ssDNA, or dsDNA (23). Inclusion of one of the flanking RGG domains gives the RRM the ability to bind RNA with a K_d of nearly 100 nM (when corrected for protein activity), which is the same as that for the RGG–ZnF–RGG domain (18, 44, 50). In short, each of these two domains (RGG–RRM and RGG–ZnF–RGG) can bind RNA and DNA with the same affinity as the full-length protein in vitro (18).

Whether both domains are necessary for RNA binding in vivo has been difficult to establish. Domain deletions seriously impair binding on the basis of in vivo cross-linking and pull-down assays. However, because the protein appears to possess strong interdomain interactions, deletion of an entire domain could have indirect effects through structural destabilization (18, 53). Similarly, published point mutations in the RRM have been interpreted as demonstrating that RNA binding is important for cellular function; however, note that these mutations are in the hydrophobic core of the RRM, which may affect protein folding (56).

Distribution and Specificity of RNA–FET Protein Interactions In Vivo

Several observations suggest that FET proteins may be relatively promiscuous in their interactions with RNA (38, 57). First, on the basis of cross-linking experiments, FET proteins bind many thousands of RNAs in the cell (37, 51, 54, 55, 57, 58). Second, there is little to no similarity between several different published RNA motifs for FET proteins binding (50, 51, 58–60). Third, a SELEX study of FUS by the Moreau–Gachelin lab (50) identified a prevalent GGUG motif; however, all sequences identified were GU rich, arguing against the uniqueness of this motif, and half of the sequences bound lacked the GGUG motif. Fourth, a cross-linking immunoprecipitation sequencing (CLIP-seq) study of FUS by the Yeo lab (58) has probably come closest to saturating signals from in vivo targets and provides significant evidence of proteins oligomerizing along introns. This last point seems to argue that specificity may be difficult to observe because, as with heterogeneous nuclear ribonucleoprotein A1 (hnRNPA1), one binding event potentiates binding to adjacent sites that may lack any preferred binding motif.

Whereas FET proteins may lack strong specificity of RNA sequence recognition, at least the FUS protein shows patterns of distribution along mRNAs. By cross-linking, FUS binding sites are enriched in 5′ untranslated regions (UTRs). The Yeo lab (58) has shown a fivefold enrichment for 5′ UTRs over what would be expected on the basis of their length. Also, Ishigaki et al. (55) showed that averaged FUS CLIP-seq signals are highest near 5′ ends and steadily diminish toward 3′ ends. This pattern was also observed by Schwartz et al. (37); more CLIP-seq reads piled up near the 5′ ends of mRNAs. This enrichment for 5′ UTRs is especially noteworthy because standard CLIP-seq protocols are biased against the detection of 5′ ends of mRNAs because they possess a methyl-G cap, which inhibits adaptor ligation. The reason for this enrichment of 5′ UTRs may be that FET proteins transition early during transcription from binding to the polymerase to binding the elongating pre-mRNA (see the section titled Role of FET Proteins in Transcription, below). Alternatively, FET proteins may be binding truncated RNA transcripts and helping to sequester them within the nucleus.

Two labs have suggested that FUS also shows some enrichment in binding 3′ UTRs. The Yeo lab (58) found threefold enrichment of 3′ UTR sequences over what would be expected on the basis of their length. Ishigaki et al. (55) showed enrichment for introns and 3′ UTRs when normalized for transcript length; however, this result seems inconsistent with the averaged pattern of binding included in the same figure, with more reads near the TSS and fewer toward the 3′ end. However, both the Yeo lab (58) and the Ule lab (57) note binding of FUS along 3′ UTRs of specific mRNAs, particularly genes with alternative polyadenylation sites. A reasonable interpretation is that FET proteins may associate with particular 3′ UTRs to affect 3′-end processing or mRNA function in the cytosol (see the section titled FET Protein Effect on RNA Processing, below).

FUS has also been proposed to preferentially bind introns (51, 54, 55, 57, 58). This idea is based on the observation that a large number of FUS-associated sequence reads in CLIP-seq experiments are within introns (51, 57). However, introns constitute nearly 90% the length of the average mammalian gene; FUS binding to introns may not be preferred over other regions of the pre-mRNA. A reasonable model is that FET proteins are indiscriminately loaded along the nascent transcript, binding both introns and exons in a transient manner, and are then removed from most mRNAs either before nuclear export or shortly after transport to the cytosol (58).

Two papers (57, 58) have noted a prominent “sawtooth” pattern of FUS enrichment on particular long introns, and also along certain long exons such as 3′ UTRs (58). In a sawtooth pattern, FUS signals accumulate at a 5′ splice site and diminish gradually throughout the length of a long intron, then sharply increase at the next 5′ splice site or even at the 5′ end of a long exon, such as a 3′ UTR. The mechanism of a sawtooth pattern of binding is likely that some sequence or event in transcription triggers FUS to load onto the pre-mRNA at a particular site, and then the cooperative binding properties of FUS promote the protein to oligomerize along the RNA in a 5′-to-3′ direction.

Several important questions about FET proteins’ interactions with nucleic acids remain unanswered. First, what is the in vivo significance, if any, of FET protein affinity for ssDNA or dsDNA? Second, do both the RRM and RGG domains contribute to FET protein affinity

for RNA in vivo, or does one domain dominate? To answer this question, a detailed map of the RNA-binding surface of these proteins is needed to target mutations that specifically disrupt RNA binding without denaturing the entire domain or the protein itself. Third, which in vivo functions of FET proteins are affected by RNA binding? To find the answer, investigators will need specific separation-of-function mutations similar to those described for the second question. Fourth, does any yet-to-be-defined specificity in binding contribute to in vivo targeting of FET proteins toward regulating the transcription or splicing of particular mRNAs? Such a hidden specificity could be one of the sequence motifs previously described or a specificity acquired through interactions with other RNA- and DNA-binding proteins. Fifth, do FET proteins associate primarily with full-length mRNA transcripts or with 5′ truncations created by premature abortion of transcription?

Self-Assembly of FET Proteins

Another striking biochemical property of FET proteins is their oligomerization to form higher-order self-assemblies. These fibrous assemblies have been observed with recombinant proteins in the presence or absence of RNA by changes in turbidity (16, 18, 61). Higher–molecular weight assemblies of recombinant FUS protein have also been observed by formaldehyde cross-linking, in which they run as larger species on SDS-PAGE (18). FET fibers grown with recombinant proteins are also readily visible by fluorescence, transmission electron, and atomic force microscopies (16–18, 61, 62).

The precise nature of FET protein higher-order interactions is unclear but appears to be related to the β-sheet structures that β-amyloids can form. In support of this idea is the finding that FUS and its two assembly domains form mostly β-strand structures, as determined by circular dichroism spectroscopy (18). Moreover, desiccated fibers of recombinant FUS protein (J.C. Schwartz, D.B. McKay & T.R. Cech, unpublished data) or the LC domain alone (16) give X-ray diffraction at 4 and 10Å, characteristic of stacked β-sheet structures such as those in amyloid fibers, although the desiccation required for diffraction could drive the proteins into a very stable β-zipper structure that is biologically irrelevant. Evidence that FUS interactions lack highly stable β-amyloid structures is that the protein fibers readily dissolve in SDS (15, 16) and stain only weakly with the amyloid-specific dye thioflavin T (18, 61). Whereas high FUS protein concentration can be used to drive the formation of fibrous assemblies, RNA binding allows assembly formation at more physiological concentrations (Figure 3) (18).

The ability of FET proteins to form fibrous assemblies arises from two types of domains: the LC and RGG domains. Both domains in isolation form fibers that are visible by microscopy (16–18). The most studied of these assembly domains are the LC domains. The LC domains of each FET protein contain numerous [S/G]Y[S/G] repeats. The tyrosines in this repeated motif are required for recombinant protein to form fibrous assemblies, because mutation of two or more of the repeated tyrosines abolishes fiber formation (17). TAF15 is unique in that, in addition to the [S/G]Y[S/G] motif, it also possesses five SYD repeats, which appear to endow the domain with stronger self-assembly interactions (17).

The second type of assembly domain in FET proteins is the C-terminal RGG–ZnF–RGG domain. Fibrous assemblies have been shown only for the RGG–ZnF–RGG domain of FUS.

Whether the equivalent domains of TAF15 and EWSR1 (18) also form fibrous assemblies has not been explored. The RGG domains are the least conserved regions between members of the FET family. TAF15 lacks the first RGG domain situated between the LC and RRM domains (Figure 2b). TAF15 also has the most identical repeats of the motif DR(G)$_n$YGG in its C-terminal domains (CTDs). FUS possesses simple RGG repeats and a more degenerate repeated DRGG[F/Y]G motif. EWSR1's RGG motifs are broken up by many prolines, which would be predicted to disrupt the secondary structure. EWSR1 RGG domains do mediate self-association, but whether the assemblies are fibrous is unknown (63).

An unresolved issue is whether FET proteins form these types of fibers in cells. At a minimum, genetic experiments suggest that some type of related structure does form and has functional consequences. Fibers of recombinant FET proteins bind RNA Pol II through its CTD (17, 18) and mutations in the LC domain of FUS that abolish the ability to form fibers also abolish the ability to promote transcription in vivo (17). FET proteins also are incorporated in vivo into the fibrous poly-Q assemblies of the HTT and ataxin proteins (62). In ALS patients, FUS proteins with mutations in their nuclear localization signal (NLS) accumulate in the cytoplasm and form aggregates that are visible in histological and immunofluorescence staining and that sediment upon high-speed centrifugation (21, 64–69). When expressed in yeast, FUS protein goes into cytoplasmic aggregates; these aggregates are only marginally reduced by deletion of either the LC or RGG domain, suggesting that each of these domains contributes to self-assembly (61, 70).

Many questions remain about the ability of these proteins to form fibrous self-assemblies. Do these fibrous assemblies form in vivo? Are they homogeneous (i.e., with a single protein component) or heterogeneous assemblies (i.e., with a mixture of LC domain proteins) in vivo? How do FET protein fibers contribute to each protein's in vivo functions? Fibers form spontaneously at high concentrations and upon RNA binding, but what is it in cells that regulates the disassembly of fibers? Do FET protein fibers interact with structural assemblies (e.g., lamin, actin, tubulin) in vivo and contribute to the structural integrity or organization within the cell and nucleus? What are the physiologically important differences between normal and pathological FET protein assemblies? What is the biological significance of phosphorylation of the LC domain of FET proteins?

FET Protein Interactions

Another property of FET proteins is their interaction with numerous other cellular proteins. Coimmunoprecipitation and direct binding experiments have revealed hundreds of protein–protein interactions involving FET proteins (71). For FUS, these interacting partners include the U1 small nuclear ribonucleic particles (snRNPs), Gemin proteins, Sm proteins, SR proteins, hnRNP proteins, other RNA-binding proteins, and transcription-related proteins (52). FET proteins also interact with the microRNA (miRNA)-processing protein Drosha, as well as many miRNAs, and may play a role in miRNA processing and stability (72, 73). Public databases reveal large overlaps between the interactomes of FET proteins (71). Indeed, the FET proteins form robust interactions with one another (51, 52, 74). These interactomes are consistent with the implication that FET proteins are involved in transcription and RNA processing.

Moreover, the individual biochemical properties of FET proteins are likely coupled, possibly through an allosteric mechanism. Supporting this hypothesis, FUS binds the proteins CBP, p300, and RNA Pol II in an RNA-dependent manner (37, 53). Additionally, FUS forms protein assemblies in an RNA-dependent manner, suggesting that RNA binding promotes or stabilizes an alternative structural organization for the protein (18). Lastly, the LC and RGG–ZnF–RGG domains interact with each other in *trans* in an RNA-dependent manner (53). Analogous allosteric regulation of nucleic acid binding proteins that triggers self-assembly has been proposed for the bacterial SgrAI and eukaryotic IreI proteins (75, 76).

Important questions about the coupling of FET protein biochemical properties remain. Are these properties linked by allostery or some other mechanism? Are contacts between the LC and RGG domains in *trans* maintained in the full-length proteins? The RGG domains both bind RNA and form self-assemblies; can these domains perform both functions simultaneously in the context of the full-length protein, or are they mutually exclusive?

ROLE OF FET PROTEINS IN TRANSCRIPTION

FET Proteins Can Affect Transcription

The most-studied cellular function of FET proteins, at the mechanistic level, is the regulation of transcription. Initial evidence that the FET proteins can affect transcription was provided by the increases or decreases in the mRNAs for numerous genes found by RNA-seq or microarray experiments in cells following knockdown of FET proteins (34, 37, 51, 54, 55, 58, 77–79).

Several lines of evidence demonstrate that the effects of FET proteins on mRNA levels are at least partly due to direct changes in transcription levels. First, FUS can affect the gene distribution and modification status of RNA Pol II. Specifically, ChIP-seq has found that FUS is associated with thousands of genes and is highly enriched near the transcription start site (TSS) (37). When FUS is knocked down, RNA Pol II accumulates near the TSS for genes in which FUS is localized to the TSS (37). The alteration in RNA Pol II distribution upon FUS knockdown probably occurs due to either an increase in transcriptional pausing or a failure to clear stalled polymerases from the TSS (37). Additional evidence for a direct role in transcription is that FET proteins physically interact with several transcription components, including RNA Pol II (17, 18, 22, 37, 80–82). Finally, in cell lysates, addition of FUS can stimulate RNA Pol II transcription and inhibit RNA Pol III transcription (79).

FET proteins were also suggested to enhance transcription because genomic translocations of the LC domain of FET proteins observed in leukemia and sarcomas are involved in the creation of a transcriptional activator (44, 45, 49, 83, 84). In these cases, the fusion of the strong FET protein promoter and the LC domain to a DNA-binding domain from ERG, CHOP, or FLI1 creates a potent oncogene (13, 14, 85). Additional evidence that the LC domain can have transcription activation activity is that an engineered fusion of LC to the DNA-binding domain of Gal4 can recruit RNA Pol II and activate transcription (17, 45, 86–88). Cells expressing the oncogenic fusions of FUS or EWSR1 show both activation and repression of numerous gene targets, foreshadowing the diversity of mechanisms by which

FET proteins can affect transcription (see the section titled FET Proteins and Cancer, below) (19, 89, 90).

Consistent with FUS playing a role in transcription is the observation that FUS preferentially localizes to active chromatin. Immunofluorescence studies have found that the FET homolog in *D. melanogaster*, cabeza, is sequestered to actively transcribed regions with loose chromatin compaction on polytene chromosomes (46). During meiosis, FUS protein coats autosomes but not X or Y chromosomes, which are held transcriptionally silent (91). Moreover, on the basis of immunofluorescence, FUS is not bound to chromatin during mitosis, while transcription is off (36).

FET Proteins Affect Transcription by Multiple Mechanisms

FET proteins have been suggested to both activate and silence transcription. This duplicity is likely the result of the multiple mechanisms by which FET proteins affect transcription.

Directing binding of RNA Pol II by FET protein fibers.—One mechanism by which FET proteins affect transcription is by directly binding RNA Pol II, which may recruit the polymerase and/or modulate its phosphorylation status. In this model, either noncoding RNAs expressed near gene promoters or nascent pre-mRNA transcripts can serve as the seed to promote FET proteins to oligomerize. Once higher-order assemblies are formed around TSSs, they interact with the CTD of RNA Pol II. This interaction has two functional consequences: (*a*) RNA Pol II is more efficiently recruited to the TSS and (*b*) the CTD is protected from premature Ser2 phosphorylation, which stimulates the transition of the polymerase from initiation to active elongation. Subsequently, higher-order assemblies may disassemble and leave a promoter upon posttranslational modification of the FET protein, either phosphorylation of the LC domain or other modifications.

Evidence suggests that the ability of FET proteins to bind RNA Pol II, specifically the CTD, is promoted by the oligomerization of the LC domain into fibers (17, 18). For example, the LC domain of FET proteins can form fibers in vitro, and mutations that disrupt fiber formation also disrupt the ability of the LC domain to promote transcription in vivo using a GAL4–LC fusion protein (17, 22, 92). Moreover, the interaction between FUS and the CTD is RNA dependent (37), and this RNA dependence appears to arise from the ability of RNA to promote oligomerization of FUS (18). Taken together, these observations suggest a general model wherein FET proteins regulate transcription by being recruited to promoters by local RNA transcripts, forming an oligomer fiber that is capable of interaction with the RNA Pol II CTD and can recruit more polymerases to the gene’s promoter, then modulating access of the CTD to kinases and thereby regulating the transition from initiation to elongation (Figure 4a).

FUS regulation of the phosphorylation status of Ser2 on the CTD of RNA Pol II was observed by ChIP-seq of the Ser2-phosphorylated RNA Pol II with or without small interfering RNA knockdown of FUS (37). This regulation is especially pronounced in HEK293T/17 cells (37). The kinases P-TEFb and CDK12 phosphorylate the CTD on Ser2 to help the transition to elongation and ultimately signal for efficient splicing and polyadenylation. FUS specifically inhibits Ser2 phosphorylation by the kinases P-TEFb and

CDK12 in both in vivo reactions and in reactions performed with recombinant purified proteins in vitro (37). Another kinase, the transcription factor II human (TFIIH)-associated CDK7 kinase, phosphorylates the CTD on Ser5 and signals for initiation of transcription and capping of the new mRNA. FUS does not inhibit Ser5 phosphorylation in vivo, nor does it inhibit TFIIH phosphorylation of the CTD in vitro. One explanation for this difference in phosphorylation may involve the way in which FUS interacts with the CTD. The CTD may bind along the stacked β-sheets of FUS assemblies to occlude Ser2 recognition by the Ser2 kinase P-TEFb or CDK12, but leave Ser5 exposed for targeting by the Ser5 kinase CDK7 (17). In this model, FET proteins, or FUS in particular, may affect the transition of RNA Pol II from the initiation phase to the elongation phase by controlling the accessibility of CTD to be phosphorylated by P-TEFb or CDK12.

Because FET proteins can be phosphorylated and phosphorylation of the LC domain prevents oligomerization (16, 40, 41, 93), which is required for CTD interaction, FET phosphorylation events may affect the ability of FET proteins to regulate transcription (17, 18). For example, phosphorylation of Ser266 affects the ability of EWSR1 fusion proteins to influence transcription (94).

Other protein modifications also modulate FET protein function. The LC domain of EWSR1 is modified by *O*-GlcNAcylation. Reduction of *O*-GlcNAcylation with small-molecule inhibitors reduces the expression level of the fusion protein EWSR1–FLI1 and represses the expression of genes regulated by EWSR1–FLI1 (95). Finally, methylation of EWSR1 or TAF15 by protein arginine *N*-methyltransferase 1 (PRMT1) causes these FET proteins to relocalize to the cytoplasm and subsequently reduces their ability to regulate transcription in the nucleus (96–98).

Phosphorylation of the CTD on Ser2 or Ser5 may inhibit CTD–LC interactions in vitro (17). However, full-length FUS and EWSR1 bind the phosphorylated forms of RNA Pol II in pull-down assays from cell lysates or using recombinant purified proteins (37, 81, 99). One possible explanation for these different results is that the oligomeric form of the LC domain strongly prefers unphosphorylated CTD, but other regions of FUS can bind the CTD independently of its phosphorylation status.

Direct interactions with other transcription factors.—FET proteins also directly bind and promote or inhibit the activity of several transcription factors. FUS prevents transcription activation of the factor Spi-1 by binding Spi-1's DNA-binding domain and preventing DNA recognition (100). FUS interacts with nuclear hormone receptors, including retinoid X receptor, estrogen receptor, thyroid hormone receptor (TR), and glucocorticoid receptor. For TR, addition of FUS promotes a much stronger activation than does stimulation with TR alone (92). FUS interacts with the nuclear factor κB (NF-κB) factor p65 and activates p65-dependent transcription in a reporter assay (101). FUS binds and inhibits CBP and p300 histone acetyltransferases. FUS recruitment to the in vivo target gene *CCND1* correlates with reduced histone acetylation and reduced transcription. Interestingly, recruitment of FUS seems to depend on the expression of noncoding RNAs in the CCND1 promoter, expression of which is induced by DNA damage (53). Additionally, EWSR1 binds

and activates the transcriptional activity of transcription factors Oct-4, CBP, and HNF4α (77, 78). EWSR1 inhibits the activity of retinoic acid receptor (Figure 4b) (77).

The diversity of transcription factors at different promoters and the multiple mechanisms by which FET proteins can affect transcription provide a reasonable explanation for why FET proteins show gene-specific increases or decreases in transcription activity. Several important questions about FET proteins' role in transcription remain. Is there specificity or redundancy in the functions of different FET proteins on transcription? Do FET proteins regulate primarily interactions between RNA Pol II and other transcription regulators, or do they have direct effects on RNA Pol II in vivo? What is the mechanism by which some genes avoid regulation by individual FET proteins? If FET proteins were found to possess any sequence specificity in vivo, this finding would provide one simple explanation for targeting of specific genes for regulation. Is there a common function of FET proteins for every gene they associate with, which subsequently activates or represses transcription on the basis of downstream effects of other local transcription factors? Or do FET proteins possess two distinct mechanisms for interacting with genes, one activating and one repressing?

FET PROTEINS AND CANCER

The ability of FET proteins to stimulate transcription directly connects to their roles in tumor promotion. Our treatment of the role of FET proteins in cancer is relatively brief, as more extensive reviews can be found elsewhere (13, 14, 19, 85).

FET protein translocations involved in cancer always involve the fusion of the NTD of a FET protein to the DNA-binding domain of a transcription factor. The C-terminal parts of these fusion proteins come from a number of transcription factors (13). Translocations are observed in Ewing sarcoma, small round cell tumor, bone sarcoma, myxoid liposarcoma, clear cell sarcoma, myxoid chondrosarcoma, fibromyxoid sarcoma, and acute leukemia (13, 19, 85). In fact, half of all fusion proteins observed in sarcomas involve one of the FET proteins (13). More than 90% of Ewing sarcomas involve a translocation in EWSR1 (19). More than 85% of myxoid liposarcomas have a translocation involving FUS (14, 85).

Each of the FET fusion proteins is a powerful oncogene that is sufficient to promote tumorigenesis for a specific tissue (44, 45, 48, 49, 77, 83, 84, 87–90, 102–105). Even a synthetic fusion protein involving the LC domain of the *Drosophila* protein cabeza is transformative (46). The unique translocation of a FET protein to each transcription factor specifically promotes tumorigenesis in a particular tissue (102, 103). For example, mice expressing the FUS–CHOP fusion protein develop only liposarcoma and not tumors of different tissues (102). Furthermore, through the use of reporter gene transcription assays, FET fusion proteins appear to be transcription activators (45, 48, 87, 88, 92, 97, 101, 106, 107). In contrast, genome-wide studies confirm that equal numbers of genes are activated or silenced by expression of either FUS or EWSR1 fusion proteins (19, 89, 90).

In conclusion, broad questions remain about the role of FET proteins in oncogenesis. Are repeated translocations within the same exons due to evolutionary selection or a preferred mechanism for translocation in cells? Is there a core set of genes targeted by FET fusion

proteins for each tumor type? What is the basis for the cell-type specificity of transformation by FET fusion proteins? Is there a role for alternative splicing in cell transformation?

FET PROTEIN EFFECTS ON RNA PROCESSING

FET proteins affect RNA-processing events. Evidence for a role of FET proteins in splicing is that a knockdown of FUS (55, 57, 58) or EWSR1 (108) alters splicing for many gene products, as analyzed by RNA-seq. There is no bias for exon inclusion or exclusion. Changes in splicing can be validated by semiquantitative reverse transcription polymerase chain reaction assays (58). In addition, RNA-seq of cells with FUS depletion shows multiple changes in the site of polyadenylation (37).

The RNA-processing functions of FET proteins could be linked to their role in transcription. RNA splicing and polyadenylation are thought to largely occur cotranscriptionally (1, 2, 6), and FET proteins could affect these processes by altering the loading of splicing factors such as SR proteins onto the polymerase (82, 99, 109). Another model is that FET proteins affect the rate of transcription elongation, which can also affect splicing (37, 110). Consistent with the idea that at least part of FET proteins' effects on splicing is due to transcriptional effects, FUS, EWSR1, and the transcription factors with which they interact have similar effects on RNA splicing (81, 99, 100).

Some evidence suggests that FUS may also directly affect splicing through interactions with splicing factors or the pre-mRNA itself. First, FET proteins are also implicated in splicing because they bind introns, as well as other parts of the mRNA, based on numerous CLIP-seq studies (51, 55, 57, 58). At a minimum, the interaction between FET proteins and pre-mRNAs can alter the availability of splice sites and binding sites for other factors. In addition, because FET proteins can interact physically with many hnRNP proteins, FET protein binding to pre-mRNAs could more broadly alter nascent mRNP assembly and splice-site accessibility.

Additional evidence that FET proteins affect splicing is the finding that in many cases transfection of FET proteins into cells can alter splicing patterns—although, for the popular E1A splicing assay, whether this is due to effects on transcription remains to be determined (80, 81, 99). FUS does affect β-globin pre-mRNA splicing in HeLa cell lysates (50, 100, 109). In that case, the β-globin pre-mRNA assay shows effects on splicing by immunodepleting and adding back recombinant FUS into cell lysates, which offers direct evidence for a role for FUS in splicing. This will be an interesting area for further investigation (109).

Finally, FET proteins interact with the splicing machinery. Most notably, FET proteins bind SR proteins (80, 82, 109) and the U1 snRNP complex (32, 35, 52, 58, 111). One report argues that FET proteins bind the snRNP but not the U1 RNA itself (35). U1 is one of the most prominent RNAs that copurifies with FUS protein, and potential cross-link sites between FUS and U1 have been identified in CLIP-seq data (52, 58). FUS binds hnRNP proteins, which also affect splicing (32, 45, 52, 82, 109, 112). Also, note that FUS regulates Ser2 phosphorylation on the CTD of RNA Pol II. This particular modification regulates the

interactions of splicing factors with RNA Pol II and splicing itself (110, 113). FUS and EWSR1 also interact with SMN proteins, suggesting they may affect snRNA biogenesis (52, 63, 114, 115).

Questions remain about the role of FET proteins in RNA processing. Are FET protein effects on RNA processing due to direct interactions with RNA and RNA-processing factors and/or downstream effects of changes in transcription itself? Do FET proteins help load splicing and RNA-processing factors onto the polymerase at the initiation of transcription? Do FET proteins make any specific interactions with a pre-mRNA that might specifically regulate splicing or RNA processing?

NUCLEAR–CYTOPLASMIC SHUTTLING OF FET PROTEINS

Although FET proteins are predominantly nuclear, multiple lines of evidence show that FET proteins cycle in and out of the nucleus. FET protein shuttling is important because mutations in the NLS of FUS both increase cytosolic FUS protein and lead to the neurodegenerative diseases ALS and FTLD (21, 116, 117). Heterokaryons, fusions of mouse and human cells, provide strong evidence for FET protein shuttling. In a heterokaryon cell, proteins that are trapped in the nucleus, such as hnRNPC, remain only in the original nucleus. Proteins that cycle, such as hnRNPA1 and FUS, are equally likely to be imported to either nucleus; therefore, the human proteins are observed to accumulate in the mouse nucleus (38).

The shuttling of FET proteins suggests a “life cycle” wherein FET proteins first interact with the transcription machinery and, in some cases, the nascent pre-mRNA, followed by nuclear export of FET proteins in conjunction with the mature mRNP. An unresolved issue is the extent to which FET proteins are exported with mature mRNA and what differences exist between exported FET proteins and the bulk of the protein resident in the nucleus. Once in the cytosol, FET proteins are released from their bound mRNAs by one of the following: mRNP remodeling components, posttranslational modifications that decrease RNA binding, displacement by elongating ribosomes, or ultimately degradation of the bound mRNA. Cytoplasmic FET proteins are then reimported into the nucleus upon transportin recognition of a nontraditional nuclear localization signal (PY-NLS) found at the C terminus of FET proteins (118–120). Modifications near this PY-NLS sequence, including arginine methylation and tyrosine phosphorylation, alter the cytoplasmic accumulation of the FET proteins (96–98, 118, 120–123). Arginine methylation of FUS or EWSR1 causes these proteins to accumulate in the cytoplasm and prevents their nuclear functions (97, 98, 120–122).

In some cell types, or in response to environmental cues, FET protein levels are increased in the cytosol. For example, examination of 35 different tissues for FUS distribution showed significant cytoplasmic accumulation of FUS in several tissue types (65). Similarly, in neurons, FUS protein is localized to dendritic spines, where many mRNAs are stored in a translational repressed state for later local translation in response to synaptic activity. This finding suggests that FUS is important for mRNA trafficking along dendrites (124–126). $FUS^{-/-}$ mice show significantly reduced dendritic spines (126). During cell adhesion, FET

proteins also accumulate in cytoplasmic spreading initiation centers (SICs), along with several hnRNP proteins (112, 127). SICs occur near newly formed focal adhesions between cell membranes and an adherent surface. SICs incorporate many proteins involved in integrin-mediated adhesion, such as RACK1 and vinculin (112). One intriguing possibility is that SICs, which are known to contain ribosomal RNA, might also contain mRNA associated with FUS, and FUS might play a role in controlling SICs' cytoplasmic location or translation.

Evidence that FUS controls translation in the cytosol has come from the analysis of mRNPs associated with the tumor suppressor adenomatous polyposis coli (APC) in cell protrusions (128). In this case, FUS appears to be associated with mRNAs targeted to cell protrusions, and in the absence of FUS, those mRNAs are translated less efficiently. Thus, FET proteins may also modulate mRNA function in the cytosol; this area is ripe for further investigation.

FET proteins can accumulate in cytoplasmic stress granules, which are cytoplasmic mRNP granules containing translationally silenced mRNA that is associated with some translation initiation factors and RNA-binding proteins. The accumulation of FET proteins in stress granules depends on both their concentration in the cytosol and stress, which triggers stress granule formation by the inhibition of translation. For example, overexpression of FUS and Taf15 proteins by transient transfection can lead to the spontaneous formation of stress granules (127). In contrast, endogenous FUS protein accumulates in stress granules only during osmotic stress and remains nuclear during oxidative stress, heat shock, or endoplasmic reticulum (ER) stress (129). However, when the FUS NLS is mutated, the cytosolic FUS concentration is increased, and FUS then accumulates in stress granules in response to multiple stresses (64, 67, 129, 130).

FET PROTEINS AND NEURODEGENERATIVE DISEASE

FET proteins have been implicated in neurodegeneration in a manner that is at least partly related to their cytoplasmic shuttling. Extensive reviews of the role of FET proteins in neurodegenerative diseases are available elsewhere (20, 31, 131–134). Mutations in FUS are currently tied for the third leading cause of the neurodegenerative disease ALS, and they account for 5% of familial and 1% of sporadic cases (31, 132–134). FUS attracted particular attention when mutations were discovered to cause ALS. This finding, along with evidence that mutations in the RNA-binding protein TDP-43 are associated with neurodegenerative disease, began a trend implicating perturbations in RNA processing as contributing to neurodegeneration (20, 68, 69, 131). TAF15 mutations have been associated with only a few ALS cases, and no mutations have been confirmed for EWSR1 (135). Mutations in FUS also cause or associate with FTLD, which is related to ALS (116, 117).

In ALS patients, FUS accumulates in cytoplasmic inclusion bodies, which include additional markers of stress granules (136, 137). Strikingly, all three FET proteins are found in cytoplasmic aggregates in the neurons of patients with FTLD (116). Mutations in FUS that trigger neurodegenerative disease cluster in the NLS but may also be distributed throughout the protein (138). Several mutations near a putative nuclear export sequence within the first RGG of FUS appear in familial ALS (139), as do numerous mutations identified throughout

the protein in sporadic ALS (138). Although all of these mutations are predicted to be deleterious, the extent to which they cause pathology remains to be determined.

The accumulation of FET proteins in cytoplasmic aggregates and the fact that some disease-causing mutations limit nuclear import have led to a two-hit model for FUS malfunction in neurodegeneration (120). In this gain-of-function model for pathology, mutations limiting FUS nuclear import lead to a cytoplasmic pool of protein, which can aggregate into stress granules during times of stress. The pathological consequence of FET protein accumulation in stress granules is unknown (130). One possibility is a gain-of-function effect in which FET protein aggregates may sequester other key regulators or trigger abnormal signaling pathways and alter cell physiology in a manner leading to cell death (131).

An alternative, but not mutually exclusive, model is that FUS contributes to neurodegeneration due to the loss of normal FUS function. For example, aggregates of FUS, whether nuclear or cytoplasmic, may deplete the cell of functional FUS protein (64, 67, 119, 140, 141). Cells expressing a mutant FUS show a loss of Gemini of coiled bodies (Gems), consistent with a loss of FUS function (52, 115). Defects in RNA splicing detected in ALS patients' brains are consistent with a loss of function for FUS (58). Moreover, a zebrafish FUS knockout mutant demonstrates neurodegenerative phenotypes, and reintroduction of ALS-causing mutant FUS fails to rescue these phenotypes, suggesting a loss of function (142). Similarly, a mouse expressing a severe ALS-causing mutant, FUS R521C, shows numerous deficits consistent with a loss of FUS function (143). Finally, in ALS patient–derived fibroblasts, defects in phosphorylation of RNA Pol II and localization of RNA Pol II within the nucleus, consistent with a loss of FUS function, have also been observed (141).

That most mutations in FUS or TAF15 causing ALS or FTLD are located in the NLS sequence strongly suggests that nuclear–cytoplasmic shuttling is important in FET protein function. A long list of questions remains about the role this function plays in FET protein biology: Do FET proteins perform predominantly different functions in different tissues depending on their nuclear or cytoplasmic distribution? Does the presence of the same PY-NLS on several interacting partners of FET proteins speak to a common molecular pathway for their function, or are they merely the result of convergent evolution? Do FET proteins perform fundamentally similar roles (perhaps a structural role) in each of the subcellular locations (transcriptionally active chromatin, dendritic spines, SICs) in which they accumulate? How does the mislocalization of FET proteins that have disease-causing mutations in their NLS affect each of their various nuclear and cytoplasmic functions?

FET PROTEINS AND DNA DAMAGE REPAIR

FET proteins are also implicated in DNA damage repair. This function of FET proteins is interesting because it may contribute to pathology in neurodegenerative diseases. A knockdown of either FUS or EWSR1 expression in cell culture causes deficiencies in DNA damage recovery, as measured by the comet-tail assay or by cell-colony survival following treatment with ionizing radiation (108, 144–146). FUS or EWSR1 knockout in cells and mice leads to accumulation of DNA breaks and high sensitivity to ionizing radiation (91,

147–149). In fact, merely overexpressing the ALS-causing mutant FUS R521C also causes a defect in DNA damage recovery, as observed by the comet-tail assay (143).

FUS is one of the earliest proteins recruited to DNA lesions, as observed by immunofluorescence following laser microirradiation (144, 145). In contrast, EWSR1 accumulates in nucleoli following DNA damage by UV irradiation (108). Phosphorylated FUS binds dsDNA breaks and Holliday junctions (40). A loss of FUS inhibits or delays the recruitment of the DNA repair factors histone deacetylase 1 (HDAC1), γH2AX, phosphorylated ATM, and DNA-PK to sites of DNA damage (144). EWSR1 interacts with DNA-PK and PARP1 in a DNA damage–dependent manner (150). FUS is phosphorylated by ATM following DNA damage (40); however, inhibitors of this kinase still allow FUS recruitment to sites of DNA damage (145). An inhibitor of the poly(ADP-ribose) polymerase, PARP1, does inhibit FUS recruitment to sites of DNA damage (145). Interestingly, both FUS and EWSR1 undergo phosphorylation by protein kinase C, a pro-apoptotic kinase also involved in the DNA damage response (94, 151). Additionally, FUS protein is phosphorylated by the fusion kinase BCR/ABL, which in turn promotes binding to ssDNA (93). This phosphorylation may regulate the POM activity of FUS and EWSR1 proteins or the promotion of invasion of dsDNA by ssDNA in cells (41–43).

Lastly, FUS binds upregulated noncoding RNAs following DNA damage by ionizing radiation. For the gene *CCND1*, the recruitment of FUS to noncoding RNAs expressed from the gene promoter is coordinated with inhibition of CBP/p300-dependent acetylation of histones and a reduction in *CCND1* gene expression (53). The regulation of CBP/p300 by FUS can be reconstituted in vitro. Pull-down and activity assays suggest that the NTD and CTD of FUS work in concert to accomplish this function, reportedly through the following allosteric mechanism. The LC domain is sequestered by the RGG–ZnF–RGG domain until the latter binds RNA. Upon RNA binding, the LC domain is released to bind and inhibit CBP and p300.

In short, much remains to be learned about the role of FET proteins in DNA damage repair. Important questions include the following: What is the relationship between transcription and DNA damage repair with respect to FET protein function in each? How do DNA damage–repair factors recruited by FET proteins know whether the targeted site is a site of transcription or a DNA lesion? Could DNA damage–repair factors possibly also be recruited to TSSs by FET proteins and perform a function there? Do EWSR1 and TAF15 mimic FUS function at sites of DNA damage? Are these functions redundant or unique? What role, if any, do posttranslational modifications have in FET protein response to DNA damage? Do FET proteins aid in strand annealing at sites of dsDNA breaks in a manner analogous to reported POM activities? Does impairment of FUS protein DNA damage–repair functions contribute to the pathology of ALS and FTLD?

SUMMARY

FET proteins are abundant nuclear RNA-binding proteins that are structurally related to hnRNP proteins. They bind RNA with broad sequence specificity, as evidenced by the large number of RNAs that are bound by FET proteins in vivo. FET proteins regulate both

transcription and RNA processing, and the mechanisms by which protein–RNA, protein–DNA, and protein–protein interactions contribute to this regulation are the subject of much current research. FET proteins also have DNA damage–repair functions. FET proteins have prion-like domains of low amino acid sequence complexity, which can promote higher-order assembly and fiber formation. An attractive model posits that the controlled formation of fibrous assemblies is critical to some or all normal FET cellular functions, but that uncontrolled aggregation (e.g., due to mutation or posttranslational modification) is pathogenic in neurodegeneration. Protein aggregation may contribute to disease both by toxic gain of function and by depletion of the normal functions of the FET proteins. Finally, in sarcomas the fusion of a FET LC domain to a new DNA-binding domain leads to inappropriate transcriptional activation that drives oncogenesis. Thus, the biochemical properties of the FET proteins are contributing to our understanding of both their biological functions and their roles in disease.

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LITERATURE CITED

1. Eberle AB, Visa N. 2014. Quality control of mRNP biogenesis: networking at the transcription site. Semin. Cell Dev. Biol 32C:37–46
2. Saguez C, Olesen JR, Jensen TH. 2005. Formation of export-competent mRNP: escaping nuclear destruction. Curr. Opin. Cell Biol 17:287–93 [PubMed: 15901499]
3. Moore MJ, Proudfoot NJ. 2009. Pre-mRNA processing reaches back to transcription and ahead to translation. Cell 136:688–700 [PubMed: 19239889]
4. Hilleren P, Parker R. 1999. Mechanisms of mRNA surveillance in eukaryotes. Annu. Rev. Genet 33:229–60 [PubMed: 10690409]
5. Mitchell SF, Parker R. 2014. Principles and properties of eukaryotic mRNPs. Mol. Cell 54:547–58 [PubMed: 24856220]
6. Müller-McNicoll M, Neugebauer KM. 2013. How cells get the message: dynamic assembly and function of mRNA–protein complexes. Nat. Rev. Genet 14:275–87 [PubMed: 23478349]
7. Castello A, Fischer B, Eichelbaum K, Horos R, Beckmann BM, et al. 2012. Insights into RNA biology from an atlas of mammalian mRNA-binding proteins. Cell 149:1393–406 [PubMed: 22658674]
8. Piñol-Roma S, Dreyfuss G. 1992. Shuttling of pre-mRNA binding proteins between nucleus and cytoplasm. Nature 355:730–32 [PubMed: 1371331]
9. Piñol-Roma S, Dreyfuss G. 1993. hnRNP proteins: localization and transport between the nucleus and the cytoplasm. Trends Cell Biol. 3:151–55 [PubMed: 14731609]
10. Lau JS, Baumeister P, Kim E, Roy B, Hsieh TY, et al. 2000. Heterogeneous nuclear ribonucleoproteins as regulators of gene expression through interactions with the human thymidine kinase promoter. J. Cell Biochem 79:395–406 [PubMed: 10972977]
11. Tomonaga T, Levens D. 1995. Heterogeneous nuclear ribonucleoprotein K is a DNA-binding transactivator. J. Biol. Chem 270:4875–81 [PubMed: 7876260]
12. Miau LH, Chang CJ, Shen BJ, Tsai WH, Lee SC. 1998. Identification of heterogeneous nuclear ribonucleoprotein K (hnRNP K) as a repressor of C/EBPβ-mediated gene activation. J. Biol. Chem 273:10784–91 [PubMed: 9553145]
13. Riggi N, Cironi L, Suva ML, Stamenkovic I. 2007. Sarcomas: genetics, signalling, and cellular origins. Part 1: The fellowship of TET. J. Pathol 213:4–20 [PubMed: 17691072]

14. Tan AY, Manley JL. 2009. The TET family of proteins: functions and roles in disease. J. Mol. Cell Biol 1:82–92 [PubMed: 19783543]
15. Han TW, Kato M, Xie S, Wu LC, Mirzaei H, et al. 2012. Cell-free formation of RNA granules: Bound RNAs identify features and components of cellular assemblies. Cell 149:768–79 [PubMed: 22579282]
16. Kato M, Han TW, Xie S, Shi K, Du X, et al. 2012. Cell-free formation of RNA granules: Low complexity sequence domains form dynamic fibers within hydrogels. Cell 149:753–67 [PubMed: 22579281]
17. Kwon I, Kato M, Xiang S, Wu L, Theodoropoulos P, et al. 2013. Phosphorylation-regulated binding of RNA polymerase II to fibrous polymers of low-complexity domains. Cell 155:1049–60 [PubMed: 24267890]
18. Schwartz JC, Wang X, Podell ER, Cech TR. 2013. RNA seeds higher-order assembly of FUS protein. Cell Rep. 5:918–25 [PubMed: 24268778]
19. Kovar H 2011. Dr. Jekyll and Mr. Hyde: the two faces of the FUS/EWS/TAF15 protein family. Sarcoma 2011:837474 [PubMed: 21197473]
20. Lagier-Tourenne C, Cleveland DW. 2009. Rethinking ALS: the FUS about TDP-43. Cell 136:1001–4 [PubMed: 19303844]
21. Mackenzie IR, Rademakers R, Neumann M. 2010. TDP-43 and FUS in amyotrophic lateral sclerosis and frontotemporal dementia. Lancet Neurol. 9:995–1007 [PubMed: 20864052]
22. Bertolotti A, Lutz Y, Heard DJ, Chambon P, Tora L. 1996. hTAF(II)68, a novel RNA/ssDNA-binding protein with homology to the pro-oncoproteins TLS/FUS and EWS is associated with both TFIID and RNA polymerase II. EMBO J. 15:5022–31 [PubMed: 8890175]
23. Liu X, Niu C, Ren J, Zhang J, Xie X, et al. 2013. The RRM domain of human fused in sarcoma protein reveals a non-canonical nucleic acid binding site. Biochim. Biophys. Acta 1832:375–85 [PubMed: 23200923]
24. Xu RM, Jokhan L, Cheng X, Mayeda A, Krainer AR. 1997. Crystal structure of human UP1, the domain of hnRNP A1 that contains two RNA-recognition motifs. Structure 5:559–70 [PubMed: 9115444]
25. Ding J, Hayashi MK, Zhang Y, Manche L, Krainer AR, Xu RM. 1999. Crystal structure of the two-RRM domain of hnRNP A1 (UP1) complexed with single-stranded telomeric DNA. Genes Dev. 13:1102–15 [PubMed: 10323862]
26. Iko Y, Kodama TS, Kasai N, Oyama T, Morita EH, et al. 2004. Domain architectures and characterization of an RNA-binding protein, TLS. J. Biol. Chem 279:44834–40 [PubMed: 15299008]
27. Shamoo Y, Krueger U, Rice LM, Williams KR, Steitz TA. 1997. Crystal structure of the two RNA binding domains of human hnRNP A1 at 1.75Å resolution. Nat. Struct. Biol 4:215–22 [PubMed: 9164463]
28. Reijns MA, Alexander RD, Spiller MP, Beggs JD. 2008. A role for Q/N-rich aggregation-prone regions in P-body localization. J. Cell Sci 121:2463–72 [PubMed: 18611963]
29. Decker CJ, Teixeira D, Parker R. 2007. Edc3p and a glutamine/asparagine-rich domain of Lsm4p function in processing body assembly in *Saccharomyces cerevisiae*. J. Cell Biol 179:437–49 [PubMed: 17984320]
30. Gilks N, Kedersha N, Ayodele M, Shen L, Stoecklin G, et al. 2004. Stress granule assembly is mediated by prion-like aggregation of TIA-1. Mol. Biol. Cell 15:5383–98 [PubMed: 15371533]
31. King OD, Gitler AD, Shorter J. 2012. The tip of the iceberg: RNA-binding proteins with prion-like domains in neurodegenerative disease. Brain Res. 1462:61–80 [PubMed: 22445064]
32. Calvio C, Neubauer G, Mann M, Lamond AI. 1995. Identification of hnRNP P2 as TLS/FUS using electrospray mass spectrometry. RNA 1:724–33 [PubMed: 7585257]
33. Takahama K, Kino K, Arai S, Kurokawa R, Oyoshi T. 2011. Identification of Ewing's sarcoma protein (EWS) as a G-quadruplex DNA- and RNA-binding protein. FEBS J. 278:988–98 [PubMed: 21244633]
34. Tan AY, Riley TR, Coady T, Bussemaker HJ, Manley JL. 2012. TLS/FUS (translocated in liposarcoma/fused in sarcoma) regulates target gene transcription via single-stranded DNA response elements. PNAS 109:6040–35

35. Hackl W, Lührmann R. 1996. Molecular cloning and subcellular localisation of the snRNP-associated protein 69KD, a structural homologue of the proto-oncoproteins TLS and EWS with RNA- and DNA-binding properties. J. Mol. Biol 264:843–51 [PubMed: 9000615]
36. Alliegro MC, Alliegro MA. 1996. A nuclear protein regulated during the transition from active to quiescent phenotype in cultured endothelial cells. Dev. Biol 174:288–97 [PubMed: 8631501]
37. Schwartz JC, Ebmeier CC, Podell ER, Heimiller J, Taatjes DJ, Cech TR. 2012. FUS binds the CTD of RNA polymerase II and regulates its phosphorylation at Ser2. Genes Dev. 26:2690–95 [PubMed: 23249733]
38. Zinszner H, Sok J, Immanuel D, Yin Y, Ron D. 1997. TLS (FUS) binds RNA in vivo and engages in nucleo-cytoplasmic shuttling. J. Cell Sci 110:1741–50 [PubMed: 9264461]
39. Perrotti D, Iervolino A, Cesi V, Cirinna M, Lombardini S, et al. 2000. BCR-ABL prevents c-Jun-mediated and proteasome-dependent FUS (TLS) proteolysis through a protein kinase CβII–dependent pathway. Mol. Cell. Biol 20:6159–69 [PubMed: 10913197]
40. Gardiner M, Toth R, Vandermoere F, Morrice NA, Rouse J. 2008. Identification and characterization of FUS/TLS as a new target of ATM. Biochem. J 415:297–307 [PubMed: 18620545]
41. Guipaud O, Guillonneau F, Labas V, Praseuth D, Rossier J, et al. 2006. An in vitro enzymatic assay coupled to proteomics analysis reveals a new DNA processing activity for Ewing sarcoma and TAF(II)68 proteins. Proteomics 6:5962–72 [PubMed: 17106916]
42. Bertrand P, Akhmedov AT, Delacote F, Durrbach A, Lopez BS. 1999. Human POMp75 is identified as the pro-oncoprotein TLS/FUS: Both POMp75 and POMp100 DNA homologous pairing activities are associated to cell proliferation. Oncogene 18:4515–21 [PubMed: 10442642]
43. Baechtold H, Kuroda M, Sok J, Ron D, Lopez BS, Akhmedov AT. 1999. Human 75-kDa DNA-pairing protein is identical to the pro-oncoprotein TLS/FUS and is able to promote D-loop formation. J. Biol. Chem 274:34337–42 [PubMed: 10567410]
44. Crozat A, Aman P, Mandahl N, Ron D. 1993. Fusion of CHOP to a novel RNA-binding protein in human myxoid liposarcoma. Nature 363:640–44 [PubMed: 8510758]
45. Zinszner H, Albalat R, Ron D. 1994. A novel effector domain from the RNA-binding protein TLS or EWS is required for oncogenic transformation by CHOP. Genes Dev. 8:2513–26 [PubMed: 7958914]
46. Immanuel D, Zinszner H, Ron D. 1995. Association of SARFH (sarcoma-associated RNA-binding fly homolog) with regions of chromatin transcribed by RNA polymerase II. Mol. Cell. Biol 15:4562–71 [PubMed: 7623847]
47. Bertolotti A, Melot T, Acker J, Vigneron M, Delattre O, Tora L. 1998. 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 18:1489–97 [PubMed: 9488465]
48. Ohno T, Ouchida M, Lee L, Gatalica Z, Rao VN, Reddy ES. 1994. 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 9:3087–97 [PubMed: 8084618]
49. Ichikawa H, Shimizu K, Hayashi Y, Ohki M. 1994. An RNA-binding protein gene, TLS/FUS, is fused to ERG in human myeloid leukemia with t(16;21) chromosomal translocation. Cancer Res. 54:2865–68 [PubMed: 8187069]
50. Lerga A, Hallier M, Delva L, Orvain C, Gallais I, et al. 2001. Identification of an RNA binding specificity for the potential splicing factor TLS. J. Biol. Chem 276:6807–16 [PubMed: 11098054]
51. Hoell JI, Larsson E, Runge S, Nusbaum JD, Duggimpudi S, et al. 2011. RNA targets of wild-type and mutant FET family proteins. Nat. Struct. Mol. Biol 18:1428–31 [PubMed: 22081015]
52. Yamazaki T, Chen S, Yu Y, Yan B, Haertlein TC, et al. 2012. FUS–SMN protein interactions link the motor neuron diseases ALS and SMA. Cell Rep. 2:799–806 [PubMed: 23022481]
53. Wang X, Arai S, Song X, Reichart D, Du K, et al. 2008. Induced ncRNAs allosterically modify RNA-binding proteins in *cis* to inhibit transcription. Nature 454:126–30 [PubMed: 18509338]

54. Colombrita C, Onesto E, Megiorni F, Pizzuti A, Baralle FE, et al. 2012. TDP-43 and FUS RNA-binding proteins bind distinct sets of cytoplasmic messenger RNAs and differently regulate their post-transcriptional fate in motoneuron-like cells. J. Biol. Chem 287:15636–47
55. Ishigaki S, Masuda A, Fujioka Y, Iguchi Y, Katsuno M, et al. 2012. Position-dependent FUS–RNA interactions regulate alternative splicing events and transcriptions. Sci. Rep 2:529 [PubMed: 22829983]
56. Daigle JG, Lanson NA Jr, Smith RB, Casci I, Maltare A, et al. 2013. RNA-binding ability of FUS regulates neurodegeneration, cytoplasmic mislocalization and incorporation into stress granules associated with FUS carrying ALS-linked mutations. Hum. Mol. Genet 22:1193–205 [PubMed: 23257289]
57. Rogelj B, Easton LE, Bogu GK, Stanton LW, Rot G, et al. 2012. Widespread binding of FUS along nascent RNA regulates alternative splicing in the brain. Sci. Rep 2:603 [PubMed: 22934129]
58. Lagier-Tourenne C, Polymenidou M, Hutt KR, Vu AQ, Baughn M, et al. 2012. Divergent roles of ALS-linked proteins FUS/TLS and TDP-43 intersect in processing long pre-mRNAs. Nat. Neurosci 15:1488–97 [PubMed: 23023293]
59. Takahama K, Oyoshi T. 2013. Specific binding of modified RGG domain in TLS/FUS to G-quadruplex RNA: Tyrosines in RGG domain recognize 2′-OH of the riboses of loops in G-quadruplex. J. Am. Chem. Soc 135:18016–19 [PubMed: 24251952]
60. Ray D, Kazan H, Cook KB, Weirauch MT, Najafabadi HS, et al. 2013. A compendium of RNA-binding motifs for decoding gene regulation. Nature 499:172–77 [PubMed: 23846655]
61. Sun Z, Diaz Z, Fang X, Hart MP, Chesi A, et al. 2011. Molecular determinants and genetic modifiers of aggregation and toxicity for the ALS disease protein FUS/TLS. PLOS Biol. 9:e1000614 [PubMed: 21541367]
62. Doi H, Okamura K, Bauer PO, Furukawa Y, Shimizu H, et al. 2008. RNA-binding protein TLS is a major nuclear aggregate–interacting protein in huntingtin exon 1 with expanded polyglutamine-expressing cells. J. Biol. Chem 283:6489–500 [PubMed: 18167354]
63. Shaw DJ, Morse R, Todd AG, Eggleton P, Lorson CL, Young PJ. 2010. Identification of a self-association domain in the Ewing's sarcoma protein: a novel function for arginine–glycine–glycine rich motifs? J. Biochem 147:885–93 [PubMed: 20211855]
64. Vance C, Scotter EL, Nishimura AL, Troakes C, Mitchell JC, et al. 2013. ALS mutant FUS disrupts nuclear localization and sequesters wild-type FUS within cytoplasmic stress granules. Hum. Mol. Genet 22:2676–88 [PubMed: 23474818]
65. Yamaguchi A, Kitajo K. 2012. The effect of PRMT1-mediated arginine methylation on the subcellular localization, stress granules, and detergent-insoluble aggregates of FUS/TLS. PLOS ONE 7:e49267 [PubMed: 23152885]
66. Mackenzie IR, Munoz DG, Kusaka H, Yokota O, Ishihara K, et al. 2011. Distinct pathological subtypes of FTLD-FUS. Acta Neuropathol. 121:207–18 [PubMed: 21052700]
67. Bosco DA, Lemay N, Ko HK, Zhou H, Burke C, et al. 2010. Mutant FUS proteins that cause amyotrophic lateral sclerosis incorporate into stress granules. Hum. Mol. Genet 19:4160–75 [PubMed: 20699327]
68. Vance C, Rogelj B, Hortobagyi T, De Vos KJ, Nishimura AL, et al. 2009. Mutations in FUS, an RNA processing protein, cause familial amyotrophic lateral sclerosis type 6. Science 323:1208–11 [PubMed: 19251628]
69. Kwiatkowski TJ Jr, Bosco DA, Leclerc AL, Tamrazian E, Vanderburg CR, et al. 2009. Mutations in the FUS/TLS gene on chromosome 16 cause familial amyotrophic lateral sclerosis. Science 323:1205–8 [PubMed: 19251627]
70. Ju S, Tardiff DF, Han H, Divya K, Zhong Q, et al. 2011. A yeast model of FUS/TLS-dependent cytotoxicity. PLOS Biol. 9:e1001052 [PubMed: 21541368]
71. Saito R, Smoot ME, Ono K, Ruscheinski J, Wang PL, et al. 2012. A travel guide to Cytoscape plugins. Nat. Methods 9:1069–76 [PubMed: 23132118]
72. Ballarino M, Jobert L, Dembele D, de la Grange P, Auboeuf D, Tora L. 2013. TAF15 is important for cellular proliferation and regulates the expression of a subset of cell cycle genes through miRNAs. Oncogene 32:4646–55 [PubMed: 23128393]

73. Morlando M, Dini Modigliani S, Torrelli G, Rosa A, Di Carlo V, et al. 2012. FUS stimulates microRNA biogenesis by facilitating co-transcriptional Drosha recruitment. EMBO J. 31:4502–10 [PubMed: 23232809]
74. Thomsen C, Grundevik P, Elias P, Stahlberg A, Aman P. 2013. A conserved N-terminal motif is required for complex formation between *FUS*, *EWSR1*, *TAF15* and their oncogenic fusion proteins. FASEB J. 27:4965–74 [PubMed: 23975937]
75. Korennykh AV, Egea PF, Korostelev AA, Finer-Moore J, Zhang C, et al. 2009. The unfolded protein response signals through high-order assembly of Ire1. Nature 457:687–93 [PubMed: 19079236]
76. Lyumkis D, Talley H, Stewart A, Shah S, Park CK, et al. 2013. Allosteric regulation of DNA cleavage and sequence-specificity through run-on oligomerization. Structure 21:1848–58 [PubMed: 24055317]
77. Araya N, Hirota K, Shimamoto Y, Miyagishi M, Yoshida E, et al. 2003. Cooperative interaction of EWS with CREB-binding protein selectively activates hepatocyte nuclear factor 4–mediated transcription. J. Biol. Chem 278:5427–32 [PubMed: 12459554]
78. Lee J, Rhee BK, Bae GY, Han YM, Kim J. 2005. Stimulation of Oct-4 activity by Ewing's sarcoma protein. Stem Cells 23:738–51 [PubMed: 15917470]
79. Tan AY, Manley JL. 2010. TLS inhibits RNA polymerase III transcription. Mol. Cell. Biol 30:186–96 [PubMed: 19841068]
80. Yang L, Embree LJ, Hickstein DD. 2000. TLS–ERG leukemia fusion protein inhibits RNA splicing mediated by serine–arginine proteins. Mol. Cell. Biol 20:3345–54 [PubMed: 10779324]
81. Chansky HA, Hu M, Hickstein DD, Yang L. 2001. Oncogenic TLS/ERG and EWS/FLI-1 fusion proteins inhibit RNA splicing mediated by YB-1 protein. Cancer Res. 61:3586–90 [PubMed: 11325824]
82. Das R, Yu J, Zhang Z, Gygi MP, Krainer AR, et al. 2007. SR proteins function in coupling RNAP II transcription to pre-mRNA splicing. Mol. Cell 26:867–81 [PubMed: 17588520]
83. Rabbitts TH, Forster A, Larson R, Nathan P. 1993. Fusion of the dominant negative transcription regulator *CHOP* with a novel gene *FUS* by translocation t(12;16) in malignant liposarcoma. Nat. Genet 4:175–80 [PubMed: 7503811]
84. Panagopoulos I, Aman P, Fioretos T, Hoglund M, Johansson B, et al. 1994. Fusion of the *FUS* gene with *ERG* in acute myeloid leukemia with t(16;21)(p11;q22). Genes Chromosomes Cancer 11:256–62 [PubMed: 7533529]
85. Law WJ, Cann KL, Hicks GG. 2006. *TLS*, *EWS* and *TAF15*: a model for transcriptional integration of gene expression. Brief Funct. Genomics Proteomics 5:8–14
86. Zhang D, Paley AJ, Childs G. 1998. The transcriptional repressor ZFM1 interacts with and modulates the ability of EWS to activate transcription. J. Biol. Chem 273:18086–91 [PubMed: 9660765]
87. May WA, Lessnick SL, Braun BS, Klemsz M, Lewis BC, et al. 1993. The Ewing's sarcoma *EWS/FLI-1* fusion gene encodes a more potent transcriptional activator and is a more powerful transforming gene than *FLI-1*. Mol. Cell. Biol 13:7393–98 [PubMed: 8246959]
88. Bertolotti A, Bell B, Tora L. 1999. The N-terminal domain of human TAFII68 displays transactivation and oncogenic properties. Oncogene 18:8000–10 [PubMed: 10637511]
89. Hancock JD, Lessnick SL. 2008. A transcriptional profiling meta-analysis reveals a core *EWS–FLI* gene expression signature. Cell Cycle 7:250–56 [PubMed: 18256529]
90. Zou J, Ichikawa H, Blackburn ML, Hu HM, Zielinska-Kwiatkowska A, et al. 2005. The oncogenic TLS–ERG fusion protein exerts different effects in hematopoietic cells and fibroblasts. Mol. Cell. Biol 25:6235–46 [PubMed: 15988032]
91. Kuroda M, Sok J, Webb L, Baechtold H, Urano F, et al. 2000. Male sterility and enhanced radiation sensitivity in *TLS*$^{-/-}$ mice. EMBO J. 19:453–62 [PubMed: 10654943]
92. Powers CA, Mathur M, Raaka BM, Ron D, Samuels HH. 1998. TLS (translocated-in-liposarcoma) is a high-affinity interactor for steroid, thyroid hormone, and retinoid receptors. Mol. Endocrinol 12:4–18 [PubMed: 9440806]

93. Perrotti D, Bonatti S, Trotta R, Martinez R, Skorski T, et al. 1998. TLS/FUS, a pro-oncogene involved in multiple chromosomal translocations, is a novel regulator of BCR/ABL-mediated leukemogenesis. EMBO J. 17:4442–55 [PubMed: 9687511]
94. Deloulme JC, Prichard L, Delattre O, Storm DR. 1997. The prooncoprotein EWS binds calmodulin and is phosphorylated by protein kinase C through an IQ domain. J. Biol. Chem 272:27369–77 [PubMed: 9341188]
95. Bachmaier R, Aryee DN, Jug G, Kauer M, Kreppel M, et al. 2009. *O*-GlcNAcylation is involved in the transcriptional activity of EWS–FLI1 in Ewing's sarcoma. Oncogene 28:1280–84 [PubMed: 19151750]
96. Jobert L, Argentini M, Tora L. 2009. PRMT1 mediated methylation of TAF15 is required for its positive gene regulatory function. Exp. Cell Res 315:1273–86 [PubMed: 19124016]
97. Araya N, Hiraga H, Kako K, Arao Y, Kato S, Fukamizu A. 2005. Transcriptional down-regulation through nuclear exclusion of EWS methylated by PRMT1. Biochem. Biophys. Res. Commun 329:653–60 [PubMed: 15737635]
98. Belyanskaya LL, Delattre O, Gehring H. 2003. Expression and subcellular localization of Ewing sarcoma (EWS) protein is affected by the methylation process. Exp. Cell Res 288:374–81 [PubMed: 12915128]
99. Yang L, Chansky HA, Hickstein DD. 2000. EWS.Fli1 fusion protein interacts with hyperphosphorylated RNA polymerase II and interferes with serine–arginine protein-mediated RNA splicing. J. Biol. Chem 275:37612–18 [PubMed: 10982800]
100. Hallier M, Lerga A, Barnache S, Tavitian A, Moreau-Gachelin F. 1998. The transcription factor Spi-1/PU.1 interacts with the potential splicing factor TLS. J. Biol. Chem 273:4838–42 [PubMed: 9478924]
101. Uranishi H, Tetsuka T, Yamashita M, Asamitsu K, Shimizu M, et al. 2001. Involvement of the pro-oncoprotein TLS (translocated in liposarcoma) in nuclear factor κB p65–mediated transcription as a coactivator. J. Biol. Chem 276:13395–401 [PubMed: 11278855]
102. Pérez-Losada J, Sánchez-Martín M, Rodríguez-García MA, Pérez-Mancera PA, Pintado B, et al. 2000. Liposarcoma initiated by FUS/TLS-CHOP: The FUS/TLS domain plays a critical role in the pathogenesis of liposarcoma. Oncogene 19:6015–22 [PubMed: 11146553]
103. Ichikawa H, Shimizu K, Katsu R, Ohki M. 1999. Dual transforming activities of the FUS (TLS)–ERG leukemia fusion protein conferred by two N-terminal domains of FUS (TLS). Mol. Cell. Biol 19:7639–50 [PubMed: 10523652]
104. Prasad DD, Ouchida M, Lee L, Rao VN, Reddy ES. 1994. TLS/FUS fusion domain of TLS/FUS–erg chimeric protein resulting from the t(16;21) chromosomal translocation in human myeloid leukemia functions as a transcriptional activation domain. Oncogene 9:3717–29 [PubMed: 7970732]
105. Martini A, La Starza R, Janssen H, Bilhou-Nabera C, Corveleyn A, et al. 2002. Recurrent rearrangement of the Ewing's sarcoma gene, *EWSR1*, or its homologue, *TAF15*, with the transcription factor CIZ/NMP4 in acute leukemia. Cancer Res. 62:5408–12 [PubMed: 12359745]
106. Kuroda M, Wang X, Sok J, Yin Y, Chung P, et al. 1999. Induction of a secreted protein by the myxoid liposarcoma oncogene. PNAS 96:5025–30 [PubMed: 10220412]
107. Braun BS, Frieden R, Lessnick SL, May WA, Denny CT. 1995. Identification of target genes for the Ewing's sarcoma EWS/FLI fusion protein by representational difference analysis. Mol. Cell. Biol 15:4623–30 [PubMed: 7623854]
108. Paronetto MP, Minana B, Valcarcel J. 2011. The Ewing sarcoma protein regulates DNA damage–induced alternative splicing. Mol. Cell 43:353–68 [PubMed: 21816343]
109. Meissner M, Lopato S, Gotzmann J, Sauermann G, Barta A. 2003. Proto-oncoprotein TLS/FUS is associated to the nuclear matrix and complexed with splicing factors PTB, SRM160, and SR proteins. Exp. Cell Res 283:184–95 [PubMed: 12581738]
110. Muñoz MJ, Pérez-Santangelo MS, Paronetto MP, de la Mata M, Pelisch F, et al. 2009. DNA damage regulates alternative splicing through inhibition of RNA polymerase II elongation. Cell 137:708–20 [PubMed: 19450518]

111. Leichter M, Marko M, Ganou V, Patrinou-Georgoula M, Tora L, Guialis A. 2011. A fraction of the transcription factor TAF15 participates in interactions with a subset of the spliceosomal U1 snRNP complex. Biochim. Biophys. Acta 1814:1812–24 [PubMed: 22019700]

112. de Hoog CL, Foster LJ, Mann M. 2004. RNA and RNA binding proteins participate in early stages of cell spreading through spreading initiation centers. Cell 117:649–62 [PubMed: 15163412]

113. Bres V, Yoh SM, Jones KA. 2008. The multi-tasking P-TEFb complex. Curr. Opin. Cell Biol 20:334–40 [PubMed: 18513937]

114. Todd AG, Morse R, Shaw DJ, Stebbings H, Young PJ. 2010. Analysis of SMN-neurite granules: Core Cajal body components are absent from SMN–cytoplasmic complexes. Biochem. Biophys. Res. Commun 397:479–85 [PubMed: 20515655]

115. Tsuiji H, Iguchi Y, Furuya A, Kataoka A, Hatsuta H, et al. 2013. Spliceosome integrity is defective in the motor neuron diseases ALS and SMA. EMBO Mol. Med 5:221–34 [PubMed: 23255347]

116. Mackenzie IR, Neumann M. 2012. FET proteins in frontotemporal dementia and amyotrophic lateral sclerosis. Brain Res. 1462:40–43 [PubMed: 22261247]

117. Neumann M, Bentmann E, Dormann D, Jawaid A, DeJesus-Hernandez M, et al. 2011. FET proteins TAF15 and EWS are selective markers that distinguish FTLD with FUS pathology from amyotrophic lateral sclerosis with *FUS* mutations. Brain 134:2595–609 [PubMed: 21856723]

118. Leemann-Zakaryan RP, Pahlich S, Grossenbacher D, Gehring H. 2011. Tyrosine phosphorylation in the C-terminal nuclear localization and retention signal (C-NLS) of the EWS protein. Sarcoma 2011:218483 [PubMed: 21647358]

119. Zhang ZC, Chook YM. 2012. Structural and energetic basis of ALS-causing mutations in the atypical proline–tyrosine nuclear localization signal of the Fused in Sarcoma protein (FUS). PNAS 109:12017–21 [PubMed: 22778397]

120. Dormann D, Madl T, Valori CF, Bentmann E, Tahirovic S, et al. 2012. Arginine methylation next to the PY-NLS modulates Transportin binding and nuclear import of FUS. EMBO J. 31:4258–75 [PubMed: 22968170]

121. Scaramuzzino C, Monaghan J, Milioto C, Lanson NA Jr, Maltare A, et al. 2013. Protein arginine methyltransferase 1 and 8 interact with FUS to modify its sub-cellular distribution and toxicity in vitro and in vivo. PLOS ONE 8:e61576 [PubMed: 23620769]

122. Tradewell ML, Yu Z, Tibshirani M, Boulanger MC, Durham HD, Richard S. 2012. Arginine methylation by PRMT1 regulates nuclear–cytoplasmic localization and toxicity of FUS/TLS harbouring ALS-linked mutations. Hum. Mol. Genet 21:136–49 [PubMed: 21965298]

123. Belyanskaya LL, Gehrig PM, Gehring H. 2001. Exposure on cell surface and extensive arginine methylation of Ewing sarcoma (EWS) protein. J. Biol. Chem 276:18681–87 [PubMed: 11278906]

124. Fujii R, Grossenbacher-Zinchuk O, Jamari I, Wang Y, Zinchuk V, Takumi T. 2009. TLS-GFP cannot rescue mRNP formation near spines and spine phenotype in TLS-KO. Neuroreport 20:57–61 [PubMed: 18989236]

125. Fujii R, Takumi T. 2005. TLS facilitates transport of mRNA encoding an actin-stabilizing protein to dendritic spines. J. Cell Sci 118:5755–65 [PubMed: 16317045]

126. Fujii R, Okabe S, Urushido T, Inoue K, Yoshimura A, et al. 2005. The RNA binding protein TLS is translocated to dendritic spines by mGluR5 activation and regulates spine morphology. Curr. Biol 15:587–93 [PubMed: 15797031]

127. Andersson MK, Ståhlberg A, Arvidsson Y, Olofsson A, Semb H, et al. 2008. The multifunctional FUS, EWS and TAF15 proto-oncoproteins show cell type–specific expression patterns and involvement in cell spreading and stress response. BMC Cell Biol. 9:37 [PubMed: 18620564]

128. Yasuda K, Zhang H, Loiselle D, Haystead T, Macara IG, Mili S. 2013. The RNA-binding protein Fus directs translation of localized mRNAs in APC-RNP granules. J. Cell Biol 203:737–46 [PubMed: 24297750]

129. Sama RR, Ward CL, Kaushansky LJ, Lemay N, Ishigaki S, et al. 2013. FUS/TLS assembles into stress granules and is a prosurvival factor during hyperosmolar stress. J. Cell Physiol 228:2222–31 [PubMed: 23625794]

130. Li YR, King OD, Shorter J, Gitler AD. 2013. Stress granules as crucibles of ALS pathogenesis. J. Cell Biol 201:361–72 [PubMed: 23629963]
131. Ramaswami M, Taylor JP, Parker R. 2013. Altered ribostasis: RNA–protein granules in degenerative disorders. Cell 154:727–36 [PubMed: 23953108]
132. Blokhuis AM, Groen EJ, Koppers M, van den Berg LH, Pasterkamp RJ. 2013. Protein aggregation in amyotrophic lateral sclerosis. Acta Neuropathol. 125:777–94 [PubMed: 23673820]
133. Polymenidou M, Lagier-Tourenne C, Hutt KR, Bennett CF, Cleveland DW, Yeo GW. 2012. Misregulated RNA processing in amyotrophic lateral sclerosis. Brain Res. 1462:3–15 [PubMed: 22444279]
134. Ibrahim F, Nakaya T, Mourelatos Z. 2012. RNA dysregulation in diseases of motor neurons. Annu. Rev. Pathol. Mech. Dis 7:323–52
135. Ticozzi N, Vance C, Leclerc AL, Keagle P, Glass JD, et al. 2011. Mutational analysis reveals the *FUS* homolog *TAF15* as a candidate gene for familial amyotrophic lateral sclerosis. Am. J. Med. Genet. B 156:285–90
136. Shelkovnikova TA, Robinson HK, Southcombe JA, Ninkina N, Buchman VL. 2014. Multistep process of FUS aggregation in the cell cytoplasm involves RNA-dependent and RNA-independent mechanisms. Hum. Mol. Genet 23:2511–26 [PubMed: 24412933]
137. Baron DM, Kaushansky LJ, Ward CL, Sama RR, Chian RJ, et al. 2013. Amyotrophic lateral sclerosis–linked FUS/TLS alters stress granule assembly and dynamics. Mol. Neurodegener 8:30 [PubMed: 24090136]
138. Deng H, Gao K, Jankovic J. 2014. The role of *FUS* gene variants in neurodegenerative diseases. Nat. Rev. Neurol 10:337–48 [PubMed: 24840975]
139. Ticozzi N, Silani V, LeClerc AL, Keagle P, Gellera C, et al. 2009. Analysis of *FUS* gene mutation in familial amyotrophic lateral sclerosis within an Italian cohort. Neurology 73:1180–85 [PubMed: 19741215]
140. Ito D, Seki M, Tsunoda Y, Uchiyama H, Suzuki N. 2010. Nuclear transport impairment of amyotrophic lateral sclerosis–linked mutations in FUS/TLS. Ann. Neurol 69:152–62 [PubMed: 21280085]
141. Schwartz JC, Podell ER, Han SSW, Berry JD, Eggan KC, Cech TR. 2014. FUS is sequestered in nuclear aggregates in ALS patient fibroblasts. Mol. Biol. Cell 25:2571–78 [PubMed: 25009283]
142. Kabashi E, Bercier V, Lissouba A, Liao M, Brustein E, et al. 2011. FUS and TARDBP but not SOD1 interact in genetic models of amyotrophic lateral sclerosis. PLOS Genet. 7:e1002214 [PubMed: 21829392]
143. Qiu H, Lee S, Shang Y, Wang WY, Au KF, et al. 2014. ALS-associated mutation *FUS-R521C* causes DNA damage and RNA splicing defects. J. Clin. Investig 124:981–99 [PubMed: 24509083]
144. Wang WY, Pan L, Su SC, Quinn EJ, Sasaki M, et al. 2013. Interaction of FUS and HDAC1 regulates DNA damage response and repair in neurons. Nat. Neurosci 16:1383–91 [PubMed: 24036913]
145. Mastrocola AS, Kim SH, Trinh AT, Rodenkirch LA, Tibbetts RS. 2013. The RNA-binding protein fused in sarcoma (FUS) functions downstream of poly(ADP-ribose) polymerase (PARP) in response to DNA damage. J. Biol. Chem 288:24731–41 [PubMed: 23833192]
146. Lee HJ, Yoon C, Schmidt B, Park DJ, Zhang AY, et al. 2013. Combining PARP-1 inhibition and radiation in Ewing sarcoma results in lethal DNA damage. Mol. Cancer Ther 12:2591–600 [PubMed: 23966622]
147. Li H, Watford W, Li C, Parmelee A, Bryant MA, et al. 2007. Ewing sarcoma gene *EWS* is essential for meiosis and B lymphocyte development. J. Clin. Investig 117:1314–23 [PubMed: 17415412]
148. Hicks GG, Singh N, Nashabi A, Mai S, Bozek G, et al. 2000. Fus deficiency in mice results in defective B-lymphocyte development and activation, high levels of chromosomal instability and perinatal death. Nat. Genet 24:175–79 [PubMed: 10655065]

149. Sugawara T, Oguro H, Negishi M, Morita Y, Ichikawa H, et al. 2010. FET family proto-oncogene *Fus* contributes to self-renewal of hematopoietic stem cells. Exp. Hematol 38:696–706 [PubMed: 20412831]

150. Brenner JC, Feng FY, Han S, Patel S, Goyal SV, et al. 2012. PARP-1 inhibition as a targeted strategy to treat Ewing's sarcoma. Cancer Res. 72:1608–13 [PubMed: 22287547]

151. Yoshida K 2008. Nuclear trafficking of pro-apoptotic kinases in response to DNA damage. Trends Mol. Med 14:305–13 [PubMed: 18539531]

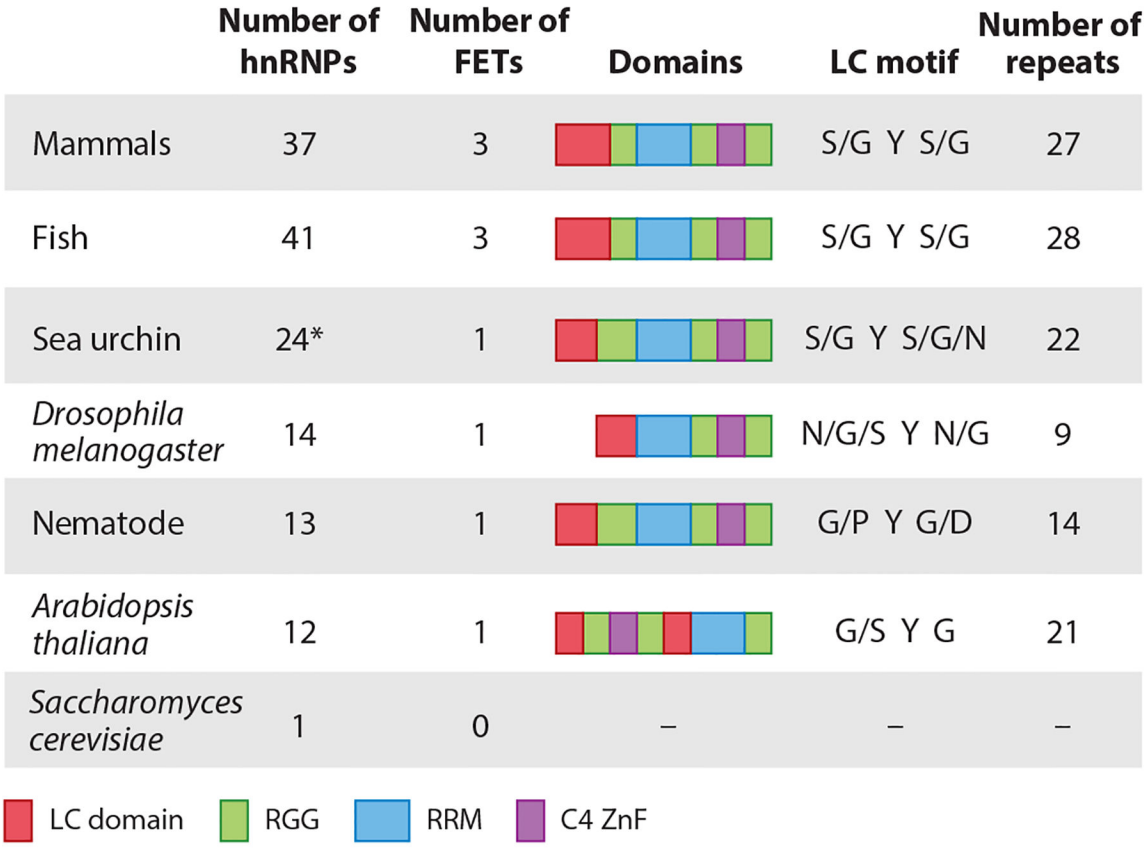

Figure 1.

Conservation of FET proteins throughout multicellular organisms. The diversity of FET proteins expands in multicellular organisms in parallel to the expansion of heterogeneous nuclear ribonucleoprotein particle (hnRNP) proteins. The domain composition of the FET proteins is consistent, although—at least for *Arabidopsis thaliana*—the order of the domains is somewhat altered. Low-complexity (LC) domains, with their repeated LC motif, are conserved, and the number of motif repeats can vary. RGG domains contain repeats of Arg–Gly–Gly. The asterisk indicates that there are 24 sea urchin hnRNP proteins, although tubeworms are reported to possess only 16 hnRNP proteins. Abbreviations: C4 ZnF, zinc-finger domain anchored by four cysteine residues; RRM, RNA recognition motif.

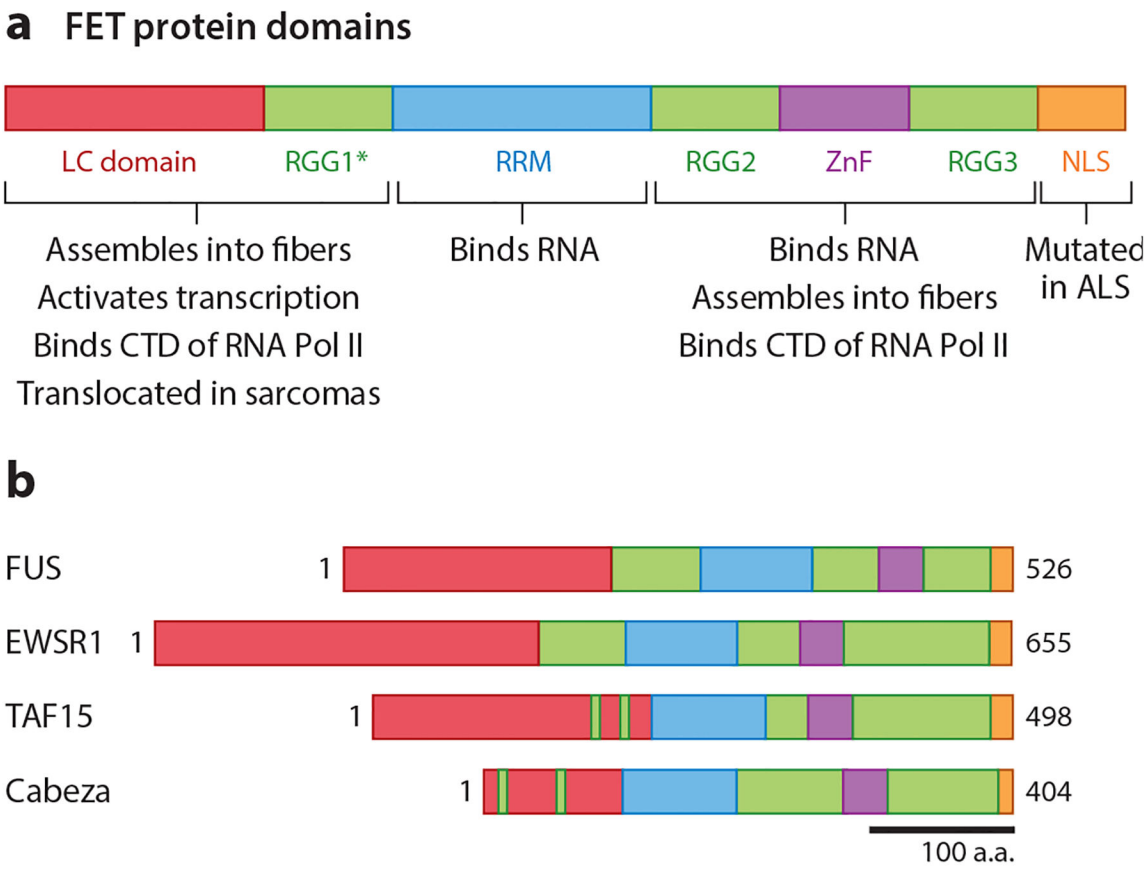

Figure 2.

(*a*) Summary of the activities and characteristics associated with the domains of FET proteins. The asterisk indicates that the first RGG domain is not very apparent in the protein TAF15 or cabeza and is limited to two RGG motifs within the low-complexity (LC) domain. (*b*) The relative size of domains (color coded as in panel *a*) for FUS, EWSR1, TAF15, and cabeza. Abbreviations: ALS, amyotrophic lateral sclerosis; CTD, C-terminal domain; NLS, nuclear localization signal; RNA Pol II, RNA polymerase II; RRM, RNA recognition motif; ZnF, zinc-finger domain.

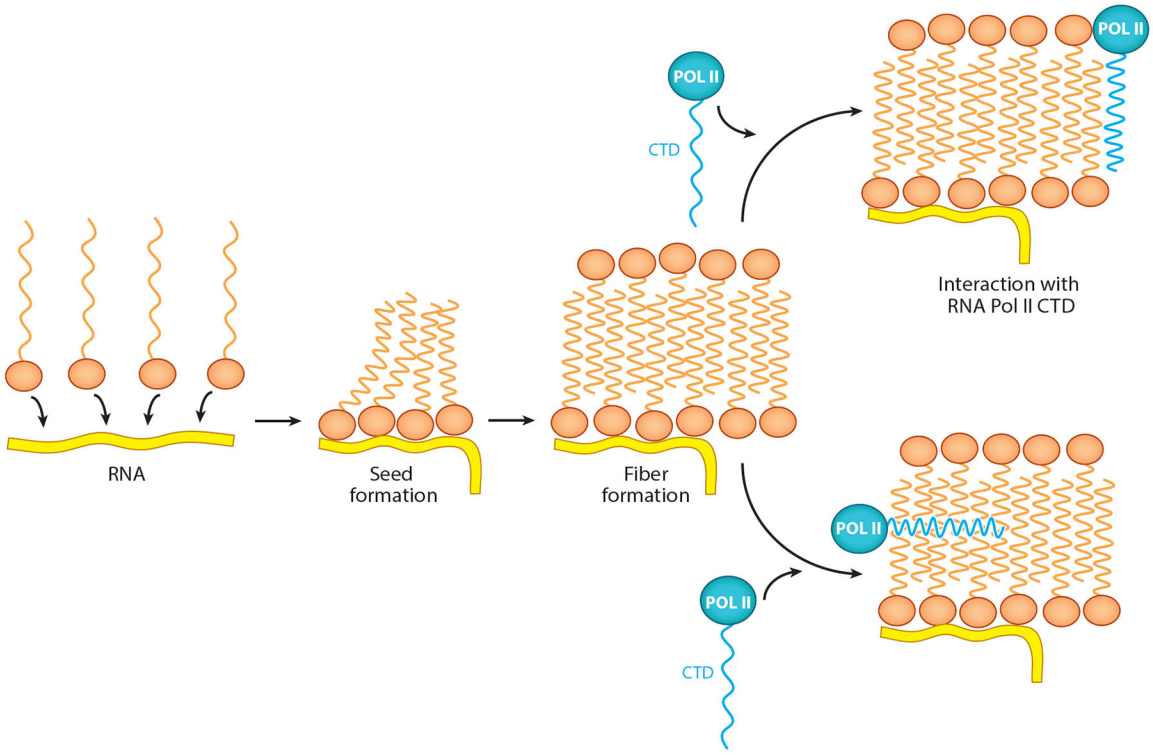

Figure 3.

Model for RNA-nucleated assembly of FUS proteins and recruitment of RNA polymerase II (Pol II). FUS binds RNA highly cooperatively. The FUS–RNA complex forms the seed for fiber growth. FUS fibers are composed of a seed of FUS protein bound to RNA and FUS proteins not bound to RNA. The C-terminal domain (CTD) of RNA Pol II may interact with this fiber either by intercalating into the growing fiber or by binding alongside the fibrous structure.

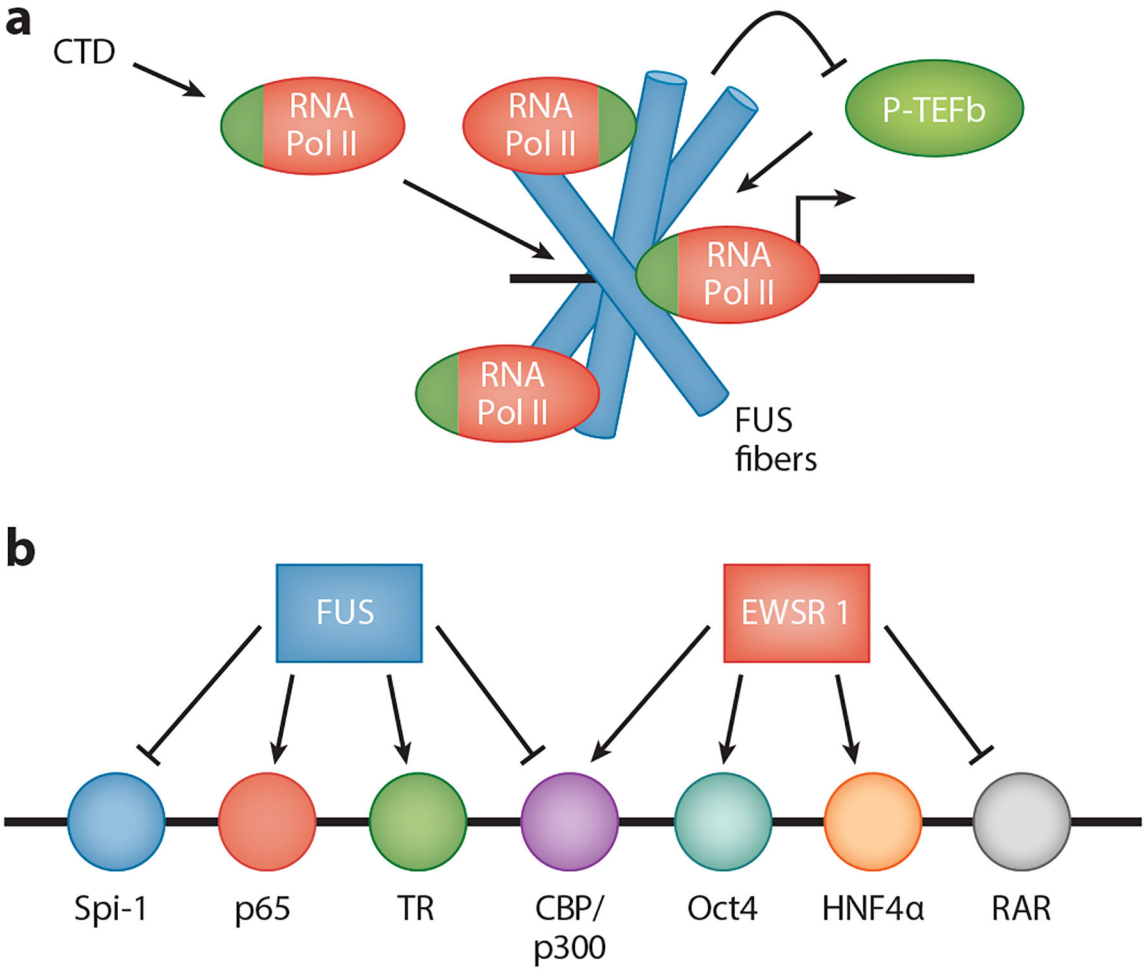

Figure 4.

Two mechanisms by which FET proteins affect transcription. (*a*) FET proteins, based on the local concentration of RNA transcripts, may form higher-order assemblies near the promoters and transcription start sites of genes. These assemblies recruit more RNA polymerase II (RNA Pol II) through interactions with the C-terminal domain (CTD) and protect the CTD from premature phosphorylation at position Ser2. (*b*) FUS and EWSR1 interact with several transcription factors to stimulate or repress their activity.