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Molecules in focus

# Fused in sarcoma/translocated in liposarcoma: A multifunctional DNA/RNA binding protein

Shu Yang[a], Sadaf T. Warraich[a,b], Garth A. Nicholson[a,b,c], Ian P. Blair[a,b,*]

[a] Northcott Neuroscience Laboratory, ANZAC Research Institute, NSW, Australia
[b] Sydney Medical School, University of Sydney, NSW, Australia
[c] Molecular Medicine Laboratory, Concord Hospital, Sydney, NSW, Australia

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ABSTRACT

The fused in sarcoma/translocated in liposarcoma (FUS/TLS) gene was initially identified as a component of a fusion pro-oncogene resulting from a chromosomal translocation seen in liposarcomas. FUS/TLS belongs to a sub-family of RNA binding proteins, encoding an N-terminal serine–tyrosine–glycine–glutamine (SYGQ) region, an RNA recognition motif (RRM) flanked by glycine rich (G-rich) regions, a cysteine$_2$/cysteine$_2$ zinc finger motif and multiple RGG repeats. The FUS/TLS protein interacts with RNA, single stranded DNA and double stranded DNA, and is involved in unique functions in mRNA processing and transport, transcriptional regulation and maintenance of genomic stability. Recently, several mutations in this gene have been found in amyotrophic lateral sclerosis (ALS) patients. The mutant forms of FUS/TLS exhibit similar pathology to other ALS causative genes, including aberrant cytoplasmic inclusions and an increased FUS/TLS cytoplasmic to nuclear ratio. The FUS/TLS mutations identified in ALS patients suggests that altered RNA metabolism may play a role in ALS pathogenesis.



## 1. Introduction

Mammalian gene expression comprises a series of integrated processes including transcription, RNA synthesis, mRNA processing, translation and post-translational processing of the protein. These processes are connected by a group of structurally similar DNA/RNA binding proteins. One of these proteins is the fused in sarcoma/translocated in liposarcoma (FUS/TLS), a member of the FUS/Ewing's sarcoma/TATA-binding protein-associated factor (FET) (formerly known as TET (TLS/E/T)) protein family. Originally identified in human myxoid liposarcomas in 1993, the *FUS/TLS* gene plays a critical role in the formation of fusion proteins related to a variety of cancers, including acute myeloid leukaemia and Ewing's tumor (Law et al., 2006). FUS/TLS serves multiple unique functions in RNA splicing, RNA transport and formation of fusion oncoproteins. Recently, several *FUS/TLS* mutations have been found in amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD) patients, implicating a pathogenic role of this protein in neurodegenerative disease (Kwiatkowski et al., 2009; Vance et al., 2009; Lagier-Tourenne et al., 2010).

## 2. Structure

The *FUS/TLS* gene (approximately 12 kb) consists of 15 exons and 14 introns (Morohoshi et al., 1998). Its putative promoter has features of a housekeeping gene promoter, including an absence of TATA boxes (Aman et al., 1996). *FUS/TLS* was initially reported as a component of the fusion gene, TLS-CCAAT enhancer-binding homologous protein (CHOP), resulting from a chromosome translocation t(12;16)(q13.3;p11.2) seen in myxoid liposarcomas (Crozat et al., 1993). *FUS/TLS* also fuses to the erythroblastosis virus E26 oncogene homologue (ERG) gene as a result of a translocation t(16;21)(p11;q22) seen in acute myeloid leukaemia (Law et al., 2006). Introns 5, 7 and 8 of *FUS/TLS* are the translocation breakpoints in both diseases (Morohoshi et al., 1998). Several characteristic features, including multiple EcoRI sites and TG repeats, may contribute to the DNA rearrangements in these three introns (Morohoshi et al., 1998).

The full-length human *FUS/TLS* cDNA encodes a 526 amino acid protein (53 kDa) (Crozat et al., 1993). The FUS/TLS protein shares highly homologous protein domains with other FET family members, including an N-terminal serine–tyrosine–glutamine–glycine (SYGQ) rich region, a glycine rich (G-rich) region, a central conserved RNA recognition motif (RRM), a cysteine$_2$/cysteine$_2$ zinc finger motif and C-terminal arginine–glycine–glycine (RGG) motifs. The structure and functional domains of FUS/TLS are shown in Fig. 1.

* Corresponding author at: Northcott Neuroscience Laboratory, ANZAC Research Institute, Hospital Road, Concord Hospital, Sydney, NSW 2139, Australia. Tel.: +61 2 9767 9118; fax: +61 2 9767 9101.
*E-mail address:* iblair@med.usyd.edu.au (I.P. Blair).

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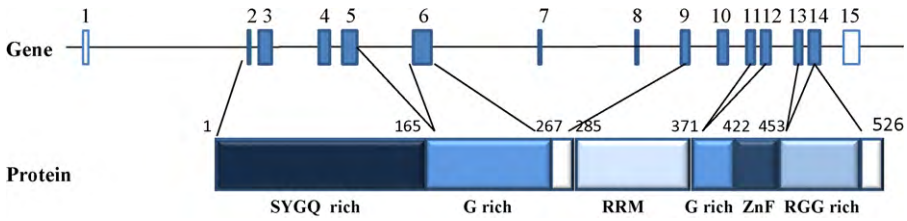


**Fig. 1.** Genomic and structural organization of human FUS/TLS gene and protein. *FUS/TLS* gene is encoded by 15 exons that cover an 11.6 kb region on chromosome 16p11.2. Protein coding exons (filled boxes) and non-coding exons (open boxes) are drawn to scale. SYGQ rich: serine–tyrosine–glycine–glutamine rich domain; G rich: glycine rich domain; RRM: RNA recognition motif; ZnF: cysteine$_2$/cysteine$_2$ zinc finger motif; RGG rich: arginine–glycine–glycine rich domain.

## 3. Expression, cellular location and regulation

The human *FUS/TLS* gene is constitutively activated and ubiquitously expressed in human tissues and cultured cell lines, including the heart, brain, placenta, lung, liver, kidney, pancreas, spleen, thymus and prostate, but is absent from cardiac endothelium, cardiac muscle cells and melanocytes (Aman et al., 1996; Andersson et al., 2008; Morohoshi et al., 1996). The expression level of FUS/TLS is heterogeneous among different tissues. The *FUS/TLS* gene is downregulated in differentiated human embryonic stem cells, differentiated neuroblastoma cells and neutrophil cells, but upregulated in acute myeloid leukaemia patient peripheral blood samples (Andersson et al., 2008; Mills et al., 2000). This may indicate a role of FUS/TLS in the promotion and maintenance of cell proliferation.

The subcellular localization of the FUS/TLS protein is cell-type dependent. FUS/TLS is mainly a nuclear protein, but it is also present in the cytoplasm of many cells, except hepatocytes, where FUS/TLS is only present in the cytoplasm (Andersson et al., 2008). Cell lines stably transfected with FUS/TLS show similar localization to the endogenous protein. In contrast, cells transiently transfected with FUS/TLS or cells exposed to environmental stresses form cytoplasmic aggregates. The FUS/TLS aggregates co-localize with stress granules containing microRNAs and translationally silenced mRNA (Andersson et al., 2008). Stress granules are regulators of post-transcription, mRNA decay and the availability of microRNAs, indicating that FUS/TLS may serve similar functions.

FUS/TLS is degraded by a proteasome-dependent process without detection of ubiquitination (Perrotti et al., 2000). In hematopoietic cells containing the breakpoint cluster region gene/Abelson murine leukaemia viral oncogene (BCR-ABL) fusion gene, the FUS/TLS protein turnover is dependent on protein kinase β II (PKCβII) and nuclear factor c-Jun (Perrotti et al., 2000). Phosphorylation by PKCβII inhibits FUS/TLS proteolysis (Perrotti et al., 2000). Mutations in the phosphorylation site (serine 256) also alter FUS/TLS proteolysis (Perrotti et al., 2000). Although FUS/TLS is not usually found ubiquitinated, it associates with an ubiquitinated protein, possibly heterogeneous nuclear ribonucleoprotein (hnRNP) A1, and targets to the proteasome pathway as a complex (Perrotti et al., 2000). c-Jun is likely to play a positive role in promoting the degradation of the FUS/TLS–hnRNP A1 complex (Perrotti et al., 2000).

## 4. Biological functions

### 4.1. FUS/TLS in DNA/RNA binding

The C-terminal RNA recognition motif (RRM), together with the flanking G-rich regions, is thought to be crucial for RNA binding (Burd and Dreyfuss, 1994). Initial studies suggested these motifs preferentially bind GGUG RNA motifs, both in vivo and in vitro (Crozat et al., 1993; Prasad et al., 1994; Zinszner et al., 1997). However, Zinszner et al. (1997) suggests that the RRM is not solely responsible for RNA targeting, as a truncated FUS/TLS protein lacking RRM still recognizes RNA. Indeed, the FUS/TLS zinc finger motif

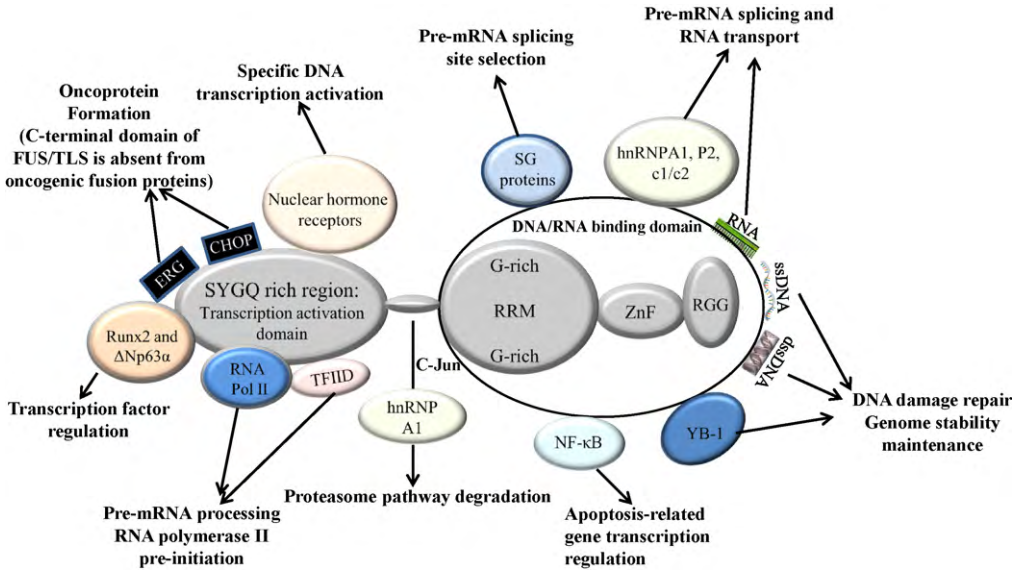


**Fig. 2.** Biological functions of FUS/TLS. FUS/TLS contains two functional domains: the N-terminal transcription activation domain (SYGQ region) and the C-terminal DNA/RNA binding domain. The N-terminal domain of FUS/TLS is involved in transcription regulation and pre-mRNA processing, whereas the C-terminal domain of FUS/TLS directly interacts with RNA and DNA, playing roles in pre-mRNA processing, mRNA splicing and genomic stability maintenance. Several interaction partners of FUS/TLS and their functions are shown. RNA pol II: RNA polymerase II.