TDP-43 and FUS/TLS yield a target-rich haul in ALS

Aaron D Gitler

Genome-wide approaches are used to discover the RNA-binding targets of the amyotrophic lateral sclerosis (ALS) disease protein FUS/TLS. These studies reveal some shared targets with another ALS-linked RNA-binding protein, TDP-43, suggesting common pathogenic mechanisms.

The pathogenesis of ALS has been an enduring mystery. This relatively common adult-onset neurodegenerative disorder, also known as Lou Gehrig's disease, is characterized by progressive motor neuron loss, leading to paralysis and, eventually, death. There is no cure and few effective treatments for this truly devastating disease. Emerging pathologic and genetic evidence has converged on altered RNA processing in ALS pathogenesis. Mutations in two RNA-binding proteins, TAR DNA-binding protein 43 (TDP-43) and fused in sarcoma (FUS), also known as translated in liposarcoma (TLS), cause some forms of inherited ALS[1]. The degenerating motor neurons of most patients with sporadic ALS exhibit mislocalization of TDP-43, and motor neurons of patients with *FUS* mutations show FUS/TLS cytoplasmic accumulation. There has been intense focus on deciphering how these two RNA-binding proteins contribute to disease. In this issue of *Nature Neuroscience*, Lagier-Tourenne *et al.* present a genome-wide compendium of FUS RNA targets[2]. Furthermore, they define a subset of targets that are shared by TDP-43 and FUS and that may be the most critical for ALS pathogenesis.

The discovery of the contributions of TDP-43 and FUS to ALS caused a paradigm shift in the understanding of ALS mechanisms. Because TDP-43 and FUS are both RNA-binding proteins, it immediately became apparent that a top priority should be to define all of the RNAs with which they associate, as this would provide a toehold for understanding TDP-43's and FUS's normal cellular functions and suggest potential consequences of misregulation in disease. TDP-43 was the first to be tackled. Using next-generation sequencing approaches coupled with affinity purification of RNA-protein complexes, independent groups produced genome-wide maps of all of the TDP-43–binding sites in pre-mRNAs from mouse brain[3] and human post-mortem brain tissue affected by fronto-temporal dementia[4]. These studies not only generated a comprehensive survey of TDP-43–binding sites, but also illuminated several new and unexpected facets of TDP-43 biology. Among these is the observation that pre-mRNAs with exceptionally long introns (>100 kb) are enriched in TDP-43–binding sites. Reduction of TDP-43 levels in the mouse nervous system by antisense oligonucleotides revealed that TDP-43 is crucial for maintaining the levels of these mRNAs[3]. TDP-43 was also found to autoregulate its own levels, providing an explanation for how this can become impaired in disease and unleash a potentially disastrous cascade of TDP-43 cytoplasmic mislocalization and aggregation.

Having established an atlas of TDP-43 binding, the authors turned their attention to FUS/TLS. TDP-43 and FUS/TLS are very similar proteins, so would FUS/TLS bind to the same RNAs as TDP-43 or would it seek out a completely distinct repertoire of binding targets? If there were shared targets, could either TDP-43 or FUS/TLS compensate for the loss of the other? To examine these questions, Lagier-Tourenne *et al.*[2] used cross-linking and immunoprecipitation coupled with high-throughput sequencing to define the RNA targets of FUS/TLS from adult mouse and human brain. These independent analyses identified >5,500 pre-mRNAs harboring FUS/TLS-binding sites. Notably, most of these binding sites were conserved between the mouse and human experiments, demonstrating highly conserved features of the FUS/TLS-bound RNAs. Like those of TDP-43, most of the FUS/TLS-bound sites were located in introns. However, there was also a significant enrichment for FUS/TLS binding in exons and untranslated regions. In contrast with the binding pattern of TDP-43, there was an intriguing 'sawtooth' pattern of FUS/TLS binding across pre-mRNAs that harbor long introns (>100 kb). Such sawtooth binding patterns indicate that FUS/TLS is co-transcriptionally deposited along long introns and reveal a potential role of FUS/TLS in co-transcriptional regulation of splicing of these long pre-mRNAs.

What is the effect of FUS/TLS binding to these RNA targets *in vivo*? To answer this question, Lagier-Tourenne *et al.*[2] determined the global effects of FUS/TLS depletion. They used antisense oligonucleotides to deplete FUS/TLS *in vivo* from the adult mouse nervous system and assessed the effects of this treatment on the levels of the identified FUS/TLS targets. FUS/TLS depletion caused some of these targets to be upregulated and others to be downregulated. However, the downregulated genes showed a much larger change in expression following FUS/TLS depletion than the upregulated genes, and those downregulated genes tended to be much longer (at least three times as long) and harbor many more FUS/TLS-binding sites than the genes that were unaffected or upregulated by FUS/TLS depletion. These data are consistent with the downregulated genes being direct FUS/TLS targets, whereas the upregulated genes are more likely to be indirectly affected by FUS/TLS loss. It seems that a main function of FUS/TLS is to bind and stabilize its direct targets, especially those pre-mRNAs with exceptionally long introns.

With the lists of validated TDP-43– and FUS/TLS-regulated targets in hand, the authors focused on the subset regulated by both proteins. Each protein alone regulated the expression or splicing of hundreds of

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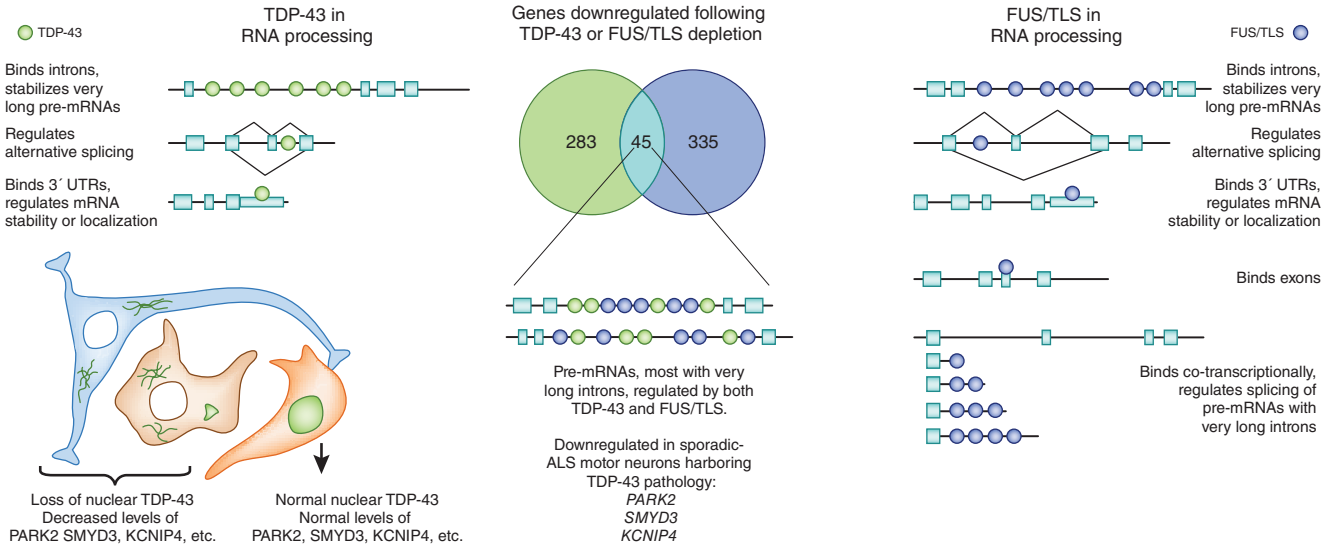

Figure 1 Intersecting roles of TDP-43 and FUS/TLS in RNA processing. The RNA-binding proteins TDP-43 and FUS/TLS have multiple roles in RNA processing. Both appear to bind pre-mRNAs with very long introns, helping to maintain the levels of these transcripts. In addition, FUS/TLS is co-transcriptionally deposited along very long genes and helps to regulate splicing. Hundreds of genes are downregulated following TDP-43 or FUS/TLS depletion (Venn diagram). However, only 45 genes are jointly regulated by both TDP-43 and FUS/TLS, and most of these genes harbor very long introns. The levels of at least three of these TDP-43– and FUS/TLS-regulated genes (*PARK2*, *SMYD3* and *KCNIP4*) are downregulated in sporadic ALS motor neurons harboring TDP-43 pathology (loss of TDP-43 from the nucleus and formation of cytoplasmic inclusions).

genes, but focusing on just the genes in common between FUS/TLS and TDP-43 permitted the authors to winnow down the list to 41 upregulated genes and 45 downregulated genes. The authors tested the effects of simultaneously downregulating TDP-43 and FUS/TLS to determine whether the proteins work in parallel or synergistically. Notably, they found that depleting both TDP-43 and FUS/TLS had no greater effect on the expression levels of the genes that they tested than the individual knockdowns, indicating that they act in parallel to regulate these genes. Because the result suggests a shared mechanism of neurodegeneration caused by misregulation of TDP-43 or FUS/TLS function, this observation is potentially one of the most important aspects of the study and has important implications for ALS in humans. The identification of shared regulatory functions for TDP-43 and FUS/TLS can hopefully be leveraged to develop therapeutic strategies aimed at restoring function of these genes whose regulation depends on both proteins.

The authors concluded the study by extending their results to human disease. They focused on three specific mRNA targets whose levels are regulated by TDP-43 or FUS/TLS, *PARK2* (parkin), *SMYD3* and *KCNIP4*, all of which contain long introns. These specific targets were of immediate interest because they are all known to be critical for neuronal function. In addition, mutation of *PARK2* has been implicated as a cause of the neurodegenerative disorder Parkinson's disease. Analyzing autopsy tissue from ALS patients and unaffected control individuals, the authors found that motor neurons harboring TDP-43 cytoplasmic aggregates contain lower levels of these three proteins (parkin, SMYD3 and KCNIP4) than neighboring motor neurons that lack TDP-43 pathology. This result is consistent with these targets being misregulated as a consequence of loss of TDP-43 protein from the nucleus as part of the disease process. The authors speculate that the loss of TDP-43 or FUS/TLS from the nucleus could contribute to disease by affecting the regulation and splicing of these key target genes. It is the dysregulation of these joint TDP-43 and FUS/TLS mRNA targets, especially those with long introns, including genes involved in synapse formation and maintenance, that are likely most critical to disease pathogenesis.

The results of Lagier-Tourenne *et al.*[2] predict that, in cells with defects in TDP-43 or FUS/TLS function as a result of mutation and/or aggregation in disease, there is reduced function of parkin, SMYD3, KCNIP4 and perhaps other targets, which explains why these neurons degenerate and thus constitutes a possible mechanism of pathogenesis. Realistically, it is unlikely that reducing the levels of a single target of TDP-43 and FUS/TLS would be the sole trigger of neurodegeneration following loss of TDP-43 or FUS/TLS function in human disease. On the contrary, Lagier-Tourenne *et al.*[2] propose that the combined reduction of this intersecting group of TDP-43 and FUS/TLS RNA targets, occurring in affected cells, will have detrimental effects on these cells (**Fig. 1**), rather than any one of them singlehandedly causing the progression of disease in humans.

The work of Lagier-Tourenne *et al.*[2] and others has provided a treasure trove of new insight into the targets of TDP-43 (refs. 3–6) and FUS/TLS[2,7–10]. Many opportunities and challenges lie ahead. In addition to TDP-43 and FUS/TLS, other RNA-binding proteins with similar properties are emerging in ALS and related disorders[11–13]. How do the RNA targets of these RNA-binding proteins compare to those of TDP-43 and FUS/TLS? Conversely, in a way, Lagier-Tourenne *et al.*[2] have provided us a sort of primer for the critical RNA targets whose dysregulation is associated with ALS (such as *PARK2*, *SMYD3* and *KCNIP4*). What other secrets are hidden in this common set of targets? Reverse engineering could be used to scour these dysregulated RNAs for predicted binding sites of new RNA-binding proteins, which could then be functionally interrogated for a role in ALS and related diseases. Finally, the recent discovery of massive expansions of a hexanucleotide GGGGCC repeat in a non-coding region of the *C9ORF72* gene as a major cause of ALS[14,15] further connects RNA processing to disease pathogenesis, and the race is now on to identify both the RNA-binding protein(s) engaging pathogenic GGGGCC repeats and the network of RNAs whose regulation is affected by this mutation. How will they compare to the TDP-43 or FUS/TLS targets identified by Lagier-Tourenne *et al.*[2]

and others[3–10]? It is very likely that the answers to these questions will provide even more insight into ALS pathogenesis and illuminate new paths toward therapies.

COMPETING FINANCIAL INTERESTS
The author declares no competing financial interests.

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New twist on orphan receptor GPR88 function

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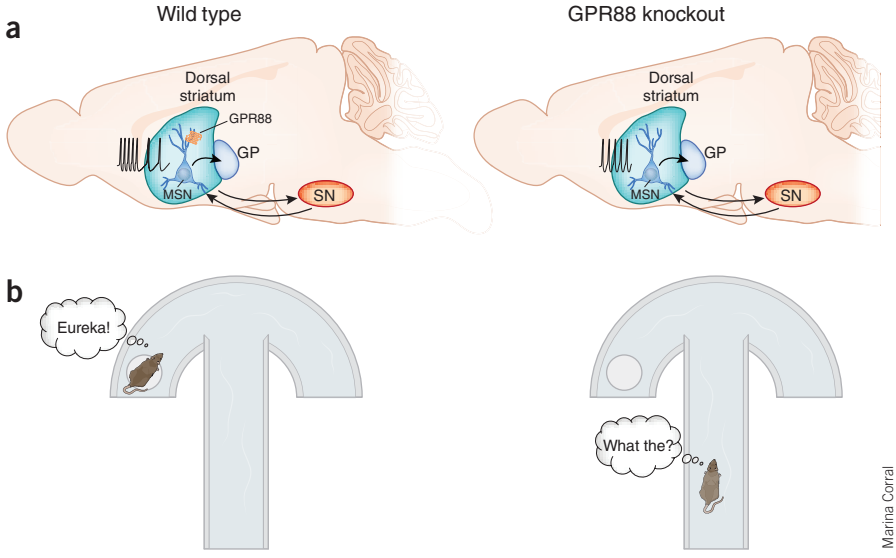
Ligands for G protein–coupled receptor 88 (GPR88) have not yet been found. A new study finds that GPR88 is important in the physiology of dorsal striatal projection neurons, as well as in behaviors involving this brain region.

Finding the proper role for orphan G protein–coupled receptors (GPCRs) is often a long and painful process that brings to mind the difficult journey of Dickens' iconic orphan Oliver Twist to find his place in the world. A case in point is GPR88, which is designated as an orphan receptor because no ligand that interacts with the receptor has as yet been identified[1]. Nonetheless, the enrichment of GPR88 in the striatum[1,2], sensitivity of receptor expression to antidepressant treatments[3], and genetic linkage to schizophrenia[4], has stimulated interest in its roles in striatal physiology and behaviors involving this brain region. Dorsal striatal circuitry contains a predominance of GABAergic medium spiny neurons (MSNs) that inhibit downstream nuclei of the basal ganglia, a feature shared by other striatal-like forebrain regions that is distinct from the glutamatergic projections arising in cortical-like structures. The molecular profile of MSNs is also distinct from forebrain glutamatergic projection neurons. Indeed, neurotransmitter receptors and intracellular signaling molecules of various types are highly enriched in MSNs[2]. Among these are G proteins such as Golf[5], signaling molecules such as REM2 (ref. 2), and the adenosine 2A (A2A) GPCR[6]. Defining the roles of these striatum-enriched proteins, including GPR88, should help us to identify molecular networks that act in concert to influence basal ganglia circuitry and action control.

In a study appearing in this issue of *Nature Neuroscience*[7], Quintana and colleagues used gene-targeted mice lacking GPR88 and viral-based re-expression of the protein to determine the receptor's role in MSN physiology, synaptic transmission, action control and action learning. Constitutive knockout of GPR88 led to increased locomotion, decreased performance and impaired learning on a motor skill test, and slower learning and poorer performance in cued escape behaviors (**Fig. 1**). When the activity of putative MSNs was examined in awake mice in a familiar environment, an overall increase in neuronal firing was observed in the knockout mice, with regular firing mainly at moderate frequencies and decreases in irregular burst firing (**Fig. 1**).

Examination of MSN electrophysiology in brain slices revealed decreased tonic GABAergic inhibition and decreased responses to applied and synaptically released GABA in the GRP88 knockout mice. In contrast, glutamatergic excitatory synaptic



Marina Corral

Figure 1 Knockout of GPR88 leads to hyperexcitation and more regular firing patterns in striatal MSNs, as well as impairment in cued response learning. (**a**) Schematic diagram of forebrain showing striatal connections and MSN activity. With normal levels of GPR88 expression (left) in MSNs, firing of the neurons varies between low frequency and short bursting modes. Loss of GRP88 (right) strengthens synaptic efficacy at inputs from cortex and decreases GABAergic signaling[7]. MSN firing becomes stable at moderate frequencies with an overall increase in firing rate in the knockout mice. GP, globus pallidus; SN, substantia nigra. Arrows indicate MSN projections to substantia nigra and back, and to globus pallidus. (**b**) Schematic diagram of the cued water U maze task used by Quintana *et al.*[7]. Mice lacking GPR88 (right) show impaired task acquisition in relation to wild-type mice (left). Deficits in learning of other cued response and avoidance tasks were also observed in the GPR88 knockout mice.

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