



The Critical, and Multiple, Roles of RNA in ALS and Related Diseases

While a complete understanding of the pathogenesis of ALS and related diseases remains elusive, evidence is growing that RNA dysregulation may play a central role, and that therapies targeting RNA may have significant potential to interrupt the disease process before it cascades beyond control.

TDP-43, FUS, and Other ALS-linked Proteins Bind RNA

The earliest strong evidence that ALS, as well as frontotemporal dementia, involve RNA dysregulation came from the discovery that the RNA-binding protein TDP-43 was a major component of aggregates found in the cytoplasm of dying neurons in 90% of cases of ALS and over half of FTD cases. The significance of the finding was amplified by the discovery that mutations in the gene encoding TDP-43 accounted for a small portion of familial ALS, and rare cases of FTD. Mutations in a second RNA-binding protein, called FUS, also cause ALS. Other RNA binding proteins are also implicated as rare causes of disease, including senataxin, angiogenin, ataxin-2, and heterogeneous nuclear ribonucleoprotein A1 (hnRNPA1).

TDP-43 is a primarily nuclear protein, but an important part of its function is to shuttle in and out of the nucleus, interacting with messenger RNAs, splicing factors, and other components of the RNA processing and transport machinery. TDP-43 is an inherently aggregation-prone protein, and the fact that it forms cytoplasmic aggregates has suggested that a key pathogenic step in the disease is a reduction in the transport of the protein back into the nucleus, allowing its cytoplasmic concentration to surpass its threshold for aggregation. In this model, a decline in nuclear TDP-43 disrupts the processing of its hundreds of RNA binding partners, potentially accounting for the widespread transcriptional dysregulation observed in the disease. Overexpression or underexpression of one or more of the affected genes may then push the affected cell along the path leading to neurodegeneration.

The C9orf72 Mutation Creates RNA Aggregates and Dipeptide Repeat Proteins

A role for RNA in ALS and FTD became even more likely with the discovery of the C9orf72 mutation, now the most common genetic cause of ALS, accounting for 40% or more of familial cases, and 6% of sporadic cases, along with a large portion of familial FTD. The function of the normal gene is unknown. The mutation is an expansion of a GGGGCC hexanucleotide repeat region, from fewer than 30 units to hundreds or even thousands. The expansion is transcribed into RNA, which forms aggregates (“foci”) in motor neurons. The foci have been shown to bind a variety of proteins, including transcription factors, RNA-binding proteins, and components of the nuclear pore. A major hypothesis of C9orf72 pathogenesis proposes that sequestration of one or more of these proteins begins the pathogenic cascade resulting in neurodegeneration. A precedent for this mechanism is found in myotonic dystrophy, in which RNA aggregates bind the transcription factor muscleblind, accounting for many of the tissue-specific effects seen in the disease.

A second potential pathogenic mechanism triggered by the RNA expansion arises from “repeat-associated non-standard” (RAN) translation of the expanded RNA. This translation, which occurs without a start codon, is carried out in all possible reading frames in both directions, producing a set of dipeptide repeat proteins (DPRs). Early evidence suggests that one or more of these DPRs is pathologic, through unknown mechanisms.

Very recently, researchers have shown that neurons expressing the C9orf72 mutation have significant



defects in nuclear transport, which may be tied to sequestration of transport factors. This may help explain the aberrant cytoplasmic localization and aggregation of TDP-43, at least in C9orf72-related disease, and suggests nuclear transport defects may contribute to other forms of ALS



Gene-regulating MicroRNAs are Implicated in ALS

In recent years, a new class of small RNAs has been discovered to play an important role in regulating gene expression. Called microRNAs (miRNAs), they are 21 to 23 nucleotides long, and bind in a sequence-specific manner to messenger RNAs, promoting their degradation or transcript inhibition. A single miRNA species may bind to and regulate the transcripts of hundreds of genes. To date, no single miRNA has been definitively implicated as a cause ALS, but it is possible that the widespread gene dysregulation seen in the disease is due in part to miRNA effects. In addition, TDP-43 binds to miRNAs, further suggesting they may play a role in disease. One well-characterized miRNA, miR155, has been linked to worsening of neuroinflammation in ALS and other disorders. MiR155 is a proinflammatory miRNA, promoting expression of multiple genes, and is elevated in ALS.

RNA-based Therapeutic Strategies

The two most common genetic forms of ALS, due to mutations in the C9orf72 and SOD1 genes, are dominantly inherited, suggesting that silencing their expression is likely to be therapeutic. Antisense therapy is advancing to clinical trials for both forms of the disease, and anti-SOD1 antisense has already been shown to be safe in ALS patients. As currently developed, therapy involves intrathecal delivery of a short nucleotide sequence complementary to the mRNA sequence of the mutant gene. This antisense oligonucleotide (ASO) is chemically modified to prevent its degradation. Once within the cell, the ASO binds to the target mRNA, triggering cell defense mechanisms that degrade the mRNA, releasing the ASO to bind again. Also under development are genes for ASO molecules delivered via adeno-associated virus, a safe and cell type-specific gene vector. ASO targeting of microRNAs may also be therapeutic.

There is much that remains to be learned about RNA dynamics in ALS and FTD, but there is a growing sense in the field that, with the recognition of RNA as a significant contributor to ALS pathogenesis, it may be possible to develop therapies that have a good chance of succeeding at slowing disease progression. Perhaps just as significant is the speed at which the understanding of RNA's role, and the designing of new therapeutic approaches based on it, has developed. This is a strong indicator that, even if the current understanding must be refined and the current approaches rethought, those refinements are likely to be made far more rapidly than we have seen in the past.

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