News & Views

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The minor spliceosome could be the major key for FUS/TLS mutants in ALS

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Despite its name, minor spliceosome alterations are often involved in human disease origin. Work by Reber *et al* (2016) in this issue of *The EMBO Journal* now demonstrates a connection between minor spliceosome components and FUS/TLS, one of the major proteins aggregating in the brain of patients affected by amyotrophic lateral sclerosis (ALS). This finding has important implications as it extends the spectrum of diseases where minor spliceosome plays a role. It may also represent a new opportunity for specific therapeutic targets.

See also: **S Reber *et al***

An ancient and famous Latin saying used to be "ubi major minor cessat", loosely translated as "when there is the major, the minor is neglected". However, a very different story is described by Reber *et al* (2016) as they for the first time find the ALS-linked RNA-binding protein, FUS/TLS, to act via the so-called minor spliceosome.

Cellular control of RNA metabolism together with autophagic and proteasome pathways represents two major research fields in current neurodegenerative research. The reason is that many genes causative of ALS/FTD spectrum disorders have been found to encode proteins that regulate various aspects of RNA processing (splicing, stability, transport, translation) or express important components of the autophagic and lysosomal machinery (Hardy & Rogaeva, 2014).

With regard to RNA processing, splicing represents an essential gene regulatory mechanism for all eukaryotes. The purpose of this process is to join portions of newly transcribed pre-mRNAs in order to obtain a mature mRNA that can be exported to the cytoplasm and correctly translated. Most importantly, exons can be selectively included or excluded from most transcripts, resulting in alternative splicing (Stamm *et al*, 2005). All these processing steps are mediated by several hundred RNA-binding proteins (RBPs) that must act in close cooperation to ensure that the biological information is correctly translated from genes to proteins. Recent research has uncovered that RBPs play a key role in neuronal development and synaptic functions (Nussbacher *et al*, 2015) and that mutations or dysfunctions in the expression of these proteins can lead to disease.

Among RBPs, the two most representative members that are found aggregated in the brain of ALS/FTD patients are two heterogeneous ribonucleoproteins (hnRNPs) called TDP-43 and FUS/TLS (Ratti & Buratti, 2016). As expected, aberrant aggregation of these proteins can lead to a huge number of changes at all levels of RNA metabolism. Several loss- and gain-of-function mechanisms have been proposed based on cellular and animal models of disease, but the pathomechanisms involved in the origin and progression of aggregation-linked disease are still a matter of debate (Lee *et al*, 2012). Amidst these, however, alterations at the level of pre-mRNA splicing following aggregation of RBPs in neuronal cells represent a very likely possibility.

Up until now, RNA splicing research mediated by TDP-43 and FUS has mainly focused on the major spliceosome. Indeed, several studies have analyzed this issue in depth and we now have a wide view of events that can be misregulated in neuronal cells following the aberrant aggregation of TDP-43 or FUS/TLS in disease. Nonetheless, providing a definitive answer to this question has not proved easy, as work with both TDP-43 and FUS/TLS has resulted in little overlap among the different datasets (Buratti *et al*, 2013; Orozco & Edbauer, 2013). Most importantly, all reports published to date have been focused on events mediated by the major spliceosome (Fig 1).

The work published by Reber *et al* (2016) abruptly shifts the attention to the possible role played by FUS/TLS in controlling the minor spliceosome. Minor spliceosomes are highly conserved in evolution and can be found in many plants, fungi, and animals. As the word suggests, minor spliceosome introns account for a very small minority of all introns found in our genes. For example, in humans it has been estimated that $< 0.5\%$ of introns are spliced through the minor pathway. Intriguingly, however, mutations in minor intron splicing have been associated with onset of many human diseases, especially at the neurodegenerative level (Turunen *et al*, 2013).

At the compositional level, the minor spliceosome differs from the major spliceosome because a set of specific snRNPs (U11, U12, U4atac, and U6atac) replace their respective major spliceosomal counterparts (U1, U2, U4, and U6). At the functional level, the minor spliceosome is currently thought to function similarly to the much better studied major spliceosome, except that U11 and U12 snRNPs form a stable di-snRNP and assemble in a single step (Turunen *et al*, 2013; Wahl & Luhrmann, 2015).

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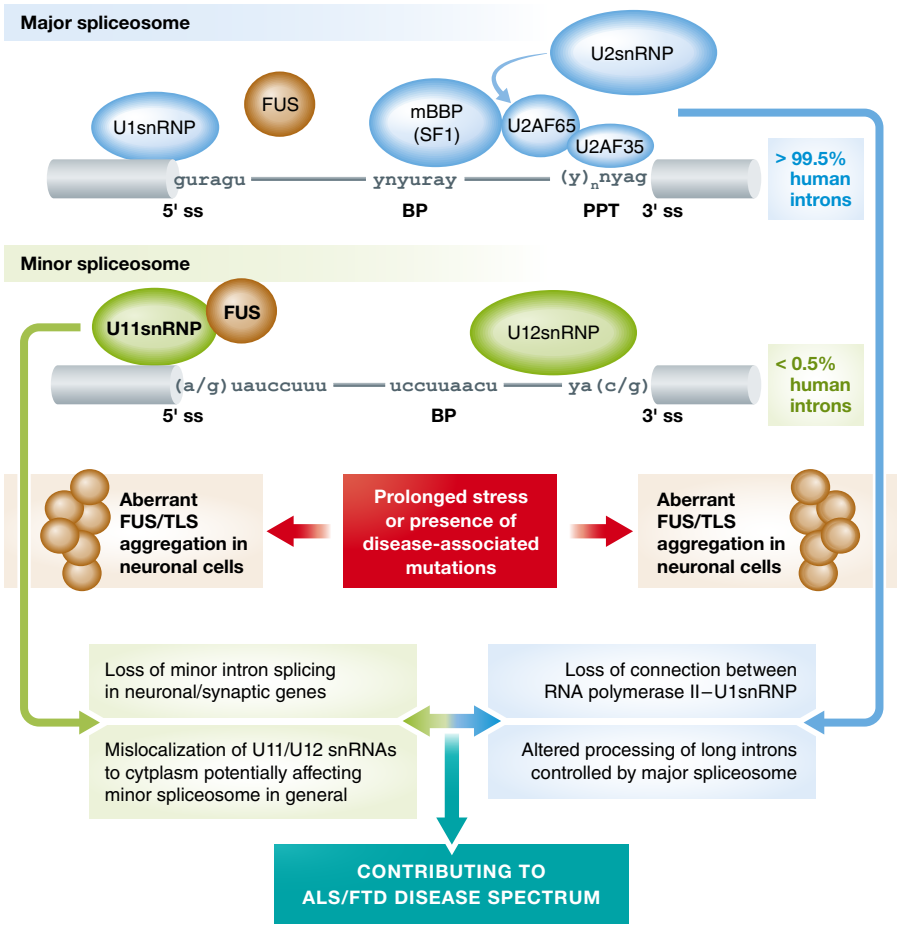

Figure 1. Schematic representation of minor and major spliceosomal introns with consensus sequences of 5′ splice sites (5′ss), 3′ splice sites (3′ss), polypyrimidine tract (PPT), and branch point sequences (BP).

The binding sites of the various small nuclear ribonucleoproteins (snRNPs) are also shown. In major introns, FUS/TLS binds to the nascent transcripts in a largely unspecific manner. In minor introns, FUS/TLS binds directly to U11snRNP and influences splicing in selected genes. The consequences of aberrant FUS/TLS aggregation in cells on both spliceosomal systems are highlighted.

The work by Reber and colleagues used mass spectrometry to identify the U11snRNP minor spliceosome component as a specific interactor of FUS/TLS (Fig 1). This interaction was highly functional, and expression and splicing of minor intron-containing genes was strongly altered in FUS-knockout SH-SY-5Y cells. The work has also characterized the way FUS can affect minor spliceosome action in several ways. First, by showing that when FUS is recruited to mRNAs using the MS2 system, it can act to promote minor spliceosome assembly through its QGSY-rich region. Second, by seeing that FUS/TLS behaves like many other classical hnRNP proteins and depending on binding location in pre-mRNAs can either promote or suppress minor intron splicing. Finally, the authors show that a well-studied disease-associated mutation in FUS/TLS (P525L) fails to promote minor intron splicing in FUS-controlled genes and, even more dramatically, that it also traps U11 and U12 snRNAs in the cytoplasm. This is potentially very important for disease as it implies that FUS alterations can potentially alter all minor intron processing events in a cell, not just the limited subset directly bound by this protein.

These observations spark several questions with regard to disease that will require further research. First and foremost is identifying which events are directly connected with disease. This task should presumably be easier than looking for similar disease-related events associated with major spliceosome due to the much lower number of introns involved. Secondly, once these events are identified what can be done to restore their normal level? In this respect, it is important to note that RNA-based therapeutic applications are quickly becoming a clinical reality in crossing the gap between laboratory bench and bedside (DeVos & Miller, 2013).

In the meantime, as we wait for further developments, the take-home message of the report by Reber *et al* (2016) regarding neurodegeneration in general is that when pre-RNA splicing is concerned, no contributions can be neglected, neither major nor minor.

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