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SPOTLIGHT

All roads lead to TDP43: Convergent mechanisms of TDP43 autoregulation

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TDP43 is an essential RNA-binding protein with fundamental ties to neurodegenerative disorders. In this issue, Hasegawa-Ogawa and colleagues (https://doi.org/10.1083/jcb.202406097) describe a new mechanism for regulating TDP43 function, involving alternatively spliced variants that inhibit TDP43 via dominant-negative activity.

Amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) are pernicious neurodegenerative diseases with distinct signs and symptoms. Despite the clinical disparities, both conditions share a common neuropathological signature, marked by cytoplasmic mislocalization and aggregation of the RNA-binding protein and splicing factor TDP43 (1, 2). Solidifying the link between TDP43 and ALS/FTD, familial forms of disease caused by mutations in *C9ORF72*, *GRN*, and *TARDBP* (the gene encoding TDP43) all result in TDP43 pathology and clinical presentations of ALS, FTD, or both (3). TDP43 dysfunction, illustrated by the accumulation of abnormally spliced transcripts harboring unannotated or cryptic exons, is also observed in both diseases (4). As such, a comprehensive understanding of the factors governing TDP43 localization and function is crucial not just for mapping the pathogenesis of ALS and FTD but also for developing truly effective disease-modifying therapies.

The *TARDBP* transcript undergoes alternative splicing to generate multiple isoforms, including but not limited to the full-length (fl) mRNA encoding canonical TDP43 (5, 6, 7, 8). Many of these events are triggered by TDP43 itself through a process known as autoregulation or regulated unproductive splicing and translation (RUST) (9): TDP43-dependent splicing of its own transcript generates truncated isoforms that are unstable and poorly translated, eventually leading to downregulation of TDP43 at the protein level (10). In this issue, Hasegawa-Ogawa et al. provide convincing evidence that alternatively spliced TDP43 isoforms are not simply transcriptional dead-ends; instead, they actively inhibit flTDP43 post-translationally via a dominant-negative mechanism (11). In so doing, the authors highlight an additional dimension of TDP43 regulation, influenced not just by TDP43 but by other RNA-binding proteins.

All of the alternatively spliced *TARDBP* isoforms generated during autoregulation effectively reduce the amount of flTDP43-encoding mRNA, curtailing the amount of newly synthesized flTDP43 protein (6). As the typical half-life of TDP43 is ∼48 h in neurons (12, 13), this process would require several days to functionally regulate TDP43 activity. Nevertheless, given the exquisite sensitivity of neurons to fluctuations in TDP43 levels (12), this degree of control is likely to be insufficient.

As indicated by Hasegawa-Ogawa et al. and others (8), truncated variants of TDP43 fill this gap by acutely inhibiting flTDP43 splicing activity. While much of exon 6, encoding the TDP43 C-terminal prion-like domain, is missing from these variants, in-frame splicing events within exon 7 result in three unique C termini, labeled MP13, MP18, and MP20 (Fig. 1 A). Upon translation, these transcripts produce proteins sharing the flTDP43 N-terminus, which is crucial for TDP43 oligomerization, but lack the canonical prion-like domain required for efficient splice repression. The result is dominant-negative inhibition of flTDP43 splicing activity, accomplished via hetero-oligomerization between flTDP43 and each of the truncated TDP43 variants. Consistent with this, the authors show that overexpression of MP20, and to a lesser extent MP18, blocks flTDP43's ability to repress the inclusion of cryptic exons in TDP43 substrates (11). Previous studies suggest that truncated variants (primarily MP18) are short-lived and rapidly degraded by the ubiquitin–proteasome system, macroautophagy, and chaperone-mediated autophagy (8, 14), such that their regulatory function is likely to be short-lived (∼1–2 days). This timeline aligns well with the normalization of flTDP43 levels associated with reduced flTDP43 synthesis and physiological clearance mechanisms (13).

TDP43 is not the only protein capable of acting through this regulatory pathway, however (Fig. 1 A). Hasegawa-Ogawa et al. find that TDP43 is also regulated by related RNA-binding proteins, including heteronuclear RNP (hnRNP) A1, hnRNP K, and fused in sarcoma (FUS). Notably, there are important differences in alternatively spliced *TARDBP* transcripts generated by each of these factors. For instance, TDP43 itself preferentially enhances the production of MP18, while hnRNP A1 shifts splicing toward MP13, and hnRNP K induces MP20 expression. Conversely, FUS acts through hnRNP K to regulate TDP43 splicing but also blocks the translation of truncated TDP43 variants (11). The authors also uncovered significant differences in the relative abundance of truncated

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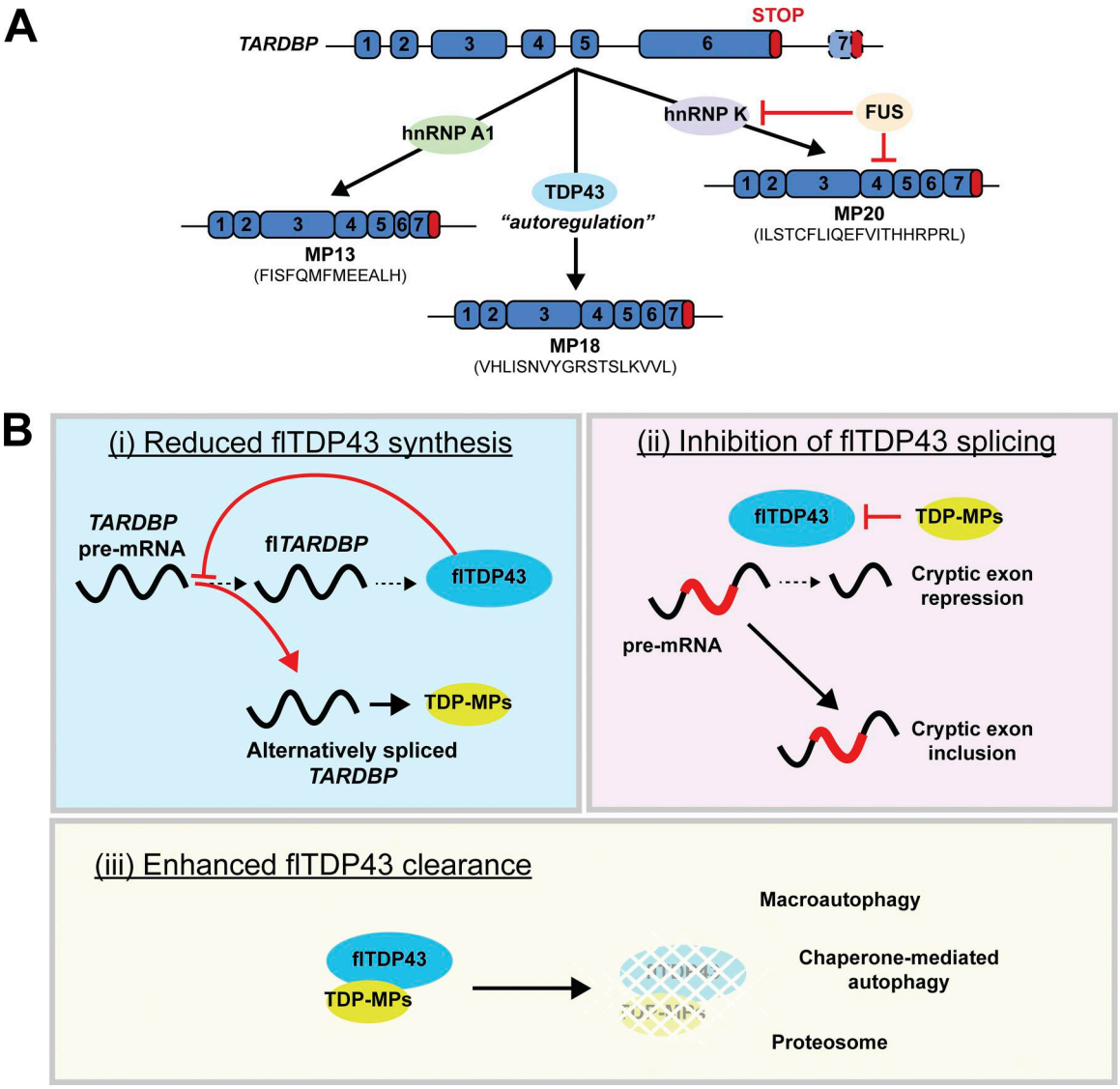

Figure 1. **TDP43 is regulated through transcriptional and posttranslational mechanisms. (A)** *TARDBP* splicing is regulated not just by TDP43 but by other RNA-binding proteins. The *TARDBP* transcript undergoes alternative splicing to generate multiple isoforms through a process tightly controlled by several RNA-binding proteins. hnRNP A1 promotes production of marginal peptide (MP)13 (C-terminal tail: FISFQFMEEALH). Via conventional autoregulation, TDP43 facilitates splicing of MP18 (C-terminal tail: VHLISNVYGRSTSLKVVL). hnRNP K instead generates MP20 (C-terminal tail: ILSTCFLIQEFVITHHRPRL), which is inhibited both directly and indirectly by FUS. **(B)** TDP43 activity is regulated through three independent mechanisms. TDP43 marginal peptides (TDP-MPs) modulate flTDP43 function by (1) reducing flTDP43 synthesis—TDP43 binds *TARDBP* pre-mRNA to inhibit flTDP43 splicing, while simultaneously enhancing the generation of alternatively spliced isoforms, particularly MP18; (2) dominant-negative inhibition of flTDP43—TDP-MPs bind flTDP43 via the intact N terminus but cannot participate in splicing, thereby blocking the repression of cryptic exons by flTDP43; and (3) enhancing flTDP43 clearance—TDP-MPs are unstable and promote flTDP43 degradation through their physical association with flTDP43.

TDP43 variants in mouse versus human tissue (11). In combination with the fact that each truncated variant displays distinct dominant-negative effects on flTDP43 splicing activity, these observations imply a complex and highly nuanced regulatory network controlling flTDP43 levels and activity: TDP43 regulation is not simply a function of TDP43 itself but is instead accomplished in conjunction with co-expressed RNA-binding proteins in a species-specific manner.

This work raises several fundamental questions, each with important implications for our understanding of TDP43 function and its relationship to disease. Building on observations from previous studies (6, 8, 14, *Preprint*), Hasegawa-Ogawa et al. show that truncated TDP43 variants regulate TDP43 activity through three independent mechanisms (Fig. 1 B): (1) reducing the amount of flTDP43-encoding mRNA, (2) dominant-negative inhibition of flTDP43 splicing activity, and (3) accelerated clearance of flTDP43. While (1) is transcriptional in nature, (2) and (3) are posttranslational and require hetero-oligomerization of truncated TDP43 variants with flTDP43. As such, mechanisms (2) and (3) are therefore highly dependent on RNA stability and export to the cytosol for translation. While MP18-encoding transcripts are unstable and degraded by nonsense-mediated mRNA decay (8), less is known about MP13 and

MP20. Additionally, since the truncated variants differ from one another only at the C terminus, the unique sequences in these domains determine their ability to regulate flTDP43 splicing activity, perhaps by influencing protein half-life, localization (nuclear vs cytoplasmic), and/or solubility. While the authors comprehensively catalog the impact of MP18 and MP20 on flTDP43, the consequences of MP13 expression still remain mysterious. Moreover, given their shared propensity for dominant-negative regulation of flTDP43 activity, it is also possible that unchecked expression of truncated TDP43 variants could contribute to the loss of flTDP43 function observed in ALS/FTD.

Lastly, the species-specific differences in TDP43 regulation observed by Hasegawa-Ogawa et al. are intriguing. Specifically, the relatively low level of flTDP43-encoding transcripts in mice implies more effective regulation of flTDP43. This is further supported by the relative abundance of MP18 in mice, a variant that is preferentially produced by TDP43 but not other RNA-binding proteins (11). It remains to be seen whether humans do, in fact, display inefficient regulation of flTDP43 activity in comparison with other species, and whether TDP43 regulation is affected by age and other risk factors tied to neurodegenerative diseases. If so, then rational manipulation of these pathways could represent a novel and potentially effective strategy for restoring TDP43 function and preventing neuron loss in ALS, FTD, and related conditions marked by TDP43 pathology.

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