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Research report

TDP43 and RNA instability in amyotrophic lateral sclerosis

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ABSTRACT

The nuclear RNA-binding protein TDP43 is integrally involved in RNA processing. In accord with this central function, TDP43 levels are tightly regulated through a negative feedback loop, in which TDP43 recognizes its own RNA transcript, destabilizes it, and reduces new TDP43 protein production. In the neurodegenerative disorder amyotrophic lateral sclerosis (ALS), cytoplasmic mislocalization and accumulation of TDP43 disrupt autoregulation; conversely, inefficient TDP43 autoregulation can lead to cytoplasmic TDP43 deposition and subsequent neurodegeneration. Because TDP43 plays a multifaceted role in maintaining RNA metabolism, its mislocalization and accumulation interrupt several RNA processing pathways that in turn affect RNA stability and gene expression. TDP43-mediated disruption of these pathways—including alternative mRNA splicing, non-coding RNA processing, and RNA granule dynamics—may directly or indirectly contribute to ALS pathogenesis. Therefore, strategies that restore effective TDP43 autoregulation may ultimately prevent neurodegeneration in ALS and related disorders.

1. Introduction

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disorder in which the progressive loss of motor neurons results in paralysis and respiratory failure (Bruijn et al., 2004). There is no effective disease-modifying therapy for ALS, and its heterogeneous biochemical, genetic, and clinical features complicate the identification of therapeutic targets. However, it is increasingly clear that RNA dysregulation is a key contributor to ALS pathogenesis. Over the past decade, disease-associated mutations have been identified in genes encoding multiple RNA-binding proteins participating in all aspects of RNA processing (Kapeli et al., 2017). Among these is TDP43, a nuclear protein integrally involved in RNA metabolism. Although mutations in the gene encoding TDP43 (*TARDBP*) account for only a small proportion of the disease burden (2–5%), cytoplasmic TDP43 mislocalization and accumulation are observed in >90% of individuals with ALS (Neumann, 2009). Moreover, mutations in several other ALS-associated genes—including *C9orf72* (Murray et al., 2011), *ANG* (Seilhean et al., 2009), *TBK1* (Van Mossevelde et al., 2016), *PFN1* (Smith et al., 2015), *UBQLN2* (Deng et al., 2011), *VCP* (Johnson et al., 2010), and *hnRNPA2/B1* (Kim et al., 2013)—result in TDP43 pathology. This convergence heavily implicates TDP43 and TDP43-dependent RNA processing in neurodegenerative disease. In this review, we examine how TDP43 dysregulation impacts RNA metabolism, in particular the maintenance of RNA stability, and how these downstream events may contribute to ALS pathogenesis.

2. Mechanisms of TDP43 autoregulation

TDP43 is an essential protein involved in several RNA processing events, including splicing, transcription, and translation. Since TDP43 recognizes UG-rich sequences present within approximately one third of all transcribed genes (Polymenidou et al., 2011; Tollervey et al., 2011; Sephton et al., 2011), it is uniquely able to influence the processing of hundreds to thousands of transcripts. In keeping with these fundamental functions, the level and localization of TDP43 are tightly regulated and critical for cell health. TDP43 knockout mice die early in embryogenesis, and partial or conditional knockout animals exhibit neurodegeneration and behavioral deficits that correlate with the neuroanatomical pattern of TDP43 ablation (Kraemer et al., 2010; Wu et al., 2012; Iguchi et al., 2013; Sephton et al., 2010). Additionally, sustained TDP43 overexpression results in neurodegeneration in primary

Abbreviations: ALS, amyotrophic lateral sclerosis; 3′ UTR, 3′ untranslated region; TDPBR, TDP43 binding region; EEJ, exon-exon junction; EJC, exon junction complex; NMD, nonsense-mediated decay; RUST, regulated unproductive splicing and translation; RAN translation, repeat-associated non-AUG translation; ncRNA, non-coding RNA; lncRNA, long non-coding RNA; miRNA, microRNA; RISC, RNA-induced silencing complex; SG, stress granule; P-body, processing body.

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neuron (Barmada et al., 2010), mouse (Swarup et al., 2011; Wils et al., 2010), rat (Dayton et al., 2013; Tatom et al., 2009), Drosophila (Voigt et al., 2010; Li et al., 2010), zebrafish (Kabashi et al., 2010; Schmid et al., 2013), and primate models (Uchida et al., 2012; Jackson et al., 2015a,b), providing convincing evidence that too little or too much TDP43 is lethal.

Despite the observed sensitivity of neurons and other cell types to long-term changes in TDP43 protein levels, TDP43 expression and localization are dynamically regulated in the short-term by physical injury and other cellular stressors (Moisse et al., 2009; Swarup et al., 2012; Johnson et al., 2011). This pattern of expression suggests that TDP43 may be important for orchestrating the response to acute injury and eventual recovery. However, even relatively minor (~2-fold) persistent changes in TDP43 levels are sufficient to drive neurodegeneration (Janssens et al., 2013; Wegorzewska and Baloh, 2011; Barmada et al., 2010, 2014), indicative of a coping response that over time becomes ineffective and eventually detrimental to cell health.

Similar to systems employed by related RNA-binding proteins, TDP43 regulates its own expression through an intricate negative feedback loop (Fig. 1). At high levels, TDP43 recognizes sequences within the 3′ untranslated region (UTR) of its own transcript (the TDP43 binding region, or TDPBR) (Bhardwaj et al., 2013; Ayala et al., 2011), triggering alternative splicing within the 3′ UTR (Tollervey et al., 2011; Polymenidou et al., 2011), mRNA destabilization, and reduced protein expression (Ayala et al., 2011; Polymenidou et al., 2011; Xu et al., 2010). Two separate mechanisms may account for this destabilization.

In the first, association of TDP43 with the TDPBR induces the removal of two alternative introns (6 and 7) within the last exon of the *TARDBP* mRNA transcript (Polymenidou et al., 2011; Koyama et al., 2016). These splicing events create perceived exon-exon junctions (EEJs) with subsequent deposition of exon-junction complexes (EJCs), structures composed of eukaryotic initiation factor 4A-III, Magoh, Y14, UPF2 and UPF3. During the process of translation, scanning ribosomes typically displace EJCs at EEJs upstream of a stop codon. Translation is stalled when the ribosome encounters a stop codon, allowing association of the SURF complex (SMG1, UPF1, and eRF1 and 2) with the ribosome. When an EJC is present >50 nt downstream of the stop codon, factors within the EJC (i.e., UPF2) may interact with UPF1 in the SURF complex, triggering UPF1 phosphorylation and nonsense-mediated mRNA decay (NMD) (Ivanov et al., 2008; Popp and Maquat, 2013). In support of this model, knockdown of UPF1—an essential NMD factor (Popp and Maquat, 2013; Sun et al., 1998; Medghalchi et al., 2001)—increased the expression of constructs carrying the *TARDBP* 3′ UTR, while exogenous TDP43 reduced their expression (Polymenidou et al., 2011; Barmada et al., 2015).

This mechanism of autoregulation by RNA-binding proteins is not unique to TDP43, and forms the basis for a cascade labeled **r**egulated **u**nproductive **s**plicing and **t**ranscription (RUST) that is also utilized by the splicing factors PTB and SC35 (Wollerton et al., 2004; Sureau et al., 2001; Ni et al., 2007; Lareau et al., 2007; Dredge et al., 2005). Like TDP43, these proteins recognize sequences present within the 3′ UTR of their respective transcripts, resulting in splicing and EJC deposition downstream of the canonical stop codon. This, in turn, causes RNA destabilization via NMD, and an overall reduction in protein levels. An analogous mechanism is responsible for the regulation of FUS, a nuclear RNA-binding protein whose cytoplasmic mislocalization and accumulation are implicated in ALS, much like TDP43 (Lagier-Tourenne and Cleveland, 2009; Lagier-Tourenne et al., 2012; Kwiatkowski et al., 2009; Vance et al., 2009). FUS and TDP43 share basic structural and functional elements, including a glycine-rich low complexity domain that harbors ALS-associated mutations. FUS also binds its own transcript, resulting in exclusion of exon 7 and a shift in the reading frame (Zhou et al., 2013). This shift uncovers a premature stop codon in exon 8, leading to destabilization of the

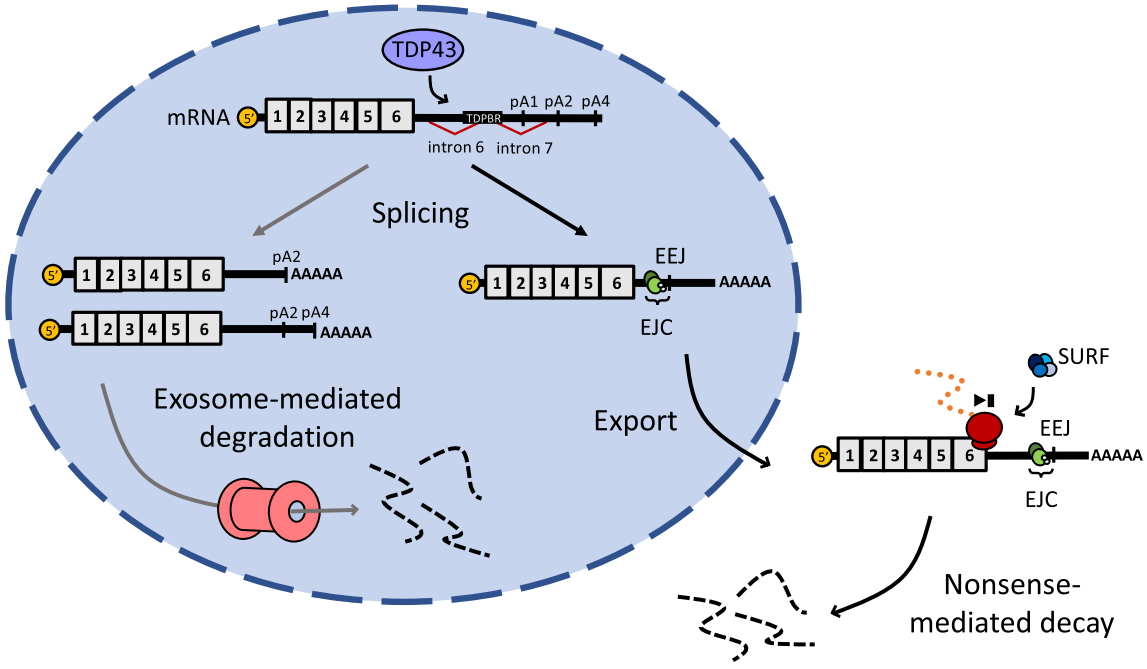

Fig. 1. TDP43 autoregulation. TDP43 may destabilize its own mRNA transcript through two distinct mechanisms. In the first (gray arrows), TDP43 protein recognizes the TDP43 binding region (TDPBR) within the 3′ UTR of its own transcript, stimulating the removal of alternative intron 7 and the primary polyadenylation site (pA1) contained within the intron. Spliced transcripts are preferentially retained in the nucleus and targeted for exosome-mediated decay. In the second mechanism (black arrows), the removal of introns 6 and/or 7 creates exon-exon junctions (EEJs) and the assembly of exon junction complexes (EJCs). The transcript is then exported to the cytoplasm. During the first or pioneer round of translation, the ribosome pauses at the stop codon, allowing the association of the SURF complex with the ribosome. Factors within the downstream EJC interact with UPF1 in the SURF complex, triggering UPF1 phosphorylation and nonsense-mediated mRNA decay.

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