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Repeat RNA toxicity drives ribosomal RNA processing defects in SCA2.

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Spinocerebellar Ataxia type 2 (SCA2) is an autosomal dominant ataxia with no effective treatments. It is the second most prevalent form of dominant ataxia, accounting for approximately 15% of cases worldwide[1, 2]. Clinical features of SCA2 include gait ataxia, tremor, abnormal eye movements, peripheral neuropathy, and other multisystem features. SCA2 neuropathology is characterized by olivopontocerebellar atrophy with marked loss of Purkinje cells, and inferior olivary, pontocerebellar, and substantia nigra neuron loss[3–6].

SCA2 belongs to a growing class of nucleotide repeat expansion disorders arising from unstable repeats in the genome[7, 8]. In this issue of Movement Disorders, Pan P. Li et al. add to an expanding body of literature indicating that repeat expansion disorders are often characterized by plural molecular pathogenic mechanisms[9].

SCA2 is caused by expansion of CAG trinucleotide repeats in the first exon of the *ATXN2* gene, encoding a polyglutamine (polyQ) repeat containing protein. In individuals with SCA2, repeat numbers are in the range of 37–39 repeats, as opposed to a normal range of 13–23 repeats[10]. Intermediate repeat lengths of 27–33 CAG repeats are associated with increased risk for Amyotrophic Lateral Sclerosis (ALS)[11]. The exact function of ATXN2 protein is not yet known, but it is implicated in RNA processing and translation, stress granule assembly, endoplasmic reticular (ER) calcium response, and cytoskeletal reorganization[12]. The expanded CAG repeat in ATXN2 encodes an abnormal stretch of polyglutamine (polyQ) in the N-terminal region. In multiple polyQ disorders, these canonically translated repeat harboring proteins show gain of function toxicities separate from their normal cellular functions[13]. These pathogenic protein species may exert their effects via multiple proximate mechanisms[14]. In the case of the polyQ disorder Huntington disease (HD), haploinsufficiency may also contribute to neurodegeneration[7].

A second general molecular mechanism in nucleotide expansion disorders is the phenomenon of repeat-associated Non-AUG (RAN) translation, which gives rise to toxic repeat peptides due to aberrant translation from expanded repeat regions. RAN translation, however, from the expanded SCA2 transcript appears to be minimal and may not be a significant pathogenic process in SCA2[15].

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Pan P. Li et al. evaluated a third potential pathogenic molecular mechanism involving repeat containing RNA transcripts. In some nucleotide expansion disorders, such as Type 1 Myotonic Dystrophy, expanded repeats exert neurotoxicity at the RNA level by disrupting mRNA processing. One of the mechanisms of RNA mediated toxicity is sequestration of key RNA binding proteins (RBPs), making them unavailable for their normal cellular functions[16]. RNA toxicity is often accompanied by formation of RNA foci. These authors find that expanded sense *ATXN2* RNA transcripts cause neurotoxicity and disrupt ribosomal RNA processing. They present evidence that overexpression of an expanded *ATXN2* transcript, not undergoing RAN translation, shows increased Caspase 3/7 activity in human neuroblastoma cells, and shows increased toxicity in nuclear condensation assays performed with primary mouse cortical neurons. The authors go on to show that the mutant transcripts bearing either 58 or 104 CAG triplets form more RNA foci when transfected into cells. RNA foci were also detected in cerebellar Purkinje cells of SCA2 transgenic mice and in one out of five postmortem human patient brains.

To examine the possibility of RBP sequestration, the authors performed *in vitro* pull down of the mutant *ATXN2* transcript followed by Mass Spectrometry. They identified a number of RBPs with a predominant nuclear and nucleolar localization pattern, suggesting the nucleus as the possible site for aberrant interactions. Several of the sequestered RBPs are critical for maturation of the small rRNA subunit. The authors selected TBL3 (transducing β-like protein 3) for further analysis due to its known interaction with the expanded Huntingtin transcript, implicating a possible common disease mechanism in HD and SCA2. With a series of elegant biochemical studies, the authors demonstrate that TBL3 binds to the aberrant hairpin structure formed by continuous CAG repeats in the expanded repeat *ATXN2* transcript.

The yeast homolog of TBL3 plays a role in 35S rRNA processing and 18S rRNA biogenesis[17]. Based on these findings, Pan P. Li et al tested the levels of 45S pre-rRNA (human equivalent of yeast 35S pre-rRNA) and the ratios of mature 18S and 28S rRNA after TBL3 knockdown in HEK293T cells. They observed an increase in 45S pre-rRNA levels and a decrease in the ratio of mature 18S and 28S rRNA, suggesting defective rRNA processing and maturation, respectively, in the absence of TBL3. Similar trends, albeit without statistical significance, were observed in postmortem HD and SCA2 brains. Defects in ribosome biogenesis are reported in HD, accompanied by nucleolar aggregates[18, 19]. This study opens up lines of investigation into the possible role of ribosomal abnormalities in another neurodegenerative disorder[20]. Taken together, the report by Pan Li. P et al., along with their earlier finding of a toxic antisense *ATXN2* transcript, presents evidence for combinatorial protein-RNA driven pathology in SCA2 (Fig.1).

Neurodegenerative disorders such as SCA2 typically exhibit age-related penetrance. The observation that ribosome biogenesis may be affected in SCA2 is intriguing and with implications for what might happen in aging brains, characterized by decreased global protein translation[21, 22]. Many age-associated neurodegenerative disorders are due to dysfunctions in core components of the translation machinery[23]. Ribosomal proteins, as well as rRNA levels, are affected during healthy aging[24] and could conceivably change ribosomal assembly as well as mRNA translation dynamics. These age-associated changes

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could influence ribosomal and proteasomal subunit stoichiometry, altering global protein homeostasis[25]. These age-related changes might make neurons more susceptible to the toxic effects of expanded repeat transcripts.

Another characteristic aspect of neurodegenerative disorders, such as SCA2, is degeneration of specific neuronal subtypes. Single-cell transcriptomic analyses of the aging brain are beginning to unravel tissue and cell type specificities for components of the translation machinery, such as ribosomal proteins[26, 27], suggesting distinct post-transcriptional outcomes based on neuronal cell type. This might explain the susceptibility of neuronal subtypes in specific disease contexts. In addition, high-energy neuronal subtypes, such as dopaminergic neurons, are particularly susceptible to mitochondrial dysfunction, an emerging factor in multiple neurodegenerative disorders.[28, 29]

Moreover, the most affected transcripts in the aging human cortex are involved in synaptic function[30]. Since activity-mediated dynamic regulation of mRNA translation at synapses is a key factor for neuronal function and survival[31], sequestration of crucial molecules, such as TBL3 as reported here, could be particularly impactful for synaptic mRNA translation. Ultimately leading to neuronal death, the initial manifestations would be synaptic dysfunction and degeneration, which are widely believed to be common features of many neurodegenerative disorders.

Within the context of an aging neuronal environment, the accumulation of expanded repeat containing RNA and proteins could trigger cascades of events that affect both arms of the peptide life cycle - synthesis and degradation - precipitating neurodegeneration[32].

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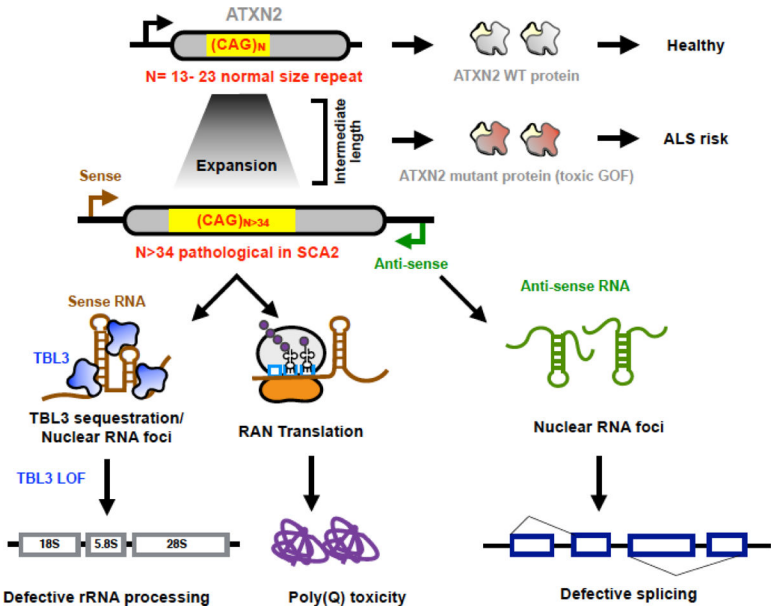

Figure 1. Multiple modes of SCA2 pathogenesis.

Normal CAG repeat number in the *ATXN2* gene generates a WT protein that participates in a host of cellular functions. The intermediate repeat length is associated with ALS while the expanded CAG repeats in the *ATXN2* gene causes toxicity by RNA binding protein sequestration, Repeat-associated Non-AUG translation or via the *ATXN2*- Antisense (AS) transcript. Each of these aberrant processes cause dysregulation of downstream molecular pathways and could collectively contribute to neurodegeneration.