RANTing about *C9orf72*

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A noncoding repeat expansion in the *C9orf72* gene is the most common genetic cause of frontotemporal dementia and amyotrophic lateral sclerosis. In this issue of *Neuron*, Ash et al. (2013) show that despite being noncoding the repeats are translated, leading to widespread neuronal aggregates of the translated proteins.

Frontotemporal dementia (FTD) and amyotrophic lateral sclerosis (ALS) are related neurodegenerative diseases that share clinical features, neuropathological findings, and underlying genetic causes. FTD is the second most common form of young-onset dementia (Harvey et al., 2003; Ratnavalli et al., 2002) and is characterized by selective involvement of the frontal and/or temporal cortices leading to behavioral changes or language impairment. ALS is clinically characterized by loss of upper and lower motor neurons leading to weakness, paralysis, and ultimately death due to respiratory failure. Both FTD and ALS are neuropathologically characterized by the presence of neuronal inclusions containing TDP-43 protein (Neumann et al., 2006). They can co-occur within a single individual or in families with genetically inherited FTD/ALS suggesting they form a disease spectrum (Lillo and Hodges, 2009). An expanded hexanucleotide (GGGGCC) repeat in a noncoding region of the chromosome 9 open reading frame 72 (*C9orf72*) gene was recently shown to be the most common genetic cause of both FTD and ALS (c9FTD/ALS) (DeJesus-Hernandez et al., 2011; Renton et al., 2011). The high frequency of *C9orf72* repeat expansion in FTD/ALS has generated great interest in the underlying disease mechanism, of which several non-mutually exclusive possibilities exist. One potential disease mechanism is termed RNA gain of function and is based on evidence from other diseases caused by large expansions of noncoding repeats such as myotonic dystrophy (Cooper et al., 2009). In these diseases, the repeat RNA is transcribed but aggregates in the nucleus in discrete structures termed RNA foci. The RNA foci sequester RNA-binding proteins and it is loss of these RNA-binding proteins which ultimately leads to disease. This mechanism has been shown to directly cause specific aspects of the myotonic dystrophy phenotype (Cooper et al., 2009). In favor of this mechanism is the presence of RNA foci in c9FTD/ALS patient neurons (DeJesus-Hernandez et al., 2011). Another possibility is loss of function of C9orf72 protein. Although little is known about C9orf72 protein function, bioinformatic approaches have recently shown that it is structurally related to DENN proteins, a class of GDP/GTP exchange factors (GEFs) that activate Rab-GTPases, suggesting it may have a role in vesicular trafficking (Levine et al., 2013; Zhang et al., 2012). In support of the loss-of-function mechanism is the finding that the level of GGGGCC repeat-containing transcripts in patient brain is decreased (DeJesus-Hernandez et al., 2011; Gijselinck et al., 2012). The findings reported in this issue of *Neuron* by Ash et al. (2013) now raise a third possibility that newly identified protein aggregates may have a role in disease pathogenesis.

Pathological analysis of c9ALS/FTD cases show that they contain TDP-43-positive inclusions but could be distinguished from other FTD/ALS cases on the basis that they all exhibit additional p62- and ubiquilin-positive pathology (Al-Sarraj et al., 2011; Brettschneider et al., 2012; Pikkarainen et al., 2010). This additional pathology is particularly evident because of the lack of TDP-43 pathology in the granular cell layer and CA4 subregion of the hippocampal formation and the granular cell layer of the cerebellum. Small p62-positive "star-like" neuronal cytoplasmic inclusions are also observed. The nature of these TDP-43-negative inclusions is of great interest given the insight into neurodegenerative diseases that has come from the identification of aggregating proteins. The key findings from this new study are the identification of novel aggregating proteins and their remarkable specificity for c9ALS/FTD.

Equally intriguing is the mechanism by which these aggregating proteins are formed. The proteins are generated by translation of the expanded non-coding GGGGCC repeats. Translation occurs via a recently described phenomenon termed repeat-associated non-ATG translation (RAN translation) (Zu et al., 2011). RAN translation was first shown for expanded CAG repeats, which cause a range of neurodegenerative diseases including Huntington's disease and several spinocerebellar ataxias (Orr and Zoghbi, 2007). It was clearly demonstrated in a molecular biology tour de force that CAG repeat expansions lacking ATG codons are translated in all three reading frames, leading to production of polyglutamine, polyalanine, and polyserine tracts (Zu et al., 2011). RAN translation was dependent on repeat length as a minimum of 58 CAG repeats was required for translation in all three frames. Importantly, RAN translation products were observed in protein aggregates in patients with expanded CAG repeats, confirming their in vivo relevance (Zu et al., 2011).

Armed with this information Ash et al. (2013) set out to determine whether RAN translation occurred in the presence of the expanded *C9orf72* GGGGCC repeats. Antibodies were generated by pooling peptides corresponding to the

three possible reading frames, poly-(glycine-proline), poly-(glycine-alanine), and poly-(glycine-arginine). The antibodies generated showed high affinity to poly-(glycine-proline) with minimal binding to the other polypeptides and were termed C9RANT antibodies. Remarkably, these antibodies revealed the presence of widespread C9RANT-positive neuronal aggregates in c9FTD/ALS that were similar in shape and distribution to the previously observed p62-positive, TDP-43-negative inclusions. No staining of inclusions was observed in 120 cases of other neurodegenerative diseases, including those with TDP-43, Aβ, α-synuclein, tau, and CAG-repeat pathology, confirming the specificity of C9RANT to c9FTD/ALS. The formation of translated products confirms that the GGGGCC repeats are transcribed and presumably transported out of the nucleus for translation. Importantly, these findings have been concomitantly reported by other researchers (Mori et al., 2013a).

These new findings raise a number of interesting questions. Perhaps the most important is the disease relevance of the RAN translation products. The next key step will be to clarify whether they play a pathogenic role or are simply innocent byproducts of the expanded GGGGCC repeats. It will also be interesting to establish the mechanism by which RAN translation occurs. In the case of CAG repeats, it was shown to be dependent on the formation of RNA hairpin structures (Zu et al., 2011). The recent demonstration that GGGGCC-repeat RNA forms G-quadruplexes (Fratta et al., 2012), a structure shown to have a role in translation initiation (Morris et al., 2010), suggests other mechanisms may also be possible. Other interesting questions include the relative contribution of the three different RAN translation products to neuronal dysfunction, the minimum repeat length required for GGGGCC RAN translation to occur in vivo and the mechanism by which the GGGGCC repeat containing transcripts exit the nucleus, which could involve hnRNP A3 (Mori et al., 2013b). Finally, as the authors propose, the specificity of the GGGGCC RAN translation products warrant further investigation into their utility as potential biomarkers for c9FTD/ALS.

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