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Review

Stress granules at the intersection of autophagy and ALS

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ABSTRACT

Amyotrophic lateral sclerosis (ALS) is a progressive, fatal disease caused by loss of upper and lower motor neurons. The majority of ALS cases are classified as sporadic (80–90%), with the remaining considered familial based on patient history. The last decade has seen a surge in the identification of ALS-causing genes – including *TARDBP* (TDP-43), *FUS*, *MATR3* (Matrin-3), *C9ORF72* and several others – providing important insights into the molecular pathways involved in pathogenesis. Most of the protein products of ALS-linked genes fall into two functional categories: RNA-binding/homeostasis and protein-quality control (*i.e.* autophagy and proteasome). The RNA-binding proteins tend to be aggregation-prone with low-complexity domains similar to the prion-forming domains of yeast. Many also incorporate into stress granules (SGs), which are cytoplasmic ribonucleoprotein complexes that form in response to cellular stress. Mutant forms of TDP-43 and FUS perturb SG dynamics, lengthening their cytoplasmic persistence. Recent evidence suggests that SGs are regulated by the autophagy pathway, suggesting a unifying connection between many of the ALS-linked genes. Persistent SGs may give rise to intractable aggregates that disrupt neuronal homeostasis, thus failure to clear SGs by autophagic processes may promote ALS pathogenesis.

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1. Introduction

ALS is the most common adult-onset motor neuron disorder, typically striking in the fifth to seventh decades of life, though

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juvenile disease also exists. It is characterized by rapid degeneration of motor neurons, and subsequent atrophy of innervated muscle groups. Death is generally secondary to failure of respiratory muscles (Ravits and La Spada, 2009; Turner et al., 2013). ALS occurs globally in all races, ethnic and socioeconomic groups. There are no pharmacological interventions for the underlying molecular pathogenesis (Miller et al., 2012).

Neurodegenerative diseases often share two clinicopathological properties. First, by definition, they affect highly translationally-active neurons preferentially to other cell types; second, they are often associated with mutations in components of protein-quality control (PQC) (Hetz et al., 2009; Kabashi and Durham, 2006). It is perhaps not surprising that perturbations to PQC pathways would have significant impact on cells that are both especially translationally active and long-lived. In the case of ALS, a number of different proteins and metabolic pathways have been linked to pathogenesis, but issues of proteostasis (*e.g.* protein folding, aggregation and quality-control) appear to be the most common pathogenic theme (Andersen and Al-Chalabi, 2011; Renton et al., 2014). Many ALS-associated proteins have intriguing properties with regard to self-association, aggregation-propensity, and interaction with cytoplasmic stress granules (SGs) (Bosco et al., 2010; Colombrita et al., 2009; Daigle et al., 2013; Dewey et al., 2012; Guo et al., 2011; Liu-Yesucevitz et al., 2010; McDonald et al., 2011; Sun et al., 2011; Vance et al., 2013). Other ALS-associated proteins have explicit functions in PQC pathways, including autophagy. Below we discuss the intersections between protein aggregation, SGs and autophagy in ALS pathogenesis.

2. Stress granules – discrete stress-induced cytoplasmic sites of ribonucleoprotein accumulation

Ribonucleoprotein (RNP) granules are cellular sites dedicated to RNA processing. Well-characterized types of RNP granules include transport RNPs, processing bodies (P-bodies) and stress granules (SGs); all of which have distinct roles in mRNA regulation (Anderson and Kedersha, 2008; Kedersha et al., 2005). Transport RNPs ensure localized neuronal translation of RNAs by facilitating their transport along cytoskeletal elements while maintaining temporary translational repression (Kiebler and Bassell, 2006). SGs and P-bodies are phenotypically similar, non-membrane-bound, discrete cytoplasmic structures visible by light microscopy (Buchan and Parker, 2009; Guil et al., 2006). They contain many of the same proteins, but each has exclusive constituents; P-bodies are enriched for proteins involved in RNA degradation, while SGs are preferentially composed of translation initiation factors (Reineke and Lloyd, 2013). Thus, P-bodies are classified as foci of RNA breakdown and turnover, and SGs are thought to be sites of paused translation initiation and global translation repression (Anderson and Kedersha, 2008; Li et al., 2013; Parker and Sheth, 2007; Thomas et al., 2011). Both SGs and P-bodies have the ability to exchange mRNAs with bulk cytoplasm depending on cellular conditions (Decker and Parker, 2012).

The formation of SGs is believed to be a conserved, protective response to various cell stresses. Some example stresses include: oxidative (Anderson and Kedersha, 2002, 2008; Bosco et al., 2010; Daigle et al., 2013), mitochondrial (Buchan et al., 2011; Chalupnikova et al., 2008; Stoecklin et al., 2004), proteasomal (Fournier et al., 2010; Mazroui et al., 2007) and viral (Emara and Brinton, 2007; Raaben et al., 2007). Interestingly, many external stimuli/stressors do not induce SG formation in mammalian cells, suggesting SGs are a specific response not common to all stress (Kedersha et al., 1999). There are several proposed means by which they exert their protection. SGs may offer direct protection for certain mRNAs from damaging stressors (Kedersha and Anderson, 2002). Alternatively, SGs may sequester unwanted mRNAs, preventing their translation, such as viral RNAs during infection (Beckham and Parker, 2008), or less critical mRNAs during stress conditions (Li et al., 2013; Unsworth et al., 2010; Wolozin, 2012). Thus, SGs may offer prioritization of specific protein products (Scheu et al., 2006). More generally, SGs may decrease protein stress through the global repression of translation by binding mRNAs that would otherwise be translated. The apparently causal role of phosphorylated eIF2α in facilitating SG formation supports this hypothesis, as eIF2α has an established role in translation repression (Kedersha et al., 1999).

SGs contain polyadenylated mRNAs, translation initiation factors, small ribosome subunits and several RNA-binding proteins (Anderson and Kedersha, 2008; Daigle et al., 2016). Putative SG functions all demand the intimate association of these components within a discrete cytosolic space, removed from the majority of cellular machinery. Importantly, RNPs containing specific mRNAs are critical for transport and localized translation in neuronal dendrites. Different types of RNPs (SG, P-body, transport) share similar components (Decker and Parker, 2012), thus neurons may be particularly sensitive to disruption of RNP homeostasis.

SGs are assembled and disassembled through the formation and dissolution of a “liquid-liquid phase-separated state”, in which the components that form SGs “demix” from the bulk solution to create a unique micro-environment. This transient phase-separated state presumably allows for a rapid, reversible response to stress (Elbaum-Garfinkle et al., 2015; Lin et al., 2015; Molliex et al., 2015). Several proteins, as well as mRNA, are implicated in driving the physical phase separation (Zhang et al., 2015). The protein TIA1, for example, is critical to the early stages of SG assembly. TIA1 has three amino-terminal RNA recognition motifs and a carboxy-terminal domain, which has low-complexity composition similar to the intrinsically-disordered domains that drive yeast prion proteins to form self-propagating amyloid fibrils. In fact, a peculiarity about many of the proteins that are both linked to ALS and SG formation is they possess yeast prion-like domains (discussed below). Substitution of this domain of TIA1 with the actual prion-forming domain of yeast prion protein Sup35, results in a restoration of SG formation, which is lost following native TIA1 prion-like domain deletion (Gilks et al., 2004).

The evolutionary conservation of SGs in eukaryotic cells indicates that they serve critical cellular functions. However, the promiscuous, *en masse* sequestration of mRNA transcripts in cytosolic granules would clearly have dramatic implications for cell survival. As with any metabolic pathway, SG formation must be balanced with mechanisms to ensure disassembly. Intracellular component turnover relies on multiple pathways, including autophagy and ubiquitin-mediated proteolysis (Ciechanover, 1994; Cuervo et al., 2005; Glickman and Ciechanover, 2002; Levine et al., 2008; Reed, 2003).

3. Autophagy – a mechanism for clearing protein aggregates

Autophagy is a well-studied system for disposal of a variety of intracellular species. First identified in the context of hormone studies in rats, it has since been appreciated as a mechanism for nearly all eukaryotic cells to dispose of a wide variety of intracellular components deemed unnecessary or maladaptive (Deter et al., 1967; Gomes and Scorrano, 2013). Autophagy involves an autophagosome, a double-membrane bound structure that forms from extant membrane-bound organelles (Chan and Tang, 2013). The autophagosome engulfs regions of the cytosol and fuses with the lysosome to become the autophagolysosome where its contents are catabolized (Deter et al., 1967; Gomes and Scorrano, 2013). This membrane-enclosed mechanism is sometimes more