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RNA binding proteins and the genesis of neurodegenerative diseases

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Recent advances in neurodegenerative diseases point to novel mechanisms of protein aggregation that revolve around the unique biology of RNA binding proteins. RNA binding proteins normally are present in the nucleus. Under conditions of cell stress these RNA binding proteins translocate to the cytoplasm where they form stress granules, which function in part to sequester specialized transcript and promote translation of protective proteins. Studies in humans show that pathological aggregates occurring in ALS, Alzheimer's disease and other dementias co-localize with stress granules. One increasingly appealing hypothesis is that mutations in RNA binding proteins or prolonged periods of stress causes formation of very stable, pathological stress granules. The consolidation of RNA binding proteins away from the nucleus and neuronal arbors into pathological stress granules might impair the normal physiological activities of these RNA binding proteins causing the neurodegeneration associated with these diseases. Conversely, therapeutic strategies focusing on reducing formation of pathological stress granules might be neuroprotective.

We have recently identified a new type of molecular pathology in Alzheimer's disease (AD) that derives from the aggregation of RNA binding proteins (RBPs), forming RNA-protein complexes that include stress granules (SGs) [1]. These SGs progressively accumulate in the brains of transgenic models of tauopathy, as well as massively accumulate in subjects with AD and other neurodegenerative diseases [1] [2]. Some SGs (e.g., those positive for RNA binding protein TIA-1) co-localize with tau pathology, while other SGs (e.g., those positive for G3BP) often identify neurons that lack tau pathology. In order to understand how these RBPs might link to disease, it is useful to examine the unique biology of RBPs.

mRNA binding proteins (RBPs) facilitate mRNA trafficking from the nucleus to the cytoplasm as part of the biological machinery that regulates mRNA metabolism, such as RNA translation, transport and decay [3]. The functions of RBPs can generally be divided into nuclear and cytoplasmic activities, each of which constitutes two very large fields of literature. In the nucleus RBPs regulate mRNA maturation, including splicing, RNA helicase activity, RNA polymerase elongation and nuclear export [4]. In the cytoplasm RBPs regulate RNA transport, silencing, translation and degradation [3]. One of the most striking aspects of RBP function is their ability to reversibly aggregate to form RNA granules, which coalesce through binding of the low complexity, glycine rich domains [5, 6]. Multiple different types of cytoplasmic granules exist, including transport granules, stress granules and P-bodies. RNA degradation is mediated by a type of RNA granule, termed the P-body [7]. Transport granules move transcripts from the soma into the dendritic,

and axonal arbors; axonal RBPs are more apparent during development and regeneration [8]. The presence of large numbers of RNA transport granules in neurons might explain why RBP dysfunction causes neuronal diseases. RBP complexes also mediate the process of activity dependent protein synthesis, which is critical element contributing to synaptic plasticity, habituation and memory [9]. These granules interface with the micro-RNA system, since both microRNA and RBP regulate protein synthesis [10, 11]. The interaction of microRNA with RBP adds an additional layer of regulatory control.

Under stressful conditions mRNA binding proteins consolidate mRNA in cytoplasmic compartments, termed the stress granules (SGs) (Fig. 1); this recruitment is mediated by multiple proteins, including T-cell intracellular antigen 1 (TIA-1), RasGAP-associated endoribonuclease (G3BP), eukaryotic initiation factor 3 (eIF3) and poly-A binding protein (PABP) [12]. The process of SG formation is best understood for pathways mediated by phosphorylation of eIF2 α . Stressful conditions prompt phosphorylation of eIF2 α at serine 51, which inhibits formation of a complex containing eIF2, GTP and tRNA [13]. Four different pathways lead to eIF2 α phosphorylation: Oxidative stress (mediated by the kinase HRI), nutrient limitation or proteasomal dysfunction (mediated by the kinase GCN2), viral infection (mediated by the kinase PKR) and endoplasmic reticular stress (mediated by the kinase PERK) [14, 15]. SG formation is also regulated through independent pathways such as cleavage of tRNA by angiogenin or inhibition of ribosomal scanning [16, 17]. Stress also induces many RBPs to translocate from the nucleus to the cytoplasm. So, during stressful conditions, capped mRNA remains bound to the pre-initiation complex, which contains the other elongation factor binding proteins EF-4A, E and G. This mRNA-protein complex is bound by SG associated proteins, including eIF3, poly-A and nucleating RBPs, such as TIA-1, TIAR, TTP or G3BP [18, 19]. The SGs are initially small, but increase in size as the RBPs form cross-links by binding to each other through the glycine rich protein aggregation domains.

An increasing number of mutations associated with neurodegenerative diseases are associated with RBPs. For instance, mutations in TDP-43, FUS, ataxin-2, matrin-2, hnRNPA1/B2, optineurin and angiogenin are all linked to amyotrophic lateral sclerosis [2] [20]. The interaction between pathology of SGs is easy to envision for RBPs that exhibit disease-linked mutations. Disease linked mutations in these proteins generally increase the tendency of the protein to aggregate. Since these proteins normally exist in equilibrium between dispersed, soluble proteins and aggregated, insoluble complexes, aggregation-accelerating mutations would shift the equilibrium, leading to increased SG formation and formation of the stable, long-lived protein aggregates that we associate with disease pathology. The presence of more aggregates and/or aggregates with enhances stability might also increase formation of secondary mature SGs around the aggregates, much like the normal evolution of SGs. This process of SG growth is analogous to cross-seeding, and normally forms the basis of SG maturation. Pathological cross-seeding has been shown in cell culture for polyglutamine rich proteins, such as huntingtin [21]. This same process of accelerated cross-seeding might occur in the brain for other pathological proteins, more generally. The formation of persistent, insoluble protein aggregates might shift the equilibrium of RBPs towards aggregation, leading to accelerated, long-lived SG formation. These highly insoluble aggregates could also serve as a nidus for further aggregation of SGs,

by cross-linking with other RBPs and also binding RNA as part of the process of SG maturation.

Thus, a shift in the binding equilibrium for RBPs can lead to excessive RNA granule formation. The shift leads to enhanced SG formation, with resulting formation of persistent SGs, which we hypothesize to be the substrate for much of the intracellular pathology that accumulates in neurodegenerative diseases. Recent studies by our group and that of the Taylor group reveal that mutations in RBPs, such as TDP-43, cause readily apparent changes even before pathology forms. We recently examined RNA transport granules, which travel along dendrites. In neurons expressing TDP-43 carrying disease-linked mutations, RNA transport granules are larger and move slower [22] [23]. This highlights mutation related changes in RNA granules that could set the stage for subsequent disease. The innate biology build from reversible aggregation combined with the enhanced tendency of neurons to form cytoplasmic RNA granules (because of their strong need for mRNA transport) appears to provide the substrate for subsequent pathological aggregation and the resulting neurodegenerative disease.

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Figure 1. Sodium arsenite induces stress granules co-positive for TIA-1 and PABP

Human SH-SY5Y cells (top panels, basal conditions) were treated with 0.5 mM sodium arsenite (Ars) for 30 min (middle panels) or arsenite plus 10 μ g/ml cycloheximide (CHX, lower panels). Arrowheads denote stress granules co-positive for TIA-1 and PABP. Scale bar = 2 μ m.