P ion, 11:313–322, 2017
Published with license by Taylor & Francis
ISSN: 1933-68961933-690X online
DOI: 10.1080/19336896.2017.1356559

EXTRA VIEWS

More stressed out with age? Check your RNA granule aggregation

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AB RAC . Low complexity (LC) prion-like domains are over-represented among RNA-binding proteins (RBPs) and contribute to the dynamic nature of RNA granules. Importantly, several neurodegenerative diseases are characterized by cytoplasmic "solid" aggregates formed by mainly nuclear RBPs harboring LC prion-like domains. Although RBP aggregation in disease has been extensively characterized, it remains unknown how the process of aging disturbs RBP dynamics. Our recent study revealed that RNA granule components including 2 key stress granule RBPs with LC prion-like domains, PAB-1 and TIAR-2, aggregate in aged *Caeno habdi i elegan* in the absence of disease. Here we present new evidence showing that sustained stress granule formation triggers RBP aggregation. In addition, we demonstrate that mild chronic stress during aging promotes mislocalization of nuclear RBPs. We discuss the consequences of aberrant interactions between age-related RBP aggregation and disease-associated RBP aggregation. In particular, we show that FUST-1 and PAB-1 co-localize in aberrant cytoplasmic accumulations. Significantly, long-lived animals with reduced insulin/IGF-1 signaling abrogate stress granule RBP aggregation through activation of the transcription factors HSF-1 and DAF-16. We evaluate the different mechanisms that could maintain dynamic

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Received June 7, 2017; Revised July 6, 2017; Accepted July 10, 2017

Extra View to: Lechler MC, Crawford ED, Groh N, Widmaier K, Jung R, Kirstein J, Trinidad JC, Burlingame AL, David DC. Reduced insulin/IGF-1 signaling restores the dynamic properties of key stress granule proteins during aging. Cell Rep 2017; 10;18(2):454-467; https://doi.org/10.1016/j.celrep.2016.12.033

stress granules. Together these findings highlight how changes with age could contribute to pathogenesis in neurodegenerative diseases and disruption of RNA homeostasis.

KE ORD . RNA-binding proteins, protein aggregation, prion-like domains, aging, stress granules

INTRODUCTION

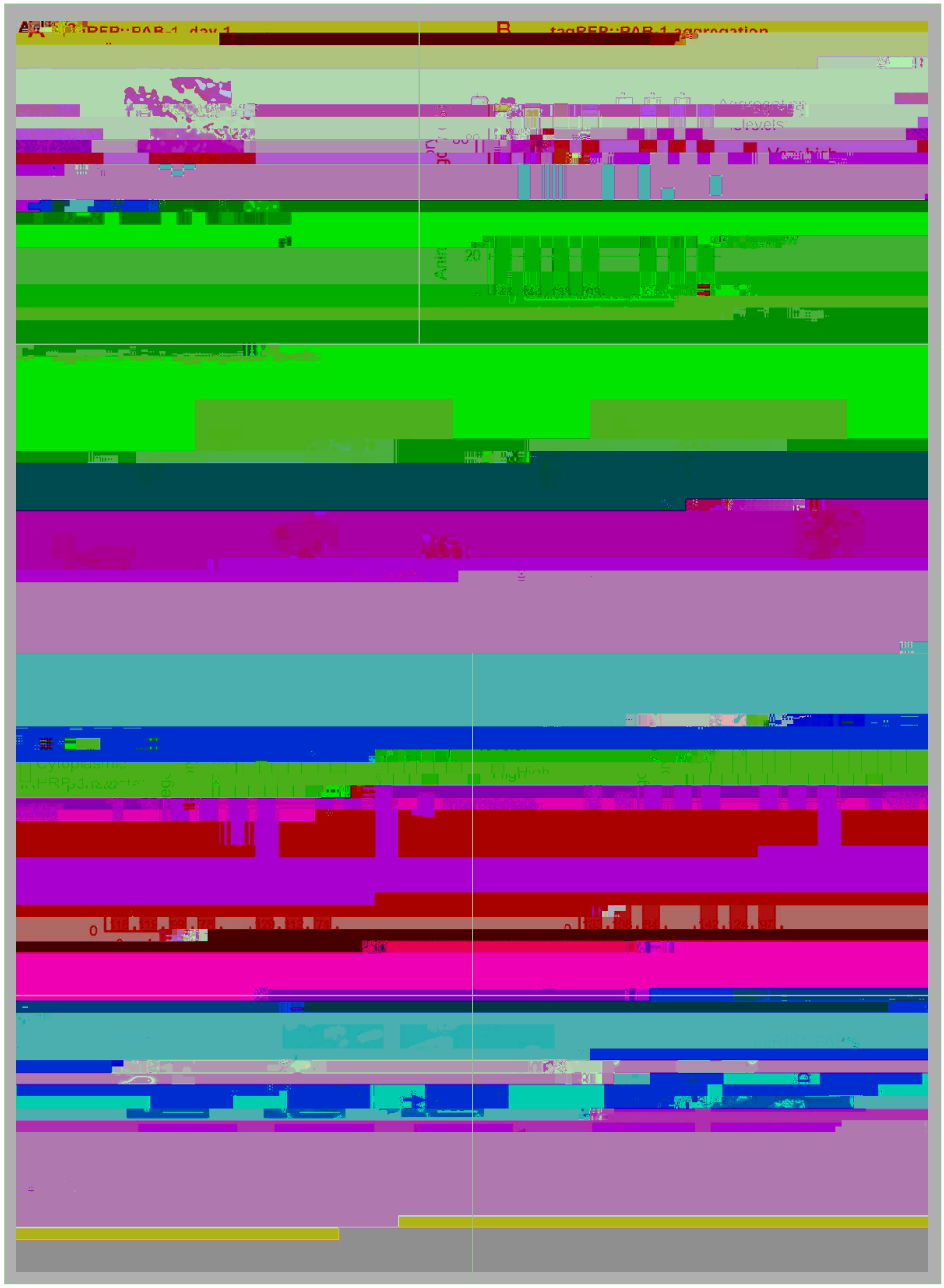
A number of diseases including certain neurodegenerative disorders are characterized by the presence of pathological highly intractable or "solid" protein aggregates formed by one or several distinct proteins. In the last decade, the list of proteins identified in aggregates associated with disease has been considerably extended with the addition of several RNA-binding proteins (RBPs). These include RBPs such as TDP-43 and FUS observed in aggregates of patients with amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD).[1-3] RBPs associated with disease contain a low complexity (LC) "prion-like" domain similar to sequences identified in yeast prion proteins.[4,5] Highlighting the importance of this domain, familial cases of these diseases are frequently related to aggregation-promoting mutations in the LC prion-like domain.[6,7] In a non-disease context, RBPs with LC prion-like domains are key components of RNA granules. Depending on their composition, RNA granules such as stress granules, P-bodies, P-granules and neuronal granules perform different functions in the cell. RNA granules are highly dynamic membrane-less organelles and their assembly is mediated by association of RBPs through their LC prion-like domains and subsequent recruitment of RNA and associated proteins by RNA-binding domains.[8-10] In particular, weak interactions built between LC prion-like domains in RBPs promote a liquid-liquid phase separation in vitro consistent with the observed liquid-like proprieties of RNA granules in vivo.[11-14] Considering the special nature of the interactions between RBPs with LC prion-like domains and the growing number of RBPs forming hallmark aggregates in different neurodegenerative disorders, we hypothesized that aberrant aggregation of RBPs and RNA granules could be an important problem that the organism needs to actively avoid especially during aging.

RBPs with LC Prion-Like Domains and RNA Granules are Associated with High Propensity to Aggregate with Age in C. elegans

Importantly, we found that animals with higher levels of PAB-1 aggregation were smaller, less motile and shorter lived than animals without aggregation.[20] These results demonstrate that aggregation of sgRBPs is potentially toxic and could accelerate the aging process.

Sustained Stress Granule Formation Triggers sgRBP Aggregation

In vitro studies have shown that liquid droplets formed by purified RBPs with LC prion-like domains will eventually transition into a more solid state over time, thus impairing their disassembly.[11-14] Solidification of liquid droplets and concomitantly the formation of fibrils was enhanced by disease-related mutations promoting aggregation and repeated cycles of phase separation.[11,13] These findings lead us to the hypothesis that sustained stress granule formation could enhance their aggregation in vivo. 24h after continuous mild stress at 25°C, confocal analysis revealed the formation of abundant stress granules in day-1-old adults (Fig. 1A). Already on day 2, these animals started to form large PAB-1 aggregates visible at low-magnification. Overall, PAB-1 aggregation in a population of *C. elegan* grown at 25°C versus 20°



inclusions of mainly nuclear-localized RBPs. Therefore RBP mislocalization from the nucleus to the cytoplasm is a key step toward pathogenesis. We investigated whether the aging process triggers mislocalization of nuclear RBPs. For this, we selected HRP-1, a nuclear RBP with a LC prion-like domain, which we identified in our proteomic analysis to become highly insoluble with age. Significantly, the human homologs of HRP-1, hnRNPA1 and hnRNPA3 form aberrant cytoplasmic inclusions in multisystem proteinopathy and they are also found to co-aggregate in inclusions in C9orf72 mutation-associated ALS/FTLD.[6,23] Using a fluorescent-tagged HRP-1, we confirmed its primary nuclear localization (Fig. 1E and 1F) and we observed that HRP-1 is

not a normal constituent of cytoplasmic stress granules as it remained in the nucleus upon acute heat stress (2 hour heat shock at 32°C, data not shown). Next we tested if aging modulates HRP-1 localization. In *C. elegan* maintained in standard conditions at 20°

but also in other pathological aggregates identified in Alzheimer disease and Huntington's disease.[21] Moreover our results strongly suggest that sgRBPs are not passive players in neurodegenerative disease.

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We found that sgRBP aggregation is efficiently prevented onp3381vlseaot p338153(idependvent)-549.9lone-
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and HSP110 chaperones act together to promote protein disaggregation.[34,35]

DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

No potential conflicts of interest were disclosed.

FUNDING

This work was supported by funding from the DZNE and a Marie Curie International Reintegration Grant (322120 to DCD)

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