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Pour Some Sugar on TDP(-43)

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In this issue of *Molecular Cell*, McGurk et al. (2018) identify how poly(ADP-ribose) binding tunes the phase behavior of the ALS disease protein TDP-43, uncovering the molecular events underlying its aggregation in disease and illuminating a novel therapeutic target.

Neurodegenerative diseases are almost always associated with the accumulation of clumps of proteins in the brains of patients with these disorders (Aguzzi and O'Connor, 2010). Different diseases have different proteins that aggregate in different parts of the brain. Defining the cellular and molecular events that cause certain proteins to aggregate in each disease will provide insight into what initiates pathogenesis and might suggest avenues for therapeutic intervention. Two neurodegenerative diseases, amyotrophic lateral sclerosis (ALS) and frontotemporal degeneration (FTD), are both associated with the aggregation of the RNA-binding protein TDP-43 (Neumann et al., 2006). TDP-43 is normally located in the nucleus, where it regulates a variety of RNA-processing events, including alternative splicing. Yet something goes wrong in ALS and FTD, resulting in TDP-43 leaving the nucleus and accumulating in the cytoplasm as phosphorylated insoluble aggregates (Dormann and Haass, 2011).

In recent years it has become clear that several ALS proteins such as TDP-43 can undergo so-called phase transitions, and this process has been suggested to contribute to both their biological function as well as their aggregation propensity in disease (Boeynaems et al., 2018). More specifically, TDP-43 and other ALS proteins converge on membrane-less organelles called stress granules (SGs). While these granules only form during times of cellular stress and retain highly dynamic liquid-like characteristics, it is believed that persistent SGs could potentially seed pathological aggregation of TDP-43 via a liquid-to-solid switch during SG maturation. Alternatively, TDP-43 could also directly transition from a diffuse to an aggregated state (Figure 1A). Notably, both paths have been observed in the test tube (Johnson et al., 2009; Wang et al., 2018), yet how this process is mediated in a cellular environment remains elusive.

In this issue of *Molecular Cell*, McGurk et al. (2018) investigate the mechanisms that lead to TDP-43 mislocalization and aggregation. To begin, they used a strategy to downregulate genes throughout the fly genome by RNA interference to screen for genes that modify toxicity associated with TDP-43 accumulation. They discovered that downregulating the *tankyrase* gene potently ameliorated neurodegeneration in their TDP-43 fly model. Conversely, upregulating the gene worsened the TDP-43-dependent degenerative phenotype. *Tankyrase* encodes the enzyme poly(ADP-ribose)-transferase, which attaches polymers of ADP-ribose to proteins. The addition of poly(ADP-ribose) (PAR) to proteins (called PARylation) plays a key role regulating various cellular pathways. As it does not seem like TDP-43 is itself PARylated, the authors next wondered whether TDP-43 could bind PAR. Using a series of biochemical assays, they found that TDP-43 can indeed bind PAR *in vitro* and *in vivo* through a region of its nuclear localization sequence (NLS). Interestingly, reducing tankyrase levels seems to correlate with increased nuclear TDP-43. Thus, it seems plausible that there may be competition between nuclear import factors and PARylated proteins for binding to TDP-43's NLS, but this remains to be tested experimentally.

To define how PAR affects TDP-43, the authors next investigated if PAR binding could affect TDP-43 liquid-liquid phase separation (LLPS). First, in the test tube, they found that TDP-43 LLPS depends on the presence of the PAR-binding domain, and phase separation is promoted by addition of PAR (Figure 1B). Interestingly, disease-associated C-terminal TDP-43 fragments that lack both the N-terminal domain (important for oligomerization; Wang et al., 2018) and the PAR-binding domain rapidly formed aggregates. They next extended their studies to mammalian cells and found that TDP-43 showed behavior strikingly similar to that in the test tube. Wild-type TDP-43 localizes to SGs, which contain PARylated proteins, upon arsenic stress (Figure 1B). Yet both a TDP-43 mutant unable to bind PAR and C-terminal TDP-43 fragments formed assemblies that did not colocalize with SGs (Figure 1C). Moreover, the latter TDP-43 assemblies were hyperphosphorylated, which is one of the defining hallmarks of ALS/FTD pathology. While TDP-43-containing SGs were reversible upon recovery from stress, the hyperphosphorylated aggregates remained stable. The authors observed a time-dependent reduction of TDP-43 dynamics within SGs, leading them to ask what happens if SGs mature under persistent stress? When they stressed cells for longer periods of time, remarkably, they observed that SGs dissolve, yet leave behind stable and hyperphosphorylated TDP-43 aggregates. Likewise, incubating cells with low levels of arsenite, insufficient for SG formation, also induced the formation of pathological TDP-43 assemblies. Collectively, these data suggest that SGs act as a sort of safe harbor that ushers in cytoplasmic TDP-43 under times of stress and prevents its pathological conversion. However, hiding out for too long in SGs can be detrimental, leading to pathological aggregation.

By using different stress regimens and an array of TDP-43 mutant constructs, McGurk et al. elegantly provide for the first time convincing evidence for at least two pathways leading to the formation of

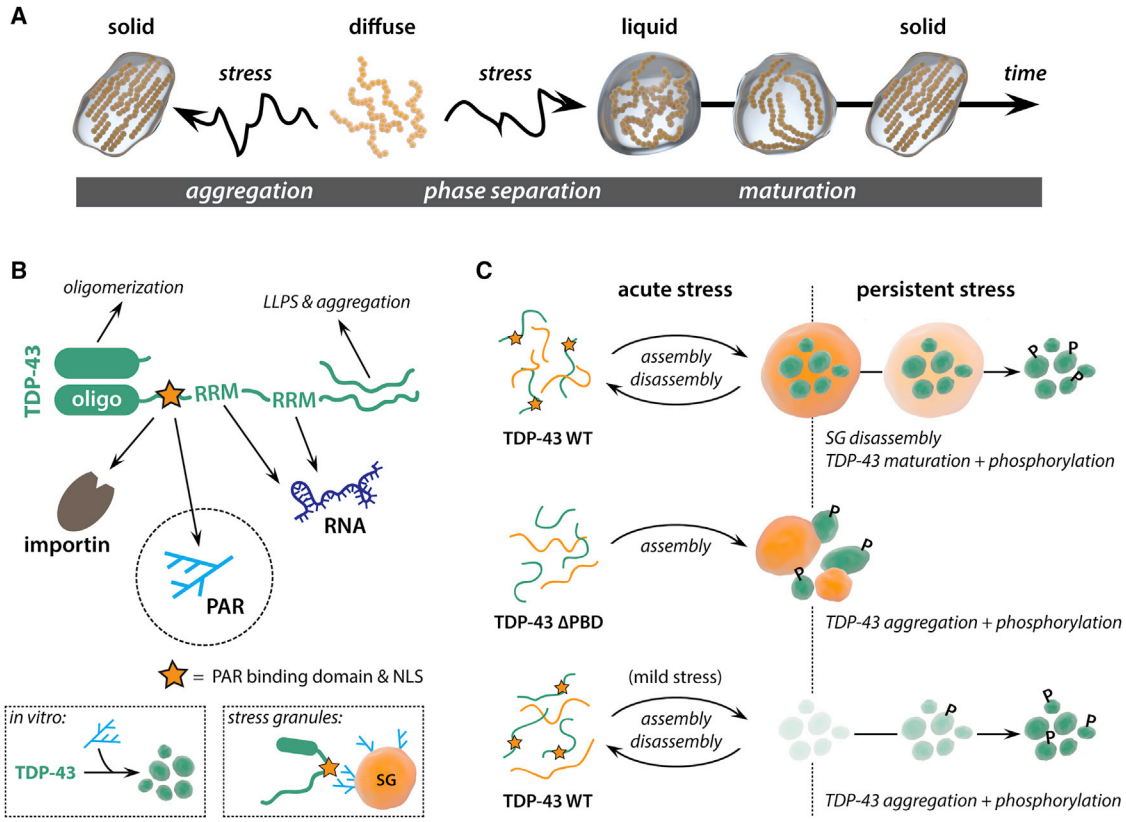

Figure 1. The Road to TDP-43 Aggregation

(A) Two non-mutually exclusive hypotheses can explain the origin of pathological TDP-43 aggregates. On one hand, TDP-43 can move from a dispersed state to a solid state due to misfolding and aggregation events. On the other hand, TDP-43 can exist in an initial liquid-like phase-separated state, which subsequently matures to the pathological solid state.

(B) Different TDP-43 domains play varying roles in regulating its phase behavior and aggregation propensity. This study identifies the TDP-43 NLS as a PAR-binding domain. PAR binding promotes TDP-43 LLPS in the test tube and recruits TDP-43 to PARylated stress granules in cells.

(C) TDP-43 pathological aggregates can arise via three different routes. First, TDP-43 can undergo a liquid-to-solid switch within persistent stress granules, which subsequently dissolve, leaving behind hyperphosphorylated TDP-43 aggregates. Second, defects in PAR binding of TDP-43 mutants or C-terminal cleavage fragments prevent its recruitment to stress granules, instantaneously pushing it toward the path of aggregation and hyperphosphorylation. Third, TDP-43 can aggregate independently from stress granule formation under conditions of mild chronic stress.

disease-relevant TDP-43 pathology in a cellular setting. First, upon cellular stress TDP-43 is recruited to SGs, preventing its hyperphosphorylation and allowing for its turnover after the stress dissipates. This finding shows that SGs may act early on to shield and protect aggregation-prone proteins and to promote their dynamic and reversible properties. This aggregation-buffering activity of SGs may stem from their association with (un)conventional chaperones (Alberti et al., 2017; Guo et al., 2018) and their high RNA content (Maharana et al., 2018). Is the role of binding PARylated proteins simply to help localize TDP-43 to SGs, or could this be involved in solubilization/aggregation buffering as well? Based on the authors' *in vitro* and *in vivo* data so far, it does not seem like PAR directly prevents TDP-43 from aggregating, but rather serves as a "homing signal" for TDP-43, directing it to SGs. It is intriguing to speculate that the kinase responsible for TDP-43 hyperphosphorylation may also be blocked from TDP-43 binding, once the latter is recruited to the condensed phase. Despite this apparent buffering activity of SGs, chronic stress allows TDP-43 to mature to an irreversible pathological state, which coincides with SG dissolution. Hence, persistent SGs may nonetheless provide a seed for TDP-43 aggregation. Second, when TDP-43 fails to be recruited to SGs, under conditions of mild chronic stress or in the case of disease-associated C-terminal fragments lacking the PAR-binding domain, the protein is immediately shunted to its pathological aggregated state.

Understanding which molecular events lead up to protein aggregation provides us key insights into the early steps of neurodegenerative diseases and presents an important therapeutic window to halt the pathological cascade. In a powerful translational extension of the work, the authors find that treating cells with tankyrase inhibitors, which have been developed as cancer therapeutics, can reduce cytoplasmic accumulation of TDP-43 in mammalian SGs, likely by inhibiting PARylation of SGs and allowing TDP-43 to shuttle back to the nucleus. This suggests that these compounds could be interesting therapeutic options for ALS/FTD, as they potentially prevent cytoplasmic sequestration and additionally promote nuclear import—preventing, in one fell swoop, the two key steps

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on the path to pathological TDP-43 aggregation.

DECLARATION OF INTERESTS

A.D.G. has been a consultant for Aquinnah Pharmaceuticals, Prevail Therapeutics, and Third Rock Ventures.

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Set Phasers to Cleave: PIWI Cleavage Directs All piRNA Biogenesis

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In this issue of *Molecular Cell*, Gainetdinov et al. (2018) show that PIWI proteins direct both piRNA biogenesis and piRNA function in most animals.

PIWI-interacting RNAs (piRNAs) have been monikered the "dark matter of the genome," and with reason. They are small RNAs that bind an Argonaute protein of the PIWI subclade (Cox et al., 1998), but everything else about them is quite distinct from other small regulatory RNAs such as microRNAs. piRNAs, along with PIWI proteins, are expressed in the germline of essentially all sexually reproducing animals. Many piRNAs target complementary retrotransposons for silencing via "classic" small-RNA-dependent Argonaute-directed cleavage. Their mysterious biogenesis drew a lot of attention right from the start: they were Dicer independent (Vagin et al., 2006) and many of them arose in an imprecise manner from genomic areas called piRNA clusters.

Studies over the past decade have revealed a complicated biogenesis route. One of the earliest observations was that piRNAs with complementary sequence overlap engage in an amplification loop called ping-pong that utilized PIWI cleavage (Brennecke et al., 2007) (Gunawardane et al., 2007). With notable prescience, Hannon and colleagues in 2007 proposed a model for piRNA production involving a single cleavage generating the 5′ end (preferably at a uridine), followed by incorporation into a PIWI, followed by 3′ end generation (Aravin et al., 2007). Studies over the next 10 years would validate and tweak this model and uncover enzymes responsible for piRNA processing, in addition to PIWI: the MITOPLD (Zuc in fruit flies) endonuclease; the 3′-5′ exonuclease PNLDC1 (Nibbler in fruit flies) that trims 3′ ends of piRNAs; and the MOV10L1 (Armitage in fruit flies) RNA helicase (Huang et al., 2017). Investigations of MOV10L1 revealed that endonucleolytic cuts of the long precursor transcript occur in a 5′-3′ direction to generate intermediate piRNA precursor fragments to be bound by PIWI proteins for processing (Vourekas et al., 2015). Working independently, the Zamore, Brennecke, and Pillai labs discovered that these cuts were successive and achieved through the so-called phasing (or inchworming) mechanism, which is the consecutive, tail-to-head generation of piRNAs by Zuc-dependent cleavage of long precursors (Han et al., 2015) (Mohn et al., 2015) (Homolka