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Poly(ADP-ribose) Engages the TDP-43 Nuclear-Localization Sequence to Regulate Granulo-Filamentous Aggregation

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Supporting Information

ABSTRACT: TAR DNA-binding protein of 43 kDa (TDP-43) forms granulo-filamentous aggregates in affected brain regions of >95% of patients with ALS and ∼50% of patients with frontotemporal degeneration (FTD). Furthermore, in disease, TDP-43 becomes N-terminally truncated resulting in protein deposits that are mainly composed of the C-terminal prion-like domain (PrLD). The PrLD is inherently aggregation-prone and is hypothesized to drive protein aggregation of TDP-43 in disease. Here, we establish that the N-terminal region of the protein is critical for rapid TDP-43 granulo-filamentous aggregation. We show that the biopolymer poly(ADP-ribose), or PAR, inhibits granulo-filamentous aggregation of TDP-43 by engaging PAR-binding motifs (PBMs) embedded in the TDP-43 nuclear-localization sequence. We demonstrate that progressive N-terminal truncation of TDP-43 can decelerate aggregation kinetics and promote formation of thread-like filaments. Thus, the N-terminal region and the PBMs of TDP-43 promote rapid granulo-filamentous aggregation and antagonize formation of thread-like fibrils. These findings illustrate the complexity of TDP-43 aggregation trajectories.

Amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration with ubiquitin-positive inclusions (FTLD-U) are two fatal neurodegenerative disorders characterized by the presence of insoluble aggregates of TAR DNA-binding protein of 43 kDa (TDP-43) in affected brain regions.[1,2] To date, most of the disease-causing mutations in TDP-43 occur in the C-terminal prion-like domain (PrLD).[3] PrLDs are intrinsically disordered regions that can switch from unfolded states to self-templating fibril forms such as the amyloid-like cross-β fibrils.[3−7] The majority of TDP-43 aggregates in ALS/FTLD-U patients have the appearance of granular filaments, but a subset have amyloid-like qualities.[8−11] Full-length TDP-43 forms granulo-filamentous aggregates *in vitro* that can transition into thread-like fibrils.[4,12,13] This transition is promoted by certain disease-linked mutations in the PrLD, including Q331K.[12] An emerging hypothesis is that the PrLD of TDP-43 may drive the protein aggregation observed in disease.[12]

PrLDs have also been implicated in liquid−liquid phase separation (LLPS), a process by which proteins condense into reversible liquid droplets.[14−16] Of interest are the ALS-linked proteins hnRNPA1, FUS, and TDP-43 which undergo LLPS *in vitro*.[4,16−20] We uncovered that the biopolymer poly(ADP-ribose) (PAR) potently promotes TDP-43 LLPS *in vitro*[20] and that PAR is elevated in ALS motor neuron nuclei.[21] PAR is generated by poly(ADP-ribose) polymerases (PARPs),[22] and inhibitors of various PARPs (PARP-1, PARP-2, PARP-5a, and PARP-5b) mitigate cytoplasmic aggregation of TDP-43 and TDP-43-associated toxicity to primary neurons and in *Drosophila*.[20,21] These findings raised the possibility that PAR may directly regulate TDP-43 aggregation.

To determine if PAR could impact TDP-43 aggregation, we purified full-length human TDP-43 with a His_6-SUMO solubility tag[23] (Figures S1A and S2A). At physiological concentrations of TDP-43 protein,[24] cleavage of the His_6-SUMO tag with ubiquitin-like specific protease (Ulp1) induced TDP-43 aggregation over a 200 min period (Figure 1A). The addition of PAR to His_6-SUMO-TDP-43-WT

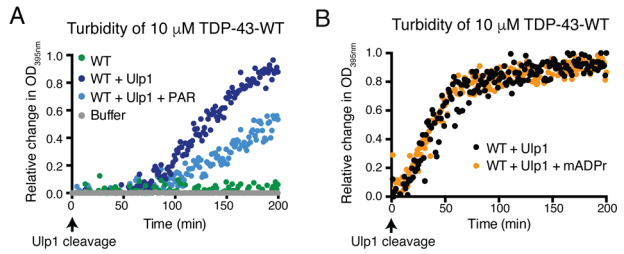

Figure 1. PAR inhibits TDP-43 aggregation. (A) Ulp1-cleavage of His_6-SUMO-TDP-43-WT increased optical density (OD). Co-incubation with 6 μM PAR reduced the optical density of TDP-43-WT. (B) Mono(ADP-ribose) (mADPr, 6 μM) had no effect on the optical density of TDP-43-WT.

significantly reduced TDP-43-WT aggregation (Figure 1A, Figure S2B,C), while mono(ADP-ribose) had no effect (Figure 1B). Our previous studies established that LLPS of TDP-43 can occur in the presence of a crowding reagent and is promoted by PAR.[20] We examined TDP-43-WT by differential interference contrast (DIC) microscopy; before cleavage with and without PAR, the protein remained diffuse and did not form any visible micron-sized aggregates (Figure S3A). However, 30 min after Ulp-1 cleavage, we observed the

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formation of spherical droplets that appeared to coalesce into solid structures after a further 30 min (Figure S3B). Our present data indicate that under conditions that lack a crowding reagent, PAR reduces filamentous aggregation of TDP-43.

The nuclear-localization sequence (NLS) of TDP-43 is a region of intrinsic disorder[25] (Figure S1B) and is critical for physically binding to PAR and as well as LLPS of TDP-43 *in vitro*.[20] In contrast to cleaved His_6-SUMO-TDP-43-WT, cleaved His_6-SUMO-TDP-43-ΔPAR-binding motif (PBM) (Figure S4A) exhibited decelerated aggregation kinetics (Figure 2A) and took over 18 h to aggregate (Figure 2B).

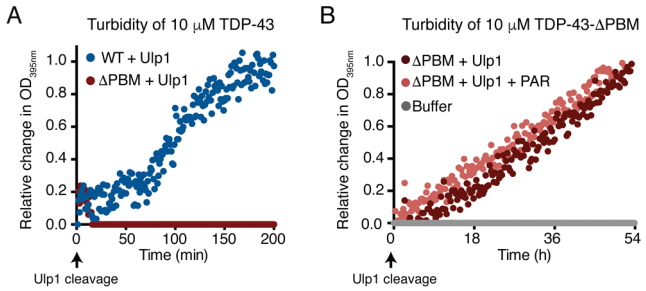

Figure 2. PAR-binding motifs enable rapid TDP-43 aggregation. (A) Compared to TDP-43-WT, the TDP-43-ΔPAR-binding motif (PBM) did not aggregate in the same time frame. (B) TDP-43-ΔPBM aggregated over 54 h. PAR (6 μM) had no effect on the optical density of TDP-43-ΔPBM.

The addition of PAR had no effect on the aggregation of TDP-43-ΔPBM (Figure 2B and Figure S4B). Examination of TDP-43-ΔPBM before cleavage revealed no preformed micron-sized aggregates (Figure S3A). Thus, the N-terminal region of TDP-43, and specifically the PBMs, enables rapid aggregation of TDP-43, and PAR engages PBMs within the NLS to reduce TDP-43 aggregation.

Transmission electron microscopy (TEM) revealed that cleavage of the His_6-SUMO tag from both TDP-43-WT and TDP-43-ΔPBM led to the formation of granulo-filamentous aggregates (Figure 3A), consistent with previous TEM studies and of TDP-43 aggregates in human tissue.[8,10,12] PAR did not drastically alter the structure of the TDP-43-WT or TDP-43-ΔPBM aggregates (Figure 3A). However, PAR significantly reduced the overall size of the TDP-43-WT aggregates, while having no effect on the size of the TDP-43-ΔPBM aggregates (Figure 3B). Indeed, PAR promoted retention of TDP-43-WT in the supernatant fraction after low-speed centrifugation (Figure 3C and Figure S5). Thus, we propose that PAR reduces granulo-filamentous aggregation of TDP-43 via an interaction with PBMs embedded within the NLS.

In ALS and FTLD-U, splicing defects and proteolytic cleavage can elicit formation of TDP-43 C-terminal fragments that contain the PrLD.[26−28] As the C-terminal fragments of TDP-43 either contain a partial PAR-binding region (TDP-43-C35) or lack the PAR-binding region (TDP-43-C25) (Figure S1A), we examined the aggregation kinetics of these two C-terminal fragments. Strikingly, the ability of TDP-43-C35 and TDP-43-C25 to form turbid aggregates was, like TDP-43-ΔPBM, reduced compared to TDP-43-WT (Figure 4A). Examination by TEM revealed that TDP-43-C35 formed granulo-filamentous aggregates, whereas TDP-43-C25 formed granulo-filamentous aggregates and thread-like fibrils (Figure 4B). The TDP-43-C25 aggregates were unreactive to the

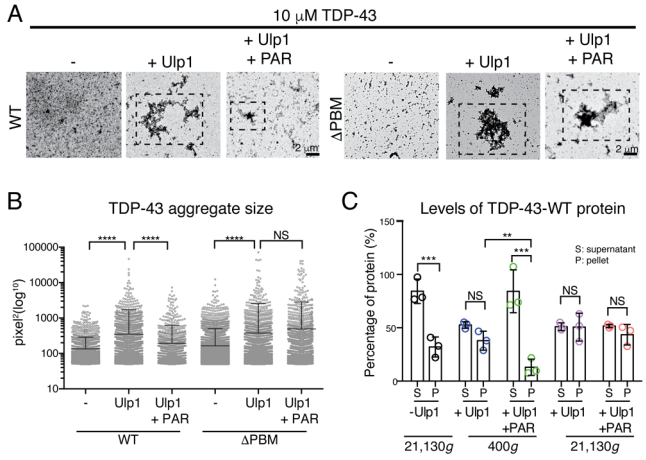

Figure 3. PAR reduces TDP-43 aggregation. (A) Ulp1 cleavage of HIS_6-SUMO-TDP-43-WT and HIS_6-SUMO-TDP-43-ΔPBM led to granulo-filamentous aggregation (hatched boxes). PAR (6 μM) reduced aggregate size of TDP-43-WT and had no effect on TDP-43-ΔPBM (hatched boxes). (B) Quantification of aggregate size. Mean (±SD), one-way ANOVA ($P < 0.0001$), and Kruskal−Wallis test. (C) PAR (6 μM) reduced the amount of TDP-43-WT in the pellet fraction at 400*g* (Figure S5). Mean (±SD), two-way ANOVA, and Tukey's test.

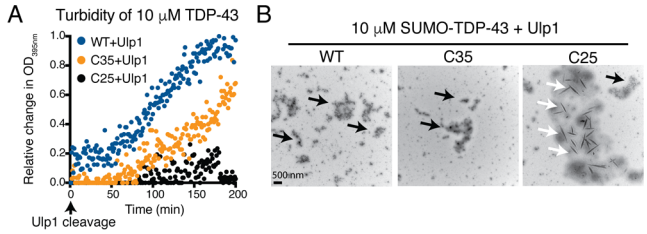

Figure 4. C-terminal fragments of TDP-43 have altered aggregation properties. (A) The increase in optical density of TDP-43-C35 and TDP-43-C25 was reduced compared to TDP-43-WT. (B) TDP-43-WT, TDP-43-C35, and TDP-43-C25 formed granulo-filamentous protein aggregates (black arrows). TDP-43-C25 also formed thread-like aggregates (white arrows).

amyloid diagnostic dye Thioflavin T (Figure S6). Combined, these data reveal that the N-terminal portion of TDP-43 contributes to granulo-filamentous aggregation and antagonizes the transition into thread-like oligomers.

Here, we show that N-terminal portions of TDP-43 contribute to granulo-filamentous aggregation. Our data indicate that PAR interacts with PBMs embedded within the NLS of TDP-43 to reduce granulo-filamentous aggregation. Defining the mechanism by which PAR binding reduces TDP-43 aggregation will require further study. Regions within the N-terminal domain of TDP-43 regulate self-oligomerization.[25,29−32] Thus, PAR-binding to the NLS adjacent to the N-terminal domain may physically block interactions that contribute toward aggregation. In disease, TDP-43 aggregates appear to be predominantly granulo-filamentous. Thus, agents that antagonize contributions from the N-terminal region of TDP-43 could have therapeutic utility. However, as oligomerization is essential for TDP-43 function,[25,29−32] agents that prevent this functional oligomerization could be detrimental. Understanding under what circumstances functional versus toxic TDP-43 assemblies form,[33] how they differ, and how they

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are resolved will help develop therapeutic strategies to selectively target toxic assemblies.

■ ASSOCIATED CONTENT

Supporting Information

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TDP-43 protein domains, protein purification analysis, turbidity assay, analysis of the sedimentation assay, and detailed materials and methods (PDF)

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Notes

The authors declare no competing financial interest.

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