

Biophysical methodologies used to assess the aggregation, phosphorylation, & antibody inhibition mechanisms of ALS-associated protein, TDP-43

Good Health & Well-Being

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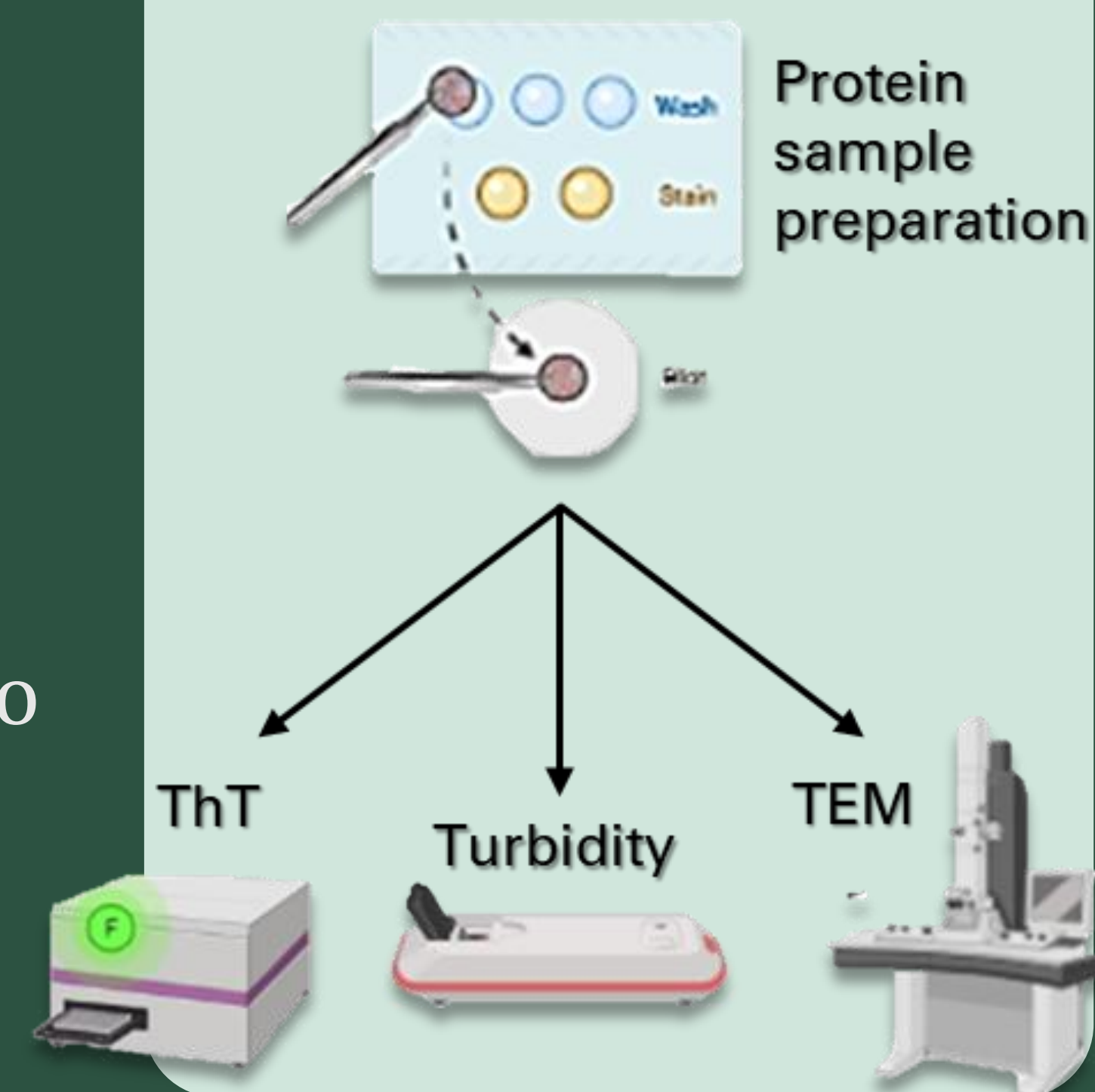
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Introduction / Methods

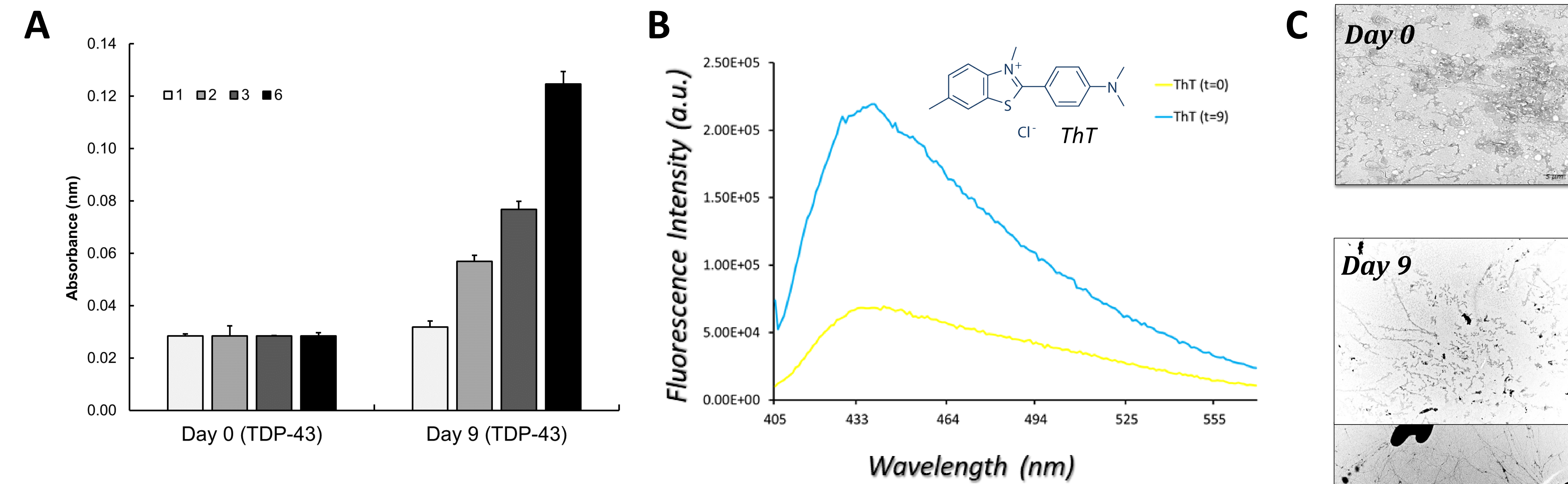
- The onset/progression of neurodegeneration (ALS, dementias) linked to TDP-43 proteinopathies
- Hyperphosphorylation and aggregation: Main contributors to the pathology of some neurodegenerative diseases
- Targeted antibodies for *in vitro* TDP-43 to determine effectiveness of inhibition studies

Methodology:

- Aggregation:** Induced by agitation/stress
- Phosphorylation:** Induced by protein kinases



Results



Turbidity (A):

- Full-length, phosphorylated TDP-43 (1 μ M)
- Basic turbidity measurements were recorded as a function of time (395nm, 9 days total) to measure aggregation

ThT (B):

- Fluorescence emission intensity of ThT at λ_{Em} = 495 nm as a function of time (days 0 and 9)
- Controls contain only buffer or ThT dye

TEM Analysis (C):

- TEM images of phosphorylated TDP-43 protein after 0 and 9 days
- Distinct and dense fibrils formed after 9 days of incubation

Conclusions

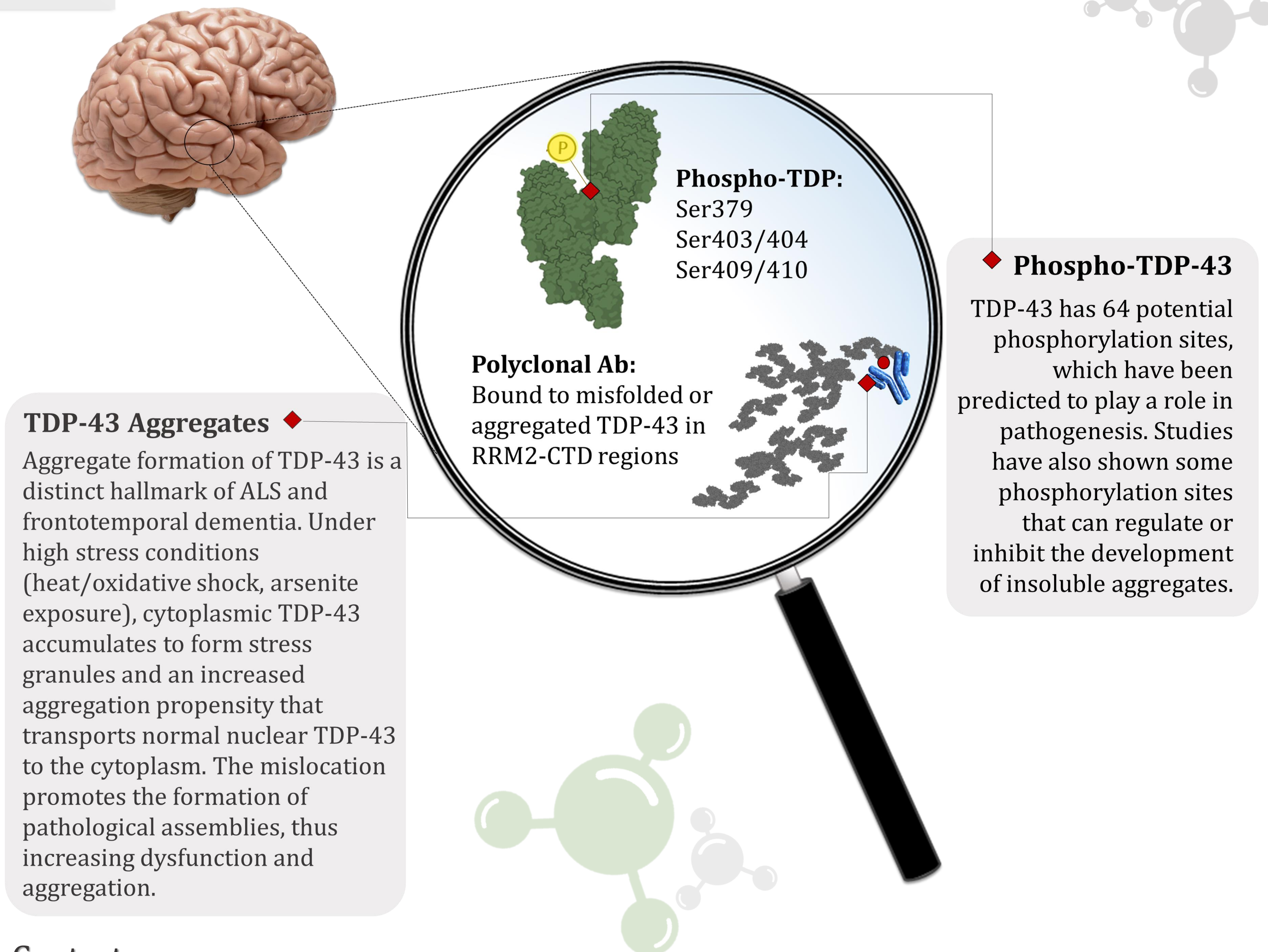
- Novel aggregation mechanisms were discovered in TDP-43, further supporting the aggregation prone nature of the protein.
- Future structural analyses required to determine the secondary structures of each amino acid domain within the protein (i.e., distinguishing between α -helices and β -sheet networks).
- This preliminary data encourage the future of TDP-43 proteinopathies and its role on neurodegeneration.

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