TDP-43 LIQUID-LIQUID PHASE SEPARATION AND PHOSPHORYLATION STUDY

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TAR DNA-binding protein 43 (TDP-43) is a multifunctional protein primarily localizing in the cell nucleus, where it binds to RNA and DNA [1]. It consists of 414 amino acids and is composed of four domains: N-terminal domain, two RNA-binding domains (RRM1 and RRM2), and low-complexity prion-like C-terminal domain [2]. RRM domains are flanked by nuclear export and import sequences, thereby enabling protein shuttling between the nucleus and cytoplasm [3]. In both the nucleus and cytoplasm, TDP-43 can form condensates through liquid-liquid phase separation (LLPS) – a biophysical process involved in the formation of membraneless organelles [4]. Due to LLPS, protein cleavage, mutations, and posttranslational modifications, such as hyperphosphorylation, TDP-43 can aggregate and form insoluble neurotoxic species, as well as disrupting downstream cellular processes [5]. TDP-43 aggregates are the hallmarks of several neurodegenerative diseases: amyotrophic lateral sclerosis and frontotemporal lobar degeneration [2]. However, latest studies have shown that phosphorylation of TDP-43 can greatly suppress the protein's propensity to undergo LLPS and slow TDP-43 aggregation into amyloid fibrils [6]. Therefore, we aim to identify key kinases implicated in neurodegeneration that can phosphorylate TDP-43 and elucidate their impact on its LLPS and aggregation.

In this study, we initially optimized the purification of full-length TDP-43. This protein is prone to degradation and forms aggregates after cleavage of the purification tag, making it difficult to purify. For LLPS studies, TPD-43 with mCherry fluorescent protein tag was purified and later used for fluorescent microscopy imaging. The kinetics of LLPS were analyzed by measuring the sample's turbidity with or without molecular crowder polyethylene glycol (PEG). For the phosphorylation assay, four different kinases were used: CK1α, PKA, PKR, and GSK3β. The phosphorylation effect was evaluated with SDS-PAGE electrophoresis.

The results demonstrate that TDP-43 is more prone to form condensates in the presence of molecular crowder. Higher PEG concentration resulted in more aggregates. For phosphorylation studies, we suggest that CK1α and PKR kinases were the most effective. However, we observed aggregates in almost all kinase treated TDP-43 samples, with fewer aggregates found in sample treated with CK1α. To further investigate these processes, we will optimize LLPS conditions and perform a more comprehensive analysis of kinase efficiencies and aggregate species.

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