Title: “LLPS in functional amyloids may unveil novel therapeutic targets for neurodegenerative diseases”

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Abstract:

Liquid-liquid phase separation (LLPS) is a protein condensation process driven by multivalent interactions that promotes the assembly of proteins into membraneless biocondensates. An increasing number of amyloid-forming proteins have been recently found to undergo LLPS, emerging as a likely element in amyloid assembly regulation. In pathological amyloids, LLPS is an initial step of the toxic pathway as they mature into an irreversible aggregated state. However, functional amyloids phase separate to control their physiological functions. According to that, analyzing the differences between functional and pathological LLPS may open new alternative therapies and the discovery of new therapeutic targets. Here, we have explored the sequence-driven molecular determinants behind the functional aggregation of human CPEB3 (hCPEB3), an RNA-binding protein key for memory persistence that functionally switches between a phase separated repressor and an aggregated activator state.

We found that the intrinsically disordered region (IDR) of hCPEB3 encodes both an amyloidogenic and a phase separation domain, separated by a connecting poly-A-rich region. The hCPEB3 amyloid core is composed by a hydrophobic region, instead of the Q-rich stretch found in *Drosophila*. The hCPEB3 phase separation domain relies on hydrophobic interactions with ionic strength dependence, and its droplet ageing process leads to a liquid-to-solid transition with the formation of a non-fibril-based hydrogel surrounded by starburst droplets. Furthermore, we describe a regulatory mechanism between both processes, by which LLPS compartmentalizes hCPEB3 amyloid assembly within clustered droplets, regulating its aggregation in space and time in a condensation-based pathway. This mechanism provides a therapeutic window to develop anti-amyloidogenic compounds based on droplet stability and LLPS regulation.