CURE-ALS
Mechanisms regulating RNP granule functionality, dynamics and disease association

Liquid-liquid phase separation can create functionally distinct membraneless compartments consisting of proteins and RNAs, which have major roles in cellular organization and physiology. RNA-binding proteins (RBPs) with domains of low sequence complexity (LC) are the key players that mediate the process of phase separation in cells. Recent data suggests that aberrant phase transitions of these proteins may be closely tied or even causative to the pathogenesis associated with diseases such as amyotrophic lateral sclerosis.

Thus, understanding how physiological phase transitions give rise to dysfunctional RNPs and eventually pathological RBP-containing aggregates will be key to understand a range of neurodegenerative diseases. We studied several human LC domain-containing RBPs that are associated with age-related neurodegenerative diseases. We show that *in vivo* these proteins phase separate to form liquid, membraneless compartments. Using an *in vitro* “aging” assay, we demonstrate that reconstituted RNP granules have a strong drive to convert from a liquid to an aggregated, pathological state. We identify and discuss several different mechanisms that regulate the conversion into pathological aggregates.

In summary, we have gained important insights into the molecular rules and principles underlying the formation of RNP granules by the process of phase separation. Most importantly, we have revealed a molecular grammar that determines the driving forces for phase separation and the forces and amino acids that govern a transition from a liquid to a solid disease state. These findings provide us with important knowledge to understand the mechanisms underlying disease-associated mutations in RNA-binding proteins. Our results also suggest that we will soon be able to control phase separation with targeted genetic and chemical approaches.