ABSTRACT

INVESTIGATING INTRACELLULAR TRANSTHYRETIN AGGREGATION AND ITS CELLULAR REGULATION

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Marquette University, 2026

Many age-related diseases are associated with protein aggregates. These protein aggregates can range from relatively unstructured aggregates to highly ordered amyloid structures, and their accumulation within cells is often detrimental. Transthyretin amyloidosis (ATTR) is a fatal systemic disease associated with extracellular amyloid deposits formed by the transthyretin (TTR) protein. Similar to other age-related diseases, extracellular TTR aggregates are internalized into cells, as evidenced by studies in cell lines, mouse models, and patient samples. However, intracellular TTR is understudied and the cellular mechanisms that regulate TTR inside cells is unknown. To address these gaps, this dissertation uses *Saccharomyces cerevisiae*, or baker's yeast, as a genetically tractable system to study intracellular TTR aggregation and its regulation. My work shows that TTR expression in yeast results in aggregate species that lack the canonical detergent resistance typically associated with amyloid, suggesting that intracellular TTR can form unstructured, non-amyloid aggregates. Additionally, the biochemical profile of TTR in yeast is similar to TTR isolated from patient plasma, suggesting TTR may have the innate ability to form these unstructured, non-amyloid aggregates. Using biochemical and microscopy approaches, I also investigated how several molecular chaperones influence intracellular TTR. Here, I show that the Hsp70 molecular chaperones limit the population of high molecular weight TTR, raising the possibility that Hsp70 may suppress TTR aggregation. Lastly, I provide evidence that TTR is a stress responsive protein that exhibits liquid-liquid phase separation qualities, similar to other disease-associated aggregating proteins in response to cellular stress. Overall, this dissertation provides evidence that TTR forms unstructured, stress-responsive aggregates inside cells, which is in contrast to the canonical extracellular amyloid structure found in patients. Furthermore, Hsp70 may be a critical cellular factor that limits this intracellular TTR aggregation. Overall, I propose that the age-related decline of Hsp70 levels may enable unstructured, stress-responsive intracellular TTR aggregates in ATTR patients. Taken together, this work advances our understanding of TTR aggregation beyond its canonical extracellular amyloid state and provides insight into potential cellular regulators that could be targeted to suppress TTR aggregation in disease.