ABSTRACT

INVESTIGATING THE ROLE OF CHAPERONES AND SEQUESTRASES IN MISFOLDED PROTEIN SEQUESTRATION

Sarah Rolli, B.S.

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Protein homeostasis, or proteostasis, is responsible for a folded and functional proteome and is essential for cell survival. Proteins can misfold during stress or because of mutation. The accumulation of misfolded proteins is observed in many diseases, including Huntington's disease. Misfolded proteins will clump together into inclusions, which can be sequestered to membrane-less compartments by proteostasis mechanisms. Several proteins, including chaperones and sequestrases, have been identified as "sorting factors" that target inclusions and misfolded proteins to different protein quality control (PQC) compartments. The juxtanuclear quality control (JUNQ) compartment forms in the nucleus-vacuole junction in yeast cells, and several soluble model misfolded proteins are sequestered to the JUNQ. The insoluble protein deposit (IPOD) forms in the periphery of yeast cells and is where insoluble proteins can be sequestered. The role of sorting factors in sorting between the JUNQ and IPOD and how disease-associated misfolded proteins are sequestered is still unclear. We used two model misfolded proteins to study how the sorting of soluble misfolded proteins to the JUNQ or IPOD occurs in yeast. We found that Hsp70s Ssa1 and Ssa2, Sti1, Btn2, and Hsp42 are critical for sorting soluble temperature-sensitive or terminally misfolded proteins. We confirmed that Sti1's sorting factor function is dependent on its co-chaperone function with Hsp70, and we propose that Sti1-Hsp70 recognize misfolded proteins upon misfolding to target them to PQC compartments or for degradation. We then investigated how a disease-associated protein is sorted to PQC compartments. We used mutant huntingtin exon 1 protein (mHTT), implicated in Huntington's disease, as it is not inherently toxic to yeast. Using a toxic and a non-toxic version of mHTT, we determined that the non-toxic construct is sorted to the IPOD as previously shown, but it can localize to the JUNQ in a Sis1, Hsp90, and Btn2-dependent mechanism. Toxic mHTT does not get sorted to a PQC compartment, suggesting that toxic mHTT may trigger proteostatic collapse and prevent sequestration. Overall, we find that sorting factors for the JUNQ recognize different types of misfolded proteins, and Sti1-Hsp70 are likely the sorting switch that allows for differential sorting to PQC compartments.