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Substrate specificity of Hsp104 chaperone protein from *Saccharomyces cerevisiae.*

Proteins serve manifold functions in every cell, such as catalysis, transport, signaling and regulation. After synthesis in linear form polypeptides fold into three dimensional structure which is essential for protein function. Complex network of chaperone proteins assist folding in ATP-dependent process. Heat, free radicals and other cellular stresses affect proper folding and influence structure of already folded proteins causing partial unfolding and aggregation. Protein aggregates are also believed to accumulate as a natural cause of cell aging. In yeast cells cooperation of two groups of chaperones, namely Hsp70 and Hsp100 is crucial for protection against protein unfolding and aggregation. Ssa1 chaperone, Hsp70 family member, in assistance of Hsp40 family members recognizes protein aggregates and recruits Hsp104 chaperone, which is responsible for disaggregation by disentangling single polypeptides from aggregate. In the next step polypeptides are either folded into native structure or degraded. Cooperation of Hsp70 and Hsp104 is also essential for metabolism of prion amyloids and it determines their inheritance in yeast population. Growing body of evidence suggests that Hsp104 serves functions unrelated to aggregates derived from thermally denaturation of proteins and prions. In this study I switched activity of Hsp104 from disaggregation to proteolysis and analyzed changes in yeast proteome using mass spectrometry to identify substrates of Hsp104 protein. This experimental approach revealed that substrates of Hsp104 are functionally related to Krebs cycle, glycogen metabolism, glycolysis and ATP synthesis. Due to high exposure to reactive oxygen species these proteins undergo carbonylation which leads to their aggregation. Hsp104 binds aggregated, carbonylated proteins what can lead to their import into mitochondria and ongoing degradation. Obtained data suggest that Hsp104 serves as one of the factors protecting yeast cells against accumulation of carbonylated proteins, a hallmark of cell aging.