Heterologous Expression Studies of a Hypothetical Human Protein in Yeast Reveal a Potential Metazoan Disaggregase


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INTRODUCTION: All living organisms contain an elaborate system of protein chaperones that regulates and maintains protein homeostasis in the cell. When this system is disrupted, proteins can lose their native structure and form toxic aggregates, which have been implicated in more than 40 human pathologies. To eliminate aggregates, many organisms, such as plants, fungi, bacteria and protists, have a clear protein disaggregase. Inexplicably, no such disaggregase has yet been identified in metazoans. However, genomic analysis of several metazoan species has revealed the presence of a possible disaggregase, as judged by its high conservation and prevalence across species, and similarity with bona fide disaggregases. MATERIALS AND METHODS: We cloned several variants of the human ortholog of the candidate disaggregase into yeast expression vectors and screened for function by complementation in yeast knockouts of hsp104 and trk1. In addition, we performed in vivo protein aggregation tests by expressing the potential disaggregase in the presence of the yeast [PSI+] and [RNQ+] prions, and analyzed the results by fluorescence microscopy, SDD-AGE, filter trap, and growth assays, as appropriate. RESULTS AND DISCUSSION: In our yeast complementation assays of hsp104 and trk1, we observed an increase in thermotolerance and growth rate, respectively, when expressing different N-terminal truncations of the potential disaggregase, but not the wild-type protein. This effect can be explained by the fact that this region contains a mitochondrial targeting signal, and its deletion changes the subcellular localization of the protein from the mitochondria to the cytosol. We also observed suppression of the yeast prion [PSI+] by high overexpression of the candidate protein, whereas moderate overexpression of all truncations, but not the complete protein, with the prion forming protein, Rnq1, actually increased the amount of RNQ polymers. This effect was correlated with a dramatic change in the subcellular localization of [RNQ+], even in the presence of expressing the full-length version of the potential disaggregase. CONCLUSIONS: Our results demonstrate that overexpression of this protein in yeast increases thermotolerance and can complement a potassium transport defect. In addition, it may be acting as a disaggregase against the yeast [PSI+] and [RNQ+] prions, supporting its role as a metazoan disaggregase.

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