2014

Molecular Chaperone Tools for Use Against Neurodegenerative Diseases

Matthew Tinkham

University of Rhode Island, matthew_tinkham@my.uri.edu

Creative Commons License

This work is licensed under a Creative Commons Attribution-No Derivative Works 4.0 License.

Follow this and additional works at: http://digitalcommons.uri.edu/srhonorsprog

Part of the Amino Acids, Peptides, and Proteins Commons, Biochemistry Commons, Cell Biology Commons, Investigative Techniques Commons, and the Molecular Biology Commons

Recommended Citation


http://digitalcommons.uri.edu/srhonorsprog/357

This Article is brought to you for free and open access by the Honors Program at the University of Rhode Island at DigitalCommons@URI. It has been accepted for inclusion in Senior Honors Projects by an authorized administrator of DigitalCommons@URI. For more information, please contact digitalcommons@etal.uri.edu.
1. Abstract
A noted characteristic found in several neurodegenerative disorders, including Alzheimer’s Disease, Huntington’s Disease, and Parkinson’s Disease, is the formation of amyloids. Amyloids are composed of misfolded proteins that aggregate into insoluble fibers. The accumulation of amyloid plaques in the brain is associated with neuronal deterioration and loss of function. Understanding the molecular mechanisms behind amyloid formation is crucial for the development of therapeutic strategies to combat diseases associated with amyloid deposits. In this study, we explore the potential of molecular chaperone tools to mitigate amyloid formation in yeast via proteolysis. We focused on the Sup35 protein, a translation termination factor, as a model system to study amyloid formation and clearance. The Sup35 prion phenotype in yeast is associated with the accumulation of amyloid deposits, which is linked to the formation of fibrillar aggregates. By examining the role of molecular chaperones, we aimed to identify novel chaperone-protease complexes that can enhance amyloid clearance in yeast. Our results suggest that the combination of molecular chaperones and proteases offers significant potential for the treatment of amyloid aggregation in neurodegenerative diseases.

2. The [PSI+] prion is a self-replicating amyloidogenic protein that forms a fiber
The Sup35 protein in yeast can aggregate into amyloid fibers.
- sup35 is a gene that encodes a translation termination factor, which is responsible for release of nascent proteins.
- Sup35 misfolds and forms amyloid in vivo and in vitro.
- Amyloids are formed from the conversion of normal proteins into misfolded prions, which promote amyloid deposits.
- The presence of the Sup35+[psi+] conformation is referred to as the [PSI+] prion phenotype.
- The [PSI+] phenotype is associated with the formation of intracellular amyloid deposits. (Kohnen and Derkx, Journal of Biological Chemistry, 1995).
- The [PSI+] phenotype is a useful tool for yeast genetic studies, allowing for the generation of amyloidogenic prions.

3. Hsp104 is an ATP-dependent chaperone protein that disassembles amyloid fibers
Hsp104 hexamer side view
- Hsp104 hexamer side view
- Hsp104, a member of the Hsp104 family of proteins, is a hexameric A/R ATPase found in Saccharomyces cerevisiae.
- Hsp104 disassembles, refolds, and detoxifies misfolded and aggregated proteins.
- Aggregated proteins are released by threading through the channel of Hsp104.
- This disassembly activity is accomplished in conjunction with other Hsp104 family members, including Hsp90 and Hsp70.

4. Bacterial chaperone proteases from E. coli
A) ClpXP is an ATP-dependent protease
- ClpXP is composed of two subunits: ClpP and ClpX.
- ClpP is an ATP-dependent molecular chaperone with ubiquitin activity.
- ClpX recognizes and transfers substrates into the chamber of the bound protease ClpP.
- ClpP-degrades proteins in the chamber and releases small peptides.
- ClpXP recognizes and degrades substrates with specific recognition signals.

5. Goals of Project
a) Express bacterial chaperone proteases in yeast
b) Test if bacterial chaperone proteases can cure the [PSI+] prion phenotype by degrading amyloid fibers
c) Generate ClpXP and Lon mutant proteins with enhanced amyloid recognition

6. Amplification and Cloning of Lon protease and ClpXP
- To express the bacterial chaperone proteases in yeast, we isolated genomic DNA from E. coli.
- We amplified chaperone and Lon gene sequences by polymerase chain reaction (PCR).
- The desired restriction sites were added to the amplified DNA sequences to introduce the gene into the yeast genome.
- The amplification was confirmed via gel electrophoresis.

7. ClpXP and Lon genes were cloned into yeast expression vector pYES2
A) clpXP genes were inserted between HindIII & BamHI
B) lon gene was inserted between EcoRI & XbaI
- Two restriction enzyme sites were added to xfp3I, and xfp3II was added at the start of the gene.
- These restriction sites matched up with sites for digestive and ligation reactions to insert the gene into the plasmid.

8. Transformation of bacterial genes into yeast
- Yeast was transformed with plasmids containing bacterial genes via electroporation.
- Yeast parental strains are both unable to synthesize uracil. The pYES2 vector contains the gene required for uracil synthesis.
- The absence of uracil in the growth media serves as a selective marker for the cells to maintain the pYES2 plasmid.

9. Visualizing the [PSI+] Phenotype
- The red phenotype is caused by termination of the adenine biosynthesis gene ade2, which results in an accumulation of red pigment.
- The presence of [PSI+] allows for a read-through of the nonsense codon in the ade2 allele. This prevents the accumulation of red pigment, which is a product of adenine biosynthesis, resulting in a red phenotype.
- Cleavage of Sup35 occurs when amyloid deposits are removed, allowing native Sup35 to function in translation termination. This is detected by a color conversion from white to red colonies.

10. Test if expression of chaperones in yeast cures [PSI+]
- [PSI+] cells expressing Hsp104 appear red, indicating that Hsp104 overexpression cures the [PSI+] phenotype.
- [PSI+] expressing ClpXP appear white, similar to the vector control, suggesting that this strain is not cured of [PSI].
- These results suggest that ClpXP does not recognize and degrade the [PSI+] amyloid.

11. Conclusions and Future Studies
- Expression of Hsp104 from a plasmid cures [PSI+] in yeast, via disassembly of amyloid fibers
- ClpXP expression is not sufficient to cure [PSI+] in yeast

Future Work:
- Perform random mutagenesis of clpXP to generate mutants with enhanced recognition of [PSI].
- Screen random mutants for enhanced prion curing by monitoring for a color change from white to red.
- Express Lon in [PSI+] and [psi-] yeast strains to determine if Lon degrades amyloids, then perform random mutagenesis of Lon to generate and screen mutants with enhanced recognition of [PSI].

12. Acknowledgements
This research was supported by the Undergraduate Research Initiative at the University of Rhode Island.

Matthew Tinkham1, and Jodi L. Camberg1
1Department of Cell and Molecular Biology, University of Rhode Island, Kingston, RI