Mapping the β-Sheet Structure of the Yeast Prion Sup35 through Creation of Targeted Mutant Forms

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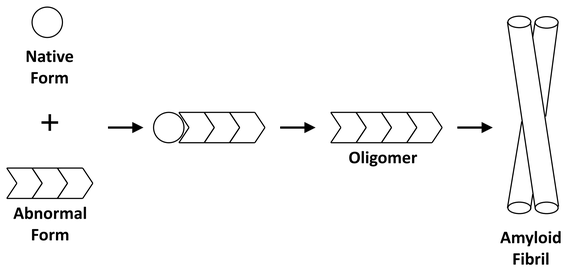
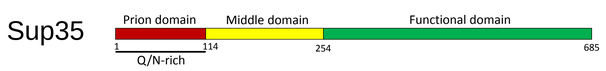
BACKGROUND

Proteins are made based on a DNA sequence template and fold into distinctive shapes or conformations. **Amyloids** are proteins that can fold into a normal form and an abnormal form. **Prions** are infectious amyloids that can further cause normally-folded versions to assume the amyloid shape. Prions are present in many eukaryotes including humans, cows, sheep, and the baker's yeast, *Saccharomyces cerevisiae*, among other species. Yeast has several prion proteins including Ure3 and Sup35, the focus of this study. **Yeast prions** are usually Q and N rich and like all amyloids, are composed of β-sheets. It has been long appreciated that the Sup35 protein in yeast has substantial β-sheet structure in its N terminal "prion domain" when in its amyloid form. However, the exact dimensions or interactions of the β-strands within the β-sheets are poorly understood. Furthermore, there are few hydrophobic and charged amino acids residues in this region. In this study we are testing the effect of adding one lysine amino acid (a charged amino acid) at several sites in Sup35 on the likelihood of prion formation and stability, with the goal of helping to map β-sheet formation in the *S. cerevisiae* Sup35 prion protein.

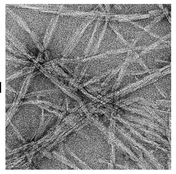
PRIONS ARE INFECTIOUS AMYLOIDS



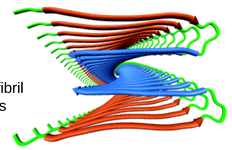
YEAST PRIONS



Electron micrograph of Sup35 amyloid fibrils



Model of Sup35 amyloid fibril structures



E.D. Ross, *et al.*, (2004) *Mol. Cell. Biol.*, 24: 7206-13

R. Tycko (2004) *Curr. Opin. in Struct. Biol.*, 14: 96-103

FURTHER READING

MacLea, K.S. and Ross, E.D. Strategies for identifying new prions in yeast. *Prion*, 5(4):1-6, 2011.

MATERIALS AND METHODS

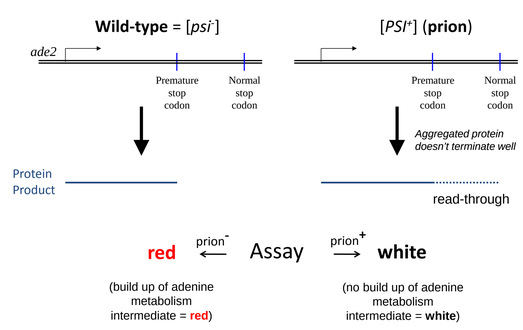
HOW DO WE DETECT PRIONS?

Sup35 **recognizes stop codons**

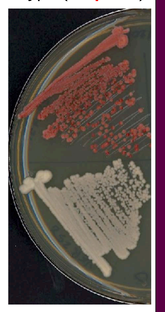
Sup35 in prion form (*[PSI⁺]*) is **non-functional**

Without functional Sup35, stop codons are sometimes **"read through"**

Read through stop codon = **full-length Ade2 protein**



Wild-type (**no prion**)



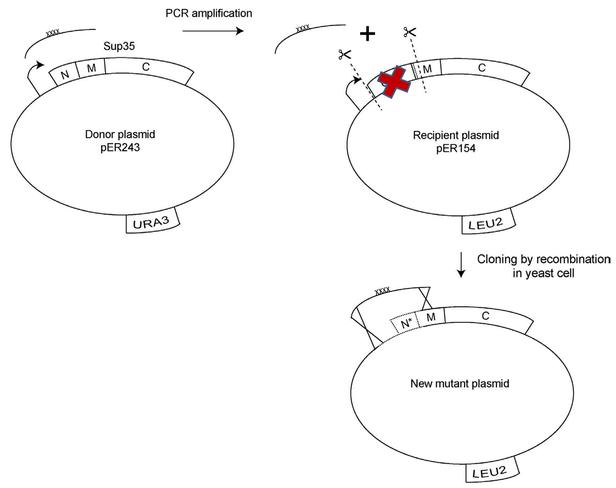
Sup35 **prion** form

CREATING MUTANT VERSIONS OF THE *SUP35* PRION GENE

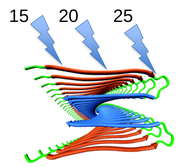
To study effects of mutation on β-sheet formation in the Sup35 protein, experiments were conducted in a strain of yeast expressing a "super-prion-forming" *SUP35* gene.

This *SUP35* variant was subjected to 3 additional point mutations within the prion forming domain, adding the charged amino acid Lysine (K) at three distinct locations.

PCR-based mutagenesis was employed to generate a full-length "2-piece" PCR product encoding mutated versions of the prion domain of *SUP35*.



These mutant PCR products were transformed into a prion-negative yeast strain and colonies were selected on the basis of nutritional markers (*LEU2*). PCR was employed to amplify the *SUP35* gene from each clone, followed by DNA sequencing by Genewiz, Inc. (New Jersey).



Introduced mutations were designed to hit at different points (residue 15, 20, 25) in the β-strands and potentially have greater/lesser effects on prion structure, stability, etc.

RESULTS

Figure 1. From left to right, 500 base pair ladder, construct 20, construct 25. This is the final PCR before transformation. The lower bands of construct 20 and 25 were cut out, purified and used in the transformation process.

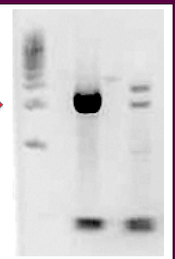


Figure 2. Yeast with a successful transformation of construct 25 plated on a sc-leu plate. Colonies from this and the other plates were picked, pre-amplified by PCR in the *SUP35* gene region, and were sent off to be sequenced.

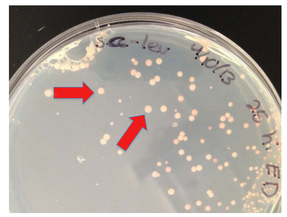
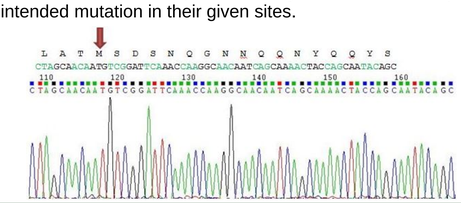


Figure 3. Representative DNA sequencing results from a single white colony from construct 25. The red arrow indicates where the sequence for the Sup35 gene begins, indicating the plasmid was successfully integrated into the *SUP35* gene. However, despite the successful transformation, none of the yeast constructs sequenced contained the intended mutation in their given sites.



CONCLUSIONS AND FUTURE PLANS

None of the selected yeast colonies sequenced contained the intended lysine insertions; however, different colonies from the same yeast plates could be correct and should be evaluated.

If those yeast colonies do have the mutation we could then use those colonies to assess the different properties each mutation has on prion properties of the Sup35 protein. We intend to create versions with multiple insertions as well.

If we do not find any colonies with the indicated mutations we could either start over making the mutants or design lysine insertions in different locations. Similarly we could try making constructs with an amino acid other than lysine. For example, we have already acquired reagents to introduce prolines, which are known to break β-sheet structures.

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