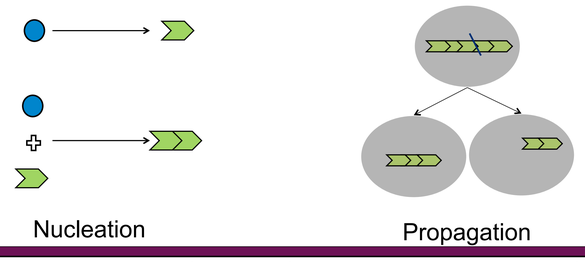
Targeted mutagenesis of the oligopeptide repeat domain of the yeast prion Sup35

James D. Knox, Emily Davis, and Kyle MacLea
Department of Biology, Linfield College, McMinnville, OR

BACKGROUND

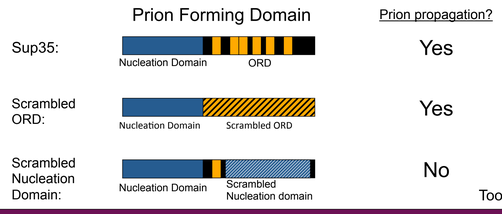
The formation of prions in the baker's yeast, *Saccharomyces cerevisiae*, is determined by amino acid composition rather than the primary sequence of amino acids. The infectious amyloid proteins known as prions undergo nucleation and propagation, two distinct activities critical for prion formation. The ability for prions to be transferred from cell to cell, or propagate, is of interest not only in yeast prions but also in prion diseases such as the mammalian spongiform encephalopathies. Prion formation has been widely studied in yeast prions, however, the fundamental mechanisms behind the specific process of propagation of prions from cell to cell are not yet understood. In the most well-studied yeast prion, the prion form *[PSI⁺]* of Sup35, a domain of 5 ½ degenerate oligopeptide repeats called the oligopeptide repeat domain (ORD), has been shown to be important for prion propagation and to have a distinct amino acid composition as compared to the nucleation domain region. A library mutagenesis experiment has identified amino acids that favor or disfavor prion propagation in yeast cells. To confirm the results of the random library mutagenesis experiment, we generated several clones in which a portion of the ORD (the fourth oligopeptide repeat) was replaced with defined sequences expected to propagate or fail to propagate.

Propagation and Nucleation of Yeast Prions



Nucleation is the conversion of a normally folded protein to an abnormal, prion form which occurs spontaneously, or when a prion converts the normally folded protein. Propagation is the breaking of amyloid fibrils, which allows the prion to be passed from mother to daughter cells.

Sup35 and the Effects of Scrambling on Propagation

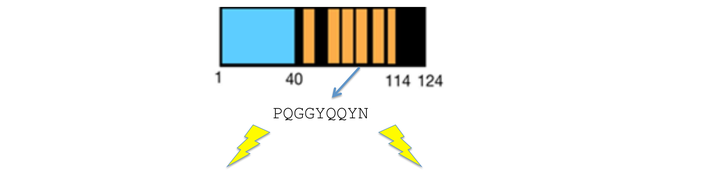


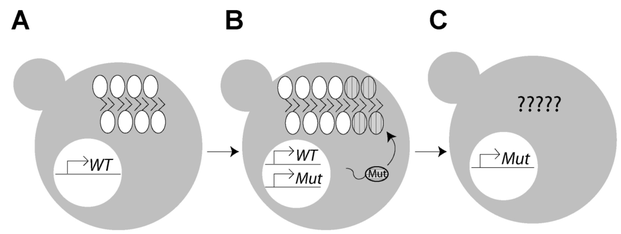
The ORD of the Prion Forming Domain is 5 ½ short peptide repeats (consensus sequence: P/Q)QGGYQ(Q/S)YN) which are important in prion propagation. The amino acid sequence of the ORD can be scrambled and will still successfully propagate. If the ORD is replaced with a scrambled nucleation domain, then propagation fails. This implies that it is the amino acid composition which is important.

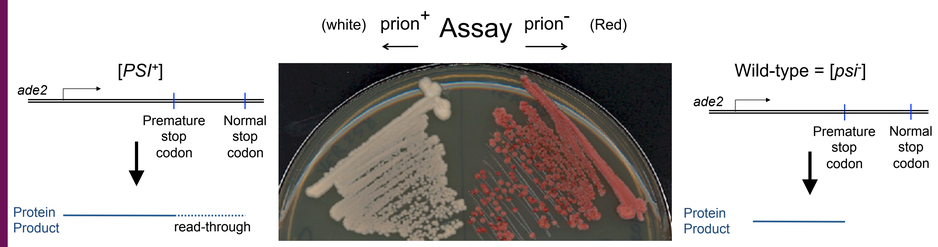
MATERIALS AND METHODS

Frequency

Groupings	Prion Positive	Prion Negative	Odds Ratio	P-Value
Aromatic (FWY)	12.7	7.7	1.74	0.12
Hydrophobic (FILMV)	31.7	23.2	1.54	0.06
Positive (KR)	6.3	13.0	0.45	0.09



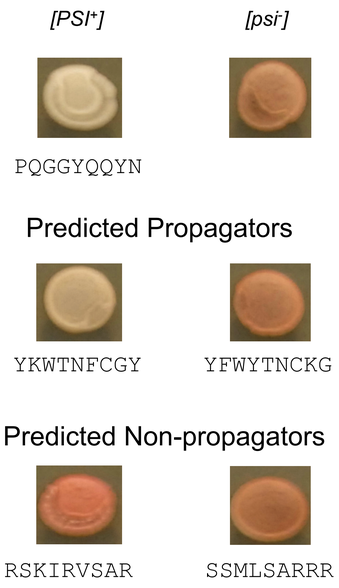




Oscherovich *et al.*, 2004

Based on a previous library mutagenic experiment the amino acids which favor and disfavor prion propagation were determined. The sequences expected to propagate included mainly aromatics and hydrophobics, while those predicted to not propagate included positively charged residues. To determine our sequence's ability to propagate, it was introduced into a cell with a wildtype plasmid which starts the initial nucleation of the Sup35 prion. The wildtype plasmid is then removed, allowing us to observe our mutants' ability to properly propagate. The Sup35 protein in its normal, non-prion form acts as the translational release factor. To serve as a reporter for prion formation in the yeast, an artificial stop codon is placed in the *ade2* gene of the yeast. When plated on minimal adenine prions that successfully propagate will read through the premature stop codon and will grow into white colonies. Prions that fail to propagate will create truncated Ade2 protein, resulting in a build up of an adenine metabolism intermediate and will grow into red colonies. To ensure that the white phenotype was a result of a prion, the colonies will be treated with GuHCl, which cures prions, causing the white phenotype to turn red.

RESULTS AND DISCUSSION



The clones depicted had their sequences verified by sequencing. The sequences chosen for the predicted propagators were the same amino acids arranged in a different order. These results suggest that because one of the predicted propagators was successful, while the other wasn't, the primary amino acid sequence may play some role in the propagation of prions. In the normal Sup35 prion, the sequence of amino acids has aromatics spaced apart, similar to the amino acid sequence in our successful propagator. By clumping together the charged residues, it could be interrupting normal propagation.

CONCLUSIONS AND FUTURE PLANS

We successfully generated all four constructs which were verified with sequencing. The sequences we expected to propagate were the same amino acids in a different order, suggesting that the order may have some influence over the ability to propagate. For the future we plan on investigating how this order may affect the ability to propagate.

LITERATURE CITED

MacLea, KS, Paul, KR, Ben-Musa, Z, Gruca, M, and Ross, ED. Different amino acid composition requirements for prion formation and propagation in the *[PSI⁺]* yeast prion. *Manuscript in preparation.*

Oscherovich, L, Cox, B, Tuite M, and Weissman, J. 2004. Dissection and design of yeast prions. *PLoS Biology*. 4: 442-451.

Toombs, JA, Liss, NM, Cobble, KR, Musa, ZB, Ross, ED. 2011. *[PSI⁺]* Maintenance is dependent on the composition not primary sequence, of the oligopeptide repeat domain. *PLoS One* 6:1-10.

James Knox
Emily Davis