Determining Amino Acid Propensity for Propagation of the Prion Sup35 in *Saccharomyces cerevisiae*

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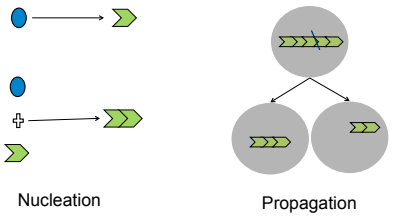
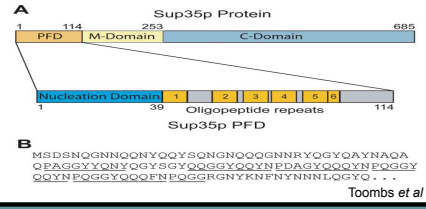
BACKGROUND

Proteins are built based on a DNA sequence template and fold into distinctive shapes or conformations. Amyloids are beta sheet-rich proteins that can fold into two stable states, a normal form and an abnormal form. Prions, the infectious amyloids, are present in many eukaryotes including humans, cows, sheep, and the baker's yeast, *Saccharomyces cerevisiae* and can cause the conversion of native protein structure into amyloid form. Unusually, yeast prion proteins have been shown to form amyloid structure on the basis of amino acid composition, independent of each protein's primary structure. The most well-studied prion protein in yeast, Sup35, consists of a prion-forming domain, a middle domain, and a functional domain. Within the prion-forming domain there is a glutamine/asparagine-rich (Q/N-rich) tract and a region of 5 ½ degenerate oligopeptide repeats (the oligopeptide repeat domain or ORD). The Q/N tract has been previously implicated in prion nucleation and the ORD was shown to be necessary for prion propagation. In this study we generated a library of yeast cells carrying varying, randomly-generated, mutations in the fourth repeat of the Sup35 ORD region and on this basis we have been able to assign propensity values for each amino acid to favor or disfavor prion propagation.

Propagation and Nucleation of Yeast Prions



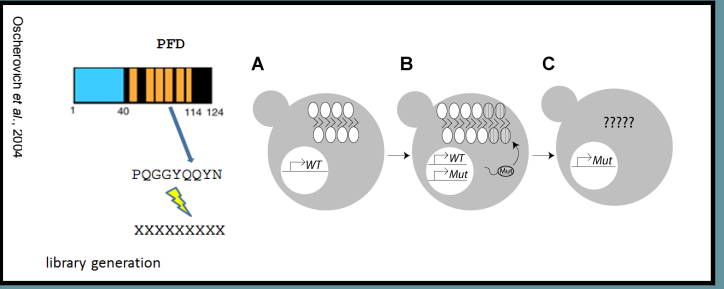
Sup35 Structure and Functional Domains



Toombs *et al* ., 2011

MATERIALS AND METHODS

Creating Mutants and Evaluating Sup35 Mutant ORD Propagation Ability



Using PCR mutagenesis we created a library of yeast clones in which the fourth repeat of Sup35 was replaced with a random sequence of amino acids. Cells containing this mutant library of plasmids (but no prion) were mated with cells containing the wildtype plasmid and the [*PSI*$^+$] prion ensuring that we measure prion propagation rather than *de novo* formation. The wildtype plasmid was removed by 5-FOA treatment and the ability of the mutant proteins to continue to propagate the existing prion was evaluated.

RESULTS AND DISCUSSION

	Frequency			
Amino Acid	**Prion Positive**	**Prion Negative**	**Odds Ratio**	***p*-Value**
Alanine (A)	4.8	5.1	0.93	1.000
Cysteine (C)	7.9	5.6	1.47	0.493
Aspartic Acid (D)	5.6	3.7	1.53	0.424
Glutamic Acid (E)	1.6	3.2	0.48	0.494
Phenylalanine (F)	5.6	3.2	1.76	0.396
Glycine (G)	11.9	8.3	1.49	0.342
Histidine (H)	1.6	3.2	0.48	0.494
Isoleucine (I)	6.3	4.6	1.40	0.616
Lysine (K)	2.4	1.4	1.73	0.673
Leucine (L)	9.5	6.0	1.64	0.281
Methionine (M)	4.0	1.9	2.19	0.281
Asparagine (N)	5.6	3.2	1.76	0.396
Proline (P)	3.2	4.2	0.75	0.774
Glutamine (Q)	0.0	1.9	0.00	0.300
Arginine (R)	4.0	11.1	0.33	0.025
Serine (S)	8.7	13.9	0.59	0.171
Threonine (T)	4.0	7.4	0.52	0.247
Valine (V)	6.3	6.9	0.91	1.000
Tryptophan (W)	2.4	2.8	0.85	1.000
Tyrosine (Y)	4.8	2.3	2.11	0.222
Groupings				
Hydrophobic (FILMV)	31.7	22.7	1.59	0.074
Aromatic (FWY)	12.7	8.3	1.60	0.196
Charged (DEKR)	13.5	19.3	0.65	0.183
Positive (KR)	6.3	12.5	0.47	0.094

Translated Mutant Sequences in the 4th ORD Repeat

Prion Positive	Sequence	Prion Negative	Sequence
4RW1	SFTSWWGCT	4RR1	RRGGVCSIG
4RW2	PGDRFNNYG	4RR2	SSSDPWWLC
4RW3	IFCMGNSPC	4RR3	TSVSSGFYS
4RW32	LIKWTVVTF	4RR5	PNPRSSLRN
4RW35	CNSNGYSDG	4RR7	SRSHLEINA
4RW45	VAFGLCGLP	4RR8	TARTMDHTQ
4RW51	NAIADILSV	4RR9	TLYCSGTIS
4RW52	YCGKMSRGD	4RR10	SLEAFSRPQ
4RW53	MCVAHHCCG	4RR13	SFGIASCCP
4RW54	SSDMISGGL	4RR16	TGCAGSRET
4RW56	FIDLLYLLE	4RR18	GTRSVLSNF
4RW57	RAIYLYMSF	4RR19	KMVHERWGS
4RW64	TLVVGDCVR	4RR20	RGFRLPWAV
4RW67	GIANKPELR	4RR21	NVYIDGTCI
		4RR23	CGVYSAVAT
		4RR24	SRPPHNSPV
		4RR25	VISLDVSAC
		4RR26	RMMCYVGTR
		4RR27	STHGFVQHE
		4RR28	GVIEILRCH
		4RR29	ERGRTLKDG
		4RR30	NAIARRQLR
		4RR31	CKLSDLRVT
		4RR32	DRSWTFWDR

Arginine residues in the fourth repeat disfavored prion propagation ($p<0.05$). This trend is also seen in a similar study of the third and fifth repeats. The fourth repeat results also suggest that hydrophobic residues (FILMV) may favor prion propagation, which is also seen in the third and fifth repeats (MacLea *et al*., *in preparation*).

CONCLUSIONS AND FUTURE PLANS

We plan to expand our library to increase our statistical significance for the amino acid propensity values. We will also design specific mutants based on the amino acid propensity values to test if we can predict prion propagation.

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.*

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