Sporadic Distribution of Prion-Forming Ability of Sup35p from Yeasts and Fungi

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ABSTRACT Sup35p of *Saccharomyces cerevisiae* can form the [PSI+] prion, an infectious amyloid in which the protein is largely inactive. The part of Sup35p that forms the amyloid is the region normally involved in control of mRNA turnover. The formation of [PSI+] by Sup35p's from other yeasts has been interpreted to imply that the prion-forming ability of Sup35p is conserved in evolution, and thus of survival/fitness/evolutionary value to these organisms. We surveyed a larger number of yeast and fungal species by the same criteria as used previously and find that the Sup35p from many species cannot form prions. [PSI+] could be formed by the Sup35p from *Candida albicans*, *Candida maltosa*, *Debaromyces hansenii*, and *Kluyveromyces lactis*, but orders of magnitude less often than the *S. cerevisiae* Sup35p converts to the prion form. The Sup35s from *Schizosaccharomyces pombe* and *Ashbya gossypii* clearly do not form [PSI+]. We were also unable to detect [PSI+] formation by the Sup35ps from *Aspergillus nidulans*, *Aspergillus fumigatus*, *Magnaporthe grisea*, *Ustilago maydis*, or *Cryptococcus neoformans*. Each of two *C. albicans SUP35* alleles can form [PSI+], but transmission from one to the other is partially blocked. These results suggest that the prion-forming ability of Sup35p is not a conserved trait, but is an occasional deleterious side effect of a protein domain conserved for another function.

PRIONS are infectious proteins, proteins that are altered in such a way that they can instruct the unaltered form of the same protein to undergo the same alteration. Vertical or horizontal transmission of the altered form to a new individual restarts the process of converting the unaltered form to the altered form. If the presence of the altered form has some toxic or other effect, or if the absence of the unaltered form is detectable, then a phenotype or disease is produced (reviewed in Liebman and Chernoff 2012; Wickner *et al.* 2013). Most prions are an amyloid form of a normally nonamyloid protein. Amyloid is a linear polymer of a single-protein species, composed largely of β-sheets, with the β-strands perpendicular to the long axis of the filaments.

In yeast, as in other organisms, infection means horizontal transmission to a neighboring cell, not necessarily related to the cell that is the source of the infection. Perhaps because of yeast's tough cell wall, neither the RNA viruses of yeast nor yeast prions leave one cell to travel through the medium and enter another cell. Rather, they are passed from cell to cell by mating and were first found as nonchromosomal genetic elements. This horizontal spread (infection) is conveniently shown by cytoduction (cytoplasmic mixing), in which two cells mate, but do not fuse their nuclei, which separate in the subsequent cell division. However, the resulting daughter cells with the parental nuclei each have mixed cytoplasms. If one parent strain carried a prion and the other not, both daughter cells will be found to carry it. A self-propagating amyloid that is not passed from cell to cell by this cytoduction process is not infectious. Prions of yeast have several diagnostic properties that distinguish them from viruses or plasmids (Wickner 1994). Overproduction of the prion protein increases the frequency with which the prion arises *de novo*. Curing of the [PSI+] amyloid-based prion by growth in the presence of guanidine (Tuite *et al.* 1981), an inhibitor of Hsp104 (Ferreira *et al.* 2001; Jung and Masison 2001; Jung *et al.* 2002), is an extremely useful test.

The [PSI+] nonchromosomal gene (Cox 1965) is a prion (infectious protein) of Sup35p (Wickner 1994), a protein whose normal functions include translation termination (Frolova

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et al. 1994; Stansfield *et al.* 1995) and regulation of mRNA turnover (Hoshino *et al.* 1999). Sup35p has three domains, N (residues 1–123), M (124–253), and C (254–685) (N terminal, middle, and C terminal). N and M are dispensable for the essential translation termination function, which is carried out by the C domain (Teravanesyan *et al.* 1993), but N and M do function in the regulation of mRNA turnover, interacting with the poly(A)-binding protein and the poly(A)-degrading enzyme (Funakoshi *et al.* 2007). The N domain is necessary and sufficient for propagation of many variants of the [PSI+] prion (Teravanesyan *et al.* 1994; Bradley and Liebman 2004; Chang *et al.* 2008), but the M domain is important as well (Liu *et al.* 2002; Bradley and Liebman 2004; Bateman and Wickner 2012). The prion form of Sup35p is a filamentous β-sheet-rich polymer (amyloid) of this protein (Glover *et al.* 1997; King *et al.* 1997; Paushkin *et al.* 1997; King and Diaz-Avalos 2004; Tanaka *et al.* 2004). Solid-state NMR studies of infectious amyloid of Sup35NM show a folded parallel in-register β-sheet architecture of most of the N domain (Shewmaker *et al.* 2006; Shewmaker *et al.* 2009). Both solid-state NMR data (Shewmaker *et al.* 2006, 2009) and hydrogen–deuterium exchange data (Toyama *et al.* 2007) show that some part of the M domain also is highly structured, apparently an in-register parallel β-sheet. Other amyloid-based prions of yeast include [URE3], an amyloid form of the Ure2 protein, a transcription regulator of nitrogen catabolism genes (Wickner 1994; Edskes *et al.* 1999; Brachmann *et al.* 2005), and [PIN+], a prion of Rnq1p, whose normal function is not known (Derkatch *et al.* 1997, 2001; Sondheimer and Lindquist 2000). Like Sup35p, infectious amyloid of both Ure2p and Rnq1p have a folded in-register parallel β-sheet architecture (Baxa *et al.* 2007; Wickner *et al.* 2008).

In addition to *Saccharomyces cerevisiae*, the Sup35p (or parts thereof) of several other yeasts have been shown capable of forming [PSI+] prions in *S. cerevisiae*. Replacing the *S. cerevisiae* Sup35 NM or N domain with the corresponding Sup35p domain of *Pichia methanolica* (Chernoff *et al.* 2000; Kushnirov *et al.* 2000; Santoso *et al.* 2000), *S. paradoxus*, *S. bayanus*, *S. kudriavzevii*, or *S. mikatae* produces fusion molecules that can form [PSI+] prions in *S. cerevisiae* (Chen *et al.* 2007; Afanasieva *et al.* 2011). Likewise, replacing the *S. cerevisiae* N domain with that of *Kluyveromyces lactis* or *Candida albicans* produced fusion proteins that showed prion-like behavior in *S. cerevisiae* (Santoso *et al.* 2000). Using full-length foreign Sup35ps, Nakayashiki *et al.* (2001) showed that the *K. lactis* Sup35p could form prions in *S. cerevisiae* or, importantly, in *K. lactis* itself. There are no previous reports of Sup35s from any species that cannot form a [PSI+] prion in *S. cerevisiae*.

The fact that Sup35s of other species can form [PSI+] has been interpreted to imply that this prion must be *advantageous* to yeast (Santoso *et al.* 2000; True and Lindquist 2000; Harrison *et al.* 2007). Of course, without a defined benefit of the prion, such an inference must be considered tentative, as the prion domain of Sup35p has a clear nonprion function (Funakoshi *et al.* 2007). While beneficial phenotypes of [PSI+] formation have been reported (Eaglestone *et al.* 1999; True *et al.* 2004), they have not been reproducible (True *et al.* 2004; Namy *et al.* 2008), even using the identical strains. Moreover, the common occurrence of lethal and near-lethal variants of [PSI+] (McGlinchey *et al.* 2011) and the rare occurrence of [PSI+] in the wild (Chernoff *et al.* 2000; Resende *et al.* 2003; Nakayashiki *et al.* 2005; Halfmann *et al.* 2012) implies that [PSI+] is generally detrimental to its host (Nakayashiki *et al.* 2005; Masel and Griswold 2009; Kelly *et al.* 2012).

Most species of *Saccharomyces* and *C. albicans* have Ure2p's that are capable of forming a [URE3] prion (Baudin-Baillieu *et al.* 2003; Edskes and Wickner 2002; Edskes *et al.* 2009, 2011; Engel *et al.* 2011). However, the Ure2p of *S. castellii* cannot form [URE3] in *S. cerevisiae* (Edskes *et al.* 2009), and those of *K. lactis* (Safadi *et al.* 2011) and *C. glabrata* (Edskes and Wickner 2013) cannot do so in either *S. cerevisiae* or their native hosts. The Ure2p prion domain is important for stability against degradation of Ure2p *in vivo* (Shewmaker *et al.* 2007), a function whose preservation may justify the risk of developing near-lethal [URE3] variants (McGlinchey *et al.* 2011).

Unlike Ure2p, Sup35p is present from yeast to humans. We sought to explore the [PSI+] prion-forming ability of a wider range of species than had been previously examined in order to assess whether prion-forming ability is indeed conserved.

Materials and Methods

Strains and media

A phylogenetic tree of the yeast and fungal species used can be found in Figure 1. Genotypes of *S. cerevisiae* strains used are listed in Supporting Information,Table S1 and Table S2. Standard yeast media and methods were used throughout (Sherman 1991). In all experiments yeast were grown at 30°.

Cloning of SUP35

Genomic DNA was isolated after growth in YPAD medium using Plant DNAzol reagent (Invitrogen) or the MasterPure yeast DNA purification kit (Epicentre). PCR was performed using KOD hot start DNA polymerase (EMD Millipore) or PfuUltra II fusion hot start DNA polymerase (Agilent Technologies). Several independent PCR reactions were performed and the products sequenced in order to identify possible PCR-induced mutations. A *Bam*HI site was introduced immediately 5′ of the start codon. The C-terminal domain of *S. cerevisiae* Sup35p starts at M254. Alignment of Sup35 proteins from other species with the *S. cerevisiae* protein identified the amino acids corresponding to M254 (Figure S1 and Figure S2). To generate Sup35NM constructs the codon corresponding to M254 in *cerevisiae* was changed to a TAA stop codon and an *Xho*I site was placed immediately 3′. The introns present in the *Schizosaccharomyces pombe* and *Neurospora*

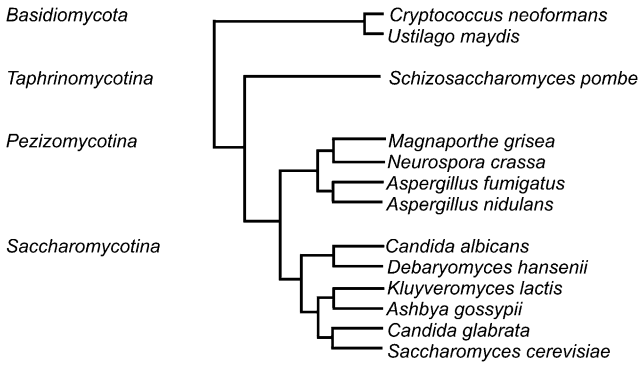


Figure 1 Phylogenetic tree of the species studied. This tree is topologically accurate but does not accurately portray evolutionarily distances (Marcet-Houben and Gabaldon 2009).

crassa SUP35NMs were removed (Figure S3). To generate fusion proteins, the 5′ domains of *SUP35* homologs, up to the codon corresponding to *S. cerevisae* M254, were linked seamlessly by PCR to the C domain of *S. cerevisiae SUP35* starting with codon M254. Directly 3′ of the *S. cerevisiae SUP35* stop codon an *Xho*I site was engineered. The *SUP35*-NM and *SUP35*NM+C fragments were cloned as *Bam*HI–*Xho*I fragments into the *S. cerevisae* expression vectors p1103 (*LEU2 CEN* P_{SUP35}), pH317 [*LEU2* 2μ P_{GAL1}; (Edskes and Wickner 2000)], and pH610 [*TRP1* 2μ P_{GAL1}; (Kryndushkin *et al.* 2011)]. p1103 was created by replacing the *Nhe*I–*Bam*HI-bordered *ADH1* promoter from pH124 (Edskes *et al.* 1999) with a 452-nt *SUP35* 5′-UTR PCR fragment also flanked by *Nhe*I and *Bam*HI (Table S3).

SUP35 integration constructs

A SUP35 promoter fragment of 452 nt was amplified by PCR with flanking *Nhe*I and *Bam*HI restriction sites. A *SUP35* 3′-UTR fragment starting 6 nt upstream of the stop codon and ending 399 nt downstream of the stop codon was amplified by PCR. TRP1 containing 278 nt 5′-UTR and 46 nt 3′-UTR was amplified by PCR with the addition of flanking loxP sites (loxP sequence: ATAACTTCGTATAGCATACATTATACGAAGTTAT). The three fragments were combined in pTIF2 (Edskes *et al.* 2009) creating *Nhe*I–P_{SUP35}–*Bam*HI–*Xho*I–LoxP–TRPI–LoxP–*SUP35* 3′-UTR–P*me*I. In the integrative vector pCW042 a *Bsm*BI site is present between *Nhe*I and P_{SUP35} and between *SUP35* 3′-UTR and *Pme*I. In the integrative vector pHK025 a *Pme*I site is present between *Nhe*I and P_{SUP35}. *SUP35NM+C* fragments were cloned as *Bam*HI/*Xho*I fragments into the same window of pCW042 or pHK025 (Table S3).

Sup35NM–GFP constructs

*SUP35*NM fragments were amplified by PCR creating a *Bam*HI site immediately upstream of the ATG start codon. Following the last codon of *SUP35NM* the sequence AGCGGCCGC was added. This creates a *Not*I site and would generate the peptide SGR when translated. *SUP35NM* fragments bordered by *Bam*HI and *Not*I sites were ligated into the same window of pH327 (Edskes *et al.* 1999) creating a *SUP35NM*–GFP fusion separated by the codons for S,G, and R. The fusion ORF was moved as a *Bam*HI–*Xho*I fragment into the same window of plasmid pH317 [*LEU2* 2μ P_{GAL1}; (Edskes and Wickner 2000)].

Bacterial expression constructs

SUP35NM fragments were amplified by PCR creating a *Nhe*I site at the ATG start codon. Six histidine codons were added at the 3′ end followed by a TAA stop codon and an *Xho*I site. The *Aspergillus nidulans SUP35NM* codon Y89 was changed from TAT to TAC to remove an internal *Nde*I site. PCR fragments were cloned into the *Nde*I–*Xho*I window of pET21a (+) (EMD Millipore). Plasmid constructs are listed in Table S3 and described further in File S1.

[PSI+] induction

Yeast cells were transformed (Geitz and Schiestl 2007) with the inducing plasmids or the vector control and plated on SD medium. Transformants were inoculated into 4 ml SD medium and grown for 2 days to saturation. A total of 3×10^7 cells were used to inoculate 4 ml minimal medium with 2% galactose and 2% raffinose as the only carbon sources and grown overnight. Cells were washed with water, diluted to 3×10^7 cells/ml, and plated in 10-fold dilutions on minimal medium lacking adenine. Colonies were counted after 5 and 6 days of incubation at 30°. To check for dilution accuracy and viability a calculated 100 cells were plated on YPAD medium. In all the experiments we found ~100 colonies on these viability test plates, indicating that overexpression of Sup35 was not extremely toxic under these conditions (Bradley *et al.* 2002).

Cytoductions

A cycloheximide-resistant *CYH2* allele (Q38K) was amplified by PCR from strain L2598 (Taneja *et al.* 2007). The PCR product was transformed into *S. cerevisiae* and after overnight recovery in YPAD, transformants were selected on YPAD plates containing 3 μg/ml cycloheximide. Cycloheximide-resistant strains were made rho° by growth on YPAD containing 30 μg/ml ethidium bromide. Donor cells placed in grids on plates lacking adenine were grown for 2 days at 30°. A lawn of rho°, cycloheximide-resistant, recipient cells was grown for 7 hr on a YPAD plate at 30°. Donor cells were transferred by replica plating onto the lawn of recipient cells and the mating mixture was incubated for 24 hr at 30°. Cytoductants were recovered by replica plating to YPG plates containing 3 μg/ml cycloheximide. After growth at 30° the adenine phenotype was checked by replica plating to SD medium lacking adenine but containing 10 μg/ml cycloheximide.

Prion curing

Cells were cured of the [PSI+] prion by growth on media containing 3–5 mM guanidine hydrochloride. Prion- and non-prion-containing cells can conveniently be identified on the same plate by the accumulation of red pigment in the *ade2-1* cells lacking [PSI+] grown on adenine limiting

Table 1 [PSI+] generation by SUP35NM homologs in a plasmid based system

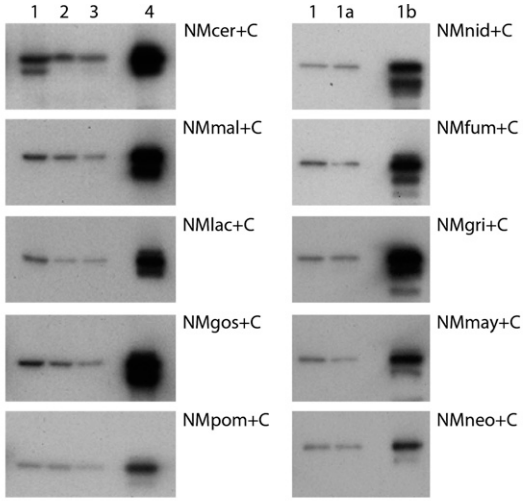
Source of *SUP35NM*	Strain	Inducing plasmid		Ade+/ 10^6 cells	*n*	Cytoduct. recipient	Total Ade+ isolates tested	Isolates giving Ade+ cytoductants
S. cerevisiae	YHE1227	pH610	vector	348	4	YHE1409	48	46
		pH952	NM cer	31,000	4		48	43
		pH1337	NM cer + C	26,700	4		48	41
C. albicans-1	YAG1	pH610	vector	17	4	YHE1430	48	0
		pH952	NM cer	23	4		47	2
		pH1361	NM alb1	33,000	4		48	19
		pH1344	NM alb1 + C	9,700	4		48	15
C. albicans-2	YAG2	pH610	vector	80	4	YHE1431	48	6
		pH952	NM cer	80	4		48	2
		p987A	NM alb2	55,000	4		48	46
		pH1343	NM alb2 + C	38,000	4		48	42
K. lactis	YAG2	pH610	vector	69	4	YHE1435	48	0
		pH952	NM cer	73	4		48	0
		pAG105	NM lac	63	4		48	0
		pH1345	NM lac + C	45	4		96	0
D. hansenii	YAG9	pH610	vector	2	4	YHE1432	48	0
		pH952	NM cer	6	4		48	0
		pAG184	NM han	4,620	4		188	75
		pH1346	NM han + C	22,000	4		48	27
C. maltosa	YAG11	pH610	vector	6	4	YHE1434	48	0
		pH952	NM cer	6	4		48	0
		pCW025	NM mal	7,500	4		47	2
		pH1347	NM mal + C	2,800	4		48	0
A. gossypii	YAG12	pH610	vector	4	4	YHE1433	48	0
		pH952	NM cer	3	4		47	0
		pAG56	NM gos	3	4		80	0
		pH1349	NM gos + C	1	4		25	0
A. nidulans	YAG6	pH610	vector	2	3	YHE1459	48	0
		pH952	NM cer	7	4		47	0
		pAG178	NM nid	2	4		47	0
		pH1348	NM nid + C	9	4		47	0
A. fumigatus	YAG4	pH610	vector	40	4	YHE1411	48	0
		pH952	NM cer	47	4		48	0
		pH976	NM fum	52	4		48	0
		pH1339	NM fum + C	40	4		48	0
M. grisea	YAG3	pH610	vector	3	4	YHE1412	35	0
		pH952	NM cer	5	4		48	0
		p992A	NM gri	2	4		26	0
		pH1341	NM gri + C	2	4		28	0
S. pombe	YAG7	pH610	vector	1	4	YHE1436	23	0
		pH952	NM cer	3	4		34	0
		pAG173	NM pom	1	4		8	0
		pH1359	NM pom + C	1	4		7	0
U. maydis	YHE1228	pH610	vector	5	4	YHE1413	29	0
		pH952	NM cer	7	4		42	0
		pH951	NM may	5	4		48	0
		pH1338	NM may + C	8	4		48	0
C. neoformans	YAG14	pH610	vector	3	4	YHE1410	18	0
		pH952	NM cer	2	3		14	0
		p990A	NM neo	3	4		13	0
		pH1340	NM neo + C	1	4		11	0

The indicated *S. cerevisiae* strain carried the indicated *SUP35NMspecies+C* gene with the *S. cerevisiae SUP35* promoter on a plasmid as well as an inducing plasmid with *SUP35NMcer* (pH952) or *SUP35NMspecies* or *SUP35NMspecies+C* (or just the vector), each with the *GAL1* promoter. Cells were grown in galactose to induce prion formation by overproduction of the fusion protein or NM and plated on –Ade. Clones were counted at 6 days and the results shown are the average of *n* experiments. The "total" number of Ade+ isolates was used as cytoduction donors, the cytoduction recipients also having a plasmid-based *SUP35*, strain YHE1407 with the corresponding plasmid with P_{SUP35}*SUP35NMspecies+C CEN LEU2* (Table S2). In plating cells expressing Sup35NM from *U. maydis*, *A. fumigatus*, *M. grisea*, *C. neoformans*, or *A. nidulans*, there was a significant background of tiny red colonies. However, some such colonies were also tested by cytoduction and did not have the properties of a [PSI+] isolate.

Table 2 Overproduction of Sup35NMspeciesGFP measured by fluorescence of whole cells

Plasmid	NM–GFP	Fold expression
pH317	Vector	1
pAZ018	GFP	41
pAZ017	*S. cerevisiae*	16
pAZ15	*K. lactis*	33
pH1301	*C. albicans1*	1
pH1302	*C. albicans2*	7
pH1303	*U. maydis*	33
pH1304	*A. fumigatus*	68
pH1305	*C. neoformans*	22
pH1306	*D. hansenii*	18
pH1307	*N. crassa*	39
pH1308	*C. glabrata*	16
pH1309	*C. maltosa*	2
pH1310	*A. gossypii*	40
pH1311	*S. pombe*	45
pH1312	*A. nidulans*	50
pH1313	*M. grisiae*	64

Plasmids used to overexpress Sup35NMspecies for prion induction were modified to encode GFP at the C terminus of the Sup35NM. Strain HJK088 carrying these plasmids was grown under the same conditions used to induce [PSI+] in the same strain. Fold expression was measured by GFP fluorescence (see *Materials and Methods*) compared to cells carrying the vector alone. Expression of Sup35NMs that did not produce [PSI+] was at least as good as those that did.



1 : Dextrose YHE1223 with CEN LEU2 P_{SUP35} SUP35 NMx+C
1a: Dextrose YHE1223 with CEN LEU2 P_{SUP35} SUP35 NMx+C and 2μ TRP1 P_{GAL1} SUP35 NMx+C
1b: Galactose YHE1223 with CEN LEU2 P_{SUP35} SUP35 NMx+C and 2μ TRP1 P_{GAL1} SUP35 NMx+C
2 : Dextrose YHE1223 SUP35 NMx+C integrated
3 : Dextrose YHE1223 SUP35 NMx+C integrated and 2μ LEU2 P_{GAL1} SUP35 NMx+C
4 : Galactose YHE1223 SUP35 NMx+C integrated and 2μ LEU2 P_{GAL1} SUP35 NMx+C

Figure 2 Western blots of Sup35NMspecies+C. Cells of indicated genotype were grown on dextrose or galactose, and extracts made and Western blots prepared as described in Methods. "x" is the species that is the source of NM. cer = *S. cerevisiae*, mal = *C. maltosa*, lac = *K. lactis*, gos = *A. gossypii*, pom = *S. pombe*, nid = *A. nidulans*, fum = *A. fumigatus*, gri = *M. grisea*, may = *U. maydis*, neo = *C.* neoformans. (1) Dextrose YHE1223 with p*CEN LEU2* P_{SUP35} *SUP35NMx+C*. (1a) Dextrose YHE1223 with pCEN LEU2 P_{SUP35} SUP35NMx+C and 2μ pTRP1 P_{GAL1} SUP35NMx+C. (1b) Galactose YHE1223 with pCEN LEU2 P_{SUP35} SUP35NMx+C and 2μ TRP1 P_{GAL1} SUP35NMx+C. (2) Dextrose YHE1223 *SUP35NMx+C* integrated. (3) Dextrose YHE1223 *SUP35NMx+C* integrated and 2μ *LEU2* P_{GAL1} *SUP35NMx+C*. (4) Galactose YHE1223 *SUP35NMx+C* integrated and 2μ *LEU2* P_{GAL1} *SUP35NMx+C*.

medium. On this YES medium (Edskes *et al.* 2009) [PSI+] cells form white colonies.

GFP fluorescence

Cells expressing GFP fusion proteins were examined by fluorescent microscopy using a Zeis Axiovert 200. Quantitation of the fluorescent signal of the cell population was performed using a Molecular Devices SpectroMax M5 multi-mode microplate reader. Cells were grown as described for the [PSI+] inductions and washed three times with water, and 300 μl of 5×10^7 cells/ml was pipetted in Costar 3915 plates. Fluorescence was measured using an excitation wavelength of 485 nm, emission wavelength of 510 nm, and a cut off filter set at 495 nm.

Protein expression and amyloid formation

Expression plasmids were transformed into BL21-CodonPlus (DE3)-RIPL cells (Agilent Technologies); transformants were inoculated in LB medium at an OD_{600} of 0.1 and grown until an OD_{600} of 0.4, at which point IPTG was added to 1 mM. Four hours later cells were harvested, resuspended in lysis buffer (15 ml/500 ml culture; 8 M guanidine hydrochloride, 150 mM NaCl, 100 mM Tris–HCl pH 8.0, 10 mM imidazole, one tablet protease inhibitor without EDTA, Roche), and incubated for 1 hr at 4°. Lysates were cleared by centrifugation at 30,000 rpm for 45 min in a SW45Ti rotor and the supernatant was mixed with 12 ml Ni-NTA (Qiagen), resuspended in equilibration buffer (8 M urea, 0.1 M Tris–HCl pH 8.0, 150 mM NaCl). Ater 4 hr gentle shaking at 4° the slurry was added to a column (Bio-Rad) and washed with 250 ml wash buffer (8 M urea, 0.1 M Tris–HCl pH 8.0, 150 mM NaCl, and 20 mM imidazole). Proteins were eluted with 12 ml buffer (8 M urea, 0.1 M Tris–HCl pH 8.0, 150 mM NaCl, 200 mM imidazole). Proteins were desalted using PD-10 columns (GE Healthcare) and concentrated to 0.5 mg/ml in 100 mM sodium phosphate pH 7.4 and 0.1 M NaCl using Amicon Ultra-15 filters. Preparations were incubated at room temperature with rotation for 1 week followed by 3 days without rotation.

Electron microscopy

Amyloid fibril suspensions were adsorbed to a carbon-coated copper grid for 2 min, washed for 1 min with H_2O, stained for 1 min with 3% uranyl acetate, blotted, and air dried. The stained samples were examined using an FEI Morgagni transmission electron microscope (FEI, Hillsboro, OR) operating at accelerating potential of 80 kV.

Western blotting

To check Sup35p expression levels, yeast cells were grown to log phase in minimal glucose medium and harvested by centrifugation. If galactose induction was used to express proteins, cells were transferred to minimal galactose medium and grown for one doubling before harvesting. Protein lysates were prepared in buffer (8 M urea, PBS pH 7.4, 1 mM PMSF, protease inhibitor cocktail tablet, Roche) using a mini-beadbeater-8 (BioSpec). Western blots were probed

Table 3 [PSI+] generation by SUP35NM homologs integrated at the genomic SUP35 locus

Source of *SUP35NM*	Strain	Inducing plasmid		Ade+/ 10^6 cells	*n*	Cytoduct. Recipient	Total Ade+ isolates tested	Isolates giving Ade+ cytoductants
S. cerevisiae	HJK088	pH317	vector	31	8	YAZ013	44	0
		pHK006	NM cer	23,900	8		47	45
		pH1294	NM cer + C	82,900	8		47	45
C. albicans-1	HJK089	pH317	vector	143	8	YHE1387	144	0
		pHk006	NM cer	162	8		144	0
		pHK007	NM alb1	1,850	8		144	12
		pH1295	NM alb1 + C	1,470	8		144	3
K. lactis	HJK092	pH317	vector	79	8	YHE1425	144	0
		pHK006	NM cer	136	8		144	0
		pHK016	NM lac	169	8		144	0
		pH1296	NM lac + C	637	8		144	7
C. maltosa	HJK122	pH317	vector	13	4	YHE1389	48	0
		PHK006	NM cer	23	4		48	0
		pHK012	NM mal	32	4		48	0
		pH1299	NM mal + C	24	4		95	0
A. gossypii	HJK111	pH317	vector	77	4	YHE1391	48	0
		pHK006	NM cer	115	4		48	0
		pHK018	NM gos	129	4		48	0
		pH1300	NM gos + C	84	4		48	0
S. pombe	HJK110	pH317	vector	24	3	YHE1383	48	0
		pHK006	NM cer	41	4		47	0
		pHK010	NM pom	24	4		48	0
		pH1297	NM pom + C	12	4		48	0

The indicated *SUP35NMspecies+C* fusion genes were integrated into strain YHE1223 replacing the *sup35::kanMX* marker, and pJ533 (*CEN URA3 SUP35cer*) was eliminated. *SUP35* homologs not listed could not be integrated into the genome of this strain. In general these are the same *SUP35* homologs that showed poor suppression of *ade2-1*. An inducing plasmid with *SUP35NMcer* (pH952), *SUP35NMspecies*, or *SUP35NMspecies+C* (or just the vector), each with the *GAL1* promoter, was introduced and cells were grown in galactose to induce prion formation by overproduction of the fusion protein or NM. Cells were plated on –Ade, clones were counted at 6 days, and the results shown are the average of *n* experiments. Cytoduction recipients have the same *SUP35* integrated at the genomic *SUP35* locus. The cytoduction recipients are not isogenic as they were created by meiotic crosses. The "total" number of Ade+ isolates were used as cytoduction donors.

with Sup35 C-terminal monoclonal antibody BE4 produced by Virivan Prapapanich (Bagriantsev and Liebman 2006).

Results

[PSI+] is assayed by the partial impairment of Sup35p's translation termination function (Cox 1965). The *ade2-1* allele is a TAA (ochre) termination codon early in the *ADE2* open reading frame and *SUQ5* is a weak serine-inserting tRNA ochre suppressor mutation (Liebman *et al.* 1975). The suppressor tRNA is in competition with the translation termination complex (Sup35p–Sup45p), and *SUQ5* is so weak a suppressor that only if the termination complex is impaired, by Sup35p being largely tied up in amyloid filaments in this case, is there sufficient suppression of the *ade2-1* nonsense mutation to allow cells to grow in the absence of adenine. The substrate of Ade2, phosphoribosylaminoimidazole, forms a red pigment whose accumulation reflects the degree of termination. The presence of [PSI+] can be distinguished from other mutations that produce an Ade+ phenotype in such strains because [PSI+] is efficiently cured by growth in the presence of 3 mM guanidine, an inhibitor of Hsp104, and because [PSI+] is efficiently transferred by cytoplasmic mixing (cytoduction).

Choice of species

The Sup35p N-terminal domains of a wide range of species are rich in glutamine and asparagine residues, but there are yeast and fungal prions with prion domains that are not Q/N rich (Balguerie *et al.* 2003; Suzuki *et al.* 2012), so we did not restrict our search to Q/N-rich Sup35ps (Figure S1). We examined a wide range of ascomycete yeasts, filamentous ascomycetes, and basiomycete yeasts, important model organisms and significant pathogens whose genomic sequences were available (Figure 1).

Both N and M of the *S. cerevisiae* Sup35p determine prion formation/propagation and thus should be viewed as a unit. Assaying N without M might not be biologically relevant. The border between M and C is easily delineated because of the strong sequence identity in the C-terminal domains (Figure S2). The border between N and M is much harder to pin down because of the lack of sequence identity.

Except for *S. pombe*, all NM domains of the Sup35 proteins have amino acid composition similarities with the protein of *S. cerevisiae*: (a) nearly all the aromatic residues are present in the N-terminal part of NM and (b) most of the aliphatic and charged residues are in the C-terminal parts of the NM domain (Table S5). The *A. nidulans*, *N. crassa*, *Magnaporthe grisea*, *Aspergillus fumigatus*, *Ashbya gossypii*, *Cryptococcus neoformans*, and, to a lesser degree, *Debaromyces hansenii* proteins have a clustering of charged residues in their N-terminal domains. In the Sup35 protein of *S. pombe*, the aromatic residues are still limited to the N-terminal part of NM, but the aliphatic and charged residues are spread throughout the protein.

We decided to create fusion proteins in which the NM domains of fungal Sup35p homologs were fused to the C-terminal domain of *S. cerevisiae*. As the C-terminal domain of Sup35p is sufficient to perform the essential translation termination function, fusion proteins would be predicted to affect the mechanics of this function less than full-length fungal homologs. The Sup35NM domains of several species were fused to *S. cerevisiae* Sup35C in a series of plasmid constructs. Introns were removed from the NM domains of the *SUP35NM* of *S. pombe* and *N. crassa* (Figure S3). These fusion proteins are designated Sup35NMspecies+C (*e.g.*, Sup35NMcer, Sup35NMhan, Sup35NMalb1, or Sup35NMalb2 for the *S. cerevisiae*, *D. hansenii*, or the two *C. albicans* alleles examined here, respectively, and Sup35NMhan+C, etc., where linked to the *S. cerevisiae* Sup35C domain). Each such construct was used to replace pJ533 (*CEN URA3 S.cerevisiae SUP35*) in a strain with a deletion of the chromosomal locus (*sup35*::*kanMX*). In each case, the fusion protein was sufficiently active to allow growth, but the fusions of *N. crassa* and *C. glabrata* Sup35NMs with the *S. cerevisiae* Sup35C-made cells partially Ade+, indicating a partially inactive fusion protein. These cases were necessarily excluded from further study.

Induction of [PSI+] using plasmid-based strains

Overproduction of a prion protein induces the *de novo* formation of the prion (Chernoff *et al.* 1993; Wickner 1994), an effect amplified by deletion of the nonprion part of the molecule (Masison and Wickner 1995; Kochneva-Pervukhova *et al.* 1998). Strains carrying the fusion plasmids were transformed with a second plasmid expressing Sup35NMcer, Sup35NM of the other species, or Sup35NM of the other species fused to Sup35Ccer (= Sup35NMspecies+C), each under control of the inducible *GAL1* promoter (Table 1). For the *S. cerevisiae SUP35*, the frequency of Ade+ colonies increased ~100-fold by overexpression of either Sup35NMcer or Sup35NMcer+C. As expected, cytoduction from each of 48 Ade+ isolates to a recipient with the same Sup35p sequence resulted in transmission of the Ade+ phenotype in nearly all cases, indicating that these clones were [PSI+]. Note that even the Ade+ clones formed without overexpression of Sup35cer were nearly all [PSI+] as judged by the high-frequency transmission of the Ade+ phenotype by cytoduction. Similar robust induction of Ade+ clones by the appropriate Sup35NMspecies or Sup35NMspecies+C, with transmission by cytoduction from many Ade+ isolates, was observed for the *C. albicans* Sup35NMalb1 and Sup35NMalb2 and *D. hansenii* Sup35NMhan (Table 1). Sup35NMmal+C showed substantial induction of Ade+ colonies by overproduction of Sup35NMmal or Sup35NMmal+C, but only 2 of the 95 isolates tested after specific induction showed transmission by cytoduction. Thus, while nearly all of the Ade+ isolates without induction with Sup35cer were [PSI+], none, or almost none, of those with Sup35NMalb1,2+C, Sup35NMhan+C, or Sup35NMmal+C were [PSI+species], although all three can rarely form prions. This suggests that conversion to the prion form is much less frequent for Sup35p from these species than for the *S. cerevisiae* Sup35.

Table 4 Transmission barrier of [PSI+alb2] to Sup35NMalb1

	Recipient NMalb1		Recipient NMalb2	
Donor	Ade+	Total	Ade+	Total
NMalb1				
YHE1464	24	24	24	24
YHE1465	23	24	24	24
YHE1466	24	24	24	24
NMalb2				
YHE1496	1	24	22	24
YHE1497	2	24	24	24
YHE1498	20	24	22	24
YHE1499	0	24	24	24

NMalb1 donor strains are derived from HJK089. Strains YHE1464 and YHE1465 are [PSI+alb1] inductants obtained after NMalb1 overexpression using pHK007 while strain YHE1466 was obtained after NMab1+C overexpression using pH1295. NMalb2 donor strains are derived from YAG2. Strains YHE1498 and YHE1499 are [PSI+alb2] inductants obtained after NMalb2 overexpression using p987A while strains YHE1496 and YHE1497 were obtained after NMalb2+C overexpression using pH1343. The NMalb1 recipient strain is YHE1430 and the NMalb2 recipient strain is YHE1431. Cells were allowed to mate on YPAD for 7 hr and cytoductants were identified after growth to single clonies on YPG+3mM cycloheximide.

The fusion Sup35s from *K. lactis*, *A. gossypii*, *A. nidulans*, *A. fumigatus*, *M. grisea*, *S. pombe*, *Ustilago maydis*, and *C. neoformans* showed neither induction of Ade+ colonies by overexpression of the corresponding Sup35NM nor transfer of the Ade+ phenotype from any of the few Ade+ colonies arising (Table 1). As a check that plasmids constructed to overproduce Sup35NMspecies were in fact doing so, we measured GFP fluorescence from the same constructs but with GFP added to their C termini. Sup35NM–GFP constructs for species whose Sup35NM did not form [PSI+] all showed strong overproduction on galactose medium (Table 2). A second approach to testing for protein overproduction, useful on the Sup35NMspecies+C proteins without including the GFP appendage, is Western blot of extracts (Figure 2). Using a monoclonal antibody to Sup35Ccer prepared by Virivan Prapapanich (Bagriantsev and Liebman 2006), we found that the Sup35NMspecies+C proteins were dramatically overproduced in all cases (Figure 2).

Integrated SUP35NMspecies+C strains

To assure a more stable source of Sup35p, and thus to more closely simulate the natural environment in which a [PSI+] prion exists, we constructed integrated versions of the hybrid genes producing Sup35NMspecies+C proteins to test again for prion formation. We integrated versions of the hybrid genes with NM segments from *C. albicans* (*SUP35alb1*), *K. lactis*, *C. maltosa*, *A. gossypii*, and *S. pombe*. A robust induction of Ade+ colony formation was observed on overproduction of NM for *S. cerevisiae* (>1000-fold), a very real (10-fold) increase for SUP35NMalb1+C, and only a marginal increase for Sup35NMlac+C (Table 3). Nearly all Ade+ clones from the all-*S. cerevisiae* Sup35 were infectious, but only a small minority of those from Sup35NMalb1+C or Sup35NMlac+C were

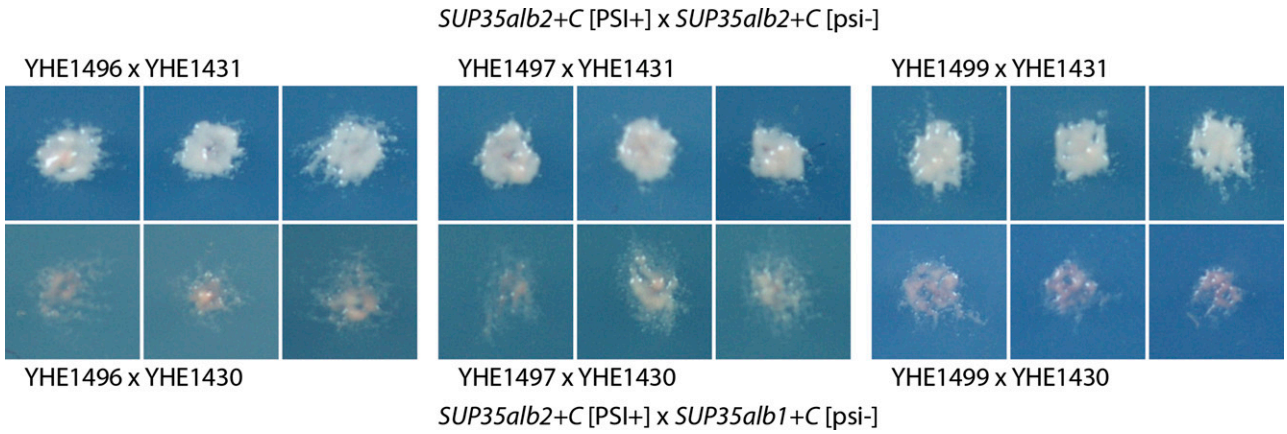


Figure 3 *SUP35alb2+C/SUP35alb1+C* heterozygous diploids lose [PSI+alb2]. Three [PSI+alb2] *SUP35alb2+C* isolates YHE1496, YHE1497, and YHE1499 (see Table 4) were mated with the [psi−] *SUP35alb2+C strain* 1431 or the *SUP35alb1+C* strain 1430. Diploids were isolated and replicaplated to –Ade plates. Most heterozygous diploids were Ade− or weakly Ade+ (below) but all homozygous diploids were Ade+ (above).

transmitted (Table 3). All seven transmissible Sup35NMlac+C isolates were also guanidine curable, confirming that they were [PSI+lac]. Sup35NMmal+C showed no significant increase in Ade+ clones and no evidence of infectivity of those that did arise. As in the plasmid-based experiments above, Sup35NMgos+C and Sup35NMpom+C showed no evidence of either induction or transmission by the few Ade+ clones arising.

To retest and confirm the prion properties of Ade+ colonies isolated using the plasmid system, we cytoduced Ade+ isolates into strains carrying the same hybrid *SUP35* gene on a plasmid or integrated at the normal *SUP35* chromosomal site (Table S4). We found that for those species for which specific induction of Ade+ colonies was observed in the plasmid-based system, only a fraction showed transmission by cytoduction using either the plasmid-based recipient or the integrated recipient. No transmission was observed from the few Ade+ colonies appearing with Sup35NMlac+C, Sup35NMgos+C, or Sup35NMpom+C.

The SN100 strain of *C. albicans*, a yeast found naturally only as a diploid, had two distinct *SUP35* alleles, differing only in their M domains (Figure S4). Each could form [PSI+alb], but cytoduction from a *SUP35alb2+C* [PSI+alb2] strain to a *SUP35alb1+C* [psi-] recipient showed only inefficient transmission of the prion (Table 4). This intraspecies barrier was observed, to different extents, for each of the four [PSI+alb2] variants tested. This intraspecies transmission barrier may partially prevent [PSI+alb] appearance or transmission in a heterozygous diploid, like the decreased appearance and transmission of human prions to individuals heterozygous for the M/V polymorphisms at PrP residue 129 (Mead *et al.* 2003). Indeed, diploids formed by mating *SUP35alb2+C* [PSI+alb] × *SUP35alb2+C* [psi-] are all [PSI+alb2] (48 Ade+ of 48), but heterozygous diploids formed by mating *SUP35alb2+C* [PSI+alb2] × *SUP35alb1+C* [psi−] are mostly [psi−] (*e.g.*, 17 of 48) or weakly [PSI+alb] (30 of 48), with only a few heterozygous diploid clones being fully [PSI+alb] (1 of 48) (Figure 3). The asymmetry of the transmission barrier is reminiscent of asymmetry in the species barrier for [URE3] between Ure2p of *S. cerevisiae* and that of *S. bayanus* (Edskes *et al.* 2009) or the intraspecies barriers between polymorphs of the Sup35p of *S. cerevisiae* (Bateman and Wickner 2012). Because Sup35alb1 and Sup35alb2 differ only in the M domain, this result is also of interest as a new example of a central role of the Sup35M domain in [PSI+] transmission.

Filament formation by Sup35NMspecies

It is likely that a wide array of proteins, perhaps any protein, can form amyloid under some conditions (reviewed in Vendruscolo *et al.* 2011). Furthermore, many amyloidoses are not infectious. Nevertheless, we determined whether the Sup35NMs studied here could form fibrils in neutral buffer with rotation, conditions under which the *S. cerevisiae* Sup35NM readily forms infectious amyloid. We purified His-tagged Sup35NMs from each species (except *K. lactis*, which was unsuccessful). We found that Sup35NMs of *S. cerevisiae*, *D. hansenii*, and *C. albicans* (Sup35alb1 and Sup35alb2) could form filaments *in vitro* and prions *in vivo* (Figure 3 and Table 1). In contrast, Sup35NMs of *A. fumigatus*, *U. maydis*, *S. pombe*, and *C. neoformans* could form neither amyloids nor prions (Figure 4 and Table 1). Although *C. maltosa* did rarely form prions, it did not form amyloid under our conditions. The *A. gossipyii*, *A. nidulans*, and *M. grisea* Sup35NMs did form fibrils *in vitro*, but did not form prions *in vivo*.

Discussion

It is possible that by using *S. cerevisiae* to examine the prion-forming ability of a foreign protein, one has detected prions made by a protein that cannot do so in its native environment. Similarly, a protein unable to form a prion in *S. cerevisiae* may do so in cells of its own species. However, the Hsp104–Hsp70–NEF chaperone disaggregation apparatus from *S. pombe*, or even *Escherichea coli*, can propagate yeast prions in *S. cerevisiae*, suggesting that prions are using for their propagation a facility provided by a wide range of

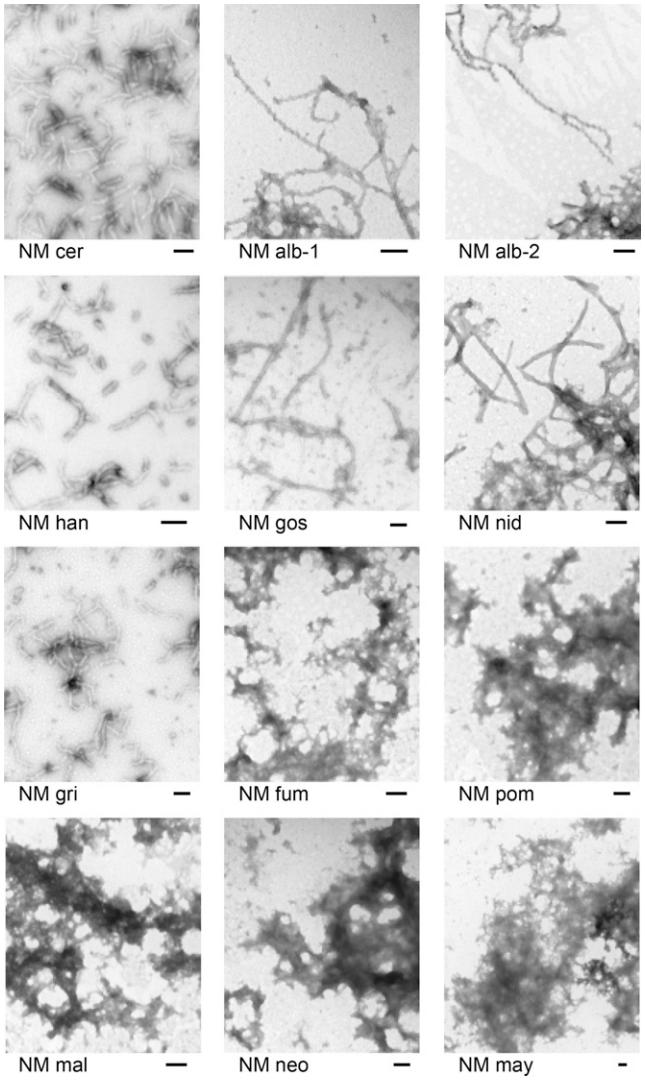


Figure 4 Amyloid formation by Sup35NM domains. Preparations of the indicated Sup35NM's were allowed to form filaments and examined by electron microscopy as described in *Materials and Methods*.

organisms (Reidy and Masison 2012; Reidy *et al.* 2013). The prion domains of Ure2p and Sup35p form prions *in vivo* more readily than do the corresponding full-length proteins (Masison and Wickner 1995; Kochneva-Pervukhova *et al.* 1998). Thus, using fragments of proteins fused to *S. cerevisiae* Sup35MC or Sup35C raises the possibility that prions are observed that would not occur were the full-length (non-hybrid) protein examined. Up to the present, the use of *S. cerevisiae* as a test bed for prion formation has not led to inconsistencies, but chaperones, the Btn2/Cur1 anti-[URE3] systems and other factors may well be crucial to prion-forming ability and must vary in their properties between species. Only a few cases have been tested in their native environment, largely because of the effort needed to develop the genetic tools in each species.

The induction of prion formation by overproduction of the prion protein (Wickner 1994), particularly by the prion domain (Masison and Wickner 1995; Kochneva-Pervukhova *et al.* 1998), is a reliable diagnostic criteria for a prion. All yeast amyloid-based prions are cured by guanidine (Tuite *et al.* 1981), but guanidine also induces mitochondrial mutations (Villa and Juliani 1980), so this agent must be used with caution. A prion must be infectious, by definition, so demonstrating horizontal transmission, most easily by cytoduction, is essential.

Previous studies of prion formation by the Sup35N of *C. albicans* did not include studies of infectivity (Santoso *et al.* 2000; Chien and Weissman 2001; Resende *et al.* 2002). We find that on overexpression of the *C. albicans* fusion protein, there was a dramatic increase in Ade+ colonies, and a portion of these were indeed infectious. The Sup35 of *K. lactis* formed aggregates in *K. lactis* that made cells carrying *ade1–14* become Ade+ (Nakayashiki *et al.* 2001). We found that *S. cerevisiae* expressing Sup35NMlac+C showed only weak specific induction of Ade+ colonies, and only a small fraction of these showed infectivity. *D. hansenii* Sup35NM+C gave robust, specific induction of Ade+ colonies, with transmission of the Ade+ phenotype by cytoplasmic mixing, indicating [PSI+han] formation. Sup35NMmal+C gave substantial specific induction of Ade+ clones in the plasmid system, but not for the integrated gene. Only a small fraction of the Ade+ clones transmitted their phenotype to plasmid-based or integrated recipients. Except for very inefficient prion formation of Sup35NMalb1,2+C when Sup35NMcer was overexpressed, no cross seeding was observed.

Prion formation did not occur with the Sup35NMspecies+C fusion proteins from *A. gossypii* or *S. pombe*, using either the plasmid-based system or the integrated hybrid gene. There was no specific induction of Ade+ clones, and the few clones that did arise were not transmissible, indicating that they were not due to prions. We saw no evidence of prion formation with the fusion proteins from *A. nidulans*, *A. fumigatus*, *M. grisea*, *C. neoformans*, or *U. maydis*, but the incomplete complementation by the Sup35NMx+C's in this group may have obscured a very rare occurrence of [PSI+]. As has been found for [URE3] formation by Ure2p from various organisms, ability to form [PSI+] is sporadically distributed among species, not necessarily conserved. Even some Q/N-rich N domains do not form [PSI+].

Although the sequence of the NM domains are not conserved, there is a remarkable conservation of amino acid composition (Table S5). In all proteins, the N domain can be identified due to overrepresentation of N + Q and the aromatic amino acids (F, Y, and W). The M domain can be identified from the overrepresentation of the charged residues (D, E, K, R), the aliphatic residues (I, L, V), and S + T. The notable exception is the *S. pombe* sequence, as charged and aliphatic residues are distributed throughout. However, aromatic residues are still clustered in the N-terminal domain. When the *S. pombe* sequence is analyzed with those of other *Schizosaccharomyces* species (Kuramae *et al.* 2006), a border can be discerned separating typical N and M domains (Table S6). Apparently, this amino acid compositional distribution is important for the normal functioning of these domains.

The sequence of the prion domains of Sup35p and Ure2p are not conserved and in fact show far more rapid variability in evolution than does the remainder of the molecules (Jensen *et al.* 2001; Edskes and Wickner 2002; Baudin-Baillieu *et al.* 2003; Bateman and Wickner 2012). The sequence changes produce barriers to transmission of [PSI+] within *S. cerevisiae* (Bateman and Wickner 2012), as well as between species for both prions (Chen *et al.* 2007; Edskes *et al.* 2009). Here we show that deletions in the *C. albicans* Sup35M domain produce an intraspecies transmission barrier. These domains have nonprion functions (Funakoshi *et al.* 2007; Shewmaker *et al.* 2007), but one can explain the rapid sequence changes as selected to produce these transmission barriers, much as sequence changes in PrP have been selected to produce resistance to transmission of human prion diseases (Mead *et al.* 2009). A majority of [PSI+] isolates in one study are severely toxic or lethal (McGlinchey *et al.* 2011), providing sufficient reason why prion resistance might be selected.

Acknowledgments

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Sporadic Distribution of Prion-Forming Ability of Sup35p from Yeasts and Fungi

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File S1

Supplementary Information

Plasmid descriptions.

pET21a(+) (EMD Millipore) and pBC KS+ (Agilent Technologies) were obtained.

Yeast expression constructs:

p1103 (*LEU2 CEN* P_{SUP35}) was created by replacing the NheI-BamHI bordered ADH1 promoter from pH124 (EDSKES *et al.* 1999) with a 452 nt SUP35 5' UTR PCR fragment also flanked by NheI and BamHI.

pH317 (*LEU2* 2μ P_{GAL1}; (EDSKES and WICKNER 2000))

pH610 (*TRP1* 2μ P_{GAL1}; (KRYNDUSHKIN *et al.* 2011))

pH327 (*LEU2 CEN* P_{URE2} *URE2*-GFP; (EDSKES *et al.* 1999))

SUP35 integration constructs:

pTIF2 (EDSKES *et al.* 2009).

pCW029 The *SUP35* promoter was amplified by PCR from p1103 adding a 3' BsmBI site. NheI-BsmBI-P_{SUP35}-BamHI was cloned into the EcoRV site of pBC KS+. BsmBI cleaves outside its recognition sequence, in this case in the *SUP35* promoter sequence.

pCW033 NheI-BsmBI-P_{SUP35}-BamHI from pCW029 cloned into the NheI/BamHI window of pTIF2 (EDSKES *et al.* 2009).

pCW037 *TRP1* containing 278 nt 5' UTR and 46 nt 3' UTR and was amplified by PCR from pH610 with the addition of flanking loxP sites. loxP sequence: ATAACTTCgTATAgCATACATTATACgAAgTTAT. A *SUP35* 3' UTR fragment starting 6 nt. upstream of the stop codon and ending 399 nt. downstream of the stop codon was amplified by PCR using genomic *S. cerevisiae* DNA. The two PCR products were fused by PCR. XhoI-LoxP-TRPI-LoxP- SUP35 3' UTR- BsmBI-PmeI-Bam HI was cloned into the EcoRV site of pBC KS+.

pCW042 XhoI-LoxP-TRPI-LoxP-SUP35 3' UTR-BsmBI-PmeI-BamHI from pCW037 was ligated into the XhoI-BglII window of pCW033 creating NheI-BsmBI-P_{SUP35}-BamHI-XhoI-LoxP-TRPI-LoxP-SUP35 3' UTR-BsmBI-PmeI

pHK002 XhoI-loxP-TRP1-loxP-SUP35 3' UTR-PmeI-BamHI was amplified from pCW042 and cloned into the EcoRV site of pBC KS+.

pHK004 NheI-PmeI- P_{SUP35}-BamHI was amplified from pCW033 and cloned into the EcoRV site of pBC KS+.

pHK021 NheI-PmeI- P_{SUP35}-BamHI from pHK004 was transferred as a NheI-BamHI fragment into the same window of pTIF2.

pHK025 XhoI-loxP-*TRP1*-loxP-SUP35 3' UTR-PmeI-BamHI was transferred as a XhoI-BamHI fragment into the XhoI-BglII window of pHK021.

Table S1. Strains of *S. cerevisiae* and other yeasts and fungi.

Strain	Genotype	Source
YHE1223 = DM780-1D/pJ533	*MAT*α *ura3 leu2 his3 ade2-1 SUQ5 kar1 trp1 sup35::kanMX* pJ533 (*URA3 CEN* P_{SUP35}*SUP35cer*	Dan Masison, NIH
RW3385	*MAT***a** *kar1 ura2 his-* [PIN+]	
YHE1407	*MAT***a** *ura3 leu2 lys2 ade2-1 trp1 SUQ5 kar1 sup35::kanMX* pJ533 (*URA3 CEN* P_{SUP35}*SUP35*) cyh2^R ρ^o	This work
HJK001	*MAT***a** *ura3 leu2 his3 trp1 kar1 ade2-1 SUQ5* [pin-] [psi-]	From 779-6A (D. Masison, NIH)
column 3, Table S2	YHE1223 *SUP35NMspecies+C-loxp-TRP1-loxp*	
column 4, Table S2	*MAT***a** *ura3 leu2 lys2 ade2-1 SUQ5 kar1 trp1 SUP35NMspecies+C-loxp-TRP1-loxp*, ρ^o	
S288C	*Saccharomyces cerevisiae*	
SN100	*Candida albicans*	Sandy Johnson, UCSF (NOBLE and JOHNSON 2005)
ATCC56498	*Kluyveromyces lactis*	
ATCC362390	*Debaromyces hansenii*	
NRRL Y17677	*Candida maltosa*	
FGSCA4	*Aspergillus nidulans*	
37A	*Candida glabrata*	June Kwon-Chung, NIH (MIYAZAKI *et al.* 1998)
FGSC2489	*Neurospora crassa*	
70-15 / ATCC34873	*Magnaporthe grisea*	Genomic DNA was a gift from Ralph Dean, NC State
B-5233	*Aspergillus fumigatus*	Genomic DNA was a gift from Janyce Sugui, NIH (TSAI *et al.* 1998)
ATCC38366	*Schizosaccharomyces pombe*	
ATCC8717	*Ashbya gossypii*	
B3501A / ATCC34873	*Cryptococcus neoformans*	June Kwon-Chung, NIH
521	*Ustilago maydis*	Genomic DNA gift from Michael Feldbrugge, Heinrich Heine Univ., Dusseldorf, Germany

Table S2. Strains in columns 1) and 2) are strains YHE1223 and YHE1407 with the corresponding plasmid from column 2) of Table S3 replacing pJ533. Strains in column 3) were derived from strain YHE1223 by integrative recombination of *SUP35NMspecies+C* replacing *sup35::kanMX.* Strains in column 4) were derived from strain HJK001 by integration of the corresponding *SUP35NMspecies+C* construct, followed by meiotic crosses.

Species source of Sup35NM	1) SUP35 deletion strain with LEU2 CEN P_{SUP35} NM+C	2) For cytoduction: *sup35*Δ strain with LEU2 CEN P_{SUP35} NM+C	3) SUP35 integrated strain	4) For cytocuction: SUP35 integrated strain
S. cerevisiae	YHE1227	YHE1409	HJK088	YAZ013
C. albicans1	YAG1	YHE1430	HJK089	YHE1387
C. albicans2	YAG2	YHE1431		
K. lactis	YAG5	YHE1435	HJK092	YHE1425
D. hansenii	YAG9	YHE1432		YHE1385
C. maltosa	YAG11	YHE1434	HJK122	YHE1389
A. nidulans	YAG6	YHE1459		
C. glabrata	YAG10			
N. crassa	YAG13			
M. grisea	YAG3	YHE1412		
A. fumigatus	YAG4	YHE1411		
S. pombe	YAG7	YHE1436	HJK110	YHE1383
A. gossypii	YAG12	YHE1433	HJK111	YHE1391
C. neoformans	YAG14	YHE1410		
U. maydis	YHE1228	YHE1413		

Table S3. Plasmids used in this study.

Species	LEU2 CEN P_{SUP35} NM+C	TRP1 2μ P_{GAL1} NM	TRP1 2μ P_{GAL1} NM+C	LEU2 2μ P_{GAL1} NM-GFP	LEU2 2μ P_{GAL1} NM	LEU2 2μ P_{GAL1} NM+C	pET21a(+) NM-His6	Integrative NM+C
S. cerevisiae	pH953	pH952	pH1337	pAZ17	pHK006	pH1294	p1339	pCW046
C. albicans1	p988A	pH1361	pH1344	pH1301	pHK007	pH1295	pH1167	pCW047
C. albicans 2	p981A	p987A	pH1343	pH1302			pH1168	pCW048
K. lactis	pAG132	pAG105	pH1345	pAZ015	pHK016	pH1296	pH1177	pCW051
D. hansenii	pAG187	pAG184	pH1346	pH1306	pHK011	pH1298	pH1179	pCW055
C. maltosa	pAG87	pCW025	pH1347	pH1309	pHK012	pH1299	pH1172	pCW057
A. nidulans	pAG175	pAG178	pH1348	pH1312			pH1178	pCW053
C. glabrata	pAG91	pAG64		pH1308			pH1171	pCW056
N. crassa	pAG199	pAG192		pH1307			pH1174	pCW059
M. grisea	pH1342	p992A	pH1341	pH1313			pH1169	pHK027*
A. fumigatus	pH977	pH976	pH1339	pH1304			pH1170	pCW050
S. pombe	pH1358	pAG173	pH1360	pH1311	pHK010	pH1297	pRMpombe	pCW052
A. gossypii	pH1350	pAG56	pH1349	pH1310	pHK018	pH1300	pH1173	pCW058
C. neoformans	p991A	pH990A	pH1340	pH1305			pH1175	pCW060
U. maydis	pH950	pH951	pH1338	pH1303			pH1176	pHK026*

Backbone plasmids:

p1103: *LEU2 CEN* P_{SUP35}

pH317: *LEU2* 2μ P_{GAL1} (EDSKES and WICKNER 2000)

pH610: *TRP1* 2μ P_{GAL1} (KRYNDUSHKIN *et al.* 2011)

pCW042: *SUP35* integrative constructs

pHK025: *SUP35* integrative constructs marked with *

Table S4. Cytoduction of [PSI+han] and [PSI+mal] into plasmid-based and integrated recipients. Using the plasmid-based system, we isolated putative [PSI+species] and used these as donors to strains expressing the same hybrid Sup35s either on a plasmid or integrated at the normal *SUP35* locus. 'Total' indicates the number of Ade+ isolates tested by cytoduction, and 'Ade+' shows the number which showed transmission to the recipient.

			Ade+	Recipient	Ade+	Total	Recipient	Ade+	Total
			per 10^6	plasmid			integrated		
C. albicans-1	pH610	vector	17	YHE1430	0	48	YHE1387	0	48
YAG1	pH952	NM cer	23		2	47		0	47
	pH1361	NM alb1	33,000		19	48		8	48
	pH1344	NM alb1+C	9,700		15	48		11	48
K. lactis	pH610	vector	69	YHE1435	0	48	YHE1425	0	48
YAG2	pH952	NM cer	73		0	48		0	48
	pAG105	NM lac	63		0	48		0	48
	pH1345	NM lac+C	45		0	96		0	96
D. hansenii	pH610	vector	2	YHE1432	0	48	YHE1385	0	46
YAG9	pH952	NM cer	6		0	48		0	47
	pAG184	NM han	4,620		75	188		83	192
	pH1346	NM han+C	22,000		27	48		31	48
C. maltosa	pH610	vector	6	YHE1434	0	48	YHE1389	0	48
YAG11	pH952	NM cer	6		0	48		0	48
	pCW025	NM mal	7,500		2	47		5	143
	pH1347	NM mal+C	2,800		0	48		3	143
A. gossypii	pH610	vector	4	YHE1433	0	48	YHE1391	0	48
YAG12	pH952	NM cer	3		0	47		0	47
	pAG56	NM gos	3		0	80		0	80
	pH1349	NM gos+C	1		0	25		0	25
S. pombe	pH610	vector	1	YHE1436	0	23	YHE1383	0	23
YAG7	pH952	NM cer	3		0	34		0	35
	pAG173	NM pom	1		0	8		0	8
	pH1359	NM pom+C	1		0	7		0	7

Table S5 Amino acid composition (%) of NM domains.

			N	Q	N+Q	D+E+ K+R	F+Y+W	I+L+V	A+G	S+T	P
S. cerevisiae	1-123	**N**	16	28	45	4	19	1	22	4	5
	M124-253	**M**	5	5	10	42	1	15	8	16	6
C. albicans-1	1-141	**N**	13	38	51	5	15	2	17	6	3
	M142-299	**M**	2	4	6	30	1	9	21	23	9
C. albicans-2	1-141	**N**	13	38	51	5	15	2	17	6	3
	142-291	**M**	2	5	7	32	1	10	19	21	9
K. lactis	1-137	**N**	15	28	43	4	17	0	25	4	4
	M138-267	**M**	4	4	8	38	1	13	14	21	5
D. hansenii	1-132	**N**	18	27	45	7	17	2	17	8	3
	M133-271	**M**	3	4	7	32	1	11	19	22	8
C. maltosa	1-144	**N**	13	36	49	5	15	3	16	6	6
	M145-282	**M**	1	4	5	29	1	13	21	20	10
A. gossypii	1-142	**N**	10	27	37	6	17	3	25	7	4
	E143-259	**M**	1	1	2	39	0	14	21	18	7
S. pombe	1-232	**NM**	4	6	11	22	5	10	19	19	13
A. nidulans	1-113	**N**	6	23	29	7	18	4	25	6	9
	S114-264	**M**	1	3	5	26	0	9	29	19	11
A. fumigatus	1-138	**N**	5	22	27	7	14	4	25	8	12
	T139-269	**M**	2	2	3	21	0	9	29	28	9
M. grisea	1-142	**N**	6	29	36	6	14	3	28	6	6
	A143-292	**M**	1	2	3	32	0	9	32	15	9
C. neoformans	1-101	**N**	2	23	25	6	17	2	24	6	20
	A102-301	**M**	2	1	3	21	1	12	33	19	12
U. maydis	1-103	**N**	9	32	41	0	17	2	26	5	8
	A104-294	**M**	1	2	3	21	0	12	30	24	10
N. crassa	1-138	**N**	7	30	37	7	15	3	27	5	5
	N139-292	**M**	1	4	5	29	0	13	28	14	10
C. glabrata	1-126	**N**	17	50	66	6	22	0	21	2	4
	M127-251	**M**	2	5	7	38	1	10	6	14	5

Table S6. Amino acid composition (%) of NM domains from Schizosaccharomyces species.

			N	Q	N+Q	D+E+ K+R	F+Y+W	I+L+V	A+G	S+T	P
S. pombe	1-232	**NM**	4	6	11	22	5	10	19	19	13
	1-83	**N**	8	13	22	11	12	7	17	16	12
	K84-232	**M**	2	3	5	28	1	12	28	21	13
S. octosporus	1-217	**NM**	3	10	12	20	5	9	20	18	13
	1-80	**N**	6	15	21	9	11	8	19	19	11
	K81-217	**M**	1	7	7	28	1	10	21	18	14
S. cryophilus	1-234	**NM**	3	9	12	22	4	9	19	19	12
	1-82	**N**	5	17	22	13	10	5	13	20	11
	K83-234	**M**	3	5	7	27	1	11	22	19	13
S. japonicus	1-233	**NM**	3	6	9	21	6	10	25	18	11
	1-86	**N**	6	12	17	10	13	7	23	19	8
	Q87-233	**M**	1	2	3	28	1	12	26	17	12

S. cerevisiae

MSDSNQGNNQQNYQQYSQNGNQQQGNNRYQGYQAYNAQAQPAGGYYQNYQGYSGYQQGGYQQYN
PDAGYQQQYNPQGGYQQYNPQGGYQQQFNPQGGRGNYKNFNYNNNLQGYQAGFQPQSQGMSLND
FQKQQKQAAPKPKKTLKLVSSSGIKLANATKKVGTKPAESDKKEEEKSAETKEPTKEPTKVEEP
VKKEEKPVQTEEKTEEKSELPKVEDLKISESTHNTNNANVTSADALIKEQEEEVDDEVVND

C.albicans-1

MSDQQNTQDQLSGAMANASLNGDQSKQQQQQQQQQQNYYNPNAAQSFVPQGGYQQFQQFQPQQQQQQYG
GYNQYNQYQGGYQQNYNNRGGYQQGYNNRGGYQQNYNNRGGYQGYNQNQQYGGYQQYNSQPQQQQQQQSQ
GMSLADFQKQKTEQQASLNKPAVKKTLKLAGSSGIKLANATKKVDTTSKPQSKESSPAPAPAPAASASTS
ASQEEKKEEKEAAATTPAAAPETKKETSAPAETKKEATPTPAAKKESTPTPAAATKKESTPVSNSASVAT
ADALVKEQEDEIDEEVVKD

C. albicans-2

MSDQQNTQDQLSGAMANASLNGDQSKQQQQQQQQQQNYYNPNAAQSFVPQGGYQQFQQFQPQQQQQQYG
GYNQYNQYQGGYQQNYNNRGGYQQGYNNRGGYQQNYNNRGGYQGYNQNQQYGGYQQYNSQPQQQQQQQSQ
GMSLADFQKQKTEQQASLNKPAVKKTLKLAGSSGIKLANATKKVDTTSKPQSKESSPAPAPAPAAFASQE
EKKEEKEAAAATPETKKETSAPAETKKEATPTPAAKKESTPVSNSASVATADALVKEQ
EDEIDEEVVKD

K. lactis

MSDQQNQDQGQGQGYNQYNQYGQYNQYYNQQGYQGYNGQQGAPQGYQAYQAYGQQPQGAYQGYNPQQAQG
YQPYQGYNAQQQGYNAQQGGHNNNYNKNYNNKNSYNNYNKQGYQGAQGYNAQQPTGYAAPAQSSSQGMTL
KDFQNQQGSTNAAKPKPKLKLASSSGIKLVGAKKPVAPKTEKTDESKEATKTTDDNEEAQSELPKIDDLK
ISEAEKPKTKENTPSADDTSSEKTTSAKADTSTGGANSVDALIKEQEDEVDEEVVKD

D. hansenii

MSDDQQYNQDKLSQDFQNTSIGSGEQQQQSYQQYQQQPQQNNFNANSAPTFTPSGQQGGYQGGYQGGYQG
GYQNYSQGGYQNYNQGYQNYNQGYQGYQNNNRGGYNNYNNRGGYNNYNNYNQQDQQPVQNQGMSLADFQK
QQNAQANLNKPKKTLKLASSSGIKLANAKKAPETESKEATPAATEKEATPAATEKEATPAATEKEATPAA
TEKETTPAPAKKEATPASVKSESKPASKSTSKVATKESTPVTSDKVIQEQVDEVDEEVVKD

C. maltosa

MSNPQDQLSNDLANASISGDQSKQPQQQQPQQQQPYFNPNQAQAFVPTGGYQQFQPQQQQQYGGGYQQNYT
QYQAGGYQQNYNNRGGYQQNYNNRGGYQQNYNNRGGYQQQQQQQYQAYNPNQQYGGYQAYNPQQQQQQQT
QSQGMSLADFQKQKAEQQASLNKPAVKKTLKLASSSGIKLANATKKVDTAKPAASKEASPAPKDEEASAE
PEAKKESTPVPASSSPAPAAADSTPAPVKKESTPTPSVASKSAPVSASASVVTADALAKEQEDEVDEEVV
KD

A. gossypii

MSEEDQIQSQGNDQGQSQAKDQGQNQGQGQQNFGQYYNPSNFQNYQGYVPQGGYQAYGQQAGGYQGYAQY
NQQAGGYQGYQGYQQYNPAQAGYQGYQQYNAQGGYQSYKQYNSQPQGNRKGNQSYGYGQGQSATAPVTLN
NFEKGTVPNATAPKPKKTLKLASSSGIKLVGAKKPVAKKEEAKAEEPTKEEPKSSAEGAPKSEDATASSE
DKAVPSIEKLSISEADTAKKDTADAAGATSSDALIKEQEDEVDEEVVKD

A. nidulans

MANQTPDSWEDELSRQTEGVNLNAQSRPQPQAPSFHPGAASFQPGAAAFVPGQQFQPYGGYPQYGQYGQG
YGGYQGYDQQQAYGQYGAYGQQPGGYNQIYNQNYGNYQQQQQFSQKPRQAAPAAAAPAQPAPKPASNAAP
KAKVLSIGGASDSPSAPKTKVLSIGTPTPASTTPSSDSGSLADAKGPAAVEAASKVTAAKAVEKTEKKAE
QKAAASGKSSPAPSGRNSPGRSSPSRAELAKEKRDADAVAAEQKADVDEETLKE

A. fumigatus
MANQTPDSWEDELSKQTEGVNLNARGQYRPQAQAPSFHPGAASFQPGAPSFVPGQTYQQYGGGYPQYGQY
GGYPAYDQQQQGFGQYGAYAQQPGGYNQIYNNQYGGYNQHQQQQYTQPPRQAAPVATQAPSAPAQPAQTA
PKPASTASAAPVLSIGGASSSSAAPKTKVLSIGTPSPASNTPSGTTTPGDTMGSAAADAAAKVTASKAIE
KTEKKAAASGKSSPTPTASGRSSPGRSSPSRGEGGKTGRDANAVALEQQADVDEETLKE

M. grisea
MSNLNSWEDDPAAQDENLARQAQQQMNLGRGGQPAQQGGFRAGASTFQPGANSFQPGAQSFQPGQPYGGG
YAPQYQQQQQYYGGQGYGQYGQQYGGQQQSHGQYGQGYGSVYGQGGYGQQGYNNQYSNYQNQQAQQQQPQ
QQAKPTPTIAKRPDAASGSAAPAADLNKPIATKEGGTKVLSIGGDAPKPKAKVLSIGTPAAAKEPAKKEE
DVKKEEAAKKEAQAKPQAGAKAAAAKAIEKTGGDASASGKTSPSPSSGRSSPTRGAKAAAARDASAVEKE
QTADVDEETLKE

S. pombe
MASNQPNNGEQDEQLAKQTSKLSMSAKAPTFTPKAAPFIPSFQRPGFVPVNNIAGGYPYAQYTGQGQNSN
SPHPTKSYQQYYQKPTGNTVDEDKSRVPDFSKKKSFVPPKPAIPKGKVLSLGGNTSAPKSTKPISISLGG
TKAPTTTKPAAPAAQSKTETPAPKVTSESTKKETAAPPPQETPTKSADAELAKTPSAPAAALKKAAEAAE
PATVTEDATDLQNEVDQELLKD

U. maydis
MNPNAPSFGGFNPNASGFVPGGQQQQGQQQQGGTPYGQQGAYYQQGFGQQQQQQQGFNGGYNSQYQQYQQ
YQQQPYGGGYAQQGFNQPGFSNQYAQQGFNGLPARPAASGSNQPPARPAQPAAATSDVASRKPVSISIGG
GAKPAAPAASDAPRKPVSISIGGGAKPAATTADKSDAPRKPVSISIGGAKKPEAAKEAVSAATKAEISVH
SAAVSAVKDTEAPSSTAVTAPSSRSDSPAPSAAAASSSAATASKAESKVASLSSKPVATERATTNADQIL
AEASKVTDEETLKD

C. neoformans
MSGQQPPSFNPGAFEFRPGQAPFAPRQQQPFDPYGQQQGGYPQYGQYGQQQGYPQYGQYGGYPQQQGYPV
PGAPGAGPRAYQPPQARNVQGFQPPSFSSSPAPPPDTKAPAGKPVSLSIGGGGAPKAAPSLSIGGGGAPK
AAPSLSIGGAPKAAPSLSIGGAPKAAPSLSIGGAKAAPSLSIGGKKEEKKEEKEASSKSSPKPAAPTPKP
ADAPAAKSEAASAPVSAAEKKAEKAVPLTSDAQGKVVAAETSAASPAKSGASTPVATVSTSTTNFSKVSA
KNDAEAIYREQNLAGDAALRD

C. glabrata
MSDPNQQQQQQQQQQQQQQGNYQQYYQNYGQQNFQPQQGYQQYQQFQNYQPQQGYQQYQNYQQGGYQNYQ
QGGYQNYQQGGYQGGRGGYQGGRGRGGYKNYNNRNNYNNQNSGYQNYQQQQQPAQGMTLDSFQQQQEQKS
EPAPKPKKTLKLVSSSGIKLANATKKEEPKKEENKQEEPAKEAKKDEETKKEETTSTESKPEATEDVSKK
MEKLDVKDTKEKEATPSQEVSSADALIKEQEDEVDEEIVND

N*. crassa*
MSGNVQNNWEEAADQDERLARQTQQQMNINAGTFRPGAAAFTPGAPSFTPGQFAAPGFTPQYQQQYYGGA
QQGYGGGYPQYGQQGYGQYNNQQQQGYGAVYGQQGYNQGYAGQQQQQQQQQYGGYQQNQGYQQRQQQNR
DAPKPAPQIVKRPEQPAAQAQPKADAPKTAAAPVKVLSVGGDAPAKVLSIGGDAPKPAAKVLSISGTAPA
KEEPKKEAAKKEGTAEAAAKVTATKAVQKTESAAASGRTSPAPSSGRASPSAAKSGNKVSRDVDAVEKDI
QSADVDEDTLKE

Figure S1 Sequences of NM domains used in this work.

Yellow amino acids changed into TAA stop codon.

```
S. cerevisiae        TNNANVTSADALIKEQEEEVDDEVVNDMFGG-------KDHVSLIFMGHVDAGKSTMGGN
C. glabrata          QE---VSSADALIKEQEDEVDEEIVNDMFGG-------KDHVSIIFMGHVDAGKSTMGGN
K. lactis            STGG-ANSVDALIKEQEDEVDEEVVKDMFGG-------KDHVSIIFMGHVDAGKSTMGGN
A. gossypii          ADAAGATSSDALIKEQEDEVDEEVVKDMFGG-------KDHVSIIFMGHVDAGKSTMGGN
C. maltosa           SASASVVTADALAKEQEDEVDEEVVKDMFGG-------KDHVSIIFMGHVDAGKSTMGGN
C. albicans          SNSASVATADALVKEQEDEIDEEVVKDMFGG-------KDHVSIIFMGHVDAGKSTMGGN
D. hansenii          TKESTPVTSDKVIQEQVDEVDEEVVKDMFGG-------KDHVSIIFMGHVDAGKSTMGGN
C. neoformans        FSKVSAKNDAEAIYREQNLAGDAALRDLYGENVKDTNIKSHLNIIFTGHVDAGKSTMGGQ
U. maydis            --------ADQILAEASKVTDEETLKDLFGE--KSDELKSHLNIVFIGHVDAGKSTMGGN
S. pombe             ---------------LQNEVDQELLKDMYGK--------EHVNIVFIGHVDAGKSTLGGN
N. crassa            GNKVSRDVDAVEKDIQSADVDEDTLKEIYG--------KEHMNIIFIGHVDAGKSTLGGA
M. grisea            AKAAAARDASAVEKEQTADVDEETLKEVYG--------KEHMNIIFIGHVDAGKSTLGGS
A. nidulans          ELAKEKRDADAVAAEQKADVDEETLKEIYGE------KKEHVNIVFIGHVDAGKSTLGGS
A. fumigatus         EGGKTGRDANAVALEQQADVDEETLKEIYGE------KKEHVNIVFIGHVDAGKSTLGGS
                                        M domain  |  C domain
```

Figure S2 The border between Sup35M (middle) and Sup35C (C-terminal, conserved, translation termination function) domains. The alignment of protein sequences shown here was used to select the site (highlighted in yellow) of the residue made a terminator for expressing Sup35NM from different species from a *GAL* promoter to induce prion formation, or in bacteria to prepare the peptide for amyloid formation. The same site was used in fusing the species Sup35NM to the *S. cerevisiae* C domain for expression from a plasmid or to integrate.

Schizosaccharomyces pombe

```
      G  *  *  S  A  K  *  R  *  T  G  *  A  T  R  Q  A  D  F  *   F1
   M  A  S  N  Q  P  N  N  G  E  Q  D  E  Q  L  A  K  Q  T  S      F2
    W  L  V  I  S  Q  I  T  V  N  R  M  S  N  S  P  S  R  L  L    F3
1   ATGGCTAGTAATCAGCCAAATAACGGTGAACAGGATGAGCAACTCGCCAAGCAGACTTCT   60

      T  V  D  V  C  K  G  T  Y  V  Y  A  *  S  C  S  L  H  S  F   F1
   K  L  S  M  S  A  K  A  P  T  F  T  P  K  A  A  P  F  I  P      F2
    N  C  R  C  L  Q  R  H  L  R  L  R  L  K  L  L  P  S  F  L    F3
61  AAACTGTCGATGTCTGCAAAGGCACCTACGTTTACGCCTAAAGCTGCTCCCTTCATTCCT   120

      F  P  T  Y  V  F  *  L  E  H  H  P  Y  V  L  R  *  L  L  V   F1
   S  F  Q  R  T  F  F  N  *  N  T  I  H  M  S  *  D  D  C  L      F2
    L  S  N  V  R  F  L  I  R  T  P  S  I  C  L  E  M  I  A  C    F3
121 TCTTTCCAACGTACGTTTTTTAATTAGAACACCATCCATATGTCTTGAGATGATTGCTTG   180

      L  S  K  K  I  I  N  F  L  L  G  P  G  F  V  P  V  N  N  I   F1
   F  *  V  K  K  L  L  T  S  C  *  A  P  D  L  Y  L  *  T  I      F2
    F  K  *  K  N  Y  *  L  L  A  R  P  R  I  C  T  C  E  Q  Y    F3
181  TTTTAAGTAAAAAAATTATTAACTTCTTGCTAGGCCCCGGATTTGTACCTGTGAACAATA   240

      A  G  G  Y  P  Y  A  Q  Y  T  G  Q  G  Q  N  S  N  S  P  H   F1
   L  L  V  V  I  L  M  P  N  I  Q  A  K  D  K  T  A  T  R  H      F2
    C  W  W  L  S  L  C  P  I  Y  R  P  R  T  K  Q  Q  L  A  T    F3
241 TTGCTGGTGGTTATCCTTATGCCCAATATACAGGCCAAGGACAAAACAGCAACTCGCCAC   300
```

Neurospora crassa

```
   M  S  G  N  V  Q  N  N  W  E  E  A  A  D  Q  D  E  R  L  A      F1
    C  P  E  T  F  R  T  T  G  R  R  L  P  T  R  M  S  A  W  R    F2
     V  R  K  R  S  E  Q  L  G  G  G  C  R  P  G  *  A  P  G  A   F3
1   ATGTCCGGAAACGTTCAGAACAACTGGGAGGAGGCTGCCGACCAGGATGAGCGCCTGGCG   60

   R  Q  T  Q  Q  Q  M  N  I  N  A  G  T  F  R  P  G  A  A  A      F1
    A  R  P  S  N  R  *  T  S  T  L  V  P  S  A  P  V  P  L  P    F2
     P  D  P  A  T  D  E  H  Q  R  W  Y  L  P  P  R  C  R  C  L   F3
61  CGCCAGACCCAGCAACAGATGAACATCAACGCTGGTACCTTCCGCCCCGGTGCCGCTGCC   120

   F  T  P  G  A  P  S  F  T  P  G  Q  F  A  A  P  G  F  T  P      F1
    S  P  P  A  P  P  P  S  P  P  A  S  S  P  L  P  A  S  L  P    F2
     H  P  R  R  P  L  L  H  P  R  P  V  R  R  S  R  L  H  S  P   F3
121 TTCACCCCCGGCGCCCCCTCCTTCACCCCCGGCCAGTTCGCCGCTCCCGGCTTCACTCCC   180

   Q  Y  Q  Q  Q  Y  Y  G  G  A  Q  Q  G  Y  G  G  G  Y  P  Q      F1
    S  T  S  S  N  I  T  A  A  P  S  R  A  T  A  V  A  T  P  S    F2
     V  P  A  A  I  L  R  R  R  P  A  G  L  R  R  W  L  P  P  V   F3
181 CAGTACCAGCAGCAATATTACGGCGGCGCCCAGCAGGGCTACGGCGGTGGCTACCCCCAG   240

   Y  G  Q  Q  G  Y  G  Q  Y  N  N  Q  Q  Q  Q  G  Y  G  A  V      F1
    T  A  S  R  A  T  D  S  T  T  T  S  S  S  R  A  M  A  L  F    F2
     R  P  A  G  L  R  T  V  Q  Q  P  A  A  A  G  L  W  R  C  L   F3
241 TACGGCCAGCAGGGCTACGGACAGTACAACAACCAGCAGCAGCAGGGCTATGGCGCTGTT   300
```

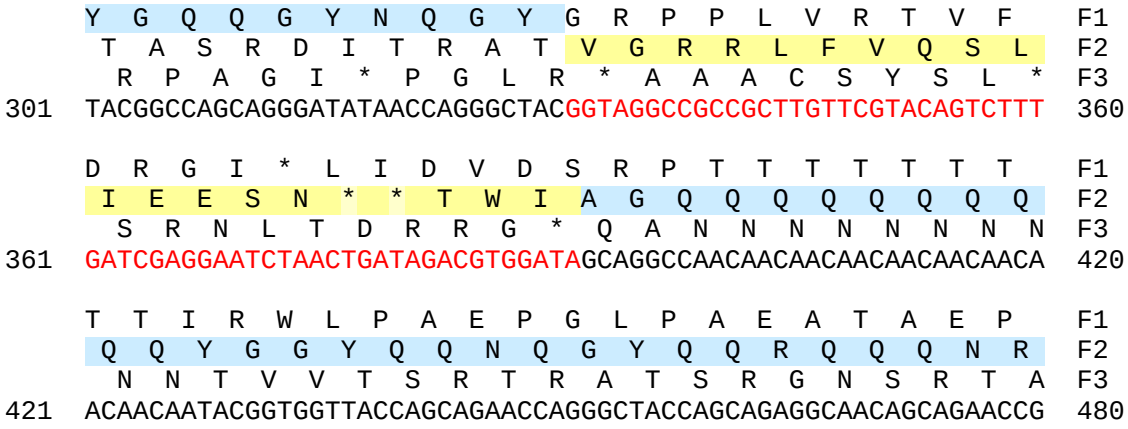

Figure S3 Introns (**ATGC**) removed from NM domains of S. pombe and N. crassa *SUP35*.

```
NMalb1          MSDQQNTQDQLSGAMANASLNGDQSKQQQQQQQQQQNYYNPNAAQSFVPQGGYQQFQQF  60
NMalb2          MSDQQNTQDQLSGAMANASLNGDQSKQQQQQQQQQQNYYNPNAAQSFVPQGGYQQFQQF  60
                ************************************************************

NMalb1          QPQQQQQQYGGYNQYNQYQGGYQQNYNNRGGYQQGYNNRGGYQQNYNNRGGYQGYNQNQQ 120
NMalb2          QPQQQQQQYGGYNQYNQYQGGYQQNYNNRGGYQQGYNNRGGYQQNYNNRGGYQGYNQNQQ 120
                ************************************************************

NMalb1          YGGYQQYNSQPQQQQQQQSQGMSLADFQKQKTEQQASLNKPAVKKTLKLAGSSGIKLANA 180
NMalb2          YGGYQQYNSQPQQQQQQQSQGMSLADFQKQKTEQQASLNKPAVKKTLKLAGSSGIKLANA 180
                ************************************************************

NMalb1          TKKVDTTSKPQSKESSPAPAPAPAASASTSASQEEKKEEKEAAATTPAAAPETKKETSAP 240
NMalb2          TKKVDTTSKPQSKESSPAPAPAPAAFAS----QEEKKEEKEAAAAT----PETKKETSAP 232
                ************************ **    ************:*    **********

NMalb1          AETKKEATPTPAAKKESTPTPAAATKKESTPVSNSASVATADALVKEQEDEIDEEVVKD  299
NMalb2          AETKKEATPTPAAKKESTPTPATATKKESTPVSNSASVATADALVKEQEDEIDEEVVKD  291
                **********************:*********************************
```

Figure S4 Sequence alignment of NMalb1 and NMalb2.

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