Prions in Yeast

Susan W. Liebman*,[1] and Yury O. Chernoff[†]
*Department of Biochemistry and Molecular Biology, University of Nevada, Reno, Nevada 89557, and [†]School of Biology, Georgia Institute of Technology, Atlanta, Georgia 30332

ABSTRACT The concept of a prion as an infectious self-propagating protein isoform was initially proposed to explain certain mammalian diseases. It is now clear that yeast also has heritable elements transmitted via protein. Indeed, the "protein only" model of prion transmission was first proven using a yeast prion. Typically, known prions are ordered cross-β aggregates (amyloids). Recently, there has been an explosion in the number of recognized prions in yeast. Yeast continues to lead the way in understanding cellular control of prion propagation, prion structure, mechanisms of *de novo* prion formation, specificity of prion transmission, and the biological roles of prions. This review summarizes what has been learned from yeast prions.

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[1]Corresponding author: University of Nevada, Reno, 1664 N. Virginia St., Mail Stop 330, Reno, NV 89557. E-mail: suel@uic.edu

PRIONS are self-propagating and transmissible protein isoforms. The initial awareness of prions came from mammalian diseases, such as sheep scrapie, human Creutzfeldt-Jacob, and bovine spongiform encephalopathy ("mad cow" disease). These diseases are transmitted by an altered infectious conformational isoform (PrP^{Sc}) of a normal cellular protein (PrP^{c}). PrP^{Sc} forms cross-β aggregates (amyloids) and is infectious because it captures PrP^{c} molecules and converts them into PrP^{Sc} (Prusiner 1982; Colby and Prusiner 2011). More than 30 other human diseases (including Alzheimer's, Parkinson's, and Huntington's diseases) are associated with amyloid formation by various proteins, and for some of them transmissibility has been demonstrated at the cellular level (Aguzzi and Rajendran 2009).

While there is no protein with homology to PrP^{c} in yeast, several yeast proteins have now been shown to exist either in a normal soluble or in a transmissible amyloid form. These different states of the same protein cause distinct phenotypes. Furthermore, each prion protein can generally form different types of infectious aggregates with distinct conformations and distinct associated phenotypes, called prion variants. Since soluble molecules conform to the specific prion variant conformation of the aggregates that they join, normally only one prion variant of a given protein propagates stably in a single yeast culture.

Yeast prions provide a model system for studying mechanisms of amyloid formation and propagation that are applicable to mammalian and human diseases. They also manifest themselves as heritable cytoplasmic elements and, in this way, provide a mechanism of inheritance that operates at the level of protein conformation rather than nucleotide sequence. The growing number of examples of prions and other amyloid proteins indicates that they may have an important biological role.

Evidence for Prions

In 1994, on the basis of new and old data, Reed Wickner (Wickner 1994) proposed that the previously known yeast non-Mendelian heritable [*URE3*] and [PSI^{+}] elements (Cox 1965; Aigle and Lacroute 1975) are, respectively, prion forms of the Ure2 and Sup35 proteins. This revolutionary hypothesis at once explained why (1) the phenotypes of [*URE3*] and [PSI^{+}], respectively, correspond to "loss-of-function mutations" in *URE2* or

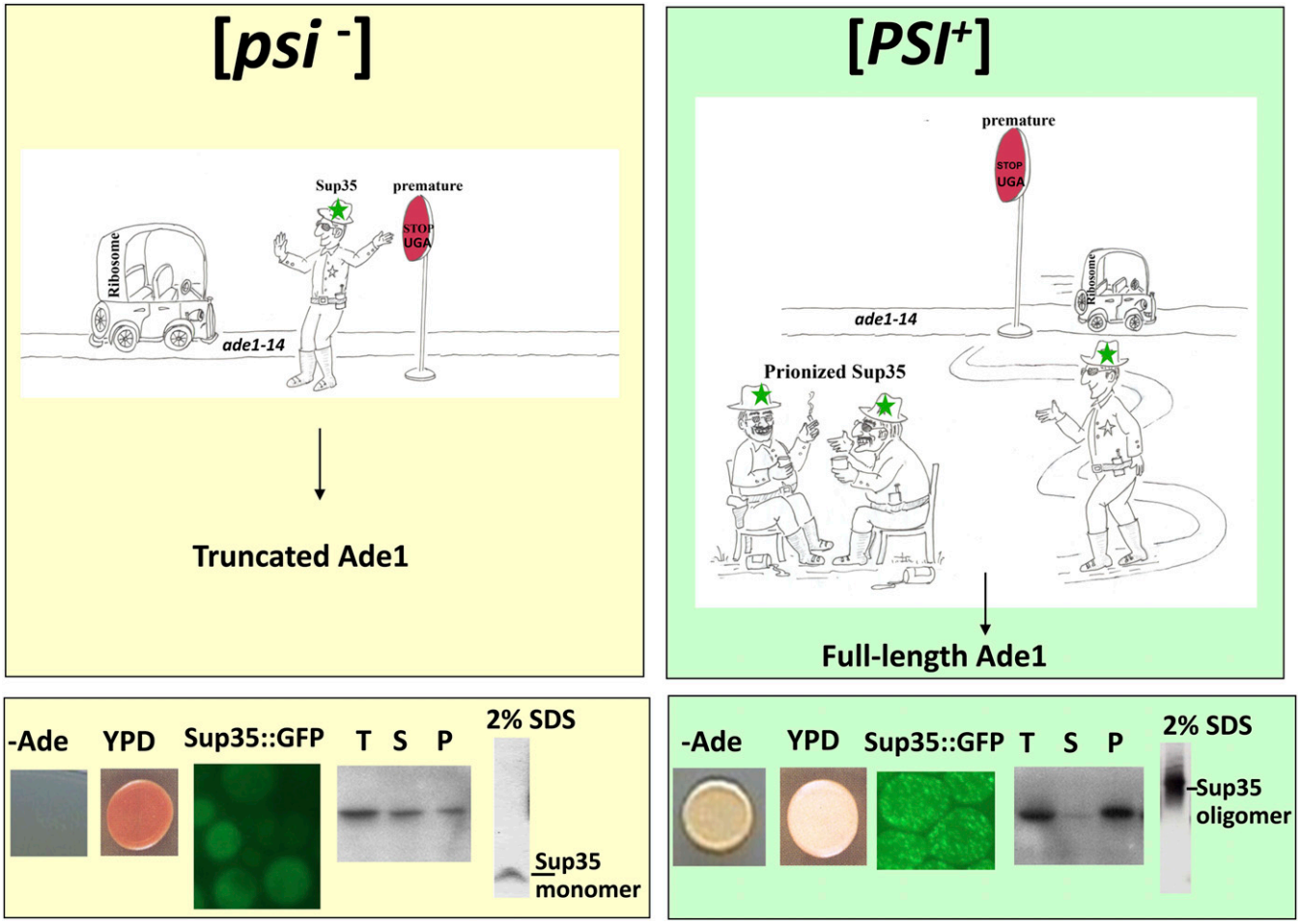

Figure 1 [*PSI*+] phenotypes. In the absence of the prion (left), the Sup35 release factor—shown as a conscientious cop—causes the ribosome to stop at the premature stop codon in the *ade1-14* mutation. This leads to the release of a truncated Ade1 protein, preventing the cell from synthesizing adenine. In the presence of the [*PSI*+] prion, shown as corrupted cops playing cards and drinking, the conscientious Sup35 is drawn into the party and the ribosome can read-through the stop codon, allowing cells to make some full-length Ade1. Thus, as shown below the cartoon, the [*psi*−] cells are unable to grow on –Ade plates and accumulate a red intermediate on complex YPD medium, while the [*PSI*+] cells grow on –Ade and are white on YPD. Sup35 tagged with GFP is diffuse in [*psi*−] cells but forms aggregates (*e.g.*, many small parties) in [*PSI*+] cells. When total cell lysates (T) are separated into supernatant (S) and pellet (P) fractions and Western blots made of boiled SDS acrylamide gel separations are developed with Sup35 antibody, Sup35 is found largely in the supernatant in [*psi*−] lysates but mostly in the pellet in [*PSI*+] lysates. When lysates are separated on agarose gels, following room-temperature incubation with 2% SDS, Sup35 runs as a monomer in [*psi*−] cells but mostly as oligomers in [*PSI*+] cells.

SUP35; (2) [*URE3*] and [*PSI*+] are inherited in a cytoplasmic fashion; (3) propagation of [*URE3*] and [*PSI*+] requires the respective presence of the Ure2 or Sup35 prion domains (without these domains there will be no prion seeds); (4) unlike loss of a virus or other cytoplasmic nucleic acid, loss of [*URE3*] and [*PSI*+] is reversible (the prion protein still present in the non-prion cell can reform the prion); and (5) transient Ure2 or Sup35 overproduction, respectively, induces the *de novo* appearance of [*URE3*] or [*PSI*+] (excess prion protein enhances the chance that some of it will misfold to form a prion seed, as described below). A great deal of additional evidence has now accumulated to support this hypothesis.

Prions often have loss-of-function phenotypes

Assuming that the function of the normal cellular protein is compromised to some extent when it forms a prion aggregate, the prion phenotype would reflect this loss of function. As an example, Figure 1 illustrates the phenotypic differences between yeast cells with the non-prion *vs.* prion forms of the translational termination factor Sup35. In the presence of the prion, the translational termination activity of Sup35 is compromised (loss of function) so cells terminate translation less efficiently at nonsense codons (Cox 1965). Likewise, [*URE3*] (Aigle and Lacroute 1975) and several other yeast prions (see *Prion Diversity* and Table 1) cause loss-of-function phenotypes. Ure2, a regulator of nitrogen catabolism, prevents uptake of allantoate (a potential nitrogen source) in the presence of other nitrogen sources, and of ureidosuccinate (USA), an intermediate in uracil biosynthesis, which closely resembles allantoate. Thus inactivation of Ure2 by mutation or by formation of the [*URE3*] prion allows *ura2* mutant cells that cannot synthesize USA to grow on –Ura by

Table 1 Proven amyloid-based prions

Prion	[*PSI*$^+$][a]	[*PIN*$^+$]/[*RNQ*$^+$][b]	[*URE3*][c]	[*SWI*$^+$][d]	[*OCT*$^+$][e]	[*MOT3*][f]	[*ISP*$^+$][g]
Protein determinant	Sup35	Rnq1	Ure2	Swi1	Cyc8	Mot3	Sfp1
Native function	Translation termination	Unknown	Nitrogen regulation	Transcriptional regulation	Transcriptional regulation	Transcriptional regulation	Transcriptional regulation
Prion phenotype	Loss of function	Heterologous prion appearance	Loss of function	Loss of function	Loss of function	Loss of function	Opposite of loss of function
Infectivity of fibers	Yes	Yes	Yes	Yes	ND	Yes	ND
Amyloid	Yes	Yes	Yes	Yes	ND	Yes	ND
QN-rich domain	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Variants isolated	Yes	Yes	Yes	ND	ND	ND	ND
Overproduction induces	Yes	ND	Yes	ND	Yes	Yes	Yes
Cured by *hsp104Δ*	Yes	Yes	Yes	Yes	Yes	Yes	No
Cured by GuHCl	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Found in wild	No	Yes	No	ND	ND	ND	ND

[a] From Cox (1965); Chernoff *et al.* (1993, 1995, 2000); Doel *et al.* (1994); Ter-Avanesyan *et al.* (1994); Wickner (1994); Derkatch *et al.* (1996); Glover *et al.* (1997); King *et al.* (1997); Resende *et al.* (2003); King and Diaz-Avalos (2004); Tanaka *et al.* (2004); and Nakayashiki *et al.* (2005).
[b] From Derkatch *et al.* (1997, 2001); Sondheimer *et al.* (2001); Bradley *et al.* (2002); Resende *et al.* (2003); Nakayashiki *et al.* (2005); and Patel and Liebman (2007).
[c] From Lacroute (1971); Wickner (1994); Moriyama *et al.* (2000); Schlumpberger *et al.* (2001); Brachmann *et al.* (2005); and Nakayashiki *et al.* 2005).
[d] From Du *et al.* (2008, 2010) and Crow *et al.* (2011).
[e] From Patel *et al.* (2009).
[f] From Alberti *et al.* (2009).
[g] From Volkov *et al.* (2002) and Rogoza *et al.* (2010).

taking up USA (Lacroute 1971). [*URE3*] can also be scored by a variety of other assays that do not require the presence of a *ura2* mutation (Moriyama *et al.* 2000; Schlumpberger *et al.* 2001; Brachmann *et al.* 2006; Hong *et al.* 2011).

In addition to the loss-of-function phenotypes, prions can also gain new functions. For example, as described below (see *Heterologous prion cross-seeding*), the presence of the prion form of the Rnq1 protein, called [*PIN*$^+$] (or [*RNQ*$^+$]) can enhance the chance that another prion will form *de novo*. Also, the presence of prions can be associated with toxicity (see *Biological Effects of Prions*).

Prions are inherited in a non-Mendelian fashion

Since prion aggregates capture and convert non-prion protein into the prion conformation, prion traits are dominant and have a chance of being inherited by all meiotic progeny. In addition, since prions seeds are located in the cytoplasm, they are efficiently transferred by cytoduction. Cytoduction entails the fusion of donor and recipient cells' cytoplasms without nuclear fusion and when the "donor" nucleus is lost (Conde and Fink 1976; Zakharov and Yarovoy 1977). Thus non-prion recipient cells become infected with prion when cytoduced with a prion-containing "donor" cell (Cox 1993).

Prion variants

The fact that isolates of mammalian prion diseases in otherwise genetically identical animals showed different stable and reproducible characteristics was a challenge for the "protein only" model of prion phenomena as this result suggested an explanation by viral mutations (Dickinson *et al.* 1968; Bruce and Dickinson 1987; Manuelidis 2003). The finding that the [*PSI*$^+$] prion also had different heritable states termed "variants" (Derkatch *et al.* 1996) paralleled the observations in mammals. However, yeast prion variants could not be explained by mutations as different variants could be induced by overproduction of the same protein in the same host.

Different [*PSI*$^+$] variants were associated with inherently different ratios of aggregated *vs.* non-aggregated Sup35 protein and therefore caused different degrees of loss of function (Figure 2). Thus, in the presence of the *ade1-14* nonsense marker, different variants of [*PSI*$^+$] cause distinct levels of translational readthrough resulting in characteristic levels of growth on –Ade medium and accumulation of red pigment associated with lack of Ade1. [*PSI*$^+$] variants that have a larger *vs.* smaller proportion of aggregated Sup35 protein and thus cause more *vs.* less translational readthrough are, respectively, called strong *vs.* weak [*PSI*$^+$] (Zhou *et al.* 1999; Uptain *et al.* 2001). When cells containing different variants of the same prion are mated, the prion variant that replicates more quickly and thus is more highly aggregated takes over the population (Bradley *et al.* 2002; Tanaka *et al.* 2006). Once a variant is established, it typically appears to be stable (Derkatch *et al.* 1996; Kochneva-Pervukhova *et al.* 2001). However, strong variants may rarely appear spontaneously in the weak variant background, and this could be facilitated by chemicals that selectively cure weak but not strong [*PSI*$^+$] (Shorter 2010).

Interestingly, different mutations in the prion domain have distinct effects on the phenotype of particular [*PSI*$^+$] variants (Derkatch *et al.* 1999; King 2001; Disalvo *et al.* 2011; Verges *et al.* 2011). Variants of other yeast prions, namely [*PIN*$^+$] (Bradley *et al.* 2002) and [*URE3*] (Schlumpberger *et al.* 2001), have also been described.

Correspondence between prions and amyloid aggregates

Considerable evidence indicates that the prion form of most proteins is an amyloid aggregate. The prion *vs.* non-prion forms of these proteins are protease K resistant and are

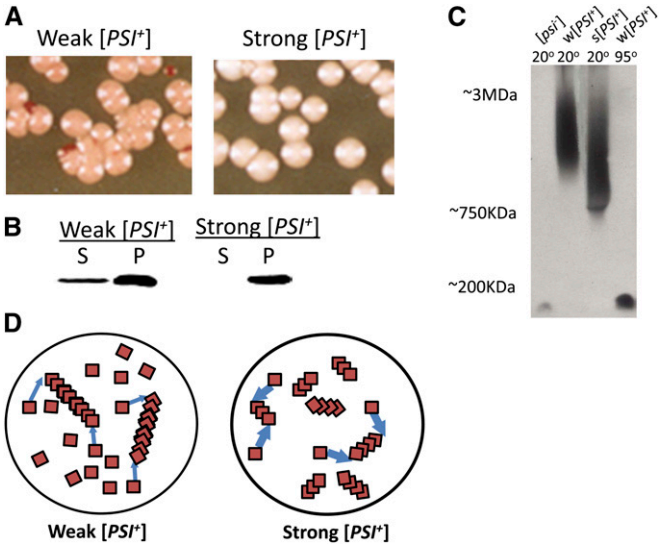

Figure 2 Differences between weak and strong [*PSI*$^+$] prion variants. (A) Colony color differences. The weak [*PSI*$^+$] strain is pink and gives rise to red ([*psi*$^-$]) colonies more frequently than the strong [*PSI*$^+$] strain that is whiter. (B) Levels of soluble Sup35 in weak *vs.* strong [*PSI*$^+$] strains. Shown is a Western blot of lysates from [*psi*$^-$] and [*PSI*$^+$] strains, separated by centrifugation into supernatant (S) and pellet (P) fractions and probed with Sup35 antibody. There is more soluble (and functional) Sup35 available in weak *vs.* strong [*PSI*$^+$] cells. (C) Size comparison of SDS-resistant Sup35 prion polymers determined with semidenaturing agarose gel electrophoresis. Lysates of [*psi*$^-$], weak (w) and strong (s) [*PSI*$^+$] cells were treated with 2% SDS at the indicated temperatures and run on a 1.5% agarose gel. Sup35 runs as a monomer in [*psi*$^-$] lysates and as a polymer smear in [*PSI*$^+$] lysates. The average molecular weight of polymers is larger in w[*PSI*$^+$] compared to s[*PSI*$^+$]. When [*PSI*$^+$] lysates are boiled, Sup35 polymers break down into monomers (see text and Derkatch *et al.* 1996; Patino *et al.* 1996; Kryndushkin *et al.* 2003; Bagriantsev *et al.* 2006). (D) Cartoon of weak *vs.* strong [*PSI*$^+$]. A few large Sup35 aggregates in weak [*PSI*$^+$] provide very few ends and so capture soluble Sup35 inefficiently. Many small Sup35 aggregates in strong [*PSI*$^+$] provide many ends that efficiently capture soluble Sup35.

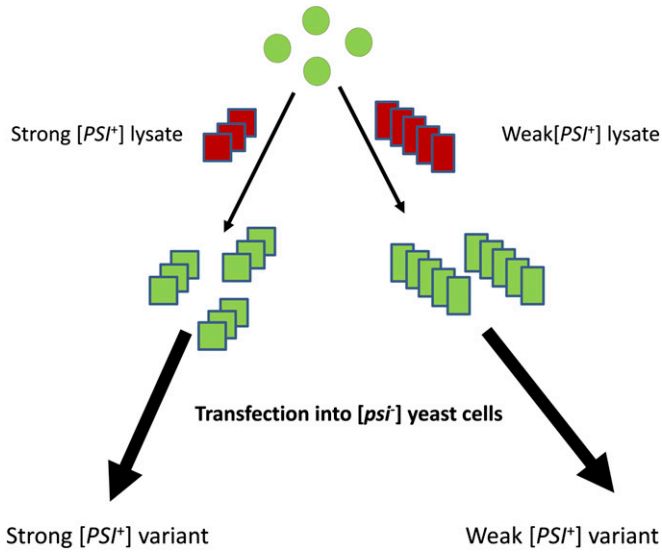

Figure 3 Proof of the "protein only" model for the Sup35 prion. A construct containing Sup35 PrD (green circles) was synthesized in and purified from *Escherichia coli*. Aggregated Sup35 protein (red rectangles) was purified from strong (smaller polymers composed of smaller squares) and weak (larger polymers composed of larger rectangles) [*PSI*$^+$] lysates by using a tagged PrD-derived Sup35 fragment. These aggregates were re-iteratively used to "seed" the *in vitro*-produced protein to eliminate any initial seed from the fibers. Resulting fibers were then transfected into [*psi*$^-$] yeast cells. This cartoon is based on King and Diaz-Avalos (2004). A slightly different version of the experiment produced the same conclusion in a back-to-back publication (Tanaka *et al.* 2004).

found preferentially in the pellet *vs.* supernatant fractions of cell lysates (Masison and Wickner 1995; Patino *et al.* 1996; Paushkin *et al.* 1996). Fusions of these prion proteins to fluorescent tags are diffuse in non-prion cells, but form punctate fluorescent dots in cells with the corresponding prion (Patino *et al.* 1996; Edskes *et al.* 1999). When lysates treated with detergent at room temperature are fractionated by centrifugation or passed through a filter, prion aggregates are precipitated or trapped by the filter because they are detergent resistant (Scherzinger *et al.* 1999). The detergent treatment dissolves large prion aggregates into polymers that can be separated on an agarose gel. The size range of the polymers found in cells is characteristic for different prion variants (Kryndushkin *et al.* 2003; Bagriantsev *et al.* 2006; Liebman *et al.* 2006). Interestingly, stronger [*PSI*$^+$] variants have smaller polymers than weaker [*PSI*$^+$] variants. The reasons for this will be discussed below (see *Requirements for Prion Propagation: Shearing and Segregation*). Additional *in vitro* and *in vivo* evidence that prions form amyloids is described in *Models of prion structures*.

Transfection of prions

Proof of "protein-only" infection by a prion required that purified prion aggregates added to a cell would cause infection. This was first demonstrated with Sup35 in *Saccharomyces cerevisiae* (Sparrer *et al.* 2000) and prion protein HET-s in the fungus *Podospora anserina* (Maddelein *et al.* 2002). However, since overexpression of a prion protein even if it is not in the infectious prion conformation will also induce *de novo* prion appearance at a high frequency (Wickner 1994; Masison and Wickner 1995; Derkatch *et al.* 1996), it was essential to distinguish infection from *de novo* induction. Since *de novo* prion appearance will include a variety of prion variants, the definitive proof required a demonstration that the prion protein infection was variant specific (Liebman 2002).

This was first done simultaneously by two groups. The C. King group (King and Diaz-Avalos 2004) used a tagged Sup35 fragment purified from cells propagating different [*PSI*$^+$] variants to seed *in vitro* fiber formation with bacterially expressed Sup35. These fibers, when sheared and introduced into [*psi*$^-$] cells, reproduced the initial [*PSI*$^+$] variants (Figure 3). In another version of this experiment, J. Weissman's group (Tanaka *et al.* 2004) used a bacterially expressed Sup35 fragment incubated at different temperatures to make fibers with distinct conformations that, when transfected into [*psi*$^-$] yeast, produced specific variants of [*PSI*$^+$]. Likewise, Ure2 fibers seeded *in vitro* with variant-specific

Table 2 Effects of chaperones and co-chaperones on prion propagation

Chaperone		Effect on [*PSI+*]		Effect on other prions	
Family	Protein/subfamily	Excess	Depletion or inactivation	Excess	Depletion or inactivation
Hsp100	Hsp104[a]	Cures	Cures	None	Cures all but [*ISP+*]
Hsp70	Ssa(1–4)[b]	Destabilizes Protects from ↑ 104	Destabilizes	Cures [*URE3*] (Ssa1, not Ssa2)	Cures [*URE3*] (Ssa2)
	Ssb(1,2)[c]	Aids ↑ 104 Destabilizes	Protects from ↑ 104	ND	ND
Hsp40	Sis1[d]	Aids ↑ 104	Antagonizes	ND[e]	Cures [*PIN+*]
	Ydj1[f]	ND	Does not cure	Cures [*URE3*][e]	Cures [*SWI+*]
	Apj1[g]	ND	ND	Compensates for *ydj1Δ*[e]	ND
Hsp90	Hsp82[h]	No effect	Protects from ↑ 104	ND	ND
NEF (for 70)	Sse1[i]	ND	Cures	Cures [*URE3*]	Cures [*SWI+*], [*URE3*]
	Fes1[i]	ND	Cures	Cures [*URE3*]	ND
Co-70/90	Sti1[j]	ND	Protects from ↑ 104	ND	ND
	Cpr7[j]	ND	Protects from ↑ 104	ND	ND

[a] From Chernoff *et al.* (1995); Derkatch *et al.* (1997); Moriyama *et al.* (2000); Du *et al.* (2008); Alberti *et al.* (2009); Patel *et al.* (2009); and Rogoza *et al.* (2010).
[b] From Newnam *et al.* (1999, 2011); Jung *et al.* (2000); Schwimmer and Masison (2002); Roberts *et al.* (2004); Allen *et al.* (2005); Sharma and Masison (2008); Mathur *et al.* (2009); and Sharma and Masison (2011).
[c] From Chernoff *et al.* (1999); Kushnirov *et al.* (2000b); Chacinska *et al.* (2001); and Allen *et al.* (2005).
[d] From Moriyama *et al.* (2000); Bradley *et al.* (2002); Sharma *et al.* (2009); and Hines *et al.* (2011).
[e] Overexpression of J-domain from Sis1, Ydj1, or Apj1 antagonizes [*SWI+*].
[f] From Sondheimer *et al.* (2001); Higurashi *et al.* (2008); Hines *et al.* (2011); and Kirkland *et al.* (2011).
[g] From Hines *et al.* (2011).
[h] From Newnam *et al.* (1999) and Reidy and Masison 2011).
[i] From Jones *et al.* (2004); Kryndushkin and Wickner (2007); Sadlish *et al.* (2008); and Hines *et al.* (2011).
[j] From Moosavi *et al.* (2010) and Reidy and Masison (2011).

[*URE3*] cell extracts infected [*ure-o*] cells with the corresponding [*URE3*] variant (Brachmann *et al.* 2005). *In vitro*-made fibers of a number of other yeast prions have also been shown to infect cells with the corresponding prion (see Table 1).

Requirements for Prion Propagation: Shearing and Segregation

Role of Hsp104 in prion propagation

While prion proteins can generate and propagate an amyloid state *in vitro* in the absence of any other cofactors, *in vivo* propagation of yeast prions depends on the chaperone machinery. The Hsp104 chaperone, a homohexameric AAA ATPase, is required for the propagation of [*PSI*$^+$] (Chernoff *et al.* 1995). Deletion of *HSP104* eliminates [*PSI*$^+$], and dominant negative *HSP104* mutations antagonize [*PSI*$^+$]. Hsp104 is also required for propagation of the other proven amyloid-based yeast prions (see Chernoff 2007; Rikhvanov *et al.* 2007; Romanova and Chernoff 2009; Reidy and Masison 2011), with the exception of [*ISP*$^+$] (Rogoza *et al.* 2010) and, possibly, the [*PHI*$^+$] prion that is based on an artificially engineered derivative of Sup35 (Crist *et al.* 2003). The effects of Hsp104 on yeast prions are summarized in Table 2.

Hsp104 and its bacterial ortholog, ClpB, are implicated in disaggregation of stress-damaged proteins (see Glover and Lum 2009). It was proposed that Hsp104 promotes fragmentation of prion fibers into smaller seeds, initiating new rounds of prion propagation (Paushkin *et al.* 1996; Kushnirov and Ter-Avanesyan 1998). Indeed, a decrease in Hsp104 activity results in the accumulation of larger Sup35 prion aggregates (Wegrzyn *et al.* 2001; Satpute-Krishnan *et al.* 2007), composed of longer SDS-resistant polymers (Kryndushkin *et al.* 2003). Excess Hsp104 promotes, rather than counteracts, propagation of Sup35 prion variants with abnormally large aggregates, generated by altered Sup35 protein (Borchsenius *et al.* 2001) or selected at high levels of Hsp104 (Borchsenius *et al.* 2006).

These data are consistent with the view that the crucial role of Hsp104 in prion propagation is prion fragmentation. One possibility is that Hsp104 breaks prion polymers by pulling individual protein molecules from the middle of the polymer through the central pore of the Hsp104 hexamer. This is how Hsp104 and its bacterial ortholog, ClpB (Weibezahn *et al.* 2004; Glover and Lum 2009), solubilize aggregated stress-damaged proteins. Indeed, a modified version of Hsp104, HAP, or the modified Hsp104-ClpB chimera, 4BAP, which contains a docking site for the inactive bacterial protease ClpP and is able to capture protein molecules pulled from aggregates, can capture Sup35 in [*PSI*$^+$] cells (Tessarz *et al.* 2008; Tipton *et al.* 2008). On the other hand, the location of some Hsp104 mutations that affect prion

propagation, but not the solubilization of stress-damaged proteins and thermotolerance, along the lateral channel of the hexamer rather than central pore (see Romanova and Chernoff 2009) suggests that the lateral channel interacts with prions (Kurahashi and Nakamura 2007). Some data indicate that Hsp104 alone can promote fragmentation of prion fibers *in vitro* (Shorter and Lindquist 2004), while results of other groups disagree (Inoue *et al.* 2004; Krzewska and Melki 2006). In any case, *in vivo* effects of Hsp104 are strongly influenced by other chaperones, as discussed below.

Overproduction of Hsp104 causes loss of [*PSI*$^+$] (Chernoff *et al.* 1995) but not of the other known prions. It was proposed that excess Hsp104 eliminates [*PSI*$^+$] by disaggregating prions to monomers (Paushkin *et al.* 1996; Kushnirov and Ter-Avanesyan 1998). Indirectly, this hypothesis is supported by the observation that Sup35 overproduction, leading to an increase in aggregate size, partly ameliorates the curing effect of excess Hsp104 (Borchsenius *et al.* 2006). Also, *in vitro*, a huge excess of Hsp104 leads to the "remodeling" of Sup35-based amyloids, resulting in the loss of their ability to transmit the prion state via transfection, while Ure2-based amyloids retain infectivity (Shorter and Lindquist 2006). Another possibility could be that excess Hsp104 prevents Sup35 monomers from efficiently joining larger polymers. However, the reverse effect is also possible: if Hsp104 overproduction causes an increase rather than a decrease in the size of Sup35 polymers, this might impair prion propagation as well. Indeed, weak [*PSI*$^+$] variants that are less efficiently fragmented by Hsp104 than by strong [*PSI*$^+$] under normal conditions (see *Role of polymer growth and fragmentation in determining differences between prion variants*) are more sensitive to Hsp104 overproduction than to strong [*PSI*$^+$] (Derkatch *et al.* 1996). Also, Hsp104 overproduction results in an increase in the size of the remaining Sup35 polymers as soluble Sup35 monomers accumulate (Kryndushkin *et al.* 2003). However, this latter result could also be explained if the larger molecular weight polymers are retained due to their greater resistance to Hsp104. Finally, since [*PSI*$^+$] curing by excess Hsp104 requires the N-terminal region of Hsp104 that is not required for prion propagation (Hung and Masison 2006), it appears that Hsp104-mediated shearing is not sufficient for prion curing by excess Hsp104. Overall, the mechanism by which excess Hsp104 antagonizes [*PSI*$^+$] and the reasons for differential sensitivities of yeast prions to excess Hsp104 remains unclear. One hypothetical model will be discussed below (see *Prion segregation at cell division*).

Hsp104 is conserved in many organisms other than *Saccharomyces*, although multicellular animals do not appear to have an orthologous cytoplasmic protein (see Rikhvanov *et al.* 2007; Romanova and Chernoff 2009). However, mammalian cells do exhibit induced thermotolerance (Li *et al.* 1995), which is controlled by Hsp104 in other organisms. The ability of Hsp104 to support prion propagation is conserved in some but not all species: Hsp104 from *Candida albicans* (Zenthon *et al.* 2006) but not from *Schizosaccharomyces pombe* (Senechal *et al.* 2009) supports the propagation of [*PSI*$^+$] in *S. cerevisiae*. Intriguingly, the *C. albicans* Sup35 protein can acquire a prion state in *S. cerevisiae* cells (Santoso *et al.* 2000; Resende *et al.* 2002), while the *S. pombe* Sup35 protein lacks a prion domain (Ito *et al.* 1998; Kong *et al.* 2004).

Role of other Hsps

The yeast Hsp70 and Hsp40 chaperones are also implicated in prion propagation (see Rikhvanov *et al.* 2007; Romanova and Chernoff 2009; Reidy and Masison 2011). Data on Hsp70 effects are summarized in Table 2. Yeast contains two major cytosolic Hsp70 subfamilies, namely Ssa (working with the Hsp40 co-chaperones Ydj1 and Sis1) and Ssb (working with Hsp40-Zuo1 and Hsp70-related co-chaperone Ssz1). Ssa is encoded by four genes (constitutive *SSA2*, moderately expressed and stress-inducible *SSA1*, and strictly stress-inducible *SSA3* and *SSA4*), of which at least one must be present for viability. Ssb, encoded by two genes, *SSB1* and *SSB2*, is nonessential, not heat inducible, ribosome associated, and implicated in folding of nascent polypeptides. Remarkably, Ssa and Ssb exhibit opposite effects on the [*PSI*$^+$] prion (Chernoff *et al.* 1999; Newnam *et al.* 1999; Allen *et al.* 2005): Ssa overproduction partly protects [*PSI*$^+$] from curing by excess Hsp104, while Ssb overproduction enhances curing, and deletion of both *SSB* genes, *ssb1Δ ssb2Δ*, counteracts curing (Chernoff *et al.* 1999). Ssa overproduction (Newnam *et al.* 1999; Allen *et al.* 2005) or *ssb1Δ ssb2Δ* deletion (Chernoff *et al.* 1999) also increases translational readthrough in [*PSI*$^+$] strains and promotes *de novo* [*PSI*$^+$] formation (see *De Novo Prion Formation*). Experiments with chimeric proteins indicate that the peptide-binding domain of Hsp70 is responsible for the differences in the effects of Ssa and Ssb on [*PSI*$^+$] (Allen *et al.* 2005).

Interestingly, overproduction of Ssa may also antagonize [*PSI*$^+$] propagation, as seen for some [*PSI*$^+$] variants, formed by altered (Borchsenius *et al.* 2001) or wild type Sup35 (Borchsenius *et al.* 2006) that are aided (rather than cured) by excess Hsp104, and for other [*PSI*$^+$] variants in the presence of overproduced Sup35 (Allen *et al.* 2005) and/or in the presence of another prion, [*PIN*$^+$] (Mathur *et al.* 2009). In the latter case, the [*PSI*$^+$] curing effect of excess Ssa was linked to an increase in the size of cytologically detectable Sup35 aggregates, leading to decreased transmissibility of these aggregates in mitotic divisions. At a molecular level, excess Ssa increases both the size of Sup35 polymers and the proportion of non-aggregated Sup35 (Allen *et al.* 2005). Ssa physically interacts with Sup35 (Allen *et al.* 2005) and was identified as a major non-Sup35 component associated with [*PSI*$^+$] aggregates *in vivo* (Bagriantsev *et al.* 2008). Excess Ssb also antagonizes weak variants of [*PSI*$^+$] upon prolonged propagation (Kushnirov *et al.* 2000b; Chacinska *et al.* 2001) or other [*PSI*$^+$] variants in the presence of excess Sup35 (Allen *et al.* 2005).

Mutation in *SSA1* was shown to antagonize [*PSI*$^+$] propagation (Jung *et al.* 2000), and deletion of *SSA2*, responsible

for the major fraction of Ssa in exponentially growing cells, destabilizes a weak [*PSI*$^+$] variant (Newnam *et al.* 2011). Overall, effects of the *ssa* mutations on [*PSI*$^+$] resemble effects of Hsp104 overproduction (see Reidy and Masison 2011). It appears that consequences of chaperone action on Sup35 aggregates depend on the balance between Hsp104 and Ssa, rather on the amount of each of these proteins *per se*. Indeed, [*PSI*$^+$] is destabilized following short-term heat shock when Hsp104 levels increase more quickly than levels of other Hsp's including Ssa, while longer incubation at high temperature, resulting in partial restoration of the Hsp104/Ssa balance, leads to restoration of [*PSI*$^+$] stability (Newnam *et al.* 2011). Deletions of individual *SSA* genes increase [*PSI*$^+$] destabilization by short-term heat shock and impair [*PSI*$^+$] recovery after longer heat shock, confirming the role of Hsp104/Ssa balance in prion maintenance during and after stress.

Each member of the Ssa family acts on [*PSI*$^+$] in the same direction when it is overexpressed (Allen *et al.* 2005); however, differential effects are detected when each Ssa protein is expressed individually in cells lacking Ssa proteins (Sharma and Masison 2008). Ssa proteins also differ from each other in their effects on other prions. For example, [*URE3*] is cured by Ssa1 overproduction but not by Ssa2 overproduction (Schwimmer and Masison 2002), with a single amino acid change at position 83 being responsible for these differences (Sharma and Masison 2011). Mutation in *SSA2* also antagonizes [*URE3*] (Roberts *et al.* 2004).

Regarding the effects of Hsp40 proteins, much of our knowledge comes from studying prions other than [*PSI*$^+$] (see Table 2). The evidence implicating Ydj1 (Moriyama *et al.* 2000; Sharma *et al.* 2009; Hines *et al.* 2011) and Sis1 (see below) in prion propagation is unambiguous. As Sis1 is essential (see Rikhvanov *et al.* 2007), its effect on prions were studied with mutants, internal deletions, or transient depletions, rather than complete disruptions. Intact Sis1 is required for the maintenance of [*PIN*$^+$] (Sondheimer *et al.* 2001) and [*URE3*] (Higurashi *et al.* 2008). Sis1 also aids in [*PSI*$^+$] propagation (Higurashi *et al.* 2008) and promotes [*PSI*$^+$] curing by excess Hsp104 (Kirkland *et al.* 2011). Sup35 capture by the above-mentioned "trapping" derivative of Hsp104, 4BAP, depends upon Sis1 (Tipton *et al.* 2008). This suggests that Sis1 is responsible for recruiting Hsp104 and possibly Ssa to prion polymers. However, the substrate-binding region of Sis1 is dispensable for [*PSI*$^+$] propagation, contradicting this model (Kirkland *et al.* 2011). Whatever the specific mechanisms of interactions, it is clear that members of both Hsp70 and Hsp40 chaperone families, apparently working together with Hsp104, play crucial roles in prion propagation.

Less is known about whether chaperones other than Hsp104, Hsp70, and Hsp40 influence prions. Elimination or overproduction of the Hsp70 nucleotide exchange factors (NEFs) Fes1 or Sse1 (Jones *et al.* 2004; Kryndushkin and Wickner 2007; Sadlish *et al.* 2008; Hines *et al.* 2011) affects [*PSI*$^+$], [*URE3*], and [*SWI*$^+$] prions. Mutation analysis suggests that NEFs act on prions via regulating Ssa. Nucleotide exchange is needed for Hsp70 to release substrates. Thus it seems that Ssa acts on prions by binding and releasing them, just as it binds and releases other misfolded proteins. Chemical inhibition of Hsp82, a yeast counterpart of the Hsp90 chaperone, or deletions of the genes encoding the Hsp70/Hsp90 co-chaperones, Sti1 or Cpr7, counteract [*PSI*$^+$] curing by excess Hsp104 (Moosavi *et al.* 2010; Reidy and Masison 2011); however, these effects could be mediated by Ssa, as Hsp82 deficiency increases Ssa levels.

Importantly, Hsp104, Hsp70-Ssa, and Hsp40s (Sis1 and Ydj1) represent the major complex involved in disaggregation and refolding of stress-damaged proteins (Glover and Lindquist 1998; Glover and Lum 2009). Hsp70 and Hsp40 components of this complex are conserved in other organisms, including humans (see Rikhvanov *et al.* 2007), suggesting that these data likely have implications for mammalian amyloids as well. It is remarkable that the same chaperone machinery is employed in protection against environmental stresses and in modulating amyloid propagation. Apparently, effects of molecular chaperones on prions are based on the same enzymatic activities that are involved in their interactions with other misfolded and/or aggregated proteins. However, the highly ordered nature of prion aggregates increases their resistance to the Hsps' action and therefore alters consequences of the aggregate/Hsp interaction. Instead of eliminating an aggregate, Hsps (at least at certain levels or, more likely, in certain ratios) promote fragmentation, which multiplies aggregated seeds and thus facilitates prion propagation. Such a unique response to chaperone action makes aggregates capable of behaving in a prion fashion *in vivo*. Therefore, it is this specific mode of interaction with the chaperone machinery that makes a yeast protein aggregate a prion.

Role of polymer growth and fragmentation in determining differences between prion variants

During prion propagation, mature protein molecules (rather than only newly synthesized ones) can be remodeled to join pre-existing prion polymers (Satpute-Krishnan and Serio 2005). Efficiency of polymer fragmentation by chaperones relative to polymer growth explains phenotypic differences between prion variants (Tanaka *et al.* 2006). Polymers of strong [*PSI*$^+$] variants are readily fragmented and therefore produce a larger number of prion units per cell. As termini of prion polymers are active in attracting new protein molecules to the polymers, a larger number of polymers results in the more efficient immobilization of newly synthesized Sup35 protein into polymers. In contrast, polymers of weak [*PSI*$^+$] variants are less efficiently fragmented, resulting in fewer polymer ends and less efficient capture of new Sup35 molecules (see also Figure 2D). This explains why weaker [*PSI*$^+$] variants are characterized by a larger average polymer size (Kryndushkin *et al.* 2003) and a higher proportion of non-aggregated Sup35 protein (Zhou *et al.* 1999; Uptain *et al.* 2001), leading to a less severe defect in termination (Derkatch *et al.* 1996) when compared to stronger [*PSI*$^+$]

variants. Sensitivity to chaperones depends on the physical properties of polymers controlled by the conformation of the prion aggregate. Apparently, a large amyloid core (Toyama *et al.* 2007) makes weak prion variants more physically stable and less accessible to Hsps than strong prion variants with a smaller amyloid core. Thus, biologically weak prion variants (as judged from phenotype and transmissibility) are based on polymers that are physically stronger. Amyloids that are absolutely stable and rigid *in vivo* would not produce new "seeds" and therefore would not be expected to behave as prions.

Prion segregation at cell division

The mitotic stability of prions requires that prions segregate to daughter cells. Guanidine hydrochloride (GuHCl), a compound that blocks prion propagation, has been employed to analyze prion segregation in mitosis. GuHCl was initially described as an antagonist of [*PSI*$^+$] (Tuite *et al.* 1981; Cox *et al.* 1988) and was later shown to antagonize all other known amyloid-based yeast prions (reviewed in Cox *et al.* 2007; Halfmann and Lindquist 2010). The effect on [*PSI*$^+$] is best understood. [*PSI*$^+$] curing by millimolar concentrations of GuHCl occurs only in proliferating cells (Eaglestone *et al.* 1999; Byrne *et al.* 2007). GuHCl neither prevents Sup35 aggregation nor destroys aggregates (Ferreira *et al.* 2001; Ness *et al.* 2002). Rather, it blocks the fragmentation of existing prion units, thereby preventing the generation of new prion units. This leads to the dilutions of prion units as the cells divide, and eventually daughter cells do not inherit any prion units. GuHCl antagonizes Hsp104-induced thermotolerance *in vivo* (Ferreira *et al.* 2001; Jung and Masison 2001) and inhibits the ATPase activity of Hsp104 *in vitro* (Grimminger *et al.* 2004), suggesting that its effect on prions is also primarily due to inhibition of Hsp104. This was confirmed by the identification of a mutation in Hsp104 that makes [*PSI*$^+$] much less sensitive to the curing effect of GuHCl (Jung *et al.* 2002). However, differences in kinetics of [*PSI*$^+$] loss in the presence of GuHCl and after direct Hsp104 inactivation by genetic manipulations (Wegrzyn *et al.* 2001; Chernoff 2004b) suggest that the picture could be more complex. Indeed, the [*ISP*$^+$] prion, which does not require Hsp104 for its propagation, is curable by GuHCl (Rogoza *et al.* 2010). Thus it appears that GuHCl also acts on other targets influencing prion propagation, in addition to Hsp104. Whatever the molecular specifics of GuHCl action, its ability to block the generation of new proliferating prion units (termed "propagons") can be used to count the number of propagons in a yeast cell.

The number of propagons in a cell can be derived from the number of cell divisions needed for prion loss in the presence of GuHCl or by determining the number of cells that retain a [*PSI*$^+$] seed in a colony derived from a single [*PSI*$^+$] cell grown in the presence of GuHCl (Cox *et al.* 2003, 2007). One caveat with these methods is that seeds are preferentially retained by mother cells (Byrne *et al.* 2009). While cell-to-cell variation in propagon numbers (from several to more than a thousand per cell) was uncovered in yeast cultures, strong prion variants are characterized by a larger average number of propagons per cell, compared to weak prion variants. This agrees with the fragmentation model (see above, *Role of polymer growth and fragmentation in determining differences between prion variants*) and accounts for differences in mitotic stability.

Yeast cultures bearing a weak [*PSI*$^+$] variant exhibit asymmetric accumulation of larger prion polymers in aged cells (Derdowski *et al.* 2010). It was proposed that larger polymers are less likely to be transmitted to a daughter cell (bud) during mitosis. Loss of weak [*PSI*$^+$] in the first cell division after heat shock (Newnam *et al.* 2011) also preferentially occurs in daughters. The size of prion polymers is increased during heat shock, and this may contribute to the asymmetry. However, it seems unlikely that increased polymer size *per se* represents a mechanical threshold for transmission, as even much larger intracellular structures are transmitted from the mother cell to the bud. It is worth noting that non-prion protein aggregates produced during heat shock, *e.g.*, agglomerates of oxidatively damaged proteins, are also preferentially accumulated in the mother cell (Aguilaniu *et al.* 2003; Liu *et al.* 2010). Hsp104 binds these agglomerates and plays a crucial role in their mother-specific accumulation (Erjavec *et al.* 2007; Tessarz *et al.* 2009). Decreased diffusion of the larger aggregates through the budneck, coupled with more efficient solubilization of aggregates in the bud, was suggested as an explanation for the asymmetry (Zhou *et al.* 2011). However, other evidence indicates that at least some aggregates either are trapped in a scaffold of actin cables in the mother cell or are subject to active retrograde transport back to the mother from the growing bud, involving the polarisome and the actin cytoskeletal network (Liu *et al.* 2010, 2011). Cytoskeletal structures are linked to prion segregation as well. For example, weak variants of [*PSI*$^+$] are destabilized after prolonged disruption of actin cytoskeleton by latrunculin A (Bailleul-Winslett *et al.* 2000), and deletion of the gene coding for actin assembly protein Lsb2 increases [*PSI*$^+$] destabilization by heat shock (Chernova *et al.* 2011). Overexpression of Btn2 or Cur1, yeast homologs of mammalian microtubule-associated Hook proteins involved in organelle transport (Walenta *et al.* 2001), cures [*URE3*] prion, possibly by impairing its segregation (Kryndushkin *et al.* 2008).

One possibility is that Hsp104, working in a stoichiometric combination with Ssa and its Hsp40 co-chaperones, fragments prion polymers *in vivo*, while Hsp104 in imbalance with Ssa directs the association of prion polymers with the cytoskeletal networks, resulting in the mother cell-specific retention and/or retrograde transport. Thus interplay between polymer fragmentation, diffusion into the daughter cell, retention by the mother cell, and/or retrograde transport back to the mother cell regulates prion segregation. In this model, Hsp104 promotes the retention and/or retrograde transport of aggregates when it cannot break them efficiently. Therefore, larger polymers that are less sensitive

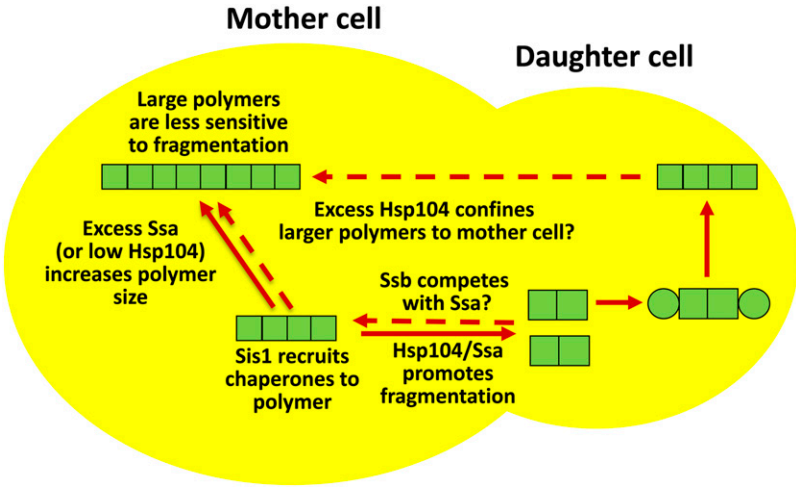

Figure 4 Regulation of [*PSI*+] propagation and segregation by chaperones. Solid lines represent proven effects—dashed lines, hypothesized effects. Sis1 is responsible for prion recognition and chaperone recruitment, while Hsp104 promotes prion fragmentation and resulting prion propagation when working together with Ssa. Also, Hsp104 is postulated to promote prion retention in the mother cell and/or retrograde transport from daughter to mother cell, leading to asymmetric segregation in cell divisions when present in imbalance with Ssa. Ssb counteracts the effects of Ssa. See more detailed comments in *Prion segregation at cell divisions.*

to Hsp104-mediated breakage would be more likely to be accumulated in the mother cell. Such a process would be adaptive as it protects daughter cells from aggregates at the expense of the aged mothers. It is possible that the same mechanism contributes to [*PSI*$^+$] curing by plasmid-mediated overproduction of Hsp104 (see above) and that the N-terminal domain of Hsp104, required for curing, is involved in the interactions promoting prion retention and/or retrograde transport. Further experiments are needed to prove or disprove this model. The role of Hsp104 in [*PSI*$^+$] propagation is summarized on Figure 4.

Structural Organization of Prions

Prion domains

Yeast prion proteins contain regions, termed "prion domains" (PrDs), that are required for formation and propagation of the prion state and can maintain the prion state even without the rest of the protein (Ross *et al.* 2005b; Inge-Vechtomov *et al.* 2007). When the major cellular function of the prion protein is known, PrD is typically dispensable for this function. However, PrDs may have functions other than prion formation; *e.g.*, Sup35 PrD (Sup35N) is implicated in interactions with poly (A)-binding protein, influencing mRNA stability (Hosoda *et al.* 2003). Interestingly, Sup35 also contains a middle region (Sup35M) linking PrD to the C-proximal release factor domain. Sup35M is enriched in charged residues and is suspected of helping to maintain a balance between aggregated and non-aggregated states, possibly via interaction with Hsp's (Liu *et al.* 2002). Indeed, Sup35M interacts with Hsp104 *in vitro* and is involved in [*PSI*$^+$] curing by excess Hsp104 *in vivo* (Helsen and Glover 2012). Yeast PrDs may confer a prion state to a different protein when fused to it artificially. Features of some yeast PrDs are shown in Figure 5.

Generally, the yeast prion PrDs known to date are intrinsically disordered in solution and QN-rich. Typically, they are more N- than Q-rich (*e.g.*, Alberti *et al.* 2009), and "minimal" PrDs may contain no Qs (Crow *et al.* 2011). Substitution of Qs for Ns increases, while substitution of Ns for Qs decreases, prion propagation by a given protein (Halfmann *et al.* 2011). "Scrambled" PrDs of Ure2 or Sup35, maintaining amino acid composition but not exact sequence, are typically capable both of generating amyloid *in vitro* [albeit with altered rates (Liu *et al.* 2007)] and prion *in vivo* and of propagating the prion state, indicating that amino acid composition plays the primary role in prion properties (Ross *et al.* 2004, 2005a). Mutational analysis of a short amino acid stretch within a certain "scrambled" Sup35 PrD suggested that prion propagation propensity could be increased by exclusion of "order-promoting" residues (even if they have amyloidogenic potential) and enrichment with "disorder-promoting" residues (Toombs *et al.* 2010). The universality of these rules is still to be determined. The N-proximal PrD region of *S. cerevisiae* Sup35 includes an N-terminal QN-rich stretch, located within the first 40 amino acids, and a region of 5.5 imperfect oligopeptide repeats (ORs), which somewhat resembles repeats of mammalian PrP and are located between positions 41 and 97. The PrD fragment required for aggregation is shorter than the fragment needed for efficient propagation of the prion state (Borchsenius *et al.* 2001) and is primarily confined to the QN-rich stretch (Osherovich *et al.* 2004). It was proposed that the Sup35 (as well as New1) PrD can be divided into "aggregation" (QN stretch) and "propagation" (ORs) elements (Chernoff 2004a; Osherovich *et al.* 2004) and that the propagation element is involved in interaction with Hsp104 (see *Requirements for Prion Propagation: Shearing and Segregation*). OR expansion increases *de novo* [*PSI*$^+$] generation (Liu and Lindquist 1999) although Ure2 or "scrambled" Sup35 PrDs lack ORs, indicating that ORs are not necessary for interaction with the chaperones responsible for prion propagation (Ross *et al.* 2005b; Toombs *et al.* 2011). Perhaps ORs are frequently associated with prions because the duplication events that generate them also extend the size of the regions with the amino acid compositions conducive to prion formation.

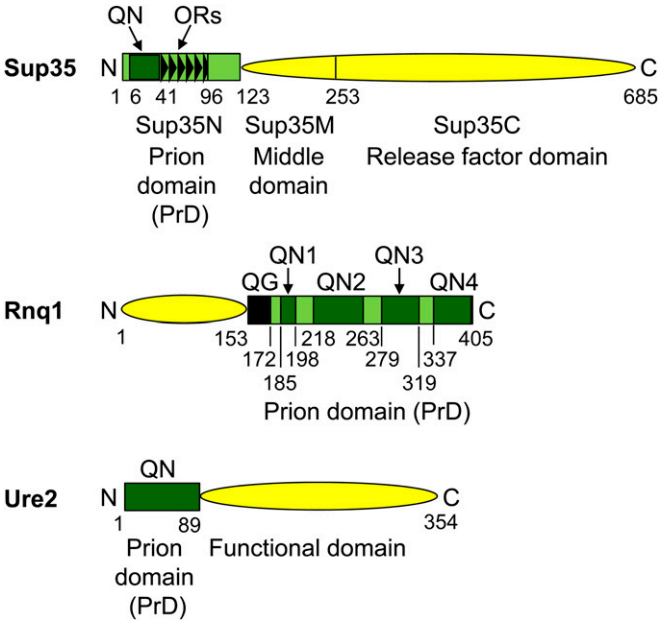

Figure 5 Examples of yeast prion domains. Prion domains are shown as green rectangles and non-prion regions as yellow ellipses. QN corresponds to a glutamine/asparagine-rich region; ORs to oligopeptide repeats (in Sup35); QG to polymorphic glutamine/glycine repeats (in Rnq1); and N and C to amino- and carboxyl-termini of proteins, respectively. Numbers indicate amino acid positions. Boundaries between the Sup35N and Sup35M regions are shown arbitrarily (different publications place them between amino acid residues 100 and 137).

In the case of the Rnq1 protein, four QN-rich stretches were found within the PrD (Kadnar *et al.* 2010). While none of these stretches was essential for prion propagation, two of four stretches were each shown to support prion maintenance if retained alone. Multiple stretches exhibit a cooperative effect on prion maintenance: one stretch, not capable of maintaining the prion state on its own, was needed to propagate some but not the other [*PIN*$^+$] variants, and this was confirmed by mutagenesis experiments (Bardill and True 2009; Stein and True 2011). The mosaic organization of Rnq1 PrD confirms that different sequence elements contribute to prion properties.

Models of prion structures

To fully understand how prions form and propagate, we must know the structure of both the prion and the non-prion states of the protein. In addition, an appreciation of how prion variants arise from different heritable structures requires a comparison of these different structures. Here we concentrate on efforts to determine the structure of protein aggregates in their prion state.

Unfortunately, the traditional approaches of X-ray crystallography and solution NMR are not suitable to solve the structure of prions because their filamentous nature prevents them from forming crystals, and they are too large to be soluble. Instead, solid-state NMR (*e.g.*, Shewmaker *et al.* 2006), H/D exchange (*e.g.*, Toyama *et al.* 2007), electron paramagnetic resonance (Tanaka *et al.* 2004, 2005), and fluorophore labeling (Krishnan and Lindquist 2005) have been used to investigate the structure of yeast prions.

Considerable controversy exists on this topic. The following reviews favor different prion structural models (Tessier and Lindquist 2009; Bockmann and Meier 2010; Wickner *et al.* 2010; Wickner *et al.* 2011).

Divide and conquer—determining the structure of prion and non-prion domains separately: The task of determining the structure of prions is made easier if one assumes that the prion and non-prion domains do not have a major effect on each other's structure. Indeed, to date, most studies have separately investigated the structures of prion and non-prion domains (Bousset *et al.* 2001a,b; Umland *et al.* 2001; Tanaka *et al.* 2004, 2005; Krishnan and Lindquist 2005; Shewmaker *et al.* 2006, 2009a; Toyama *et al.* 2007; Wickner *et al.* 2008a; Chen *et al.* 2009; Engel *et al.* 2011). However, this approach has been questioned because Cys scanning and disulfide bond data suggest that the prion and non-prion domains of Ure2 interact when Ure2 fibers are made under native conditions (Fay *et al.* 2005). Also, the fact that mutations in non-prion domains can affect prion generation and propagation suggests that there might be some interaction between the domains (Masison and Wickner 1995; Maddelein and Wickner 1999; Shibata *et al.* 2009; Chen *et al.* 2011; Kabani *et al.* 2011; Kurahashi *et al.* 2011).

Despite these observations, interactions between prion and non-prion domain regions appear to have minor, if any, effects on their structures. Indeed, non-prion domains have been shown to retain their structure and activity even within the prion aggregate (Baxa *et al.* 2002, 2004, 2011; Bai *et al.* 2004; Krzewska *et al.* 2007; Zhang *et al.* 2008; Zhang and Perrett 2009). More importantly, amyloid fibers of PrD fragments are infectious when transfected into non-prion-containing cells (King and Diaz-Avalos 2004; Tanaka *et al.* 2004; Brachmann *et al.* 2005; Diaz-Avalos *et al.* 2005; Patel and Liebman 2007; Du *et al.* 2008, 2010; Alberti *et al.* 2009). Although it has been hypothesized that the fibers induce prion formation *de novo* by titrating away chaperones rather than by seeding (Bousset *et al.* 2010), retention of specific prion variants after transfection indicates that the *in vitro*-generated PrD material is in the infectious prion conformation. Studies demonstrating that the introduction of even a single Sup35NM fiber into a cell can cause the appearance of [*PSI*$^+$] (see figure S1 in Tanaka *et al.* 2006) are consistent with the idea that fibers, rather than some contaminating PrD structures, are the infectious material. This validates the relevance of the models derived from studies of PrD amyloid fibers.

Yeast prions are amyloid: *In vitro data:* A purified prion protein or fragments containing its PrD often form mixtures of amyloid fibers with various morphologies (Glover *et al.* 1997; Diaz-Avalos *et al.* 2005). Some fibers are twisted, others straight, and the twists have different radii and stiffnesses. These fibers bind dyes indicative of amyloid and

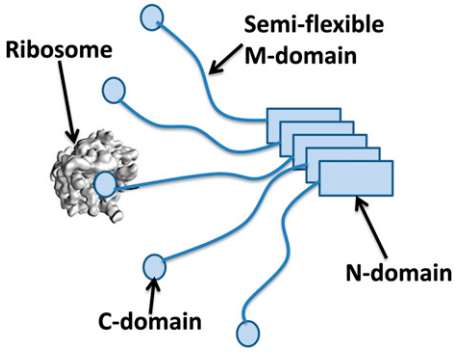

Figure 6 The cross section of a Sup35 fiber. The rectangles represent the N domains in the core. Extended and flexible M domains connect the core to the C domains that have enough space to interact with ribosomes (shown to scale). Adapted from Baxa *et al.* (2011).

have the 4.7-Å X-ray diffraction reflection characteristic of a cross-β structure and diagnostic of amyloid (Glover *et al.* 1997; King *et al.* 1997; Schlumpberger *et al.* 2000; Kishimoto *et al.* 2004; Baxa *et al.* 2005; Castro *et al.* 2011). There is also some evidence that Sup35 prion domain fibers seeded by different [*PSI* $^{+}$] variants have slightly different mass per unit length, all hovering around one molecule per 4.6 Å (Diaz-Avalos *et al.* 2005). Indeed, a larger core region (residues 2–73) was solvent-protected in fibers made at 37° (corresponding to a weak [*PSI*$^{+}$] variant) *vs.* a smaller core for fibers made at 4° (corresponding to a strong [*PSI*$^{+}$] variant) (Toyama *et al.* 2007). Recently, fibers in the weak *vs.* strong [*PSI*$^{+}$] conformation have been shown to have an approximately two-fold difference in average stiffness (Castro *et al.* 2011). Thus it appears that fiber properties differ for different prion variants.

Using cryo-electron microscopy (cryo-EM) and scanning transmission EM, both Ure2 and Sup35-1-61-GFP fibers were found to have a central small core with globular appendages. Fibers made of only PrD lack the globular appendages. When fibers made of full-length protein were digested with protease, the core region that remained undigested corresponded to the PrD (Baxa *et al.* 2003; Baxa *et al.* 2005; Diaz-Avalos *et al.* 2005; Kryndushkin *et al.* 2011a). Recently, Sup35 fibers have been found to have an ~8-nm core with a 65-nm cloud of the globular C-region domain extending far enough from the core to allow the C domain to interact with the ribosome (Figure 6) (Baxa *et al.* 2011).

Despite the above evidence, controversy over whether the prion form of Ure2 is amyloid remains (Bousset *et al.* 2002b, 2004; Ripaud *et al.* 2004; Fay *et al.* 2005; Redeker *et al.* 2007). A minority view is that the Ure2 globular domain is part of the fiber core because this domain is more ordered in *in vitro* Ure2 fibers made of full-length Ure2 *vs.* the PrD alone (Loquet *et al.* 2009).

In vivo data: While it is more difficult to prove the existence of amyloid inside cells, fiber-like structures resembling amyloids have been detected *in vivo*. Ure2 fibers were identified in [*URE3*] cells overexpressing Ure2 with EM (Speransky *et al.* 2001). [*PSI*$^{+}$] and [*PIN*$^{+}$], but not [*psi*$^{-}$] or [*pin*$^{-}$], cells were shown to be stained by the dye thioflavin-S (Kimura *et al.* 2003) that binds amyloid. EM analysis of Sup35 polymers isolated from [*PSI*$^{+}$] lysates showed them to be composed of ~20-nm-wide barrels and other larger structures (Bagriantsev *et al.* 2008). The fluorescent rings and dots formed in the process of prion induction by overexpressed Sup35-GFP (see *Prion induction by overproduction*) were shown to be made of fibrils (Tyedmers *et al.* 2010). Also, fibrils that look like those formed *in vitro* have been seen in [*PSI*$^{+}$] cells by EM in large dot-and-line aggregates as well as in diffuse structures in the cytoplasm (Kawai-Noma *et al.* 2010).

Specific models: *Parallel in-register β-sheets:* Since amyloids were known to be composed of β-sheets, the finding that scrambling the amino acid sequence of Sup35 and Ure2 PrDs did not destroy their ability to form a prion (Ross *et al.* 2004, 2005a; Toombs *et al.* 2010) led Wicker and associates (Ross *et al.* 2005b) to propose that the prion structures were parallel in-register β-sheets. According to this model, the β-sheets in the PrD of each molecule are aligned with identical residues stacked on top of each other. This forms the amyloid core with the globular non-prion domains hanging off the core. The model nicely explains the data because all the PrD molecules of the same scrambled version would contain the identical scrambled sequence, so all amino acids that favor β-structures would still be available to align and form parallel in-register β-sheets (Figure 7A).

Indeed, several mass-per-unit-length measurements of fibers containing the Sup35 and Ure2 PrDs indicate about one molecule per 4.7 Å as predicted by the stacked architecture of the β-sheets in the parallel in-register model (Baxa *et al.* 2003, 2011; Diaz-Avalos *et al.* 2005; Chen *et al.* 2009). The final evidence in support of this model for yeast prions comes from solid-state NMR data for *in vitro*-generated infectious fibers of Sup35NM, Rnq1 PrD, and Ure2 PrD and fibers made of Ure2 PrDs with shuffled sequences. The method was to specifically label one or a few amino acids with ^{13}C and to then measure the distance to the nearest labeled residue on a different molecule. For a parallel in-register β-sheet, this measurement will be 4.7 Å (the distance between the β-strands as mentioned above). For any other type of β-sheet, the distances will be larger. One difficulty with this approach is that the number of residues that can be specifically labeled is limited because PrDs are so rich in glutamines and asparagines. Nonetheless, most of the residues examined were within the 4.7-Å distance of the identical residue on a different molecule, strongly supporting the parallel in-register model (Chan *et al.* 2005; Fayard *et al.* 2006; Baxa *et al.* 2007; Shewmaker *et al.* 2008, 2009b; Wickner *et al.* 2008a,b; Chen *et al.* 2009).

A given prion domain is hypothesized to form several parallel in-register β-sheets interspersed with non-β-sheet

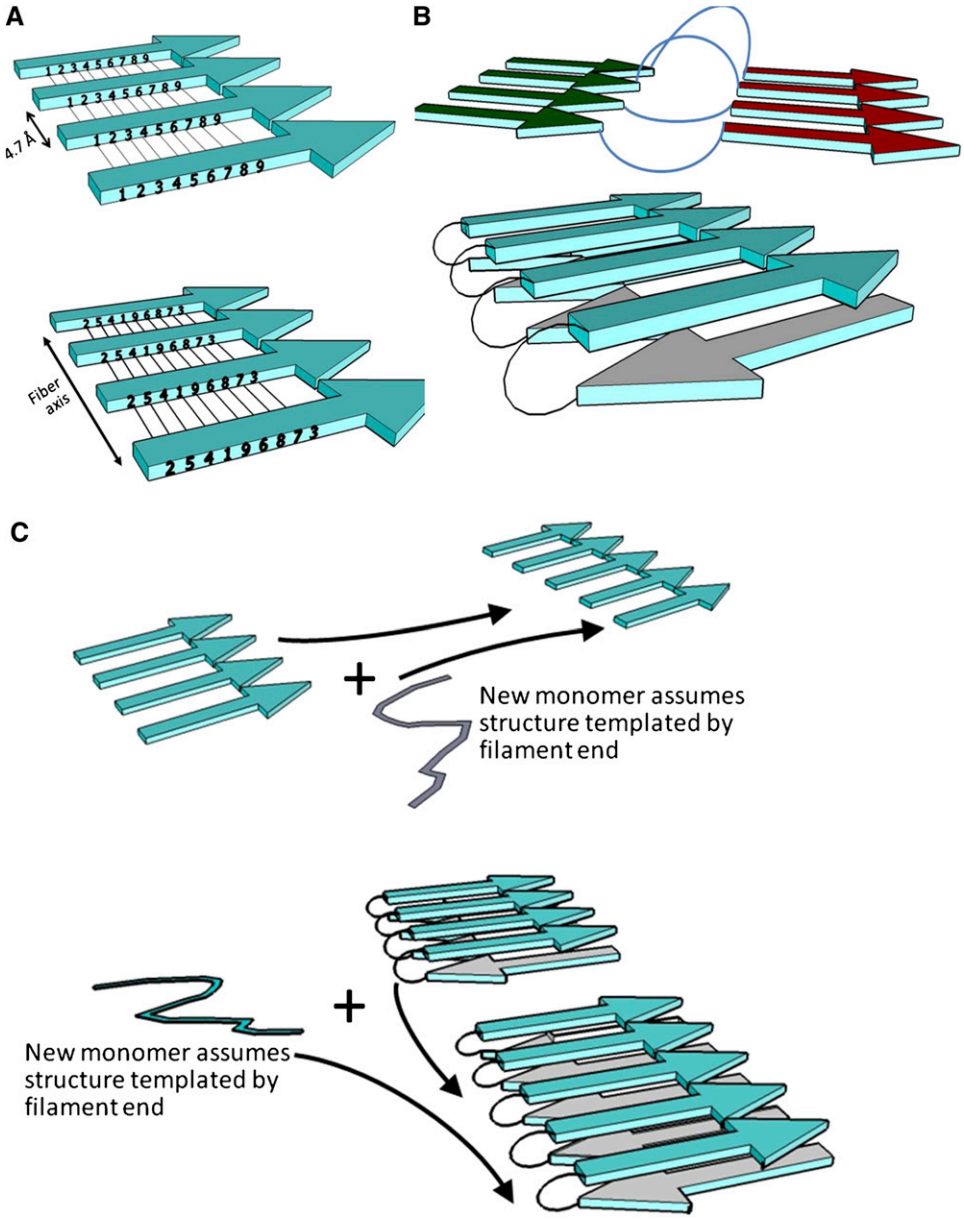

Figure 7 Predictions and ramifications of the parallel in-register β-sheet model. (A) Amino acid shuffling (unshuffled amino acid residue numbers are shown) is not predicted to destroy a parallel in-register structure. Interactions indicated by thin black line hold the peptide chains in register. (B) Prion variants could differ in the position of β-sheets, non-β-sheet loops, and steric zippers. (Top) Two β-sheets separated by a loop and color-coded to correspond to Figure 8 with red (head), blue (central), and green (tail) regions of the prion domain. (Bottom) The β-sheets fold back to form a steric zipper. (C) A parallel in-register structure could template variant structure. (Top) The prion variant is folded in a single β-sheet. (Bottom) This same molecule in a different variant shows the single β-sheet broken into two β-sheets with a loop separating them and the β-sheets folding back to form a steric zipper. These two different variant structures template new monomers to assume the structure of the fiber that they are joining.

loops. These non-β-sheet loops can account for the residues that are not within the 4.7-Å distance (Figure 7B). Also, the different β-sheets are proposed to interact with each other to form a "steric zipper" (Figure 7B, bottom) in which the side chains of the residues in the opposing β-sheets interdigitate, forming tight van der Waals bonds named "steric zippers." Such steric zippers have been seen for crystals of short peptides made of amyloid sequences, including peptides from Sup35 PrD (Nelson *et al.* 2005; Sawaya *et al.* 2007; Van Der Wel *et al.* 2007).

The lengths of the β-sheets and loops are proposed to differ in, and be the basis for, differences between prion variants. Indeed, Sup35NM prion variants formed *in vitro* differ in the length of the region protected from H/D exchange, which likely corresponds to the β-rich amyloid core (Toyama *et al.* 2007). Larger regions were protected in fibers formed at 37° (weaker prion variant) compared to fibers formed at 4° (stronger prion variant). This agrees with the higher physical stability of weaker *vs.* stronger prion variants (see above, *Requirements for Prion Propagation: Shearing and Segregation*). Once a fiber forms with a set of β-sheets, steric zippers, and loops that represent a particular prion variant, new monomers that join the fiber are expected to be templated to form the same β-sheets, steric zippers, and loops (Figure 7C). The inclusion of different PrD segments into different components of the structure may explain the different effects of specific PrD structural elements on Rnq1 prion propagation (Bardill and True 2009; Kadnar *et al.* 2010) (see *Prion domains*) and on the specificity of [*PSI*$^+$] prion transmission (Chen *et al.* 2010) (see below, *Sequence Specificity of Prion Transmission and Transmission Barriers*).

One concern with the solid-state NMR data are that the widths of the lines (each of which represent an atom in a particular environment in the aggregates) of the Sup35, Rnq1, and Ure2 PrD spectra were much broader than expected. This suggests either that the samples are composed of a mixture of fibers with similar but different conformations (possibly a mixture of different prion variants) or that there is some disorder in the fibers, *e.g.*, "breathing" on their ends giving rise to non-β-sheet loops of different sizes (Bockmann and Meier 2010).

More support for the parallel in-register β-sheet model has recently appeared from a study of Ure2 prion domain fibers using site-directed spin labeling and electron paramagnetic resonance (Ngo *et al.* 2011). This study also provides evidence that a portion of the β-sheet region is more solvent-protected than the rest, suggesting that the β-sheets are organized in inner and outer cores that may differ in different prion strains.

β-Helix: Other *in vitro* evidence supports a β-helix model for Sup35 PrD (Kishimoto *et al.* 2004; Krishnan and Lindquist 2005). According to this model, each rung of the β-helix surrounds an empty central cavity (Figure 8).

Krishnan and Lindquist (2005) labeled Cys residues, which they introduced throughout the Sup35NM sequence and which did not alter prion function, with fluorescent dyes responsive to solvent exposure. The solvent-protected core identified by this approach encompassed some (residues 36–86) or most (residues 21–121) of the N domain, depending upon whether the fibers were primarily of the strong [*PSI*$^{+}$] variant (made at 4°) or primarily of the weak [*PSI*$^{+}$] variant (made at 25°), respectively. The core domains defined by this method are shorter than the region predicted to be part of the Sup35NM parallel in-register β-sheets. Even shorter core regions were deduced from H/D exchange data (Toyama *et al.* 2007). However, the parallel in-register structure could in principle be reconciled with these results if the edges of the β-sheet domains dynamically expand and contract. This "breathing" might prevent the β-sheet domains from being solvent-protected, but still allow the detection of weak intermolecular self-interactions with solid-state NMR.

According to the β-helix model, only rungs at the top (head) and bottom (tail) of the solvent-protected region would have intermolecular contacts. Indeed, peptide array experiments have identified sites within head and tail regions of the Sup35 PrD as primary sites of intermolecular interactions (Tessier and Lindquist 2007), although it is not clear if interactions uncovered by this approach are identical to those involved in amyloid formation. By labeling individual Cys residues with fluorophores that respond to the presence of nearby dye, Krishnan and Lindquist (2005) detected intermolecular interactions only between residues located in the head and tail regions, and not between residues in the central PrD region. To address the concern that the large fluorophores might alter the prion structure, the authors demonstrated that disulfide bonds between Cys residues in the head region or in the tail region enhanced or did not alter the rate of amyloid formation, while disulfide bonds in the central region were inhibitory. These data could also be consistent with the parallel in-register β-sheet model if the Cys residues in the central region fell within a non-β-sheet loop. Likewise, the finding that interactions in the head and tail regions are crucial for initiating amyloid aggregation is consistent with both models. However, there is no easy explanation for the faithful reproduction of prion variants by the β-helix model, as in this model newly joining PrD initially interacts with the pre-existing structure only at one end.

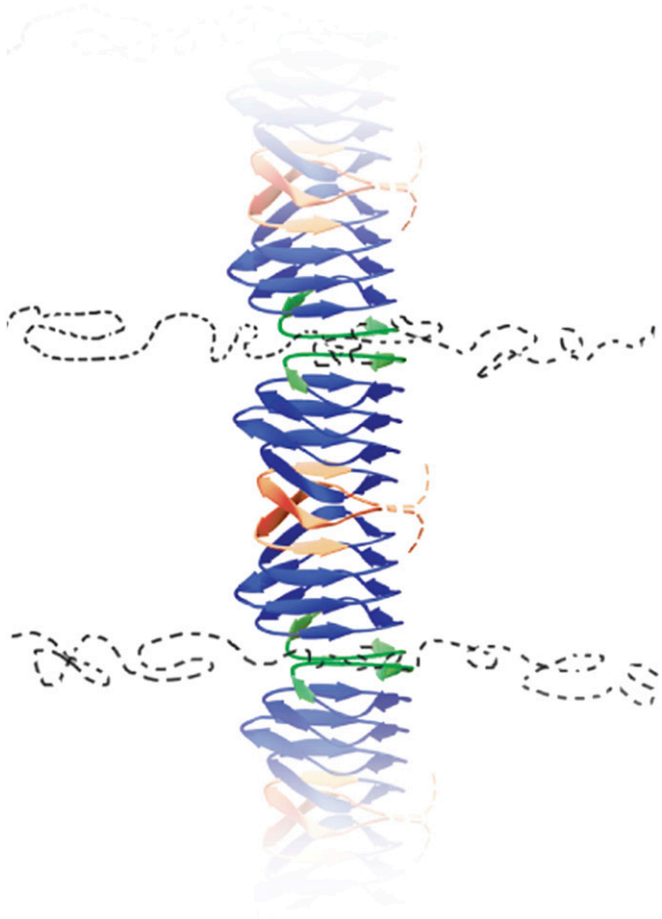

Figure 8 β-Helix model of Sup35NM fiber structure. The head and tail regions of the N domain have intermolecular contacts shown as head to head (red) and tail to tail (green). The central (blue) region of the N domain makes no intermolecular contacts. The M domain (dashed loops) is shown as a flexible region hanging off the core fiber. Reprinted with permission from Krishnan and Lindquist (2005).

An important clue to distinguish between the β-helix and parallel in-register models is the 8- to 10-Å reflection in the X-ray diffraction pattern, which is predicted only by the parallel in-register β-sheet model. While this reflection is generally agreed to be present in dried fibers, it has been reported to be missing in hydrated fibers, suggesting that the dried fibers and hydrated cellular prion could be in different confirmations (Bousset *et al.* 2002a, 2003, 2004; Fernandez-Bellot *et al.* 2002; Kishimoto *et al.* 2004). However, two groups have found this reflection to be associated even with hydrated prion fibers (Shewmaker *et al.* 2009a; Wang *et al.* 2011).

So far, all structural data for yeast prions has been obtained with *in vitro*-generated fibers, and no approach has produced a structure at atomic resolution. Also, only one or two variants have been studied in each set of experiments. Among non-yeast amyloids, there are examples of both parallel in-register β-sheets (*e.g.*, human Aβ, amylin, and α-synuclein) and possible β-helices (*e.g.*, bacterial curli) (Shewmaker *et al.* 2009b). It is very possible that different yeast prions, or even different variants of the same prion, may have very different structures. Indeed, a mutation in Aβ can lead to the formation of a predominantly antiparallel, rather than a typical parallel, β-sheet (Tycko *et al.* 2009).

Structure of a well-defined fungal prion, [HET-s]: The [HET-s] prion of the fungus *P. anserina* (Saupe 2011) is the only prion whose structure is known at the level of atomic resolution (Van Melckebeke *et al.* 2010). Although the Het-s PrD is not QN-rich, there are a lot of other similarities with the yeast prions. Het-s fibers have an amyloid core with globular appendages (Ritter *et al.* 2005; Sen *et al.* 2007). The core is made of the PrD and is protease resistant and infectious (Maddelein *et al.* 2002), supporting the globular decoration model. The Het-s PrD structure is the same whether fibers are made of only PrD or of the complete protein (Wasmer *et al.* 2009). Unlike the solid-state NMR data for the yeast prions, the data for the Het-s PrD contain very narrow bands, indicative of a single structure with little disorder (Bockmann and Meier 2010; Van Melckebeke *et al.* 2010). This may be because there are no variants of the [Het-s] prion; indeed, no variants have been reported.

The [Het-s] prion domain structure combines elements of both the β-helix and the parallel in-register β-sheet models. It has modified parallel in-register β-sheets in the shape of a left-handed β-solenoid that surround an empty central cavity. There are two windings per molecule leading to a mass per unit length of one molecule per 9.4 Å (Sen *et al.* 2007; Mizuno *et al.* 2011) rather than the 4.7 Å seen for the yeast prions. There are eight β-strands per molecule (Figure 9). Strands 1a and 3a, 1b and 3b, 2a and 4b, 2b and 4b are pseudodirect repeats in amino acid sequence that align with their pseudorepeat partner in parallel and in register. Additional molecules align so that all the pseudorepeat β-strands form parallel in-register sheets. Three of these sheets define a hydrophobic triangular core while the fourth points away from the core. The two β-sheet layers per molecule are connected by a flexible linker. As in globular proteins, hydrophobic residues are found pointing into the core while polar residues are on the surface.

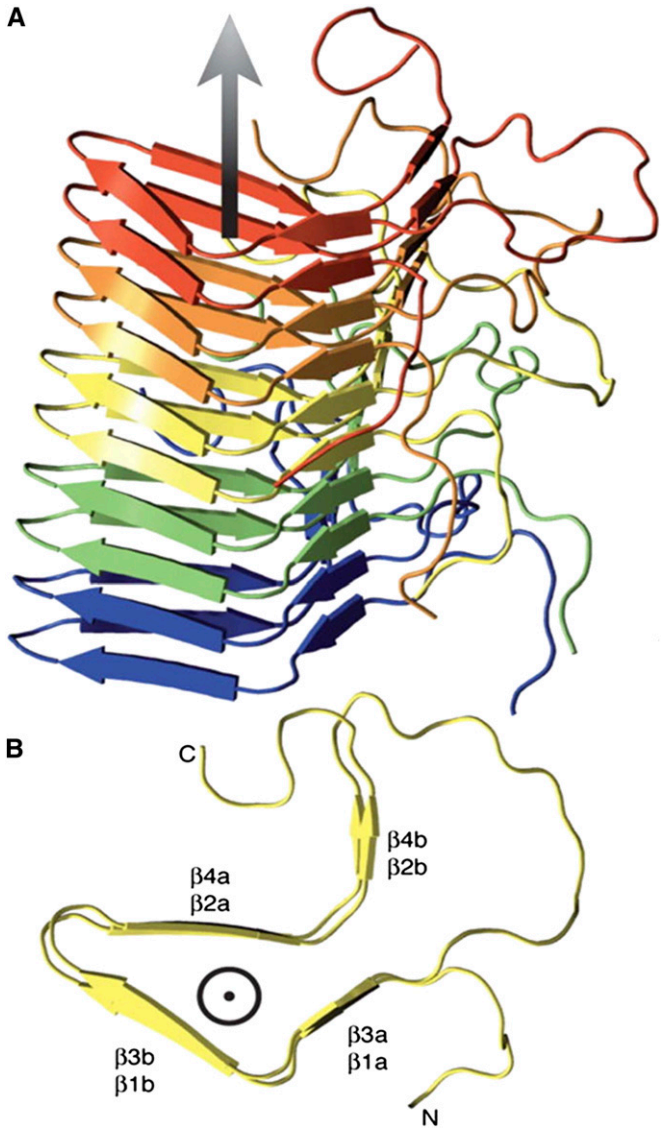

Figure 9 β-Solenoid structure of HET-s (218–289) fibrils showing modified parallel in-register β-sheets. The arrow indicates the fiber axis. (A) Side view of five molecules of the fiber. (B) Top view of yellow molecule from A. β3 and β4 form modified parallel in-register β-sheets with their pseudorepeats, β1 and β2, respectively. Reprinted from figure 2 of Wasmer *et al.* (2008).

De novo Prion Formation

Prion induction by overproduction

Transient overexpression of a variety of prion proteins has been shown to dramatically increase (in some cases, as much as 3000-fold or more) the chance that the overexpressed protein will form a prion seed *de novo* (Chernoff *et al.* 1993; Wickner 1994; Derkatch *et al.* 1996; Alberti *et al.* 2009; Patel *et al.* 2009). Indeed, transient overexpression of just a PrD (or a portion of PrD) can cause this effect and is often more effective than overproduction of the entire protein. One reason overproduction could induce prion formation is that the increase in protein level could make it more likely for misfolding events to occur, *e.g.*, because of an insufficient supply of chaperones. At higher local concentration it would also be easier for monomers to find each other and aggregate. PrDs may also be more likely to misfold when they are not in the context of the complete protein. Also, the increased protein levels may cause misfolded protein to escape degradation by proteolytic pathways.

Still, overproduction *per se* is not always sufficient for prion formation. For some prions, the frequency of prion induction by transient overproduction changes dramatically, depending upon the presence of other (heterologous) prions or prion-like aggregates. The best-studied and most dramatic example of this is the induction of [*PSI*$^+$], which is greatly enhanced by the presence of the [*PIN*$^+$] prion, other QN-rich prions, or QN-rich proteins in an aggregated state (Derkatch *et al.* 1997, 2001; Derkatch and Liebman 2007). [*PIN*$^+$] also enhances the *de novo* appearance of [*URE3*], although effects are much less dramatic (Bradley *et al.* 2002), and increases the induction of the non-QN rich *Podospora* prion [Het-s] about twofold in yeast (Taneja *et al.* 2007). When [*PSI*$^+$] was induced by Sup35 overproduction at a lower frequency in a [*psi*$^-$ *pin*$^-$] background, each [*PSI*$^+$] cell was shown to have also picked up a *de novo*-formed [*PIN*$^+$] prion that likely facilitated [*PSI*$^+$] appearance (Derkatch *et al.* 2000). However, the [*PIN*$^+$] prion *per se* is not required for [*PSI*$^+$] formation, as [*PSI*$^+$] could also form *de novo* even in strains that lack the [*PIN*$^+$]-forming protein, Rnq1 (Chernova *et al.* 2011; Z. Yang, J. Hong, I. L. Derkatch, and S. W. Liebman, unpublished results). Since, as explained below, other prions or prion-like aggregates may substitute for [*PIN*$^+$], it is possible that another aggregate helped [*PSI*$^+$] to appear in these cases.

Heterologous prion cross-seeding

[*PIN*$^+$] was first identified as a non-Mendelian factor that enhanced the appearance of [*PSI*$^+$] and had prion-like properties (Derkatch *et al.* 1997, 2000) and was then shown to be a prion form of Rnq1 (Derkatch *et al.* 2001). A separate study identified Rnq1 as a prion-forming protein on the basis of a similarity of amino acid composition to Sup35 PrD (Sondheimer and Lindquist 2000; Sondheimer *et al.* 2001). Since *rnq1Δ* strains did not enhance [*PSI*$^+$] induction, it was clear that the [*PIN*$^+$] prion phenotype was not due to inactivation of Rnq1. Furthermore, other prions or overexpression of other QN-rich proteins did confer the Pin$^+$ phenotype to yeast cells (Derkatch *et al.* 2001, 2004; Osherovich and Weissman 2001; Meriin *et al.* 2002). This led to the hypotheses that the [*PIN*$^+$] prion might (1) titrate away cellular factors that inhibit [*PSI*$^+$] prion formation and/or (2) provide an initial nucleus to cross-seed the *de novo* prion aggregation of the heterologous Sup35 QN-rich protein (Derkatch *et al.* 2001; Osherovich and Weissman 2001) (see Figure 10). However, candidates for [*PIN*$^+$]-sequestered factors that inhibit prion formation have not been identified despite several genetic screens (Osherovich and Weissman 2002). On the other hand, there is significant evidence in support of the cross-seeding model. Purified Rnq1 PrDs can form fibers *in vitro* (Glover *et al.* 1997; King *et al.* 1997; Patel and Liebman 2007; Vitrenko *et al.* 2007), and the presence of these fibers enhances the fibrillization of Sup35 PrD and vice versa (Derkatch *et al.* 2004; Vitrenko *et al.* 2007). Likewise, yeast Sup35 PrD overexpressed in bacteria formed amyloid fibers, but only if another QN-rich

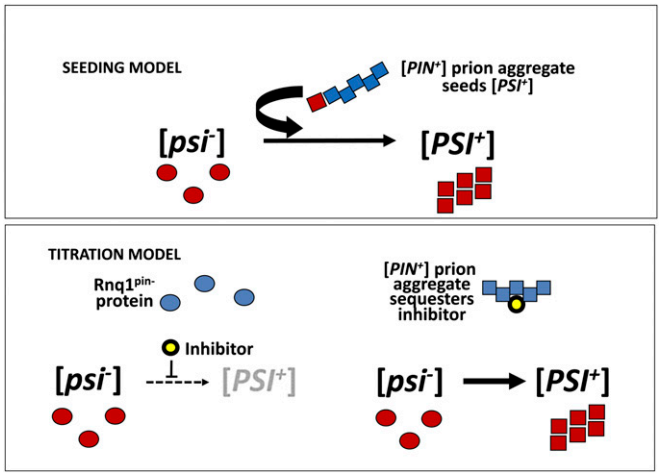

Figure 10 Titration and cross-seeding models. (Top) The [*PIN*$^+$] directly seeds the [*psi*$^-$] Sup35 to aggregate as a prion. (Bottom, left) [*PSI*$^+$] formation is prevented by an inhibitor. (Right) The [*PIN*$^+$] prion sequesters the inhibitor, allowing Sup35 to aggregate to form [*PSI*$^+$] (modified from Derkatch *et al.* 2001).

amyloid was already present (Garrity *et al.* 2010). In addition, cross-seeding can be imitated artificially by fusing Sup35 (or Ure2) PrDs to Rnq1 PrD: such fusions induced [*PSI*$^+$] (or [*URE3*]) even when expressed only at a low level, but this was completely dependent upon [*PIN*$^+$] (Choe *et al.* 2009). Also mutations in the Rnq1 prion domain that specifically alter the ability of [*PIN*$^+$] to promote the appearance of [*PSI*$^+$] have been isolated (Bardill and True 2009).

The *de novo* induction of [*PSI*$^+$] by transiently overproduced Sup35 (or its PrD) in the presence of [*PIN*$^+$] goes through several stages (Figure 11). First, amyloid-like detergent-resistant Sup35 polymers accumulate (Salnikova *et al.* 2005). When Sup35-based constructs are fused to GFP, the aggregated protein is initially seen as large filamentous (ring-like) assemblies at the cell periphery. The appearance of peripheral rings is increased in nondividing cells, and these rings are transient: they later collapse into smaller internal rings that surround the vacuole. Finally, cells with rings give rise to daughter cells with dot-like aggregates, characteristic of [*PSI*$^+$] (Zhou *et al.* 2001; Ganusova *et al.* 2006; Mathur *et al.* 2010). If fluorescently tagged Sup35 is overproduced in a cell with established [*PSI*$^+$], the typically detectable numerous small prion aggregates, distributed throughout the cell, are replaced by one or a few large dots or clumps, but rings do not appear. Remarkably, similar induction aggregate morphologies have been observed for other prions (Derkatch *et al.* 2001; Alberti *et al.* 2009), including the non-QN-rich *Podospora* prion, [Het-s], when induced in yeast (Mathur *et al.* 2010). Peripheral Sup35-GFP rings overlap cortical actin patches, and both peripheral and internal rings accumulate actin assembly proteins (*e.g.*, Sla1 and Sla2) (Ganusova *et al.* 2006). This suggests a role for the actin cytoskeleton in ring assembly and/or collapse. Indeed, alterations of the actin cytoskeleton influence both ring formation and [*PSI*$^+$] induction by excess Sup35 as

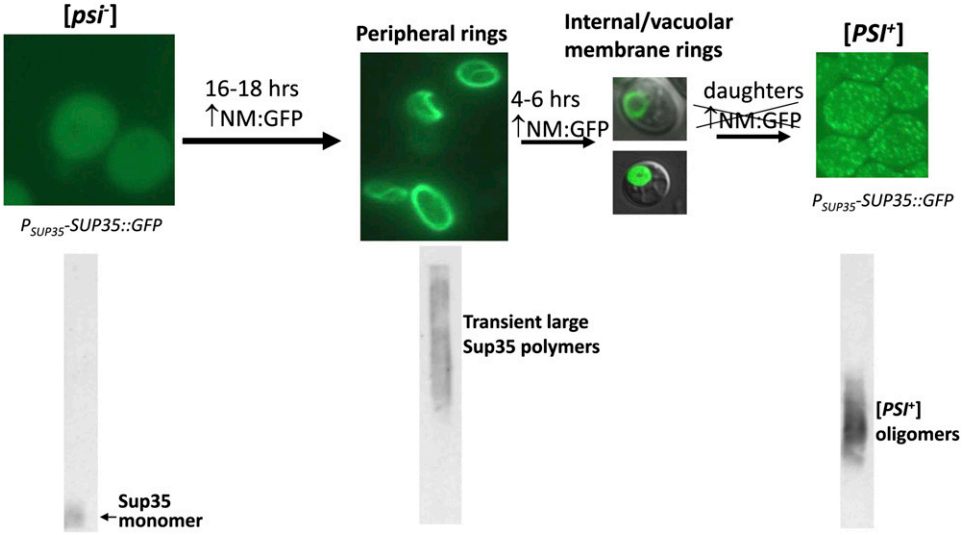

Figure 11 Stages during induction of [*PSI*$^+$] by overexpression of Sup35NM-GFP. The figure shows [*PSI*$^+$] induction in the presence of [*PIN*$^+$]. Similar stages occur if [*URE3*] or overexpressed prions such as QN-rich proteins substitute for [*PIN*$^+$]; however, in the absence of any such factor, these events do not occur. After ~16–18 hr of Sup35NM-GFP (NM-GFP) overexpression, fluorescent rings or meshes appear at the cell periphery during stationary phase. By 22–24 hr of overexpression, many of the rings collapse around the vacuole. During the time period when rings are visible, very large detergent-resistant oligomers of Sup35 appear. Daughter cells initially inherit prion aggregates that are too small to see under the fluorescent microscope. If expression of Sup35NM-GFP is turned off, but synthesis of endogenous Sup35 labeled with GFP remains, daughter cells later exhibit the small but visible aggregates characteristic of [*PSI*$^+$] cells. Cells in which endogenous Sup35 has been tagged with GFP (P_{SUP35}*SUP35:GFP*) are shown. (Bottom) Western blots of semidenaturing agarose gel electrophoresis (samples treated with 2% SDS at room temperature and run on agarose). Sup35 first appears as monomers; later, the transient large aggregates appear; and, finally, in the stable [*PSI*$^+$] progeny, characteristic [*PSI*$^+$] oligomers are present. Adapted from Salnikova *et al.* (2005) and Mathur *et al.* (2010).

described below. It was hypothesized that actin cytoskeletal networks assemble misfolded proteins into quality control deposits and, in this way, promote initial prion formation (Ganusova *et al.* 2006).

Ring structures followed by dots also appear when Sup35NM-GFP is constitutively overproduced, *e.g.*, with a GPD promoter (Tyedmers *et al.* 2010). EM indicated that, while both the rings and prion dots are composed of fiber bundles, the fibers in rings are very long while those in dots are highly fragmented. Furthermore, lysates of cells with rings can efficiently infect cells with [*PSI*$^+$] if the fibers are sheared. Rings detected during constitutive overproduction overlapped the preautophagosomal markers, characteristic of the insoluble protein deposit (IPOD). In cells with proteolysis defects, IPOD is formed as a single vacuole-associated structure that collects irreversibly aggregated and oxidatively damaged proteins (Kaganovich *et al.* 2008). Rnq1 has been found in IPOD, although it is not known if Rnq1 was in the prion state in the cells used for this study. It was proposed that [*PSI*$^+$] formation is initiated at the IPOD (Tyedmers *et al.* 2010). However, it is not yet clear if a single IPOD is present in nonstressed cells. It is possible that dispersed preautophagosomal proteins first interact with cytoskeleton-associated aggregates (and/or Rnq1), while formation of IPOD-like structures occurs at subsequent stages. The continuous overexpression of Sup35NM-GFP in the Tyedmars *et al.* (2010) study makes it possible that the majority of cells examined inherited Sup35 rings from the previous generation, making such persistent rings different from the peripheral rings formed *de novo* when Sup35NM-GFP is overproduced transiently.

It is necessary to note that the *de novo* induction of some prions, *e.g.*, [*MOT3*$^+$], by PrD overproduction does not appear to depend on the presence of pre-existing prions (Alberti *et al.* 2009). For other prions, the requirement for a pre-existing nucleus for their efficient *de novo* formation can be overcome. Indeed, overproduction of certain Sup35 PrD-containing fragments with a short extension of hydrophobic residues (Derkatch *et al.* 2000) or with an addition of the expanded polyglutamine stretch (Goehler *et al.* 2010) allows efficient *de novo* induction of [*PSI*$^+$] in [*pin*$^-$] cells.

Spontaneous and environmentally induced prion formation

The rate with which prions appear *de novo* without infection or overexpression varies dramatically for different prions and is very dependent upon the environment. [*PIN*$^+$] enhances not only [*PSI*$^+$] induction by overproduced Sup35 (or Sup35 PrD) but also the spontaneous appearance of [*PSI*$^+$] in the absence of overproduction (Derkatch *et al.* 1996, 1997, 2000, 2001).

Rates of spontaneous [*PSI*$^+$] formation have been difficult to measure because Mendelian nonsense suppressors that mimic the [*PSI*$^+$] phenotype arise at a higher frequency than *bona fide* [*PSI*$^+$]. However, a fluctuation test combined with determining the proportion of GuHCl curable (*i.e.*, prion) colonies among all suppressor colonies recovered determined the rate of spontaneous *de novo* appearance of [*PSI*$^+$] to be 7.1×10^{-7}/generation for a [*pin*$^-$] strain (Allen *et al.* 2007) and 5.8×10^{-7}/generation in a strain whose [*PIN*$^+$] status was not mentioned (Lancaster *et al.* 2010). The frequencies of the spontaneous appearance of [*URE3*], in a strain with unknown [*PIN*$^+$] status (Maddelein and Wickner 1999), and of [*PIN*$^+$] (Sideri *et al.* 2011) were estimated as 2×10^{-6} and 2×10^{-2}, respectively. The high frequency of spontaneous [*PIN*$^+$] appearance may explain why [*PIN*$^+$] is found in nature (see below, *Biological Effects of Prions*).

Prolonged incubation in the cold has been reported to enhance the spontaneous appearance of [*URE3*] (M. Aigle data, confirmed and cited in Chernoff *et al.* 1995), [*PSI*$^+$], and [*PIN*$^+$] (Derkatch *et al.* 2000). [*PSI*$^+$] is also induced by other long-term (rather than short-term) stresses (Tyedmers *et al.* 2008) that may be related to the accumulation of misfolded proteins and/or alterations of Hsp levels during the stress. Elimination of ribosome-associated peroxiredoxins, Tsa1 and Tsa2, results in the increased induction of [*PSI*$^+$] by oxidative stress (Sideri *et al.* 2010) because methionine oxidation in Sup35 apparently promotes aggregation (Sideri *et al.* 2011). Once again, [*PIN*$^+$] is required for [*PSI*$^+$] induction in *tsa1Δ tsa2Δ*, although formation of [*PIN*$^+$] can also be induced by oxidative stress (but not by oxidation of Rnq1 itself) in this strain.

Other host effects on prion formation

Aside from the effects of other prion proteins described above, the cellular control of *de novo* prion formation is not yet well understood. Chaperones that play a crucial role in prion propagation (see above, *Requirements for Prion Propagation: Shearing and Segregation*) also modulate *de novo* prion appearance; however, it is not always possible to conclude whether these chaperones act at the stage of initial prion formation *per se* or influence propagation and/or detection of the newly formed prions. Alterations of the heat-shock factor (Hsf), which regulates Hsp expression, influence *de novo* [*PSI*$^+$] appearance. Depending on the Hsf domain altered, these mutations can increase or decrease the frequency of [*PSI*$^+$] appearance and change the spectrum of the *de novo*-induced [*PSI*$^+$] variants (Park *et al.* 2006). Hsp104 was implicated in the promotion of amyloid formation by excess Sup35NM *in vitro* (Shorter and Lindquist 2004), although this effect could be due to multiplying the initially formed nuclei via fragmentation. *In vivo*, excess Hsp104 also promotes *de novo* induction of the [*URE3*] prion in the presence (but not in the absence) of the [*PIN*$^+$] prion, possibly via sheering [*PIN*$^+$], thereby increasing the abundance of the [*PIN*$^+$] nuclei (Kryndushkin *et al.* 2011b). Ssa overproduction increases [*PSI*$^+$] induction by excess Sup35 (Allen *et al.* 2005), while deletion of both *SSB* genes increases both overproduction-induced and spontaneous [*PSI*$^+$] formation (Chernoff *et al.* 1999). Therefore, Ssb depletion manifests itself as a "protein mutator," increasing the frequency of heritable conformational changes in other proteins. As Ssb is implicated in the folding of nascent polypeptides, it may antagonize the accumulation of misfolded protein, providing a substrate for prion nucleation. However, dependence of the effects of Ssa overproduction and Ssb depletion on the presence of a pre-existing nucleus (*e.g.*, [*PIN*$^+$] prion) indicates that these chaperones do not directly control the nucleation step. Overproduction of Sse1, a nucleotide exchange factor for Ssa, promotes *de novo* [*PSI*$^+$] induction, while deletion of *SSE1* inhibits it and allows formation of only unstable very weak prion variants (Fan *et al.* 2007). In contrast, excess Ssa, Ydj1, or Sse1 antagonizes induction of the [*URE3*] prion (Kryndushkin *et al.* 2011b). All of these effects are [*PIN*$^+$]-dependent.

Alterations in the ubiquitin system, which is involved in protein degradation, also influence *de novo* [*PSI*$^+$] formation. [*PSI*$^+$] induction by excess Sup35 is more efficient at increased ubiquitin levels and is reduced by a decrease in the levels of free ubiquitin (*i.e.*, in strains defective for a deubiquitinating enzyme) (Chernova *et al.* 2003). Deletion of *UBC4*, which encodes one of the major yeast ubiquitin-conjugating enzymes, increases both [*PSI*$^+$] resistance to curing via an excess of chaperone Hsp104 and *de novo* [*PSI*$^+$] formation (Allen *et al.* 2007). Notably, the increase of [*PSI*$^+$] formation by *ubc4*Δ is independent of the presence of any other prion, although it requires the presence of the Rnq1 protein, even though in a non-prion state. The simplest explanation for the *ubc4*Δ effect would be that a defect in ubiquitination prevents degradation of misfolded Sup35, thereby increasing its abundance and conversion into a prion. However, no evidence for direct ubiquitination of Sup35 was found. On the other hand, *ubc4*Δ increases the level of Ssa chaperone associated with Sup35 (Allen *et al.* 2007). Thus, alterations in the ubiquitin system may influence prions via auxiliary factors.

Several mutations and deletions influencing [*PSI*$^+$] induction by excess Sup35 have been reported (Bailleul *et al.* 1999; Ganusova *et al.* 2006; Tyedmers *et al.* 2008; Manogaran *et al.* 2011). Most of these include components of the stress response pathways, ubiquitin-proteasome system, intracellular trafficking networks, and actin cytoskeleton. Mutation in actin or deletions of the genes coding for the actin assembly proteins Sla1, Sla2, or End3 (Bailleul *et al.* 1999; Ganusova *et al.* 2006), as well as Las17, Sac6, or Vps5 (Manogaran *et al.* 2011), decrease both formation of aggregated structures and *de novo* [*PSI*$^+$] induction. In contrast, deletions of genes coding for actin assembly proteins Arf1 and Bem1, vesicle-trafficking protein Bug1, and the regulator of osmotic response and actin polymerization Hog1 reduced [*PSI*$^+$] induction without affecting filament formation, indicating that these proteins probably act at later stages in the pathway (Manogaran *et al.* 2011). Protein Lsb2/Pin3, containing the QN-rich domain and shown to substitute for [*PIN*$^+$] when overproduced (Derkatch *et al.* 2001), is capable of aggregation and [*PSI*$^+$] induction only when it is associated with the actin cytoskeleton via interactions with Las17 (Chernova *et al.* 2011). Notably, Lsb2 is a short-lived protein, rapidly induced during heat shock and other stresses, and then degraded via the ubiquitin system. It was shown to be involved in protection of a weak [*PSI*$^+$] variant from destabilization by heat shock (see *Requirements for Prion Propagation: Shearing and Segregation*) and may mediate effects of stresses and the ubiquitin system on prion induction (Chernova *et al.* 2011).

Overall, *de novo* prion formation, when induced by overproduction of Sup35 or its PrD, appears to be a multi-step process that involves the concentration of misfolded protein in quality control deposits (possibly including IPOD-like

structures), followed by subsequent conversion into a prion state. Thus, quality control deposits may act as prion induction sites. Initial aggregate assembly appears to be driven by cytoskeleton-associated proteins, while other proteins may influence prion conversion. Filamentous ring-like agglomerates of misfolded Sup35 are cytotoxic (Zhou *et al.* 2001; Ganusova *et al.* 2006; Manogaran *et al.* 2011); thus, conversion of a misfolded protein into a prion may help to ameliorate toxicity when protein degradation fails to eliminate aggregates. It is possible that a similar pathway is activated when misfolded proteins are accumulated during stress (Chernova *et al.* 2011). Notably, [*PSI*$^+$] prions are frequently unstable when they first appear. Over time stable prion variants emerge although some variants remain unstable indefinitely (Derkatch *et al.* 1996, 2000; J. Sharma and S. W. Liebman, unpublished results).

Sequence Specificity of Prion Transmission and Transmission Barriers

Evolution of prion domains

Yeast PrDs typically evolve faster than regions of the same proteins that are responsible for their major cellular functions. The Sup35C regions in the nearest relatives, *Saccharomyces paradoxus* and *S. cerevisiae* (Scannell *et al.* 2007), are 100% conserved, while the Sup35N and Sup35M regions, respectively, are 94 and 87% conserved (Jensen *et al.* 2001; Chen *et al.* 2007). Nonetheless, comparison between these species confirms that the Sup35N sequence remains under selective pressure (Jensen *et al.* 2001). Sup35 orthologs from genera other than *Saccharomyces* exhibit an even higher divergence in the Sup35N and M regions, with only 30–40% amino acid identity between *S. cerevisiae* and distantly related genera such as *Pichia* or *Candida* (for reviews, see Inge-Vechtomov *et al.* 2007; Bruce and Chernoff 2011). At such divergence, only conservation of some patterns of amino acid composition and organization of Sup35NM regions (such as the presence of the QN-rich stretch and oligopeptide repeats in Sup35N and enrichment of Sup35M by charged residues) can be clearly seen. In contrast, the Sup35C regions remain clearly aligned. Despite PrD divergence, most Sup35 proteins of even distantly related yeast species that have been tested are capable of forming a prion in *S. cerevisiae*. A likely exception is the Sup35 protein of *Schizosaccaromyces pombe*, which apparently lacks a PrD. Prion formation by Sup35 proteins of non-*S. cerevisiae* origin in their native environments has not yet been systematically studied, although aggregates of endogenous Sup35 in *K. lactis* have been reported (Nakayashiki *et al.* 2001). In the case of Ure2, most proteins of heterologous origin can also form a prion in *S. cerevisiae*; however, some, *e.g.*, the Ure2 protein of *Saccharomyces castellii* (Edskes and Wickner 2002; Edskes *et al.* 2009) or *K. lactis* (Safadi *et al.* 2011), were unable to do so. It was also shown that *Saccharomyces bayanus* Ure2 can form a prion in its native environment while *S. paradoxus* Ure2 cannot (Talarek *et al.* 2005). Overall, these results indicate that the ability to form a prion state is generally conserved across long evolutionary distances; however, it can be lost in specific cases. It remains unclear to what extent this ability is realized by the respective proteins in their native proteomes.

Prion species barrier at high levels of sequence divergence

Each particular amyloid fiber typically incorporates only molecules of its specific sequence. The ability of amyloid proteins to form homogenous polymers depends on a high level of sequence identity between the units of a polymer and a newly captured protein molecule. In mammals, even transmission of the prion state to certain homologous proteins from closely related species is inefficient, resulting in the so-called "species barrier" (Moore *et al.* 2005; Collinge *et al.* 2006). If a species barrier is overcome, this may lead to cross-species prion transmission, *e.g.*, in the case of "mad cow" disease transmitted to humans.

In yeast, species barriers were initially detected between the *S. cerevisiae* Sup35 protein and its orthologs from the distantly related species, *e.g.*, *Pichia methanolica* or *C. albicans*, whose PrDs show only 30–40% amino acid identity with *S. cerevisiae* (Chernoff *et al.* 2000; Kushnirov *et al.* 2000a; Santoso *et al.* 2000; Zadorskii *et al.* 2000). These heterologous proteins do not coaggregate due to divergence of their QN-rich regions (Santoso *et al.* 2000). Chimeric PrD, composed of portions of the *S. cerevisiae* and *C. albicans* QN-rich regions, exhibited a "promiscuous" prion behavior, indicating that each QN-rich fragment works independently (Chien and Weissman 2001; Foo *et al.* 2011). Heterologous coaggregation with their *S. cerevisiae* counterpart was reported for the Sup35 orthologs of *K. lactis* and *Yarrowia lipolytica*, that are less divergent from *Saccharomyces* than *Candida* and *Pichia* (Nakayashiki *et al.* 2001). However, it was not clear whether or not coaggregation is followed by transmission of the prion state.

Prion species barrier at low levels of sequence divergence

A prion species barrier was also observed at short phylogenetic distances, *e.g.*, among Ure2 proteins from various species of the genus *Saccharomyces* (Edskes and Wickner 2002; Baudin-Baillieu *et al.* 2003; Talarek *et al.* 2005; Edskes *et al.* 2009). In these studies, barriers were detected for some but not all species combinations. Different [*URE3*] prion variants generated by protein with the same sequence could exhibit different cross-species transmission patterns (Edskes *et al.* 2009).

The most detailed information about the specificity of prion transmission between closely related proteins has been obtained for the Sup35 orthologs from the species of the *Saccharomyces sensu stricto* group, including *S. cerevisiae*, *S. paradoxus*, *S. mikata*, *S. kudriavzevii*, and *S. bayanus* (see Scannell *et al.* 2007). Sup35 PrDs of the *Saccharomyces sensu stricto* clade exhibit from 77 to 94% amino acid identity (Jensen *et al.* 2001; Chen *et al.* 2007). This is similar to

the range of variation observed for vertebrate prion proteins. Both complete Sup35 proteins and chimeric constructs with a heterologous PrD region were studied (Chen *et al.* 2007, 2010; Afanasieva *et al.* 2011; see also Bruce and Chernoff 2011). *SUP35* genes of different origins (or chimeric genes) were substituted for *S. cerevisiae SUP35* by plasmid shuffle in a [*PSI*$^+$] *S. cerevisiae* cell. Some data were also confirmed by exposing [*psi*$^-$] cells to nonhomologous [*PSI*$^+$] seeds using cytoplasm exchange (cytoduction) in the *S. cerevisiae* genotypic environment. Coaggregation of proteins containing heterologous PrDs with endogenous Sup35 was detected *in vivo*, depending on the species combination and [*PSI*$^+$] variant (Chen *et al.* 2007, 2010; Afanasieva *et al.* 2011). However, impairment of cross-species prion transmission was detected even in some combinations where coaggregation was observed. Therefore, the prion species barrier in yeast can be controlled at steps other than the physical association of heterologous proteins. As in the case of Ure2 (see above), the Sup35 species barrier depends not only on sequence divergence, but also on the particular prion variant. Asymmetry of cross-species prion transmission was also detected in some combinations; *e.g.*, prion transfer was inefficient from *S. cerevisiae* to *S. bayanus* PrD but efficient in the opposite direction (Chen *et al.* 2010). Major parameters of the transmission barrier were reproduced *in vitro* by using purified NM fragments of the *S. cerevisiae*, *S. paradoxus*, and *S. bayanus* Sup35 proteins. With one exception, *in vitro* results followed *in vivo* data (Chen *et al.* 2007).

Experiments with chimeric PrDs have surprisingly shown that different regions of PrD (and not necessarily the QN-rich stretch) are primarily responsible for the species barrier in different combinations (Chen *et al.* 2010). Moreover, naturally occurring polymorphisms in the non-QN-rich portion of Sup35N or in Sup35M may generate prion transmission barriers even within the *S. cerevisiae* species (Bateman and Wickner 2012). Notably, transmission barrier is not directly proportional to sequence divergence (Chen *et al.* 2010; Bruce and Chernoff 2011). These data, in agreement with previous observations in mammalian systems (Prusiner *et al.* 1990), clearly show that the identity of specific sequences rather than the overall level of PrD homology is crucial for prion transmission.

Transmission barriers generated by mutations

Transmission barriers between yeast prion proteins can also be generated by mutations. Substitutions within Sup35 PrD, *e.g.*, the dominant negative G58D (*PNM2* in the oligopeptide repeat region), prevent transmission of some but not other prion variants from wild type to mutant protein (Cox 1994; Doel *et al.* 1994; DePace *et al.* 1998; Derkatch *et al.* 1999; King 2001; Disalvo *et al.* 2011; Lin *et al.* 2011). Although the mechanisms are not entirely clear, it is known that the *PNM2*-dependent transmission barrier is modulated by the Hsp104 chaperone dosage: it becomes more pronounced when the Hsp104 dosage is increased, while decreasing the Hsp104 dosage partly overcomes the barrier (DiSalvo *et al.* 2011). Apparently sequence differences generate metastable heteroaggregates that are more sensitive to the disaggregating activity of Hsp104. A similar mechanism was proposed to explain the decreased conversion of heteroaggregates into stably maintained prions for combinations of the divergent *Saccharomyces sensu stricto* Sup35 proteins (Afanasieva *et al.* 2011). *PNM2*-containing heteroaggregates also show asymmetric distribution to mother cells in cell divisions (Verges *et al.* 2011). This could also be explained by differential sensitivity to Hsp104. Another dominant negative mutant, in the QN-rich region of the Sup35 PrD, Q24R, reduces the ability of the protein to be converted to the prion by wild-type Sup35 aggregates (DiSalvo *et al.* 2011).

For the Rnq1 protein, transmission barriers were shown to be generated by deletions of QN-rich regions (Kadnar *et al.* 2010). Thus, transmission specificity is controlled not only by the identity of amino acid sequences, but also by the length of cross-interacting regions. This agrees with previous observations that shortened PrD fragments can "poison" propagation of the [*URE3*] prion (Edskes *et al.* 1999).

Fidelity of cross-species prion conversion

Rare instances of prion transmission to highly divergent PrDs, *e.g.*, from *P. methanolica* to *S. cerevisiae*, resulted in multiple prion variants (Vishveshwara and Liebman 2009). This could be explained by rare nucleation of *de novo* amyloid formation by the aggregate of a divergent protein, similar to cross-seeding by nonhomologous proteins with PrDs of similar amino acid compositions (see *Spontaneous and environmentally induced prion formation*).

Transmission of the prion state between Sup35 or Ure2 proteins with closely related or mutationally altered PrDs sometimes resulted in prions with altered phenotypic patterns. Notably, reverse transmission of the prion state back to the original protein restored the original prion patterns in most (Edskes *et al.* 2009; Chen *et al.* 2010; Lin *et al.* 2011) but not all (*e.g.*, *S. cerevisiae/S. bayanus*) cases (Chen *et al.* 2010). As the frequency of reverse prion transmission in this combination was reasonably high, the appearance of new prion variants could not be explained simply by nonspecific *de novo* nucleation. The data suggest that conformational fidelity during prion transmission is controlled at both genetic (sequence identity) and epigenetic (prion variants) levels. Notably, the OR region controls both the transmission barrier and the conformational infidelity in the *S. cerevisiae/S. bayanus* combination.

Overall, the data suggest that both specificity and conformational fidelity of prion transmission in yeast are determined by relatively short amino acid stretches, rather than by the whole PrD (Chernoff 2008; Bruce and Chernoff 2011). These stretches could correspond to sequences that initiate intermolecular interactions, resulting in the formation of the cross-β amyloid core. Indeed, stronger [*PSI*$^+$] variants need shorter portions of PrD for their propagation, both *in* vivo (Shkundina *et al.* 2006) and *in vitro* (Chang *et al.* 2008). The locations of the specificity stretches could

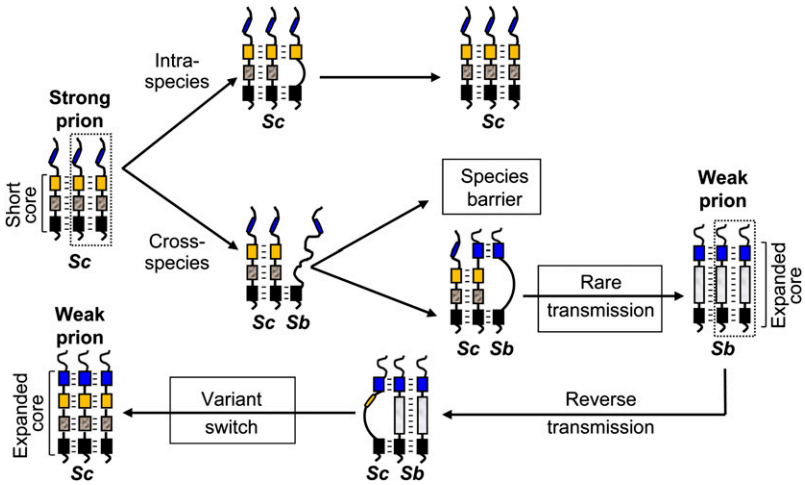

Figure 12 Model for species barrier and variant switch. Cross-β regions are shown as boxes. Specificity stretches are colored. Dashes represent bonds. *Sc* and *Sb* refer to *S. cerevisiae* and *S. bayanus*, respectively. Multiple prion variants could be generated due to multiple secondary specificity stretches. Model is modified from Bruce and Chernoff (2011).

control the variant-specific prion patterns by determining the position and size of the amyloid core(s). In the case of a variant switch (*e.g.*, the *S. cerevisiae/S. bayanus* combination), the interaction between altered specificity stretches is probably too weak to form the cross-β structure. However, alternative stretches located at different positions could occasionally be employed, resulting in the formation of an amyloid core at a different location and/or size. In such a scenario, the ability of prion variants either to be faithfully propagated by a heterologous protein or to undergo a conformational switch depends on the level of identity of the specificity stretches that define amyloid cores in these variants (Figure 12).

Antagonism between heterologous prions

Although, as described in the previous sections, heterologous prions enhance each other's appearance, the newly established prion does not require the presence of the heterologous prion once it has been established (Derkatch *et al.* 2000). Rather, it is often the case that a prion will destabilize a heterologous prion. For example, certain variants of [*PIN*$^+$] destabilize weak [*PSI*$^+$] (Bradley and Liebman 2003). While the mechanism of destabilization is unknown, [*PIN*$^+$] causes [*PSI*$^+$] aggregates to become larger, and enlarged aggregates are harder to transmit to daughters (Mathur *et al.* 2009). Also, [*PSI*$^+$] and [*URE3*] destabilize each other (Bradley *et al.* 2002; Schwimmer and Masison 2002). Overexpression of fragments of the Ure2 prion domain cure [*URE3*] (Edskes *et al.* 1999), and overexpression of certain fragments of the Rnq1 prion domain in the presence of [*PIN*$^+$] inhibits propagation of [*PSI*$^+$] and [*URE3*] (Kurahashi *et al.* 2008). Finally, overexpression of Rnq1with certain mutations in the non-prion domain causes [*PSI*$^+$] aggregates to enlarge and be lost (Kurahashi *et al.* 2011). Indeed, overexpression of a large number of QN-rich proteins destabilize pre-existing [*PSI*$^+$] and [*URE3*] prions apparently by interfering with the ability of the prion aggregates to be sheared (Z. Yang, J. Hong, I. L. Derkatch, and S. W. Liebman, unpublished results).

Prion Diversity

Prions of normal yeast proteins

In the past few years, it has become clear that prion phenomena are much more widespread than previously thought. Seven currently proven amyloid-based yeast prions and their properties are summarized on Table 1. All have QN-rich domains, although this is biased by the screening criteria used (see below). Curiously, four of the six prions with known functions are involved in transcriptional regulation. The prion aggregates generally appear in the cytoplasm, but one prion, [*ISP*$^+$], exhibits nuclear aggregates (Rogoza *et al.* 2010). [*PSI*$^+$] and [*URE3*] were initially identified as prions on the basis of their unusual genetic properties (Wickner 1994). Later, [*PIN*$^+$]/[*RNQ*$^+$] was identified as a prion (see *Heterologous prion cross-seeding*). Other proteins were identified as possible prion candidates on the basis of sequence algorithms derived from known QN-rich prion domains (Michelitsch and Weissman 2000; Santoso *et al.* 2000). Some of the QN-rich domains from these proteins are able to confer a prion state to a chimeric reporter construct. A recent large-scale screen (Alberti *et al.* 2009) identified 19 new potential PrDs that are capable of replicating as a prion in cells when fused to a reporter. It is likely that many of these domains can also confer a prion state to their native proteins, although an example exists of a protein (New1) that contains a PrD region conferring a prion state to a reporter but does not appear to form a prion on its own (Santoso *et al.* 2000; Osherovich *et al.* 2004). Indeed, undoubtedly, there are more prions because the screen was not exhaustive, the initial sequence criteria were biased (so that one independently identified prion protein, Sfp1, was excluded on this basis), and some prion proteins (Cyc8 and Mot3) did not work with the Sup35MC-based reporter used.

An earlier screen for proteins that, when overexpressed, substitute for [*PIN*$^+$] in promoting the appearance of [*PSI*$^+$], identified 11 candidates (Derkatch *et al.* 2001) with QN-rich domains, including Ure2 and the above-mentioned New1, also proven to possess such a property in an independent

article (Osherovich and Weissman 2001). The Alberti *et al.* (2009) candidates overlap with some of these proteins, and two of them, the chromatin remodeler Swi1 and the global transcriptional regulator Cyc8, have now been shown to, respectively, form the [*SWI*$^+$] (Du *et al.* 2008) and [*OCT*$^+$] (Patel *et al.* 2009) prions. However, other proteins from this screen, *e.g.*, Pin3/Lsb2, do not appear to propagate as a typical stable prion (Chernova *et al.* 2011).

The [*ISP*$^+$] prion was first detected because it counteracted the nonsense suppression associated with certain mutations (Aksenova *et al.* 2006). It was later identified as a prion form of the global transcriptional regulator, Sfp1, which has a QN-rich domain (Rogoza *et al.* 2010). Antisuppression was probably due to increased abundance of a reporter transcript; in contrast to five other proven prion proteins with known functions, the [*ISP*$^+$] phenotype is different from a loss of Sfp1 function. [*ISP*$^+$] is transferred by cytoduction less efficiently than other prions, probably due to the nuclear location of [*ISP*$^+$] prion aggregates. Also, propagation of [*ISP*$^+$] also does not require Hsp104, which is needed for cytoplasmic prions (see *Requirements for Prion Propagation: Shearing and Segregation*).

In addition to the amyloid-based prions, other transmissible protein-based phenomena have been described in yeast. The [*GAR*$^+$] prion is propagated by a complex between two proteins, Std1 (a glucose-signaling protein) and Pma1 (a plasma membrane proton pump). This atypical prion causes growth on glycerol in the presence of a nonmetabolizable glucose analog as a dominant non-Mendelian trait. Propagation of [*GAR*$^+$] is not dependent upon Hsp104, and the Std1-Pma1 prion complex does not show the characteristics of amyloid (Brown and Lindquist 2009).

Another transmissible protein-based phenomenon is based on self-catalyzed protein processing rather than on protein aggregation. The protein involved is protease B, which can spontaneously generate a persisting self-activated state, termed [β], in yeast cells that are missing the functionally redundant protease A protein (Roberts and Wickner 2003).

Other non-Mendelian elements of a yet-unknown nature, but with prion-like behavior, have also been reported in yeast. One example is [*NSI*$^+$], a non-Mendelian nonsense suppressor that is detectable only in the absence of the Sup35N prion domain. [*NSI*$^+$] possesses the genetic characteristics of a classical yeast prion, although its protein determinant is unknown (Saifitdinova *et al.* 2010).

Artificial and heterologous prions that can propagate in yeast

Several studies have employed fusions of known or suspected PrDs to GFP, Sup35C, Ure2C, or glucocorticoid receptor reporters (Patino *et al.* 1996; Li and Lindquist 2000; Sondheimer and Lindquist 2000; Alberti *et al.* 2009, 2010). These chimera often, but not always, behave as prions. Some aggregation-prone sequences, *e.g.*, polyQ stretches of non-yeast origin (Osherovich *et al.* 2004) or oligopetide repeats of the mammalian prion protein PrP (Parham *et al.* 2001; Dong *et al.* 2007; Tank *et al.* 2007), can substitute for all (if expanded) or part of Sup35 PrD without disrupting its prion properties. Interestingly, a non-QN-rich PrD of the *Podospora* Het-s protein, fused with GFP, was shown to propagate as a prion in yeast, making it likely that non-QN-rich endogenous yeast prion proteins also exist (Taneja *et al.* 2007). Indeed, see Suzuki *et al.* (2012), published while this review was in press.

Biological Effects of Prions

Prion-associated toxicity

Amyloids and amyloid-like inclusions are associated with diseases in humans and other mammals, such as Alzheimer's, Parkinson's, and Huntington's diseases; amyotrophic lateral sclerosis; type II diabetes; transmissible spongiform encephalopathies ("mad cow" disease, Creutzfeldt-Jacob disease, etc.); and others (Aguzzi and O'Connor 2010). In yeast, the presence of the [*URE3*] prion, or a combination of the [*PSI*$^+$] prion with the tRNA suppressor *SUQ5* (Eaglestone *et al.* 1999; Jung *et al.* 2000; Schwimmer and Masison 2002), can induce the stress response. Overproduction of Sup35 or its PrD is toxic to [*PSI*$^+$] strains (Chernoff *et al.* 1992) and at high levels to [*PIN*$^+$] strains, in which *de novo* [*PSI*$^+$] induction is efficient (Derkatch *et al.* 1997), but not to strains lacking any prions. Likewise, Rnq1 overproduction is toxic to [*PIN*$^+$] strains (Douglas *et al.* 2008). Overproduced Sup35 PrD in a [*PSI*$^+$] cell sequesters full-length Sup35 into prion aggregates, and overproduced full-length Sup35 sequesters another release factor, Sup45, contributing to toxicity (Vishveshwara and Liebman 2009). Indeed, some *sup45* mutants (Kiktev *et al.* 2007, 2011) or a heterozygous *sup45* deletion (Dagkesamanskaya and Ter-Avanesyan 1991) are lethal or sublethal in a [*PSI*$^+$] background. Although some variants of [*PSI*$^+$] do not impact exponential yeast growth (Eaglestone *et al.* 1999), other [*PSI*$^+$] variants are toxic unless rescued by the Sup35 derivative (Sup35C) that lacks the PrD and therefore cannot be sequestered (McGlinchey *et al.* 2011). Some variants of the [*URE3*] prion also decrease growth (McGlinchey *et al.* 2011). Some Hsp104 mutations result in [*PSI*$^+$]-dependent cytotoxicity (Gokhale *et al.* 2005). Overall, existing evidence indicates that at least some [*PSI*$^+$] and [*URE3*] variants are detrimental to yeast (see Wickner *et al.* 2011). It was hypothesized (Bateman and Wickner 2012) that sequence polymorphisms causing prion transmission barriers arose to prevent acquisition of harmful prions.

Prions as susceptibility factors for polyQ disorders

Prion variants that do not cause toxicity on their own may become toxic in combination with other factors. For example, fragments of the human huntingtin protein with the expanded polyQ stretch, associated with Huntington's disease, are toxic to yeast strains containing an endogenous

prion, such as [*PIN*$^+$] (Meriin *et al.* 2002) and/or [*PSI*$^+$] (Gokhale *et al.* 2005). Heterologous pre-existing aggregates promote polyQ aggregation in yeast (Osherovich and Weissman 2001; Meriin *et al.* 2002), and [*PIN*$^+$] apparently mediates sequestration of some actin assembly proteins (Meriin *et al.* 2003), while [*PSI*$^+$] mediates sequestration of Sup45 (Gong *et al.* 2012; Kochneva-Pervukhova *et al.* 2012) in the presence of polyQ aggregates. This leads to a cytotoxic defect, establishing yeast as a model for Huntington's disease (see Duennwald 2011; Mason and Giorgini 2011) and raising the intriguing possibility that variations in the onset of Huntington's disease in humans, recorded even among individuals with the same length of polyQ stretch, could be partly explained by variations in the composition of endogenous QN-rich aggregates in human cells.

Facts and hypotheses about biologically positive roles of prions in yeast and other fungi

The finding that most proteins can form amyloids *in vitro* suggests that the amyloid conformation is an ancient protein fold (Chiti and Dobson 2009). Indeed, positive biological roles of amyloids include scaffolding of melanin polymerization (Fowler *et al.* 2007), storage of peptide hormones (Maji *et al.* 2009), protection from stress (Iconomidou and Hamodrakas 2008), silk production (Romer and Scheibel 2008), substrate attachment (Gebbink *et al.* 2005), biofilm formation (Wang *et al.* 2008), and a proposed connection to long-term memory (Si *et al.* 2003, 2010; Heinrich and Lindquist 2011). [Het-s] *Podospora* was the first prion shown to provide a biological advantage to its host (Coustou *et al.* 1997; Wickner 1997). [Het-s] controls vegetative incompatibility, an adaptive trait, by causing death of [Het-S] mycellium at the position of contact (see Saupe 2007, 2011). Initially, only [*PIN*$^+$] and not [*PSI*$^+$] and [*URE3*] was found in any natural or industrial *Saccharomyces* strains (Chernoff *et al.* 2000; Resende *et al.* 2003; Nakayashiki *et al.* 2005). However, a more extensive search recently identified [*PSI*$^+$] in ~1.5% of strains (Halfmann *et al.* 2012). Notably, all of these [*PSI*$^+$] strains were also [*PIN*$^+$], suggesting that [*PIN*$^+$] promotes [*PSI*$^+$] generation in the wild as well as in the laboratory. If transcriptional regulators form prions, that could potentially result in regulatory switches that are adaptive. One intriguing candidate is Mot3, which regulates cell-wall biosynthesis genes, shows a high frequency of spontaneous conversion between prion and non-prion states (Alberti *et al.* 2009), and is found in a significant fraction of wild strains (Halfmann *et al.* 2012). About one-third of the natural and industrial *Saccharomyces* strains tested exhibit phenotypes that are curable by transient inactivation of Hsp104 (Halfmann *et al.* 2012), indicating that yet-unidentified Hsp104-dependent prions are widespread in wild yeast and may contribute to adaptation. Readthrough of termination codons caused by [*PSI*$^+$] was proposed to broaden the adaptation spectrum in changing environments via increasing phenotypic variability (True and Lindquist 2000; True *et al.* 2004). This agrees with the hypothetical consequences of translational ambiguity in general (*e.g.*, see Inge-Vechtomov *et al.* 1994) and is supported by mathematical simulations (Masel and Bergman 2003; Masel 2006; Griswold and Masel 2009). [*PSI*$^+$] indeed increases the growth of some laboratory or wild strains in certain conditions (True and Lindquist 2000; True *et al.* 2004; Halfmann *et al.* 2012), although these effects are genotype-specific, and no consistent effect of [*PSI*$^+$] on adaptation to novel environments has been detected (Joseph and Kirkpatrick 2008). Some [*PSI*$^+$]-mediated phenotypes could be due to suppression of nonsense mutations occurring in the genomes of the respective strains and/or due to induction of stress-defense genes (Eaglestone *et al*. 1999; Schwimmer and Masison 2002). It is not known to what extent [*PSI*$^+$] influences termination at natural stop signals that are protected by context (Bonetti *et al.* 1995). However, [*PSI*$^+$] modulates at least one naturally occurring mistranslation phenomenon, frameshifting in the antizyme gene, responsible for feedback regulation of polyamine biosynthesis (Namy *et al.* 2008). This explains some of the [*PSI*$^+$]-mediated phenotypes, although their adaptive role remains unclear (see Chernoff 2008).

Notably, pathogenic and beneficial effects of a prion are not mutually exclusive. Considering prion formation as a mutation occurring at the protein level, with prion variants analogous to alleles (Chernoff 2001), one could expect different effects on fitness for different prion variants, in the same way as mutations with deleterious or beneficial effects may arise in a single gene. Environmental changes may affect selection in the following way: if normal function of a prion protein becomes deleterious under certain conditions, decrease of this function in a prion form would become adaptive. Thus, prions could be helpful in certain conditions and be eliminated otherwise.

It is also possible that, in some cases, not the prion *per se* but rather the process leading to its formation could be beneficial. For example, aggregates formed during unfavorable conditions may be beneficial due to their increased resistance to degradation (see Chernoff 2007). While such aggregates are normally disassembled by chaperones upon return to normal conditions, prions could arise as by-products of such a process. Indeed, stress granules, assemblies that protect pre-initiation mRNA complexes during stress, are formed with participation of a protein containing a prion-like QN-rich domain (Gilks *et al.* 2004). Filamentous aggregates (rings) of overproduced Sup35 protein are toxic (see *De Novo Prion Formation*), while prion formation may ameliorate this toxicity, becoming a "lesser of two evils" (Ganusova *et al.* 2006). Possibly, similar events may occur if misfolded protein is accumulated during stress.

While the complete spectrum of biological effects of prions is yet to be uncovered, a growing number of prion examples indicate that the impact of prions on the host biology is significant and can no longer be ignored in models of evolution.

Perspectives

At the time at which the precursor of this YeastBook series (the second three-volume Cold Spring Harbor monograph series entitled *The Molecular and Cellular Biology of Yeast Saccharomyces*) was published in 1992, the basis of the mysterious [*URE3*] and [*PSI*$^+$] non-Mendelian factors was unknown. Today, their identification as prions has established the entirely new field of protein-based inheritance. Along with other epigenetic phenomena, prions have made it increasingly clear that knowing the sequence of the genome does not tell the whole story. Thus, a key remaining issue is the biological role of prions, including the contribution of protein-based components toward the transmission of heritable traits. Yeast will continue to lead the way toward understanding this and other fundamental properties of prions. By the time the next encyclopedia of yeast biology is published we will hopefully know how prion seeds are dispersed during cell division, what the structural basis for prion variants is, and what rules govern the specificity of their reproduction. Since amyloids and prions are found in many organisms, the importance of these findings will extend well beyond yeast and may help to develop therapeutic and prophylactic treatments for human protein-misfolding diseases.

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