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# Yeast and Fungal Prions: Amyloid-handling Systems, Amyloid Structure and Prion Biology

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## SUMMARY

Yeast prions (infectious proteins) were discovered by their outré genetic properties, and have become important models for an array of human prion and amyloid diseases. A single prion protein can become any of many distinct amyloid forms (called prion variants or strains), each of which is self-propagating, but with different biological properties (e.g., lethal vs. mild). The folded in-register parallel β sheet architecture of the yeast prion amyloids naturally suggests a mechanism by which prion variant information can be faithfully transmitted for many generations. The yeast prions rely on cellular chaperones for their propagation, but can be cured by various chaperone imbalances. The Btn2/Cur1 system normally cures most variants of the [URE3] prion that arise. Although most variants of the [PSI+] and [URE3] prions are toxic or lethal, some are mild in their effects. Even the most mild forms of these prions are rare in the wild, indicating that they too are detrimental to yeast. The beneficial [Het-s] prion of *Podospora anserina* poses an important contrast in its structure, biology and evolution, to the yeast prions characterized thus far.

### Keywords

Sup35; Ure2; [PSI+]; [URE3]; Btn2; anti-prion system; protein gene; chaperone; [Het-s]

## INTRODUCTION

A protein altered form that can convert the normal form of the protein into the same altered form can be an infectious protein, if it has a way to move from one individual to another. Amyloids, linear polymers of largely β-sheet structure, are one way that this can happen (but not the only way). The classical mammalian prion diseases are the uniformly fatal transmissible spongiform encephalopathies (TSEs), based on self-propagating amyloid of the PrP protein (reviewed in (Prusiner 2004; Aguzzi and Lakkaraiu 2015)). Humans are also subject to a number of more common non-infectious amyloidoses such as Alzheimer's disease, Parkinson's disease, type II diabetes mellitus and others. The yeast and fungal prions were first detected as non-chromosomal genes, conferring nonsense suppression ([PSI+] in *Saccharomyces cerevisiae*, (Cox 1965)), derepressed nitrogen regulation ([URE3]

in *S. cerevisiae*, (Lacroute 1971)) and heterokaryon incompatibility ([Het-s] in *Podospora anserina*, (Rizet 1952)). Only much later were they shown to be prions (Wickner 1994; Coustou et al. 1997), but their status as genes remains true, with vertical as well as horizontal transmission of information. As will be detailed below, the yeast prions can have heritable alleles, some as stable as chromosomal gene alleles, and will (rarely) mutate from one allele to another.

We will see that these genes (prions) are based on proteins templating their conformation for other protein molecules, in analogy to the templating of sequence by DNA and RNA molecules. Self-propagating, self-templating amyloids are the basis of these prions, linear filamentous polymers of the respective prion protein. A mechanism has been proposed for the templating (Wickner et al. 2008; Wickner et al. 2013), based on their known architecture (Shewmaker et al. 2006; Baxa et al. 2007; Wickner et al. 2008) and the sequence-independence of prion-forming ability (Ross et al. 2004; Ross et al. 2005).

Yeast and fungal prions fit the definition of 'epigenetic phenomenon', namely, “mitotically and/or meiotically heritable changes in gene function that cannot be explained by changes in DNA sequence” (Riggs et al. 1996). Like the chromatin-based epigenetic phenomena, the amyloid-based prions have provided an alternative means of transmission of heritable/infectious information.

Yeast and fungal prions have proven to display many phenomena that parallel the mammalian TSEs, and these parallels will be detailed below. However, the many tools available to the yeast geneticist have facilitated the rapid development of the yeast prion field, resulting in rapid progress in this area, in spite of the relatively modest number of research groups involved. In some cases, models proposed in the TSE field (like the existence of prions!) were first proven for yeast prions. In other cases (such as the extensive role of chaperones and the Btn2/Cur1 prion-curing system) the yeast system has led the way.

## DISCOVERY OF YEAST PRIONS

That [URE3] and [PSI+] were prions of Ure2p and Sup35p, respectively was first evident from their unusual genetic properties, each inconsistent with [URE3] or [PSI+] being DNA or RNA replicons, but actually expected for a prion (Wickner 1994)(Figure 1). These genetic criteria were a) infectivity, as shown by non-chromosomal inheritance; b) reversible curability: each prion could be efficiently cured, but would (rarely) re-appear in the same strain; c) overproduction of the corresponding protein dramatically increased the frequency of the prion's appearance; and d) the phenotype of the prion was essentially that of a recessive mutant in the corresponding chromosomal gene for the prion protein, and yet this chromosomal gene was necessary for the propagation of the corresponding prion (Wickner 1994)(reviewed in (Wickner et al. 2015)). That amyloid of the respective prion proteins is the basis of the [PSI+] and [URE3] prions (Fig. 2) was shown by the protease - resistance of Ure2p in [URE3] strains (Masison and Wickner 1995), the aggregated state of Sup35p in [PSI+] cells (Patino et al. 1996; Paushkin et al. 1996), and of Ure2p in [URE3] cells (Edskes et al. 1999), and amyloid formation by Sup35p (King et al. 1997), seeded by extracts of [PSI+] cells (Glover et al. 1997; Paushkin et al. 1997), and of Ure2p (Taylor et al. 1999). Of

course, infectivity of amyloid produced in vitro from the corresponding recombinant prion protein, but not by the non-amyloid form, is definitive (Maddelein et al. 2002; King and Diaz-Avalos 2004; Tanaka et al. 2004; Brachmann et al. 2005), but not useful as a screening procedure. Other methods of finding prions will be discussed below.

## PRION DOMAINS

The N-terminal Q/N rich regions of Ure2p (Masison and Wickner 1995; Masison et al. 1997) and Sup35p (TerAvanesyan et al. 1994) are each sufficient for the propagation of the [URE3] and [PSI+] prions, respectively (Figure 3). The Ure2p prion domain can do so in the complete absence of the C-terminal part of the molecule, efficiently receiving the prion from the full length Ure2p, propagating the prion and transmitting again to the full length Ure2p (Masison et al. 1997). Deletions in the C-terminal domain of Ure2p can dramatically elevate the efficiency of conversion to the prion form, *in vivo* (Masison and Wickner 1995) and *in vitro* (Taylor et al. 1999). Sup35p and HET-s prion formation is also elevated by deletion of regions outside the prion domain (Kochneva-Pervukhova et al. 1998; Balguerie et al. 2003), presumably by destabilizing the native structure of the prion domain. Modifications of the Sup35M (middle) domain also affect prion propagation by affecting the ability of Hsp104 to access the filaments for cleavage (Helsen and Glover 2012). This region, enriched for charged amino acids, separates the N-terminal prion forming domain from the essential C-terminal domain.

The prion domain of Ure2p is important for the stability of the molecule against protein degradation, so that without the prion domain, nitrogen catabolite regulation is defective (Shewmaker et al. 2007). The Sup35p prion domain also has non-prion functions, being important in regulating general mRNA turnover through its interaction with the polyA binding protein and polyA-degrading enzymes (Hosoda et al. 2003; Funakoshi et al. 2007). This region also affects localization of polysomes, directing some to associate with the tubulin cytoskeleton (Li et al. 2014). Thus, prion domains have non-prion functions.

Ross et al. showed that amino acid composition, not sequence, determines the ability of a segment to form a prion, so that randomly shuffling the amino acids in the Ure2p or Sup35p prion domains did not eliminate their prion-forming ability (Ross et al. 2004; Ross et al. 2005). Ross and coworkers have dissected the nature of this composition requirement. and can rather accurately predict prion forming ability (Toombs et al. 2010; Cascarina and Ross 2014). Indeed, it was possible to design prion domains *de novo*, synthesize the genes, and show that the 'designer' protein could be a prion *in vivo* (Toombs et al. 2012).

## THE ROLL CALL OF PRIONS (Table 1)

PrP is a non-essential mammalian cell surface protein of uncertain function whose self-propagating conversion to an amyloid form underlies the TSEs. These TSEs include scrapie of sheep, bovine spongiform encephalopathy, human Creutzfeldt-Jakob disease (in its many forms) and chronic wasting disease of elk and deer. The absence of effective treatment for these conditions and the enormous cost in time and money in their study makes the yeast and

fungal prion systems particularly attractive for understanding the prion phenomena and how cells deal with them.

Sup35p is a subunit of the translation termination factor of *S. cerevisiae*, and its conversion to a self-propagating amyloid form results in the prion [PSI+], with deficiency of this factor producing frequent read-through of the termination codons of many yeast genes (see references above)(Fig. 2). The prion is usually assayed by read-through of premature termination codons in *ADE1* or *ADE2* for adenine biosynthesis. In addition to enabling selection of the [PSI+] prion, the red color of *ade1* or *ade2* colonies enables differentiation of 'strong' (white) or 'weak' (pink) prion variants (see below) based on the efficiency of the read-through (inversely related to the available free Sup35p) as well as the bright red clones that have lost [PSI+].

The [URE3] prion of *S. cerevisiae* is an amyloid form of Ure2p (see references above), a negative regulator of transcription of genes encoding the enzymes and transporters for poor nitrogen sources (Cooper 2002; Magasanik and Kaiser 2002). Ure2p is active when a good nitrogen source is available, inactive when only a poor nitrogen source is supplied or when Ure2p is in the prion form (Fig. 2). A *DAL5-ADE2* fusion gene is usually used to detect or select [URE3] (Schlumpberger et al. 2001; Brachmann et al. 2005). Dal5p is the allantoate transporter and its expression is closely controlled by Ure2p (Rai et al. 1987).

The prion amyloid of Rnq1 (rich in N and Q residues) is called [PIN+] (for [PSI+] - inducibility) or [RNQ+] (Derkatch et al. 1997; Sondheimer and Lindquist 2000; Derkatch et al. 2001) (Fig. 2). [PIN+] dramatically increases the frequency with which [PSI+] arises by relatively rarely serving as a nidus for Sup35p amyloid formation. Rnq1p has no known function (Sondheimer and Lindquist 2000). In detecting the basis of the [PIN+] prion, it was shown that overproduction of other Q/N-rich proteins could also stimulate [PSI+] generation (Derkatch et al. 2001; Osherovich and Weissman 2001). This led to the discovery of several other prions, including [SWI+], [OCT+] and [MOD5+] (Du et al. 2008; Patel et al. 2009; Suzuki et al. 2012).

[SWI+] is an amyloid-based prion of Swi1p (Du et al. 2008), a subunit of a chromatin remodeling complex. Like *swi1* mutants, [SWI+] strains are deficient in utilizing non-fermentable carbon sources such as raffinose, galactose, glycerol or sucrose.

[OCT+] is an amyloid-based prion of Cyc8p, a subunit (with Tup1p) of a transcription repressor (Patel et al. 2009). Like *cyc8* mutants, [OCT+] cells are slow growing, with defects in mating and sporulation, and are derepressed for expression of Cyc7p, allowing growth on lactate of *cyc1* mutants (Patel et al. 2009).

[ISP+] (opposite of [PSI+]) is a prion of Sfp1p, a transcriptional regulator of ribosomal protein genes and ribosome biogenesis genes (Rogoza et al. 2010). [ISP+] strains show decreased read-through of nonsense codons (the opposite of [PSI+]) in strains with certain mis-sense mutations in *sup35* and *sup45* as a result of increased expression of Sup35p. Sfp1p forms largely intra-nuclear aggregates in [ISP+] cells and, presumably as a result, is transmitted by cytoplasmic mixing only at a low frequency (Rogoza et al. 2010).

Mot3p is a transcription factor that represses genes for adaptation to hypoxic conditions, and positively regulates some genes involved in resisting osmotic stress (Martinez-Montanes et al. 2013). Mot3p can form an amyloid-based prion, [MOT3+] that enhances filamentous growth on poor nitrogen sources (Holmes et al. 2013).

Mod5p is tRNA isopentenyltransferase, a modifier of anticodon-adjacent residues of several tRNAs affecting their decoding properties (Dihanich et al. 1987). Unlike other yeast amyloid-based prions, Mod5p does not have a Q/N-rich region, but nonetheless forms an amyloid-based prion, [MOD+] and was found by its ability to produce a Pin-like effect (ability to enhance [PSI+] generation) (Suzuki et al. 2012). The core of the infectious amyloid of Mod5p is a 24 residue region with 6 charged residues, unlike the charge-poor prion domains of other yeast prion cores (Suzuki et al. 2012). It will be of interest to know the structure of the amyloid underlying this prion. The presence of the [MOD+] prion enhances growth in the presence of azole anti-fungal drugs, probably by allowing greater ergosterol synthesis. The prion impairs growth in the absence of the drug to a similar degree (Suzuki et al. 2012).

The [Het-s] prion of the filamentous fungus *Podospora anserina* is based on amyloid of the HET-s protein (Coustou et al. 1997; Maddelein et al. 2002)(Fig. 2). This prion is named for its requirement for heterokaryon incompatibility based on the polymorphic *het-s / het-S* locus (Rizet 1952). Fungal clones are generally not completely separate cells (unlike cells in a clone of yeast), but rather have connections through perforated septa partially separating cells in a mycelium. These perforations allow passage of organelles and other cell contents from cell to cell, making the clone like one large polynucleated cell. When two clones of *P. anserina* grow toward each other, there is fusion of hyphae from each clone with those of another clone. The first trial hyphal fusions test whether the two clones are identical at about a dozen polymorphic loci scattered about the fungal genome (*het* loci). A difference at any one of these loci results in death of the cells that underwent the trial fusion, and a barrier to further fusion. These heterokaryon incompatibility systems are designed to protect against harmful viruses and plasmids. One such polymorphic locus is *het-s*, with 2/3 of wild isolates having the *het-s* allele and 1/3 having the *het-S* allele. When *het-S* and *het-s* clones meet, the proper incompatibility is only shown if the HET-s protein (product of *het-s*) is in the prion form.

Not all prions are based on amyloid. The [BETA] prion is simply the active form of the vacuolar protease B of *S. cerevisiae*. Normally the inactive protease B precursor protein is activated by cleavage by protease A (Jones 1991). However, in the absence of protease A (*pep4* mutant), mature active protease B itself can activate its own precursor (Zubenko et al. 1982). As a result, in a *pep4* mutant, active protease B can act as a prion, with all the expected properties (Roberts and Wickner 2003). This system is somewhat artificial, because the prion property is only evident in the *pep4* mutant, but in that condition, [BETA] is critical for survival in stationary phase and necessary for sporulation (Roberts and Wickner 2003). Several other non-chromosomal genetic elements have been proposed to be prions, but will require further work to confirm their status.

It is not out of place to briefly discuss what is and is not a prion. When Prusiner defined the word "prion" in 1982 (Prusiner 1982), many were at pains to argue that there was/ could be no such thing as a prion. But once we discovered yeast prions in 1994, this attitude changed to "prion envy", a phenomenon that continues to the present. The defining features of prions are a) infectivity and b) the protein-only requirement. As the preceding example illustrates, amyloid is not part of the definition, but is rather a frequent (but not invariant) property of most prions. An amyloid that is not transmissible from one individual to another is not a prion, even if it is self-propagating. Even less qualified is an amyloid whose formation and destruction is regulated by some cellular condition or environment. To be a prion, a protein must have a non-prion form that is quite stable in the absence of the prion form. If the protein, once formed, automatically falls into the amyloid form, for example, then there is no possibility of infection.

## PRION VARIANTS/STRAINS AND TRANSMISSION BARRIERS

Prions are poorly transmitted between species, a phenomenon called the species barrier, and first recognized as an extended incubation period on first transmission of scrapie from sheep to goats (compared to sheep-to-sheep or goat-to-goat transmission) (Cuille and Chelle 1939). In some cases the barrier to transmission of TSEs is absolute. The basis of the species transmission barrier was shown to be the PrP amino acid sequence itself (Prusiner et al. 1990), and was one of the most convincing lines of early evidence for the prion model of scrapie because it suggested that the PrP molecule was the information-carrying molecule.

[PSI+] can be formed by the Sup35 prion domains (N) of *Candida albicans, Pichia methanolica* and *Kluyveromyces lactis*, fused to the *S. cerevisiae* Sup35MC (the non-prion part) and expressed in *S. cerevisiae* (Chernoff et al. 2000; Kushnirov et al. 2000; Santoso et al. 2000). Full length Sup35p's, also expressed in *S. cerevisiae*, from *Candida maltosa, Debaryomyces hansenii, K. lactis*, and *Zygosaccharomyces rouxii* could also form [PSI+] (Nakayashiki et al. 2001). Transmission barriers among these species were evident, although, of course, these species do not mate in the wild. Haploids of different species of *Saccharomyces* can mate with each other to produce healthy diploids, although their meiotic products are usually inviable. Transmission barriers between the Ure2p's of *Saccharomyces* species for transmission of [URE3] were also observed (Edskes and Wickner 2002; Edskes et al. 2009), as were barriers for the transmission of [PSI+] between the Sup35's of several *Saccharomyces* species (Chen et al. 2007). These barriers were based on interspecies sequence differences in the prion domains (Chen et al. 2007; Edskes et al. 2009).

Within *S. cerevisiae*, there are essentially three groups of Sup35p prion domain sequences (Resende et al. 2003; Bateman and Wickner 2012). These sequence differences produce substantial transmission barriers among these three polymorphs (Bateman and Wickner 2012). Rnq1p polymorphs are also found in wild *S. cerevisiae* strains and these too produce transmission barriers, or in some cases, complete inability to have a [PIN+] prion (Kelly et al. 2012).

A single peptide with a single amino acid sequence can form any of a large number of prion 'variants' or 'strains'. This phenomenon, long known in mammalian prions (e.g., (Bruce et

al. 1991)), was first recognized in yeast prions by Derkatch and Liebman studying [PSI+] (Derkatch et al. 1996), and has been now documented in [URE3] and [PIN+] as well (Schlumpberger et al. 2001; Bradley et al. 2002; Brachmann et al. 2005). This phenomenon suggests that there must be a mechanism by which a prion can template its own structure, but since the sequence of the protein was the same in different prion variants, this must mean that conformation can be templated. Evidence for conformational differences between different strains/variants of a given prion has been found in several systems (Bessen and Marsh 1992; Toyama et al. 2007; Chang et al. 2008), but only with the elucidation of the architecture of the yeast prions has a possible mechanism for this template emerged (see below). The array of prion variants is surprisingly large (Table 2), a fact which at one time led many to doubt the existence of prions because there was not even a hypothesis about how protein conformation could be transmitted from molecule to molecule. Mammalian prion variants can be distinguished by (at least) incubation period, regions of the brain typically affect, neurological symptoms and signs, degree of protease-resistance of PrP in diseased tissue and ability to infect other species than the species of origin. Yeast prion variants have been classified by strength of the prion phenotype (strong vs. weak), stability of prion propagation, reaction to overproduction or deficiency of various chaperones and cofactors, toxicity to the host (lethal, toxic or mild), sensitivity to antiprion systems (such as Btn2/Cur1), ability to cross intraspecies or interspecies transmission barriers, or ability to propagate in an array of mutant prion proteins (Table 2).

How stable are prion variants? Some experiments using interspecies transfer of TSEs have suggested remarkable stability as long as the transmission barrier to the other species was not too great (reviewed by (Bruce 2003)). In other cases, crossing the species barrier selected a distinct variant that, when returned to the original species showed a different incubation period (e.g. (Kimberlin et al. 1987). Similar results have been found in yeast. Different [URE3] variants show different barriers to transmission to other *Saccharomyces* species's Ure2p (Edskes et al. 2009). Having overcome a species barrier, [URE3] variants retain their ability to propagate in their host of origin, in contrast to variants arising *de novo* in the new host (Edskes et al. 2009). If the species barrier to transmission is too high, as between *S. cerevisiae* and *Pichia methanicola*, the accuracy of transmission breaks down and rare mistemplated variants are selected (Vishveshwara and Liebman 2009).

The three polymorphs of Sup35p in wild strains of *S. cerevisiae* produce [PSI+] transmission barriers between polymorphs, called intraspecies barriers (Bateman and Wickner 2012). The extent of these barriers depends dramatically on the [PSI+] variant being tested (Bateman and Wickner 2012). Remarkably, simple extensive mitotic propagation of one strain carrying one of these variants gives rise to each of the other variants (Bateman and Wickner 2013). Since no selective pressure was applied, this result implies the existence of a mixture of variants that resulted in segregation of relatively pure isolates. However, further extensive propagation (without selection) of each of the segregated variants produced, again, each of the other variants, implying that there must be a prion mutation process that continues to renew the heterogeneity of variants within a single cell (Bateman and Wickner 2013). This "prion cloud" model was first proposed to explain the existence of prion variants in mice (Collinge and Clarke 2007), and evidence consistent with this idea were obtained using prion variants resistant to an anti-prion drug (Li et al. 2010), but in those experiments

induction of variant mutation by the drug could not be eliminated. This phenomenon suggests that the treatment of amyloid diseases may be complicated by the selection of drug-resistant variants, similar to the problems in treating microbial infectious diseases. The non-selective segregation and mutation of [PSI+] variants at the prion level (Bateman and Wickner 2013) resembles the neutral genetic drift model of Kimura so prominent in explaining DNA-level evolution (Kimura 1968).

## STRUCTURE OF INFECTIOUS AMYLOID OF YEAST AND FUNGAL PRIONS

Amyloid is a filamentous unbranched polymer of proteins or peptides containing mostly β sheet structure, with the β strands perpendicular to the long axis of the filament. The major types of β sheet are anti-parallel, parallel in-register, parallel out-of-register and β helix (Fig. 4A) (Tycko 2006). Several human pathologic amyloids have been shown to be parallel in-register (reviewed in (Tycko and Wickner 2013).

**[Het-s]**Infectious amyloid of the HET-s prion domain forms a single structure as indicated by sharp peaks on multidimensional solid-state NMR (Ritter et al. 2005; Wasmer et al. 2008). The structure is a β helix with each molecule comprising two turns of the helix (Fig. 4A)(Wasmer et al. 2008). In addition to the detailed solid-state NMR data (Ritter et al. 2005; Wasmer et al. 2008), mass per length data showed ~0.5 molecules per 4.7 angstroms, the distance between two β strands (Sen et al. 2006). This means that each molecule comprises two β strands along the long axis of the filament, exactly as expected for the β helix model (Figure 4A). In a β helix structure, at least half of the hydrogen bonds supporting the structure are intramolecular. Intramolecular bonds will be kinetically favored, and so it is likely that they form first, before intermolecular bonds, and fix the conformation of the monomer. Over a longer time frame, monomers then probably stack to form the filaments. This could explain why there is only one conformer, and only one prion variant.

### Yeast prion amyloids.

The prion domains of Ure2p or Sup35p could be shuffled (simply re-ordering the amino acids without changing the amino acid content) and yet still generate prions (in all five shuffles of each prion domain) (Ross et al. 2004; Ross et al. 2005). However, prion propagation requires near sequence identity between donor and recipient molecules (e.g. (Prusiner et al. 1990)), and in some cases, even a single amino acid difference can block prion propagation (Priola et al. 1994; Santoso et al. 2000; King 2001). This seeming paradox led to the suggestion that the amyloid underlying these prions has an in-register parallel architecture (Ross et al. 2005)(Fig. 4B). Amyloids of Ure2p and Sup35p are known to have β sheet structures (Glover et al. 1997; King et al. 1997; Taylor et al. 1999). If the arrangement of β strands were antiparallel, or β helix, or parallel but out of register, the sequence specificity for propagation would have to be due to an interaction of complementarity between residues on the new molecule joining the end of the filament, and the largely non-identical residues of the last molecule already on the end of the filament. Shuffling the sequence would almost always destroy such complementarity and thus destroy prion-forming potential. However, if the structure were in-register parallel β sheet, then a residue on the molecule newly joining the end of the filament would interact with the

identical residue of the molecule already present on the end of the filament. Shuffling the sequence would leave the same residues interacting with their identical partner, but just in a different order along the peptide chain (Ross et al. 2005).

Using selective labeling schemes of infectious amyloid of the prion domains of Sup35p, Ure2p and Rnq1p, and solid-state NMR, it was shown that the architecture of each is, indeed, an in-register parallel β sheet (Shewmaker et al. 2006; Baxa et al. 2007; Wickner et al. 2008; Gorkovskiy et al. 2014) (Fig. 5). The prion amyloid of the *Candida albicans* Ure2p prion domain also has this architecture (Engel et al. 2011). As predicted from the shuffling experiments, shuffled prion domains also had the same architecture (Shewmaker et al. 2008). These experiments show that the distance between a particular residue in the prion domain and the same residue on the next molecule in the filament is about the 4.7 angstrom distance between β strands. These results rule out antiparallel β sheets, the β helix, and parallel out-of-register structures (Fig. 4A). Electron microscopy showed that the sheets must be folded several times along the long axis of the filaments (Fig. 4 a). This architecture was supported by mass per length measurements of each of these filaments, which all show a single molecule per 4.7 angstroms length along the filament (Baxa et al. 2003; Diaz-Avalos et al. 2005; Chen et al. 2009). These measurements specifically rule out β-helix models which predict 0.5 or less of a molecule per 4.7 angstroms, as observed in amyloid of HET-s (Sen et al. 2006) (see Fig. 4A). Electron spin resonance measurements of Ure2p amyloid confirm the in-register parallel β sheet model (Ngo et al. 2011).

Recently, site-specific labeling was used to confirm the in-register parallel architecture and to find some potential locations of folds in the sheet of the Sup35p prion domain (the fold locations could differ in different prion variants as discussed below) (Gorkovskiy et al. 2014). Electron spin resonance experiments have similarly suggested the locations of folds in Ure2p amyloid (Ngo et al. 2012). Wong and King have used chemical cross-linking of amyloid filaments made of a modified Sup35p short prion domain seeded by filaments purified from either of two different [PSI+] prion strains to determine the location of folds in the β sheets of the filaments (Wong and King 2015). They detected clear differences between the filaments seeded by different variants. Importantly, the filaments generated in vitro produced only the respective original variants when transfected into yeast cells implying that the templating was faithful (Wong and King 2015). These results support the folded in-register parallel β sheet model and promise to clarify more detail than has been possible so far.

A detailed atomic-level structure of the yeast prion amyloids has so far been impossible because of inherent disorder in the filament preparations and the relatively large size of the minimal infectious peptides. However, some assignments of residues in the Sup35 prion domain have been possible with confirmation of the β sheet secondary structure of this region (Luckgei et al. 2013).

The folded in-register parallel architecture found for the yeast prions studied so far is similar to that found for nearly all of the human pathological amyloids, including Aβ peptide (Alzheimer's disease), amylin (type II diabetes), alpha-synuclein (Parkinson's disease) and

others (reviewed in (Tycko 2011)). Yeast prions have thus become important model systems for the very common human amyloidoses.

## YEAST PRION AMYLOID STRUCTURE EXPLAINS INHERITANCE OF CONFORMATION

A parallel in-register β sheet amyloid has rows of identical amino acid side chains extending along the long axis of the filaments (Figure 5B). The register is maintained by favorable interactions among the identical side chains. For example, a row of glutamine residues can form a row of hydrogen bonds joining the amide groups of their side chains and extending the length of the filament. The same structure can form with asparagine, serine or threonine residues. A line of any hydrophobic residues will be stabilized by hydrophobic interactions between the side chains. Charged residue side chains would be unfavorable, and there are (probably for this reason) very few charged residues in the prion domains of known prion proteins with this architecture.

The folded in-register parallel β sheet architecture of yeast prion domains leaves relatively little flexibility for variation, although a single peptide sequence can form amyloid with any of many different detailed structures, each self-propagating. The location and size of peptide chain loops at the locations of the folds, and the extent of the parallel in-register structure could well differ among prion variants (Fig. 5A). We have suggested that the same favorable interactions between identical side chains that keep the structure in-register must be the main factor allowing conformational templating by prion amyloids (Ross et al. 2005; Wickner et al. 2008; Wickner et al. 2013). The molecule joining the end of the amyloid filament will adjust its turns to be in the same location as those of the last molecule on the end of the filament in order to have these favorable hydrogen bonds and hydrophobic interactions that are found between molecules already in the filament (Figure 5B). This is the only model that has been proposed so far for the central event in prions, the templating of protein conformation.

## PRION BIOLOGY

While most amyloids in humans are pathogenic, functional amyloids in various species have been described. Pmel17 is a melanosome protein that functions in an amyloid form, and is important for melanin biogenesis, (Fowler et al. 2006). Fish embryos (Podrabsky et al. 2001) and fungal cell surfaces (Mackay et al. 2001) use amyloids as a protective layer. Proteins forming a gel in the zona pelludica of mouse eggs are amyloids (Egge et al. 2015), and regulated amyloid formation of Rim4p in *S. cerevisiae* controls stages of meiosis (Berchowitz et al. 2015). Are some prions functional?

### The [Het-s] prion of *Podospora anserina*

The *het-s/het-S* system has at least two, and probably three effects on its host, *P. anserina*. The HET-s protein must be in the amyloid ([Het-s] prion) form in order that a *het-s/het-S* heterokaryon show the proper incompatibility with programmed death of the heterokaryon cells and formation of a barrier to further hyphal fusions (Rizet 1952; Coustou et al. 1997).

As mentioned earlier, the heterokaryon (vegetative) incompatibility reaction is a self/non-self recognition system seen in most or all filamentous fungi whose function is to limit the spread of harmful viruses and plasmids (Caten 1972; Hartl et al. 1975; Debets et al. 1994). In fact the *het-s/het-S* system can block transmission of a harmful plasmid in *Podospora* (Debets et al. 2012). It was thus proposed that this was the first case of a functional prion (Coustou et al. 1997; Wickner 1997). However, in contrast to this effect on the mitotic heterokaryon formation, [Het-s] has another, less favorable, effect, this on the meiotic cycle. Mating of female *het-s* [Het-s] strains with male *het-S* strains results in a high frequency selective death of *het-S* meiotic segregants (Bernet 1965; Dalstra et al. 2003). Crossing *het-s* [Het-s*] (no prion) with *het-S* strains does not produce this spore lethality. This is an example of meiotic drive, a gene or locus promoting its spread in the population, not by favoring fitness of the individual, but by impairing inheritance of other alleles at that locus. Segregation Distorter of *Drosophila melanogaster* and the "t locus" of mice are well known examples. The meiotic drive effect of [Het-s] favors the inheritance of the *het-s* allele and could result in elimination of the *het-S* allele. However, the heterokaryon incompatibility function of the *het-s/het-S* locus is most effective if the two alleles are present at roughly equal frequency in the population (Debets et al. 2012). That 2/3 of wild strains have the *het-s* genotype is explained as a balance between these two effects (Debets et al. 2012). As expected for a beneficial infectious element, [Het-s] is present in 92% of wild *het-s* strains (Debets et al. 2012).

Adjacent to the *het-s/S* gene is *nwd2*, encoding a gene with similarity to a family of pathogen recognition proteins of plants and animals, called STAND proteins, and including an N-terminal domain similar to the prion domain of HET-s. The *nwd2* gene is disrupted in all *het-s* strains, but intact in *het-S* strains (Debets et al. 2012). It is proposed that an as yet unknown ligand of NWD2 can induce amyloid formation by NWD2 and that, like the cell death induced by the interaction of HET-S with the prion amyloid of HET-s, the amyloid of NWD2 induces HET-S to cause cell death (Paoletti and Saupe 2009). Evidence for this reaction has been obtained, although the nature of the normal signal remains unknown (Daskalov et al. 2015). It is proposed that from this NWD2 : HET-S interaction evolved the [Het-s] prion by successive disruption of *nwd2*, and mutation of *het-S* to *het-s* (Daskalov and Saupe 2015).

These three biological effects of the *het-s/S* locus have combined to shape its evolution. This system is so far unique in being a demonstrated beneficial prion, but with side-effects whose details and implications are continuing to be elucidated.

## Yeast Prions.

**Can they be beneficial?—**Following the discovery of the beneficial [Het-s] prion, it was reported that the yeast [PSI+] prion made cells resistant to the effects of high temperature or high ethanol concentration and that [PSI+] was thus an advantage to yeast (Eaglestone et al. 1999). A subsequent study compared five [PSI+] and [psi−] isogenic strain pairs and did not find protection against heat or ethanol by [PSI+], nor any consistent protection by [PSI+] under any condition (True and Lindquist 2000). However, in about 1/4 of the conditions where [PSI+] made a difference in a particular strain pair, the [PSI+] strain grew better (True

and Lindquist 2000). It was inferred that [PSI+] could help yeast evolve by helping some strains under some conditions. Another group, using the same strains, could not reproduce these results (Namy et al. 2008). A later study, reporting that in the rare [PSI+] wild strains, the prion aided cell growth under certain conditions (Halfmann et al. 2012) was again not confirmed using the same strains (Wickner et al. 2015). Thus, it remains controversial whether [PSI+] is beneficial under any but the sort of artificial conditions used in the assay (read-through of a premature termination codon in a biosynthetic gene).

It may be impossible to resolve the issue of whether [PSI+] or [URE3] are beneficial or detrimental by laboratory tests. Even finding [PSI+] or [URE3] to be reproducibly beneficial under some condition, would not imply that such condition constitutes a significant part of the *S. cerevisiae* ecological niche. A report that certain stress conditions induce the appearance of [PSI+] also included the finding that under most such conditions, [PSI+] was detrimental (Tyedmers et al. 2008), and the stress-induction could not be repeated (Kelly et al. 2012; Westergard and True 2014).

**Can [PSI+] and [URE3] be toxic/lethal?—**[PSI+] and [URE3] can be quite detrimental or even lethal: most [URE3] isolates slow growth noticeably (Schwimmer and Masison 2002) and most variants are highly toxic (McGlinchey et al. 2011). Isolating [PSI+] in the presence of minimal expression of Sup35C (lacking the prion domain) allowed detection of lethal and near-lethal variants of [PSI+], which were, again, more common than the mild form of the prion (McGlinchey et al. 2011). Note that the studies reporting benefits of yeast prions only considered the mildest variants (Eaglestone et al. 1999; True and Lindquist 2000; Halfmann et al. 2012). A realistic estimate of the fitness value of a cell's forming a prion must weigh the potential benefits against the real risks of prion formation. *[PSI+], [URE3], [PIN+] and [SWI+] in wild strains*. Even the uniformly fatal mammalian prion diseases are not rare in the wild. Chronic Wasting disease of deer and elk are epidemic in many parts of the US (Saunders et al. 2012). Like other lethal infectious viral or bacterial diseases, their infectivity outruns their destruction of their hosts. If there were an infectious agent that actually benefited its host, infectivity and host effects would both act to spread the agent in the population, and it would soon be found in most isolates. The [Het-s] prion, found in 92% of wild *het-s* isolates (Debets et al. 2012), is a clear example of this general idea. Therefore, an infectious agent that is rare in the wild must be detrimental to its host. Examining the incidence of [PSI+] and [URE3] in the wild is a measure of how beneficial/detrimental are the mildest, most stable variants of these prions, and gives only an upper limit on any potential positive effect on the fitness of maintaining prion-forming ability.

Two early studies found no [PSI+] isolates among a few wild strains, but did find two [PIN+] strains (Chernoff et al. 2000; Resende et al. 2003). A further study of 70 wild isolates found none with [PSI+] or [URE3], but again did detect [PIN+] in 11 of the strains (Nakayashiki et al. 2005). The selfish RNA viruses, L-BC, 20S and 23S RNA and the DNA plasmid 2 micron DNA, encode only factors promoting their own replication and segregation, but were found in 8, 14, 1 and 38 of the 70 strains, respectively (Nakayashiki et al. 2005). The 2 micron DNA plasmid was particularly useful because studies by three groups have shown that this parasitic plasmid slows cell growth by 1-3% (Futcher and Cox 1983; Mead et al. 1986; Futcher et al. 1988; Kelly et al. 2012). The 2 micron DNA plasmid

is somewhat unstable in mitosis, and can only arise *de novo* over geologic time, whereas nonlethal [URE3], [PSI+] and [PIN+] arise about once in $10^6$ cells. Nonetheless, these three prions are less widespread, implying that even their mildest variants must have a greater detrimental effect than the 1-3% growth slowing shown for 2 micron DNA (Kelly et al. 2012). Another larger study of 690 wild isolates found 9 [PSI+] strains (Halfmann et al. 2012), statistically indistinguishable from the 0 of 70 in the earlier report (Nakayashiki et al. 2005). The incidence of [PIN+] was slightly lower, at 6%.

Although the low frequency of [PIN+] indicates, by this comparison with the 2 micron DNA plasmid, that [PIN+] is detrimental, the frequency of its occurrence in nature is still greater than the frequency of its arising *de novo*. The spread of [PIN+] could be a result of selection for an advantageous trait determined by [PIN+] or it could be a result of outcross mating (Kelly et al. 2014). As predicted by the latter explanation wild [PIN+] strains tend to show evidence of having arisen from outcross matings, namely, heterozygosity for common polymorphic alleles (Kelly et al. 2014). The report that 1/3 of wild strains had guanidine -curable (and thus prion-determined) traits could not be reproduced with a sample of these strains (Kryndushkin et al. 2013). The [SWI+] prion was also absent from the 70 strain collection, indicating that it too has a negative effect on fitness (Bateman and Wickner 2012).

**Species Distribution of Prion-forming Ability.—**The N-terminal domains of Sup35s of several species have been found capable of substituting for the *S. cerevisiae* Sup35p prion domain in forming a prion in *S. cerevisiae* cells: Sup35N from *Candida albicans, Kluyveromyces lactis, Pichia methanolica* and several species of *Saccharomyces* (Chernoff et al. 2000; Kushnirov et al. 2000; Santoso et al. 2000; Nakayashiki et al. 2001; Chen et al. 2007; Afanasieva et al. 2011). These results were interpreted as implying conservation of a desirable function (prion formation) selected by evolution. However, a wider survey of Sup35s from yeasts and fungi showed that the Sup35s of many species, including *Schizosaccharomyces pombe, Ashbya gossypii, Aspergillus nidulans, Aspergillus fumigatus, Magnaporthe grisea, Ustilago maydis* and *Cryptococcus neoformans* could not form prions by the same test (Edskes et al. 2014). Of course, the presence of a trait in many species does not imply that that trait is a function conserved for the organism's benefit. Occasional broken limbs is a trait widely found in vertebrates.

[URE3] formation by full length Ure2p from other *Saccharomyces* species has likewise been tested in *S. cerevisiae*, and most can do so (Edskes and Wickner 2002; Baudin-Baillieu et al. 2003; Edskes et al. 2009), but that of *Saccharomyces castellii* cannot (Edskes et al. 2009). The Ure2p of *Kluyveromyces lactis* is unable to form [URE3] in either *S. cerevisiae* or in *K. lactis* itself (Safadi et al. 2011). The Ure2p of *Candida albicans* can form [URE3] in either *S. cerevisiae* or in *Candida glabrata*, but the Ure2p of *C. glabrata* is unable to form the prion in either *S. cerevisiae* or its native host, *C. glabrata* (Edskes et al. 2011; Engel et al. 2011; Edskes and Wickner 2013). Notably, the Ure2p of *C. glabrata* is very close in sequence to that of *S. cerevisiae*, but cannot form a prion, while the more distantly related *C. albicans* Ure2p can form a prion. This is not what is expected for conservation of prion formation.

All of the studies comparing the putative prion domains (which do not always form prions) of Sup35p and Ure2p have shown that the rate of evolutionary change is far greater for these domains than for the remainder of the molecule (e.g. (Santoso et al. 2000; Edskes and Wickner 2002; Bateman and Wickner 2012)). Because even a single amino acid difference between the prion protein of the infection donor and recipient can, in some cases, block propagation of a prion (e.g. (Priola et al. 1994; Santoso et al. 2000; King 2001), the accumulation of mutations in the prion domains can be viewed as selected to block infection of the organism with a potentially lethal disease. Kuru is an epidemic form of Creutzfeldt-Jakob disease that occurred among the Fore people of New Guinea as a result of their ritual funereal cannibal custom (Collinge and Alpers 2008). This epidemic led to the emergence of the PrP G127V mutation because it confers complete resistance to the disease (Asante et al. 2015). The polymorphs found among *S. cerevisiae* strains in the Sup35p prion domain likewise produce a barrier to transmission of [PSI+] and are likely to have been selected for this reason (Bateman and Wickner 2012). The prion domains of Ure2p and Sup35p are known to have important functions (see above), and so have not been simply lost. Their composition has likely been selected to minimize the frequency of prion formation given the constraints of these non-prion functions. Paul et al. found that proteins with composition somewhat similar to that of prions could be converted to prions by a very small number of changes, suggesting that their composition has been selected to minimize prion formation (Paul et al. 2015).

It remains possible that there is some special niche of yeast in which one or more of these prions is helpful for the host, but a general role in aiding stress-tolerance (Eaglestone et al. 1999) or evolvability (True and Lindquist 2000), as has been proposed by others, seems unlikely. Some have proposed a 'bet hedging' role for prion generation under stress (Newby and Lindquist 2013), but the prevalence of lethal or highly toxic [URE3] and [PSI+] variants makes prion generation a bad bet for the cell.

**Other yeast prions.—**Pseudohyphal growth is a highly regulated process which allows starved cells to search for nutrients (Gimeno et al. 1992). The [MOT3+] prion can affect development of pseudohyphal growth by the absence of Mot3p's normal regulatory effect on *FLO11* transcription (Holmes et al. 2013). While it is argued that [MOT3+] is thus adaptive, it would seem that, like other prions affecting gene expression, [MOT3+] simply reduces the flexibility of cells to adapt to changing conditions. [MOT3+] was found in six of 96 isolates (Halfmann et al. 2012). Other yeast prions (Table 1) have not yet been surveyed in wild strains or studied in enough detail to know if they are advantageous or detrimental.

It is often argued that prions or retrotransposons are desirable agents because they generate the diversity that natural selection needs in order that organisms may evolve in competition with other organisms or adapt to changing environments. It should be noted that diversity is not in short supply in the biological world. A myriad of assaults on our DNA genomes are opposed by a similarly bewildering array of repair and recombination processes to limit the changes and attempt to pass on a relatively intact version of the genome to the next generation. These repair processes are inevitably imperfect, as the diversity we see testifies, so there is no need for a 'diversifier' function. The immune system's diversifying activity may seem an exception to this rule, but because the diversifications are not inherited,

this is rather a case of differentiation of cell function. It would seem that selecting for a mutator in a sexual organism is limited by the fact that most, like the prions, produce mostly detrimental changes.

## CELLULAR SYSTEMS AFFECTING YEAST PRIONS.

### Chaperones and chaperone co-factors.

The early recognition of the critical role of the disaggregating chaperone, Hsp104 in prion propagation (Chernoff et al. 1995) opened this area as a means of learning features of prions as well as a method of studying chaperones (reviewed by (Masison and Reidy 2015)). Hsp104 is necessary for the propagation of all of the amyloid-based yeast prions (Chernoff et al. 1995; Derkatch et al. 1997; Moriyama et al. 2000; Du et al. 2008; Alberti et al. 2009; Patel et al. 2009; Suzuki et al. 2012) except [ISP+] (Volkov et al. 2002). Overexpression of Hsp104 cures [PSI+] (Chernoff and Ono 1992; Chernoff et al. 1995), but not other prions.

Millimolar guanidine HCl cures [PSI+] (Tuite et al. 1981) and indeed all of the amyloid based yeast prions. This prion curing effect is due to inhibition of Hsp104 (Ferreira et al. 2001; Jung and Masison 2001; Jung et al. 2002; Grimminger et al. 2004). Hsp104's disaggregase activity includes the ability to pull the chain of the aggregated protein through a pore formed by the hexameric Hsp104, thereby denaturing it and giving it a new chance to renature (Lum et al. 2004; Weibezahn et al. 2004). Mutations impairing this activity also impair prion propagation proportionately (Hung and Masison 2006; Tessarz et al. 2008). This suggests that Hsp104 (with collaborating factors) pull a molecule from the middle of an amyloid fiber, thereby breaking the fiber and creating new seeds/propagons (Fig. 6). [Note that 'seed' means prion propagating unit in the cell, presumably a single amyloid filament.] Based on the evidence that guanidine inhibition of Hsp104 blocks the splitting of prion amyloid (Paushkin et al. 1996; Ness et al. 2002; Kryndushkin et al. 2003), and thus the generation of new seeds, seed-counting methods were developed that have been useful in further studies (Cox et al. 2003).

Hsp104 acts with Hsp40s and Hsp70s to disaggregate proteins in vitro (Glover and Lindquist 1998), and it was soon learned that Hsp70s affect both the overproduction curing of [PSI+] by Hsp104 (Newnam et al. 1999), and are necessary for its stable propagation (Jung et al. 2000; Jones and Masison 2003; Hines et al. 2011). Hsp40s are also needed for prion propagation (Sondheimer et al. 2001; Aron et al. 2007; Higurashi et al. 2008; Tipton et al. 2008; Hines et al. 2011; Troisi et al. 2015). This collaboration was confirmed by showing that ClpB, the *E. coli* homolog of Hsp104, cannot propagate the yeast prions [PSI+], [URE3] or [PIN+] when substituted for Hsp104, but when the *E. coli* Hsp70 (DnaK) and its nucleotide exchange factor (GrpE) are also expressed, then ClpB can fulfill both the heat shock - resistance and prion propagation roles of Hsp104 (Reidy and Masison 2012). Substitution of the ClpB M domain, known to interact with Hsp70s, with that of Hsp104 also suffices to allow its functioning in yeast (Reidy and Masison 2012). The Hsp104 of *S. pombe* can substitute for that of *S. cerevisiae* without other *S. pombe* components (Reidy et al. 2013).

In addition to being required for propagation of all amyloid-based yeast prions, Hsp104, when overproduced, cures [PSI+] (and not others) (Chernoff et al. 1995). Several lines of evidence indicate that the [PSI+]-curing activity of Hsp104 is quite distinct from its prion-propagating (seed-producing) activity. Mutations in the N-terminal domain of Hsp104, or deletion of the entire N-terminal region to residue 147 does not adversely affect prion propagation or heat shock tolerance, but completely eliminates the ability of Hsp104 overexpression to cure [PSI+] (Hung and Masison 2006). Other cellular components that affect Hsp104 overproduction curing do not affect Hsp104 promoted prion propagation: the ribosome-associated Hsp70s, Ssb1 and Ssb2, assist Hsp104 overproduction curing (Allen et al. 2005); Sti1p, a cochaperone that interacts with Hsp90, Hsp70 and Hsp104, is necessary for Hsp104 overproduction curing (Reidy and Masison 2010); inhibition of Hsp90 with radicicol blocks Hsp104 curing (Reidy and Masison 2010); and increasing or decreasing ubiquitin expression increases or decreases Hsp104 curing (Chernova et al. 2003; Allen et al. 2007). Guanidine, which inhibits the "severing" activity, still inhibits Hsp104 when the chaperone is overexpressed, and yet guanidine has little effect on the efficiency of Hsp104 overproduction curing (Park et al. 2014). This data indicates that curing by Hsp104 overexpression is not a result of inhibition of the activity.

Nystrom's group has described a system that assymetrically segregates damaged (e.g. oxidized) proteins, with preferential retention in the mother cell (reviewed in (Nystrom and Liu 2014)). This system involves Hsp104 (e.g.(Erjavec et al. 2007)) and so it was possible that Hsp104 overproduction was curing [PSI+] by assymetric segregation of the prion aggregates. However, sorting of cells being cured of [PSI+] by Hsp104 overproduction curing gave equal curing of mother and daughter cells (Park et al. 2014). Further, the distribution of prion aggregates between mother and bud during Hsp104 overproduction was in proportion to their relative volumes (Park et al. 2014).

Direct observation of cells undergoing [PSI+] curing by Hsp104 overexpression indicate that the foci are being gradually dissolved (Park et al. 2014). It is suggested that this represents removal of Sup35p molecules from the ends of the filaments ("trimming"). The influence of ubiquitin and other chaperone factors on Hsp104 overproduction curing is explained by proteasome degradation of the amyloid core after trimming (Park et al. 2014).

The chaperone environment also shapes what prion variants arise and the frequency of prion generation. Ssb1 and Ssb2 are nearly identical ribosome-associated members of the Hsp70 family, and are believed to aid in the proper folding of newly synthesized proteins (Nelson et al. 1992; James et al. 1997). The Ssb's are attached to the ribosome by another Hsp70 family member, Ssz1, and an Hsp40, Zuo1p (Gautschi et al. 2001; Huang et al. 2005). Overproduction of Ssb1/2 increases the efficiency with which Hsp104 overproduction cures [PSI+] (Chernoff et al. 1999), showing that the Ssb's have anti prion effects. Deletion of both *SSB* genes results in an increase in [PSI+] generation, either spontaneously or induced by overexpression of the Sup35 prion domain (Chernoff et al. 1999), and this induction is independent of [PIN+] (Kiktev et al. 2015). Like *ssb1 ssb2* strains, mutations of *zou1* or *ssz1* also increase the rate of [PSI+] generation (Amor et al. 2015; Kiktev et al. 2015), but have the opposite effects of *ssb1 ssb2* on overproduction curing by Hsp104 (Kiktev et al. 2015). Disruption of *zou1* or *ssz1* results in freeing of much of the Ssb1/2 from the ribosomes,

where it competes with Ssa's for binding to Sup35p amyloid, thereby, it is proposed, aiding in the Hsp104 overproduction curing (Kiktev et al. 2015).

Overexpression of Hsp104 increases [URE3] prion generation by the Ure2p of *Candida albicans* by 1000-fold (Kryndushkin et al. 2011). Sis1p is necessary for propagation of all of the yeast prions tested (Higurashi et al. 2008), but not all of the protein is needed (Kirkland et al. 2011). Interestingly, deletion of either the C-terminal domain or the adjacent glycine - methionine rich region as well, resulted in a normally mild variant of [PSI+] being highly toxic or lethal (Kirkland et al. 2011). The mechanism of toxicity in these cells would be of considerable interest.

As mentioned above, inhibition of Hsp90 with radicicol inhibits curing of [PSI+] by overproduction of Hsp104. Recently, evidence has appeared that [URE3] propagation requires interaction of Hsp90 with its co-chaperone Cpr7 (Kumar et al. 2015). Deletion of either the C-terminal motif of Hsp90 that interacts with Cpr7p, or deletion of the *CPR7* gene itself results in inability to propagate [URE3] (Kumar et al. 2015). The precise role of Hsp90s and their co-chaperones in [URE3] propagation remain to be determined.

## Btn2/Cur1 cure [URE3] (not [PSI+]) by sequestering seeds

A screen for proteins whose overproduction cures [URE3] produced Btn2p and its paralog, Cur1p (Kryndushkin et al. 2008). Overproduced Btn2p or Cur1p also cure an artificial prion (Malinovska et al. 2012). Btn2p is involved in endosomal protein sorting (Kama et al. 2007) and is distantly related to human HOOK1, a member of a family of proteins involved in transport of large cargos along the microtubules (Maldonado-Baez et al. 2013). In cells in which overproduced Btn2p is curing [URE3], the aggregates of Ure2p appear collected at a single site coincident with Btn2p (Kryndushkin et al. 2008) (Fig. 7). Most [URE3] prion variants isolated in the absence of Btn2p and Cur1p can be cured by replacement of normal levels of either protein (Wickner et al. 2014). As discussed above, there are many methods of curing yeast prions, particularly by over- or under-expressing some chaperones or other cellular components, but Btn2p and Cur1p appear to be acting to cure the [URE3] prion at their normal expression levels. The distinction between which [URE3] variants are cured by normal levels of Btn2p and Cur1p and which are only cured by overexpression appears to be the seed number. Those [URE3] with low seed number (and thus a 'weak' phenotype) are cured by normal levels of Btn2p and Cur1p while the higher seed number [URE3]s are only cured on overproduction of the antiprion components (Wickner et al. 2014). Hsp42 is known to function in collecting aggregated proteins (Specht et al. 2011), and it interacts *in vivo* with Btn2p (Malinovska et al. 2012). Hsp42 was found to be important for the prion - curing action of overproduced Btn2p, but neither of Btn2p or Cur1p is dependent on the other in their curing (Wickner et al. 2014). Overproduction of Hsp42 also cures [URE3] in a process dependent on Cur1p (Wickner et al. 2014).

Btn2p also collects non-prion non-amyloid aggregates (Kryndushkin et al. 2012; Malinovska et al. 2012), however overproduced Btn2p or Cur1p do not cure a [PSI+] prion variant, although there is some degree of colocalization of Btn2p and Sup35p in such cells(Kryndushkin et al. 2008). Cur1p overproduction also increases lethality of [PSI+] in strains heterozygous for a *sup45* missense mutation (Kiktev et al. 2011), again suggesting

interaction of Cur1p with the Sup35p amyloid. The site to which Btn2p appears to bring Ure2p aggregates in a [URE3] cell is not clear at this time, and there is some disagreement about where non-prion aggregates are collected (Malinovska et al. 2012; Miller et al. 2015). Moreover, the relation of Btn2p's prion-curing activity and its endosome transport role remains to be elucidated.

There is a wide array of aggregate-accumulating sites defined recently in yeast. These include the IPOD (Kaganovich et al. 2008), JUNQ (Kaganovich et al. 2008), aggresome (Wang et al. 2009), P-bodies (Sheth and Parker 2003), Q-bodies (Escusa-Toret et al. 2013), age-related aggregates (Aguilaniu et al. 2003), Btn2p-sites (Kryndushkin et al. 2008), Hsp42-sites (Specht et al. 2011) and stress granules (Hoyle et al. 2007).

**Actin cytoskeleton, ubiquitin, heat shock and prions.—**Although prolonged growth at elevated temperatures does not cure yeast prions, a short term heat shock (30 - 60 min at 39 C) cures a weak [PSI+] variant from a substantial proportion of cells (Newnam et al. 2011). This curing occurs with an asymmetric preferential loss of [PSI+] from daughter cells (Newnam et al. 2011; Ali et al. 2014). [PSI+] propagation is also impaired by growth in the presence of lactrunculin A, a drug that disrupts the actin cytoskeleton (Bailleul-Winslett et al. 2000), or by mutation of *sla1*, a gene involved in actin cytoskeleton assembly (Bailleul et al. 1999). Sla1p interacts with the Sup35p prion domain in two-hybrid tests (Bailleul et al. 1999). Las17p is an actin nucleation promoting factor, and Lsb1 and Lsb2 are paralogous binding partners of Las17. An *lsb1* or *lsb2* deletion (or both) destabilize [PSI+] after heat shock (Chernova et al. 2011; Ali et al. 2014), and the stabilizing activity of Lsb1p requires its proteolytic processing (Ali et al. 2014). Lsb2p also has Pin+ activity when overproduced (Derkatch et al. 2001), an effect that is limited by the ubiquitin-mediated rapid turnover of Lsb2p (Chernova et al. 2011). Finally, *ubc4*Δ (a ubiquitin conjugating enzyme) or *ubp6*Δ (a ubiquitin deconjugating enzyme) result in decreased curing of [PSI+] by Hsp104 overproduction and increased generation of [PSI+] (Allen et al. 2007). These observations indicate that the ubiquitin system antagonizes [PSI+] generation, but the authors could not detect ubiquitin conjugation of Sup35p itself (Allen et al. 2007).

**Perspectives and Controversies.—**While the yeast prion world has made a strong showing in spite of being massively outnumbered by those studying human and animal prions, there are a number of outstanding controversies which will only be resolved by more work and development of better methods.

**Structure of prion amyloids.:** Although the folded in-register parallel β sheet architecture of yeast prions appears to be settled, this does not constitute an atomic level 'structure'. While HET-s naturally forms uniform filaments, such is apparently not the case for the yeast prions that have been studied thus far. Even seeding with extracts or extracted filaments from a cells with a single apparent prion variant does not appear to produce filaments with the uniformity of the HET-s filaments. However, some progress with this approach has been made (Kryndushkin et al. 2011; Frederick et al. 2014; Wong and King 2015). The approach of seeding from cells may be limited by the inherent heterogeneity of prions in the cells themselves, as shown in the 'prion cloud' phenomenon (Bateman and Wickner 2013).

**Biology.:** While it seems settled that the *Podospora anserina* prion [Het-s] is largely beneficial and the yeast prions are usually detrimental to their host, it remains possible that there are some circumstances, not yet defined, when the latter may prove to be a benefit. Some yeast prions not yet discovered or well characterized may be advantageous, or even a well studied prion may be found to be common in yeast isolated from a particular niche.

**Prion - handling systems.:** Whether Hsp104 can act alone to sever fibers, the location of Btn2p (with and without aggregates), the precise role of ubiquitin in prion propagation, the role of the many sites of aggregate accumulation in prion phenomena, … and many other issues of mechanism remain for further work. The Btn2/Cur1 system works on [URE3], but not on [PSI+] or [PIN+]; are there other systems that cure these prions (the Ssb's block [PSI+] generation)?

**New Prions.:** The large number of prions found in yeast suggests that there are many more to be found in other organisms. Biochemical methods to search for amyloids (e.g. (Kryndushkin et al. 2013)) are more widely applicable while genetic methods depend on special features of an organisms life cycle. Recent developments in the study of amyloidoses suggest that the TSEs are not the only mammalian prions (reviewed by (Jucker and Walker 2013)). Mice with apolipoprotein AII amyloidosis appear to transmit the disease to animals in the same cage (Xing et al. 2001), and amyloidosis A transmission between animals without innoculation has been shown (Zhang et al. 2008). Infectivity by inoculation for mouse models of Alzheimer's disease (Jucker and Walker 2013), and of mice with human Alzheimer's brain (Watts et al. 2014) has now been observed. Finally, evidence of iatrogenic infection with Alzheimer's disease in patients treated with human pituitary-derived growth hormone has appeared (Naunmuktane et al. 2015).

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**Box 1:**

### Abbreviations and yeast genetic nomenclature.

Yeast prions are non-chromosomal genes and therefore are shown in brackets, e.g. [URE3]. Yeast prions are dominant and so, like dominant chromosomal mutants, are shown in capitals, often italicized. Chromosomal genes are given in italics, e.g., *URE2*, and recessive (usually mutant) alleles are lower case, e.g. *ure2*. Protein products of a *S. cerevisiae* gene are shown as, for example, Ure2p. For some prions, "+" indicates the presence of the prion and "−" or "−o" its absence. For all, the absence of the prion is indicated by the name in lower case, e.g., [psi−] or [ure-o].

Sup35p = subunit of the translation termination factor and the protein that can form the [PSI+] prion.

Ure2p = negative regulator of transcription of genes encoding enzymes and transporters for poor nitrogen sources; active in the presence of a good nitrogen source. Forms the [URE3] prion.

Rnq1p = basis of the [PIN+] prion (also called [RNQ+].

TSE = transmissible spongiform encephalopathy

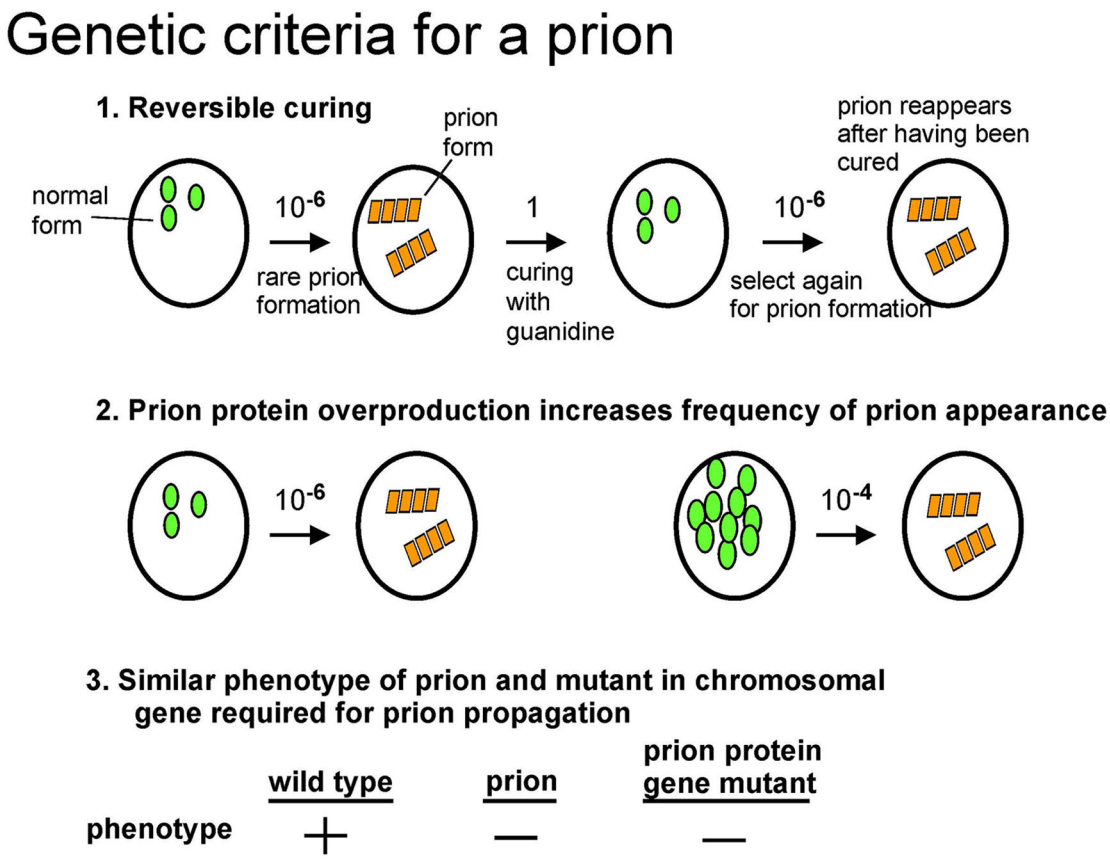


**Fig. 1. Genetic criteria for a yeast or fungal prion.**

The [URE3] and [PSI+] prions follow all three criteria (Wickner 1994) because their phenotype is often due to the deficiency of the normal protein form, but [PIN+] and [Het-s] do not (Coustou et al. 1997; Derkatch et al. 2001) because their phenotypes are due to novel effects of the amyloid form. Modified from Wickner et al., 2013.

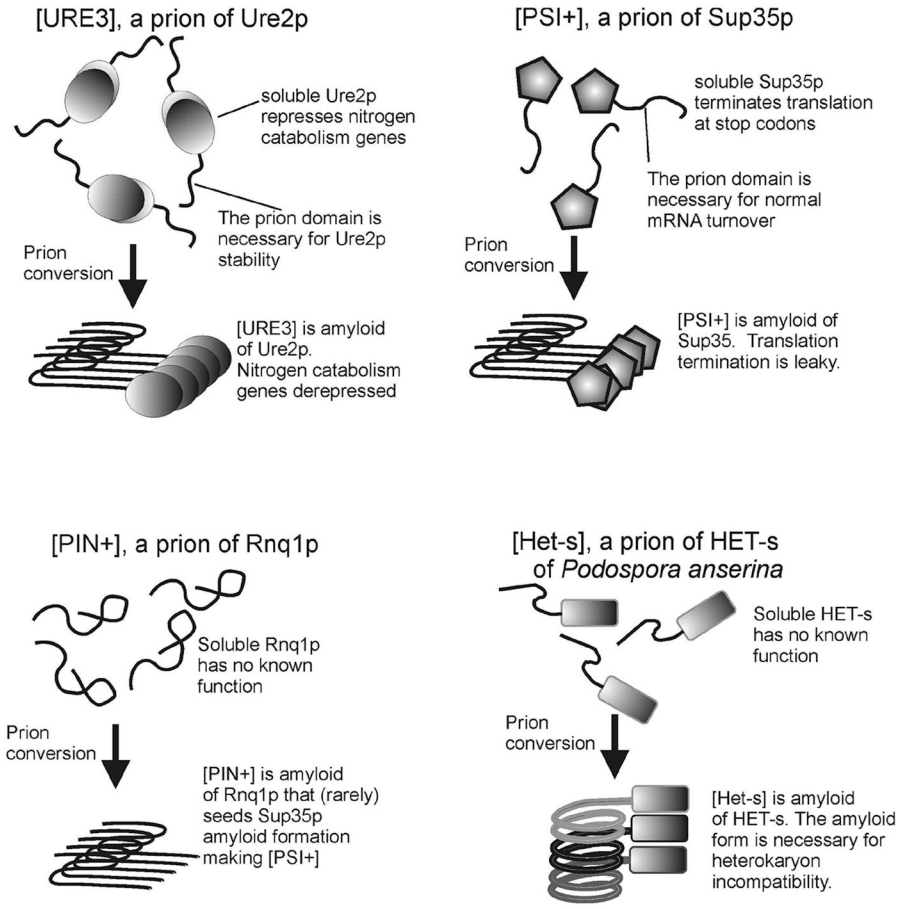


**Fig.2. Yeast and fungal prions.**

The prions [URE3], [PSI+], and [PIN+] of *S. cerevisiae* and [Het-s] of *Podospora anserina* are formed by amyloid formation of Ure2p, Sup35p, Rnq1p and HET-s, respectively. The proteins' normal functions and prion phenotypes are shown. The prion domains of Ure2p and Sup35p have normal functions, explaining their retention in evolution in spite of rare prion formation. The amyloids of Ure2p, Sup35p and Rnq1p are each folded in-register parallel β-sheet structures with one molecule per one β-strand layer along the long axis of the filament, while that of HET-s is a β-helix with one molecule per two β-strand layers. Modified from Wickner et al., 2008.

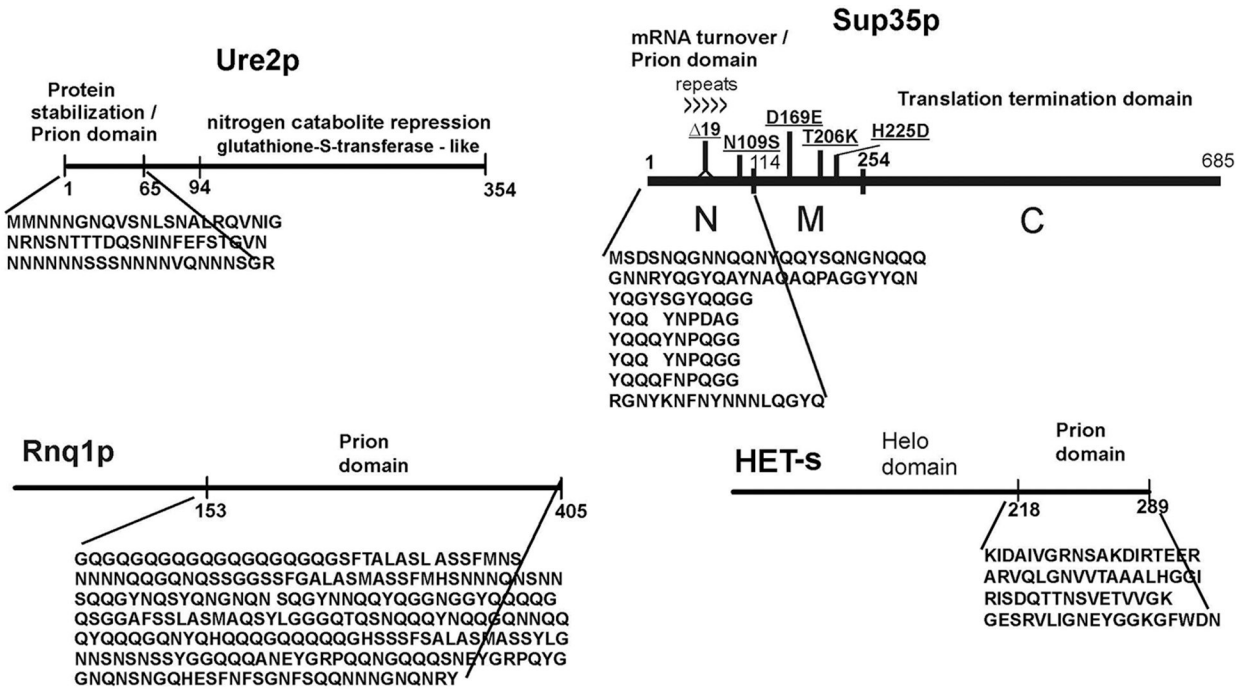


**Fig.3. Prion domains.**

The portion of the molecule that actually forms amyloid and is essential and sufficient for prion propagation is shown diagramatically. Note that the precise borders of the prion domain depend on the prion variant (e.g. (Bradley and Liebman 2004; Chang et al. 2008)). The prion domains of Ure2p and Sup35p each have non-prion functions as well. The common polymorphisms of Sup35p that give rise to intraspecies barriers (Bateman and Wickner 2012) are shown. Modified from Wickner et al., 2008.

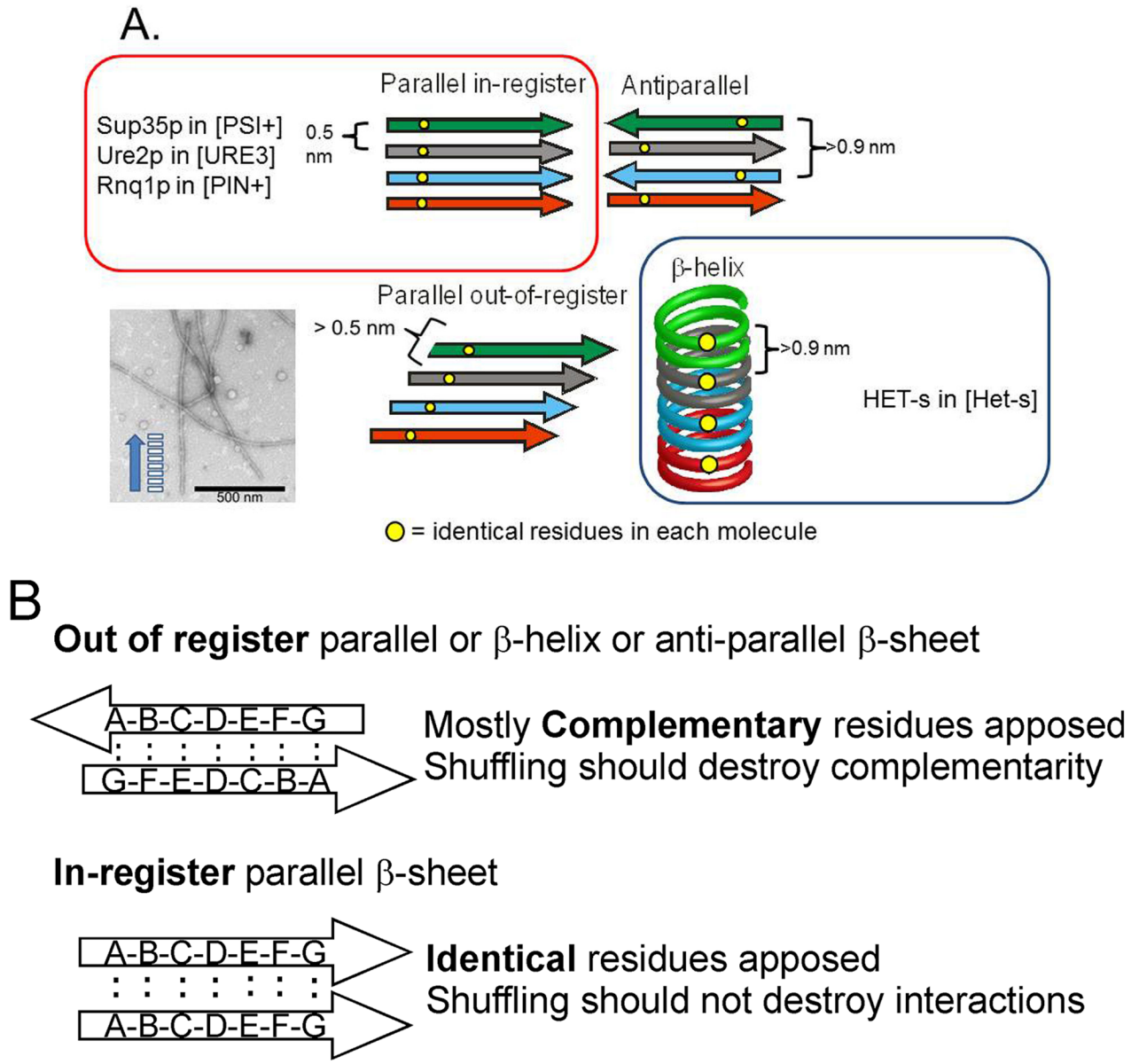


**Fig. 4. Possible amyloid β-sheet structures.**

A. Each color represents a separate molecule, and each small circle represents a particular atom, the same in each molecule. Labeling a single C atom with $^{13}C$ and using solid-state NMR to measure the distance to the nearest neighbor $^{13}C$ allows one to distinguish the in-register parallel β-sheet structure (~0.5 nm expected) from the other structures (> 0.5 nm expected). B. The strict sequence specificity for prion propagation implies an interaction between residues in the filament and those of a molecule newly joining the end. If the structure is antiparallel, β-helix, or out of register, that relationship must be one of complementarity. If the structure is in-register parallel, it is identity. Shuffling is expected to destroy the complementarity relationships (as it would with DNA strands), but not identity interactions, which could still all occur, just in a different order along the peptide chain (Ross et al. 2005). Modified from Ross et al., 2005.

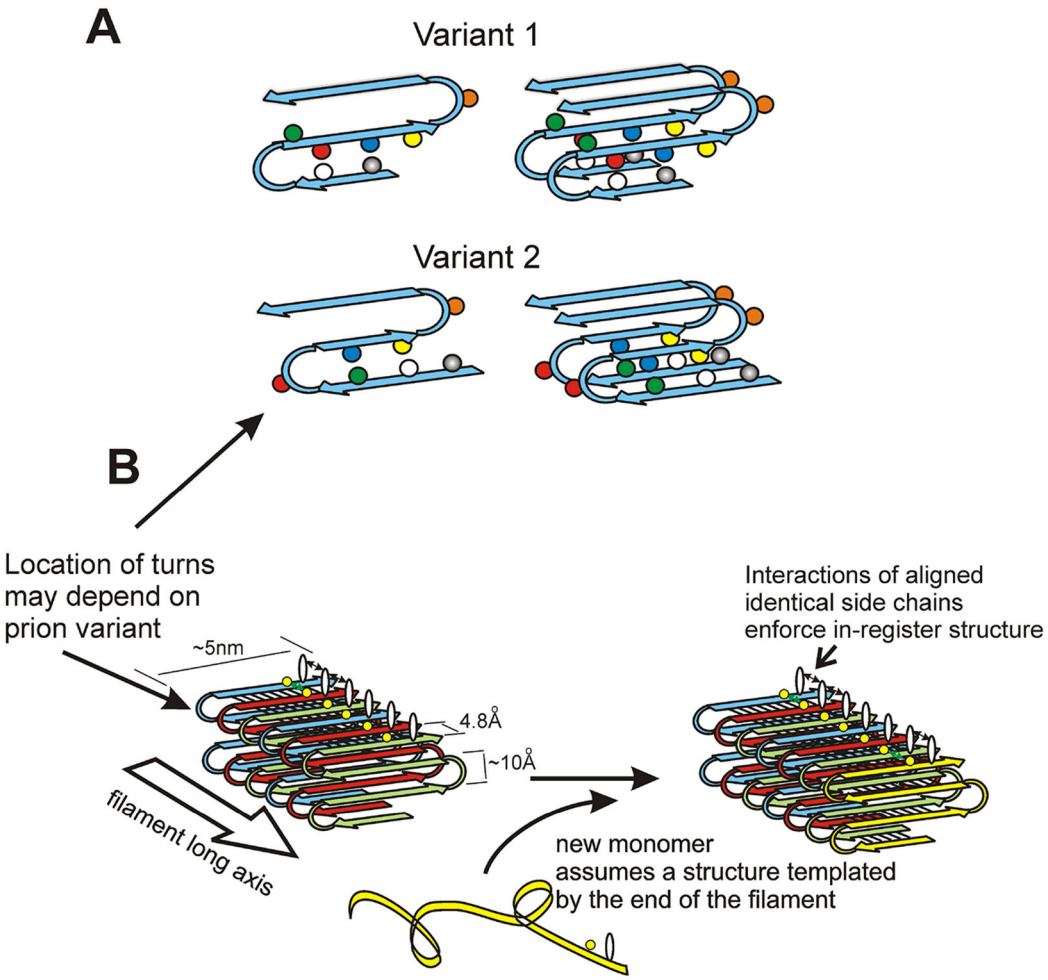


**Fig. 5. Structure of the prion amyloids suggests a mechanism of conformation templating.**

A. It is proposed that different prion variants have the folds of the β-sheet (turns in the molecules) in different locations (Wickner et al. 2008; Wickner et al. 2013). B. Molecules with the folded in-register parallel β-sheet architecture have rows of identical side chains along the long axis of the filament. Positive interactions (hydrogen bonds or hydrophobic interactions) between the identical side chains maintain the in-register architecture. In order to have those same positive interactions, the new unstructured prion domain joining the end of the filament must adopt a structure in which its turns are in the same location as those in the molecules already in the filament. This explains how prion proteins can template their conformation (Wickner et al. 2008). Modified from Shewmaker et al., 2006.

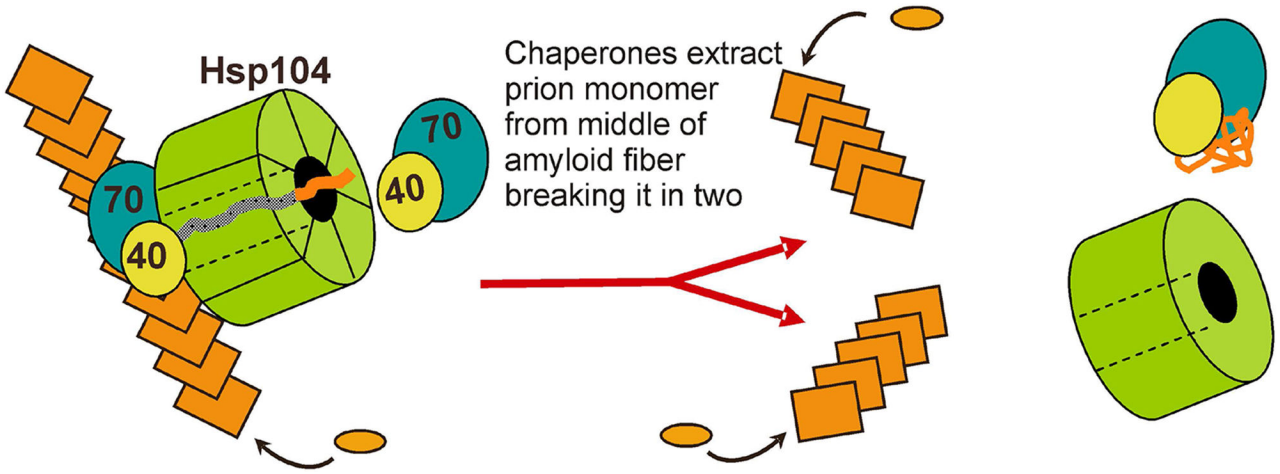


**Fig. 6. Chaperone-catalyzed filament cleavage generates new prion seeds.**

Hsp104, in collaboration with Hsp70 and Hsp40, extract a prion protein monomer from the middle of a filament, breaking the filament into two filaments, each of which can continue to grow and propagate the prion. Adapted from reference (Masison et al. 2009).

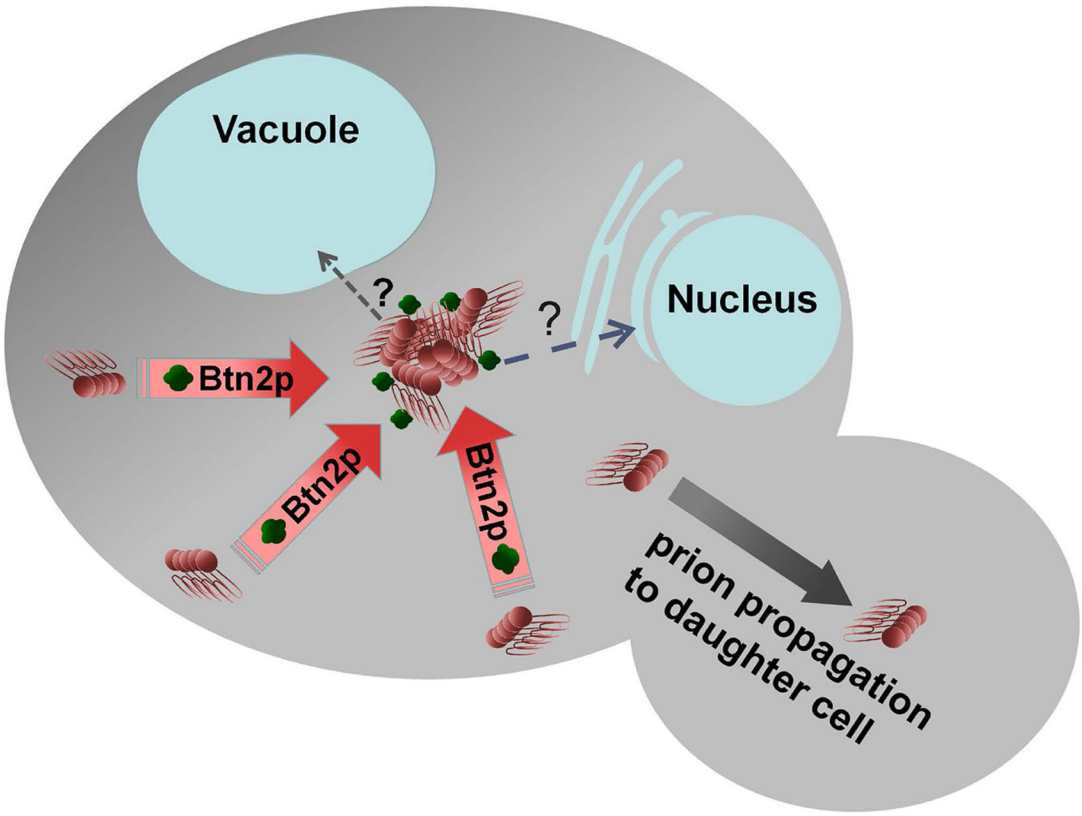


**Fig. 7. Btn2p collects protein aggregates, thereby curing the [URE3] prion.**

Btn2p, acting with Hsp42, gathers prion filaments (and other aggregates) to one place in the cell. Following cell division, one of the daughter cells is prion-free. Low seed number [URE3] prions are cured by normal levels of Btn2p, while higher propagon number [URE3]s are only cured on overproduction of Btn2p. Modified from Wickner et al., 2015.

**Table 1.**

Prions of Yeast, Fungi and Mammals

| Prion | Prion protein | Prion phenotype | Protein function | Reference |
|---|---|---|---|---|
| TSE | PrP | transmissible spongiform encephalopathy | GPI-anchored cell surface glycoprotein, function not clear | (Prusiner 2004) |
| [URE3] | Ure2p | derepressed nitrogen catabolism genes | repression of nitrogen catabolism gene transcription (e.g. *DAL5*) | (Wickner 1994) |
| [PSI+] | Sup35p | readthru of translation termination codons | subunit of translation termination factor | (Wickner 1994) |
| [PIN+] | Rnq1p | rare priming of formation of other prions | non-essential; function unknown | (Derkatch et al. 2001) |
| [SWI+] | Swi1p | deficient in utilizing non-fermentable carbon sources | chromatin remodeling | (Du et al. 2008) |
| [OCT+] | Cyc8p | slow growing, with defects in mating and sporulation | subunit (with Tup1p) of transcription repressor | (Patel et al. 2009) |
| [ISP+] | Sfp1p | antisuppressor, overproduces Sup35p | transcription factor for ribosome proteins | (Rogoza et al. 2010) |
| [MOT3+] | Mot3p | filamentous growth on low nitrogen | transcription factor for hypoxic, hyperosmotic stress | (Alberti et al. 2009) |
| [MOD5+] | Mod5p | slow growth, fluconazole-resistance | tRNA isopentenyltransferase | (Suzuki et al. 2012) |
| [BETA] | Prb1p | activation of Prb1p precursor by active Prb1p | Vacuolar protease B | (Roberts and Wickner 2003) |
| [Het-s] | HET-s | heterokaryon incompatibility in *Podospora anserina* | heterokaryon incompatibility (no non-prion function known) | (Coustou et al. 1997) |

**Table 2.**

Prion variants/strains

| Prion | Variant discriminant | Ref. |
|---|---|---|
| TSE | Incubation period | |
| | Region of brain affected | |
| | Interspecies transmission efficiency | |
| | Clinical signs: dementia, ataxia, glucose intolerance… | |
| [PSI+] | Strong/Weak: efficiency of terminator read-thru | (Derkatch et al. 1996) |
| | Stability: directly related to seed number | (Derkatch et al. 1996) |
| | Lethal/Toxic/Mild | (McGlinchey et al. 2011) |
| | Pattern of sensitivity to intraspecies transmission barriers from polymorphic Sup35 prion domain | (Bateman and Wickner 2012; Bateman and Wickner 2013) |
| | Sensitivity to interspecies barriers | (Sharma et al. 2015) |
| | Ability to propagate in various mutant prion proteins | (King 2001) |
| | Sensitivity to chaperone over/under production | (Borchsenius et al. 2006)<br>(Kushnirov et al. 2000)<br>(Lancaster et al. 2013)(Harris et al. 2014) |
| [URE3] | Strong/Weak: level of *DAL5* promoter activity | (Brachmann et al. 2005) |
| | Stability: directly related to seed number | (Brachmann et al. 2005) |
| | Toxic/Mild | (McGlinchey et al. 2011) |
| | Btn2-Cur1 Sensitive/Hypersensitive | (Wickner et al. 2014) |
| | Species barrier sensitive/resistant | (Edskes et al. 2009) |
| [PIN+] | Strong/weak: efficiency of generating [PSI+] | (Bradley et al. 2002) |
| | Pattern of [PSI+] variants generated | (Sharma and Liebman 2013) |