Published in final edited form as:
FEMS Yeast Res. 2010 December ; 10(8): 980–991. doi:10.1111/j.1567-1364.2010.00666.x.

Prion amyloid structure explains templating: how proteins can be genes

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Abstract

The yeast and fungal prions determine heritable and infectious traits, and are thus genes composed of protein. Most prions are inactive forms of a normal protein as it forms a self-propagating filamentous β – sheet - rich polymer structure called amyloid. Remarkably, a single prion protein sequence can form two or more faithfully inherited prion variants, in effect alleles of these genes. What protein structure explains this protein-based inheritance? Using solid-state NMR, we showed that the infectious amyloids of the prion domains of Ure2p, Sup35p and Rnq1p have an in-register parallel architecture. This structure explains how the amyloid filament ends can template the structure of a new protein as it joins the filament.

The yeast prions [*PSI*$^{+}$] and [URE3] are not found in wild strains, indicating they are a disadvantage to the cell. Moreover, the prion domains of Ure2p and Sup35p have functions unrelated to prion formation, indicating that these domains are not present for the purpose of forming prions. Indeed, prion forming ability is not conserved, even within *S. cerevisiae*, suggesting that the rare formation of prions is a disease. The prion domain sequences generally vary more rapidly in evolution than does the remainder of the molecule, producing a barrier to prion transmission, perhaps selected in evolution by this protection.

Keywords

prion; amyloid; in-register parallel structure

Scrapie is a uniformly lethal neurodegenerative disease of sheep that has been known in Europe since at least the 18th century (Parry, 1983) and perhaps much longer in China (Wickner, 2005). Its transmissibility to sheep and goats (Cuille & Chelle, 1936, Cuille & Chelle, 1939) and the typical brain pathology gave it, and similar diseases of humans, cattle, deer and elk, the name transmissible spongiform encephalopathy (TSE). The recent epidemic of the bovine form of this disease (Wilesmith, 1988), and its fortunately rare transmission to humans (Will, *et al.*, 1996) spotlighted these conditions, but their relation to non-infectious amyloid diseases such as Alzheimer's disease, Parkinson's disease, and type II diabetes may ultimately prove even more important. Early studies of the infectious agent were hampered by the year+ incubation periods and the expense of buying a flock of sheep for each experiment! The infection of mice with the scrapie agent improved matters (Chandler, 1961), but not until recent tissue culture infection methods (Klohn, *et al.*, 2003) has the agent assay truly become simple.

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Historically, the extreme UV-resistance of the scrapie agent first implied that any essential nucleic acid component must be much smaller than even the small RNA phages (Alper, *et al.*, 1966, Alper, *et al.*, 1967, Bellinger-Kawakara, *et al.*, 1987), and led to the suggestion that the infectious agent had no essential nucleic acid (Alper, *et al.*, 1967). Griffith then proposed what is essentially the modern 'protein-only' model (Griffith, 1967). The purification of the infectious agent showed a single major protein species, named PrP (Bolton, *et al.*, 1982, Diringer, *et al.*, 1983), and Prusiner coined the term "prion" to mean an "infectious protein", transmitting an infection without an essential nucleic acid component (Prusiner, 1982).

PrP is encoded by a chromosomal gene (Basler, *et al.*, 1986, Locht, *et al.*, 1986), the same (Carlson, *et al.*, 1986, Hunter, *et al.*, 1987) as the *Sinc* gene of mice, shown much earlier to control scrapie incubation period (Dickinson, *et al.*, 1968). The finding that the gene affected in familial human TSE disease that produces infectious material for monkeys (Masters, *et al.*, 1981) was the gene encoding PrP (Hsiao, *et al.*, 1989), and that PrP determines the species barrier (Prusiner, *et al.*, 1990) were important arguments for the prion model.

Amyloid formed from recombinant PrP is minimally infectious unless other lipid and nucleic acid components are included (Legname, *et al.*, 2004, Deleault, *et al.*, 2007, Makarava, *et al.*, 2010, Wang, *et al.*, 2010), leaving some residue of doubt about whether the TSE are truly prions. Clearly, PrP is the determinant of specificity and essential for infectivity, but it remains possible that minor RNA or lipid components contribute.

In contrast, the initial genetic evidence for yeast prions was convincing (Wickner, 1994, Masison, *et al.*, 1997): unlike nucleic acid replicons, 1) curing of prions is reversible, meaning that they arise de novo in a cell presumed to be devoid of the putative replicon; 2) overproduction of the prion protein increases the frequency of prion generation; and 3) the propagation of the prion requires a chromosomal gene (encoding the prion protein) whose mutant phenotype is similar to that of the presence of the prion. These properties posited for a yeast prion were true of [URE3] and [*PSI*$^+$] as prions of Ure2p and Sup35p, respectively (Wickner, 1994). None of the three genetic properties posited for yeast prions were known then (or even now) for TSEs. Similar evidence was obtained for the [Het-s] non-chromosomal gene of the filamentous fungus *Podospora anserina* being a prion of the HET-s protein (Coustou, *et al.*, 1997). Amyloid formed *in vitro* from recombinant prion protein efficiently transmits the corresponding prion to yeast or fungal cells (Maddelein, *et al.*, 2002, King & Diaz-Avalos, 2004, Tanaka, *et al.*, 2004, Brachmann, *et al.*, 2005, Patel & Liebman, 2007).

Studies of TSEs have revealed that many "strains" of the infectious agent could show quite distinct properties in the identical host (Bruce, 1993). One source of resistance by many to the proposals that the TSEs are infectious proteins has been the striking and consistent differences in the incubation periods, tissue distributions and other features of the disease produced by different strains of scrapie, and the lack of a plausible mechanism by which a protein could transmit its conformation from one molecule to another. Not only were there no known examples of heritable (or transmissible) traits being encoded in anything but nucleic acid sequence, but it was not clear how protein-only transmission of information could occur. For example, in reviewing the TSEs and proposing the term "prion" to describe them Prusiner (Prusiner, 1982) proposed that, if indeed the TSEs were cases of a "protein-only" infectious agent, the mechanism might be a) reverse translation, b) protein-dependent protein synthesis or c) a protein’s induction of transcription of its own gene. None of these proved to be the mechanism of the mammalian prion. While clear evidence of differences in protein conformation in different prion strains was obtained (Bessen & Marsh, 1994,

Caughey, *et al.*, 1998), no mechanism for the faithful propagation of such conformational differences was proposed (Fig. 1).

With the discovery of yeast prions (Wickner, 1994), and the existence of prion strains (or "variants") in yeast (Derkatch, *et al.*, 1996), it was clear that the variant phenomenon was general, and did not reflect a nucleic acid component of the prions. Yeast prion variants are also based on different structures (see below) -amyloids- but what are these structures, and how are they faithfully propagated?

The spectrum of prions in yeast and fungi (Table 1)

The yeast amyloid-based prions include [URE3], a prion of Ure2p, a regulator of nitrogen catabolism (Fig. 2)(Wickner, 1994); [*PSI*$^+$], a prion of Sup35p, a subunit of the translation termination factor (Wickner, 1994); [*PIN*$^+$], a prion of Rnq1p of unknown normal function (Derkatch, *et al.*, 2001); [SWI+], a prion of Swi1p, a component of the SWI-SNF chromatin remodeling complex (Du, *et al.*, 2008); [MCA], a prion of MCA1p, the yeast metacaspase homolog (Nemecek, *et al.*, 2009); [OCT+], a prion of Cyc8, a subunit of the Tup1-Cyc8 transcription repressor (Patel, *et al.*, 2009); and [MOT3] a prion of Mot3p, a transcription factor (Alberti, *et al.*, 2009). The yeast [β] prion is a self-propagating active vacuolar protease B, which can be essential for the activation of its inactive precursor protein (Zubenko, *et al.*, 1982,Roberts & Wickner, 2003). [Het-s], a prion of the HET-s protein of the filamentous fungus *Podospora anserina* (Coustou, *et al.*, 1997), is a mediator of heterokaryon incompatibility, and is unusual in that it functions in its prion form, but has no known function in its non-prion form.

Biology of yeast and fungal prions

Species barriers

Mammalian prions of one species may be unable to infect another species, or only do so with dramatically increased incubation period (reviewed in (Collinge & Clarke, 2007)). This 'species barrier' is a result of sequence differences between the PrP proteins of the respective species (Prusiner, *et al.*, 1990). A similar species barrier between yeast species has likewise been observed (Chernoff, *et al.*, 2000, Kushnirov, *et al.*, 2000, Santoso, *et al.*, 2000, Chen, *et al.*, 2007, Edskes, *et al.*, 2009).

Prion variants

Prion variants of [*PSI*$^+$], [URE3] and [*PIN*$^+$] were first observed as differences in intensity of the prion phenotype (strong or weak) and in the stability in propagation of the prion during growth (Derkatch, *et al.*, 1996, Schlumpberger, *et al.*, 2001, Bradley, *et al.*, 2002, Brachmann, *et al.*, 2005). However, [*PSI*$^+$] variants also differ in whether they can propagate in cells expressing a given mutant of the prion domain (King, 2001), a result that corresponds logically to the species barrier in mammalians. Variants of [URE3] show dramatic differences in the actual species barrier among different species of *Saccharomyces* (Edskes, *et al.*, 2009). Chaperones are important in prion propagation (reviewed by (Sharma & Masison, 2009)) and these effects also vary depending on the prion variant (Kushnirov, *et al.*, 2000). Different [*PSI*$^+$] variant amyloids show different distribution along the prion domain sequence of rates of hydrogen-deuterium exchange, indicating that as in mammals, yeast prion variants are based on self-propagating structural variants (Toyama, *et al.*, 2007).

Are yeast and fungal prions a help or a hindrance?

Heterokaryon incompatibility of fungi is like transplantation incompatibility in mammals, preventing vegetative fusion of genetically distinct fungal strains, apparently for the purpose

of blocking the spread of fungal viruses (Saupe, *et al.*, 2000). Since the [Het-s] prion mediates heterokaryon incompatibility in *Podospora* (Coustou, *et al.*, 1997), it was proposed that it may actually be a beneficial prion (Wickner, 1997). This theme was applied to prions of yeast when it was noted that certain [*PSI*$^+$] strains were more stress-resistant than the corresponding [psi-] strain (Eaglestone, *et al.*, 1999). However, further observations did not support a general stress-resistance of [*PSI*$^+$] cells (True & Lindquist, 2000). Indeed, in a screen of many phenotypes, there was, except for hypersensitivity to 5 mM Zn^{2+}, no phenotype consistently imparted by being [*PSI*$^+$], and in most cases, being [*PSI*$^+$] was detrimental (True & Lindquist, 2000). Finally, in a re-examination using the same strains it was found that less than half of the differences were reproducible (Namy, *et al.*, 2008). Even if these experiments had shown that a prion conferred a consistent benefit for yeast under some specific culture condition, this would not prove that the prion was of benefit to yeast unless a) this condition could be shown to be a significant part of the niche of this species in the wild, b) yeast carrying this prion could be isolated from that niche, and c) this benefit outweighed any detriment that would be encountered under other conditions (Partridge & Barton, 2000). It seems clear that [*PSI*$^+$] should produce inappropriate readthrough of translation termination codons of many genes, and thus cause the production of many non-functional or mis-functional proteins, so it is not surprising that no consistent advantage of being [*PSI*$^+$] has been detected.

Infectious elements, such as viruses, bacteria or prions, are easily found in the wild even if they are lethal, simply because their rate of spread out-runs the damage they do to their hosts. For example, chronic wasting disease, a prion infection of deer and elk, is found in several percent of animals in large areas of the US, and scrapie of sheep has been a problem for at least centuries (Parry, 1983), if not millenia (Wickner, 2005). Certainly if an infectious element is a benefit to its host, and is stable (like the [*PSI*$^+$] and [URE3] variants claimed to help their hosts) it will quickly spread in the population as infectivity and benefit work together. The mitochondria, which originated as a bacterium infecting another cell, is an example. Thus, if an infectious entity is NOT found in wild isolates, one can then safely conclude that it is a detriment to its host. We surveyed seventy wild yeast strains isolated from several continents and many different environments, and none had [URE3] or [*PSI*$^+$], indicating that both are diseases (Nakayashiki, *et al.*, 2005).

It is suggested that [*PSI*$^+$] formation may be induced under certain stress conditions to relieve the cells of the stress by altering translation (Tyedmers, *et al.*, 2008). However, it was found that under four of the six stress conditions inducing [*PSI*$^+$] formation, [*PSI*$^+$] was a detriment to the cells, rather than helping them survive. This indicates that the [*PSI*$^+$] induction was not an adaptive response, but may have been due to chaperones being occupied with dealing with the stress, and were not available to prevent prion formation. In the two other [*PSI*$^+$] "induction" conditions where the presence of [*PSI*$^+$] was reported to favor cell survival, the authors failed to rule out the possibility that this condition was not inducing [*PSI*$^+$] appearance but was simply selecting [*PSI*$^+$] cells already in the population before the stress, thus explaining the modestly increased fraction of surviving cells that were [*PSI*$^+$].

If the prion domains of prion proteins had no function other than prion formation, and were conserved through a long span of evolution, then one could argue that prion formation may be conserved for some purpose that we do not know about. However, prion domains of Ure2p and Sup35p each have non-prion functions and the ability to form prions is not generally conserved. The prion domain of Ure2p is important for the nitrogen regulation function of the molecule in that Ure2p is rapidly degraded if it lacks this region and nitrogen catabolite repression is leaky (Shewmaker, *et al.*, 2007). Likewise, the prion domain of Sup35p interacts with the polyA binding protein and is necessary for the normal shortening

of the polyA structure of mRNAs and thus for normal mRNA turnover (Hoshino, *et al.*, 1999, Hosoda, *et al.*, 2003). Moreover, the prion-forming ability of Sup35p is not well conserved even within *Saccharomyces cerevisiae* isolates and fully 25% of strains examined have a large deletion that prevents them from becoming [PSI^+] (Resende, *et al.*, 2003). The Ure2p prion domain is apparently conserved within *S. cerevisiae* (Edskes & Wickner, 2002), but *S. paradoxus* is unable to form the [URE3] prion (Talarek, *et al.*, 2005), and the Ure2p of *S. castelii* is unable to convert to [URE3], at least in *S. cerevisiae* (Edskes, *et al.*, 2009). Thus prion formation is sporadic, and not well conserved, and the prion domains have non-prion functions, indicating that prions are molecular malfunctions.

Human populations are polymorphic for residue 129 of PrP with about half of alleles having Val and half a Met residue. Heterozygous individuals rarely get either spontaneous or infectious CJD, leading Mead et al. to propose that this polymorphism arose to protect humans from this disease in an era when cannibalism was not rare (Mead, *et al.*, 2003). The Ure2p and Sup35p prion domains also vary more rapidly than does the remainder of the molecule (although both prion domains have non-prion functions), and this results in a species barrier between the intermating species of the *Saccharomyces* genus (Chen, *et al.*, 2007, Edskes, *et al.*, 2009). We suggested that the Q/N - rich prion domains are preserved in evolution because they have functions (protein stabilization, mRNA turnover), but that, as in the human case, they vary rapidly (within limits imposed by these functions) to prevent prion infection (Edskes, *et al.*, 2009).

The [Het-s] prion of *Podospora anserina* constitutes an interesting comparison with the yeast prions. [Het-s] is found in 80% of wild *het-s* strains (Dalstra, *et al.*, 2003), suggesting that it is beneficial to its host, as mentioned above. However, in addition to heterokaryon incompatibility, [Het-s] also determines a 'meiotic drive' system that promotes the inheritance of the prion-forming allele, *het-s*, over the *het-S* allele (Dalstra, *et al.*, 2003). Meiotic drive is a phenomenon in which a chromosomal gene promotes its own inheritance by inactivating germ cells carrying the opposite allele. The meiotic drive allele may be quite detrimental to the organism, but it can often spread in the wild by cheating on meiosis. Thus, it is not clear whether the [Het-s] prion is widespread because heterokaryon incompatibility is important to *Podospora*, or because of the meiotic drive effect. In either case, the HET-s protein has evolved to be a prion with a specific effect on the cell, and thus a specific structure.

The [β] prion is not an amyloid but is just the active form of vacuolar protease B, normally synthesized as an inactive precursor which is activated by cleavage by protease A (Jones, 1991). In the absence of protease A, active protease B can activate its own precursor (Zubenko, *et al.*, 1982)(Roberts & Wickner, 2003). A cell which lacks active protease B remains so, but can be 'infected' by transfer of cytoplasm from a cell that has it. Thus, in the absence of protease A, the active mature form of protease B is a prion, called [β] (Roberts & Wickner, 2003). [β] is important for cell survival in stationary phase and for sporulation and meiosis, both processes requiring protein turnover (Roberts & Wickner, 2003). This is clearly a case of a beneficial prion.

Shuffled prion domains can still form prions

The N-terminal prion domain of Ure2p is unstructured in the native form and changes to β-sheet in the formation of the infectious amyloid (Taylor, *et al.*, 1999, Baxa, *et al.*, 2003, Baxa, *et al.*, 2005, Pierce, *et al.*, 2005); we assume that the prion domain of Sup35p is likewise unstructure in the native form, and it certainly is largely β-sheet in the prion form (e.g. (King, *et al.*, 1997)). The C-terminal nitrogen regulation domain of Ure2p (residues ~94-354) does not change substantially (Baxa, *et al.*, 2002, Bai, *et al.*, 2004, Loquet, *et al.*,

2009), and the C-terminal part of Sup35p is likewise unaltered by amyloid formation (Krzewska, *et al.*, 2007).

In order to determine whether there were specific sequences in the Ure2p or Sup35p prion domains which were essential for prion formation, we shuffled each domain, without altering their amino acid content, inserted the shuffled domain in place of the normal prion domain, and selected prion-containing cells, either those arising spontaneously, or those induced by overproduction of the shuffled prion domain. We were surprised to find that each of the 5 shuffled Ure2ps and each of the 5 shuffled Sup35p proteins could become a prion in yeast (Ross, *et al.*, 2004, Ross, *et al.*, 2005). This remarkable result would appear, on the surface, to contradict the fact that even a single amino acid change can, in some cases, constitute a 'species barrier' and block propagation of a prion from one protein to another. For example, a single amino acid change in the Sup35p prion domain can block propagation of the usual [*PSI*$^+$] from the wild type Sup35 (Doel, *et al.*, 1994), but the mutant Sup35p can form its own [*PSI*$^+$] (Kochneva-Pervukhova, *et al.*, 1998).

Amyloid is β sheet - rich, with the β strands perpendicular to the long axis of the filaments, but amyloid can have any of four types of architecture: 1) antiparallel, 2) parallel in-register, 3) parallel out-of-register or 4) β-helix (Fig 3A). Except for the parallel in register structure (Fig. 3B), different amino acid residues are apposed and interact with each other. For such a structure to require near identical sequence for propagation, there would have to be some relation between the apposed residues, perhaps complementarity (large with small, positive with negative charge) or identity (both hydrophobic or both hydrophilic). This relation would almost certainly be lost on shuffling the amino acid sequence. In contrast, the parallel in-register architecture pairs identical residues of different molecules. In this case, shuffling the sequence would not prevent identical residues from having the same interactions; the only difference is that they would be ordered in a different sequence (Fig. 3B). Such a structure would be favored by a peptide composed of residues that have favorable interactions with themselves, such as hydrophobic or hydrophilic residues, but would not be expected if charged residues were abundant. In fact, charged residues are scarce in the known prion domains. Postulating a parallel in-register architecture for prion amyloids is not without precedent, as this structure has been demonstrated for amyloid of the Aβ peptide and Tau involved in Alzheimer's disease, the amyloid of amylin involved in type II diabetes mellitus, alpha-synuclein involved in several neurodegenerative diseases (Balbach, *et al.*, 2002,Der-Sarkissian, *et al.*, 2003,Luca, *et al.*, 2007,Margittai & Langen, 2008).

Verification of parallel in-register β-sheet architecture of yeast prion amyloids

Because of their non-crystalline nature, large size and insolubility, solid-state nuclear magnetic resonance (ssNMR) is the best method to address the structure of amyloid filaments (Tycko, 2006). Labelling the carbonyl carbon of specific groups of residues with ^{13}C provides a probe of both secondary structure of the labeled residues and of their distance from the next closest labeled residue. Compared to random coil, residues in β-sheet structure show a shift in carbonyl ^{13}C resonant frequencies to lower values, while those in α-helix shift to higher values (Wishart, *et al.*, 1991). This confirmed measurements showing that the Ure2p, Sup35p and Rnq1p prion domain amyloids were largely β-sheet structures (Shewmaker, *et al.*, 2006, Baxa, *et al.*, 2007, Wickner, *et al.*, 2008).

Distance measurements were used to distinguish the in-register parallel architecture fromβ-helix, antiparallel, or parallel out-of-register structures. The former predicts a distance of ~0.5 nm from one labeled carbonyl carbon atom to the same atom on the adjacent molecule in the filament (Fig. 3A). This is just the distance between strands in a β-sheet and if

residues of adjacent molecules are aligned (in-register) then this will be the distance observed between labeled atoms. Each of the other possible structures predict a much greater distance (Fig. 3A). The distance measurement is done using a dipolar recoupling experiment (Tycko, 2007). "Magic angle spinning" - spinning the sample at an angle of 54.74 degrees relative to the direction of the magnetic field - is used to eliminate dipole-dipole interactions (the nuclei directly interacting as two tiny magnets). Selected dipole-dipole interactions are re-established by a series of radiowave pulses. These interactions result over time in the loss of alignment of the ^{13}C nuclear spins, and thus the decay of the NMR signal. The rate of this signal decay is proportional to the inverse of the cube of the distance to the next labeled nucleus (Fig 4B).

The material for these experiments was amyloid formed *in vitro* from recombinant prion domains of Sup35p, Ure2p and Rnq1p, labeled with one amino acid carrying ^{13}C specifically in the carbonyl position. Because of their relatively large size, these peptides cannot be synthesized, and must be made in *E. coli*. Due to the vicisitudes of bacterial metabolism, it is only practical to label certain amino acids without concern about leakage of label into other residues or dilution of labeled amino acid with endogenously synthesized material. For this purpose, Leu, Ile, Val, Phe, Tyr, and Met have been most useful. For Ala-3-^{13}C, a large amount of labeled amino acid and a short labeling period produces only ~60% labeling, but not cross-labeling of other amino acids. The key point is that each labeled amyloid preparation is highly infectious for yeast, transmitting the respective prion (King & Diaz-Avalos, 2004, Tanaka, *et al.*, 2004, Brachmann, *et al.*, 2005, Patel & Liebman, 2007).

In each case, we found that the rate of signal decay reflected a distance of about 0.5 nm, consistent with a parallel in-register β-sheet architecture (Fig. 4B) (Shewmaker, *et al.*, 2006,Baxa, *et al.*, 2007,Wickner, *et al.*, 2008). However, it was critical to establish that this distance was an intermolecular distance, because we are unable to label single residues. For this purpose, we diluted the fully amino acid-labeled sample with an unlabeled sample. If the measured distance were an intramolecular distance, then we would expect no effect on the rate of signal decay (Fig. 4A). However, if we had an in-register parallel β-sheet structure, the rate of signal decay should be substantially deminished because in most cases, the neighboring molecules of the labeled molecule would be unlabeled, and decay would be promoted only by more distant ^{13}C nuclei. Indeed, as shown for example by Fig 4B, this was found in each case, showing that the nearest neighbor was in a different molecule, again indicative of the in-register parallel structure.

To finally confirm the in-register aspect, it was necessary to use Ala-3-^{13}C labeled amyloid. The side chains in a β-strand point alternately in opposite directions (Fig. 4C). Thus, while an in-register structure structure would give a distance in the dipolar recoupling experiment of about 0.5 nm, this distance is >0.8 nm if the strands are even a single residue out of register. The result was as expected for an in-register structure in each case (Fig. 4D) (Shewmaker, *et al.*, 2006,Baxa, *et al.*, 2007,Wickner, *et al.*, 2008).

That shuffled prion domains could still be prions originally suggested the in-register parallel structure (Ross, *et al.*, 2005), so we examined whether in fact the shuffled prion domains have this structure (Shewmaker, *et al.*, 2008). Indeed, by the same NMR methods used to study the normal sequences, we showed that two of the shuffled Ure2p prion domains and one shuffled Sup35p prion domain each have an in-register parallel β-sheet structure (Shewmaker, *et al.*, 2008).

Although the amyloid formed in vitro from recombinant prion domains is highly infectious for yeast, it generally produces an array of prion variants on infection (e.g. (Brachmann, *et*

al., 2005)). Corresponding to this demonstrated genetic heterogeneity, the 2-dimensional ^{13}C-^{13}C ssNMR spectra show rather broad peaks indicative of structural heterogeneity (Shewmaker, *et al.*, 2006, Baxa, *et al.*, 2007, Wickner, *et al.*, 2008). It has been found that Sup35NM filaments formed at 37C produce, on transfection into yeast, mainly a weak [*PSI*$^{+}$] variant, while fibers formed at 4C produce mainly a strong variant (Tanaka, *et al.*, 2004). Differences in the distribution of slow-exchanging amide hydrogens between the 37C and 4C filaments indicate structural differences between these preparations (Toyama, *et al.*, 2007). We used the same solid-state NMR methods to examine such preparations and found that both had an in-register parallel structure (Shewmaker, *et al.*, 2009). This study also confirmed that drying of samples did not alter their infectivity or their properties as judged by ssNMR.

Again, the [Het-s] prion of *Podospora anserina* provides an illuminating contrast to the yeast prions (Saupe, 2007). Only a single variant of [Het-s] has been described, and, correspondingly, the ssNMR peaks of a 2D ^{13}C-^{13}C homonuclear dipolar coupling experiment of amyloid of HET-s$^{217–289}$ (the prion domain) are remarkably sharp, probably sharper than for any other amyloid described, suggesting that a single well defined structure is formed (Ritter, *et al.*, 2005). This structure is a 2-turn β-helix, formed by direct partial repeats in the peptide sequence (Wasmer, *et al.*, 2008).

In-register parallel structure explains inheritance of variant information

The in-register parallel architecture was hypothesized to explain the shuffleability of prion domains of Ure2p and Sup35p (Ross, *et al.*, 2005). We then verified, using solid-state NMR, that this is in fact the architecture of the various infectious amyloids of Ure2p, Sup35p and Rnq1p (Shewmaker, *et al.*, 2006, Baxa, *et al.*, 2007, Wickner, *et al.*, 2008). We still do not know the detailed structure of any prion amyloid. But it is clear that the in-register parallel architecture can explain the inheritance of prion variant information (Wickner, *et al.*, 2007, Wickner, *et al.*, 2008, Wickner, *et al.*, 2008), as discussed below.

What holds the structure in-register?

The in-register parallel β-sheet structure (Fig. 5) is held in-register by several types of bonding between the side chains of identical residues of adjacent molecules:

- **a.** the β-zipper structure of lines of glutamine or asparagine residues consisting of hydrogen bonds between their sidechain amide hydrogen and sidechain carbonyl oxygen (Perutz, *et al.*, 1994, Chan, *et al.*, 2005, Nelson, *et al.*, 2005). This forms a strip of hydrogen bonds running the length of the amyloid filament.
- **b.** hydrogen bonds between serine or threonine hydroxyl hydrogen and hydroxyl oxygen of the same residue of the next molecule up or down the filament.
- **c.** hydrophobic interactions between identical residues.

The only interactions between identical residues that are unfavorable are between identical charged residues, and such residues are few in the known prion domains, while Gln, Asn, Ser and Thr residues are particularly abundant.

What are the differences among prion variants?

We have proposed that variants differ in the location of the turns connecting the β-strands, the extent of the β-sheet structure or, potentially, the way in which protofilaments associate. Variants of Aβ structure have either two or three protofilaments associating to form a filament (Paravastu, *et al.*, 2008), but the mass per unit length of prion domains of Ure2p,

Sup35p and Rnq1p are each one monomer per 4.7 angstroms (Baxa, *et al.*, 2003, Diaz-Avalos, *et al.*, 2005, Chen, *et al.*, 2009), making this unlikely in the yeast prion case.

How does prion amyloid assemble?

Assembly of Sup35 prion domain amyloid occurs by addition of monomers (Collins, *et al.*, 2004). Amyloid of the [URE3] prion (at least) is assembled by conversion of the unstructured prion domain in the native molecules (Pierce, *et al.*, 2005) into highly structured polymers, with the structure assumed by new monomers templated by the structure of the last monomer on the end of the filament (Fig. 5). Thus, in the same way that DNA sequence of the parental strand templates the sequence of the new strand, the conformation of a protein molecule at the end of the filament templates the conformation of a new molecule as it joins the end of the filament. Turns in the template become turns in the new molecule. Intramolecular side chain-side chain interactions perpendicular to the filament axis will also be reproduced in the new molecule. We now see a mechanism by which proteins can act as genes, but it remains to be determined exactly what are the structures of different prion variants.

Conclusions

The rapidly increasing number of yeast prions, and the in-depth studies of their mechanisms of generation and propagation, is revealing an array of pathobiological phenomena that is aiding efforts to understand mammalian prion and non-prion amyloid diseases. Our finding an in-register parallel architecture for the infectious amyloids of the prion domains of Ure2p, Sup35p and Rnq1p has clear implications for the mechanism that underly the otherwise mysterious fact that proteins are capable of templating their own self-propagating conformation.

Acknowledgments

We were fortunate to be able to collaborate with Rob Tycko (NIDDK, NIH) in our solid-state NMR studies. This work was supported by the Intramural Program of the National Institute of Diabetes Digestive and Kidney Diseases of the National Institutes of Health.

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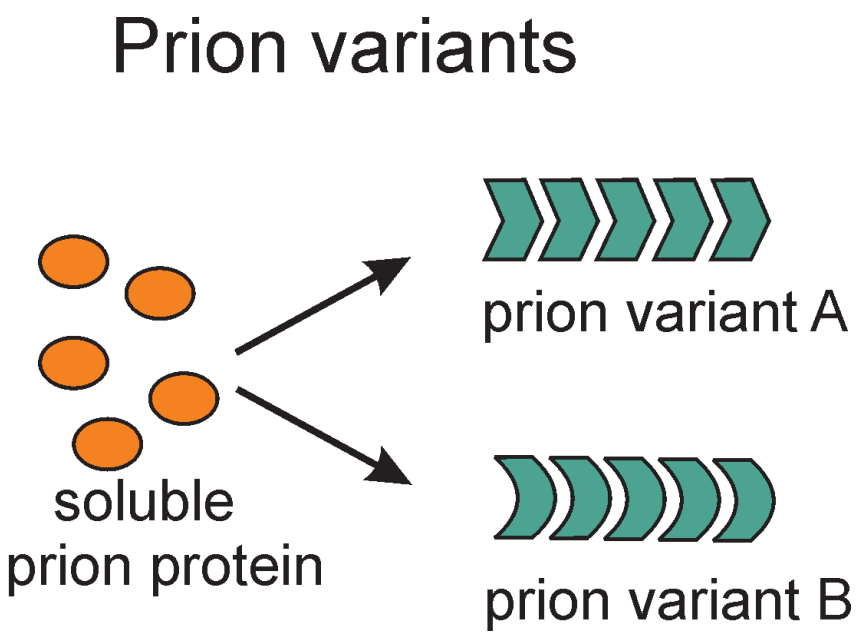

Fig. 1.

One protein sequence can produce several heritable prion variants. How can a protein structure be self-propagating? We have suggested that the in-register parallel β-sheet architecture, compatible with many different structures, is uniquely able to explain protein structure templating.

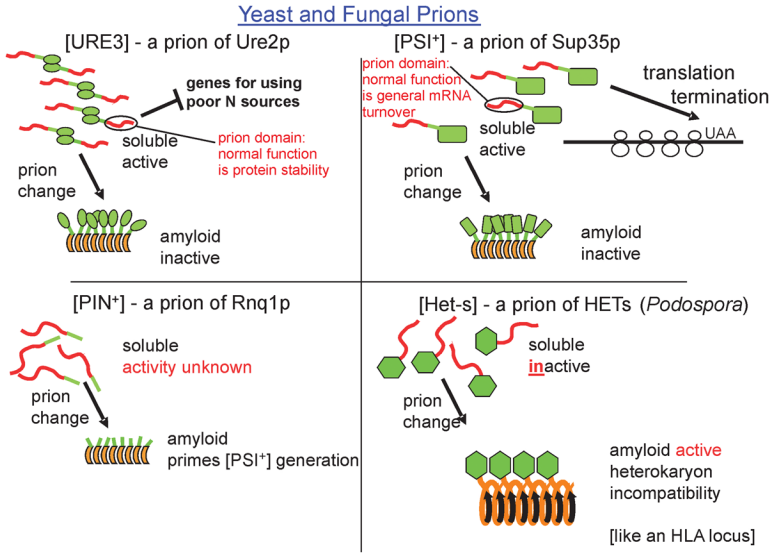

Fig. 2.

Yeast and fungal prions. The most widely studied prions of *S. cerevisiae* and *Podospora anserina* are diagramed. For the yeast prion proteins Ure2p and Sup35p, prion amyloid formation prevents normal function, thus producing a phenotype. The normal function of Rnq1p is unknown. The normal function of the HET-s protein is its prion function.

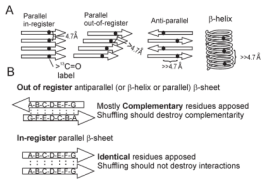

Fig. 3.

A. The four types of β-sheet architecture. Only the in-register parallel form results in a labeled atom in a residue of one molecule being about 4.7 angstroms from the same atom of the same residue of an adjacent molecule. The solid-state NMR experiments measure this distance. The large dot represents a single amino acid residue labeled with ^{13}C at its carbonyl carbon. B. If a prion domain can be shuffled and still be a prion, it is likely to have an in-register parallel architecture. The specificity of prion propagation requires that interacting residues have some relation to each other. If the architecture is antiparallel, β helix or out of register parallel, that relation would have to be one of non-identity in most cases. Shuffling the sequence would disrupt this relation. However, in the case of a parallel in-register β sheet, it is identical residues whose side-chains interact to determine the sequence-specific prion propagation. Shuffling the sequence does not prevent this same interaction, although the residues will be in a different sequence, identical residues can still interact to produce an amyloid filament.

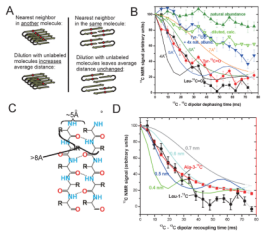

Fig. 4.

Solid-state NMR data demonstrates the in-register parallel architecture for the yeast prion amyloids - the prion domain of Rnq1 in this case (Wickner, *et al.*, 2008). The rapid decay of the NMR signal for 1-^{13}C-Tyr or 1-^{13}C-Leu labeled molecules indicates that these atoms are ~5 angstroms from their nears labeled neighbor (B). To show that that nearest neighbor is in a different molecule, labeled molecules are diluted with unlabeled molecules and the observed results (B, inverted blue triangles) are in accord with the expected results (inverted empty green triangles) as diagrammed in (A) for the nearest neighbor being in a different molecule. To determine if the structure is really in-register, methyl-^{13}C-Ala labeled molecules were examined. Even a single residue out of register would result in a slow signal decay (C). The rapid decay confirms the in-register parallel architecture (D). Similar results have been obtained for the prion domains of Sup35p (Shewmaker, *et al.*, 2006) and Ure2p (Baxa, *et al.*, 2007).

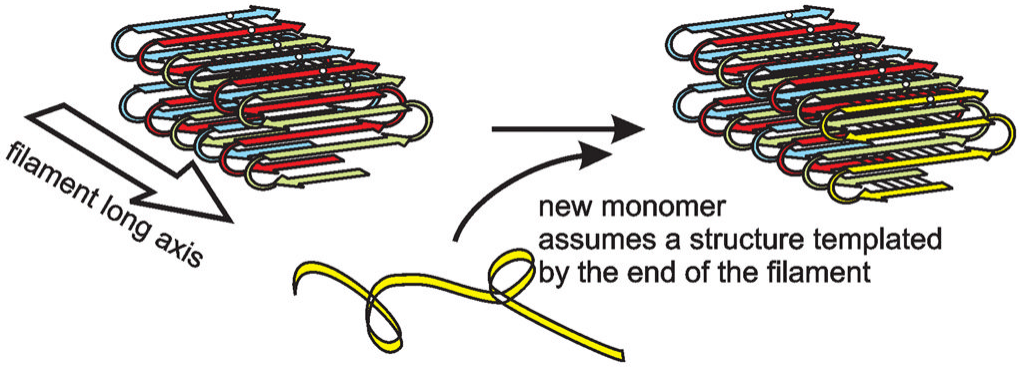

Fig. 5.

In-register parallel structure explains the ability of each yeast prion amyloid to faithfully template any of several "variant" structures. We suggest that variants differ in the locations of the turns (the folds of the sheet). Side chain–side chain interactions along the filament axis enforce the same locations for turns in the molecule newly joining the end of the filament as those in the previous molecule. The black dots represent a particular residue, say Gln46, in a prion domain sequence.

Table 1

Yeast and fungal prions.

Prion	Protein	Normal protein function	Prion manifestation	Refs.
[URE3]	Ure2p	Nitrogen catabolism: In the presence of a rich N source, Ure2p binds the positive transcription factor Gln3p, keeping it in the cytoplasm.	Inappropriate derepression of enzymes and transporters for the utilization of poor nitrogen sources.	(Lacroute, 1971) (Wickner, 1994)(Turoscy & Cooper, 1987)
[*PSI*$^+$]	Sup35p	Translation termination, mRNA turnover	Increased readthrough of translation termination codons	(Cox, 1965)(Wickner, 1994)
[*PIN*$^+$]	Rnq1p	none known	Increased frequency of generation of [PSI+] and [URE3] prions.	(Derkatch, *et al.*, 1997) (Sondheimer & Lindquist, 2000) (Derkatch, *et al.*, 2001)
[β]	Prb1p	Vacuolar protease PrB; prion form is active PrB, not amyloid	Poor sporulation, poor survival in stationary phase	(Zubenko, *et al.*, 1982)(Roberts & Wickner, 2003)
[*SWI*$^+$]	Swi1p	subunit of SWI-SNF chromatin remodeling complex	Partially defective Swi1- phenotype such as poor growth on raffinose, galactose or glycerol	(Du, *et al.*, 2008)
[MCA]	Mca1p	Metacaspase homolog. ?apoptosis?		(Nemecek, *et al.*, 2009)
[*OCT*$^+$]	Cyc8p	Transcription co-repressor	Derepressed invertase, Cyc2p, other proteins	(Patel, *et al.*, 2009)
[*MOT3*]	Mot3p	Transcription repressor of genes derepressed under anaerobiasis	Derepression of "anaerobic genes".	(Alberti, *et al.*, 2009)
[*GAR*]	Pma1p, Std1p	plasma membrane proton pump; glucose signalling	Resistance to glucose-repression	(Brown & Lindquist, 2009)
[Het-s]	HET-s	No known non-prion function	Prion form necessary for heterokaryon incompatibility	(Coustou, *et al.*, 1997)