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Prion Diseases of Yeast: Amyloid Structure and Biology

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Summary

Prion “variants” or “strains” are prions with the identical protein sequence, but different characteristics of the prion infection: e.g. different incubation period for scrapie strains or different phenotype intensity for yeast prion variants. We have shown that infectious amyloids of the yeast prions [PSI⁺], [URE3] and [PIN⁺] each have an in-register parallel β -sheet architecture. Moreover, we have pointed out that this amyloid architecture can explain how one protein can faithfully transmit any of several conformations to new protein monomers. This explains how proteins can be genes.

Introduction

‘Prion’ means ‘infectious protein’, a protein which can transmit a disease or trait horizontally without the need for an accompanying nucleic acid. Although the self-activating yeast vacuolar protease B also can be a prion [1], most prions are amyloids, the filamentous protein polymer rich in β -sheet with the β -strands perpendicular to the filament long axis. Here we restrict our attention to the amyloid - based prions.

Perhaps the greatest mystery about prions, those of yeast as well as those of mammals, is the fact that a single protein sequence can stably propagate any of several prion variants. It is not particularly amazing that one protein can have several conformations; but that either of these conformations can be faithfully transmitted from molecule to molecule is certainly surprising. How is this conformational information transmitted? This “variant mystery” was one of the reasons for early skepticism of the prion hypothesis, independent of the absence of clear proof. We have shown that infectious amyloid of the prion domains of Ure2p, Sup35p, and Rnq1p each have an in-register parallel β -sheet architecture [2-4], with folds in the β -sheet along the long axis of the filaments [5]. This architecture nearly completely specifies the structure of the filament except for the locations of the folds and the precise extent of the sheet structure. “It has not escaped our attention that” this in-register parallel architecture provides a mechanism whereby the location of the folds and extent of the sheet once established, can be transmitted faithfully to each new protein monomer as it joins the end of the chain, thus explaining the variant mystery. It is not clear that other β -sheet structures (antiparallel, β -helix or out of register parallel) could explain this central mystery.

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We will review the evidence for the in-register parallel β -sheet structure of these prion amyloids, and contrast it with the two-turn β -helix structure of the [Het-s] prion. It is likely that the different structural patterns reflect the different biology of these systems. [Het-s] is evolved to be a prion with a specific structure, while the yeast prions [PSI+] and [URE3] are apparently diseases.

The Basics

The word 'prion' means 'infectious protein', a protein able to transmit a trait or disease without a required nucleic acid. Most of the prions of yeast and fungi are amyloids. Amyloid is a filamentous protein aggregate with a cross- β sheet secondary structure, meaning that the β -strands of the β -sheet are perpendicular to the long axis of the filament. We restrict our attention to the prions [PSI+] [URE3] [PIN+] and [MCA] of *S. cerevisiae*, and [Het-s] of *Podospora anserina*, which are prions of Sup35p, Ure2p, Rnq1p, Mca1p, and HET-s, respectively [6-9]. Amyloids made from the respective protein are infectious, efficiently transmitting the prion to yeast cells [10-12][13], thus confirming a large body of evidence that yeast prions are amyloids [reviewed by [14].

Sup35p is a subunit of the translation termination factor [15,16], and Ure2p is a negative regulator of nitrogen catabolism [17,18]. The phenotypes produced by infection with [PSI+] and [URE3] [19,20] each reflect simply a deficiency of the activity of the normal soluble protein, a key to their initial identification as prions [6]. In contrast, the phenotypes produced by [Het-s] and [PIN+] are novel. [Het-s] is necessary for heterokaryon incompatibility - a sort of fungal HLA locus - in which fungal colonies differing at the *het-s*/*het-S* locus abort fusion of hyphae (cellular processes) after a trial. Clones deleted for this gene show no such phenomenon and are neutral for heterokaryon formation. While deletion of *RNQ1* produces no phenotype [21], [PIN+] allows [PSI+]-inducibility by overproduction of Sup35p [8,22]. Mca1p is a metacaspase homolog, believed by some to have a role in a yeast apoptosis phenomenon [reviewed in [23], but see [24]].

That yeast prions may have variants was first shown by Derkatch and Liebman studying the [PSI+] prion [25], with prion variants distinguishable by the intensity of their phenotype and the stability of their propagation. [PIN+] and [URE3] were later shown to have variants [12,26,27], and prion variants were found to be distinguishable by their response to overproduction of various chaperones [28] and to the species barrier phenomenon [29,30]. Interestingly, variants of the [Het-s] prion have not been described [[see chapter by Saupe]].

It is clear from studies in both mammals and in yeast that different prion strains are due to amyloids with different structures [11,31-33]. As we discuss below, the yeast prions [PSI+], [URE3] and [PIN+] have in-register parallel β -sheet architectures, including two different variants of [PSI+], but the detailed structural difference between variant structures have not yet been determined.

Prion domains

The prion properties of Ure2p, Sup35p, HET-s and Rnq1p are each determined by a restricted region of the protein. Residues 1-65 of Ure2p, 1-124 of Sup35p, 218-289 of HET-s and 153-405 of Rnq1p can propagate the prion *in vivo* or, as amyloid made *in vitro* from recombinant protein, infect yeast cells with the corresponding prion [21,34-36]. For example, a cell expressing only Ure2p¹⁻⁶⁵ is efficiently infected by cytoplasm expressing the full length protein and carrying the [URE3] prion, can propagate the prion stably, and can then efficiently infect a cell expressing the full length protein but initially lacking the prion [37]. Thus, while deletions or mutations outside the prion domain can affect prion generation or propagation, the prion domain is both necessary and sufficient.

Shuffled prions domains can still form prions: suggests in-register parallel architecture

Initially with the goal of showing that the sequence of the Ure2p prion domain is important for prion formation, we found that each of 5 random shuffles of Ure2p residues 1-89, when integrated in place of the normal Ure2 prion domain, could support prion formation and propagation in vivo and amyloid formation in vitro [38]. The same result was obtained with the Sup35p prion domain [39]. These results indicate that the amino acid composition of these domains, not their sequence, determines their ability to form prions, and that the oligopeptide repeats of the Sup35 prion domain were not critical (although the amino acids of which they are composed may be) [40].

Although only composition determines prion formation in these two cases, there is abundant evidence that propagation of a given prion depends critically on sequence. This is the famous species barrier [41]. In some cases, even a single amino acid difference can block propagation of a particular prion variant between molecules [42-44].

Amyloids are β -sheet structures, and there are several types of β -sheet (Fig. 1). The most common type in soluble proteins is the antiparallel β -sheet, in which adjacent strands run in opposite N->C and C->N directions. Parallel β -sheets are also known, and these can be in-register (as in the case of A β amyloid) or out-of-register. An in-register β -sheet has identical residues aligned between molecules, so that there will be a line of, for example valine 35's with side-chains interacting, etc. A parallel β -sheet can also be out of register (Fig. 1). A β -helix is a helical structure, with the strands of the helix in β -sheet form.

In an anti-parallel β -sheet, an out-of-register parallel β -sheet, or a β -helix, apposed amino acid residues will generally be non-identical. In order for the strict sequence identity in prion propagation to be true, apposed amino acid residues would have to have some relation to each other. They might be positive with negative charge, or large residue with small residue, or hydrophobic with hydrophobic or hydrophilic with hydrophilic. This relation between juxtaposed residues would insure that only identical (or nearly identical) sequences could propagate a given prion. If the sequence were shuffled, the complementarity would likely be lost, and with it, the ability to form a prion.

However, the parallel in-register architecture apposes identical residues (Fig. 1). If the favorable interactions between aligned identical residues allow (or favor) prion formation, then shuffling the sequence will allow the same favorable interactions to occur, just in a different order. Thus, if a protein can form a prion, and its shuffled versions can form prions, these considerations argue that the structure must be in-register parallel, not the other possibilities [40]. While no prion architectures had been elucidated at the time of this proposal, it was already known that amyloids of the A β peptide, amylin and alpha-synuclein had an in-register parallel structures [45-47].

This result also has interesting biological implications. The conservation of sequences in the prion domains of Ure2p and Sup35p [48-50] has been interpreted to imply that prion formation must be a useful function for the host cell [50]. However, the fact that shuffling the prion domain sequences does not impair prion-forming ability shows that whatever the reason for the sequence conservation, it cannot be to preserve prion-forming ability [40]. Rather, the conservation of sequence probably reflects the normal functions of these domains (see below).

Structural studies of yeast prion amyloids by solid-state NMR

X-ray crystallography and solution NMR cannot be used for studying the structure of amyloids because they cannot be crystallized and are not soluble (and are too large). Solid-state NMR [51] and electron paramagnetic resonance studies [52] are most useful, and solid-state NMR is particularly suited because the amyloid-forming peptide need not be chemically modified. Solid state NMR can measure distances between nuclei (through the efficiency of magnetization exchange), chemical environment (through the 'chemical shift') and motion (which can enhance or destroy the signal, depending on the experiment and the nature of the motion).

The first detailed structure of a naturally occurring amyloid was that of the A β peptide, studied by solid-state NMR of a variety of synthetic specifically isotopically labeled samples [45,53,54]. A β forms an in-register parallel structure in which each molecule has a hairpin conformation, and two stacks of molecules are paired with C₂ rotational symmetry. In fact, a second form of amyloid, formed by the same peptide, is a three-fold symmetric structure, with each of the stacks of hairpins having essentially the same conformation as in the two-fold structure [55] (Fig 2A).

The most detailed prion amyloid structure determined to date is that of the HET-s prion domain [56,57]. This 72 residue peptide apparently forms a single amyloid structure, and thus produces sharp peaks in a 2 dimensional ¹³C-¹³C exchange solid-state NMR experiment. Note that [Het-s] has only a single variant, implying an unique structure. Moreover, only 43 residues show signals (rapid motion of the others eliminating their signals), simplifying the spectrum [58]. Each HET-s prion domain peptide forms a two-turn β -helix with direct repeats in the sequence aligned and paired in the structure (Fig. 2B).

Infectious amyloids of the prion domains of Ure2p, Sup35p, and Rnq1p have each been shown to have an in-register parallel β -sheet architecture [2-4]. The simplest way to establish this architecture is to label one or a few residues with a ¹³C-1-amino acid, and measure the distance from the labeled atoms to their nearest neighbor labeled atom. For an in-register parallel β -sheet, this distance is about 0.5 nm, the distance between β strands in a β -sheet. For any of the other types of β -sheet, this distance will be substantially greater (dots in Fig. 1). Thus, if one knows that the structure is a β -sheet, based on X-ray fiber diffraction, the solid-state NMR can confirm this fact, prove the in-register parallel architecture, and rule out all the other possibilities. The experiment is called PITHIRDS-CT [59], and is a method of dipolar recoupling - inducing the interaction between nearby magnetic nuclei. The strength of the dipolar interaction is proportional to $1/r^3$, where r is the distance between the interacting nuclei. That the measured nearest neighbor is on a different molecule is shown by diluting the labeled sample with ~4 parts of unlabeled protein. If the nearest neighbor is on the same molecule, then the dilution has no effect, but if it is on an adjacent molecule, the measured average distance increases in a predictable manner. Another method of demonstrating an in-register parallel structure is to measure distance by transfer of magnetization from an ¹⁵N-amino group to the α -carbon of the same residue on a different molecule [60].

These prion domains are too long to be synthesized, and only certain amino acids can be specifically labeled without their label leaking into other residues or their label being diluted by endogenous synthesis. For example, Ure2p¹⁻⁸⁹ was labeled at either valine residues 9, 19, 43 and 58, or at leucine residues 12, 16, and 81 or at the alanine at residue 15. In each case the short 0.5 nm nearest neighbor distance was observed arguing strongly for the in-register parallel architecture.

If the Ure2p prion domain amyloid were a single unfolded β -sheet, the filaments would be ~23 nm wide. In fact, such filaments are only ~4 nm wide, indicating that they are folded lengthwise, as shown diagrammatically in Fig. 3.

Mass per length measurements of filaments of Ure2p and Sup35p prion domain also strongly support the in-register parallel model. For full length Ure2p, or for Ure2p¹⁻⁶⁵, Ure2p¹⁻⁸⁹ or either fused to various enzymes, careful measurements of mass per length consistently give one monomer per 4.7 angstroms [61], a result expected for the in-register parallel model. For Sup35¹⁻⁶¹ fused to GFP, infectious filaments likewise showed mass per length values of one monomer per 4.7 angstroms [62], as did Sup35NM [63]. These results again rule out β -helix models [64] as well as models for Ure2 amyloid based on interactions between C-terminal domains [65].

Structural differences between prion variants

Structural differences between prion variants of transmissible mink encephalopathy (studied in mice) were first shown by different protease - resistant cores of isolated PrP^{Sc}, the putative infectious material [31]. Genetic studies have shown that mutations in the Sup35 prion domain affect different [PSI⁺] variants differently [29]. Equivalently, variants of [URE3] show different degrees of propagation across a species barrier [30]. Extensive scanning mutagenesis indicates that the extent of the critical structure differs between [PSI⁺] variants, showing that the size of the core structure can vary substantially [66]. Hydrogen-deuterium exchange experiments also show differences between prion variants in the overall extent of protected regions between different prion variants [33]. While different prion variant amyloids have in-register parallel β -sheet architecture [67], the evidence points to differences between variants in the extent of this structure [33,66].

One limitation of structural studies (including ours) is that, although the amyloid preparations are highly infectious, it is generally unknown what fraction of the amyloid prepared *in vitro* is actually infectious, what is the particle/infectious particle ratio. Amyloids made *in vitro* from recombinant prion proteins are generally heterogeneous by genetic criteria - producing a mixture of prion variants on infection of yeast cells (e.g., [12]), but filaments of Sup35NM grown at 37C or at 4C produce predominantly one or another single prion variant on transfection [11]. As mentioned above, these different preparations have different extents core structures by H/D exchange, but both preparations show a high degree of structural heterogeneity by this criterion. If the infectious material is only a small fraction of the filaments, the structural studies may be misleading. The same criticism may be made of our solid state NMR studies. Our data reflects the large majority structure, but what fraction of the amyloid filaments are infectious is unknown. This same type of problem arises with cytological studies of aggregates of GFP-labeled prion proteins *in vitro*. While the fact that prion-containing cells have aggregates and wild type cells do not [68][69] shows that aggregation is a feature of prions, attempts to correlate details of the aggregation patterns with infectivity will generally be difficult because shorter filaments (with less fluorescence) will be more infectious per mass of protein than longer filaments. Most fluorescence comes from the larger filaments and most infectivity probably comes from the shorter ones.

Mechanism of Conformation Inheritance Inferred from Parallel In-register Structure

The in-register parallel architecture, and the presence of longitudinal folds in the sheets, and the requirement that each of many alternate prion protein conformations are faithfully propagated, led us to suggest that the locations of folds, as well as the extent of the β -sheet

structure (see above), distinguish the amyloids that underlie different variants, and to suggest a detailed mechanism of prion conformational templating [70,71]. In the parallel in-register structure, the register is maintained by favorable interactions between the aligned amino acid side chains. For example, the 'polar zipper' structure posited by Perutz [72] and observed in actual amyloids [73,74] in which glutamine side chains hydrogen bond with each other is possible only if the register is maintained. Asparagines presumably form a similar line of hydrogen bonds. A line of serines or a line of threonines could presumably also form hydrogen bonds between side chains, and a line of a hydrophobic residue could have favorable interactions between the side chains of the aligned identical residues. Of course, a line of identically charged residues would be unfavorable, and in fact few charged residues are found within the yeast prion domains. We have proposed [70,71] that the same favorable side chain - side chain interactions between identical residues that enforce the in-register structure direct the new monomer joining the end of the amyloid filament to assume the same conformation as the other molecules in the filament (Fig 3). This forces the new monomer to have turns in its structure at the same place as all the other molecules in the filament, and to have the same extent of β -sheet structure as the previous monomers. This provides the answer to the fundamental mystery of prions: how protein conformation can be inherited.

Are yeast prions adaptive or disease agents?

The biology of prions is determined by their structure, and the structure, in turn, reflects the biology. As reflected in the orthopedic analogy in the introduction, a functional amyloid (like a functional enzyme) is likely to have a specific structure optimized by evolution to carry out that function. However, a pathologic amyloid (like a denatured protein) is expected to have any of a variety of detailed structures. The yeast prions are known to have a variety of structures, as reflected in the prion variants, and wide peaks in 2D solid-state NMR experiments, but [Het-s] has only one known variant and structural homogeneity as shown by sharp peaks in 2D NMR.

Because the [Het-s] prion is involved in heterokaryon incompatibility (see The Basics), a normal process seen in many filamentous fungi that may protect cells from infection by debilitating viruses [75], we suggested that [Het-s] may therefore be a beneficial/functional prion [76]. However, [Het-s] also determines a meiotic drive phenomenon that promotes inheritance of the *het-s* allele over *het-S* [77]. Meiotic drive genes promote their inheritance by blocking inheritance of other alleles, rather than by increasing the fitness of the organism. In this case, crosses of female *het-s* [Het-s] with male *het-S* [het-o] strains result in high rates of inviability of *het-S* meiotic segregants. Which manifestation of [Het-s] is primary, and which a side-effect is not clear, but in either case, *het-s* has evidently evolved to be a prion. Thus the existence of only a single [Het-s] variant, and a single amyloid structure, are explicable by the biology of the system.

[PSI+] was proposed to make cells generally resistant to the stress of heat or high concentrations of ethanol [78], but a later report showed that there was no such general protection [79]. Rather it was found that under most of the many conditions tested, when there was a difference, [PSI+] strains showed poor growth in comparison to isogenic [psi-] derivatives, and that under no condition tested was [PSI+] consistently an advantage [79]. The latter authors surmised that [PSI+] might help yeast evolve by allowing survival under stress conditions until they could change by mutation or recombination to a more stress-resistant genotype, although such an event was not documented. Moreover, using the same strain pairs, another group could not reproduce the reported differences [80]. It was further shown that under some stress conditions, the frequency of [PSI+] formation was modestly elevated, although under other stresses, the frequency decreased [81]. Increased frequency

of [PSI⁺] under stress was interpreted as an adaptive response, although in most conditions that increased [PSI⁺] formation, being [PSI⁺] was detrimental for survival [81]. The increase of [PSI⁺] formation may be a result of anti-prion chaperones, such as Ssb1 and Ssb2 [82] being tied up with repairing the damage caused by the stress condition, and thus being unavailable to block prion formation.

Other lines of evidence indicate that [PSI⁺] and [URE3] are diseases. First, prion-forming ability is not conserved, even within *Saccharomyces*. Sequences of *SUP35* from 16 wild *S. cerevisiae* showed that 4 isolates had a large deletion in the prion domain making them unable to propagate [PSI⁺] [83]. Similarly, the Ure2p of *S. castellii* is unable to become [URE3] or to propagate the [URE3] of other *Saccharomyces* species. Second, the prion domains of Ure2p and Sup35p have well-documented non-prion functions [84-86], so these domains, even when they are conserved, cannot be said to be conserved for the purpose of prion formation. The Ure2p prion domain is important for the nitrogen regulation function of the protein because it is necessary for normal Ure2p stability against protein degradation [86]. The Sup35p prion domain is necessary for normal mRNA turnover of all messages through its interactions with polyA-binding protein and the polyA-degrading exonucleases [84,85]. A third line of evidence that [PSI⁺] and [URE3] are diseases is that the cells themselves consider these prions to be stresses. Masison's group has shown that each prion produces elevated levels of Hsp104 and the cytoplasmic Hsp70s, a typical stress-response [87,88].

Determining advantage vs. disadvantage of a prion by growth tests is quite problematic. Even if a growth advantage under some particular condition is found, it is not evidence of benefit to the cell unless it can be shown that that specific condition occurs in the wild as part of the organism's ecological niche, and that prion-carrying strains are enriched in that environment. This is particularly important if under most conditions (as in the case of [PSI⁺]) the prion is a disadvantage. One way to sum over all conditions to determine the net benefit/detriment, is to examine the incidence of the prion in the wild. Even a detrimental (or lethal) virus or prion is found in the wild because infection can outpace the damage done to the host. Wild elk and deer are often found to have the prion infection, Chronic Wasting Disease, even though it is a lethal disease. An infectious agent that is an advantage to the host will quickly spread because infectivity and selective advantage will be working in the same direction. Certainly, a prion that is not found in the wild must be a serious disadvantage to its host. Neither [URE3] nor [PSI⁺] are found in wild strains [83,89,90], indicating that they are more detrimental than any of the parasitic nucleic acid replicons.

What is the connection of amyloid structure and prion biology?

First, a protein selected in evolution to be a prion should have a single prion variant/structure, because it is selected to do a specific task. Of course, we are thinking of [Het-s], which by one view is helping the host by its role in heterokaryon incompatibility and in another view is primarily a manifestation of a 'parasitic' meiotic drive gene. In either case, the HET-s protein is selected to have a specific form, and, indeed, there has not been any report of multiple [Het-s] variants.

Accumulating evidence indicates that the [PSI⁺] and [URE3] prions are diseases, like the mammalian prion diseases. With no selection for a particular structure, it is not surprising that there are multiple prion variants, particularly now that there is an explanation of how any of several alternate conformations of the same protein molecule can be stably propagated (inherited). A knee bends in a specific way, but a leg may be broken in any of many different ways.

Second, as outlined above, the in-register parallel β -sheet architecture of the yeast prions is compatible with any of many different inherited structures, but the β -helix seems more likely to produce a single structure. The helical conformation places an added constraint, not present in the parallel in-register architecture. Further, in the parallel in-register architecture, all of the H-bonds are intermolecular except for the inter sheet interactions. In the β -helix, half or more of the longitudinal interactions between side chains are intramolecular. This should further constrain the β -helix to a smaller number of forms.

A third connection of structure and biology is in the production of amyloid toxicity. Different variants with different turn locations and different extent of structure will expose different surfaces, and presumably adsorb different proteins, producing different toxicities.

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Figure 1. Kinds of β -sheet and the implications of shuffling

A. Labeling a single site ($\text{\AA}$) allows distinguishing the different types of β -sheet. For an in-register parallel β -sheet, the distance between labeled nuclei will be ~ 4.7 angstroms. For an out-of-register parallel or antiparallel sheet or for a β -helix, the distance will be much greater as shown. This distance can be measured using solid-state NMR. B. For an anti-parallel β -sheet prion (or out-of-register parallel or β -helix), the sequence identity requirement for prion transmission would imply that there be some relation between paired amino acids. If the prion domain is shuffled, this relation would be lost. But for an in-register structure, identical residues are paired, and if the sequence is shuffled, the same identical residues could pair, although in a different order. Thus, shuffleability of a prion domain suggests that it has an in-register parallel architecture.

Figure 2. Models of amyloid structure

A. A β^{1-40} structure is parallel in-register with each peptide in a hairpin configuration and monomers stacked [45,53,54](from [51]). Two stacks (or three in a variant [55]) are aligned as shown in C₂ (or C₃) symmetry. B. The HET-s prion domain (residues 218-289) forms a two-turn β -helix with partial direct repeat sequences in the peptide aligned in the β -strands [56,57](from [57]).

Figure 3. In-register parallel structure explains inheritance of variant information

The same interactions (small black arrows and small green arrows) that hold the peptide chains in-register force a new molecule joining the filament to adopt the same conformation as the other molecules. This determines the location of the turns (folds of the sheet) and the extent of the β -sheet structure. This explains the templating of protein conformation in analogy to the templating of sequence by replicating DNA.