Yeast prions assembly and propagation

Contributions of the prion and non-prion moieties and the nature of assemblies

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Yeast prions are self-perpetuating protein aggregates that are at the origin of heritable and transmissible non-Mendelian phenotypic traits. Among these, *[PSI⁺]*, *[URE3]* and *[PIN⁺]* are the most well documented prions and arise from the assembly of Sup35p, Ure2p and Rnq1p, respectively, into insoluble fibrillar assemblies. Fibril assembly depends on the presence of N- or C-terminal prion domains (PrDs) which are not homologous in sequence but share unusual amino-acid compositions, such as enrichment in polar residues (glutamines and asparagines) or the presence of oligopeptide repeats. Purified PrDs form amyloid fibrils that can convert prion-free cells to the prion state upon transformation. Nonetheless, isolated PrDs and full-length prion proteins have different aggregation, structural and infectious properties. In addition, mutations in the "non-prion" domains (non-PrDs) of Sup35p, Ure2p and Rnq1p were shown to affect their prion properties in vitro and in vivo. Despite these evidences, the implication of the functional non-PrDs in fibril assembly and prion propagation has been mostly overlooked. In this review, we discuss the contribution of non-PrDs to prion assemblies, and the structure-function relationship in prion infectivity in the light of recent findings on Sup35p and Ure2p assembly into infectious fibrils from our laboratory and others.

Introduction

Yeast prions are self-perpetuating fibrillar aggregates that manifest as dominant traits which are cytoplasmically inherited in a non-Mendelian manner. *[PSI⁺]*, *[URE3]* and *[PIN⁺]*, which are the prion forms of Sup35p, Ure2p and Rnq1p, respectively, are the best known and the most documented yeast prions.^{1,2} The biological significance of prions for fungi in their natural ecological niches has been recently reviewed in references 1–3.

Yeast prion proteins are not homologous. The striking enrichment in proteins involved in transcriptional and translational control led to the idea that prions, in yeast, may act as epigenetic modifiers of gene expression, enabling yeasts to rapidly

(and transiently) adapt to changes in their surrounding environment.^{1–3} While this hypothesis can be successfully tested in laboratory strains and conditions, the inability to identify wild-type yeast isolates that stably propagate one of the well-known prions raises doubts on whether this is actually a real mechanism for the control of gene expression in the natural environment.⁴

Under physiological conditions, prion proteins spontaneously assemble into fibrils in vitro (Fig. 1). When re-introduced into prion-free yeast cells, these fibrils are able to induce their conversion to the prion state with high efficiency.^{5–9} Fibril formation depends on the presence of a domain, often referred to as the prion domain (PrD), essential for assembly and the conversion of the protein from the soluble, functional state to the aggregated prion state. In most cases, the PrD is dispensable for the normal function of the protein.^{10,11} Although diverse, the PrDs share common characteristics such as enrichment in glutamines and asparagines, and the presence of oligopeptide repeats (Fig. 2). These traits are reminiscent of similar motifs found in the mammalian prion PrP, responsible for Creutzfeldt-Jakob disease, sheep scrapie and other transmissible spongiform encephalopathies, as well as in proteins responsible for neurodegenerative diseases such as Huntington disease or spinocerebellar ataxia,^{12,13} which have recently been shown to have prion-like properties that may play a significant role in neurodegenerative disease progression.^{14–22}

The PrDs can be found at either the N- or C-termini of prion proteins (Fig. 2). Purified PrDs are able to assemble into amyloid-like fibrils (Fig. 1) that can convert prion-free cells to the prion state upon transformation.^{6,8,9,23–25} PrDs can assemble into different structural variants that induce the formation of different prion strains, akin to mammalian prion strains, with varying phenotypic strength, mitotic stability and susceptibility to the action of molecular chaperones.^{6,8,9} Finally, non-prion proteins acquire a propensity to aggregate when fused to PrDs.^{26–28} Altogether, experimental evidence supports a model where PrDs are the sole drivers of prion formation and propagation.

However, this model is challenged by many studies demonstrating that isolated PrDs and full-length prion proteins have different aggregation, structural and infectious properties, and that mutations in the 'non-prion' domains (non-PrDs) of yeast prion proteins affect their prion properties.^{7,11,29,30} In this review, we discuss the contribution of non-PrDs to prion assemblies and the structure-function relationship in prion infectivity in

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Figure 1. Electron micrographs of negatively stained (A) Sup35NM, (B) full-length Sup35p, (C) Ure2p (1–93) and (D) full-length Ure2p fibrils (scale bar: 0.1 μ m).

the light of recent findings on Sup35p and Ure2p assembly into infectious fibrils from our laboratory and others.

The [*PSI*⁺] and [*URE3*] Prions

The non-Mendelian [*PSI*⁺] and [*URE3*] traits were described more than three decades ago and were subsequently shown to be the prion forms of Sup35p and Ure2p, respectively.^{31–35} Sup35p (Fig. 2) is a component of the eukaryotic release factor, homologous to eRF3, which mediates stop codon recognition and translation termination in conjunction with Sup45p (eRF1). In [*PSI*⁺] cells a substantial proportion of soluble Sup35p is depleted within prion aggregates, resulting in an increased nonsense suppression phenotype due to defective translation termination. It is worth noting that de novo [*PSI*⁺] formation, but not propagation, requires the presence of [*PIN*⁺].^{36–38}

Ure2p (Fig. 2) is a negative regulator of nitrogen catabolism. When good nitrogen sources such as ammonia are present in the growth medium, Ure2p represses the Gln3p transcriptional activator that controls the expression of genes encoding enzymes and transporters required for the assimilation of poor nitrogen sources.^{39–41} In [*URE3*] cells, Ure2p is sequestered within prion aggregates and can no longer interact with Gln3p, which becomes constitutively activated. [*URE3*] cells are therefore able to metabolize poor nitrogen sources such as allantoate, and because of its structural analogy, ureidosuccinate, even in the presence of good nitrogen sources.³⁴

Both [*PSI*⁺] and [*URE3*] are cured by treatment with millimolar concentrations of guanidine hydrochloride or by deleting *HSP104*, but only [*PSI*⁺] can be cured by the overexpression of *HSP104*.^{42,43}

Biological Functions of the Prion and “Non-Prion” Domains of Sup35p and Ure2p

What is the contribution of prion domains to the normal cellular function of Sup35p and Ure2p? Several lines of evidence suggest that prion propagation and normal cellular functions are carried out by separate, well defined regions within Sup35p and Ure2p (Fig. 2). Cells in which the essential *SUP35* gene has been replaced by a *sup35* allele where the N-terminal (Sup35N; residues 1–123) or NM (Sup35NM; residues 1–253) domains have been deleted grow as well as the wild-type, suggesting that only the C-terminal domain is required for the function of Sup35p in translation termination.¹⁰ Similarly, an *ure2* allele where the N-terminal domain (residues 1–89) has been deleted can perfectly restore nitrogen catabolism regulation in a Δ *ure2* strain.¹¹ However, these strains are not able to convert to the prion state or maintain it. Conversely, the transient overexpression of Sup35N and Sup35NM or residues 1–89 from Ure2p efficiently induces [*PSI*⁺] and [*URE3*], respectively.^{11,44} Sup35N, Sup35NM and Ure2p N-terminal fragments encompassing residues 1–89 are able to assemble into amyloid-like fibrils in vitro (Fig. 1) that can efficiently induce the [*PRION*⁺] state upon transformation into [*prion*⁺] cells.^{6,9,30}

However, these boundaries have been blurred by other observations that suggest that functional and/or physical interaction between prion and ‘non-prion’ domains may contribute to both the prion and normal cellular functions of Sup35p and Ure2p. Indeed, the C-terminal domain of Sup35p is strongly conserved through evolution from yeast to mammals.⁴⁵ The N-terminal extension is present in most Sup35p/eRF3 orthologs suggesting that it plays an important, yet unclear, role in the function(s) of these proteins.^{45,46} Although highly divergent in length and sequence, the amino-acid composition of the N-terminal domain of Sup35/eRF3 proteins from yeast to mammals is often highly unusual (e.g., mostly enriched in Q/N in yeasts and P/G/S in higher eukaryotes).^{13,46,47} The only crystallographic structure of eRF3 available is that of *Schizosaccharomyces pombe*’s Sup35p which suggests that the N-terminal extension could bind and block the eRF1 binding site in the C-terminal domain (Fig. 2).⁴⁸ Accordingly, both negative and positive effects of the N-terminal domain of yeast, fungal and mammalian eRF3 proteins on translation termination, non-sense suppression and eRF1 binding were described.^{7,49–54}

Similarly, the globular C-terminal domain of Ure2p (residues 90–354) is dimeric in solution, displays homology to glutathione S-transferases (Fig. 2) and binds glutathione or related compounds with high affinity.^{39,55–57} This globular domain retains its native structure in the fibrillar form of the protein.^{58–60} While the C-terminal domain alone can support the nitrogen regulation function of Ure2p (and complement a Δ *ure2* allele), the

presence of the N-terminal domain appears to be required for tighter nitrogen catabolite repression under normal Ure2p expression levels.^{39,61,62} Ure2p displays glutathione peroxidase and glutaredoxin activities, in both the soluble and fibrillar state, and therefore contributes to cell protection against heavy metal ions and oxidant toxicity.^{63–65} However, truncations within the N-terminal domain of Ure2p increased the glutaredoxin activity,⁶⁴ suggesting that the N-terminal domain is an important feature for the proper biological function of Ure2p.⁶²

Altogether, these data suggest that yeast PrDs contribute to the normal biological function of their corresponding proteins, although the extent of this contribution may not be easily detectable under normal growth conditions. Furthermore, no evidence supports a model where the evolutionary conservation of N-terminal extensions within Sup35p and Ure2p is correlated to a necessity to maintain the prion function of these proteins. Indeed, while many (but not all) close or distant Sup35p and Ure2p orthologs have been shown to have prion forming abilities when expressed in *Saccharomyces cerevisiae*, evidence for such prion propagation in their natural hosts is rather scarce.^{66–73} This may be due to unique properties (e.g., molecular chaperones, quality-control sub-cellular compartments, etc.,) evolved by *S. cerevisiae* laboratory strains that are required for the maintenance and propagation of prions.

Why do mutations in the “non-prion” domain affect [URE3] and [PSI⁺] prion propagation? The effects of mutating Sup35p and Ure2p PrDs on the propagation of [PSI⁺] and [URE3] have been extensively documented. However, few studies attempted to investigate the effects of mutating the non-PrDs of these proteins on prion propagation.

In a recent study, we showed for the first time that a single amino-acid change within the C-terminal domain of Sup35p dramatically affects its prion properties.⁷ The substitution of threonine 341 for alanine (Sup35A) or aspartate (Sup35D) was shown to affect the efficiency of translation termination by reducing Sup35p's GTPase activity and binding to Sup45p.⁵¹ Remarkably, these substitutions induced conformation changes within soluble Sup35p that affected its rate of assembly into fibrils.⁷ Furthermore, cross-seeding between Sup35p and Sup35A was inefficient, both in vitro and in vivo, suggesting that the T341A mutation alters the structural properties of Sup35p within the fibrils and generates a new prion variant.⁷ Indeed, we were able to show that Sup35p and Sup35A fibrils have markedly

Figure 2. The modular structure of Sup35p, Ure2p and Rnq1p. PrDs are depicted in dark grey and their amino-acid composition is shown (Q residues are in red; N residues are in blue). The oligopeptide repeats within Sup35p's N-terminal domain are underlined. The non-prion domains are depicted in black and, when known, the normal biological function of the protein is indicated. The crystallographic structures of the globular C-terminal domains of Sup35p (pdb entry: 1R5N)⁴⁸ and Ure2p (pdb entry: 1G6Y)⁵⁶ were generated with PyMol (<http://pymol.org>).

different infectious properties, and that the [PSI⁺] variant propagated in cells expressing only Sup35A was weaker than that of wild-type cells.⁷

Similarly, mutations in the “non-prion” C-terminal domain of Ure2p have been described that affect [URE3] formation.¹¹ The deletion of residues 151–158 or a truncation of the extreme C-terminal residues (348–354) were found to increase [URE3] induction by two orders of magnitude.¹¹ Furthermore, an *ure2* allele lacking most of the N-terminal PrD (residues 1–65) as well as residues 151–158 and 348–354 was still able to efficiently induce [URE3] when overexpressed, whereas a *ure2* allele only lacking residues 1–65 (with or without residues 348–354) could not.¹¹ Furthermore, the deletion of residues 151–158, that have no major consequences on Ure2p structure, accelerates the nucleation, growth and fragmentation of Ure2p fibrils in vitro,⁷⁴ while the deletion of residues 221–227 resulted in a decreased ability to induce [URE3].¹¹ These mutational analyses suggest an interplay between the prion and “non-prion” domains of Ure2p.

Similarly, mutations have been identified in the “non-prion” domain of Rnq1p that destabilize the [PIN⁺] prion.⁷⁵

Three events could account for the interference of the globular C-terminal domains of Sup35p and Ure2p in the assembly process. Firstly, the C-terminal domains could interact with the N-terminal domains in the soluble form of the proteins.^{7,48,60,76} Such an interaction should impact the ensemble

Figure 3. Schematic models of how the non-prion domains may contribute to fibrillar assemblies. (A) In this first model, the PrDs (in green) drive assembly by docking the molecules on top of each other through stacks of β -sheets. The non-PrDs (in blue) either (1) lock the structure or (2) are simply organized around a central core made by the PrD. (B) In this second model, both the PrDs and non-PrDs are integral components of the fibrillar scaffold. The PrDs may (1) interact with two adjacent non-PrD molecules or (2) act as molecular glue between adjacent subunits.

of assembly-competent folding intermediates that can be populated by the prion domains of Sup35p or Ure2p. Secondly, conformational changes within the C-terminal domains of Sup35p and Ure2p may affect the packing of the prion domains during assembly. Thirdly, the globular C-terminal domain of Sup35p could be directly involved in the fibrillar scaffold as it is the case for Ure2p.^{29,58,60,76} Plausible models of how the non-PrDs of Sup35p and Ure2p may contribute to the architecture of their fibrillar assemblies are depicted in **Figure 3**. The fact that the PrDs of Sup35p and Ure2p have different assembly, structural and infectivity properties in isolation and in the context of the full-length proteins fully supports a model where the C-terminal domain is an important part of the bona fide prion.^{29,30} The consequences on the structural nature of infectious prion assemblies are discussed in the following section of this review.

Relationship between Prion Particles Structure and Infectivity

To date, a limited number of direct evidence supports the tacit assumption that prions are ‘infectious amyloids’. We review in the following section the molecular nature of the infectious form of prions and the relationship between the amyloid forms of prions that can be created and studied in vitro and the infectious form generated and transmitted in vivo.

What are amyloids and what is the evidence that yeast prions are amyloids? An amyloid is formally defined as a protein found in fibrillar form in extracellular deposits, which binds Congo Red and exhibits yellow-green birefringence in polarized light.^{77,78} This strict definition has been extended to intracellular assemblies e.g., neurofibrillary tangles that are named “amyloid-like”. The amyloid fibrils have a core region consisting of β -strands that are orientated perpendicularly to the axis of the fibril that generate the characteristic diffraction pattern of X-rays by cross- β structures. The cross- β structure is however not diagnostic of an amyloid since amorphous protein aggregates can also show enrichment in such β -structure.⁷⁹

Yeast prion domains are particularly rich in Q and N residues (**Fig. 2**). There is evidence that Q/N-rich regions function as “polar zippers” mediating protein-protein interactions by the capacity of the Q/N side chains to form networks of hydrogen bonds.⁸⁰ This however does not imply that these regions form cross- β structures even though the peptide GNN QQN Y, found within the N-terminus of the prion Sup35p, was used to generate the first high resolution X-ray structure of an amyloid fibril.⁸¹ Indeed, over 100 proteins in the yeast proteome encompassing a range of protein types including transcription factors (e.g., Snf5p) and protein kinases (e.g., Yck1p) have Q/N-rich regions.⁸²

To consider the validity of the assumption that yeast prion proteins can form amyloids, a careful analysis of both in vitro and in vivo data is required. In vitro studies have largely focused on recombinant fragments of the relevant proteins: Sup35N (1–123), Sup35NM (1–254), Ure2p (1–93) and Rnq1p (132–405) (**Fig. 2**). In vitro, Sup35N, Sup35NM, Ure2p (1–70) alone or fused with reporter proteins and Rnq1p (132–405) fibrils are of amyloid nature.^{5,23,24,29,83–86} Full-length Sup35p and Ure2p can also assemble into fibrils (**Fig. 1**).^{24,87,88} These differ significantly however from those of Sup35NM and Ure2p (1–93), respectively (**Fig. 1**). Indeed, when the assembly of Sup35NM and full-length Sup35p are compared, significant differences in the critical concentration for assembly, the minimal size of the nuclei and the cross-seeding efficiencies are observed.³⁰ These differences suggest either that Sup35NM or full-length Sup35p assemble into distinct fibrils, or that the C-terminal domain of Sup35p contributes to the assembly process (**Fig. 3**). Similarly, fibrillar Ure2p (1–93) and full-length Ure2p do not cross-seed,²⁹ suggesting that they differ structurally.

Two forms of Ure2p fibrils have been reported: one with an α -helical content, an X-ray fiber diffraction, solid state NMR, H/D exchange and proteolytic patterns that are not those of a classical amyloids;^{58,60,87,89} another that has the typical characteristics of amyloids.^{85,90} The latter form may be the consequence of fibril dehydration.⁸⁵ It is worth noting that the weak 4.7 Å reflection that characterizes amyloids observed in hydrated fibrils⁹⁰ may also originate from the contamination of full-length hexahistidine-tagged Ure2p preparations by the N-terminal fragment of the protein as observed.⁸⁶

Finally, although fusing Sup35p PrD to GFP certainly leads to the appearance of discrete molecular aggregates (‘foci’)³⁵ in [PSI⁺] cells and the overexpression of Sup35p PrD generates

“fibre-like” structures,⁹¹ the evidence that Sup35p and Ure2p can form amyloid aggregates *in vivo* is limited.

What makes an amyloid infectious? The amyloid state of a protein differs radically from its soluble state. Indeed, while β -sheet-containing polypeptides such as immunoglobulin can assemble into β -sheet rich amyloid fibrils, natively unfolded polypeptides such as α -synuclein⁹² and even α -helical polypeptides, such as myoglobin⁹³ can also do so. All amyloid fibrils have a specific type of conformational arrangement of the polypeptide backbone in common, where the β -strands extend transversely to the main fibril axis while the β -sheets are parallel to the fibrils axis.⁹⁴ In the cross- β structure, the protein chains that run orthogonal to the fibril direction are hydrogen bonded, with an interchain spacing of 0.47 nm. The peptide chains may be arranged in an antiparallel fashion, connected by reverse turns, or in a parallel fashion. Antiparallel β -sheets exhibit a crystallographic repeat that is twice the characteristic 0.47 nm intrasheet interchain spacing of all amyloids.

Amyloid fibrils grow indefinitely by incorporating the constituent polypeptide chains at their ends. As hydrogen bonding is the driving force of amyloid fibril growth, the two ends of the fibrils are expected to have identical or near identical growth rates. The limiting step in the assembly reaction is the conformational change that a polypeptide chain must undergo to become capable of being incorporated at one of the ends of the fibrils. While the sequence features that make an amyloid infectious remain relatively unclear, the ability of amyloid fibrils to grow indefinitely and their resistance to disassembly/degradation account at least in part for their ability to propagate.

What feature makes prion proteins infectious? Some insight into this question has come from introduction of fibrillar assemblies made *in vitro* into yeast cells, and the observation that the fibrils promote [*PRION*⁺] trait appearance,^{5,6,8,9} and a dissection of the so-called prion-forming region (Sup35N). The latter studies have demonstrated that a region containing a series of oligopeptide repeats (Fig. 2) is required for the propagation of the aggregated prion state of Sup35p, and the number of such repeats can influence the frequency of both *de novo* and seeded formation of the [*PSI*⁺] prion.⁹⁵ Furthermore, a single amino acid substitution in one of these repeats leads to a defect in the ability to propagate the prion form of Sup35p (i.e., [*PSI*⁺]) but not aggregation of Sup35p *per se*. Yet it has been suggested that the key feature of this region, and the equivalent PrD in Ure2p (residues 1–93), is the amino acid composition and not the primary amino acid sequence. This conclusion was reached by randomising the relevant prion domain sequences without changing the amino acid composition and demonstrating that the ‘shuffled’ PrDs could not only mediate prion propagation, but also form amyloid-like fibrils *in vitro*.^{96–99} The scrambled prions were however defective in cross seeding between the different scrambled domains in the case of Sup35p in contrast to Ure2p.⁹⁸

Is prion infectivity the consequence of their potential amyloid nature or their ability to assemble? There is a widely held view that prion propagation is a result of the assembly of prions into amyloid fibrils and that the infectious and transmissible properties

of these fibrils are the consequence of their capacity to act as seeds, i.e., elongate through the incorporation of soluble intracellular prion molecules in an irreversible manner. It is the generation of short fibrils, either by breakage of pre-existing fibrils or their *de novo* formation that is believed to drive prion propagation.

The capacity of a polypeptide to assemble cannot be used to simply describe infectivity and propagation as other protein polymers (e.g., microtubules, actin filaments, flagellin), possibly involved in the cell-to-cell transmission of epigenetic traits,¹⁰⁰ do not define phenotypic traits. Neither can the efficiency of elongation of preexisting nuclei be at the origin of the infectious properties of prions. The only property that differentiates protein polymers that are “infectious” from those that are not, is the reversibility and strength of the protein polymers generated upon assembly. Indeed, microtubules and actin filaments are intrinsically labile polymers. Their reversibility is due to an irreversible reaction (bound nucleotide, ATP or GTP hydrolysis) occurring during assembly, the only purpose of which is the destabilization of the polymer.^{101–104} Prions form highly stable polymers following a conformational change, the extent of which can be variable. The establishment of numerous hydrogen bonds, hydrophobic and van der Waals interactions between polypeptides in the same conformation generates nearly irreversible polymers as the intermolecular interactions by far outweigh the entropic energy in solution under physiological conditions. The extent of the conformational change and nature of intermolecular interactions are the two parameters that define the stability of such protein polymers, allowing the depolymerization of some under very mildly non-physiological conditions (e.g., flagellin, Sick cell hemoglobin), while very harsh conditions are needed to disassemble others (e.g., Sup35p, Ure2p and Rnq1p PrDs). Thus, while the assembly propensity appears critical for prion propagation, prion assemblies do not need to be of amyloid nature to be infectious. Indeed, the establishment of a vast number of specific interactions can lead to highly stable, quasi irreversible assemblies that grow indefinitely by incorporation of the constitutive form of prion proteins.

The different domains of yeast prions, assembly and infectivity. The evidence so far suggests that fragments of the three yeast prion proteins Sup35p, Ure2p and Rnq1p can form amyloids. However, limited evidence exists for an *in vivo* mechanism for generating such prion fragments, nor that prion protein degradation is closely associated with the *de novo* formation of the associated prion traits. Furthermore, little data is available for the full-length protein molecules and where available, this data does not necessarily indicate that the resulting fibrils are amyloid in nature. It is therefore tempting to imagine that the parts of the molecules missing in most *in vitro* studies could influence the overall structure of the fibrils formed (Fig. 3). Evidence for the involvement of the C-terminal domains of Ure2p and Sup35p in fibril formation beside that of the N-terminal domains has been brought.^{7,59,79} Of particular interest is the finding that single point mutations within the compactly folded C-terminal domain of prion molecules are involved in strain formation.⁷

Conclusions

Fibrillar full-length or truncated Sup35p, Ure2p and Rnq1p can induce the appearance of [PSI⁺], [URE3] and [PIN⁺], respectively. It is unclear whether the polymers generated in vitro physically seed the assembly of the intracellular pool of soluble prions when introduced within the cells, or induce the de novo appearance of these traits by perturbing molecular chaperones homeostasis.

Irreversible reactions designed to occur during protein assembly generate intrinsically unstable polymers that remain assembled as long as a stabilizing cap is at their ends. The loss of the caps allows the fibrils to disassemble and their constituent polypeptide to recycle. This energy consuming process allows the building of highly labile structures such as the cytoskeleton, that the cell uses to adapt to its changing environment. Although energy consuming, this system is much more economical than protein synthesis where polypeptide chains are used for a particular function only once. Highly labile, cytoskeletal proteins, although capable of polymerizing as efficiently if not better than polypeptides

involved in disease, are unsuitable for the propagation of a trait. In contrast, static polymers assembled following conformational changes are made irreversible because of the establishment of a vast number of interactions which lead to specific traits. The irreversible assembly of proteins certainly contributes to make a trait heritable. Extrinsic factor(s), e.g., partner proteins, certainly play key roles in prion trait appearance and propagation as well. The interaction of both prion and “non-prion” domains of yeast prions with partner proteins in vivo may also modulate assembly. Thus, beside the need for further structural characterization of Sup35p, Ure2p and Rnq1p assemblies isolated from cell-free extracts, it is critical to characterize the contribution of prion-partner proteins by proteomic, biochemical and structural approaches to better understand the molecular events leading to prion occurrence and propagation.

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