

Prions of Yeast and Filamentous Fungi

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Glossary

Nonchromosomal gene A gene that segregates 4+:0 in meiosis and can be transferred by cytoplasmic mixing, in contrast to chromosomal genes that segregate 2+:2– in meiosis, and are not transferred by cytoplasmic mixing.

Nonsense suppressor tRNA A mutant tRNA that recognizes a translational stop codon and inserts an amino acid thus allowing the peptide chain to continue.

Introduction and History

The word prion, meaning ‘infectious protein’ without need for a nucleic acid, was coined to explain the properties of the agent producing the mammalian transmissible spongiform encephalopathies (TSEs), although 25 years later there remains some debate if the TSEs are indeed caused by prions. The yeast and fungal prions were identified by their unique genetic properties which were unexpected for any nucleic acid replicon, but specifically predicted for an infectious protein.

[PSI] was described by Brian Cox in 1965 as a non-chromosomal genetic element that increased the efficiency of a weak nonsense suppressor transfer RNA (tRNA). [URE3] was described by Francois Lacroute as a nonchromosomal gene that relieved nitrogen catabolite repression, allowing expression of genes needed for utilizing poor nitrogen sources even when a good nitrogen source was available. The [Het-s] prion was described in 1952 by Rizet as a nonchromosomal gene needed for heterokaryon incompatibility in *Podospora anserina*. Each of these elements was later found to be a prion. The [PIN] prion was discovered in 1997 by Derkatch and Liebman in their studies of *de novo* generation of the [PSI] prion.

Genetic Signature of a Prion

Viruses of yeast and fungi generally do not exit one cell and enter another, but spread by cell–cell fusion, as in

mating or heterokaryon formation (Figure 1). Infectious proteins (prions) should likewise be nonchromosomal genetic elements. To distinguish prions from nucleic acids, three genetic criteria were proposed: (1) If a prion can be cured, it can reappear in the cured strain at some low frequency. (2) Overproduction of the protein capable of being a prion should increase the frequency of the prion arising *de novo*. (3) If the prion produces a phenotype by the simple inactivation of the protein, then this phenotype should resemble the phenotype of mutation of the gene encoding the protein, which gene must be needed for prion propagation.

All three criteria were satisfied by [PSI] and [URE3], strongly indicating, perhaps proving, that they were prions. The [Het-s] prion of *P. anserina* and the [PIN] prion of *Saccharomyces cerevisiae* were likewise proved by application of the same genetic criteria, but because their prion form produces the phenotype, rather than the absence of the normal form, they do not satisfy criterion (3).

Self-Propagating Amyloid as the Basis for Most Yeast Prions

The finding that Sup35p, Ure2p, and HET-s were protease resistant and aggregated in prion-containing cells, that these proteins (and particularly their prion domains) would form amyloid *in vitro* indicated that a self-propagating amyloid was the basis of these prions. This was confirmed by the finding that the corresponding prions were transmitted by introduction of amyloid formed *in vitro* from the recombinant proteins (see below). However, in some cases, self-modifying enzymes have the potential to become prions (see below).

Chaperones and Prions

Chaperones of the Hsp40, Hsp70, and Hsp104 groups, as well as Hsp90 co-chaperones, have been found to be clearly involved in prion propagation. Millimolar concentrations of guanidine are known to cure each of the amyloid-based prions, and the mechanism of action of guanidine curing has been shown to be specific inhibition

Au2

p0020

s0015

p0025

s0020

p0030

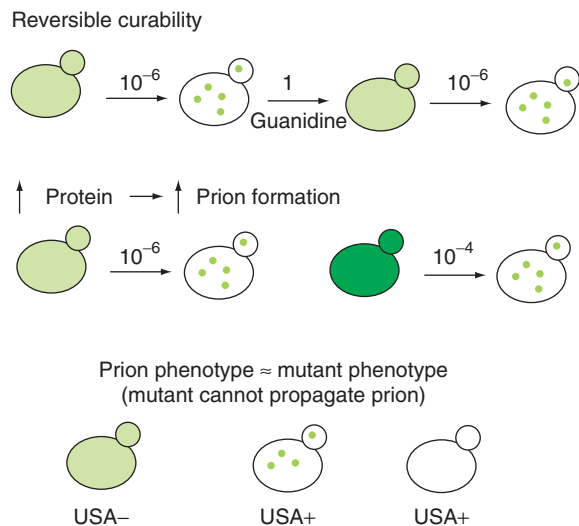


Figure 1 Genetic signature of a prion. Among nonchromosomal genetic elements, those with the three properties shown here must not be nucleic acid replicons and are almost certainly prions. However, only prions for which the prion form of the protein is inactive (such as [URE3] or [PSI]) will have property (3). The [Het-s] and [PIN⁺] prions are active forms of the HET-s protein and Rnq1 protein, respectively and have properties (1) and (2), but not (3).

of Hsp104. It is believed that at least one function of these chaperones is to break large amyloid filaments into smaller ones which can then be distributed at cell division to both daughter cells and insure the inheritance of the prion. Overexpression of some chaperones cure yeast prions, perhaps by solubilizing the filaments or perhaps by binding to the ends of filaments and preventing their elongation with new monomers. There is considerable specificity in which chaperone is needed for which prion and which chaperone can cure which prion by overexpression. The detailed mechanisms of chaperone action on prions (and amyloids in general) remain to be elucidated, but it is clear that they play an important role in these phenomena.

The Species Barrier and Prion Variants

Scrapie, a prion disease of sheep, only infects goats after a long incubation period, and subsequent goat-to-goat transmission has a much shorter incubation period. This is called the species barrier, and this barrier can in some cases be absolute, as appears to be the case between sheep and humans. The same phenomenon has now been documented in yeast, where [PSI] prions formed by the Sup35p of one species will not be transmitted to the Sup35p of another species, even though the other species' Sup35p can itself form a prion.

A single protein sequence can form several prions that are distinguishable, in yeast, by the intensity of their phenotype and the stability of their propagation. These are

called 'prion variants' and are believed to reflect different amyloid structures. Paradoxically, a similar phenomenon, long documented in the mammalian TSEs, was used as an argument against the protein-only model. Elucidation of the structure of different prion variants, and the mechanism of their faithful propagation, in some cases across species barriers, remains an important problem.

The bovine spongiform encephalopathy epidemic in the UK has brought the species barrier and prion variant phenomena together. It is clear that the 'height' of the species barrier is a function of the prion variant. Collinge views the species barrier as a reflection of the degree of overlap of possible variants of the prion proteins of the two species. If they have few common amyloid conformers (prion variants) then the barrier will be high. If each sequence can adopt nearly all of the amyloid conformers of the other, there will be little species barrier.

Formation of Prions by Sup35p and Ure2p Homologs

The C-terminal domain of Sup35 is conserved in eukaryotes with a human homolog capable of complementing the *S. cerevisiae* protein. All Sup35 proteins have N-terminal extensions, however, with limited or no sequence homology between species. N-terminal sequences from some species related to *S. cerevisiae* are capable of forming a [PSI⁺]-like prion. Ure2p is limited to the ascomycete yeasts. As with Sup35p, Ure2 proteins have a conserved C-terminal domain and a variable N-terminal domain that in general is rich in Asn and/or Glu residues. Ure2p homologs of some *Saccharomyces* yeasts can propagate [URE3] in *S. cerevisiae*.

Prion Generation, and [PIN]: A Prion That Gives Rise to Prions

One of the lines of evidence that showed [PSI] was a prion of Sup35p was that overproduction of Sup35p increased the frequency with which [PSI] arises *de novo*. However, it was found that in some strains, overproduction of Sup35p did not yield detectable emergence of [PSI]-carrying clones. Another nonchromosomal genetic element, named [PIN] for [PSI]-inducibility, was found necessary. [PIN] is a self-propagating amyloid form of the Rnq1 (rich in Asn (N) and Gln (Q)) protein, and it promotes *de novo* generation of [URE3] as well as [PSI].

Transfection with Amyloid of Recombinant Proteins

Amyloid filaments formed *in vitro* from recombinant HET-s protein, Sup35p, or Ure2p can efficiently transform cells to

the corresponding [Het-s], [PSI], or [URE3] prion. In some cases it was shown that the soluble form or non-specific aggregates of the protein were ineffective. This argues that the respective amyloids are not by-products or a dead-end stage of these prions, but are themselves the infectious material. All infectious Ure2p amyloids are larger than about 40-mer size. Amyloid formed *in vitro* is capable, for at least [PSI] and [URE3], of transmitting any of several prion variants. This implies that the amyloids can have any of several structures, a fact demonstrated by solid-state nuclear magnetic resonance (NMR) for amyloid of the Alzheimer's disease peptide, A β .

important for prion formation. The Sup35 prion domain has octapeptide repeats much like those in PrP, and deletion or duplication of these showed substantial effects on prion generation. In addition, single amino-acid changes in the prion domain of Sup35p blocked prion propagation.

To critically test whether the Ure2p prion domain had sequences essential for prion development, the entire Q/N-rich region (residues 1–89) was randomly shuffled (without changing the amino acid content) and each of five shuffled sequences were inserted into the chromosome in place of the normal prion domain. Surprisingly, each of these five shuffled sequences could support prion generation and propagation, although one was rather unstable. Each protein with the shuffled sequence could also form amyloid (Figure 3) *in vitro*. This showed that it was the amino acid content of the Ure2p prion domain that determines prion formation, and that sequence plays only a minor role.

Similarly, five shuffled versions of the Sup35p prion domain were each inserted in place of the normal sequence. Again, all five shuffled versions allowed formation and propagation of a [PSI]-like prion. It is likely that the effects of deletion or duplication of the octapeptide repeats observed on prion formation or propagation were

Shuffling Prion Domains and Amyloid Structure

The prion domains (Figure 2) of Ure2p and Sup35p are quite rich in Asn and Gln residues, and nearly the entire sequence of Rnq1p, the basis of the [PIN] prion, is rich in these amino acids. However, many Q/N-rich proteins are not capable of being prions. Thus, it was assumed that specific sequences in the known prion domains were

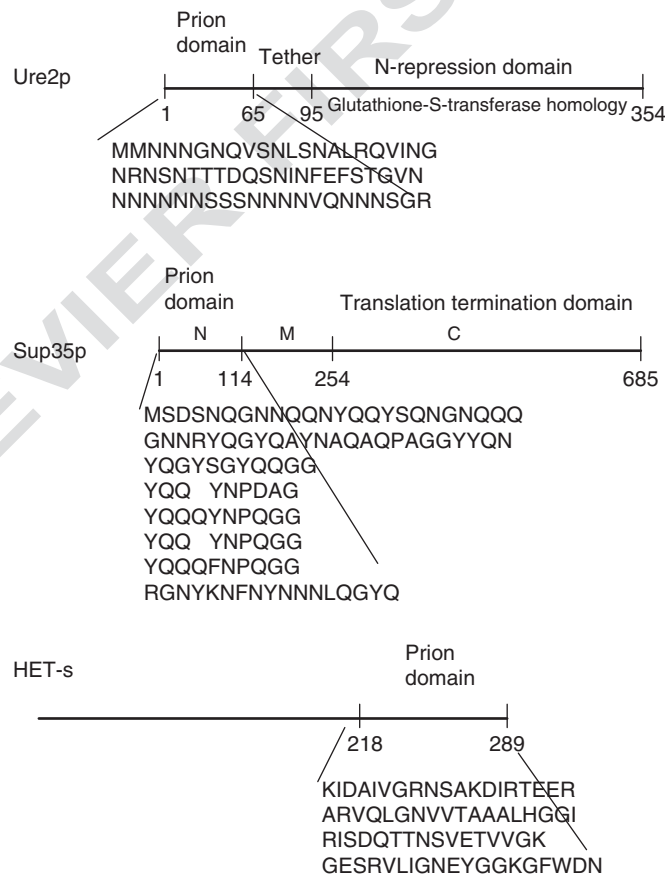


Figure 2 Prion domains. The prion domains of Ure2p, Sup35p, and HET-s are largely unstructured in the native form and in β -sheet in the prion form.

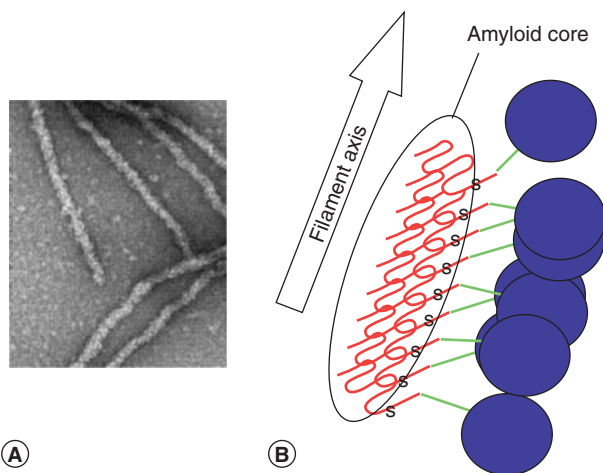


Figure 3 Amyloid. (a) Electron micrograph of amyloid of Ure2p. (b) Model of the structure of Ure2p amyloid. Red, prion domain; green, tether; blue, glutathione-S-transferase-like nitrogen regulation domain. Amyloid of recombinant HET-s, Sup35p, or Ure2p is infectious for yeast transmitting [Het-s], [PSI⁺], or [URE3], respectively. Amyloid of Ure2p is proposed to have the parallel in-register β -sheet structure shown based on the solid-state NMR studies of a fragment of the Ure2p prion domain, and the fact that the prion domain can be shuffled and still be a prion (see text).

due to changes in the length or composition of the prion domain. It appears that repeats *per se* are not important for prion generation or propagation.

Shuffleable Prion Domains Suggests Parallel In-Register β -Sheet Structure

Amyloids are β -sheet structures, but there are at least three kinds of β -sheets. Antiparallel β -sheets have adjacent peptide chains oriented in opposite directions: N \rightarrow C next to C \rightarrow N. This results in pairing of largely nonidentical residues. A β -helix also involves pairing of largely nonidentical residues, although they are within the same peptide chain. A parallel β -sheet pairs identical residues if it is in-register, but in principle, one could have an out-of-register parallel β -sheet, in which case nonidentical residues would be bonded to each other.

Prion propagation (and amyloid propagation in general) is a very sequence-specific process. For example, a single amino-acid change (at residue 138) can block propagation of scrapie in tissue culture. Humans are polymorphic at PrP residue 129 with roughly equal numbers of alleles encoding M and V. Either M/M or V/V individuals can get Creutzfeldt-Jakob disease (a human prion disease), but M/V heterozygotes cannot. Similarly, a single amino-acid change in the prion domain of Sup35p can block propagation of [PSI] from the normal sequence, but the mutant Sup35p can itself become a prion nonetheless. Thus, if a prion amyloid has an antiparallel,

parallel out-of-register, or β -helix structure, there must be some form of complementarity between bonded residues. Shuffling such a sequence would be expected to destroy the complementarity. In contrast, shuffling the sequence of a parallel in-register β -sheet would still leave identical residues paired. This suggests that prion domains that can be shuffled without destroying their prion-forming ability are forming parallel in-register β -sheets. Indeed Ure2p¹⁰⁻³⁹, a fragment of the prion domain, indeed forms amyloid with a parallel in-register β -sheet structure.

Biological Roles of Prions: A Help or a Hindrance?

In an attempt to discern whether yeast prions (Figure 4) are an advantage or disadvantage to their host organism, cell growth of isogenic [PSI⁺] and [psi⁻] strains have been carried out under a variety of conditions. To what extent the various growth conditions tested represent the normal yeast habitat seems unknowable, although [psi⁻] was an advantage under far more conditions than was [PSI⁺].

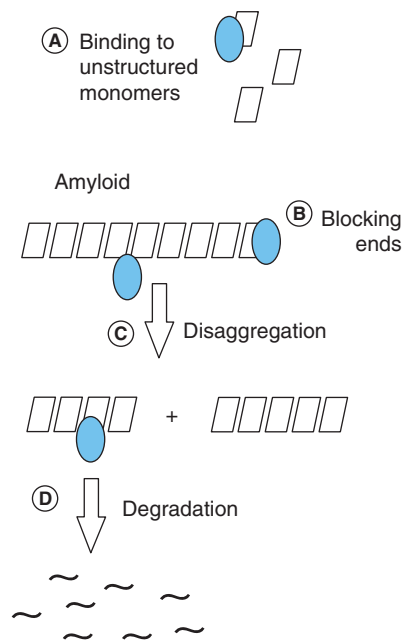
An alternative approach was to compare the frequency with which [PSI⁺] or [URE3] was found in wild strains to those of several 'selfish' RNA and DNA viruses and replicons known in *S. cerevisiae*. In any organism, an infectious element (such as a virus) may be widely distributed in spite of it causing disease in its host because the infection process overcomes and outraces the loss of infected individuals from negative selection. Certainly an infectious element, which is an advantage to its host, will quickly become widespread as selection and infection operate in the same direction. In fact, while the mildly deleterious RNA and DNA viruses and plasmids of yeast are easily found in wild strains neither [URE3] nor [PSI⁺] was found in any of the 70 wild strains examined. This indicates that [URE3] and [PSI⁺] produce disease in their hosts, and a rather more severe disease than the mild nucleic acid replicons.

The [Het-s] prion of *Podospora* appears to carry out the normal fungal function of heterokaryon incompatibility, thought to be a protection against the sometimes debilitating fungal viruses. Indeed, as one would expect for a prion with a function for the cell, 80% of wild *Podospora* isolates carry [Het-s], confirming its beneficial effects.

Unlike [PSI⁺] and [URE3], [PIN⁺] is found in wild strains at a frequency similar to that of the parasitic RNA and DNA viruses and plasmids. This suggests that [PIN⁺] is at least not as severe a pathogen as are [URE3] and [PSI⁺].

Enzyme as Prion

While most of the known prions involve amyloids, the word prion (infectious protein) is more general, requiring only that transmission by protein alone. If an enzyme



10020 **Figure 4** Chaperones and prions. Possible roles of chaperones in prion propagation are diagrammed.

is made as an inactive precursor that needs the active form of the same enzyme for its activation, then such a protein can be a prion. The vacuolar protease B (Prb1p) of *S. cerevisiae* can be such a prion in a mutant lacking protease A, which normally activates its precursor. Cells initially carrying only the inactive precursor remain so unless the active enzyme is introduced. Once a cell has some active enzyme, the autoactivation process can continue indefinitely. It is likely that other examples of this type of phenomenon will be found among the many protein kinases, methylases, acetylases, and other modifying enzymes that are known. Indeed, a protein kinase of *P. anserina* appears to be able to become a prion in this manner, producing a nonchromosomal genetic element called 'crippled growth'.

p0115 The advent of yeast prions has propelled the prion field forward, and is giving us insight into the broader field of amyloids and how they interact with cellular components. There is already evidence for a number of new prions, mostly in yeasts and fungi, in part because they are so well

suitable to genetic studies, and in part because their frequent natural mating or heterokaryon formation (in fungi) results in complete mixing and exchange of cellular proteins.

See also: Prions (00478).

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