

Minireview

## Prion variants, species barriers, generation and propagation

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### Abstract

Prion variants faithfully propagate across species barriers, but if the barrier is too high, new variants (mutants) are selected, as shown in a recent *BMC Biology* report. Protein sequence alteration can prevent accurate structural templating at filament ends producing prion variants.

Prions are infectious proteins, able to propagate and transmit the infection from one individual to another without an essential nucleic acid. In addition to this horizontal transmission, typical of the mammalian transmissible spongiform encephalopathies (TSEs), prions of fungi also transmit the infection vertically (to their offspring), and so they are proteins acting as genes, just as nucleic acids can act as enzymes (Table 1).

Most prions are amyloids - filamentous polymers high in  $\beta$ -sheet structure, usually protease resistant and with characteristic staining properties. Prion transmission occurs when donor amyloid enters the recipient cell and the equivalent recipient protein joins to the ends of the amyloid filaments, which act as a structural template, so that the recipient protein adopts (usually) the same conformation as the donor amyloid. The known prion-forming proteins of yeast and mammals are listed in Table 1.

A single prion protein sequence can form any of several biologically distinct prion 'strains' or 'variants', differentiated in mammals by incubation time, disease signs and lesion distribution, or in yeast by prion stability, phenotype

intensity or sensitivity to elevated or depressed levels of particular chaperones (reviewed in [1,2]). Different prion variants have different amyloid structures, although the exact structures are as yet unknown.

### Crossing the species barrier

Prions that are fully infectious between individuals of the same mammalian or yeast species may transmit poorly - or not at all - between species, a phenomenon called the species barrier. In spite of centuries of exposure, sheep scrapie is not known to have been transmitted to humans, but bovine spongiform encephalopathy (BSE) has (fortunately only rarely) done so. The primary determinants of the species barrier are the sequences of the potential prion proteins of the two species. However, the prion variant is also an important factor. For example, the Ure2 nitrogen regulation proteins of various *Saccharomyces* species can become prions (called [URE3]), and species barriers are seen among these [URE3]s that are dependent on the prion variant. While one variant of the [URE3] prion of species A may transmit with 100% efficiency to species B, another variant may transmit with 0% efficiency between the same two species [3].

**Table 1****Prions of mammals, yeast and the filamentous fungus *Podospora anserina***

Organism	Prion	Protein	Normal function	Prion manifestation
Mammals	TSEs	PrP	Not known	Transmissible spongiform encephalopathy
<i>Saccharomyces cerevisiae</i>	[URE3]	Ure2	Nitrogen catabolite repression	Derepression of nitrogen catabolism enzymes and transporters
	[PSI <sup>+</sup> ]	Sup35	Translation termination	Read-through of stop codons
	[PIN <sup>+</sup> ]	Rnq1	Not known	Rare seeding of [PSI <sup>+</sup> ], other prions
	[SWI <sup>+</sup> ]	Swi1	Chromatin remodeling	Poor growth on glycerol, raffinose, galactose
	[MCA]	Mca1	Metacaspase (suspected function in apoptosis)	Unknown
	[OCT <sup>+</sup> ]	Cyc8	Repression of <i>CYC7</i> and other genes	Derepression of transcription
	[MOT3 <sup>+</sup> ]	Mot3	Transcription factor	Cell-wall changes
<i>Podospora anserina</i>	[Het-s]	HET-s	Heterokaryon incompatibility; meiotic drive (as a prion)	Heterokaryon incompatibility; meiotic drive (as a prion)

These phenomena can be explained by assuming that each sequence has a range of possible conformers. A narrow overlap of conformers between donor and recipient produces a high species barrier, while a wide overlap implies a low barrier. Thus, according to this model, a specific conformer common to donor and recipient could overcome what would otherwise be a high species barrier [4]. It is likely that interactions with chaperones or other cellular factors, known to differ depending on prion variant, will be found to be at least part of some species barriers [5].

In yeast, *de novo* formation of prions can, though rarely, be primed by other prions. All of the prion-forming proteins of yeast have asparagine/glutamine-rich prion domains, and this shared structure is thought to enable prions of one of the proteins to prime filament formation by others [6].

In fact, *de novo* generation of the [PSI<sup>+</sup>] prion of *Saccharomyces cerevisiae* is almost undetectable in a strain not carrying one of the other prions. This cross-seeding produces an array of prion variants, whereas passing a species barrier usually produces a single, unchanged prion variant in the recipient.

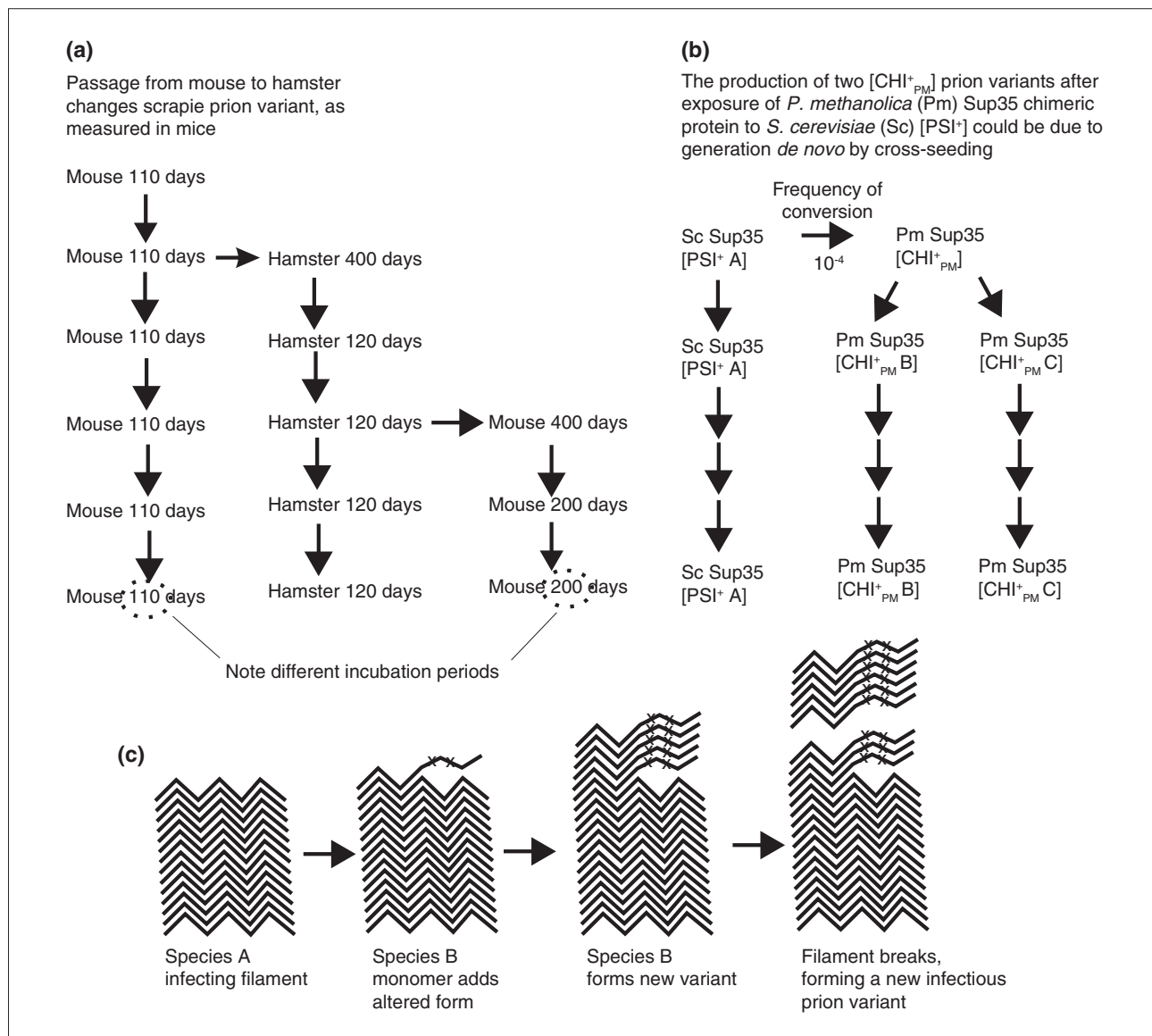
In both mammals and yeast, if a prion is successfully transmitted to a new host, the variant produced in the recipient is usually that of the donor. For example, when zoo animals were infected with BSE, and those infections were then introduced into mice, the same unique distribution of brain lesions was seen as when mice were infected with BSE directly from cows. Similarly, passing the [URE3] of one

species through Ure2p of a different species and then returning it to the original Ure2p generally produces a [URE3] prion 1 with the same properties as the original [3].

In some cases, however, infection of a new species is so inefficient - in other words, the species barrier is so high - that disease only results if a 'mutant' prion is selected that can replicate readily in the new host (Figure 1a). For example, mouse scrapie strain 139A only produces disease in hamsters after an extended incubation period (see, for example [7]). Serial passage of the infection in hamsters then eventually produced a shorter stable incubation period. However, on passage from hamsters back into mice and after the initial species barrier had subsided by a few passages, the agent had a dramatically longer incubation period than the original mouse scrapie and gave a different brain-lesion profile. The conclusion from this classic experiment was that a 'mutant' scrapie strain had been selected [7].

### Prion cross-seeding

An apparently analogous phenomenon has recently been reported in *BMC Biology* by Vishveshwara and Liebman using chimeric yeast prions [8]. The [PSI<sup>+</sup>] prion of *S. cerevisiae* is based on an amyloid form of the protein Sup35p, which normally functions as a translation termination factor (Table 1). Sup35p has a glutamine (Q)/asparagine (N)-rich amino-terminal prion domain (N) - the domain responsible for amyloid formation - a charged middle domain (M), and a carboxy-terminal domain (C), which is responsible for Sup35p's normal function of



**Figure 1**

Prion variant generation by cross-seeding could overcome species barriers to prion transmission. **(a)** An altered form (a 'mutant') of mouse scrapie strain 139A is selected by the high species barrier encountered when it is transferred to hamsters (modified from [7]). **(b)** The species barrier between the *S. cerevisiae* Sup 35 prion  $[PSI^{+}]$  and a chimeric protein with a *P. methanolica* Sup35 prion domain results in the rare generation of either of two  $[CHI^{+}_{PM}]$  prion variants of the latter on exposure to  $[PSI^{+}]$  [8]. **(c)** Schematic diagram showing partial templating by species A amyloid filament of species B protein. Species B protein sequence is incompatible with all of species A filament structure, and so assumes an altered self-propagating form - a prion variant.

translation termination. A chimeric protein made by fusing the similarly Q/N-rich N domain and the M domain of Sup35 protein of the yeast *Pichia methanolica* to the *S. cerevisiae* C domain ( $NM_{PM}-C_{SC}$ ) will act as a prion, called  $[CHI^{+}_{PM}]$ , when expressed in *S. cerevisiae* [9]. However, the considerable sequence difference between the *P. methanolica* and *S. cerevisiae* Sup35 N domains results in a

species barrier between the two N domains, so that prion transmission is rare.

Moreover, the rare prion transmission from  $[PSI^{+}]$  to  $[CHI^{+}_{PM}]$  results in at least two different prion variants of the chimera (Figure 1b) [8]. This indicates that the *S. cerevisiae* Sup35N amyloid was not able to accurately template the

chimera, although its presence certainly induced prion formation by the chimeric protein. In this case, it was probably prion generation that was induced by [PSI<sup>+</sup>], rather than transmission, although it remains possible that one of the [CHI<sup>+</sup><sub>PM</sub>] variants corresponds to the original [PSI<sup>+</sup>] variant.

The similarity between the scrapie 'mutation' phenomenon and the yeast stimulated prion generation is striking. In each case, sequence differences largely blocked duplication of the donor prion conformation, resulting in only partial templating and generation of altered prion variants. This also is presumed to be the basis of the prion priming phenomenon described above.

### What is the structural basis of variant phenomena?

The structure of infectious PrP is not yet known, but infectious amyloids of the prion domains of Ure2p, Sup35p and Rnq1p each have an in-register parallel  $\beta$ -sheet structure (see, for example, [10]). Thus, each residue of the last monomer to join the filament contacts the same residue of the preceding monomer (Figure 1c). The register is maintained by hydrogen bonds between Gln or Asn (the so-called  $\beta$ -zipper) and possibly between Ser and Thr residues. A line of hydrophobic residues down the fiber will likewise have positive interactions, helping to keep the  $\beta$ -sheet in register. The location of turns, the contacts between  $\beta$ -sheets and the extent of  $\beta$ -sheet are thus transmitted to the newly joined monomer. Combined with chain breakage to make new seeds, this templating action can explain the heritability of prion strains/variants [11]. A weakly homologous or non-homologous (but still Q/N rich) monomer might interact with part of the monomer on the end of the filament, so that only part of its conformation was fixed. The remainder may form by some stochastic interaction with another monomer identical to itself (shown schematically in Figure 1c). This could explain yeast prion cross-seeding and the 'mutation' phenomena using the known structural information.

### A prion without variants is evolved to be a prion: [Het-s]

Unlike the mammalian TSEs and the yeast prions [URE3] and [PSI<sup>+</sup>], which are all diseases, the [Het-s] prion of the filamentous fungus *Podospora anserina* is evolved to be a prion [12]. It appears to function for the host in heterokaryon incompatibility, and to be the basis of a striking meiotic drive phenomenon [13]. Which is the phenomenon and which is the 'epiphenomenon' is not yet clear, but in either case, the HET-s protein is evolved to be a prion. Only a single prion variant of [Het-s] has been described, as would be expected for a protein evolved to be a prion.

The infectivity and heritability of yeast prions and the ease of yeast manipulation as exemplified by the work of Vishveshwara and Liebman [8] make possible detailed studies of different amyloid forms, their generation and interaction with each other and with other cellular components, that would be impossible in the non-infectious amyloid diseases of mammals. Nonetheless, the findings with the prions are applicable to the non-infectious amyloid diseases that pose a burgeoning problem for our aging populations. Both the cross-seeding phenomenon, as suggested by the coincident occurrence of amyloids of A $\beta$  peptide, tau,  $\alpha$ -synuclein and others in the human amyloidoses, and the variant phenomenon, as in the different self-propagating amyloid forms of A $\beta$  [14], are apparently present in non-infectious amyloidoses.

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