The yeast prions [PSI⁺] and [URE3] are molecular degenerative diseases

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The yeast prions [URE3] and [PSI] are not found in wild strains, suggesting they are not an advantage. Prion-forming ability is not conserved, even within *Saccharomyces*, suggesting it is a disease. Prion domains have non-prion functions, explaining some conservation of sequence. However, in spite of the sequence being constrained in evolution by these non-prion functions, the prion domains vary more rapidly than the remainder of the molecule, and these changes produce a transmission barrier, suggesting that these changes were selected to block prion infection. Yeast prions [PSI] and [URE3] induce a cellular stress response (Hsp104 and Hsp70 induction), suggesting the cells are not happy about being infected. Recently, we showed that the array of [PSI] and [URE3] prions includes a majority of lethal or very toxic variants, a result not expected if either prion were an adaptive cellular response to stress.

Mammalian prions are uniformly fatal, but a lethal yeast prion would not be detected by the usual procedure, which requires growth of a colony under some selective condition. As a result, the prion variants commonly studied are quite mild in their effects. This circumstance has led to the suggestion that yeast prions actually benefit their host. Sup35p, the translation termination subunit whose amyloid becomes the [PSI⁺] prion, is essential for growth and Ure2p, the nitrogen regulation protein whose amyloid constitutes the [URE3] prion, is important for growth, with *ure2* mutants showing noticeably slowed growth.

When yeast prions were discovered,¹ we assumed they were diseases, by analogy with the mammalian diseases and the many non-prion amyloid diseases. Inactivating the essential Sup35p or the desirable Ure2p did not seem like a useful strategy. While control of either protein's activity might be advantageous, and Ure2p activity control is the key to regulation of nitrogen catabolism, prion formation is a stochastic process, so it makes control of activity of these proteins random instead of appropriate to the circumstances. The [Het-s] prion changed that picture.² Here was a prion necessary for a normal function, heterokaryon incompatibility, and we suggested that it was the first beneficial prion.³

[PSI] and [URE3] are Proposed to Help Yeast

Soon after [Het-s] appeared to be an advantage, [PSI] was proposed to protect cells from heat or high ethanol,⁴ the first proposal that yeast prions were beneficial. Further studies showed that this was not generally true, and in fact under none of the many conditions tested was [PSI⁺] generally beneficial.⁵ Under three quarters of growth conditions tested that showed a difference between [PSI⁺] and [psi⁻], cells were healthier if they were [psi⁻]. There was no growth condition tested in which [PSI⁺] was consistently beneficial across the five pairs of [PSI⁺]/[psi⁻] strains.⁵ Nonetheless, it was proposed that by producing various phenotypes, [PSI⁺] helped yeast as a “generator of diversity.” Of course, if all of these phenotypes had been detrimental under the conditions tested, there would be no benefit to the yeast in such ‘diversity’. So it was particularly damaging that another group was unable to reproduce the beneficial phenotypes with the identical strains.⁶

Lacking any obvious, consistent benefit of carrying a prion, it seems unlikely that one could demonstrate a benefit to carrying a prion based on a few marginally beneficial phenotypes. It would at least be necessary to show a correlation of beneficial phenotypes with detection of strains carrying the prion in a corresponding environment in the wild.⁷

Lindquist's proposal for a benefit for [PSI⁺] was somewhat in analogy to the “evolutionary capacitor” model she had only a few years earlier put forward to explain the appearance of developmental defects observed on inhibition or mutation of Hsp90 in *Drosophila*.⁸ It was proposed that Hsp90 suppresses the expression of accumulating mutations until a stress condition uses up the Hsp90, allows the developmental phenotypes to be expressed, and gives selection a chance to pick out a better adapted offspring.⁸ There was little effort in this report to show that the phenotypes were actually due to pre-existing mutations, as hypothesized. This “evolutionary capacitor” model now appears unlikely as recent data shows that Hsp90 is part of the piRNA (piwi-interacting) system that represses transposon activity in *Drosophila*.^{9,10} Inhibition or mutation of Hsp90 allows transposons to produce mutations, some of which are apparent as developmental defects that are not suppressed by restoration of Hsp90. Moreover, it would seem that there is no shortage of diversity for evolution to act on. Mutations are produced by cosmic rays, UV light, environmental chemicals, replication mistakes, cytosine deamination, depurination,

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Table 1. The “prion-as-benefit” view

Arguments for Functional Prions ¹⁶	But...
prions produce phenotypes, occasionally favorable	not reproducible, mostly prions are detrimental
prion domains are conserved	sequence conservation not needed for prion formation prion domains have non-prion functions
some stress conditions slightly increase frequency of prion formation	no induction of prion formation with wild type prion domain sequence the prion is usually detrimental under those inducing conditions

unavoidable ⁴⁰K in our bodies, transposon mediated rearrangements and recombination between repeat sequences. There are a myriad of DNA repair systems to reverse these effects and prevent their occurrence. None of these systems is perfect, or can be perfect, and the diversity of nature is evident. While the immune system generates diversity within each individual and malaria surface antigens generate diversity to evade host immunity, and yeast switches mating type, there are no known systems designed to produce general mutation. Even further, population geneticists have questioned the possibility of selection for evolvability.¹¹

Other arguments have been brought forward for beneficial prions. The fact that Sup35 prion domains of *Candida albicans*, *Pichia methanolica* or *Kluyveromyces lactis* could serve as prion domains in *S. cerevisiae* when fused to the cerevisiae Sup35 C-terminal (translation termination) domain was interpreted as conservation of prion-forming ability,¹²⁻¹⁵ and it was argued that this conservation implies a benefit to yeast.¹⁶ Ure2p from *C. albicans* and several *Saccharomyces* species can likewise form [URE3] in yeast.¹⁷⁻¹⁹ Of course, the conservation among animals of occasional broken legs does not imply a benefit of a broken leg.

Partial sequence conservation of the Ure2p and Sup35p prion domains has been interpreted as evidence for the usefulness of prions to yeast cells.²⁰ However, shuffling the prion domains of either protein does not prevent prion formation, indicating that it is amino acid composition, not sequence, that largely controls prion-forming ability.^{21,22} Thus the sequence conservation of these domains must reflect conservation of their non-prion functions (see below).

If [PSI⁺] could help cells survive a stressful condition until they or their progeny acquired adaptive mutations, then cells might specifically convert to the [PSI⁺] form under such a stressful condition. Indeed, it is reported that various stress conditions do affect the frequency of [PSI⁺] arising de novo, although to an extent only detectable with a mutant Sup35 prion domain that converts to prion form at elevated frequency.²³ However, the conditions that increase [PSI⁺] appearance are, in 4 of 6 cases, conditions under which being [PSI⁺] is detrimental to the cells.²³ This implies that the marginally increased frequency of [PSI⁺] appearance is probably a consequence of chaperone systems that normally prevent [PSI⁺] appearance (such as the Ssb proteins²⁴) being too occupied dealing with the stress to efficiently block [PSI⁺] generation. Arguments for an advantage of yeast prions are summarized in Table 1.

Accumulating Evidence Shows that [PSI⁺] and [URE3] are Diseases

To resolve the issue of benefit vs. detriment of [PSI⁺] and [URE3] the distribution of these prions in wild strains provides a sort of sum over all ecological niches for what the net effect is on the host. Even if it has a disastrous impact on its host, an infectious entity may be easily found in wild organisms. The virus, bacterium or prion may simply spread faster than its pathologic effects kill off its host. For example, the uniformly fatal Chronic Wasting prion disease of elk and deer is decimating populations in areas of the western and central US. If an infectious element is beneficial to its host, then infectivity and effects on the host will be working in the same direction, instead of opposing each other, and the element would rapidly spread through the population. Thus, an infectious element that is not found in wild strains must be a substantial detriment to its host.

None of several wild strains examined by Chernoff’s or Tuite’s groups had the [PSI⁺] prion, although a couple had [PIN⁺].^{12,25} We surveyed 70 wild strains and found that none had either [PSI⁺] or [URE3], although various proportions carried each of the parasitic nucleic acid replicons of yeast.²⁶ This indicates that [PSI⁺] and [URE3] are more detrimental than these parasitic viruses and plasmids. For example, 2 micron DNA slows yeast growth by ~1%,²⁷⁻²⁹ but 38 of our 70 wild strains carry the 2 micron plasmid.²⁶ This indicates that the overall detriment of the [PSI⁺] and [URE3] is substantially greater than the 1% slowing of growth shown by 2 micron DNA. The presence of [PIN⁺] in 11 of the 70 wild strains is similar to the incidence of the mildly detrimental parasitic nucleic acid replicons examined.²⁶ Thus, [PIN⁺] may simply be mildly detrimental, or have some unknown benefit under some special condition, but be generally detrimental. [PIN⁺]’s priming of [PSI⁺] generation is such a rare event (~1 in 10⁶ cells) that it is unlikely to be either good or bad for this reason.

While the Sup35p and Ure2p of some other species have been found able to be prions in *S. cerevisiae* (see above), there are also species close to cerevisiae that cannot do so. The Ure2p’s of *S. castellii* and of *Candida glabrata* cannot become [URE3] in *S. cerevisiae*,^{19,30} and the *Kluyveromyces lactis* Ure2p cannot form a [URE3] prion in *K. lactis* itself.³¹ Thus prion-forming ability appears to be sporadically distributed, but further studies will help clarify this aspect.

The prion domain of Sup35p is critical for the general mRNA turnover process,³²⁻³⁴ and the Ure2p prion domain is necessary for full nitrogen regulation activity by protecting the full length

protein from degradation.³⁵ These are functions of the respective domains when the protein is in the non-prion form. Thus, these domains are not present simply to enable prion formation. Rather, prion formation appears to be a risk that must be undergone to preserve these non-prion functions.

The conservation of sequence in the prion domains is likely based on these functions, but mutations in the prion domains are far more frequent than in the non-prion parts of the same molecules.^{13,17,18,20} These mutations produce species barriers between *S. cerevisiae* and other *Saccharomyces* species.^{30,36} Collinge has shown that the polymorphism in human PrP residue 129 (methionine/valine) protected against Kuru infection.³⁷ We suggest that these mutations are likewise selected in nature to protect the cells from 'catching' a yeast prion.

One way to assess whether these prions are advantageous or not to yeast is to ask the yeast cells themselves. Masison's group has shown that both [URE3] and [PSI⁺] induce elevated levels of the stress proteins Hsp104 and Hsp70.^{38,39} This indicates that the cells consider these prions as a stress condition.

In spite of the above data, many still maintain that [PSI⁺] and [URE3] are beneficial,^{40,41} perhaps because the strains of these prions commonly studied appear to grow well in the lab, in contrast to the uniformly fatal outcomes of the transmissible spongiform encephalopathies. A variant of [PSI⁺] that efficiently incorporated essentially all of the Sup35p into filaments would be expected to be lethal. We devised a method to detect such 'suiΨdal [PSI⁺]', expressing Sup35C, lacking the prion domain, from a (counter-selectable) *URA3* plasmid at a low level sufficient to support cell growth, but insufficient for efficient termination⁴² (Fig. 1). [PSI⁺] isolates were then tested for their ability to lose the *URA3-SUP35C* plasmid. We found that 8% of [PSI⁺] isolates were unable to grow without the minimal supply of Sup35C provided by the plasmid, and nearly half of [PSI⁺] variants were extremely slow growing without the supply of Sup35C.⁴² This implies that the mild [PSI⁺] variants usually studied do not represent the range of damage this prion can do to the host.

Spontaneous [URE3] isolates from two strains included frequent variants that grew very slowly, while a *ure2Δ* derivative of the parent strain showed no slowing of growth.⁴² In this case, the [URE3] prion was toxic to the cells, with a gain-of-function toxicity not due to simple loss of active Ure2p. These results give a more balanced view of the impact of the [PSI⁺] and [URE3] prions on their yeast host.

The [SWI⁺] and [OCT⁺] prions produce inability of cells to use certain carbon sources, phenotypes that do not seem to be

Figure 1. Scheme for isolation of SuiΨdal [PSI⁺]. (A) A [PSI⁺] derivative of a normal strain that converts all Sup35p to amyloid (suiΨdal [PSI⁺]) would be lethal because this protein is essential for growth. (B) By supplying a minimal amount of Sup35C from a counter-selectable plasmid, suiΨdal [PSI⁺] can be detected as [PSI⁺] clones that cannot afford to lose the plasmid.⁴²

particularly advantageous, although each has been put forward as aiding yeast in some way.^{43,44}

Could [PSI⁺] or [URE3] be beneficial under some as yet unknown condition? One might ask if the transmissible spongiform encephalopathies, lethal under known circumstances, are beneficial in some as yet unknown environment. Certainly a young man with a broken leg who is passed over by the army recruiter for that reason, may consider his broken leg a benefit. Further, evolution is not a finished job, and the rare detrimental prion (yeast or mammalian) may be insufficient to drive elimination of the protein domains responsible, particularly since the yeast prion domains are known to have important functions.

Table 2. The “prion-as-disease” view

Evidence that yeast prions are diseases	Reference
[PSI ⁺] and [URE3] not found in wild strains	12, 25, 26
Prion domains have non-prion functions	32–35
Cells show a stress response to prion infection	38, 39
Prion domains evolve more rapidly than non-prion domains, producing species barriers	17, 30, 36
[PSI ⁺] and [URE3] are often lethal or severely toxic	42
Prion-forming ability is not well conserved	See text

While an advantageous yeast prion may indeed be found, [PSI⁺] and [URE3] are quite plainly diseases (Table 2).

[Het-s]: Heterokaryon Incompatibility (Host Function) vs. Meiotic Drive (Disease)

The [Het-s] prion² provides an interesting contrast to the yeast prions. Heterokaryon incompatibility is a normal function of filamentous fungi, generally having nothing to do with prions.⁴⁵ Two colonies of a filamentous fungus growing toward each other will fuse their cellular processes to effectively form a single colony. But they only do so if trial fusion cells conclude that the two colonies are identical at a dozen polymorphic loci scattered about the fungal genome. Non-identity at even one locus results in death of the trial fusion cells and building a barrier blocking further fusions. This system may be designed to prevent spread of fungal viruses (reviewed in ref. 46). One of these genes is *het-s*, with alleles *het-s* and *het-S*. Only the product of *het-s* can form the [Het-s] prion (amyloid) form. The protein encoded by *het-s* must be in the prion form in order for the proper incompatibility

to be manifested. This role of [Het-s] is apparently a benefit to the cell.

However, a different manifestation of the [Het-s] prion is a meiotic drive phenomenon, plainly a detrimental ‘selfish gene’ aspect.⁴⁷ A sexual mating of female *het-s* [Het-s] strain with a male *het-S* followed by meiosis results in death of nearly all of the *het-S* segregants. This promotes the spread of the *het-s* allele, without it necessarily being of benefit to the organism. Is [Het-s] primarily a ‘selfish gene’ manifestation, essentially parasitic on the fungus, with heterokaryon incompatibility an incidental side effect? Or is the heterokaryon incompatibility the fundamental origin of [Het-s] with the meiotic drive a side-effect? It is not obvious how this issue will be resolved, but in either case, the protein encoded by *het-s* is certainly evolved to be a prion, unlike those of the known yeast prions. Probably because the HET-s protein is devoted to prion formation, there is only one [Het-s] prion variant, one optimized for its function. Amyloid of the HET-s protein reflects this, being very uniform in structure as reflected in unusually narrow peaks in solid-state NMR.⁴⁸

Conclusions

There may well be fungal or yeast prions that are advantageous. [Het-s] is currently the best candidate. There is as yet no evidence that yeast prions are adaptive for the host, and several strong lines of evidence that [PSI⁺] and [URE3] are detrimental.

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