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Prions are Affected by Evolution at Two Levels

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Abstract

Prions, infectious proteins, can transmit diseases or be the basis of heritable traits (or both), most based on amyloid forms of the prion protein. A single protein sequence can be the basis for many prion strains/variants, with different biological properties based on different amyloid conformations, each rather stably propagating. Prions are unique in that evolution and selection work at both the level of the chromosomal gene encoding the protein, and on the prion itself selecting prion variants. Here we summarize what is known about the evolution of prion proteins, both the genes and the prions themselves. We contrast the one known functional prion, [Het-s] of *Podospora anserina*, with the known disease prions, the yeast prions [PSI⁺] and [URE3] and the transmissible spongiform encephalopathies of mammals.

Keywords

amyloid; Ure2p; Sup35p; HET-s; Rnq1; parallel in-register beta sheet

Background

“Prion” means “infectious protein”, a protein able to transmit an infection without an accompanying nucleic acid. Unlike most proteins, prions transmit their protein conformation, and thus their biological properties, to new molecules of the same sequence, converting them into prions with the same conformation and properties. As a result, evolutionary pressure selects among prion variants (different amyloid conformers) which are most fit (spread or propagate the best). This is distinct from the usual evolution at the DNA level, which selects among gene/protein sequences based on their total impact on the host/population.

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Genetic nomenclature:

Yeast chromosomal genes are given as *URE2*, *SUP35*, *RNQ1*, *BTN2*, etc.

Non-chromosomal genes, such as yeast prions, are shown in brackets, such as [URE3], [PSI⁺], [PIN⁺], etc. Proteins are shown as Ure2p, Sup35p, Rnq1p, etc.

Phenotypes are shown as Ade⁺ or Ura⁻.

For the *Podospora anserina* system, the [Het-s] prion is due to the HET-s protein.

The transmissible spongiform encephalopathies (TSEs) are uniformly fatal mammalian amyloidoses of the PrP protein, a non-essential, GPI-anchored cytoplasmic membrane protein whose normal function is not yet clear. The prion concept began with evidence that the agent of scrapie, the sheep TSE, is so resistant to UV light that it likely contains no essential nucleic acid [1, 2]. The identification of PrP as the key protein essential for infectivity [3–7] supported this concept. Although the specificity of TSE prion transmission clearly resides in PrP (e.g. [8]), recent reports indicate that there is an important phospholipid component [9].

Yeast prions [URE3] and [PSI+] were long-known as non-chromosomal genetic elements [10, 11]. It was then recognized that their special genetic properties were incompatible with their being viruses or plasmids, but were just as expected for infectious proteins of Ure2p and Sup35p, respectively [12]. After curing either [URE3] or [PSI+], the prion could arise again in the cured cells [12, 13]. Transient overproduction of the respective protein could induce the formation of the prion [12, 14]. Finally, the presence of the prion had the same phenotype as deficiency of the gene for the protein, on which propagation of the prion depends [12, 15, 16]. Protease-resistance of Ure2p in extracts specifically of [URE3] strains [17], and the protease-resistance and aggregation of Sup35p specifically in [PSI+] cells [18, 19] supported the genetic evidence for [URE3] and [PSI+] as prions. The N-terminal Q/N – rich domains of Ure2p [17, 20] and Sup35p [21] were found to be responsible for the prion properties of these proteins. Finally, amyloid of these prion domains (but not the non-amyloid form) could transfect yeast cells transmitting the prion [22–24].

Ure2p (for ureidosuccinate, which is taken up by strains with the [URE3] prion or a *ure2* mutation) is a negative regulator of genes encoding enzymes and transporters for catabolism of poor nitrogen sources [25, 26]. Sup35p (for suppressor of nonsense mutations) is a subunit of the translation termination complex [27, 28]. The [PIN+] (for [PSI+]-inducibility) prion of Rnq1p (rich in N and Q) was discovered by its ability to rarely prime the generation of the [PSI+] prion, but Rnq1p has no known non-prion function [29–31]. A variety of other prions of *S. cerevisiae* have now been described, suggesting that there are many prions waiting to be discovered (Table 1).

The [Het-s] prion of the filamentous fungus *Podospora anserina* was discovered as a non-chromosomal gene necessary for one mode of heterokaryon incompatibility in this organism [32], and then shown to have the genetic properties of a prion [33]. Heterokaryon incompatibility is a system found in most filamentous fungi that limits the fusion of cellular processes of different clones to those that are closely related, as tested by identity of alleles at a number of polymorphic loci (reviewed by [34]). This involvement in a normal cellular function sets [Het-s] apart from the yeast prions, most of which are defective in some cellular function. We will see that [Het-s] provides an important counterpoint to the mammalian and yeast prions.

Prion variants in mammals and yeast

A single prion protein sequence can be the basis for a variety of biologically distinct ‘prion strains’ or ‘prion variants’. In mammals, the amino acid sequence of PrP is well conserved

(van Rheede *et al.* 2003), yet prion strains show distinguishable incubation times, different abilities to infect species other than the source of the infection, differing symptoms and signs, distinct areas of the brain affected and structural differences in the altered form of the PrP protein (reviewed in [35, 36]). In yeast, prion variants differ in the strength of their phenotype, stability of prion propagation during cell growth, prion lethality/toxicity, their response to overproduction or deficiency of various chaperones, their ability to cross transmission barriers due to prion protein sequence differences (inter-species or intra-species), sensitivity to the Btn2/Cur1 antiprion system and the biochemical and physical properties of the prion amyloids [23, 37–45].

Prion variants are relatively stably propagated, and since prion variants are based on differences in prion protein conformation (not sequence), there must be some mechanism by which the prion amyloid can act as a template to determine the conformation of a monomer newly joining the end of an amyloid filament (see below). Prion protein sequence places limits on the prion variants that are possible in a given animal, but because prion strains/variants differ in their biological properties and are each self-propagating, evolutionary forces can shape and select which variants are found in nature.

Prion domains

The ability of the N-terminal Q/N rich part of Ure2p to propagate the [URE3] prion in the complete absence of the remainder of the molecule gave rise to the notion of a prion domain [20]. A similar interpretation was made [20] of earlier work showing that the Q/N-rich N-terminal domain of Sup35p could propagate [PSI⁺] as a molecule expressed separately from the essential Sup35p C-terminal domain [21]. Calling these protein regions ‘prion domains’ tends to obscure the fact that they each have non-prion functions [46–49]. Although the N-terminal prion domains can propagate the prion without the respective C-terminal domains, the latter do affect the prion properties. The non-prion functions of these prion domains limit the ability of evolution to eliminate prion forming ability of these proteins.

Amyloid structures of infectious HET-s, Sup35p, Ure2p and Rnq1p prion domains

The HET-s protein prion domain (residues 218–289) forms infectious amyloid that has a beta-helix structure, with each monomer comprising two turns of the helix [50, 51]. The beta-helix structure of the HET-s prion domain amyloid implies that half of the main chain beta bonds are intramolecular. It is likely that these intramolecular bonds form before the intermolecular beta bonds, thus largely fixing the structure of the filament. In contrast, the infectious prion domain amyloids of the yeast prion proteins Sup35p, Ure2p and Rnq1p have a parallel in-register folded beta sheet architecture, with each monomer occupying only one layer of the structure [52–55]. Here, all of the beta bonds are intermolecular, and it is doubtful that this conformation forms as the monomer. A further (related) important difference is that while the HET-s amyloid assumes a single well-defined structure in vitro (and only has a single prion variant in vivo), the yeast prion amyloids are quite heterogenous in vitro (and can assume any of a number of variants in vivo). Both of the Sup35p amyloid variants tested are in-register folded parallel beta sheets [56], and the nature of this architecture suggests that the differences among variants must be either the location of the length-wise folds and/or the extent of the beta sheet structure.

The folded in-register parallel beta sheet architecture naturally suggests an answer to the mystery of how a single prion protein sequence, unstructured in the native form [57], can assume any of several different prion variants, each of which is self-propagating, templating its conformation and imposing it on a monomer newly joining the end of the filament. The same side chain – side chain interactions between/among identical residues along the long axis of the filament that hold the structure in-register will force the new monomer to have its bends/turns/folds at the same location along the peptide chain. Thus, as the locations of the folds in the sheet are the major difference between different prion variants, all of which have in-register parallel beta sheet architecture, this mechanism can explain the templating of conformation and hence the relatively stable propagation of prion variants. This concept has been discussed in more detail elsewhere [58–60]. At this time, this is the only hypothesis that has yet been offered to explain the stable propagation of any of the many different conformations / prion variants by a single prion protein sequence, with transmission of conformation information from molecules in the filament to the molecule joining the end of the filament.

Although several non-infectious PrP amyloids have been shown to have in-register parallel beta sheet architecture, the structure of infectious PrP^{Sc} is not yet known.

Biology of prions: TSEs vs [Het-s] vs [URE3] and [PSI+]

Prions cause numerous fatal TSEs in humans (Creutzfeldt-Jakob disease (CJD), variant CJD, i.e. human BSE, kuru) [61], livestock (scrapie in sheep, bovine spongiform encephalopathy (BSE) in cattle) [62], and wildlife (chronic wasting disease (CWD) in deer, elk and moose, feline spongiform encephalopathy (FSE) in domestic and wild cats) [63, 64]. These prion diseases can be inherited, transmitted or acquired sporadically, though horizontal transmission accounts for the vast majority of natural prion infections in sheep and wildlife species [65–67]. CWD and scrapie prions have been detected in urine, feces, milk and saliva [68–74], and these agents can remain infectious in the environment for extended periods of time in soil [75–77], water sources and housing structures [74, 78]. Furthermore, prions are deposited in muscle tissue [79, 80], and carcasses have been implicated as a viable environmental source of infectious prions [78].

As such, oral exposure is likely a major route of entry for many TSE agents, including BSE, vCJD, kuru, scrapie and CWD, although inhalation has also been shown to be an effective means of prion uptake [81]. Once ingested, prions can be transported by specialized intestinal cells across the gut epithelium [82], where they come in contact with nerve endings that can facilitate further spread. In addition, infectious agents can spread directly from the tongue to the CNS via cranial nerves [83]. Neurons, and in some cases glial cells, become infected, with infectivity often spreading along neural tracts [84]. Hematopoietic cells are also involved in prion transport from peripheral tissues into secondary lymphatic tissues, wherein the vast majority of PrP^{Sc} replication occurs (reviewed in [36]); though neuroinvasion can proceed in the absence of lymphoreticular amplification [83]. As prions replicate and accumulate in the CNS, neuropathological symptoms such as ataxia, dementia, myoclonus and behavioral abnormalities become apparent, though clinical symptoms of TSEs can vary widely. TSEs can also be distinguished based on deposition patterns of PrP^{Sc}

in neural and lymphatic tissue. For example, amyloid plaques are prevalent in neural tissue of vCJD patients, but they are rarely seen in sporadic CJD patients [61]. Likewise, scrapie and CWD-infected animals show extensive accumulation of prions in lymph nodes [85, 86], though this is typically not seen in animals with BSE infections [87].

However, infection of one species with prions from another species may produce a silent carrier state in which prions replicate in the foreign host, but too slowly to produce disease within the recipient's lifespan [88]. Mice infected with hamster prions were asymptomatic throughout their lifetimes and did not show detectable levels of PrP^{Sc}, however subsequent passage of brain material from these symptom-free mice revealed that the infectious material was still capable of rapidly inciting disease in hamsters [88].

Assessing evolutionary dynamics of prions in the wild

The low incidence of yeast prions in wild isolates implies they are detrimental

—The prions [URE3] and [SWI+] are not found in any of the 70 wild strains examined [89, 90]. The [PSI+] prion is rare in wild *S. cerevisiae* and [PIN+] is found in only 6 – 15% of wild strains [89, 91]. A beneficial prion which, like the yeast prions, is infectious by spreading to all meiotic progeny, would be found in most or all wild strains. The rarity of these yeast prions indicates that even their mildest variants are detrimental to their hosts.

Using the two micron DNA plasmid as a metric of detrimental effects of an infectious agent

—The prevalence of a non-chromosomal genetic element is determined by its frequency of generation or loss, its rate of spread and the benefit or detriment it gives to its host [92]. The yeast 2 micron DNA plasmid is inherited in the same way as yeast prions, segregating 4+:0 in meiosis, and having no extracellular route of transmission. Because its inheritance and effects on the host have been extensively studied, we used it as an internal control to measure the effects of yeast prions on their host. The frequency of loss of 2 micron DNA has been measured as about 10^{-4} per generation [93], and its generation frequency can be taken as essentially zero by comparison. The frequency of plasmid spread by outcross mating must balance the loss of its incidence in the wild because of its known detrimental effects on its host [93–96]. For the yeast prions, rates of generation and loss are easily measured, and rate of spread is largely a function of outcross mating frequency. Yeast cells normally exist in the wild in the diploid or polyploid state, heterozygous for the mating type locus and thus unable to mate. Meiosis of a diploid cell, induced by unfavorable conditions, produces haploid spores able to mate. They may mate with other spores of the same meiotic tetrad (intratetrad mating) or, since wild yeast is usually homothallic (interconverting mating type from **a** to **α**), with another cell in the same spore clone (homothallic mating), or with an unrelated cell (an outcross mating). The intratetrad or homothallic matings are unlikely to spread a prion since the mating cells will either both have or both lack the prion. Outcross matings are the main opportunity for the prion (or 2 micron DNA plasmid) to spread horizontally.

The frequency of outcross mating for *Saccharomyces paradoxus* has been estimated to be about once per 10^5 mitotic divisions [97], or, according to the authors, about once per century! Another study of *S. cerevisiae* produced an estimate of once per 5×10^4 mitotic

divisions based on inferred meiotic recombination in three unrelated strains [98]. The 2 micron plasmid is inherited and spread by the same mechanisms as are prions of yeast, all being inherited by mitotic offspring, and spread by outcross matings. Moreover, its frequency of loss or gain and, most importantly, its effect on the host has been carefully studied by several groups [93–96]. With this information, our finding that 38 of 70 wild strains carry the two micron DNA plasmid [89], we could estimate that yeast have an outcross mating approximately once per 100 mitotic divisions [96], an estimate that is 500 – 1000 fold greater than previous estimates. If outcross mating were as rare as has been reported by others, on the order of once per 10^5 mitotic divisions [97, 98], the 2 micron plasmid would be quickly lost from wild populations [96].

This value for outcross mating frequency, combined with typical *de novo* generation and loss frequencies for the prions, were then used to show that the **mildest variants of [URE3], [PSI+]** and **[PIN+]** must be >1% detrimental to the growth/survival of their hosts [96]. Another analysis, using the vastly lower mating frequency estimates predictably concluded that the net detriment of [PSI+] would be lower [99]. However, it should be emphasized that none of the analyses to date include the toxic or lethal effects of the most common variants of [PSI+] or [URE3] [43]. These analyses also do not yet consider the fact that a part of the low frequency of [PSI+] is a result of the existence of multiple Sup35p sequences in wild strains of *S. cerevisiae* [90, 100] which create barriers to prion transmission [90].

The existence of a substantial fraction of wild isolates carrying the [PIN+] prion made possible another line of investigation to distinguish spread by non-mendelian inheritance from selection based on advantage to the host [101]. If [PIN+] is mildly disadvantageous, but is found in the wild simply because it spreads by mating, [PIN+] strains should be enriched in heterozygous wild strains (compared to homozygotes) since these are formed by outcross mating. Indeed, 80% of [PIN+] strains were heterozygous for ‘public’ alleles, indicating their arising from outcross matings, whereas only 45% of [pin-] strains had such heterozygosities [101]. These results argue that [PIN+] spreading is largely due to the frequency of outcross mating.

Ability to form [URE3] and [PSI+] is distributed sporadically among yeasts and fungi—

Early reports that the Sup35p and Ure2p of some yeasts other than *S. cerevisiae* could form prions [102–104], were interpreted by some to mean that the prion forms of these proteins must be functional for the host. However, further work showed that the ability to form either of these prions is sporadically distributed [42, 105, 106]. For example, the Ure2p of *Candida albicans* can form [URE3], but that of *C. glabrata* cannot, even though the *C. glabrata* Ure2p is more closely related in sequence to the *S. cerevisiae* Ure2p [107]. Moreover, the prion domains of Ure2p and Sup35p have non-prion functions, as described above, and so the conservation of these domains need not indicate evolutionary value of the prion formation. The occasional development of emphysema is not the reason for conservation of the lung in animals.

Lethal and near lethal variants of [PSI+] and [URE3] are more frequent than mild variants—Any claim for benefit of prion generation must be weighed against the risk of developing a lethal or severely toxic prion. Because the C-terminal part of Sup35p

(Sup35C) is essential, it is expected that some [PSI⁺] variants in which the amyloid soaked up most of the Sup35p would be lethal because of a general failure of translation termination. To detect such variants, Sup35C, lacking the prion domain, was expressed at a low level from a plasmid, and [PSI⁺] variants were induced by overexpression of the prion domain and selected [43]. It was found that at least 5% of such [PSI⁺] isolates were completely unable to grow without the plasmid expressing Sup35C, and most variants could only grow extremely slowly [43]. Thus [PSI⁺] can be extremely damaging to the cell. One limitation of this study is that [PSI⁺] was (necessarily) induced by overproduction of Sup35p, and spontaneous [PSI⁺] isolates could have a higher or lower proportion of toxic/lethal [PSI⁺] (see ref. [108]). Of course, deprivation of Sup35p is only one possible mechanism for [PSI] prion toxicity, so this represents only a lower bound on the frequency of [PSI⁺] lethality/toxicity.

In a strain in which deletion of the *URE2* gene did not even slow growth, it was found that a majority of [URE3] isolates were extremely slow-growing, forming very tiny colonies. In this case, deprivation of Ure2p activity could not explain the effect on cell growth, implying that these [URE3] variants had a severe toxic effect on the cells [43]. In this case as well, the observed majority of toxic variants must be a minimum of the toxic/lethal prions formed, since lethal variants would have been lost.

The [Het-s] prion has beneficial and detrimental effects on *Podospira anserina*

—The [Het-s] prion was discovered by its requirement for the heterokaryon incompatibility effect between strains of *Podospira anserina* carrying the chromosomal *het-s* and *het-S* alleles [32]. Hyphal anastomosis is a normal function for filamentous fungi that allows cooperation between neighboring colonies of closely related strains. However, heterokaryon incompatibility limits this fusion to strains identical at around a dozen polymorphic loci scattered around the genome. This phenomenon serves to prevent infection by harmful fungal viruses and plasmids, as well as limiting conspecific parasitism.

The [Het-s] prion has a second, in this case detrimental, effect on its host. Meiotic mating female *het-s* [Het-s] cells with male *het-S* [het-o] cells results in lethality of nearly all *het-S* progeny [109]. This phenomenon constitutes a meiotic drive selection favoring inheritance of the *het-s* allele. The two lethal effects of [Het-s] both result from a mixture of HET-s and HET-S proteins in a cell with the [Het-s] prion.

Is the beneficial heterokaryon incompatibility the primary function of the [Het-s] prion or its detrimental role in meiotic drive? This issue seems impossible to resolve, but in either case, the HET-s protein seems selected by evolution to be a prion. Over 90% of wild isolates with the *het-s* genotype carry the [Het-s] prion, as expected for a functional prion [110]. Only a single prion variant has been identified for [Het-s], and the structure of the infectious amyloid of the HET-s prion domain is unique as demonstrated by the very narrow lines observed in two-dimensional solid-state NMR experiments [111]. The homogeneity of HET-s amyloid structure by solid-state NMR suggests that the protein sequence was selected to form a particular prion variant that best carries out the desired function. Perhaps it is not surprising that for this clearly functional prion, there is no known non-prion function of the HET-s protein. It is proposed that the frequency of the *het-s* allele, encoding the prion –

forming HET-s protein, is favored by the meiotic drive activity, but that its spread in the population is limited by the preference for an even distribution to carry out the heterokaryon incompatibility activity [110].

Recently, it has been suggested that the [Het-s] system has a close evolutionary relation to innate immunity and other genes of mammals, plants and other organisms [112].

As discussed above, heterokaryon incompatibility in *Podospora anserina*, as in other filamentous fungi, involves a recognition of self as a condition for the fusion of cellular processes between adjacent colonies. Several polymorphic genes (called *het* genes) prevent fusions between colonies that are not identical in each pair of alleles. Many of the proteins encoded by these *het* genes have a structure similar to mammalian and plant proteins that are involved in control of innate immunity and apoptosis, called STAND proteins (for Signal Transduction ATPase with Numerous Domains) [113]. They have a common central STAND domain (~170 residues), an N-terminal death-effector domain and a C-terminal repeat region that recognizes specific ligands [112]. Adjacent to the *Podospora* het-s/S locus is the NWD2 gene, with the STAND protein structure, but not known to be part of one of the *het* systems. Strikingly, the N-terminal 30 residues includes one copy of the HET-s prion domain repeat sequences that make up the beta sheet part of the beta helix in the prion form of the protein. It was proposed that the NWD2 protein, when it binds its (as yet unknown) ligand in its C-terminal repeat domain, can form amyloid (with its N-terminal domain) and initiate [Het-s] prion formation because its N-terminal domain is similar to that of HET-s [114]. This prediction has been verified by substituting the C-terminal repeat domain with that of another *het* gene whose ligand is known [115]. In addition, the amyloid-forming segments of either HET-s or of NWD2 can substitute for the aggregation domain of mammalian NLRP3, involved in innate immunity [116].

A functional prion, such as [Het-s], is like proteins with non-prion functions in that their sequence is selected to form a particular prion variant that best carries out the desired function. There is thus only one prion variant of [Het-s], the amyloid structure of HET-s is homogeneous based on solid-state NMR, and most wild isolates carry the prion. Perhaps it is not surprising that for this clearly functional prion, there is no known non-prion function of the HET-s protein.

This contrasts with the multiple variants of the yeast prions, heterogeneity of infectious amyloid formed in vitro (wider peaks in two dimensional solid-state NMR experiments), and the rarity of the yeast prions [PSI+], [URE3] and [PIN+] in wild strains (see above). A functional prion will be selected by evolution to have a single optimally functional form, while there are many ways that a protein can mis-fold, and, within the constraints of its normal function, evolution selects a sequence that will not often misfold (form a prion). A knee bends in a single place in a single way, but a leg bone can break in many ways.

Selection acts on prion genes and prion variants

Prions are unique in being affected by heritability and selection at two levels: the sequence of the protein is inherited in a DNA → DNA process and the prion variant itself is heritable in a protein → protein process. These selective processes will act differently depending on

the biology of the particular prion variant(s) and the prion protein. The interplay of non-prion function, prion disease and/or prion function produce complications unlike other cases of inheritance.

DNA-level selection and natural polymorphs of PrP, Ure2p, Sup35p and Rnq1p

Sequence polymorphisms of PrP, Ure2p and Sup35p have been examined in some detail for their effects on prion propagation between species (the ‘species barrier’) and within species (‘intraspecies barrier’). In some cases, a single amino acid change can block transmission of some prion variants between individuals with different prion protein sequences [90, 117–119]. Heterozygosity for the residue 129 M/V polymorphism in PrP results in a dramatic decrease in the frequency of spontaneous CJD [117] and provides relative resistance to kuru [120]. This 129 M/V polymorphism thus constitutes a balancing selection, selected, at least among the Fore, by resistance to CJD [120]. Balancing selection is a condition where heterozygotes have a selective advantage over either homozygote. The geographic widespread nature of this polymorphism suggested that this is an ancient balancing selection (~500,000 yr) and that cannibalism may have been widespread in the distant past [120]. Further studies questioned the age of this polymorphism, indicating that it may be only ~100,000 years old [122, 123]. Although most sites within the PrP genes of rodents and bovine species show negative purifying selection, some residues of rodents, monkeys, horses and dogs do show positive selection [123–126]. Positive selection may be insuring maintenance of the (still unclear) function of PrP, as well as selecting for alleles that are resistant to conversion to the prion form. Negative purifying selection may be eliminating alleles that more easily convert to the prion form or alleles that do not carry out the normal function.

Heterozygosity for another polymorphism, Lys or Glu at residue 219, is also protective for heterozygotes [127]. In addition, a G to V mutation at PrP residue 127 confers complete resistance to kuru and other forms of CJD and was selected by the kuru epidemic [119].

Signatures of selection for these prion-resistant alleles are apparent in kuru-exposed populations (linkage disequilibrium, distortion of Hardy-Weinburg equilibrium, higher relative fitness for resistant alleles), and higher prevalence of PrP 127 V and 129 M/V near the epicenter of kuru exposure further suggests that these alleles were selected in response to prion disease epidemics [128].

PrP polymorphisms that are protective against CWD have also been identified. Surveys of wild cervid populations indicated that deer heterozygous for PrP 95 Q/H and/or 96 G/S had lower risk of prion infection [129, 130]. Similarly in elk, a M/L mutation at PrP 132, which corresponds to codon 129 in humans, is associated with reduced CWD susceptibility [131, 132]. Although there is some evidence that these cervid PrP polymorphs reflect natural selection in CWD host populations (Robinson *et al.* 2012), it is not known if their frequency has increased within CWD-infected areas in response to selection. Even with high levels of CWD exposure, the effects of selection on cervid PrP genotype frequencies could take decades [133], maybe even centuries [134] to manifest. Further, selection may not elicit a strong genotype shift in cervids because PrP genotypes do not confer complete resistance, but merely extend CWD incubation period [135], and susceptible animals would therefore

have opportunities to reproduce before infection or during clinical progression [136]. As such, it has been suggested that partially resistant PrP genotypes could ultimately exacerbate CWD epidemics by prolonging the time period for dispersal-mediated spread or prion shedding into the environment [135].

Three PrP polymorphisms are associated with susceptibility to scrapie: 136 A/V, 154 H/R, and 171 H/Q/R [137]. Sheep with ARQ/ARQ, ARQ/VRQ or VRQ/VRQ haplotypes are the most susceptible, whereas homozygous ARR sheep exhibit high levels of resistance [138]. Selective breeding programs targeting the ARR PrP haplotype have been implemented to reduce scrapie susceptibility in domesticated sheep, however, genetic resistance is not absolute, as ARR animals can infrequently acquire natural scrapie [139].

Often proteins with Q/N-rich domains, resembling the prion domains of many yeast prions, are found unable to actually form prions. Paul et al. have found that in some of these cases, a small number of changes convert these domains to prion-capable, suggesting that evolution has selected for changes preventing prion formation [140]. Furthermore, in yeast, Ure2, Sup35 and Rnq1 exhibited higher rates of amino acid substitution within prion domains as compared to non-prion domains of the same protein, suggesting a higher rate of evolution for prion domains [90, 96, 141, 142]. The sequences of *SUP35* of over 70 wild isolates of *S. cerevisiae* revealed many common polymorphisms, mostly in the N and M regions known to affect prion propagation. [PSI⁺] can arise in each of these natural variants of the prion domain, but transmission of the prion arising in one Sup35p polymorph to a cell expressing only another polymorph is quite reduced [90]. Likewise, the prion domain of Rnq1 is highly variable, and similar intraspecies transmission barriers have been documented for [PIN⁺]. Several Rnq1 alleles from wild *S. cerevisiae* contained premature stop codons, and all of these variants completely abrogated [PIN⁺] propagation. Additionally, two naturally occurring Rnq1 haplotypes with small deletions and nonsynonymous mutations showed reduced [PIN⁺] transmission efficiency (Kelly *et al.* 2012). The high prevalence of these polymorphisms and their demonstrated protective effects suggest that these polymorphisms were selected to confer prion-resistance [90, 96].

Selection of prion conformers

A second level of evolution occurs because prion strains/variants propagate their properties from one individual to another, and different prion variants can produce dramatically different effects on organisms with the exact same prion protein sequence. This phenomenon is similar to the different chromatin states, some heritable, that constitute epigenetic phenomena. Indeed, if an epigenetic difference is defined as a phenotype difference without gene a sequence difference, then prions are epigenetic phenomena.

Prion variants are selected based on their own ability to propagate or be transmitted, by their effects on the host, by the chaperone systems that are needed for their propagation, by the sequences of donor and recipient prion protein, and by the cellular anti-prion systems.

The effectiveness of the natural interspecies or intraspecies barriers to [URE3] or [PSI⁺] prion transmission between cells with different Ure2p or Sup35p sequences depends on the prion variant examined [42, 44, 90]. Under conditions not selecting for one prion variant or

another, mitotic segregants of a single [PSI⁺] cell gave rise to at least 4 different transmission variants, but these, over many generations, again interconverted among themselves [44]. These experiments show that a prion can exist as a cloud of variants, with different variants segregating relative to each other, and with variant ‘mutation’ occurring under non-selective conditions. Interspecies transmission barriers have likewise been shown for both [PSI⁺] [102, 104, 143, 144] and for [URE3] [42, 107, 141].

Selection among prion variants is also evident in wild populations. Lethal and highly toxic variants of [PSI⁺] and [URE3] are a majority of isolates in laboratory conditions [43], but of course have not been found in wild strains [89, 91]. Reports that certain [PSI⁺] variants are beneficial under certain conditions [91, 145, 146] have not been reproducible [60, 146, Namy, 2008 #2178]. But were there such reproducibly beneficial [PSI⁺]s, they would be subject to selection in the wild with yeast isolates from the corresponding wild environments being expected to have an increased probability of carrying such beneficial variants. While no benefit from carrying any [PIN⁺] variant has been reported, [PIN⁺] is found in a substantial proportion of wild yeast [89, 91]. Whether [PIN⁺] is beneficial under some conditions or is simply not detrimental (the Rnq1 protein has no known function [30]) remains to be determined. As discussed above, the frequency of [PIN⁺] in wild isolates is compatible with [PIN⁺] having a mild detrimental effect and spread by outcrossed mating [96].

An antiprion system has also been described in yeast that cures a large majority of the variants of [URE3] arising [45, 147]. Btn2p and Cur1p, apparently acting with Hsp42, without overexpression of any of these components, selectively cure low seed number variants, thus constituting a selective force working to favor high seed number variants [45]. Variants are selected in the wild for relatively low pathogenicity, and for ability to resist the prion-curing effects of Btn2 and Cur1. Btn2p acts by sequestering Ure2p prion aggregates in one place in the cell at which place Btn2p itself is also concentrated [147]. Btn2p and Cur2p also can cure an artificial prion [148], but do not cure the [PSI⁺] prion [147]. Btn2p also acts to bring non-prion aggregates to one place in the cell [149, 150], so while Btn2p has an antiprion action, it is not exclusively devoted to prions.

It has been suggested that generation of a spectrum of variants benefits the cell by a sort of bet-hedging process, where some variants produce host survival under certain stress conditions, even though other variants are lethal [151]. In one study, it was reported that certain stress conditions modestly increased the frequency of [PSI⁺] arising de novo, and proposed that this was adaptive [152]. However, the authors reported that under most conditions inducing [PSI⁺] appearance, [PSI⁺] was detrimental to the cells. Moreover, we were unable to reproduce the prion-inducing effects [60]. Experimental support for this concept would require reproducible evidence that [PSI⁺] is an advantage under some conditions (see discussion above). Moreover, the predominance of detrimental prion variants of [PSI⁺] and [URE3] [43] means that the marginal prospect of marginal benefits must be weighed against the likelihood of a serious detrimental effect. This makes the proposed ‘bet-hedging’ a bad bet for the cell.

Cellular factors play a large role in prion propagation, and thus the nature of the cellular environment determines, in part, the array of prion variants that can propagate, and, potentially, even whether a particular protein can be a prion at all in a given cell. In yeast, chaperone proteins are particularly influential during prion propagation. Sse1p is a member of the Hsp110 family, and cures [URE3] when overproduced and destabilizes the same prion when deficient [153], but has a selective effect on variants of the [PSI⁺] prion when deficient [154]. A single PrP^{Sc} strain can propagate as distinct, host-specific variants when passaged through different human cell lines [155], and treatment with anti-prion compounds like quinacrine or swainsonine can result in selective amplification of drug-resistant PrP^{Sc} variants in cell culture [156, 157]. As such, it has been suggested that the cellular environment can elicit adaptive mutation in prion populations [157], and it may also enhance virulence of prion variants [158]. Béringue *et al.* [159] recently demonstrated that CWD and BSE prions propagated in the spleen of PrP transgenic mice readily overcame interspecies transmission barriers, whereas propagation in brain tissue resulted in limited cross-species transmission. Moreover, tissue-specific selection for distinct prion variants was observed in a single host, and agents amplified in the spleen were readily able to reinfect the original host [159]. These findings seem to indicate that different tissues exhibit heterogeneity in the cofactors affecting prion replication, or perhaps the existence of tissue-specific prion variants (i.e. organ tropism, discussed in Aguzzi *et al.* [36])

Perspective

We have seen that there is a duality of effects of evolutionary pressure on prion proteins and prions themselves. First, selection operates on the chromosomal gene encoding the prion protein to either favor formation of a particular functional prion (as in [Het-s]) or to prevent prion formation (as in PrP and the yeast prions [URE3] and [PSI⁺]). Second, when the disease-related prions do form, they can take an array of different forms, called ‘prion strains’ or ‘prion variants’, and these are then subject to the second layer of evolutionary pressure. In yeast, the common lethal or highly toxic variants are selected against, and only mildly detrimental variants are found in nature. If yeast prions beneficial in certain specific conditions are found, such variants will be isolatable from wild yeast in the corresponding environmental niche. Evolution has also produced at least one anti-prion system (Btm2/Cur1/Hsp42), active against the [URE3] prion, and it is expected that other such systems will be detected active against other prions.

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Abbreviations

TSE	transmissible spongiform encephalopathy
BSE	bovine spongiform encephalopathy

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Determinants of Prion Evolution

Figure 1.

Evolution and selection of prions operates on the prion gene and on prion conformers. Interactions between/among identical amino acid residues (blue circles) along the long axis of the prion fiber hold the structure in-register, and impose the same conformation on a monomer joining the end of the filament. 1. Polymorphisms in the genes that encode prion proteins can interfere with this templating process, thereby creating intraspecies or interspecies barriers to prion transmission. 2. A cloud of prion variants, comprising conformers with different biochemical and physical properties, is subjected to selection pressures elicited by different cellular environments. 3. Different variants may be segregated from each other during growth without selection. In addition the replication environment in different cells, tissues, or hosts can contribute to variant mutation, and the selection of dominant prion conformers that replicate more efficiently than other variants.

Table 1

Prions of Yeast and Mammals.

Prion	Prion protein	Protein normal function	Prion disease phenotype or function	Reference
scrapie agent	PrP	non-essential	transmissible spongiform encephalopathy	[35]
[URE3]	Ure2p	repressor in nitrogen catabolite repression	inappropriate derepression of nitrogen catabolism genes	[12]
[PSI+]	Sup35p	translation termination	inappropriate read-thru of termination codons	[12]
[PIN+]	Rnq1p	none known	rare priming of [PSI+], [URE3] or other amyloid prion	[29–31]
[Het-s]	HET-s	none known	heterokaryon incompatibility	[33]
[BETA]*	Prb1p	vacuolar protease	functional protease B, viability in stationary phase, sporulation	[160]
[SWI+]	Swi1p	chromatin remodeling	poor growth on non-glucose C sources	[161]
[OCT+]	Cyc8p	transcription repressor	inappropriate gene derepression	[162]
[MOT3+]	Mot3p	transcription repressor of <i>FLO11</i> , hypoxia genes	inappropriate derepression of <i>FLO11</i> , hypoxia genes	[163, 164]
[ISP+]	Sfp1p	transcription regulator	decreased read-thru of termination codons from overproduced Sup35p	[165]
[MOD+]	Mod5p	tRNA isopentenyl transferase	Slow growth, fluconazole resistance	[166]

PrP and HET-s form prions in mammals and *Podospora anserina*, respectively. All others are prions of *S. cerevisiae*.