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The prion hypothesis: from biological anomaly to basic regulatory mechanism

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Preface

Prions are unusual proteinaceous infectious agents that are typically associated with a class of fatal degenerative diseases of the mammalian brain. However, the discovery of fungal prions, which are not associated with disease, suggests that we must now consider the impact of these factors on basic cellular physiology in a different light. Fungal prions are epigenetic determinants that can alter a range of cellular processes, including metabolism and gene expression pathways, and these changes can lead to a range of prion-associated phenotypes. The mechanistic similarities between prion propagation in mammals and fungi suggest that prions are not a biological anomaly but instead are a new appreciated and perhaps ubiquitous regulatory mechanism.

Introduction

Cellular phenotypes are a ‘readout’ of the complex interplay of genetic and **epigenetic** determinants that ultimately define a unique proteome and thereby specify cellular identity. However, modulation of the proteome itself is emerging as a key concept in our understanding of the molecular basis of phenotypic traits. Many studies over the past two decades have highlighted how regulated changes in protein modifications, such as phosphorylation and glycosylation, contribute to cellular phenotypes by altering protein abundance, function and localization. Such changes can in turn impact on complex regulatory pathways that control cellular phenotypes. But post-translational modifications are not the whole story; changes in **protein conformation** might also explain many phenotypic switches albeit by a mechanism that is not yet fully understood.

The prototypical and perhaps most extensively characterized example of protein conformation-based, inherited phenotypic traits are those defined by proteinaceous infectious particles known as **prions**. These factors were originally identified as infectious entities associated with a group of transmissible neurodegenerative diseases in mammals^{1, 2} known as transmissible spongiform encephalopathies (TSEs) — such as Creutzfeldt–Jakob disease (CJD) and kuru in humans, scrapie in sheep and bovine spongiform encephalopathy (BSE) in cattle — the causative agent of which is resistant to treatments that damage nucleic acids.^{1, 3} The fact that prions could act as infectious agents despite the absence of a nucleic acid genome led to the formulation of the “protein-only” or prion hypothesis.^{1, 3} According to this idea, the TSE agent is a self-perpetuating **conformer** of a host protein PrP (prion protein).¹ The infectious conformer of this protein (PrP^{Sc}) was predicted to recruit and convert the normal conformer PrP^C into the PrP^{Sc} form through contacts between specific regions of the protein, thereby ‘replicating’ the agent during infection. A wealth of genetic and biochemical data now support this concept of conformational replication, leading to its near universal acceptance.

In addition to mammalian PrP, prions have also been found in two species of fungi, the yeast *Saccharomyces cerevisiae* and the multicellular fungus *Podospora anserina* (BOX 1).⁴ This intriguing collection of functionally unrelated proteins can, like PrP, individually adopt a range of physical forms and transition between these states under physiological conditions. In all systems, these physical transitions specify new phenotypes, which may result from alterations to the normal function of the protein (gain-of-function and/or loss-of-function), the cellular response to the new protein conformation and/or the rate of accumulation of the altered form. Remarkably, these alternative conformers, each with a distinct yet stable three dimensional shape, are self-replicating and can be transferred between cells or organisms, allowing the associated traits to be transmitted as infectious diseases, as occurs in mammals⁵, or inherited through cell division, as occurs in fungi.⁴ Although the mechanistic basis of prion propagation and transmission are emerging concepts in all systems, it is clear that these processes exist and ensure a level of genetic stability for prion-based epigenetic determinants that is in line with that of nucleic acid-based genetic determinants.

In this Review we examine the expanding range of cellular processes and complex phenotypes that are determined by these epigenetic elements in fungi and in mammals and discuss how the process of conformational self-replication provides a framework for understanding the molecular basis of prion-associated phenotypes. As the number of identified prion proteins continues to grow, we suggest that the prion mechanism has now moved from the realm of a disease-causing biological anomaly to one of a novel regulator of cell phenotype.

Prion-associated phenotypes in fungi

Fungal prions have been identified through classical genetic studies, sequence-based algorithms and genetic screens (Box 2)⁶⁻⁹. To date, eight proteins are confirmed fungal prions (Table 1), and at least another 20 proteins may potentially fall into this class. Remarkably, fungal prions affect the activity and/or regulation of several cellular processes, with many playing important roles in global gene regulation either at the transcriptional or post-transcriptional level (Table 1). Because of their roles in regulating the expression of genetic information, prion proteins could indirectly affect a wide range of co-regulated cellular processes.

[PSI⁺] prion-based loss-of-function phenotypes

The epigenetic [PSI⁺] determinant is the prion form of Sup35, which is required to terminate mRNA translation and to release the polypeptide chain from the ribosome. [PSI⁺] enhances the efficiency of **tRNA-mediated nonsense suppression**¹⁰ and **+1/-1 ribosomal frameshifting**¹¹ in *S. cerevisiae*, two phenotypes that could be ascribed to loss-of-function or modification-of-function mutations in Sup35. In [PSI⁺] cells, Sup35 is mainly found in large aggregates,^{12, 13} suggesting that conversion of Sup35 to its prion form compromises its activity in translation termination by sequestration. This reduction in release activity allows near cognate tRNAs to mistranslate a stop codon as sense. Sup35 proteins from many closely related *Saccharomyces* species retain the ability to behave as prions when expressed in the *S. cerevisiae* cytoplasm,¹⁴ indicating that [PSI⁺] could be a newly appreciated but conserved epigenetic mechanism for regulating both translation termination and reading-frame maintenance. This change in translational fidelity affects a broad range of cellular phenotypes either directly or indirectly.

The [PSI⁺]-dependent suppression of specific nonsense mutations generates readily assayed changes in phenotype. However, there are several complex phenotypes that are [PSI⁺] dependent but not simply attributable to suppression of a specific nonsense mutation, including altered sensitivity to a range of physical stresses such as heat¹⁵ and toxic chemical

agents.^{16, 17} Such prion-mediated effects likely reflect a more global perturbation of the proteome that could arise through specific effects on a regulatory factor or alternatively through the extension of an ORF by stop codon read-through, which could modify the function of the encoded protein or inactivate it.

Despite the existence of opposing forces that act to ensure translational fidelity, such as the context of the stop codon¹⁸ and the nonstop mRNA decay pathway,¹⁹ recent studies have uncovered support for each of these mechanisms. For example, antizyme, encoded by *OAZ1*, is a negative regulator of polyamine biosynthesis that is translated from two overlapping out-of-frame ORFs by a +1 frameshift (Figure 1a).²⁰ [*PSI*⁺] cells have increased antizyme levels and, correspondingly, reduced polyamine levels, a physiological state that may account for a large proportion of [*PSI*⁺]-dependent phenotypes.²¹ Also, the *PDE2* ORF, encoding a high-affinity cAMP phosphodiesterase, is extended in [*PSI*⁺] cells leading to a destabilization of the encoded enzyme and resulting in an increase in cAMP levels (Figure 1b).²²

Other prion-based loss-of-function phenotypes in fungi

Five of the established yeast prion proteins (Ure2/[*URE3*], Swi1/[*SWI*⁺], Cyc8/[*OCT*⁺], Mot3/[*MOT3*⁺] and Sfp1/[*ISP*⁺]; Table 1; Figure 2), normally function to co-ordinately regulate transcription. For example, Swi1²³ and Cyc8²⁴ regulate the transcription of ~400 different genes, many of which contribute to a range of key cellular pathways. The newly described [*MOT3*⁺] prion⁹ also has a broad repertoire of target genes, including those important for cell wall remodelling under anaerobic conditions and for ergosterol biosynthesis, potentially affecting vacuole function,²⁵ and Sfp1 coordinates the expression of ribosomal proteins and factors that control ribosome biogenesis.²⁶ The transcriptional effects of these factors are mediated by their association with larger protein complexes (for example, the SWI–SNF chromatin remodelling complex, the Cyc8–Tup1 and Mot3–Rox1 co-repressor complexes, and Sfp1 regulation by TORC1). The switch to the prion form may affect these associations and, in so doing, alter the expression of a large portion of the yeast transcriptome. This idea has been experimentally confirmed for strains carrying the [*OCT*⁺] prion in which the Cyc8–Tup1 repressor complex becomes inactive and genes such as *CYC7* (encoding iso-2-cytochrome C) and *SUC2* (encoding invertase) become derepressed (Figure 2a).²⁴

[*URE3*], the first fungal prion to be discovered, also exemplifies the phenotypic consequences of a transcriptional regulator – in this case the Ure2 protein – switching to a prion form. In its soluble non-prion form, Ure2 binds to two transcriptional activators (Gln3 and Gat1) in the cytoplasm, preventing their transit into the nucleus.^{27, 28} This sequestration represses the transcription of target genes and prevents the use of poor nitrogen sources while preferred sources, such as ammonia, are available (Figure 2a). In [*URE3*] cells, Ure2 is aggregated in the cytosol and cannot retain Gln3 and Gat1, resulting in activation of the transcriptional programme needed to use alternative nitrogen sources, thereby phenocopying loss-of-function *ure2* mutations. Ure2 has also been implicated in cellular responses to heavy metals and oxidative stress that are independent of its transcriptional regulatory role²⁹, so the switch to the [*URE3*] form could affect additional cellular phenotypes.

Prion-based gain-of-function phenotypes in fungi

The [*PSI*⁺] and [*URE3*] prions establish heritable loss-of-function phenotypes by inactivating their protein determinants, but there are also several examples of prion-mediated phenotypes in fungi linked with an apparent gain of function, including the well-studied [Het-s] prion of *P. anserina* and [*ISP*⁺] in *S. cerevisiae*³⁰. [Het-s] was first identified as a non-Mendelian genetic element that controlled **vegetative incompatibility**.³¹ When a

strain of the fungus carrying the [Het-s] prion meets a strain that expresses an allele of the *het-s* gene (*het-S*) that encodes a non-prion-forming form of the protein (called HET-S), the mixed **heterokaryon** formed on cell fusion dies (Figure 2b).³² This cell death blocks horizontal transmission of the prion and may represent a form of innate immunity.³³ Although the mechanism of cell death remains unclear, this event is only triggered if the [Het-s] prion is present in one of the two strains; a cross between a *het-s* strain expressing the non-prion soluble form of the protein ([Het-s*]) or a Δ *hets* strain is compatible.^{31, 32} Intriguingly, the incompatibility is dependent on the ability of Het-S to adopt a prion conformation in the presence of the [Het-s] prion, suggesting that these mixed complexes are either themselves the toxic form or indirectly induce toxicity upon co-aggregation.³⁴

An apparent gain-of-function prion phenotype is also linked to Rnq1 (rich in N and Q) of *S. cerevisiae*. Although the cellular function of Rnq1 remains unknown, the aggregated prion form of this protein (originally designated [RNQ⁺])³⁵ supports the *de novo* formation of the [PSI⁺] prion (Figure 2c).^{36, 37} On the basis of this phenotype, [RNQ⁺] is typically referred to as [PIN⁺] for [PSI⁺] inducibility and is the only prion reported to exist in non-laboratory strains of *S. cerevisiae* to date.^{38, 39} Exactly why the prion form of Rnq1 but not its soluble form can facilitate **de novo prion formation** remains an open question. However, *in vitro* studies have shown that preformed aggregates of Rnq1 can initiate the assembly of Sup35 prion polymers by a direct cross-seeding mechanism,⁴⁰ although [PIN⁺] is not required for the continued propagation of [PSI⁺] or any other prion *in vivo*.⁴¹ Remarkably, [URE3] can provide [PIN⁺] activity in the absence of Rnq1,³⁶ indicating that the same prion form can simultaneously contribute both loss-of-function and gain-of-function phenotypes.

Are fungal prions of benefit to the host?

While the prion mechanism provides a means to profoundly alter protein function and cellular phenotype in fungi, the question remains: does this process bring benefit or harm to the organism? Given the range of possible biological functions and phenotypes associated with fungal prions, the answer is likely to be equally complex. Two fungal prions, [PIN⁺]³⁸ and [Het-s]⁴² have been isolated in wild strains suggesting that their presence may be beneficial, but two others, [URE3] and [PSI⁺], have not, raising the possibility that they are deleterious.^{38, 39} While the adverse effects of [URE3] and [PSI⁺] on yeast growth under some conditions supports this view;^{29, 43} the failure to detect these prions in natural isolates may not be a sufficient criterion to discount a potentially beneficial role for these phenotypic states. In the case of [PSI⁺], Sup35 homologues in *Saccharomyces* species have retained the ability to adopt a prion conformation in a laboratory setting,¹⁴ and under these conditions, the [PSI⁺] state is beneficial in the short term (i.e. in response to chemical or physical threats)^{15, 17, 21} and perhaps in the long-term, through adaptation and ultimately evolution of new genetic traits.^{16, 17, 44, 45} A more broad and systematic survey of prion determinants in natural isolates and in diverse niches will undoubtedly shed light on the costs and benefits of these protein conformational and phenotypic switches for fungi.

Prion-associated phenotypes in mammals

While fungal prion proteins adopt self-replicating conformers that are largely benign to the host under standard laboratory conditions, the appearance of similar conformers in mammals is most typically associated with emergence of disease. Despite this clearly disadvantageous phenotype, mounting evidence suggests that prion conversions in mammals, like their fungal counterparts, are a mechanism for regulating protein function.

Gain-of-function amyloids

In addition to prions, a broader group of endogenous mammalian proteins can undergo a prion-like self-replicating change in conformation, leading to the build-up of amyloid deposits *in vivo*. These amyloidoses can be distinguished from the prion diseases by their lack of infectivity. However, recent studies have uncovered a prion-like behaviour for some of these proteins within single organisms, where the amyloid form can be propagated from one cell to a neighboring cell by exogenous transfer of these aggregates.⁴⁶ Among these proteins are the amyloids associated with Alzheimer's disease (amyloid- β),⁴⁷ Huntington's disease (polyglutamine)⁴⁸ and Parkinson's disease (α -synuclein).⁴⁹ Because these prion-like amyloids lack a complete infectious cycle, they have been referred to as '**prionoids**'.⁵⁰

How do these diseases relate to the normal function of the protein? The emerging consensus suggests that the toxicity associated with the amyloidoses represents a newly acquired activity linked to the alternative conformation.⁵¹ This hypothesis is consistent with our current understanding of the gain-of-function phenotypes of some fungal prions and the identification of proteins in bacteria and an invertebrate, for which the "normal" biological activity is associated with the amyloid form.^{52, 53} Recently, this concept of "functional" amyloid has been extended to vertebrates. For example, amyloid formation by a proteolytic fragment of the transmembrane glycoprotein Pmel17 is required for melanosome maturation and stimulates the synthesis of melanin,^{54, 55} and peptide and protein prohormones are stored in an amyloid form in pituitary secretory granules.⁵⁶ In the case of the prohormones, amyloid formation is reversible,⁵⁶ as is amyloid formation by an SH3 domain *in vitro*.⁵⁷ Therefore, the amyloid form can, in principle, act as a mechanism to regulate protein function in mammals.

PrP as a disease-causing agent in vertebrates

In the case of the TSEs, a clear and irrefutable role for PrP in the appearance, phenotypic manifestation and spread of disease has been established, primarily through studies in transgenic mice. PrP is the major constituent of biochemically enriched preparations of the TSE agent,² and mice devoid of PrP cannot replicate the infectious agent and are resistant to TSEs upon challenge.⁵⁸ In addition, strong evidence supports a direct role for PrP in clinical disease. Depletion of PrP post-infection extends incubation times and reverses both neuropathology and behavioural defects induced by the infection.^{59, 60} These observations are consistent with a functional role for PrP in TSE aetiology. But, important questions remain: what is the molecular basis of TSE-associated neurotoxicity, and how does it relate to the normal function of PrP?

In fungi, the classification of prion-associated phenotypes as gain of function or loss of function is defined in relation to the null phenotype; however, a similar comparison for PrP and the TSEs has proven more complex. Early attempts to identify a function for PrP were hampered by the absence of overt phenotypic defects in PrP-null mice and of conserved structural or sequence motifs.⁵⁸ Despite these initial setbacks, subsequent studies have shown that PrP-null mice differ from their wildtype counterparts in many activities, including circadian cycles, neuroprotection, synaptic function, lymphocyte activation, cell adhesion, stem cell renewal and proliferation, and olfaction,^{58, 61-63} and two recent studies have uncovered new roles for PrP. During zebrafish development, knockdown of either of the duplicated PrP genes induces loss of cell adhesion and altered localization of E-cadherin and Fyn Tyr kinase, phenotypes that are suppressed by expression of murine PrP.⁶⁴ In adult mice, regulated proteolysis and expression of PrP on the neuronal cell surface is required for maintenance of myelination through a non-cell autonomous route.⁶⁵ Together, these studies may suggest that PrP has a pleiotropic role *in vivo*, perhaps mediating its broad effects through an activity in cell signalling pathways.⁶⁶

Can TSE pathogenesis be linked to a loss-of-function or a gain-of-function phenotype for PrP? Our current state of knowledge suggests that the answer to that question lies somewhere in-between the two possibilities. While the absence of neurodegeneration in PrP-null mice was originally considered to be incompatible with a loss-of-function model for prion diseases, subsequent studies have shown that expression of some PrP fragments induces spontaneous neurodegeneration in PrP-null mice.^{58, 67, 68} Based on these observations, PrP toxicity during TSE infection could be explained as a loss of some PrP functions but not others.⁶⁶ Consistent with this idea, PrP must be expressed on the surface of neurons to mediate TSE pathogenesis following infection,⁶⁹⁻⁷¹ suggesting that normal localization of the protein is required to elicit neurotoxicity. However, a gain-of-function model for the TSEs, as has been suggested for the amyloidoses, cannot be ruled out at this point. In this model, conformation conversion of PrP to the prion state would be predicted to be neurotoxic to the host. The putative neurotoxic species was originally proposed to be PrP^{Sc}, which accumulates during the terminal stages of disease and in purified preparation of the infectious agent;² however, the presence of PrP^{Sc} correlates poorly with clinical disease.^{72, 73} Indeed, mice that are heterozygous for a PrP disruption progress to the terminal stage of disease more slowly than their wildtype counterparts despite the accumulation of similar levels of PrP^{Sc}.⁷⁴ Thus, any gain-of-function model for the role of PrP in the TSEs must identify a novel and biologically active species of this protein.

Conformational self-replication pathway

Although the exact mechanism by which misfolding of PrP alters the function of the protein to mediate TSE pathogenesis remains an open question, mounting evidence suggests that the process of conformational self-replication itself provides a robust framework for understanding the molecular basis of prion phenotypes. The prion hypothesis originally predicted that PrP^{Sc} catalyzed the conversion of the PrP^C in the context of a heterodimer of the two forms.^{1, 3} However, subsequent studies have revealed that soluble PrP^C binds to an oligomer of PrP^{Sc}, which stimulates remodelling upon incorporation into these complexes.⁵ Such a mechanism will progressively increase the size of prion complexes; however, without evoking a mechanism for generating new templates, this seeded polymerization process cannot account for the exponential increase in infectious titre observed over the course of disease in mammals or for the mitotic stability of prion propagation in yeast. What has emerged from mathematical models of prion propagation in both mammals and yeast is that, in addition to the seeded polymerization step, there must also be on-going polymer fragmentation to generate new templates (**'propagons'**; Figure 3).⁷⁵ This prediction is now well-supported by experimental studies with yeast prions, in which the fragmentation of prion polymers is catalysed by the molecular chaperone Hsp104 in conjunction with co-chaperones such as Hsp40 and Hsp70.⁷⁶ In mammalian cells no orthologue of Hsp104 has been described, and it remains to be established how prion complexes are fragmented.⁷⁷

Unlike a simple heterodimeric conversion mechanism, a multi-step pathway of conformational self-replication allows for the formation of intermediate states that could alter prion protein function and thereby explain some aspects of prion biology. For example, although the accumulation of PrP^{Sc} is inconsistent with a direct role for this conformer in TSE pathogenesis, TSE incubation times correlate with PrP expression level.^{74, 78, 79} How might PrP expression induce disease independently of PrP^{Sc} formation? One possibility is that a transient intermediate on the pathway to PrP^{Sc} formation mediates toxicity, a concept that may also explain pathogenesis in other non-transmissible neurodegenerative diseases^{5, 51, 80} and the stability of prion phenotypes in fungi.⁸¹ According to this idea, the rate of replication of the infectious PrP agent strongly influences the rate of formation of the transient toxic species, with efficient replication, such as that observed in the presence of

increased PrP expression, allowing the accumulation of the intermediate form to levels sufficient to cause clinical disease.

According to this dynamic model of prion phenotypes, any condition that alters the efficiency of conformational self-replication has the potential to impact prion-associated phenotypes by modulating the proportion of the protein that is found in state that determines the phenotype. Such fine-tuning, in turn, allows an expansion of the range of phenotypes that may be conferred by a single protein without an underlying change in the genetic make-up of the host. Intriguingly, this framework provides a molecular explanation for many enigmatic aspects of prion biology.

Prion strains and evolution of their phenotypes

Prion proteins in both mammals and yeast can adopt a range of self-replicating conformers known as prion strains, which are thought to confer distinct phenotypes by assembling into aggregates with different physical properties.^{5, 82, 83} These differences are believed to specify unique rates of conversion of the soluble protein to the prion state and of fragmentation of prion complexes to generate new propagons, and thereby the efficiency of conformational self-replication, providing a molecular basis for the phenotypes.⁸³

However, multiple prion strains may arise in individual mammals^{84, 85} through co-infection^{86, 87} or **strain “mutation”/adaptation**, where prion-associated phenotypes “evolve” in response to new conditions, such as the presence of compounds that interfere with prion propagation *in vivo* or to transfer to a new host.^{10, 88-92} The existence of mixtures of prion strains *in vivo* raises the possibility that prion phenotypes may not simply reflect the physical properties of a single conformer but rather the collective and dynamic behaviour of the various forms present. Indeed, prion strains have markedly different disease characteristics alone than when present in a mixture, including changes in incubation periods,^{84, 93} the efficacy of inoculation routes⁹⁴ and transmission rates to other species.^{84, 85, 93} Likewise, prion disease pathology, duration and clinical symptoms in humans are altered by the coexistence of prion strains.^{95, 96} Thus, the interplay between prion strains directly affects prion phenotypes.

How do prion strains interact to produce observable phenotypes? As prion strains can only stably persist if their rates of replication counteract processes that lead to their decline, such as degradation in mammals or dilution through cell division in yeast,^{75, 83} the relative efficiencies of conformational self-replication for the interacting strains seems to be crucial in establishing the phenotype of prion mixtures. In mammals, co-inoculation with multiple prion strains that replicate at different rates almost invariably results in selection of the faster replicating strain,^{97, 98} but when two different prion strains are introduced at different times (known as **superinfection**), the outcome is influenced by the interval between inoculations, the inoculum dosage and the routes of inoculation (see below).^{99, 100} Conditions that allow a slower replicating prion strain to establish an infection before the introduction of a faster replicating prion strain through the same route either delay or completely block superinfection. These experiments suggest that prion strains compete for a limiting host component necessary for establishing a prion infection,^{101, 102} and the competition between existing strains may similarly be affected by the efficiency with which each conformer is replicated in a given tissue.⁹⁴ Considering the process of conformational self-replication (Figure 3), this component is likely to be the non-prion state protein,¹⁰² a suggestion that is supported by studies in yeast showing that the prion strains that most efficiently incorporate non-prion state protein are phenotypically dominant (Figure 4A).^{83, 87, 103}

Prion resistance and attenuation

The physical interaction between prion proteins is an essential event in conformational self-replication. Thus, it is perhaps not surprising that variations in the amino acid sequence of prion proteins affect their phenotypes in both mammals and yeast. Naturally occurring PrP polymorphisms in animals and man alter TSE characteristics and, in extreme cases, the susceptibility to prion disease.¹⁰⁴ Emerging evidence suggests that sequence variants exert their effects by altering the efficiency of conformational self-replication, but they do so by targeting different steps in the process (Figure 4B).

The most well-characterized sequence variant of human PrP is the Met/Val 129 polymorphism.^{105, 106} Although both PrP129 homozygotes (Met/Met or Val/Val) and heterozygotes are susceptible to prion disease, the genotype at this position affects the pattern of PrP^{Sc} accumulation in the brain and the clinical symptoms, incubation period and duration of disease.¹⁰⁴ Disease progression is always more rapid in homozygotes than in heterozygotes, providing a potential molecular explanation for the over-representation of homozygotes in some TSEs.¹⁰⁴ The Met/Val129 polymorphism has no effect on the structure or stability of native PrP,¹⁰⁷⁻¹⁰⁹ but it does affect conformational self-replication *in vitro*. Under certain conditions, mixture of Met129 and Val129 PrPs (as would occur in heterozygotes) slows the rate of amyloid growth^{110, 111} and favours the formation of an oligomeric intermediate that cannot be directly converted to the amyloid state.^{110, 112, 113} Together, these observations suggest that mixtures of Met129 and Val129 variants may alter disease progression by limiting the rate of conversion of PrP to the prion form.

Heterozygous interference appears to be a general phenomenon, as both animals and humans heterozygous for other PrP alleles show a similar **overdominance**.¹¹⁴⁻¹¹⁹ Perhaps the most intriguing of these polymorphisms are Glu/Lys 219 in humans and Gln/Arg 171 in sheep, which confer resistance to TSEs.^{104, 118, 120, 121} The protective effects of these variants correlate with their ability to dominantly inhibit conformational self-replication by the other PrP alleles.^{122, 123} Notably, this dominant inhibition occurs independently of *trans*-factors, suggesting an incompatibility in the interactions between these allelic variants.^{124, 125} Although the mechanisms by which PrP sequence variants inhibit conformational self-replication are currently unclear, the alleles can be distinguished by the ratios, relative to wildtype protein, at which they become effective inhibitors.^{123, 124} According to a mathematical model, these differences in effective inhibitory concentrations may reflect a targeting of distinct events in conformational self-replication. For example, inhibitors that theoretically act by binding to the ends of linear prion complexes and blocking PrP conversion are predicted to be effective at lower concentrations than inhibitors that would interfere with other aspects of conformational self-replication such as fragmentation, which would require binding along the length of the aggregate (Figure 4B).¹²⁶

How changes in replication rates translate into altered phenotypic states requires an assessment of their effects within the context of a living organism, and the yeast prion models provide avenues to further explore these questions. Dominant inhibitory mutations have been isolated in the fungal prions and, as is the case for PrP polymorphisms, these mutations interfere with conformational self-replication to varying extents, creating a range of protein-based traits.^{127, 128} For the Sup35/[*PSI*⁺] prion, some substitutions, known as [*PSI*⁺]-no-more (*PNM*) mutations, induce prion loss, and others, known as antisuppressor (*ASU*) mutations, modestly decrease the efficiency of conformational self-replication, preserving the prion form but allowing the accumulation of soluble and functional Sup35, which reverses the prion phenotype.¹²⁷ The most extensively studied *PNM* mutant is *PNM2*, which encodes a Sup35 Gly58Asp mutant.^{128, 129} The Sup35 Gly58Asp mutant incorporates into wild type Sup35 prion complexes and can even support prion propagation on its own.^{87, 130-132} However, *PNM2* induces prion loss over many generations,^{129, 132} suggesting that

this variant interferes with the replication of prion complexes and/or their transmission to daughter cells, ideas that can be directly tested through an assessment of prion protein dynamics *in vivo*.

Species barrier

In animal models of prion infectivity, a barrier to interspecies transmission has long been appreciated^{133, 134}, but renewed interest in this phenomenon has arisen with the realization that variant CJD appeared in the human population following transfer of BSE from cattle.^{135, 136} Mechanistic studies now suggest that species barriers are the outcomes of interactions between prion strains and sequence variants of PrP, which reflect the rate of conformational self-replication in the recipient.⁸⁰

Species barriers may manifest as a complete block of disease development^{84, 137} or, alternatively, as a prolonged incubation period following first passage that is shortened on subsequent passage within the same species.^{86, 133, 134} In both cases, replication of the infectious species occurs during the asymptomatic phase,^{138, 139} but the toxic species apparently does not reach the threshold concentration required for clinical disease within the organism's natural lifespan.¹⁴⁰ This alteration to the efficiency of conformational self-replication has been linked to differences in the PrP sequences of the donor and recipient. For example, the species barriers between hamster or mink and mice are abolished by heterologous expression of the donor PrP in mice,^{84, 141} and, strikingly, this effect has been linked to identity at two PrP residues: 170 and 174.¹⁴² What is the molecular basis of this requirement for sequence compatibility? Prion protein sequence likely contributes to species barriers in multiple ways. First, it determines the efficiency of interaction between the donor and recipient proteins. Second, it defines the range of conformations that a prion protein may adopt,^{5, 143} and third, it determines the stability with which a given conformer is replicated.⁹² Indeed, the identity of residues at PrP positions 170 and 174 define the structure of a loop that can form an intermolecular interface common to amyloidogenic proteins,¹⁴⁴ and variations at position 226 correlate with the stability of PrP strains.⁹² Thus, interspecies transmissibility may be operationally defined by the ability of the recipient protein to efficiently replicate the conformation imposed by the template.

Perhaps the strongest evidence to support the idea that the efficiency of conformational replication creates the barrier to interspecies prion transmission is the observation of asymmetry in prion conversions. For example, mouse PrP can seed the formation of amyloid by Syrian hamster PrP *in vitro* but not vice versa.¹⁴⁵ A comparable asymmetry has been observed between Sup35 homologues from the yeasts *Kluyveromyces lactis*, *Saccharomyces paradoxus*, or *Saccharomyces bayanus* and *S. cerevisiae*^{14, 146, 147}, between Ure2 homologues from *S. bayanus* and *S. cerevisiae*,¹⁴⁸ and between fragments of Rnq1.¹⁴⁹ Although these reciprocal cross-seeding reactions involve the same two proteins, they differ in which protein is present in the prion form and, therefore, serves as the template for conformational conversion. Consistent with this model, there is a close correlation between seeding and structure: PrP variants, which can seed one another, spontaneously form amyloid fibres of similar secondary and quaternary structure *in vitro*,¹⁵⁰ and PrP and Sup35 molecules adopt different structures depending on the template provided.^{150, 151}

The concept of a conformational replication barrier to interspecies transmission also explains an early observation that such transitions are often accompanied by a change in prion strain.^{152, 153} The emergence of a new strain could reflect the selection of a compatible strain from a mixture present in the initial inoculum (Figure 4C)⁸⁶ or, alternately, a conversion of one conformer to another. Indeed, biological clones of prion strains undergo adaptation and selection following transfer to a new species *in vivo* and *in vitro*^{98, 154} (Figure 4C). Studies *in vitro* suggest that these transitions may proceed through

mixed complexes with heterogeneous structures, reflecting only a partial compatibility between the accessible conformations of the two proteins.¹⁵⁵ Such mixed complexes could alter the physical properties of prion aggregates and thereby the efficiency of replication in a manner analogous to that described above for dominant inhibitory mutants. Indeed, interspecies transmissions that lead to the emergence of new strains tend to require more passages to stabilize the length of the incubation period in the new host than those that retain their strain identities.^{98, 153, 154}

Conclusions

The prion hypothesis first emerged as a radical proposal to explain the transmission of a brain disease in animals. The possibility that by simply undergoing a change in its tertiary conformation, the PrP protein could switch from a benign to a self-replicating pathogenic form that induced a long-term and progressive neurodegeneration, was nothing short of heretical. Almost 40 years on, the hypothesis has turned to accepted dogma, but a wealth of recent studies now suggest that the prion concept is entering a new realm of even greater relevance, in which this process is no longer considered simply as an explanation for an unusual and invariably fatal disease. The realisation that there are a number of prions in fungi that can radically change the phenotype of the 'infected host' necessitates a rethinking of the role of prions. We would suggest that prions have evolved as epigenetic regulators of phenotype rather than as disease-causing agents. Thus, paralleling the underlying process of conformational self-replication, the prion mechanism provides a robust yet dynamic system to modulate protein function and thereby cellular phenotypes. The plasticity of this pathway, its epigenetic nature, and its potential to create a continuum of related phenotypes highlight its utility as a common regulatory process that we are only now beginning to appreciate. To date the impact of prions on species other than fungi has been restricted to the role of PrP^{Sc} in mammalian brain degeneration and death. However, the possibility that synaptic activity in sensory neurons in the brain may be regulated via a prion-like mechanism^{53, 156} provide the first clue that prion-mediated control of complex cellular processes might also exist in higher eukaryotes.

Box 1

Fungal prions, cytoplasmic inheritance and epigenetic regulation

In a genetic cross between haploid [*PRION*⁺] and [*prion*⁻] strains of *Saccharomyces cerevisiae*, typically the resulting diploid is [*PRION*⁺], and all meiotic progeny also carry the prion determinant.⁴ If the associated phenotype was controlled by a loss-of-function nuclear gene mutation then typically it would be recessive in a diploid, and a 2 [*PRION*⁺]: 2 [*prion*⁻] segregation pattern would be evident amongst the meiotic progeny. This non-Mendelian mode of inheritance shown by prions is indicative of transfer through the cytoplasm, which can also occur in other epigenetic systems, for example, mitochondrial *petite* mutations.¹⁵⁷ Inheritance of the [*PRION*⁺] determinant generally results in the establishment of a new stable genetic state that can be maintained and propagated over many generations. In most cases inheritance of the [*PRION*⁺] determinant will result in a change of phenotype when compared with the [*prion*⁻] cell (Table 1) that is not accompanied by a change in the nucleotide sequence of the prion protein-encoding gene, or for that matter, any other nuclear gene. Consequently, prions can rightly be viewed as epigenetic determinants that can affect cellular processes (see Table 1). Prion-based epigenetic systems may have evolved because they can rapidly modify a cellular phenotype in response to a changing environment without introducing a change in the sequence and function of the genome.

Box 2**Can you identify a prion protein from its amino acid sequence?**

Although prions that affect the host phenotype or cause disease have only been identified and verified in fungi and mammals, it is unlikely they are restricted to these two groups of organisms. One important question that we are now getting near to answering is whether we can identify a prion protein from its sequence. Soon after their discovery in fungi it emerged that there were some sequence features shared by the various yeast prion proteins particularly the presence of a region of the protein (the prion-forming domain; PrD) that contained an atypically high density of Gln and Asn residues and that was conformationally flexible.^{8, 9} A high density of Gln residues in a protein makes it prone to aggregate, but it is the high density of Asn residues that is crucial for prion propagation.⁹ Remarkably, it is the amino acid composition rather than its sequence that is crucial in determining prion potential.¹⁵⁸ In addition to Gln-Asn content, some prion-forming proteins, such as Sup35 and to a lesser extent Rnq1, have short oligopeptide repeats with sequence similarity to a series of octapeptide repeats found in PrP, suggesting that such sequences may be important for prion propagation.¹⁴³ One approach has identified 200 proteins in *S. cerevisiae* with this architecture, although many of these do not satisfy the genetic and/or biochemical criteria to be designated as prions.⁹ The fact that PrD of the *Podospora anserina* HET-s prion protein lacks both Asn and Gln residues, but can be efficiently propagated as the [Het-s] prion in either *P. anserina*³² or *S. cerevisiae*¹⁵⁹ suggests that alternative mechanisms must exist for propagating fungal prions.

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GLOSSARY

Conformer	One of a number of different stable tertiary structures that can be taken up by a single polypeptide chain
De novo prion formation	The establishment of the prion form of a protein without benefit of acquired prion seeds or propagons or mutation i.e. spontaneous appearance of the prion form
Epigenetic	A change in inherited phenotype resulting from a molecular event other than a change in the DNA sequence of the organism's genome e.g. DNA methylation chromatin remodelling
Heterokaryon	Coexistence of two or more genetically different nuclei in a common cytoplasm
Overdominance	A heterozygous phenotype that is outside of the range of phenotypes for either homozygous state
Prion	A structurally altered form of a protein that can impose that form on a previously correctly folded form of the same protein and

	hence propagate the prion form in either heredity (yeast prions) or infectious (mammalian PrP) manner
Prionoid	Proteins that show prion-like conformational self-replication activity but that lack infectivity
Propagon	Conformation-replicating physical entities that are transmitted from mother to daughter cells and are required to maintain the [PRION ⁺] phenotype. Seeds is commonly used as a synonym
Protein conformation	The stable, native three-dimensional shape taken up by a polypeptide chain that determines the alternative functions of the protein
+1/−1 ribosomal frameshift	A net shift in the translation of an open reading frame by one base either in the 3' direction (+1) or 5' direction (−1) directed by a specific signal in the mRNA sequence
Strain “mutation” or adaptation	The conversion of one prion conformer to another, either spontaneously or in response to a new environment
Superinfection	Sequential but temporally spaced inoculations of a host with more than one strain of an infectious agent
tRNA-mediated nonsense suppression	The translation of a termination codon by a transfer RNA with a mutation in its anticodon. This can result in partial restoration of function to the encoded protein when the codon is located within the ORF i.e. a nonsense mutation
Vegetative incompatibility	Death of a multinucleate heterokaryon arising from the fusion of two different strains of a fungus thereby preventing the transfer of cytoplasmic components from one strain to another

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Figure 1. Cellular consequences of termination codon readthrough and +1 ribosomal frameshifting mediated by the $[PSI^+]$ prion

The $[PSI^+]$ prion compromises the function of the translation termination release factor Sup35, leading to the readthrough of stop codons or translational frameshifting. This epigenetic loss of Sup35 function creates new phenotypes based on the altered translation of specific mRNA transcripts.

a. *OAZ1* in yeast consists of two overlapping open reading frames (ORF1 and YPL052w), with the latter in the +1 frame with respect to ORF1.²⁰ During translation the ribosomes can either terminate or shift into the +1 frame at the ORF1 UGA codon, thus by-passing the stop codon and continuing translation into the YPL052w ORF. This +1 frameshift increases as polyamine levels rise. The resulting fused protein, antizyme, regulates the ubiquitin-dependent degradation of ornithine decarboxylase (ODC), which mediates the synthesis of the polyamines spermidine, spermine and putrescine. In $[PSI^+]$ cells there is a significant reduction in the levels of functional release factor, leading to an increase in the pause time at the UGA codon. This in turn leads to increased levels of +1 frameshifting and hence antizyme synthesis, resulting in a reduction in ODC and polyamine synthesis when compared with a $[psi^-]$ cell.²¹

b. As a component of the cAMP-dependent protein kinase signalling system in yeast, Pde2p controls the basal levels of cAMP by hydrolysing cAMP to AMP. Readthrough of the native UGA codon gives rise to a form of Pde2p (Pde2Lp) that has no phosphodiesterase activity. Consequently $[PSI^+]$ cells have approximately two-fold higher levels of cAMP than $[PSI^-]$ cells.²²

Figure 2. Fungal prions and their associated phenotypes

Prion and non-prion conformers confer unique phenotypes by altering the normal activity of the prion protein. Several transcriptional regulators in yeast have been identified as prions. The *[URE3]* prion alters the capacity of Ure2 to associate with the transcriptional activators Gln3 and Gat1 in the cytoplasm (left), which occurs in the non-prion *[ure-o]* state, allowing these activators to translocate into the nucleus (centre), where they up-regulate genes that allow *[URE3]* cells to use the poor nitrogen source ureidosuccinate (USA) in the presence of ammonia (right). Figure kindly provided by C. Cullin (Institut de Biochimie Génétique Cellulaires, Bordeaux). The *[OCT⁺]* prion compromises the ability of the Cyc8 transcriptional repressor to associate with its co-repressor Tup1 (oval, left), leading to the upregulation of many genes, including *CYC7* (centre), which allows the use of lactate in *cyc1Δ* strains (right). Figure kindly provided by S. Liebman (University of Illinois-Chicago).

b. The *[Het-s]* prion allows the Het-s protein to acquire a new activity to induce cell death (compare left and centre) following mating with a *P. anserina* strain that expresses the Het-S allele, leading to a block in heterokaryon formation, which leads to a barrier band of cell death (arrow, right). Figure kindly provided by S. Saupe (Institut de Biochimie Génétique Cellulaires, Bordeaux).

c. The *[PIN⁺]* (also known as *[RNQ⁺]*) prion allows Rnq1p to support the *de novo* formation of the *[PSI⁺]* prion especially when the levels of Sup35 protein are artificially elevated (center and right).

Figure 3. How prions are propagated in the cell

Conformationally flexible prion proteins are converted from their normal non-prion form to the self-replicating prion form by associating with existing oligomeric complexes of the same protein in the prion form. These extended polymers may then be dissociated into smaller units (polymer fragmentation) either enzymatically in yeast or mechanically in mammals to produce transmissible oligomers (propagons). This process of conformational self-replication is limited in efficiency by the degradation of normal conformers or of dissociated prion monomers and by the formation of large, non-transmissible aggregates ($[Agg^+]$). Changes in the rates of these dynamic transitions create a range of protein-based phenotypes from a single prion protein.

Figure 4. Prion mixtures, strain competition and the species barrier

a. Mixtures of prion strains in the same cell compete for non-prion state protein. Differences in the rate of fragmentation or conversion allow strains to establish phenotypic dominance by increasing their steady-state concentrations. In extreme cases, this competition leads to loss of one strain.

b. Sequence variants of prion proteins dominantly inhibit conformational replication of other alleles by incorporating into prion complexes and inhibiting further conformational conversion or fragmentation. Variants interfering with conversion exert their effects at substoichiometric levels as they function at the site of conversion, whereas variants interfering with prion aggregate dynamics exert their effects at stoichiometric levels since they must incorporate throughout the complex.

c. Incompatibility between donor and recipient prion proteins creates a barrier to interspecies transmission. This barrier may reflect an inability of the proteins to associate (not shown) or alternately to adopt compatible conformations. For strains capable of traversing a species barrier, the recipient prion protein may adopt an identical conformation (selection), leading to the conservation of strain identity on transfer, or, alternately, may adopt a partially compatible conformation that leads to a change in strain (adaptation).

Table 1

Prions in fungi

Determinant	Protein	Cellular function	Phenotype	Ref
[<i>URE3</i>]	Ure2	Transcriptional co-repressor	Nitrogen source utilisation	7
[<i>PSI⁺</i>]	Sup35	Translation termination factor (eRF3)	Nonsense suppression Resistance to toxic compounds	12, 13
[<i>PIN⁺</i>]/[<i>RNQ⁺</i>] [*]	Rnq1	Unknown	<i>De novo</i> prion formation	35
[<i>SWI⁺</i>]	Swi1	Subunit of the SWI/SNF chromatin remodelling complex	Repression of transcription	23
[<i>OCT⁺</i>]	Cyc8	Transcriptional co-repressor	Derepression of transcription	24
[<i>MOT3⁺</i>]	Mot3	Transcriptional co-repressor	Derepression of transcription	9
[<i>MCA⁺</i>]	Mca1	Metacaspase, regulates apoptosis	None reported	6
[Het-s] ^{**}	HET-s	Unknown	Vegetative incompatibility Cell death	32
[<i>ISP⁺</i>]	Sfp1	Transcriptional activator	Translational accuracy	30

^{*} The prion form of Rnq1 was originally designated as [*RNQ⁺*]. Subsequently, Rnq1 was identified as the prion protein that can induce de novo [*PSI⁺*] formation and was dubbed [*PIN⁺*] ([*PSI⁺*] inducibility). Although other Gln-Asn-rich proteins can act as [*PIN⁺*] prions and induce de novo [*PSI⁺*] formation, it is generally assumed that [*PIN⁺*] is the same as [*RNQ⁺*].

^{**} All prions bar [Het-s] are found in *Saccharomyces cerevisiae*. [Het-s] is found in *Podospora anserina*.