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ABSTRACT Prions are infectious protein polymers that have been found to cause fatal diseases in mammals. Prions have also been identified in fungi (yeast and filamentous fungi), where they behave as cytoplasmic non-Mendelian genetic elements. Fungal prions correspond in most cases to fibrillary β-sheet-rich protein aggregates termed amyloids. Fungal prion models and, in particular, yeast prions were instrumental in the description of fundamental aspects of prion structure and propagation. These models established the "protein-only" nature of prions, the physical basis of strain variation, and the role of a variety of chaperones in prion propagation and amyloid aggregate handling. Yeast and fungal prions do not necessarily correspond to harmful entities but can have adaptive roles in these organisms.

INTRODUCTION

In 1994, a paper signed by a single author based on genetic approaches opened a decisive breach leading to a dramatic expansion of our perception of the biological significance of the prion phenomenon (1). In the following years, biochemical reconstitution and transformation established that these biological entities, originally identified and defined in the context of mammalian diseases such as Kuru or Creutzfeldt-Jacob disease, also exist in yeast as "protein-based genes" and correspond to previously described non-Mendelian genetic elements (2, 3). It is now clear that in most known cases the physical basis for prion propagation is the formation, growth, and fragmentation of an amyloid aggregate. Amyloids are ordered protein polymers with a so-called cross-β structure (4). The original definition of prions as "infectious proteinaceous particles" is imprecise enough to still be operational today but as a consequence embraces a variety of biological phenomena and structural features (5). Defining prions thus remains a nontrivial task. While a more restrictive definition is perhaps neither possible nor desirable, this general term induces some confusion and controversy. In an attempt to clarify discourse, at some point investigators in the mammalian disease-related field denied the fungal "infectious proteinaceous particles" the name of prions and proposed instead to term them "prionoids" (6). These semantic battles should not be disregarded as sterile, but rather should be taken as an indication of the variety of the biological realities that the term covers.

Prions are not necessarily harmful entities, nor do they necessarily correspond to amyloid structures (7). By some definitions they do not need to correspond to propagation of a specific conformational state (8). The situation is further complicated by the description of so-called prion domains; prion-forming domains (PFDs) are protein domains that confer the ability to other proteins to behave as prions in an *ad hoc* experimental setting. However, this observation does not necessarily imply that the native protein from which the domain is isolated also behaves as a prion (9, 10). That said, this chapter provides a description of prions and prion domains in the fungal kingdom. Our minimal operational

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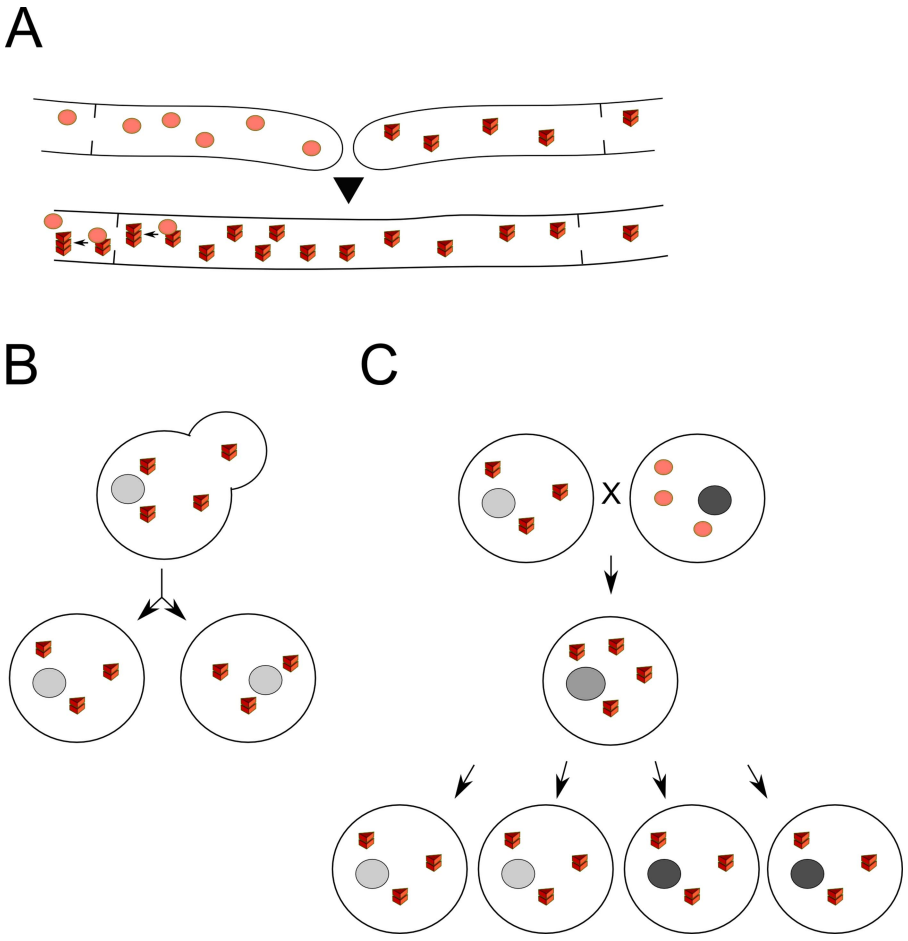
definition of a prion here will be a protein able to adopt a transmissible conformation, this conformation being transmitted vertically to mitotic (and often meiotic) progeny and horizontally between strains through cytoduction (in yeast) or somatic anastomosis (in filamentous fungi) (Fig. 1). We will more specifically focus on models for which the amyloid nature of the prion particle is known or can be suspected.

The study of prions in fungi is largely that of prions in the yeast *Saccharomyces cerevisiae*, but a prion and several PFDs have also been identified in filamentous fungi. Three historical prion models, [URE3], [*PSI*$^+$] in yeast, and [Het-s] in *Podospora anserina*, were identified and studied as non-Mendelian genetic elements before they were found to correspond to prions (11–13). [*PIN*$^+$], in turn, was identified in relation to prion curing experiments carried out on [*PSI*$^+$] strains (14). The list of fungal prions now amounts to about a dozen (15). The work on yeast prions dominates the literature in terms of sheer amount, diversity of models, and level of detail. In many aspects, yeast models and the [*PSI*$^+$] system have established the general paradigms and methods that are

FIGURE 1 Natural situations of prion propagation in fungi. **(A)** Prion propagation after hyphal anastomosis in a filamentous fungus (for instance, [Het-s] in *Podospora anserina*). The prion form is transmitted from a donor-infected strain (right) to a recipient strain (left). The prion form then converts the entire mycelium to the prion state due to cytoplasmic continuity throughout the thallus. Prion transmission also occurs in meiotic crosses with maternal inheritance (not depicted here). **(B)** Prion propagation during mitotic cell divisions in yeast. Prion seeds are transmitted from mother to daughter cells during budding. **(C)** Prion transmission during sexual crosses. In a cross between a [PRION$^+$] (left) and a [*prion*$^-$] strain (right), the resulting diploid is [PRION$^+$] and there is non-Mendelian segregation of the [PRION$^+$] character (often, but not always, with 4:0 segregation as in the example depicted here).



used in the field. Prions from filamentous fungi (that is, essentially the [Het-s] model), are considered by comparison to this paradigm, and in some aspects, but not all, they conform to it. Yeast prion systems are also intensively used to study the aggregation and toxicity and prion behavior of proteins or protein domains derived from other species. This approach has been used commonly, but not exclusively, for proteins relevant to human protein-deposition diseases (see references 16 and 17 for recent examples of this approach). This line of research will not be specifically discussed here.

PHYSIOLOGY OF PRION FORMATION AND PROPAGATION IN FUNGI

The initial discovery of prions in fungi, and immediate follow-up work, focused on cells being in one of two states: [*PRION*$^+$] and [*prion*$^-$]. Because their inheritance involves self-templating changes in protein conformation, [*PRION*$^+$]-based traits have very different patterns of inheritance in genetic crosses than DNA-based traits: their phenotypes are dominant (denoted by capital letters) and segregate to meiotic progeny in a non-Mendelian fashion (denoted by brackets). The characterization of [*PRION*$^+$] and [*prion*$^-$] states quickly moved beyond phenotypic assessment and epigenetic inheritance of the phenotype to focus on the aggregation of the prion protein itself. The aggregated state of the prion protein was followed by microscopy with fusion proteins and by sedimentation analyses that could discern soluble and insoluble, or higher molecular weight species (2, 18–20). Below we highlight specific examples that laid the foundation for this burgeoning field.

[*PSI*$^+$]: The Most Studied Fungal Prion

The prion form of the translation termination factor Sup35 (eRF3) results in inefficient termination and detectable nonsense suppression (21). Strains harboring a premature termination codon in either the *ADE1* or *ADE2* genes (the *ade1-14* or *ade2-1* alleles) are unable to synthesize adenine. The loss of either Ade1 or Ade2 proteins in an otherwise functional pathway results in the buildup of a metabolic intermediate that gives the colonies a red color. Read-through of the premature termination codon (nonsense suppression) and completion of the pathway restores the colony color to white when nonsense suppression is high or to pink when it is not as high. Thus, *ade1-14* or *ade2-1* colonies are red when cells are [*psi*$^-$] and Sup35 is soluble and translation termination is efficient. They are white when cells are [*PSI*$^+$] and Sup35 is in the prion state.

Prion Variants and Prion-Forming Domains

The sensitive phenotype associated with the [*PSI*$^+$] prion enabled the discovery of distinct [*PSI*$^+$] prion variants (22). Different heritable states of [*PSI*$^+$] were observed without any genetic change. That is, they arose from the same Sup35 protein sequence. These states were termed prion "variants" and are analogous to mammalian prion strains. These are thought to be different self-propagating structures of the mammalian prion protein PrP that are largely responsible for variation in pathologies in mammalian prion diseases (23). [*PSI*$^+$] variants are discernable phenotypically by stable and distinct colony colors that range between red and white. How white or pink the colony is correlates to how much Sup35 is in the nonfunctional prion state. This can also be assessed biochemically by the relative amount of insoluble Sup35 protein in [*PSI*$^+$] variant lysates (21, 24–26).

Prion variants are distinct self-propagating structures of a prion protein. They are derived from the same polypeptide sequence. Thus, the stable phenotypic differences are a consequence of these structural changes. Proof of that concept was provided by the generation of biophysically distinct amyloid structures *in vitro* with purified recombinant Sup35 protein, which was then used to infect [*psi*$^-$] yeast and generate phenotypically discernable [*PSI*$^+$] variants (27). Such prion variants are not unique to [*PSI*$^+$] and have been observed with [URE3] (28, 29) and [*PIN*$^+$/*RNQ*$^+$] (30–32) as well. In addition, PrP has been suggested to replicate as over 30 prion strains. Many (perhaps all) amyloidogenic proteins associated with human disease can form multiple self-replicating β-sheet-rich structures (33). In contrast to what has been described for several yeast prion models, no prion variants have been reported for the [Het-s] system. One may argue that the lack of a sensitive detection assay has prevented identification of such hypothetical variants. Yet the fact that HET-s prion fibrils are characterized by the absence of structural polymorphism further supports the notion that there are no [Het-s] variants (34).

Distinct amyloid structures formed by the same protein involve different primary structure elements. Thus, changes in protein sequence can have differential effects on the propagation of distinct prion variants. For example, mutation of an amino acid residue that is buried in the core (packed in the templating β-sheet structure) of one amyloid structure but on the outside of the core in another amyloid structure can prevent propagation of one structure but have no effect on another (21, 35–39).

Although some mutants can prevent propagation of a particular amyloid structure, the ability to establish a prion state appears to be more dependent on the overall amino acid composition of the PFD (40, 41). For most of the prion proteins identified (particularly those in *Saccharomyces*), the PFD is a long stretch of amino acids rich in glutamine and asparagine residues. These domains are typically devoid of predictable secondary structure and are thought to be intrinsically disordered. Proteins with intrinsically disordered domains are not uncommon and are generally thought to acquire a stable structure upon interaction with a cofactor or complex (42). Such domains may render the protein susceptible to aggregation, however. Indeed, a wide spectrum of human protein conformational disorders may be caused by the aggregation of proteins with intrinsically disordered domains. Many of these harbor glutamine- and asparagine-rich sequences. Initial structure/function work to investigate regions of Sup35 required for prion formation determined that the amino terminal PFD was not only necessary, but also sufficient for prion propagation (43). The prion-conferring activity was transferable to a different protein by simply fusing the PFD to it (43). This appeared to be true for other PFDs as well, such as the Ure2 (44) and Rnq1 PFDs (45).

Chaperones Modulate Prion Formation and Propagation

Yeast allowed the identification of Hsp104 as the first chaperone to alter prion propagation (46). In fact, the activity of Hsp104 is required for most prions to faithfully propagate and transmit from mother to daughter cells. Hsp104 works in conjunction with Hsp70 and Hsp40 cochaperones, primarily Ssa1/2 and Sis1. Hsp70 and Hsp40 work together to recognize misfolded or aggregated polypeptides and deliver them to Hsp104, which then unfolds substrates by ATP-dependent threading through a central pore in its hexameric structure (47–50). This system is required for the propagation of amyloid-based prions and is thought to generate smaller oligomeric amyloid seeds that not only increase the free ends for efficient propagation, but also more readily transit the mother-bud neck to maintain stable inheritance. The prion variant-specific chaperone activity requirements may stem from the ability of chaperones to recognize specific accessible sequence elements as well as the amount of energy needed to break up the amyloid structure to generate oligomeric seeds. While many of these effects are challenges that alter cellular chaperone balance in a specific, and possibly nonphysiologic, manner, one variant of the [*PSI*$^+$] prion can be cured by transient heat shock (51). In this case, prion propagation is altered by a global cellular reaction to stress that would mimic a naturally occurring chaperone response.

Prion-specific effects of chaperones have been reported as well. While Hsp104 has perhaps the most general effect on different yeast prions (though it does not affect all prions), its relationship to [*PSI*$^+$] is unique in that the overexpression of Hsp104 can cure cells of [*PSI*$^+$]. Most prions affected by Hsp104 are cured by its inhibition or deletion and are not affected by its overexpression. One plausible explanation for this relates to the necessity for activity of Sup35 for viability. *SUP35* cannot be deleted because some Sup35 activity is required for translation termination. This likely limits the array of [*PSI*$^+$] prion variants that can propagate and maintain cell growth. If Sup35 were to form a very efficiently propagating prion structure, cell viability would be compromised. Indeed, some data support this (52, 53). It is possible that the structural array of [*PSI*$^+$] variants and the requirement for a pool of soluble, functional Sup35 results in less flexibility in the amount of Hsp104 activity that can support [*PSI*$^+$] maintenance. There is some specificity in the Hsp70 involvement with Hsp104 in prion maintenance as well. Ssa1 is the Hsp70 required for the propagation of [*PSI*$^+$], while the nearly identical Ssa2 is required for the propagation of [*URE3*]. In addition, the Hsp70 nucleotide exchange factor Sse1 is required for the propagation of [*URE3*] but for only some variants of [*PSI*$^+$] (54, 55). Furthermore, some chaperones selectively impact the propagation of the [*URE3*] prion. The Hsp90/Cpr7 chaperone system is required for the propagation of [*URE3*] (56). Finally, Hsp42 overexpression also cures [*URE3*] in a manner that is dependent on a putative protein transport system that also shows some variant-specific effects on [*URE3*] propagation (57).

Prion-variant-specific effects of the chaperone machinery have also been reported. For instance, it was reported that the PFD of the prion protein Rnq1 is sufficient to propagate some [*RNQ*$^+$] variant structures, but not all. This may be primarily because there is a chaperone binding site in the non-PFD domain of Rnq1, and the binding of that chaperone, the Hsp40 Sis1, is required for the propagation of the [*RNQ*$^+$] prion (39). The reduction in Sis1 binding or activity cures the cells of the [*RNQ*$^+$] prion (58). Sis1 appears to interact tightly with Rnq1, only in the [*RNQ*$^+$] prion state (59), but interacts with every [*RNQ*$^+$] variant tested (31, 60). Moreover, Sis1 interacts with at least two regions of Rnq1 (39, 61). The function of Sis1 in [*RNQ*$^+$] propagation is tied to the activity of the Hsp104 disaggregase. Sis1 is

also essential for the propagation of other prions tested, including [*PSI*$^+$] (62), and different variants of [*PSI*$^+$] show different requirements for Sis1 activity to propagate (62). The Hsp104 chaperone was also found to be involved in the propagation of the [Het-s] prion, where the propagation rate and the number of prion particles per cell is reduced in a mutant background for Hsp104 (63). The [Het-s] prion can also be propagated in yeast (64), indicating that the chaperone machinery is able to replicate a totally alien prion. In this setting also, Hsp104 was required for prion propagation.

There is also evidence for the involvement of other cellular machineries distinct from the chaperone network in the maintenance of prions. For instance, overexpression of the Btn2 protein involved in endosomal protein sorting cures [*URE3*] (57), and autophagy protects against [*PSI*$^+$] formation (65). In addition, the actin cytoskeleton and the ubiquitination systems have also been found to modulate prion propagation (reviewed in reference 66).

FUNCTIONS AND TOXICITY

Because the prion concept was initially conceived to explain a baffling pattern of disease transmission, the term has long been associated with pathology. However, in the intervening decades many examples of functional amyloids have been reported in a wide variety of organisms. Although their evolutionary value has been controversial (67, 68), multiple lines of evidence suggest that many fungal prions can exert both positive and negative phenotypic effects, depending on strain and circumstance.

Functional versus Pathogenic Prions

Proteins with prion-like properties have been discovered in organisms ranging from yeast to mammals (15, 69). Their effects range from being necessary in non-self recognition ([Het-s] in *P. anserina*; see reference 70) to deadly (neurodegenerative diseases caused by PrP in humans, deer, and elk; see references 5 and 71). Computational analyses suggest that PFDs are ubiquitous in fungal proteomes (10, 72). Molecular characterization in *S. cerevisiae* indicates that some of these putative prions do indeed have the capacity to adopt multiple self-templating conformations under normal physiological conditions (10, 73). Their ubiquity, and the observation that fungal prions result in diverse phenotypes, some of which are adaptive (74–76), leads to a fundamental question about the biology of these epigenetic elements: Are prions purely selfish replicating elements, or can they provide some benefit to the organism? This question has been the subject of vigorous debate. Some argue that prions are often adaptive "bet-hedging" elements that can facilitate survival in fluctuating environments. Others have maintained that most fungal prions are simply diseases.

The bet-hedging hypothesis (68, 75, 77–79) is based on observations of phenotypic diversity imparted by prion switching and on the fact that rates of [*PRION*$^+$] acquisition and loss are much higher than the rate of spontaneous mutation. For example, within a [*prion*$^-$] yeast colony, a few [*PSI*$^+$] cells will appear sporadically, expressing heritable new phenotypes. If the [*PSI*$^+$] phenotype is detrimental, only a few individuals will be lost from a genetically identical population. If it is advantageous in a stressful environment, however, those few individuals might ensure survival of that genome when it would otherwise have been lost. [*PSI*$^+$] is also lost sporadically, providing a complementary survival advantage should the environment again change to favor the [*prion*$^-$] state. Thus, switching between the [*prion*$^-$] and the [*PRION*$^+$] states could enhance phenotypic diversity and promote survival of genetic lineages in fluctuating environments where they might otherwise have perished. Indeed, some have argued that repeated cycles of prion gain and loss (and indeed even spontaneous translation errors) may create a situation in which variation in the 3′-untranscribed regions (3′-UTR) of genes subject to [*PSI*$^+$]-mediated read-through have already experienced some selection (80).

Evidence supporting this hypothesis includes (i) the abundance of PFDs in fungal genomes (10, 72) and their common occurrence in regulatory proteins; (ii) prion induction and loss in stressful conditions when phenotypic variation would be most beneficial (81, 82); (iii) the existence of prion variants—akin to genic alleles (26, 30, 83–86); (iv) modular domain architecture (for example, Sup35's PFD does not appreciably affect translation termination but has been retained in fungi for ~500 million years [87]); (v) the recent appreciation that prion-based traits are common in wild yeast populations and that some of these are strongly beneficial (74); (vi) genetic assimilation of traits that are initially dependent on [*PSI*$^+$], which can be rendered independent of the prion via meiotic recombination of the cryptic genetic variation that initially drives them (74, 77)—this provides a means to separate the beneficial phenotype from the costs of the mechanism generating it; and finally (vii) because of their capacity to regulate translational fidelity throughout the transcriptome, the traits produced by [*PSI*$^+$] are genetically complex (75, 77) and

would require far longer to achieve by mutation alone (see below).

Others have argued that most fungal prions are selfish elements, diseases, or even artifacts of laboratory cultivation (53, 67, 88) and that the rare beneficial phenotypes they induce are a side effect of infection. This line of thinking posits that the evolutionary retention of prion proteins can be explained because selection has been too weak to remove them (89). Evidence supporting this hypothesis includes (i) the fact that most individual prions are present at low frequencies in natural populations (74, 88); (ii) the occasional generation of lethal [*PSI*$^+$] variants *in vivo* when the prion and translation domains are separated (53); (iii) the observation that population allele frequencies of some prions are similar to those that would be expected for deleterious elements in outcrossing populations (89); (iv) the existence of structural variants for most prions, which could reflect an absence of positive selection because at least one prion, [Het-s], shows no such variation; (v) some PFDs having additional functions that could explain their evolutionary conservation (67); and (vi) prion acquisition leading to a stress response in some genetic backgrounds (90).

These hypotheses are not mutually exclusive, and most fungal prions show some degree of antagonistic pleiotropy. That is, they are beneficial in some environments and detrimental in others. Indeed, prions are clearly not universally beneficial, or they would have been fixed in natural populations of fungi. Standing frequencies of [*PSI*$^+$] and [*MOT3*$^+$] in natural populations of yeast (74) are consistent with a modest (~1%) fitness detriment on average for these elements (89). These models necessarily make many assumptions about rates of [*PRION*$^+$] gain and loss and the frequency of outcross mating. Alternative values for these parameters, which have been observed in other studies (91), suggest a fitness benefit (69). Further investigation of these questions in the appropriate physiological context will be critical to resolve these questions. Computational modeling has also placed these arguments within a theoretical framework grounded in population genetics (79, 92, 93). These analyses suggest that [*PSI*$^+$]'s potential benefit as a bet-hedging element would not likely be sufficient to explain its evolutionary retention under nonstressed conditions. In contrast, bet-hedging would be sufficient to motivate retention of [*PSI*$^+$] in stressful environments, even with realistic rates of sex. The selective advantage calculated with these models is even stronger for another prion, [*GAR*$^+$] (see below).

Yeast Prions in (Epi)Genetic Diversity and Prions in Biotic Interactions

[*PSI*$^+$] is formed by the translation-termination factor Sup35, which ensures faithful termination by the ribosome at stop codons. The regions downstream of such stop codons (i.e., in the 3′-UTR of genes) are comparatively free to accumulate mutations because they are not under selective pressure to produce a protein (although they often are subject to selection as regulatory sequences). Acquisition of [*PSI*$^+$] causes translational readthrough of many stop codons throughout the transcriptome (94) and consequently drives the emergence of new heritable phenotypes. This process is conceptually analogous to duplicated genes that are inactivated by a premature stop codon and are retained as pseudogenes, providing a source for the generation of new functional gene products via mutation and eventual reactivation (95).

This prion-dependent phenotypic diversification is strongly dependent on genetic background. That is, a phenotype elicited by [*PSI*$^+$] in one strain background might not be elicited in another. For example, in *S. cerevisiae* strain 33G the acquisition of [*PSI*$^+$] led to increased resistance to bleomycin and sensitivity to benomyl. In contrast, acquisition of [*PSI*$^+$] led to sensitivity to bleomycin and resistance to benomyl in *S. cerevisiae* strain 5G (75). Genetic dissection established that [*PSI*$^+$]-induced changes in translational fidelity produce new phenotypes based on genetic variation that was previously silent or "cryptic" (75, 77). Most phenotypes produced by [*PSI*$^+$] are driven by multiple polymorphisms. [*PSI*$^+$] thus provides immediate access to traits that otherwise require the serial acquisition of multiple rare mutations. The degree to which this mechanism has contributed to the evolution of biological novelty remains to be established. However, the presence of [*PSI*$^+$] in wild strains of *S. cerevisiae* (74) and its capacity to fuel strong phenotypes in those backgrounds suggest that this prion has exerted an important influence on the phenotypic landscape of natural fungal isolates.

In the 2 decades since [*PSI*$^+$] was recognized as a prion, approximately 10 other fungal prions have been discovered (15). Some, such as [*OCT*$^+$], formed by the Cyc8 transcriptional repressor (96), were found serendipitously. Others, such as [*MOT3*$^+$], formed by the Mot3 transcription factor, were found in a systematic screen to identify proteins with N/Q-rich sequences that resembled three other prion proteins known at the time: Sup35, Rnq1, and Ure2 (10). Investigation of [*MOT3*$^+$] revealed that it governs the acquisition of facultative multicellularity in *S. cerevisiae*, likely through both gains

and losses of function (73). This phenotype is driven by [*MOT3*$^+$]-dependent transcription of *FLO11*, a major determinant of cell-cell adhesion, in response to nutrient deprivation. [*MOT3*$^+$] can be regulated by the environment. It is induced in response to ethanol and lost in response to hypoxia, providing some suggestion that it may be engaged in a subpopulation of cells during natural respiro-fermentative cycles (73). These phenotypes (in addition to others produced by the prion [see reference 74]) are highly strain-dependent. Thus, in addition to [*PSI*$^+$], [*MOT3*$^+$] can exert a strong influence on the phenotypic manifestation of natural genetic variation.

For decades, prions that create new traits (e.g., [*PSI*$^+$] and [*MOT*$^+$]) had not been found in wild strains of *S. cerevisiae* (88). Although these studies were limited in scope, they led to the perception that prions could be rare diseases or artifacts of laboratory cultivation. However, an examination of hundreds of wild *S. cerevisiae* isolates revealed that ~1 to 2% harbored [*PSI*$^+$] and ~6% harbored [*MOT3*$^+$] (74). These elements conferred many beneficial phenotypes under diverse selective conditions. Approximately one third of the wild strains examined in this study had heritable phenotypes that required the activity of Hsp104 to be propagated from one generation to the next. Those that were tested could also be transmitted from one strain to another through cytoplasmic mixing without transferring nuclear material. That is, these traits had prion-like patterns of propagation. Although the molecular origin of these traits remains to be identified, these experiments established that a potentially broad array of prion-like elements can influence the phenotypes of wild strains.

Switching between [*prion*$^-$] and [*PRION*$^+$] states is generally thought to be a rare event. For example, although the rates of [*MOT3*$^+$] acquisition are increased in response to ethanol, most cells still do not switch under these conditions (73). The [*GAR*$^+$] prion provides a striking exception to this paradigm. Discovered by chance decades ago in a screen for mutants that reverse glucose repression (97, 98), [*GAR*$^+$] is a protein-based element of inheritance that allows fungi (*S. cerevisiae* and other related yeast species including *Nauvozyma castellii* and *Dekkera bruxellensis*) to circumvent a normal hallmark of their biology: extreme metabolic specialization for glucose fermentation (7, 99, 100). When glucose is present, even in trace quantities, yeast will not metabolize other carbon sources. [*GAR*$^+$] allows cells to circumvent this "glucose repression," and arises at different frequencies in wild fungal isolates. These parameters strongly correlate with the ecological niche from which the strain was derived (100).

Through serendipitous contamination of a selective plate it was discovered that switching to [*GAR*$^+$] can be induced in virtually all *S. cerevisiae* cells when they are cocultured with certain species of bacteria (99). This cross-kingdom communication proceeds through an as yet unidentified small molecule secreted by the inducing bacteria. The results of this communication benefit yeast and bacteria alike. Providing an advantage for the bacteria, [*GAR*$^+$] yeast cells produce less ethanol than [*gar*$^-$] yeast cells. Providing an advantage for the yeast, [*GAR*$^+$] cells can readily metabolize complex carbohydrates and survive better in late-stage fermentations. As expected for a mechanism whose adaptive value originates from the selective pressures of life in biological communities, the ability of bacteria to induce [*GAR*$^+$] and the ability of yeast to respond to bacterial signals have been lost repeatedly during the monoculture inherent to laboratory domestication. These data suggest that [*GAR*$^+$] is a broadly conserved and often adaptive strategy to link environmental and social cues to heritable changes in metabolism.

Prion Domains in Signal Transduction in Filamentous Fungi

In the filamentous fungus *P. anserina*, the prion protein HET-s is involved in a cell death process known as heterokaryon incompatibility (70). Such incompatibility arises when a strain bearing the [Het-s] prion (in its amyloid form) and a strain expressing a different allelic variant of this protein (termed HET-S) come into contact. The [Het-s] prion is common in natural isolates of *P. anserina*, leading to the prevailing view that the prion is adaptive (66, 101). HET-s and HET-S are two-domain proteins with an N-terminal globular domain and a C-terminal prion-forming domain. Interaction with [Het-s] causes HET-S to relocalize from the cytoplasm to the cell periphery (102). Biochemical and structural studies suggest that this interaction drives conformational conversion of a HET-S PFD region into a β-solenoid fold which in turn induces a refolding of the globular HeLo domain (Het-s/LOP-B; see more on structure in the next section) to expose an ~34-residue transmembrane segment at the N-terminus of the protein. This converts HET-S into an integral membrane protein. Once it is at the membrane, HET-S oligomerizes and fuels the loss of membrane integrity in a manner resembling pore-forming toxins (103). The role of this type of conformational conversion in signaling intrinsic programmed cell death is only beginning to be appreciated. The *P. anserina* genome contains a gene encoding a Nod-like receptor protein (NWD2) adjacent to the gene

encoding HET-S (104). Strikingly, NWD2 contains an N-terminal region that is homologous to the amyloid motif of HET-s. When NWD2 binds its cognate ligand, this interaction also drives conversion of HET-S into the amyloid conformation (105).

The paradigms established by the study of HET-s appear to be echoed in programmed cell death in other fungi. The short prion motif and globular HeLo domain characteristic of HET-S are also associated with other proteins such as lipases and regulatory Nod-like receptors in other fungi. The functional and evolutionary significance of this relationship has recently been examined in the saprophytic fungus *Chaetomium globosum* (106). A cluster of genes harboring short prion motifs was examined that included a protein known as HELLP (because it contains a HeLo-like domain). HELLP also has an N-terminal transmembrane helix that is homologous to that of HET-S. Despite the evolutionary distance between this organism and *P. anserina*, HELLP behaves as a HET-S analog, relocating to the membrane upon interaction with the prion form of the short prion motif. Moreover, the HeLo-like domain of HELLP bears homology to mammalian pore-forming domains involved in necroptosis, suggesting the possibility of an ancient evolutionary relationship between these processes.

Amyloid/Prion Toxicity in Yeast

The toxicity of prion amyloids in yeast has been the subject of intense debate. Most commonly studied variants of [*PSI*$^+$] have a slight fitness defect. However, overexpression of the NM domain of Sup35 induces [*PSI*$^+$] variants with a wide spectrum of effects. Those that are lethal are normally purged from the population but can be maintained when the C-terminus of Sup35 (Sup35C, which cannot be converted into [*PSI*$^+$] but is competent for translation termination) is expressed on a plasmid (53). In these experiments ~8% of the induced [*PSI*$^+$] variants were lethal. The remaining variants were either slow-growing or nontoxic. The slow-growing variants were unstable upon loss of the Sup35C plasmid. In contrast, the nontoxic [*PSI*$^+$] variants were stable. Analogous results were seen with [URE3] in these experiments, highlighting the fact that selection has already operated on the ensemble of prion conformers typically studied in the laboratory. The molecular interactions governing prion loss remain to be fully elucidated but involve multiple chaperone activities including Hsp104, Hsp42 Btn2, and Cur1 (57).

In other cases, prion proteins are toxic only when overexpressed, and only in [*PRION*$^+$] cells. For example, Rnq1 is profoundly toxic in [*RNQ*$^+$] but not [*rnq*$^-$] cells (61). This toxicity is not caused by a general proteotoxic stress. Rather, in these cells Rnq1 sequesters the core spindle pole body component Spc42 in an insoluble protein deposit, engaging the Mad2 cell cycle checkpoint. The Hsp40 chaperone Sis1 suppresses this toxicity, but rather than inhibiting aggregation of Rnq1 it drives assembly of the protein. Interfering with this Sis1-triggered aggregation exacerbates Rnq1 toxicity. These data underscore the fact that amyloids are not always the toxic species in prion-like aggregation pathways. Molecular understanding of these distinctions, as well as the structural differences between toxic and nontoxic amyloid species stand as goalposts for future work.

FUNGAL PRION STRUCTURES

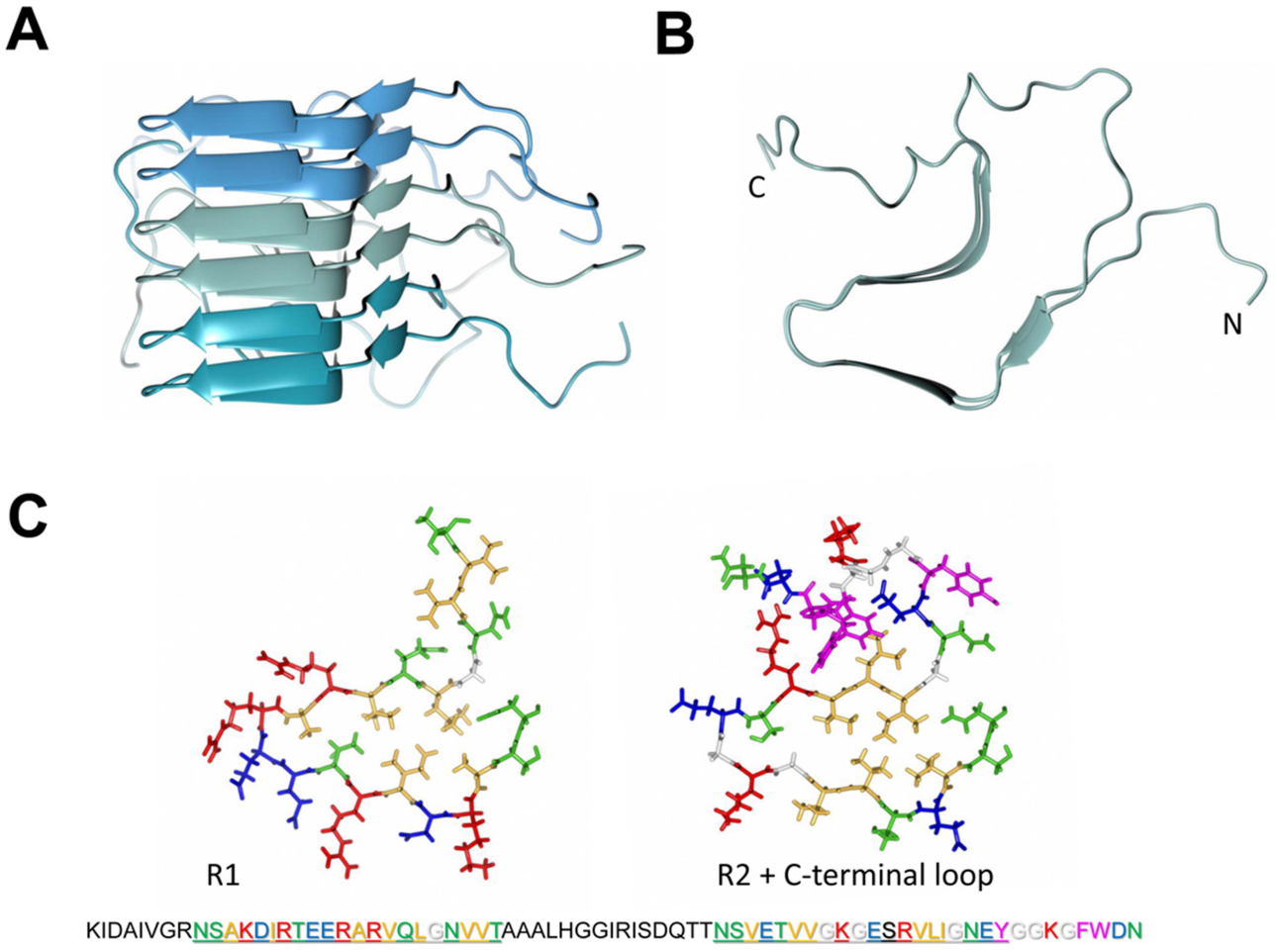
Structural characterization of amyloids, which constitute the physical basis of many fungal prions, is a considerable challenge, and one faced by the amyloid field in general. The most adapted technique to gain access to high-resolution structures of amyloid assemblies is currently solid-state nuclear magnetic resonance (NMR) (107). Other approaches include X-ray diffraction techniques that so far can only inform on the overall fold or fold modification. The Eisenberg group has also reported high-resolution X-ray crystallography structures of seven amino acid peptides derived from the Sup35 PFD assembled into nanocrystals (108), but this approach cannot be applied to the full-length PFD so far. One of the central limitations hindering progress in the structural determination of amyloids is structural heterogeneity. It appears that many amyloids, in particular disease-related amyloids but also yeast prion models, exist as mixtures of structural polymorphs displaying subtle or large differences in their amyloid architecture (109).

The [Het-s] prion is currently the only model for which a high-resolution structure is available. Solid-state NMR could be successfully applied here, because HET-s PFD fibrils are not polymorphic and lead to well-resolved solid-state NMR spectra. The HET-s PFD adopts a so-called β-solenoid structure with two rungs of β-strands per monomer. This structure resembles other β-helical structures found in soluble proteins (110). The HET-s PFD comprises two conserved 21-amino acid residue imperfect repeats connected by a poorly conserved 15-amino-acid-long loop (111). Each of these repeats forms one 4.7-Å layer of a stacked β-sheet structure (111, 112). Each repeat comprises four β-strands; the first three β-strands delimit a triangular hydrophobic core, and the fourth protrudes from the

core (34, 113) (Fig. 2). At the C-terminal end of the PFD, an aromatic loop folds back into a groove delimited by the third and fourth β-strands to form a semihydrophobic pocket (113). Two asparagine ladders, a frequent motif in amyloids, occur at the beginning of the first and fourth β-strands (N226/N262 and N243/279), and three salt bridges (K229/E265, E234/K270, and R236/E272) stabilize the stacking of the two rungs of β-strands. The inner core is composed exclusively of hydrophobic residues, with the exception of two hydroxyl residues occupying different layers, that can form a hydrogen bond inside the core (T233/S273). Glycine residues occupy the β-arches between the third and the fourth β-strand in both layers. A short C-terminal loop containing two aromatic residues (F286 and W287) folds back onto the fourth β-strand to form a semihydrophobic pocket.

The structure-function relationship in the HET-s PFD was analyzed in detail with site-directed mutagenesis approaches (111, 114, 115). The β-solenoid fold was found to be robust. The majority of the sequence alterations did not affect the global fold or the prion-forming ability. Exceptions are the glycine residues of the β-arches between the third and fourth strand of each rung, whose mutation affects the overall fold and abolishes

FIGURE 2 Structure of HET-s prion-forming domain. **(A)** Lateral view of a trimer of HET-s (218-289) in the prion amyloid conformation. Each monomer bears a different color, after pdb 2KJ3. **(B)** View from the fibril axis of one HET-s(218-289) monomer; the N- and C-terminal ends are marked, after pdb 2KJ3. **(C)** Structure of the two individual repeats of HET-s(218-289) marked R1 (position 226 to 246) and R2 (position 262 to 282) as well as the C-terminal semihydrophobic loop (position 283 to 289), after pdb 2KJ3. Amino acids are coded by chemical property (G in light gray, polar in green, hydrophobic in yellow, positively charged in red, negatively charged in blue, and aromatic in magenta). The sequence of HET-s(218-289) is given below with the same color coding in R1, R2 (underlined), and the C-terminal loop.



prion function (114). The different structural elements mentioned above (N-ladders, salt bridges, hydrophobic core, buried polar residues) contribute to various extents to the prion function, β-solenoid fold, and fibril stability and fibril formation rate. Importantly, the C-terminal aromatic loop modulates the prion-forming ability, although this region is not part of the rigid cross-β core of the fold (114). In addition to the solid-state NMR structure, a cryo-electron microscopy structure of the HET-s PFD has also been reported and largely agrees with the solid-state NMR structure (116).

Although no high-resolution structure of a yeast prion is yet available, valuable information on the overall fold of the prion amyloid state of Sup35, Ure2, and Rnq1 was obtained. Several lines of evidence converge to suggest that these yeast prions may adopt an in-register parallel β-sheet structure. Prior to its experimental validation, this organization was already hypothesized based on the experiments mentioned above, showing that shuffled Ure2 and Sup35 PFD sequences (retaining the same amino acid composition) are still able to form prions (41). It was argued that only a parallel in-register β-sheet architecture (and not alternate β-helical or antiparallel or parallel out-of-register models) could explain retention of prion formation with scrambled sequences. Using selectively labeled samples and solid-state NMR, the parallel in-register architecture was supported by dipole-dipole relaxation rates for all three models (101, 117–119). Mass-per-length measurements of prion filaments are also compatible with models in which a single protein molecule occupies one layer of the β-sheet structure (that is, 4.7 Å) (85, 120, 121). Collectively, these results are compatible with the structural model proposed by Kajava and coworkers (122) and can account for strain variation. They stand in contrast to an alternative β-helical model for Sup35 based on chemical cross-linking approaches (123). The highly degenerate nature of Sup35 fibers complicates interpretation of these data, and a full atomic-level understanding of yeast prion structure will likely require the application of new technologies to the problem.

CONCLUSION

Our understanding of prion biology is built on the rich curiosity of researchers investigating undefined epigenetic phenotypes in fungi beginning over 50 years ago. Their persistence has led to a wealth of knowledge that now impacts our understanding of biological complexity and human health in a multitude of ways. While many incredible discoveries have been made thus far, these systems lend themselves to additional advances relating to epigenetics, the biology of complex phenotypes, prion structure and replication, prion diversity, the effects of intracellular and extracellular modulators, and the impact of protein-based elements on organismal fitness and survival.

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