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PRIONS AS PROTEIN-BASED GENETIC ELEMENTS

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■ **Abstract** Fungal prions are fascinating protein-based genetic elements. They alter cellular phenotypes through self-perpetuating changes in protein conformation and are cytoplasmically partitioned from mother cell to daughter. The four prions of *Saccharomyces cerevisiae* and *Podospora anserina* affect diverse biological processes: translational termination, nitrogen regulation, inducibility of other prions, and heterokaryon incompatibility. They share many attributes, including unusual genetic behaviors, that establish criteria to identify new prions. Indeed, other fungal traits that baffled microbiologists meet some of these criteria and might be caused by prions. Recent research has provided notable insight about how prions are induced and propagated and their many biological roles. The ability to become a prion appears to be evolutionarily conserved in two cases. [PSI^+] provides a mechanism for genetic variation and phenotypic diversity in response to changing environments. All available evidence suggests that prions epigenetically modulate a wide variety of fundamental biological processes, and many await discovery.

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INTRODUCTION

Prion proteins are unique because they adopt at least two distinct conformational states, one of which, the prion form, can stimulate the nonprion conformation to convert into the prion form. The term "prion" first described the unusual proteinaceous infectious agent that causes devastating neurodegenerative diseases of mammals called transmissible spongiform encephalopathies (TSEs) (128). TSEs include mad-cow disease of cattle, scrapie of sheep and goats, as well as Creutzfeldt-Jakob disease and Kuru of humans (129). Most transmissible diseases are caused by nucleic acid–based agents; however, TSEs are probably caused by the aberrant folding of a cellular protein (PrP^C) into an infectious form (PrP^{Sc}) (129). The function of PrP^C is unknown. Although the protein-only nature of the infectious agent has not been unequivocally demonstrated, protein conformational change is closely linked to transmissibility.

The revolutionary prion concept has been extended to explain three unusual genetic elements of the yeast *Saccharomyces cerevisiae* and one of the filamentous fungus *Podospora anserina* (35, 147, 176). The term prion is no longer confined to the infectious agent of TSEs, but applies to any protein that can switch to a self-sustaining conformation. The agents of the four fungal prions are four endogenous cellular proteins, which participate in diverse biological processes and are apparently nonhomologous to each other or to the mammalian prion protein. The conformational switch to the prion state alters the protein's function and the cell's phenotype. The altered phenotypes are propagated from generation to generation as the protein in the prion state is transferred from mother to daughter cell, continuing the cycle of conformational conversion. Thus, yeast prions act as heritable protein-based genetic elements that cause biologically important phenotypic changes without any underlying nucleic acid change.

The yeast prion [*PSI*$^+$] is caused by a conformationally altered form of Sup35 (122, 123, 176), one of two proteins that comprise the translational release factor (152). Some mutant Sup35 proteins cause ribosomes to read through stop

codons at an appreciable frequency (71, 72). Such mutants suppress nonsense-codon mutations in other genes, hence their name. [*PSI*$^+$] strains also display a nonsense-suppression phenotype (38) because translational termination becomes impaired when Sup35 adopts the prion conformation (Figure 1; for nomenclature, see Figure 3). Unlike the recessive phenotype of *sup35* mutations, [*PSI*$^+$] is

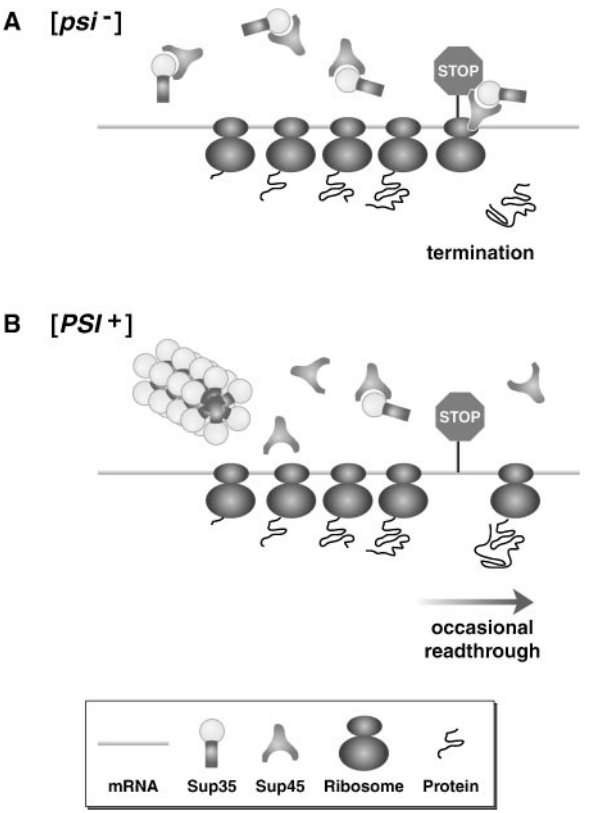

Figure 1 The effect of [*PSI*$^+$] on Sup35 and translational termination. (*A*) A complex of Sup35 (see legend at bottom) and Sup45 binds ribosomes at stop codons and mediates translational termination. Sup35 is composed of two regions, a prion-determining domain (PrD, *rectangle*) and a termination domain (Sup35C, *sphere*). In nonprion [*psi*$^-$] strains, translational termination occurs efficiently at stop codons at the ends of open reading frames, and the completed protein is released from the ribosome. (*B*) In [*PSI*$^+$] cells, most Sup35 proteins adopt the prion conformation and self-assemble into an aggregated, possibly amyloid structure (depicted as *large cylinder*). This conformational change impairs Sup35's ability to participate in translational termination and consequently, stop codons are read through occasionally, producing proteins with a C-terminal extension.

dominant, characterized by non-Mendelian inheritance, and efficiently transmitted from mother cell to daughter through the cytoplasm (38, 40). (The brackets in the name [*PSI*$^+$] denote the latter two characteristics, and the uppercase and italicized *PSI* denotes a dominant trait).

The prion [*URE3*] affects nitrogen catabolite repression. Normally, yeast growing on rich nitrogen sources such as ammonium repress production of proteins needed to metabolize poor nitrogen sources such as ureidosuccinate (158). Recessive mutants termed *ure* were isolated that utilize poor nitrogen sources in the presence of good sources (95, 96). The Ure2 protein regulates nitrogen catabolism by binding to and interfering with the transcriptional activator Gln3 (10). One dominant variant, called [*URE3*], showed non-Mendelian inheritance and was cytoplasmically transferable (2, 96). Conversion of Ure2 into its prion form renders Gln3 constitutively active, thereby eliminating nitrogen catabolite repression and enabling yeast to utilize ureidosuccinate in the presence of ammonium (176) (Figure 2).

Another yeast prion was identified by a directed computer search of yeast genome databases based upon its similarity to regions of Sup35 and Ure2. Rnq1, so named because its sequence is rich in asparagine (Asn, N) and glutamine (Gln, Q), is the protein determinant of the prion [*RNQ*$^+$] (147). Genetic, cell biological, and biochemical analyses proved [*RNQ*$^+$] is a prion, despite being caused by a nonessential protein of unknown function (45, 47, 48, 147). Moreover, [*RNQ*$^+$] is coincident with an epigenetic factor previously identified as [*PIN*$^+$] that affects [*PSI*$^+$] induction, providing the first evidence that one prion affects the appearance of another (45, 118).

The [Het-s] prion of *P. anserina* mediates heterokaryon incompatibility. Because mycelia of this fungus can fuse, resulting in cytoplasmic mixing and occasional exchange of nuclei, this organism has a mechanism to prevent heterokaryon formation between incompatible strains (134). Heteroallelism between any of as many as nine *het* loci triggers a lytic reaction that leads to cell death via an uncharacterized mechanism (134). On solid media, accumulation of these dead cells leads to a distinct, easily visible abnormal contact line, termed a barrage. There are two alleles at the *het-s* locus called *het-s* and *het-S*. The *het-s* allele encodes a protein with two different conformations: HET-s behaves as a prion and HET-s* does not (35). The *het-S* allele encodes the HET-S protein that is incompatible with [Het-s] prion strains but is compatible with neutral [Het-s*] strains (for nomenclature, see Figure 3).

Here, we review the literature on [*PSI*$^+$], [*URE3*], [*RNQ*$^+$], and [Het-s], focusing on their common characteristics, the mechanisms by which they are induced and propagated, their biological significance, and their possible roles in evolution. A comprehensive analysis of the many intricacies of these fascinating elements is beyond the scope of this review. Fortunately, many extensive reviews of individual prions are available (23, 129, 141, 143, 178) and we urge interested readers to consult them. Because many other unidentified prions probably exist (113, 143, 147), we seek to aid the reader in ascertaining whether proteins with unusual conformational

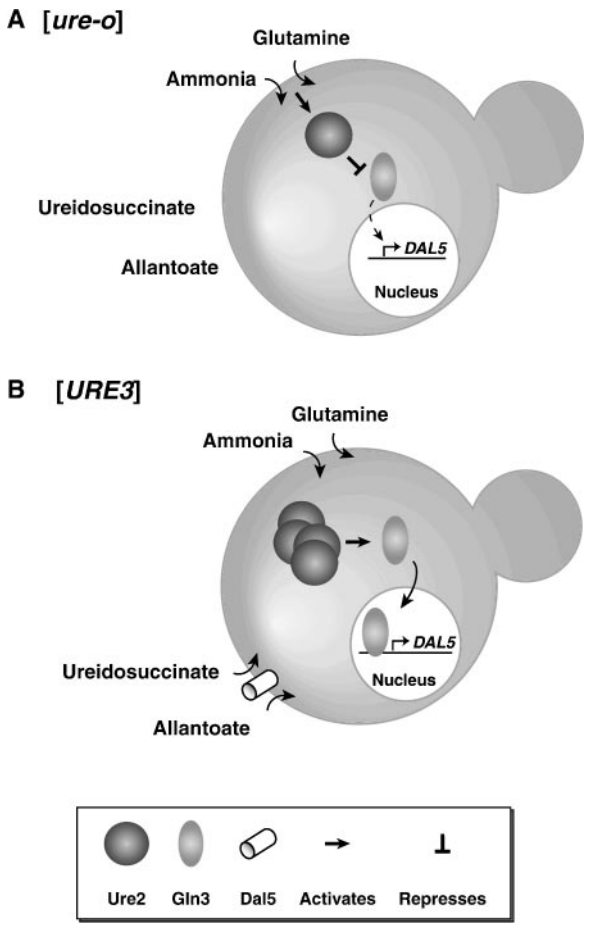

Figure 2 The effect of [*URE3*] on Ure2 and ureidosuccinate uptake. (*A*) In nonprion [*ure-o*] cells, uptake of poor nitrogen sources such as ureidosuccinate and allantoate is repressed in the presence of good nitrogen sources such as glutamine and ammonia. The availability of good nitrogen sources is relayed through Ure2 (see legend at bottom), which blocks the action of the transcription factor, Gln3. Without transcriptional activation, the allantoate transporter, Dal5, is not produced. (*B*) In [*URE3*] cells, conversion of Ure2 into its prion conformation interferes with its ability to repress Gln3. Thus, even in the presence of preferable nitrogen sources, Gln3 activates the transcription of *DAL5*. Ureidosuccinate is a structural mimic of allantoate and enters the yeast cell via Dal5.

[*PSI*$^+$] NOMENCLATURE	
Alleles	
SUP35	Wild-type gene encoding a subunit of the translation termination factor
sup35	Mutant that impairs translational termination
Protein Products	
Sup35	Full length protein that can exist in nonprion and prion states
Sup35N	N-terminal fragment required for [*PSI*$^+$], includes the QN-rich region and imperfect repeats
Sup35NM	N-terminal fragment consisting of Sup35N and the highly charged M region
Phenotypes	
[*PSI*$^+$]	Prion phenotype causes nonsense suppression of all three types of stop codons
[*psi*$^-$]	Nonprion phenotype, wild-type translational termination

[*Het-s*] NOMENCLATURE	
Alleles	
het-S	This allele does not encode a prion and is incompatible with the *het-s* allele
het-s	This allele encodes a protein that can exist in a prion and nonprion form
het-s^o	*het-s* locus disrupted by gene replacement
Protein Products	
HET-S	Protein product of the *het-S* allele, incompatible with HET-s
HET-s	Protein product of the *het-s* allele in the prion state, incompatible with HET-S
HET-s*	Protein product of the *het-s* allele in the nonprion state, compatible with HET-S or HET-s
Phenotypes	
[Het-S]	Phenotype of *het-S* allele, is incompatible with [Het-s] strains
[Het-s]	Prion phenotype of the *het-s* allele that is incompatible with [Het-S]
[Het-s*]	Neutral, nonprion phenotype of the *het-s* allele that is compatible with [Het-S] and [Het-s]

[*URE3*] NOMENCLATURE	
Alleles	
URE2	Wild-type gene involved in nitrogen catabolite repression
ure2	Mutant allele that is impaired in nitrogen catabolite repression
Protein Products	
Ure2	Full length protein that exists in nonprion and prion states
Ure2N	N-terminal fragment containing the asparagine and glutamine-rich PrD of Ure2
Ure2C	C-terminal fragment that functions in nitrogen catabolite repression
Phenotypes	
[*URE3*]	Prion phenotype, faulty nitrogen catabolite repression
[*ure-o*]	Nonprion phenotype, wild-type nitrogen catabolite repression

[*RNQ*$^+$] NOMENCLATURE	
Alleles	
RNQ1	Wild-type gene, function unknown
rnq1	Mutant with no obvious phenotype
Protein Products	
Rnq1	Full length protein that exists in nonprion and prion states
Rnq1N	N-terminal fragment, unknown function
Rnq1C	C-terminal fragment of Rnq1 containing the asparagine and glutamine-rich PrD of Rnq1
Phenotypes	
[*RNQ*$^+$]	Prion phenotype, function unknown
[*rnq*$^-$]	Nonprion phenotype, function unknown

Figure 3 Summary of fungal prion nomenclature. This is a brief guide to the nomenclature and terminology of fungal prions and their associated alleles and phenotypes. The yeast prion nomenclature is in accordance with the accepted rules for yeast nomenclature (142).

properties or genetic traits with unusual epigenetic properties are prion-like. We hope that highlighting the distinctiveness and variety of prion behavior will fuel discovery of novel prions.

CHARACTERISTICS OF FUNGAL PRIONS

The four fungal prions, particularly [*PSI*$^+$] and [*URE3*], share many characteristics (Figure 4) that are widely cited as criteria to identify novel prions. Indeed, these criteria led to the identification of other prion elements (133, 147) and undoubtedly will lead to the discovery of more. There are some notable differences among [*PSI*$^+$], [*URE3*], [*RNQ*$^+$], and [Het-s], however, making it likely that prions differing in key aspects will be found. Ultimately, to be a prion we suggest that a protein must fulfill only one requirement: It must adopt at least two stable states, one of which is self-sustaining.

Fungal Prions are Metastable

Prions occur spontaneously in laboratory fungal strains at a low frequency. [*PSI*$^+$] and [*URE3*] arise in 1 per 10^5 to 10^7 cells, depending on the genetic background (2, 38, 105, 108, 176). The frequency at which [Het-s*] converts to [Het-s] is estimated at less than 1 per 10^7 (8). Incubating cells at low temperatures increases the induction rate of [*PSI*$^+$] and [*URE3*] somewhat (23, 26, 47). Overexpressing the gene encoding a prion increases the induction rate several orders of magnitude (22, 24, 35, 50, 110, 147, 176), presumably because this increases the chance that some proteins will spontaneously adopt the prion conformation. Importantly, [*PSI*$^+$] and [*URE3*] are maintained even if the increased expression of the prion determinant is transient (24, 176). Thus, [*PSI*$^+$] and [*URE3*] are self-perpetuating, even after the levels of Sup35 and Ure2 return to normal.

Once established, most prions propagate faithfully through mitosis and meiosis and are rarely spontaneously lost, although there are some exceptions (9, 35, 40, 176). Stability differences can be attributed to genetic background (96, 108) and the specific prion (50). In general, [*URE3*] is less meiotically stable than [*PSI*$^+$] (38, 96). Some growth conditions efficiently cure fungal prions. Perhaps the most widely used is growth on 1–5 mM guanidinium hydrochloride (GuHCl), which eliminates [*PSI*$^+$], [*URE3*], and [*RNQ*$^+$] (2, 108, 165, 176) but not [Het-s] (35). Prions can usually reappear in previously cured strains.

Altering the expression of cellular proteins that affect protein folding can also cure prions. All three yeast prions require Hsp104, a molecular chaperone that resolves misfolded protein aggregates (70, 121), and are irreversibly cured when *HSP104* is deleted (26, 114, 147). [*PSI*$^+$] requires a specific amount of Hsp104, as it can also be cured by *HSP104* overexpression (26). Because no other yeast prion is cured by Hsp104 overproduction (45, 48, 114, 148), the mechanisms by which overexpression and deletion of *HSP104* cure [*PSI*$^+$] might be distinct.

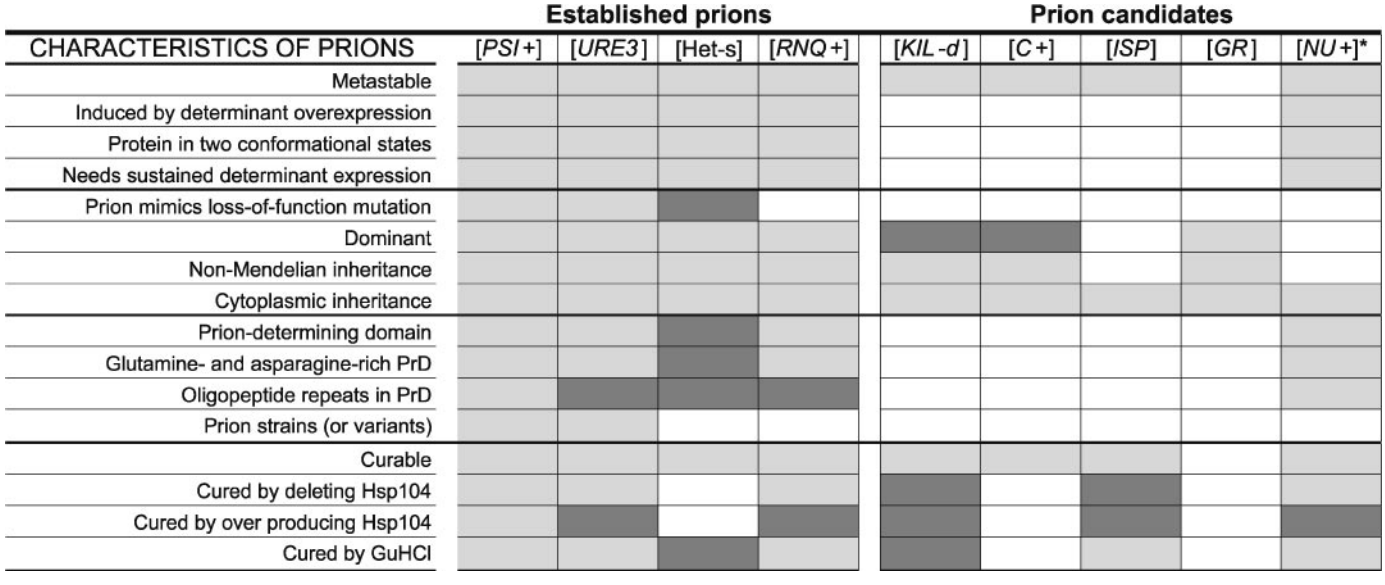

Figure 4 Comparison of the characteristics of established prions to those of candidate prions. Gray indicates that a prion or trait displays a characteristic and black indicates that it does not. White denotes no information available. *based upon the behavior of New1 PrD fused to Sup35C. See text for references.

Capacity to Convert to a Self-Propagating Conformational State

Strong support for the prion model derives, in part, from biochemical characterization of prion proteins. When crude yeast lysates are fractionated by differential sedimentation, Sup35 and Rnq1 partition differently, depending on whether the cells contain a prion. Sup35 and Rnq1 proteins are mostly soluble in [*psi*$^-$] and [*rnq*$^-$] lysates, but they are insoluble in [*PSI*$^+$] and [*RNQ*$^+$] lysates (122, 123, 147). When [*PSI*$^+$] lysates are fractionated using size-exclusion chromatography, the prion form of Sup35 is associated with high-molecular-weight fractions (123). The Sup35 prion conformation is somewhat more resistant to proteinase K digestion than the nonprion form (122, 123). Together, these studies indicate that Sup35 and Rnq1 are aggregated in the prion state but not in the nonprion state, which provides a satisfying explanation for the translational termination defect of [*PSI*$^+$] strains.

To visualize the prion conformation converting newly made protein to the prion state in living cells, Rnq1 or certain prion-determining fragments of Sup35 were fused to green fluorescent protein (GFP) and expressed in yeast. In cells without either prion, these GFP fusion proteins distribute evenly throughout the cytoplasm (122, 147). In [*PSI*$^+$] or [*RNQ*$^+$] cells, such fusion proteins quickly coalesce into discrete, cytoplasmic foci that do not appear to localize to any cellular structure (122, 147). The particulate nature of the fusions in [*PSI*$^+$] cells was confirmed by differential sedimentation (122). For [*PSI*$^+$], double labeling demonstrated the capture of newly made Sup35-GFP fusions at the sites of pre-existing Sup35 aggregates tagged with hemagglutinin (104).

Similar approaches indicated that the prion forms of Ure2 and HET-s are aggregated in prion-containing cells; however, the nature of this conformational difference is not as well understood as with Sup35 and Rnq1. In some genetic backgrounds, the prion form of Ure2 is more resistant to proteolysis than the nonprion form (111, 137, 162). Consistent with these findings, differential sedimentation and size-exclusion chromatography of yeast lysates indicate that Ure2 (57, 137) is insoluble in [*URE3*] cells but soluble in nonprion [*ure-o*] cells. Moreover, ectopically expressed Ure2-GFP fusions coalesce into fluorescent foci only in [*URE3*] (57, 137). In other yeast genetic backgrounds, however, such Ure2-GFP fusions do not coalesce detectably (62, 63), and there is no apparent difference in how Ure2 partitions upon fractionation of [*URE3*] or [*ure-o*] lysates by differential sedimentation (63). One plausible explanation is that the extent of Ure2 aggregation depends on the genetic background.

For [Het-s], detection of prion aggregates apparently depends on the expression level of HET-s and the assay used. In crude cell lysates, the prion conformation, HET-s, is more resistant to proteinase K digestion than its nonprion form (35), which suggests the prion form is conformationally different and might be insoluble. By differential sedimentation or size-exclusion chromatography of [Het-s] lysates, however, HET-s aggregates are detectable only in [Het-s] cells that highly

overexpress the prion protein (37). Similarly, HET-s-GFP fusions coalesced into fluorescent foci specifically in [Het-s] prion strains only when those fusions were highly expressed (37). At normal levels, fluorescence is diffusely distributed in the cytoplasm or is localized to vacuoles. Possibly, only a small fraction of the HET-s protein is aggregated in vivo. Alternatively, the prion conformation may be in small aggregates or preferentially degraded, thereby precluding its detection. Indeed, the steady-state level of HET-s is more than threefold lower in [Het-s] prion strains than in [Het-s*] neutral strains (37).

The conformational switch of all four fungal prions between soluble and insoluble states can be reconstituted in vitro under physiological conditions (53, 69, 146, 156). The insoluble conformation consists of highly ordered amyloid fibers (53, 69, 81, 156), remarkably similar to those associated with Alzheimer's and Huntington's diseases (17). The fiber's width varies depending on the prion and solution conditions (range: 4–22 nm) but is proportional to the molecular weight of the monomer (5, 53, 69, 81, 138, 156, 162). Fibers can be short or tens of microns long (53, 69, 162). Like other amyloids, prion fibers are rich in β-sheet structure (53, 69, 156) and exhibit a cross-β-pleated-sheet pattern by X-ray diffraction (5, 140).

Biochemical characterization of prion amyloid formation provided remarkable insight into the probable molecular mechanism by which prions arise and propagate in vivo. Just as prions arise spontaneously, but infrequently, in cells (2, 8, 38, 105, 108, 176), each prion protein forms fibers spontaneously, but slowly, in vitro (53, 69, 146, 156). Mutations that increase or decrease the rate of prion induction in vivo correspondingly affect fiber formation in vitro (44, 69, 105). Because most fungal prions are mitotically stable, some process must facilitate efficient conversion of nascent prion proteins into the prion conformation. Remarkably, small amounts of pre-existing amyloid can promote rapid conversion of unpolymerized prion proteins into fibers in vitro (53, 69, 81, 146, 156). That this reflects prion propagation in vivo is evidenced by the fact that lysates from [*PSI*$^+$] cells, but not [*psi*$^-$] cells, accelerate Sup35 fiber formation (69, 124, 168). Thus, amyloid formation in vitro serves as a model for how prions act as heritable elements in vivo.

Prions Require Sustained Expression of Their Determinant Genes, Yet Their Phenotypes Often Mimic Loss-of-Function Mutations

Although the fungal prions act as genetic elements, their maintenance depends upon continual expression of the genes encoding them (176). Poor or interrupted expression leads to the eventual loss of the prion (99, 147, 176). It is not surprising, therefore, that *SUP35*, *URE2*, and *RNQ1* are all expressed at nearly constant levels throughout the cell cycle (43, 150).

The conformational change to the prion state often decreases the protein's activity. Aggregation may sequester a prion from its substrate or impede its proper

localization within the cell. Thus, the phenotypes of prions mimic loss-of-function mutations in their prion determinants (176). As with *ure2* mutants, nitrogen catabolite repression is impaired in [*URE3*] strains (54, 96, 176). Similarly, the nonsense-suppression phenotype of [*PSI*$^+$] reflects a partial loss of Sup35 activity (122, 123). However, not all prion phenotypes mimic loss-of-function mutations. A strain carrying a prion can be phenotypically indistinguishable from one lacking the prion, particularly when no obvious phenotype is associated with its determinant. Such is the case with [*RNQ*$^+$] and [*rnq*$^-$] strains (147). The portion of a protein that facilitates prion conversion is often distinct from the portion that is enzymatically active; thus, conversion to the prion conformation may not significantly affect an enzyme's activity, particularly if its substrate is diffusable (99, 111). In one case, the phenotype associated with cells in which the determinant is disrupted is opposite that of a prion. *P. anserina het-s*^{0} strains, in which the *het-s* locus was inactivated by gene replacement, are neutral in compatibility assays (166), unlike [Het-s] prion strains, which are incompatible with [Het-S] strains.

Dominant, Non-Mendelian Segregation and Cytoplasmic Transmission

Unusual genetic properties are a hallmark of fungal prions. [*URE3*], [*PSI*$^+$], and [*RNQ*$^+$] are dominant traits that display non-Mendelian inheritance (38, 45, 48, 96). In matings between haploid [*PRION*$^+$] and [*prion*$^-$] yeast cells, the resulting diploid cells are [*PRION*$^+$], and when the diploid is sporulated all four spores are usually [*PRION*$^+$], although not always (2, 96, 100, 176). The peculiar inheritance of [*PSI*$^+$] and [*URE3*] led yeast geneticists to conclude that these elements were transmitted cytoplasmically. This was confirmed for [*PSI*$^+$] and [*URE3*] and later for [*RNQ*$^+$] by using strains that have a nuclear mutation that allows the cytoplasm of the two cells to mix upon mating but prohibits fusion of their nuclei (cytoduction) (2, 31, 40, 147). Subsequent experiments demonstrated that [*PSI*$^+$] and [*URE3*] were not caused by other cytoplasmically transferred genetic determinants, such as mitochondrial DNA, viruses, or plasmids (68, 96, 97, 164, 185).

The dominant, non-Mendelian, and cytoplasmic character of these unusual genetic elements is consistent with a self-perpetuating protein-based mechanism of inheritance. The prion conformation is self-propagating, unlike most protein aggregates. During mating, haploid yeast fuse, mixing cytoplasmic contents and allowing the prion conformation to convert proteins from the [*prion*$^-$] cytoplasm into the prion conformation. Upon cell division, the prion conformation is passed from the mother cell to the daughter cell through the cytoplasm.

The [Het-s] prion of *P. anserina* can also be transferred cytoplasmically (8, 35). Anastomosis is a fungal process, analogous to yeast cytoduction, by which hyphae fuse to create a network. This causes their cytoplasms to mix. When a [Het-s] strain undergoes anastomosis with a neutral [Het-s*] strain, the resulting mycelium contains the prion [Het-s] (8, 35). Cytoplasmic transmission also affects [Het-s] propagation during mating and meiosis. Because the female parent contributes

most of the cytoplasm, nearly all meiotic progeny of a cross between [Het-s] and [Het-s*] strains have the phenotype of the female parent (8, 134).

Prion-Determining Region (PrD)

Sup35 contains three regions that are distinguished by their function and amino acid composition (78, 93, 94, 157, 180). The amino-terminal region, termed Sup35N (amino acids 1 to 123), is unusually rich in Gln (28%) and Asn (16%) residues, but has few aliphatic amino acids (e.g., alanine and valine, 6%) (Figure 5). By comparison, the average protein has just 9% Gln and Asn (133) and 29% aliphatic residues (112). Sup35N also contains several imperfect oligopeptide PQGGYQQ_YN repeats that are similar in character to PHGGGWGQ repeats present in the mammalian prion protein, PrP (85, 105, 120). These repeats are the only immediately obvious similarity between Sup35 and PrP protein sequences. The middle region, Sup35M (amino acids 124 to 253), is highly charged, unlike the rest of the protein. Forty-one percent of the residues in this region are lysine, glutamic acid, or aspartic acid. Sup35NM is not required for viability (160); however, Sup35C (amino acids 254 to 685) is essential (91, 160). Sup35C is homologous to the translation elongation factor EF-1α, contains four putative GTP-binding sites, and is sufficient for translational termination (78, 94, 152, 180).

Ure2 and Rnq1 are also Gln- and Asn-rich proteins. Each can be subdivided into two functionally distinct regions. Ure2N [amino acids 1 to 93 (126, 162)] contains more Asn (36%) than Gln residues (11%) (Figure 5), and only 13% of its residues are aliphatic. The remainder of the protein, Ure2C (amino acids 94 to 354), encodes the nitrogen regulatory activity of Ure2 (33) and is homologous to bacterial glutathione S-transferases (GST), although it lacks GST enzymatic activity (13, 29, 33, 167). The Rnq1C (amino acids 153 to 405) has many Asn (16%) and Gln (27%) residues and contains few aliphatic residues (8%) (147). Rnq1N (amino acids 1 to 152) is not similar to any other protein and its function is unknown (147). Neither Ure2 nor Rnq1 are essential for viability (33, 147) and neither contains oligopeptide repeats similar to those of Sup35 or PrP.

Remarkably, distinct regions of Sup35, Ure2, and Rnq1, which are similar in character but not identical in sequence, enable these protein to act as prions and are called prion-determining regions (PrDs). Mutational analysis delimited the regions of Sup35 and Ure2 necessary for [*PSI*$^+$] and [*URE3*]. When N-terminal segments are deleted, [*PSI*$^+$] and [*URE3*] are irreversibly cured (111, 159). Moreover, those N-terminal fragments of Ure2 and Sup35 are sufficient to support prion induction and propagation (110, 159). Although Sup35M is not required for [*PSI*$^+$], it appears to play a role in [*PSI*$^+$] stability and Sup35 solubility (69, 104, 160). That Ure2C is dispensable for prion propagation was demonstrated by passaging the prion through strains lacking this region (110).

sup35 and *ure2* mutants that affect prion induction and propagation cluster to their N-terminal regions (44, 50, 52, 62, 105, 109, 111, 120). Most reduce the Asn and Gln content of these proteins or the number of oligopeptide repeats in Sup35,

indicating that these two characteristics are important for these regions to function as PrDs. The absolute ratio of Asn to Gln is not crucial because [*PSI*$^+$] can be induced by overproducing a fusion of a mutant Sup35N region to GFP, in which a polyGln stretch replaced an Asn- and Gln-rich stretch (44).

Because prions are induced more frequently when their determinants are overexpressed, several groups measured the efficiency with which [*PSI*$^+$] and [*URE3*] arose when various prion protein subfragments were overproduced. Elevated expression of any fragment containing Sup35N or Ure2N increases the rate of prion induction (24, 50, 83, 109–111, 176). These fragments induce prion formation much more efficiently than full-length versions expressed at similar levels (50, 83, 109, 111). Together with the mutational analysis, these studies showed that the N-terminal regions of Sup35 and Ure2 are required for [*PSI*$^+$] and [*URE3*].

Because [*RNQ*$^+$] strains are phenotypically indistinguishable from [*rnq*$^-$] strains, the region of *RNQ1* that constitutes its PrD was ascertained using a novel approach. Sondheimer & Lindquist (147) reasoned that if Rnq1C, which is rich in Asn and Gln, was the PrD, it would functionally substitute for Sup35's endogenous PrD. Indeed, they found that a [*PSI*$^+$]-like state is induced in strains expressing Rnq1C fused to SupMC.

Other studies further demonstrated that the PrDs of yeast prions are modular and transferable. Fusions of the PrDs of Sup35, Rnq1, and Ure2 to GFP can join preexisting prion aggregates in vivo (57, 122, 147). In [*PSI*$^+$], [*RNQ*$^+$], or [*URE3*] cells, expression of these fusions leads to discrete fluorescent foci; whereas, in [*psi*$^-$], [*rnq*$^-$], and [*ure-o*] cells, the fluorescence is evenly distributed throughout the cytoplasm. In another case, the PrD of Sup35 was fused to a completely unrelated protein to create a novel chimeric prion. The mammalian glucocorticoid receptor (GR), a hormone-regulated transcriptional activator, was fused to the PrD of Sup35 and expressed in a strain bearing a GR-regulated *lacZ* reporter. This fusion protein can exist in two stable but interchangeable functional states, only one of which activated transcription of the reporter (99). Importantly, the fusion protein exhibited many of the unusual genetic characteristics of known yeast prions, and it converted to the nonfunctional state on its own in a mutant [*psi*$^-$] strain in which the endogenous Sup35 PrD was deleted.

The 289-amino-acid HET-s protein is the only identified fungal prion not Asn or Gln rich (166) and without a well-defined PrD. To identify regions important for conversion to the [Het-s] prion state and for heterokaryon incompatibility, a series of *het-s* deletion mutants were ectopically expressed in a *het-s*^{0} strain (36). An N-terminal fragment (amino acids 1 to 112) is sufficient for incompatibility and for conversion to [Het-s], suggesting that it contains the PrD. Consistent with this interpretation, point mutations in this region affect prion propagation (36). Further analysis revealed that a C-terminal fragment (amino acids 86 to 289) also mediates incompatibility and conversion. Although this suggests that the 27-amino-acid region common to both fragments is the minimal PrD, a fragment lacking only that region also supports incompatibility and conversion. Thus, no specific PrD was identified. Interestingly, an even shorter N-terminal fragment (amino acids

1 to 25) propagates the [Het-s] phenotype (36) but cannot mediate heterokaryon incompatibility.

PRION CANDIDATES

Yeast and filamentous fungi have other traits with unusual genetic properties that resemble those of prions (Figure 4). Although they are not yet characterized enough to judge whether they are caused by prions, all can be transmitted cytoplasmically. Most display non-Mendelian inheritance and are metastable and curable. However, some of their characteristics, such as not being cured by GuHCl treatment, are unlike those of known prions. If any are prions, those unique characteristics will broaden the criteria for identifying prions and aid the search for novel prions.

[*KIL-d*]

The [*KIL-d*] element epigenetically regulates viral gene expression in haploid *S. cerevisiae* cells (154, 155, 175). The killer virus is composed of two double-stranded RNAs, called L-A and M, that are transmitted cytoplasmically between yeast. L-A encodes for the virus's replication machinery and is required to maintain its satellite, M, which encodes a secreted toxin that kills surrounding uninfected yeast and a pre-toxin that provides the infected cell resistance to the toxin it produces. Yeast infected with L-A and M are phenotypically described as killer (K+) and resistant to toxin (R+). Uninfected yeast and those infected with only L-A are phenotypically nonkiller (K−) and susceptible to toxin (R−) [reviewed in (177)].

Like the fungal prions, [*KIL-d*] is a cytoplasmically transmitted trait (155). It causes haploid yeast infected with killer virus (both L-A and M) to display variegated, defective killer phenotypes (K*R*), such as being defective in killing or resistance or both (e.g., K−R+, K+R−, K−R−) and losing the M satellite at a higher rate. The [*KIL-d*] element does not map to L-A or M (155), mitochondrial DNA (155), or the yeast 2-μm plasmid (154). Like known prions, [*KIL-d*] is metastable. It is lost at a frequency of 10^{-4} to 10^{-5} and arises spontaneously at a rate of 10^{-3} (154, 175).

Some characteristics of [*KIL-d*] are unlike those of established fungal prions. [*KIL-d*] is not cured by GuHCl treatment (155), is recessive in the diploid (175), and is not dependent on the molecular chaperone, Hsp104. [*KIL-d*] persists in strains where the chromosomal copy of *HSP104* is deleted or in strains overexpressing *HSP104* (154).

Peculiarly, a cell only manifests the [*KIL-d*] variegated killer phenotype after it undergoes meiosis (175). [*KIL-d*] is phenotypically cryptic in diploids and in haploids that have not passed through meiosis. Mating a [*KIL-d*] strain harboring M to a nonkiller wild-type strain results in a diploid strain with the killer and resistance phenotypes (K+R+). After the diploids sporulate, however, their haploid meiotic progeny exhibit a range of pleiotropic, defective phenotypes that are indicative of [*KIL-d*]. Backcrossing any of the defective progeny (K*R*) generates diploid

cells that are killer and resistant (K+R+). The meiotic progeny of this diploid again exhibit various degrees of killer and resistance defects (175). [*KIL-d*] can be transmitted by cytoduction, but is cryptic in the haploid cytoductants. Diploids formed by mating these cytoductants to a wild-type uninfected strain are also killer and resistant (K+R+). The meiotic progeny, however, display the range of killer and resistance phenotypes that are characteristic of [*KIL-d*] (155). Thus, [*KIL-d*] is transferred through the cytoplasm but its phenotypic expression requires cells first undergo meiosis.

The molecular determinant of [*KIL-d*] is unknown, precluding any direct biochemical assessment of whether it can adopt the prion conformation. One hypothesis is that [*KIL-d*] is a prion-like element whose phenotypic expression is "reset" by meiosis and "healed" by nuclear fusion (155). If so, the molecular determinant of [*KIL-d*] would alter expression of a nuclear chromosomal target, which epigenetically regulates M expression. Thus, the prion conformation underlying [*KIL-d*] would only access its chromosomal target during meiosis. Variation in the extent of this interaction would cause the variegated phenotypes typical of [*KIL-d*] meiotic progeny. Finally, because all [*KIL-d*] strains were isolated after ethyl methanesulfonate mutagenesis of a killer-infected yeast strain, the determinant of [*KIL-d*] may be a mutant more likely to spontaneously adopt the prion conformation, but effectively convert the wild-type protein into the prion. Some *sup35* and *ure2* mutants similarly affect [PSI^+] (105) and [*URE3*] (62).

[C^+]

Crippled growth, [C^+], is a cytoplasmically transferable trait of *P. anserina* that impairs mycelial growth, hence its name (144). It also causes abnormal hyphal morphology, pigment accumulation, shortened longevity, and reduced female fertility (144). Like known prions, [C^+] is metastable. Wild-type strains become [C^+] once they enter stationary phase. However, strains with certain ribosomal protein mutations that augment accurate translational termination become [C^+] during vegetative growth (144). Agents that stress cells, including high and low temperatures, high osmotic pressure, nutrient deprivation, and ultraviolet irradiation, cure [C^+] (144) and some also cure known prions (9, 35, 137, 145, 165). Some drugs that impair translational termination also cure [C^+] from vegetatively growing cells, suggesting that termination read through produces a factor that impairs [C^+] propagation (144). [C^+] can be efficiently transmitted during mitosis; however, it is inefficiently propagated through meiosis (144), possibly because its molecular determinant, which is unknown, is not expressed during meiosis. There is no evidence of mitochondrial DNA mutations or virus-like elements in [C^+] strains that could explain the unusual genetic behavior of this element (144).

[ISP^+]

[ISP^+], inversion of suppressor phenotype, is another prion-like trait involved in regulating translation termination in *S. cerevisiae* (169). This non-Mendelian element abrogates the nonsense-suppression phenotype of certain recessive *sup35* mutations. GuHCl treatment cures [ISP^+] strains but the element reappears

spontaneously at a high frequency in those cured strains. [*ISP*$^+$] propagation does not depend on Hsp104, however. [*ISP*$^+$] is not induced by expressing the mutant *sup35* allele and it can be propagated in strains lacking the PrD of Sup35. Moreover, Sup35 is not aggregated in [*ISP*$^+$] cells. Thus, [*ISP*$^+$] interacts with some *sup35* mutants but is not a form of [*PSI*$^+$].

[*GR*]

[*GR*] is a cytoplasmically transferable trait of *S. cerevisiae* that provides resistance to glucosamine, a glucose analog that inhibits growth on nonfermentable carbon sources (6, 60). [*GR*] is a dominant, non-Mendelian element that is not caused by mitochondrial DNA, killer virus, [*PSI*$^+$], or [*URE3*]. It is not cured by heat or cycloheximide treatment, which eliminate the killer virus (87).

GRR1 is a plausible candidate for the determinant of [*GR*] (146). Grr1 is a component of the ubiquitin proteolysis machinery and appears to regulate the glucose signaling pathway and cell cycle progression (98). Grr1 contains several runs of polyAsn residues at its N and C termini. *grr1* deletion strains exhibit pleiotropic phenotypes related to loss of degradation of key regulatory proteins, some of which, including glucosamine resistance, are also exhibited by [*GR*] strains (65, 146, 183).

[*PIN*$^+$]

[*PIN*$^+$] is non-Mendelian trait of *S. cerevisiae* that is required for efficient [*PSI*$^+$] induction when Sup35 is overproduced (47, 48). Because all yeast prions are efficiently induced when their determinant genes are overexpressed, Derkatch et al. (45) searched for genes that converted cells from [*pin*$^-$] to [*PIN*$^+$] when overexpressed. Twelve such proteins were identified (45), two of which were the prion forms of Ure2 and Rnq1 (45). Thus, having one prion in a cell increases the frequency that another will appear. The other proteins participate in diverse cellular processes. Swi1 and Cyc8 regulate transcription, Yck1 and Ste18 play roles in signal transduction, Nup116 is important for nuclear transport, and Lsm4 is involved in mRNA processing (1, 45). *NEW1*, *PIN2*, *PIN3*, and *PIN4* are uncharacterized genes. All encode Gln- or Asn-rich proteins with obvious similarity to the PrDs of Sup35, Rnq1, and Ure2. Whether any form stable prions is unclear; however, the Asn-rich region of New1 is part of the determinant of [*NU*$^+$], another prion-like element (133).

[*NU*$^+$]

Because prion domains are modular and transferable, replacing Sup35's PrD with other proteins and assaying for [*PSI*$^+$]-like behavior is a powerful method to identify new prion candidates (147). [*NU*$^+$], whose putative prion determinant is encoded by an N-terminal fragment of the *NEW1* gene fused to Sup35C, was identified in this manner (133). The New1-Sup35C fusion can adopt two

biochemically and functionally different states (133). New1 was analyzed because it is Gln and Asn rich and has several oligopeptide repeats reminiscent of those of Sup35 and PrP (133). [*NU*$^+$] can be transferred by cytoduction (118), cured by GuHCl treatment (118), and cannot be isolated in cells lacking *HSP104* (118). However, Hsp104 overproduction has no effect (119).

Although New1-Sup35C behaves like a prion, it is unclear whether the full-length wild-type New1 ever exists as a stable prion. [*NU*$^+$] cannot be supported by endogenous New1, but requires expression of New1-Sup35C (118). *NEW1* is a determinant of [*PIN*$^+$], however, suggesting that it can convert to a prion-like state (45, 118).

PRION STRAINS

One of the most controversial aspects of mammalian prions is the existence of distinct TSE strains, which differ in their disease latency periods, brain pathologies, neuropathological manifestations, and distribution of PrP^{Sc} in brain tissues (14, 16). Some clinically distinct human prion diseases are caused by mutations in the prion protein gene, *Prnp* (129). However, prion strains cannot be due to any mutations in *Prnp* or elsewhere in the mouse genome because they are passaged in the same inbred mice background. Opponents of the protein-only hypothesis argue that a viral TSE agent best explains prion strains (15, 51, 139); proponents posit that PrP^{Sc} conformational differences cause strains (20, 129, 139). Such conformational variation is present because different positions of cleavage occur upon protease treatment of infectious material from different strains [reviewed in (20, 30)]. Whether this is the underlying basis of mammalian prion strains remains unclear.

[*PSI*$^+$] and [*URE3*] can also exist as different strains (38, 50, 137), which are sometimes called "variants" to avoid confusion with the many yeast genetic backgrounds (strains) in which prions are passaged. [*PSI*$^+$] variants were discovered first and are the best characterized. *SUP35* overproduction in [*psi*$^-$] cells induces new [*PSI*$^+$] strains that exhibit a wide range of translational termination defects, despite having arisen in genetically identical cells (50). When any of these [*PSI*$^+$] variants are cured and *SUP35* is overexpressed, each [*psi*$^-$] derivative can give rise to [*PSI*$^+$] cells with the same range of termination defects. Thus, mutations in *sup35* or any other yeast gene do not cause [*PSI*$^+$] variants. They are epigenetic.

Most [*PSI*$^+$] variants are distinguished by their nonsense-suppression phenotypes and are called strong, moderate, or weak depending on their severity (50). Usually, weak variants do not spontaneously become strong or vice-versa; however, one such example was described (82). Compared to strong [*PSI*$^+$] variants, weak variants are less stable and are more easily cured by GuHCl treatment or by *HSP104* overexpression (50). Strong [*PSI*$^+$] is dominant to weak [*PSI*$^+$] in genetic crosses (49). The extent of Sup35NM-GFP aggregation differs among [*PSI*$^+$] variants. Many more strong [*PSI*$^+$] cells have punctate fluorescent foci than weak [*PSI*$^+$] cells (49). A few [*PSI*$^+$] variants can only be differentiated by their

translational termination phenotypes when certain *sup35* mutations are expressed (49, 80).

[*PSI*$^+$] variants can also be distinguished by reproducible differences in the amount of soluble Sup35 in vivo and by the efficiencies with which they mediate conversion to the prion state in vitro (168). Strong [*PSI*$^+$] cells have much less soluble Sup35 than weak [*PSI*$^+$] cells, yet the total amount of Sup35 is the same (82, 168, 188). In cell-free conversion assays, the prion proteins from some [*PSI*$^+$] variants convert Sup35 to the prion state much more efficiently than others, differing by as much as 20-fold (168). Importantly, those that convert most efficiently originate from [*PSI*$^+$] variants with the most severe termination defects and the least soluble Sup35 in vivo (168).

These results led Uptain et al. (168) to propose a model that explains the phenotypic characteristics of [*PSI*$^+$] variants in molecular terms. The severity of a variant's translational defect is determined by the amount of soluble, functional Sup35 in the cells. The less soluble Sup35, the more pronounced the termination defect. The amount of soluble Sup35 is determined by how well the prion conformation of Sup35 captures and converts soluble Sup35 to the insoluble prion state. In strong [*PSI*$^+$] variants, conversion is efficient and consequently the amount of soluble Sup35 is low. In weak variants, conversion is less efficient, more soluble Sup35 is present, and the variants exhibit milder termination defects. Although prion strains were first discovered in mammals, the different physical states of PrP in vitro have not been correlated with the phenotypes associated with prion strains, primarily because the cellular function of PrP is unknown. Thus, this model for [*PSI*$^+$] variants provides the first coherent molecular explanation for the phenotypes of prion strains.

Whereas [*PSI*$^+$] variants have been observed for decades (38, 50, 100), [*URE3*] variants were described only recently (137). Three types of [*URE3*] variants were identified when Ure2 or its PrD was overproduced in [*ure-o*] cells (137). A novel reporter system involving the adenine biosynthesis pathway was developed to detect them (137). On rich media, Ade2-deficient cells are red because they accumulate a red metabolic intermediate, whereas wild-type cells are white. When *ADE2* expression is controlled by the *DAL5* promoter, [*ure-o*] colonies are red because Ure2 keeps Gln3 in the cytoplasm, the *DAL5* promoter is not activated, and *ADE2* is not expressed. Most [*URE3*] strains are pink or white. Type A [*URE3*] variants are pink and more difficult to cure than type B variants, which are redder. Type C variants are red and unlike the other two types of variants, they have soluble Ure2 and cannot be cured by GuHCl treatment or transferred by cytoduction (137). Although the molecular basis of these variants is unknown, no differences in the prion conformation of Ure2 were detected by proteinase K digestion (137). One attractive possibility is that differences in prion conversion efficiency similar to that of Sup35 may underlie them (137, 168).

What might lead to the differences in conversion efficiencies in yeast prion variants? Prion proteins may adopt conformations with intrinsically different abilities to convert the nonprion form into the prion form. Indeed, purified Sup35NM

can form amyloid fibers with different structural states in vitro (69). Similarly, a Sup35NM chimera derived from two yeast species forms fibers with distinct physical properties in vitro related to different [*PSI*$^+$] variants in vivo (28). In the experiments of Uptain et al. (168), no conformational differences were observed between fibers nucleated by Sup35 from different wild-type [*PSI*$^+$] variants; however, in a second round of seeding these fibers did not retain their initial characteristic differences in conversion efficiency. Thus, if different Sup35 conformations cause [*PSI*$^+$] variants, they are not retained in the cell-free system. Perhaps interactions between the prions and other cellular factors, which might be stochastic in origin but self-perpetuating in nature, maintain variants in vivo. Alternatively, if prion aggregates of stronger variants were smaller and more numerous than those of weaker variants, they might convert soluble Sup35 more effectively and be propagated more faithfully in vivo, but not be sustainable in vitro.

YEAST PRIONS AS PROTEIN-BASED HERITABLE ELEMENTS

The ability of fungal prions to act as protein-based elements of inheritance depends upon two key processes: *induction*, whereby a protein spontaneously converts to the prion state, and *propagation*, which depends upon efficient, continual conversion of newly synthesized prion protein and partitioning of the prion conformation to daughter cells before cytokinesis. Since Wickner's (176) remarkable proposal that [*URE3*] and [*PSI*$^+$] are prions, an explosion of studies have provided notable insight into the mechanism of induction and propagation. Indeed, today much more is known about induction and propagation of yeast prions than mammalian prions (19).

Induction, Propagation, and Curing

Any model for prion induction must account for several general observations. The rate of spontaneous induction of [*PSI*$^+$], [*URE3*], [*RNQ*$^+$], and [Het-s] is slow, typically 10^{-5} to 10^{-7} (2, 8, 38, 105, 108, 176). However, induction frequency increases dramatically when the prion determinant or fragments containing the PrD are overproduced, depending on the level of overproduction and amino acid context of the PrD (22, 24, 35, 50, 109, 111, 147, 176). High-copy expression plasmids induce [*PSI*$^+$] more efficiently than low-copy plasmids (50). At the same copy number, plasmids expressing a certain region of Sup35N (amino acids 1 to 114) induce [*PSI*$^+$] more efficiently than other fragments (50, 80, 83).

Induction seems to involve a maturation period, during which nascent prions are particularly unstable. [*PSI*$^+$] and [*RNQ*$^+$] are unstable when newly induced and segregate [*prion*$^-$] and stable [*PRION*$^+$] clones (45, 47). Stabilizing the prion state may be more difficult for some prion-like proteins than others, and some may never complete this maturation period (45, 118). Perhaps a certain number of

conversion events must occur to cross a threshold of stabilization. Alternatively, some proteins may adopt the prion conformation more readily than others.

Tremendous insight into how prions are induced and propagated came from studying the self-perpetuating conversion of prions in vitro. Conformational conversion of prion proteins into amyloid fibers proceeds by a cooperative process, which can be subdivided into a lag phase, during which nuclei form, and a templated assembly phase, which is characterized by rapid conversion of soluble proteins as it interacts with nuclei (53, 69, 81, 138, 140, 147, 162). The lag phase can be quite long and is reminiscent of the slow rate of spontaneous prion induction in vivo. It can be shortened, or even eliminated, by adding small amounts of preformed converted protein at the onset, mimicking how pre-existing prions convert newly synthesized protein in vivo.

The processes that convert Sup35 and Ure2 into amyloid in vitro and produce [*PSI*$^+$] and [*URE3*] in vivo are certainly closely related. Addition of [*PSI*$^+$], but not [*psi*$^-$], lysates greatly accelerates conversion of Sup35NM (69, 124, 168). Mutations that increase or decrease the rate of prion induction correspondingly alter the rate of amyloid formation in vitro (44, 105). The structural characteristics of Sup35 appear to be optimized to allow conversion (135). The optimal temperature for Sup35NM assembly into amyloid is 25–30°C, coinciding with the optimal growth temperature of yeast (135). Finally, [*PSI*$^+$] can be induced, albeit inefficiently, by introducing purified, recombinant Sup35NM into the yeast cytoplasm using a liposome transformation method (149). Although the conformational state of the Sup35NM used in the protein transformation experiments could not be determined, it also accelerated Sup35NM conversion in vitro.

Although prion proteins form amyloid fibers in vitro, it is not clear if the mature prion conformation in vivo is amyloid fibers. Amyloid-like filaments were detected by thin-section electron microscopy and immunogold-labeling techniques in [*URE3*] cells that overproduce Ure2, but not in [*URE3*] cells with wild-type Ure2 levels or in [*ure-o*] cells (151). Attempts to detect Sup35 filaments in [*PSI*$^+$] cells expressing wild-type levels of Sup35 were inconclusive (A. Kowal & S. Lindquist, unpublished data). Fluorescent, ribbon-like structures are observed in [*PSI*$^+$] (187) and [*URE3*] cells (137) when prion-GFP fusions are overproduced. Thus, the prions can promote the formation of amyloid-like filaments in yeast when highly overproduced, but perhaps not under wild-type conditions. Perhaps, one or more of the structural intermediates identified during formation of prion amyloids in vitro might constitute the prion state in vivo (67, 140, 161).

Two subregions of Sup35N crucial for conversion were identified through mutations. Mutant proteins containing fewer Gln and Asn residues than wild type between amino acids 8 and 24 do not propagate [*PSI*$^+$] (44). Deletion of an oligopeptide repeat or even certain point mutations within one interfere with [*PSI*$^+$] propagation (52, 105, 120). Some mutant proteins convert too slowly to propagate the prion state (44, 105, 120). Others can dominantly interfere with incorporation of wild-type protein into the prion aggregate, and [*PSI*$^+$] is cured when those mutants are coexpressed with wild-type Sup35 (44, 52, 184).

Although no equivalent *ure2* mutants are known, [*URE3*] propagation can be efficiently impeded by highly expressing Ure2N-, Ure2C-, or Ure2-GFP fusions (57). This is a particularly perplexing phenomenon because overexpressing full-length *URE2* efficiently induces [*URE3*] (176). Adding to the complexity, those same fragments or GFP fusions do not cure when overexpressed at low levels (57). Possibly, the overproduced proteins interfere with [*URE3*] propagation by interacting with wild-type Ure2 prion aggregates. Alternatively, a factor that cures the prion, such as a molecular chaperone, may be induced, or a limiting factor necessary for [*URE3*] propagation may be consumed.

One mystery is how prions are partitioned from one cell to another. Clearly, this process is efficient. Most yeast prions are mitotically stable and [*prion*$^-$] cells rarely appear spontaneously (38, 96). It is widely assumed that the partitioning of prion particles occurs stochastically as a portion of the yeast cytoplasm containing many prion particles is passed from mother cell to daughter. However, prion aggregates might segregate by an active mechanism, perhaps analogous to organelle partitioning (18, 170). Interestingly, [*PSI*$^+$] strains lacking Sla1, a protein that interacts with Sup35 and is involved in cortical actin polymerization, are more easily cured than wild-type [*PSI*$^+$] strains (3). Sla1 may help partition prions to daughter cells, but Sup35NM-GFP fusions do not colocalize with actin patches in [*PSI*$^+$] cells (4).

A remarkable yet poorly understood feature of fungal prions is their different rates of spontaneous loss. [*PSI*$^+$] is usually more stable than [*URE3*] during meiosis (38, 96). Even the stability of prions formed from the same determinant can vary. Notably, weak [*PSI*$^+$] variants become [*psi*$^-$] more often than isogenic strong [*PSI*$^+$] variants (50). For example, most strong [*PSI*$^+$] elements segregate to 100% of meiotic progeny (38); however, one form of [*PSI*$^+$], initially called [*ETA*$^+$], segregates to only 70%–85% (100, 188). This instability may relate to the intrinsically lower prion conversion rates in weak [*PSI*$^+$] variants (168). A few *sup35* mutants, including one lacking Sup35M, reduce the mitotic stability of [*PSI*$^+$] when they replace the wild-type *SUP35* allele (12, 25, 88, 104). Usually, prions are equally stable during meiosis and mitosis, although in rare instances these can be uncoupled (74).

FACTORS THAT MODULATE PRION INDUCTION FREQUENCY Certain mutations in prion determinants or in other genes increase the rate of spontaneous prion induction. The most effective *sup35* mutant tested contains two additional oligopeptide repeats. [*PSI*$^+$] arises 5000-fold more frequently in this mutant background (105). Compared to wild-type Sup35, this mutant protein is less structured and forms amyloid fibers more readily in vitro (105, 135). A Sup35 mutant lacking some of the repeats was more structured than wild-type and underwent conversion much less efficiently (105, 135). This and other work suggest that conformational flexibility increases prion induction frequency. Several *ure2* mutants increase the rate of [*URE3*] induction, one (*h2*) as much as 1000-fold over the wild type (62). Unexpectedly, most of the 10 amino acid substitutions in h2 that affect induction

map outside the PrD. Perhaps these mutations destabilize normal Ure2 interactions with itself or with other proteins, allowing the PrD to exert a stronger influence on conversion.

[*PIN*$^+$] is a non-Mendelian element required to induce [*PSI*$^+$] efficiently but is not caused by *SUP35* and is independent of [*PSI*$^+$] (47, 48). Overproducing Sup35 readily converts [*PIN*$^+$] cells, but not [*pin*$^-$] cells, to [*PSI*$^+$] (48). [*PIN*$^+$] is unnecessary for [*PSI*$^+$] propagation and does not affect the translational defect of [*PSI*$^+$] cells (47). [*PIN*$^+$] is a dominant, cytoplasmically transferable element (45, 48) that occurs spontaneously at a low frequency and is cured by treating cells with GuHCl or deleting *HSP104* (47).

Remarkably, [*PIN*$^+$] is not caused by just one prion protein; rather, it is a susceptibility state in which the presence of any one of several different prions makes it more likely that a second prion will form (45, 118). One hypothesis is that one protein in the prion state can, with low efficiency, nucleate conversion of another to the prion state through a direct interaction (45, 118). Preliminary work with New1 and Sup35 failed to detect heterotypic interactions or cross-nucleation (118). Alternatively, a prion may titrate a factor that ordinarily impedes another prion protein's conversion. Because of their impact on [*PSI*$^+$], molecular chaperones are obvious candidates for such factors. Whether some prions can arise spontaneously without any other prion remains unclear. [*RNQ*$^+$] induction also apparently requires a [*PIN*$^+$] factor, such as [*PSI*$^+$] or [*URE3*]. Punctate fluorescent foci appear in [*PSI*$^+$][*rnq*$^-$] or [*URE3*][*rnq*$^-$] strains when Rnq1-GFP fusions are overexpressed; however, fluorescence is diffuse in [*psi*$^-$][*rnq*$^-$] or [*ure-o*][*rnq*$^-$] strains (45).

Unlike [*PIN*$^+$] determinants, some yeast proteins affect induction of specific prions. One such example is Sup45, which interacts with Sup35 to mediate translational termination (61, 66, 125, 152). Sup45 does not affect [*PSI*$^+$] propagation; however, *SUP45* overexpression inhibits [*PSI*$^+$] induction when *SUP35* is also overexpressed (46). By binding to Sup35, Sup45 may stabilize the nonprion state and thereby interfere with conversion (46). If so, this interaction need not affect prion propagation if conversion occurs before Sup35 associates with Sup45 or if subunit exchange is dynamic in Sup35-Sup45 complexes.

Sla1 is a Gln-rich protein involved in cortical actin assembly in yeast. Two-hybrid analysis identified an interaction between the Gln-rich regions of Sla1 and Sup35N that depends on *HSP104* expression (3). Sup35 and N-terminal fragments induce [*PSI*$^+$] less efficiently in *sla1* deletion strains than in wild-type strains (3). [*PSI*$^+$] propagation, [*PSI*$^+$]-mediated translational read through, and the amount of soluble Sup35 are unaffected in *sla1* deletion strains (3). Sla1 may play some role in translation because *sla1* deletion strains are sensitive to translational inhibitors (3). If Sla1 interacts with some aspect or component of translation that also interacts with Sup35, *SLA1* overexpression may titrate factors that normally limit Sup35 conversion. Alternatively, Sla1 may promote prion induction by interacting with Sup35.

As noted earlier, Hsp104 expression is required for all well-characterized yeast prions. Other chaperones can affect induction of [*PSI*$^+$], and perhaps induction of

other prions. Two Hsp70 members, *SSB1* and *SSB2*, associate with the ribosome and nascent polypeptide chains and may function in cotranslational protein folding (11, 116, 127). Intriguingly, [*PSI*$^+$] spontaneously arises 10 times more often in *ssb1, ssb2* deletion strains than in wild-type strains (27). Perhaps conformational conversion to the prion state occurs cotranslationally, and *SSB1* and *SSB2* chaperone Sup35 folding into the translationally active nonprion form.

At least one signal transduction pathway can modulate [*URE3*] induction. Mks1 negatively regulates *URE2* in the nitrogen catabolite repression cascade (58). In response to environmental cues, Mks1 acts indirectly to positively regulate *DAL5*, the permease that imports ureidosuccinate (58). Mks1 is unnecessary for [*URE3*] propagation because the prion can be transferred to a [*ure-o*] *mks1* deletion strain by cytoduction (59). However, the efficiency of [*URE3*] induction is higher in strains overexpressing *MKS1* and is several orders of magnitude lower in *mks1* deletion strains. Mks1 does not alter the steady-state levels of Ure2 (58), and the mechanism by which it modulates [*URE3*] induction is yet to be discovered.

AGENTS THAT CAN CURE FUNGAL PRIONS A great variety of chemical, environmental, and protein-based agents cure yeast prions. These agents differ in curing efficiency, action, and specificity. In principle, curing agents may impair conformational conversion, inhibit partitioning to the daughter cell, or both. [*PSI*$^+$] and [Het-s] strains are readily cured if grown on media containing various solutes at high osmotic strength (9, 35, 145, 165). In contrast, [*URE3*] is selectively cured by switching from minimal media with ureidosuccinate to rich media with other nitrogen sources (137). The observation that many agents that cure [*PSI*$^+$], such as 10% dimethylsulfoxide, 10% methanol, as well as high concentrations of potassium chloride, ethylene glycol, sodium glutamate, and glycerol, are not mutagenic was one of the first indications that [*PSI*$^+$] is not caused by a nucleic acid–based agent (39, 108, 145, 165). Although other curing agents, such as ethyl methanesulfonate and ultraviolet light, are known mutagens, they probably cure [*PSI*$^+$] by affecting *HSP104* expression rather than by mutating *SUP35* (26, 103).

GuHCl is the most frequently used prion-curing agent because it effectively cures [*PSI*$^+$] (165), [*RNQ*$^+$] (45, 48), and [*URE3*] (176). At molar concentrations GuHCl denatures proteins, but protein denaturation is unlikely to explain curing because millimolar concentrations are effective. GuHCl cures [*PSI*$^+$] only in proliferating cells and appears to block [*PSI*$^+$] proliferation without affecting preexisting prion aggregates (56). There is a lag phase of several generations before cells are cured, possibly because the numerous pre-existing prion particles are diluted with each cell division (56). It is unclear whether GuHCl cures by inactivating Hsp104 or by some other mechanism. Certainly, at low concentrations, GuHCl lowers the ATPase activity of Hsp104 in vitro (70). GuHCl also inhibits cells from acquiring thermotolerance and from refolding thermally denatured luciferase, two processes that require active Hsp104 (64, 75). However, the kinetics

by which GuHCl and *hsp104* deletion cure [*PSI*$^+$] may be different, which would suggest that these agents cure by different mechanisms (171).

The toxin latrunculin A (Lat-A) disrupts the actin cytoskeleton by sequestering monomeric actin (34), and cures some forms of [*PSI*$^+$] but not [*RNQ*$^+$] (4, 45). Because [*PSI*$^+$] can be propagated in *sla1* deletion strains (3), it is unlikely that Lat-A cures [*PSI*$^+$] by affecting Sla1. Moreover, the kinetics by which Lat-A disrupts the actin cytoskeleton and cures [*PSI*$^+$] are quite different, suggesting that different mechanisms of action underlie them (4). Because yeast cells do not divide in the presence of Lat-A, this drug may cure [*PSI*$^+$] by disrupting prion aggregates or by interfering with conversion (4).

The Role of Hsp104 in Prion Induction and Propagation

The molecular chaperone Hsp104 resolves thermally denatured proteins (70, 121). The *HSP104* gene was isolated in a genetic screen for factors that cure [*PSI*$^+$] when overproduced (26). When *HSP104* is highly expressed, most forms of [*PSI*$^+$] are efficiently cured (26). Remarkably, even transient high-level expression suffices (26). Moderate overexpression, however, does not cure efficiently, but it partially alleviates [*PSI*$^+$]-mediated nonsense suppression. Deletion of *HSP104* also cures (26). Thus, [*PSI*$^+$] propagation requires an intermediate level of Hsp104. [*URE3*] and [*RNQ*$^+$] are also cured when *HSP104* is deleted (45, 48, 114, 147). The dependence of these three prions on this protein-remodeling factor is strong evidence that prions are caused by protein-based agents.

Two models might explain why [*PSI*$^+$] requires an intermediate level of Hsp104. One postulates that Sup35 conversion proceeds through an unstable, oligomeric intermediate state promoted by Hsp104 (26, 103, 122, 140). In [*PSI*$^+$] cells, the intermediate state of Sup35 converts to the prion state when it associates with prion conformers. The intermediate state reverts to the nonprion state in [*psi*$^-$] cells and it is unlikely to form without Hsp104. Transient overexpression of *SUP35* efficiently induces [*PSI*$^+$] de novo because of the increased propensity of Hsp104 and Sup35 to interact. [*PSI*$^+$] propagation does not require Hsp104 to disaggregate Sup35 prion aggregates, although some disaggregation may occur when *HSP104* is highly overexpressed.

The other model posits Hsp104 simply disaggregates Sup35 prion aggregates, which creates many smaller aggregates that are more likely to be partitioned to the daughter cell (90, 123). Without Hsp104, fewer and larger aggregates accumulate, reducing the probability that enough prion proteins will partition to the daughter. High Hsp104 levels cure [*PSI*$^+$] cells simply because all prion aggregates are resolved. Unlike the first model, Hsp104 is not needed to convert Sup35 to the prion state.

Both models predict that Hsp104 interacts with Sup35. Stable complexes of Hsp104 with Sup35, or any other yeast prion, have not been detected; but there is indirect evidence for a transient interaction (136). Sup35 inhibits the ATPase activity of Hsp104 in vitro, and the circular dichroism spectrum of a mixture of

Hsp104 and Sup35 differs from that predicted by adding these proteins' individual spectra (136). Whether this interaction is necessary to convert Sup35 or to disrupt prion aggregates is less clear. Indeed, it may do either, depending on the presence of other chaperones. Consistent with the first model, Sup35 amyloid formation proceeds through an oligomeric state that forms without Hsp104 in vitro (69, 140). When Sup35-GFP fusions are expressed at low levels in [*psi*$^-$] *hsp104* deletion strains, no punctate fluorescent foci are visible. Expressing the fusion at higher levels overcomes the apparent requirement for Hsp104 (69). Finally, a greater proportion of Sup35 is soluble in [*PSI*$^+$] cells that moderately overexpress *HSP104* (123). This may indicate that Hsp104 disaggregates Sup35 or that Sup35 molecules are dispersed among more chaperone complexes, thereby lowering the number of oligomeric intermediates formed.

One apparent difficulty with the disaggregation model is that high *HSP104* expression does not cure [*URE3*] and [*RNQ*$^+$] (45, 48, 114). On the other hand, two observations support the disaggregation model. First, Sup35NM-GFP foci become larger and fewer, and [*PSI*$^+$] is rapidly cured when Hsp104 levels are reduced (171). Second, in a *sup35* mutant strain in which three oligopeptide repeats are disrupted, [*PSI*$^+$] is unstable and a greater proportion of Sup35 is insoluble than in wild-type [*PSI*$^+$] strains (12). One possible explanation is that large insoluble aggregates are inefficiently partitioned during cell division. Consistent with the disaggregation model, moderately overproducing Hsp104 bolstered the stability of [*PSI*$^+$] in the mutant strain.

The Role of Other Molecular Chaperones in Prion Induction and Propagation

[*PSI*$^+$] is cured inefficiently or not at all by many conditions that dramatically raise the level of Hsp104, including heat shock, or sporulation (131, 132, 145, 165). This was initially surprising because high *HSP104* overexpression cures [*PSI*$^+$] efficiently (26). Perhaps curing requires cellular division, which does not occur during heat shock, or other factors that are induced that counteract the effect of higher Hsp104 levels.

One such candidate is the heat-inducible molecular chaperone Hsp70. Yeast have four functionally redundant and nearly identical cytosolic Hsp70 proteins called Ssa1-4 (11, 41, 42). Ssa1 is constitutively expressed, but its level increases two- to threefold after heat stress (173, 174). Hsp104 overproduction cures [*PSI*$^+$] less effectively, and less Sup35 becomes soluble when Ssa1 is also overproduced (117). In contrast, overproduction of Ssb1 or Ssb2, two Hsp70 relatives that are not heat inducible (106, 172), enhance the efficiency with which Hsp104 overproduction cures [*PSI*$^+$] (27).

Although the role of chaperones in prion propagation is less well characterized for [*URE3*] than [*PSI*$^+$], there are intriguing differences. *HSP104* deletion cures [*URE3*] and [*PSI*$^+$], but *HSP104* overexpression cures only [*PSI*$^+$] (26, 114). Overproducing Ydj1, a cytosolic Hsp40, slowly cures [*URE3*] (114), but not [*PSI*$^+$] (89).

Because Hsp40s can specify the substrates of Hsp70-Hsp40 complexes (76), they may direct which prions and chaperones interact.

Despite the importance of chaperones in prion propagation, only one stable interaction between chaperones and prions has been identified. The cytosolic Hsp40 Sis1 is essential for viability and required for translational initiation (107, 186). The glycine- and phenylalanine-rich (G/F) region of Sis1 partly determines the chaperone's specificity (182). Rnq1 only binds stably to Sis1 when it is in the prion state, and [*RNQ*$^+$] cannot be propagated in a *sis1* mutant lacking the G/F region (148).

EVOLUTIONARY AND BIOLOGICAL SIGNIFICANCE OF PRIONS

Why do prions exist? At first glance, yeast prions do not appear beneficial. Indeed, [*PSI*$^+$] cells have a translation defect, and [*URE3*] cells grow slower than wild-type cells and wastefully utilize poor nitrogen sources (38, 96). Because yeast rapidly lose even mildly harmful markers, the ability to become a prion would not be conserved if prions are simply deleterious. However, the Sup35 and Ure2 homologs of many yeast species contain PrD-like regions positioned N-terminally to their functional domains (33, 92, 115, 133) (Figure 6).

To determine if Sup35NM might be adaptive, the extent of nucleotide polymorphism within many laboratory, commercial, and clinical isolates of *S. cerevisiae* in Sup35 was compared to the extent of sequence divergence of a related species, *S. paradoxus* (73). The amino acid sequences of Sup35N, M, and C are constrained to varying extents, presumably by purifying selection against mutations that change a coding sequence. Sup35C is under strong constraint, presumably because of its essential role in translational termination; Sup35NM also appears to be under selection, albeit more weakly.

What might be the function of the N-terminal PrD-like regions of Sup35 and Ure2 homologs? Certainly, the PrDs of *S. cerevisiae* are not essential for translational termination or for nitrogen catabolite repression (33, 160), although nitrogen regulation of Gln3 is somewhat diminished when the PrD of Ure2 is deleted (86). Sup35NM and the ability to form [*PSI*$^+$] is essential in yeast strains containing nonsense mutations in vital genes (84, 102). But most strains do not contain such mutations. One possibility is that the putative PrDs have functions other than being able to act as prions. Under some growth conditions, Sup35NM deletion strains have phenotypes distinct from those of [*PSI*$^+$] or [*psi*$^-$] strains (163). Sup35N may link translation to the cytoskeleton via its interaction with Sla1, or it may play a role in glucose metabolism by interacting with Reg1 and Eno2 (3). A more interesting possibility is that a putative PrD is conserved to produce a prion, and thereby epigenetically modify the function of the protein domain to which it is attached.

One line of support for this hypothesis is the observation that all yeast Sup35 homologs tested can convert to the [*PSI*$^+$] state. *Kluyveromyces lactis* Sup35 was shown to have this capacity in *K. lactis* (115). Because most other yeast species lack a facile phenotypic assay for [*PSI*$^+$], the ability of diverse *SUP35* proteins to adopt a [*PSI*$^+$]-like state was tested in *S. cerevisiae* by expressing fusions of putative PrDs from many species to *S. cerevisiae* Sup35NM or Sup35C (25, 88, 115, 133). Most could not convert the endogenous *S. cerevisiae* Sup35 to the prion state. Remarkably, all could induce and propagate a prion-like state if the corresponding heterologous *SUP35* homolog was also expressed (25, 88, 115, 133).

Strikingly, the amino acid sequences of homologus PrDs are divergent, precluding precise sequence alignment. Moreover, the ratio of Asn to Gln residues varies tremendously, even between homologous PrDs. [Compare the *S. cerevisiae* Ure2 PrD with its *K. lactis* homolog and the *Saccharomycodes ludwigii* Sup35 PrD to that of *Pichia methanolica* (Figure 6)]. Thus, these regions have acquired many changes in sequence, yet they retain both the unusual amino acid composition characteristic of the *S. cerevisiae* PrDs and the capacity to form prions. We suggest that these observations support the hypothesis that the capacity of these domains to function as prions is evolutionarily conserved. Moreover, this capacity is derived from their unusual amino acid composition, not from their absolute sequences.

The potential evolutionary significance of [*URE3*] is not obvious. However, some *ure2*, and presumably [*URE3*] strains, grow better than wild-type strains in the presence of high concentrations of Na^+, Li^+, and Mn^{2+} (181), and reach a higher biomass than wild-type strains when grown in the presence of grape juices (130). Natural populations may also benefit from being able to constitutively use a wide array of good and poor nitrogen sources, as is the case in *ure2* and [*URE3*] strains.

One interesting hypothesis for an adaptive value for [*PSI*$^+$] was that the translational read through it causes would produce a constitutive heat shock response and make cells constitutively thermotolerant (55). Although this was true in some strains, it was not true in most strains tested [(55, 163); J. Taulein, Y. Chernoff & S. Lindquist, unpublished data]. A more provocative suggestion is that [*PSI*$^+$] provides a mechanism for genetic variation and phenotypic diversity in the face of changing environments. On rich media, the growth characteristics of isogenic sets of [*PSI*$^+$] and [*psi*$^-$] strains are usually indistinguishable. However, differences in growth or survival often occur when such sets are grown under other conditions, including a variety of carbon and nitrogen sources, and in the presence of potentially toxic salts, metals, and inhibitors of diverse cellular processes including DNA replication, signal transduction, protein glycosylation, and microtubule dynamics (163). In an extensive study using more than 150 different conditions, [*PSI*$^+$] exerted a substantial effect in at least one strain background in nearly half of the conditions tested, and [*PSI*$^+$] strains grew or survived better than [*psi*$^-$] strains in about 20 of these tests (163). Remarkably, each genetic background displayed unique and diverse constellations of phenotypes in response to different environmental conditions (163).

How might this extraordinary phenotypic diversity arise? Almost certainly, it is related to the translational defect caused by [*PSI*$^+$] (163). Some stop-codon read through events might append extra amino acids to proteins, and others might activate cryptic genes or pseudo-genes that had accumulated mutations while inactive. Because [*PSI*$^+$] is spontaneously induced or cured at a low frequency in a natural population of any substantial size, some cells will be present in a [*PSI*$^+$] state and others in a [*psi*$^-$] state. The different phenotypes of these cells would increase the chance that the genome will survive when environmental conditions change. Finally, because the level of Hsp104 affects [*PSI*$^+$] propagation and is increased by environmental stress, [*PSI*$^+$] provides an intriguing and plausible mechanism for yeast to adapt to different environmental niches in response to environmental change.

If prions are beneficial, how may novel prions be created under natural circumstances? One explanation is that prion domains are modular and transferable. Sup35NM can be transferred to an unrelated transcriptional activator to create a novel prion that regulates transcriptional initiation rather than translational termination (99). This chimeric prion protein can exist in two stable, but interchangeable, functional states, which are independent of the prion conformation of Sup35. Thus, new prions may arise stochastically through recombination that appends sequences encoding PrDs to other genes in the genome.

HOW WIDESPREAD ARE PRIONS?

How many other prion proteins are there? To address this fascinating question, an algorithm was used to search the proteomes of 31 organisms for proteins containing at least 30 Gln or Asn residues within an 80-amino-acid region (113). Proteins fitting this criterion were nearly absent from the 28 archeal, thermophilic, and mesophilic bacterial proteomes examined. In contrast, they constituted a surprisingly large fraction of eukaryotic proteins, as much as several percent of the total. *S. cerevisiae* had 107 candidates, or about 1.69% of the total proteins. This estimate is consistent with another, independently obtained with a more stringent algorithm ($>$50 consecutive residues containing at least 45% Q/N and 60% polar residues) (L. Li, M. Long & S. Lindquist, unpublished data). The prion candidates are involved in diverse biological processes and include transcription and translation factors, nucleoporins, DNA- and RNA-binding proteins, and proteins involved in vesicular trafficking. Although it is unclear how many of these proteins are prions, eight were independently identified as determinants of [*PIN*$^+$] when overexpressed (45). Moreover, [*RNQ*$^+$] and [*NU*$^+$] were found using similar database searches (133, 146). Notably, some prions that are not rich in Asn and Gln, such as HET-s and PrP, are missed by such searches, suggesting that these searches are still underestimating the number of possible prion proteins.

The results of directed searches and the mere fact that prions occur in yeast, filamentous fungi, and mammals strongly suggest that prions are widely dispersed

throughout the living world and underlie a wide variety of biological processes. Prions may epigenetically modulate chromatin structure and function (101), mediate homologous chromosome pairing (153), comprise developmental switches (101), and participate in organelle inheritance.

Although the self-perpetuating mechanism of the fungal prions is closely related to amyloid formation, some as yet unrecognized prions might be propagated through entirely different mechanisms. Indeed, there are other novel self-assembling protein structures that are unrelated to amyloids (32, 77, 79). Similarly, there may be mechanisms to partition prions faithfully other than stochastic division of cytoplasmic content. Associations with cellular structures, such as organelles or outer membranes, can mediate inheritance of proteins. Indeed, one example of multigenerational cortical inheritance is the protein Rax2, which orients bud polarity in yeast (21). Another is cortical inheritance of the pattern of cilia on the surface of *Paramecium* (7, 179).

Finally, more than one prion can exist in a cell, and the presence of a prion can increase the likelihood that another will appear (45, 118). Thus, different combinations of prions within a cell might produce a wide variety of heritable phenotypes, all without necessitating any underlying changes in the genome. Clearly, identifying prions and ascertaining their roles in biology will occupy scientists for years to come.

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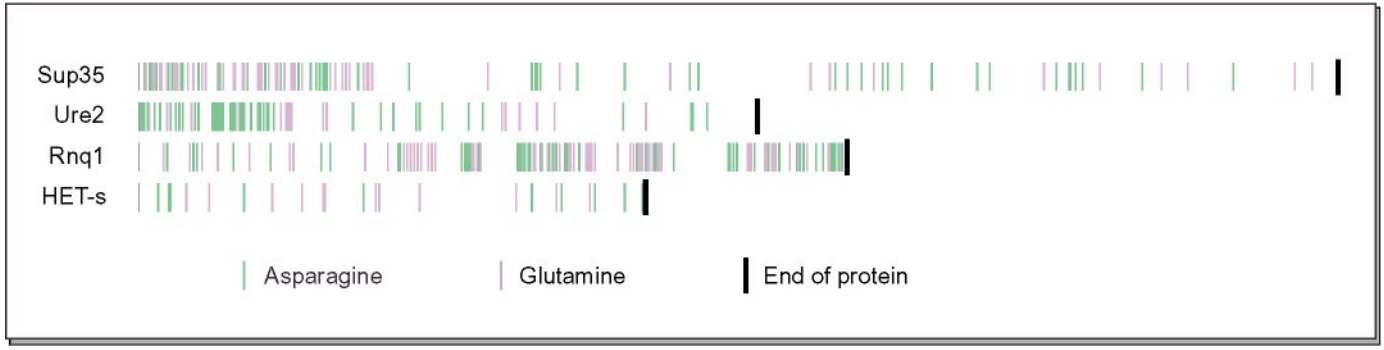

Figure 5 Comparison of the Gln- and Asn-rich regions of *S. cerevisiae* and *P. anserina* prions. Scale representation of the amino acid sequences of the four fungal prions. A *green bar* represents one Asn residue and a *purple bar* represents one Gln residue. All other amino acids are denoted as *white spaces*. The *black bar* designates the C-terminal end of each protein. GenBank accession numbers are Sup35 (NP_010457.1), Ure2 (NP_014170), Rnq1 (NP_009902), and HET-s (S16556).

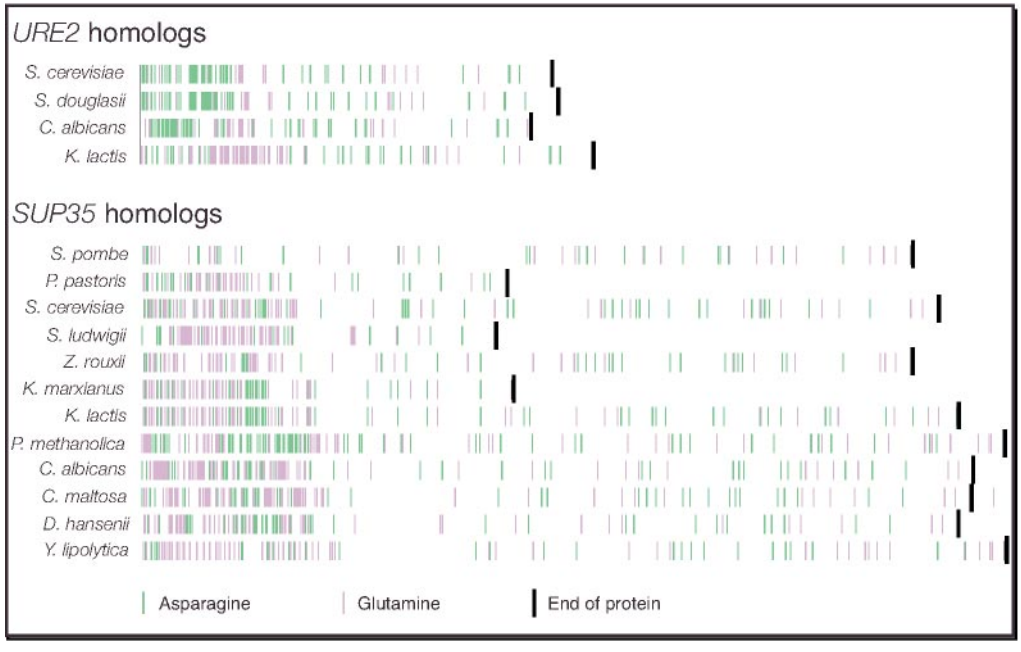

Figure 6 Comparison of the Gln- and Asn-rich regions of Ure2 and Sup35 yeast homologs. Scale representation of the amino acid sequences of the yeast homologs of Ure2 and Sup35. A *green bar* represents one Asn residue and a *purple bar* represents one Gln residue. All other amino acids are denoted as *white spaces*. A *black bar* designates the last residue in the sequence. The sequences for *Pichia pastoris*, *Saccharomycodes ludwigii*, and *Kluyveromyces marxianius* only include the regions homologous to the *S. cerevisiae* Sup35NM region. GenBank accession numbers for Ure2 are NP_014170 (*S. cerevisiae*), AAK51641 (*Saccharomyces douglasii*), AAK51643 (*Candida albicans*), and AAK51642 (*Kluyveromyces lactis*). GenBank accession numbers for Sup35 are BAA33530.1 (*Schizosaccharomyces pombe*), AAF14005.1 (*Pichia pastoris*), NP_010457.1 (*S. cerevisiae*), AAF14006.1 (*Saccharomycodes ludwigii*), BAB12684.1 (*Zygosaccharomyces rouxii*), AAF14004.1 (*Kluyveromyces marxianus*), BAB12680.1 (*Kluyveromyces lactis*), S12921 (*Pichia methanolica*), AAB82541.1 (*Candida albicans*), BAB12681.1 (*Candida maltosa*), BAB12682.1 (*Debaryomyces hansenii*), and BAB12683.1 (*Yarrowia lipolytica*).