MINIREVIEW

Rebels with a cause: molecular features and physiological consequences of yeast prions

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Abstract

Prions are proteins that convert between structurally and functionally distinct states, at least one of which is self-perpetuating. The prion fold templates the conversion of native protein, altering its structure and function, and thus serves as a protein-based element of inheritance. Molecular chaperones ensure that these prion aggregates are divided and faithfully passed from mother cells to their daughters. Prions were originally identified as the cause of several rare neurodegenerative diseases in mammals, but the last decade has brought great progress in understanding their broad importance in biology and evolution. Most prion proteins regulate information flow in signaling networks, or otherwise affect gene expression. Consequently, switching into and out of prion states creates diverse new traits – heritable changes based on protein structure rather than nucleic acid. Despite intense study of the molecular mechanisms of this paradigm-shifting, epigenetic mode of inheritance, many key questions remain. Recent studies in yeast that support the view that prions are common, often beneficial elements of inheritance that link environmental stress to the appearance of new traits.

Introduction

A hallmark feature of aging is the breakdown of homeostatic systems. Just as mechanisms that safeguard genomic integrity (e.g. telomerase activity), cell differentiation (e.g. transdifferentiation of marrow adipocytes into osteoblasts) and metabolism (e.g. mitochondrial function) decline in aging cells, so too do protein quality control systems (Lopez-Otin *et al.*, 2013). Mutations, environmental perturbations, and general stresses of aging can lead to protein misfolding and degradation, and even pathological states. Indeed, a multitude of human diseases, many age related, arise from protein-folding defects (Chiti & Dobson, 2006; Balch *et al.*, 2008).

To buffer threats to protein homeostasis, all organisms from bacteria to humans employ a conserved cohort of heat-shock proteins (HSPs). These chaperones, also known as protein-remodeling factors, catalyze the folding of newly synthesized proteins co-translationally and the re-folding of proteins that have lost their native conformations within the crowded cellular milieu (Mayer & Bukau, 2005; Taipale *et al.*, 2010). Studies from yeast have also helped elucidate a further measure that reduces the negative effects of proteins that are damaged or have formed aggregates beyond rescue by chaperones: spatial segregation and asymmetric retention in mother cells during mitotic division (Tessarz *et al.*, 2009; Liu *et al.*, 2010; Zhou *et al.*, 2011). Because many types of protein aggregates are harmful to cells, asymmetric retention is probably imposed to minimize their proliferation through cell division (Erjavec *et al.*, 2008). While the physical basis of this mechanism is still hotly debated (Liu *et al.*, 2011), the process appears to be deeply conserved in eukaryotes. For example, such asymmetric retention of polyglutamine expansion proteins has not only been observed in yeast, but also in *Drosophila* embryonic neuroblasts and mammalian cells (Rujano *et al.*, 2006).

Some protein aggregates, however, such as the self-perpetuating conformations of prion proteins, bypass asymmetric retention and are faithfully passed on from mother cells to their daughters during cell division. Prion proteins were originally discovered as the cause of the mammalian neurodegenerative disease scrapie (Griffith, 1967; Prusiner, 1982) and are now known to underpin

several other similarly devastating diseases including kuru and Creutzfeldt–Jakob disease that afflict humans, and bovine spongiform encephalopathy, or 'mad cow' disease. 'Proteinaceous infectious particles', or prions, exist stably in different conformational states, at least one of which can self-propagate over long biological timescales (Prusiner, 1982; Shorter & Lindquist, 2005). This endows prions with properties that are usually associated with nucleic acid-based elements of inheritance.

Prion-based inheritance is highly orchestrated. Just as the mitotic spindle ensures proper chromosome segregation required for faithful inheritance of nucleic acid-based traits, molecular chaperones ensure the faithful transmission of prions from mother cells to their daughters (Shorter & Lindquist, 2006). Once converted to the prion state, cells can also lose their prion conformations. Frequencies of loss of the prion state have not been measured extensively, although simulations and experimental measurements thus far suggest that they are low (Derdowski *et al.*, 2010).

In their native conformations, many yeast prion proteins regulate transcription, translation, and signaling networks (Halfmann *et al.*, 2010). As a consequence, the prion states of these proteins can create diverse new traits. These can be beneficial, detrimental, or inconsequential, depending on the particular prion, genetic background, and environmental context (Shorter & Lindquist, 2005; True & Lindquist, 2000). Despite intense interest and investigation, however, the biological relevance of prions has long remained controversial (McGlinchey *et al.*, 2011; Wickner *et al.*, 2011). Although our knowledge of this realm of inheritance is still fragmentary, studies in yeast over the past several years have helped clarify its mechanisms and scope, deepening our understanding of how prions interface with proteostasis and cell physiology to provide adaptive opportunities.

Prion characteristics

Many years ago, it was reported that loss of translation-termination fidelity – specifically, increased read-through of stop codons by ribosomes – could be inherited cytoplasmically and dominantly via an element named [*PSI*$^+$] (Cox, 1965). (Brackets denote the non-Mendelian inheritance of this element in genetic crosses and capital letters its dominance.) We now know that [*PSI*$^+$]'s unusual inheritance is the result of a 'protein-only' mechanism involving the class II translation-termination factor protein, Sup35 (aka eRF3). This essential GTPase promotes polypeptide release and ribosome recycling, in concert with other release factors. It also has an unusual ability to switch between two conformations. Its native conformation is a soluble, functional protein. Its prion conformation is a self-templating amyloid that sequesters additional copies into an inactive form apart from the ribosome. The ensuing changes to translation fidelity produce the [*PSI*$^+$] phenotype.

For prion aggregates to be propagated within cells, they must be processed into 'seeds', or small templates that can efficiently recruit soluble molecules of the same protein to aggregate. These seeds are passed from mother cells to their daughters during mitotic division, ensuring that there are templates for forming aggregates in new cells (Fig. 1a). In mating, a haploid cell will always transmit prions to the diploid. If that diploid is sporulated, all meiotic progeny typically inherit the prion template (Fig. 1b), in striking contrast to genetic mutations, which are inherited by half of such progeny. In fungi, the disassembly of prion aggregates into seeds is generally catalyzed by the Hsp104 chaperone, a member of the broadly conserved Clp family of protein chaperones (Doyle & Wickner, 2009). Hsp104 was first recognized for its critical function in thermotolerance, promoting general protein disaggregation in stressed cells (Sanchez *et al.*, 1992). Inheritance of self-replicating Sup35 prion templates (that give rise to [*PSI*$^+$]) critically depends on normal levels of Hsp104 activity. Too little Hsp104 activity results in long fibers and few seeds, preventing transmission of the phenotype, while too much Hsp104 activity resolves Sup35 fibers to a degree that they can no longer self-template (Chernoff *et al.*, 1995).

Prions have a very strong preference for self-templating naive protein molecules (those in their native fold) encoded by the same gene. They also exhibit a strong species barrier for propagation (Scott *et al.*, 1989; Bruce *et al.*, 1994; Santoso *et al.*, 2000). Prions of one species template homologs from another species poorly; efficient cross-templating only occurs if the prion-forming domain, or PrD, of the foreign species is changed sufficiently to resemble that of the templating species (Santoso *et al.*, 2000; Tanaka *et al.*, 2005). In addition, prion proteins form structurally polymorphic amyloids. Different conformations, once formed, are self-templating and yield distinct phenotypes; these heritable conformational polymorphisms are known as 'strains' (Derkatch *et al.*, 1996; King, 2001; King & Diaz-Avalos, 2004; Tanaka *et al.*, 2004). Thus, variants of these protein-based heritable elements exhibit features akin to the outcomes of different alleles of nucleic acid-based genes.

Mechanisms of propagation and inheritance

Biochemical studies of Sup35 assembly have helped to define the mechanisms of prion inheritance in yeast (Glover *et al.*, 1997; King *et al.*, 1997; Serio *et al.*, 2000;

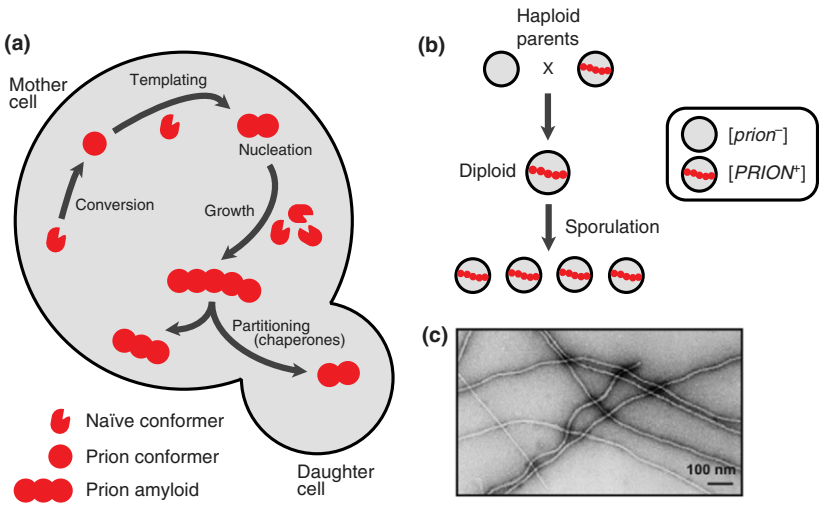

Fig. 1. Attributes of yeast prions. (a) Schematic of prion protein conversion, nucleation, growth, and partitioning. Here, partitioning (e.g. by Hsp104) is shown during budding yeast mitosis; however, this process is also required for intracellular propagation of prion aggregates. (b) Yeast prions exhibit non-Mendelian 4 : 0 (or sometimes 3 : 1) inheritance patterns, mediated by cytoplasmic transfer of prion templates. A [*PRION*$^+$] haploid cell carries the prion into the diploid upon mating; the element is then passed to all four meiotic progeny upon sporulation. (c) Electron micrograph of self-propagating [*PSI*$^+$] amyloid fibers. Image reprinted with permission from Elsevier (Halfmann *et al.*, 2010).

Tuite & Cox, 2003). Sup35's soluble, intrinsically disordered prion domain (known as NM) is converted into a self-templating amyloid via a nucleation-dependent process that proceeds by monomer addition (Fig. 1c; Bradley & Liebman, 2004; Collins *et al.*, 2004; Krishnan & Lindquist, 2005). Partitioning of this amyloid template from mother cells to their daughters is achieved by the Hsp104 disaggregase (Shorter & Lindquist, 2004, 2006). Propagation of [*PSI*$^+$] is sensitive to even minor sequence perturbations – a single amino acid substitution near the N-terminus of Sup35 can potently reduce propagation efficiency of the prion conformation (Marchante *et al.*, 2013). Tiled peptide arrays have demonstrated that small, highly specific sequence elements nucleate Sup35's conformational conversion to [*PSI*$^+$], and govern formation of distinct prion strains and species specificities (Tessier & Lindquist, 2007).

It has been proposed that many neurodegenerative diseases may in fact be infectious prion diseases (Prusiner, 2012; Jucker & Walker, 2013). Among several lines of supporting evidence, proponents cite observations that disease-associated amyloid of discrete origin (exogenous or endogenous) can propagate into previously healthy, neighboring tissue in mammalian brains (Ridley *et al.*, 2006; Li *et al.*, 2008; Clavaguera *et al.*, 2009). However, even mechanisms governing the spread of prion diseases, the transmissible spongiform encephalopathies (TSEs), are not well understood in animals. And in yeast, there is no evidence demonstrating that prions can be secreted and transferred to neighboring cells. Many bacteria, however, are coated with amyloids that are essential for virulence and the construction of biofilms, and some fungi secrete amyloids known as hydrophobins that facilitate spore dispersion and invasion of biotic and abiotic substrates (Gebbink *et al.*, 2005).

Structural features

While many proteins have the capacity to form amyloids if subjected to the right conditions (Dobson, 1999), insight into the structure of this fold has been stymied by its experimental intractability. Except for small peptides, an atomic-resolution structural model of a complete amyloid fibril exists for only one protein, the PrD of the HET-s prion from the filamentous fungus *Podospora anserina,* which forms a solenoid-shaped core of tightly packed ß-sheets (Wasmer *et al.*, 2008). This is consistent with highly ordered cross-ß-sheet structures predicted by or observed in numerous other structural studies of amyloid (Greenwald & Riek, 2010). A lower resolution, but more holistic view of prion amyloid, was achieved by hydrogen/deuterium (H/D) exchange on a pair of distinct [*PSI*$^+$] conformations (Toyama *et al.*, 2007). Both conformations shared a similar hydrophobic core, but differed markedly in the structures of their extensions, which were far more ordered in the conformation that propagated more weakly. This feature may render them less recognizable by Hsp104 for efficient partitioning to daughter cells.

Amyloids implicated in human neurodegenerative diseases are also structurally polymorphic, producing distinct strains that vary in their seeding properties and toxicities when propagated *in vivo* (Petkova *et al.*, 2005; Guo *et al.*,

2013). Remarkably, one oligomeric intermediate of [*PSI*$^+$] reacts with an antibody that was raised against oligomeric Aβ (Krishnan *et al.*, 2012), the polypeptide causatively linked to Alzheimer's disease (Hardy & Selkoe, 2002). Sup35 does not bear sequence homology to Aβ, suggesting instead that there are conserved structural features in these oligomers. In yeast, the transience of Aβ-like oligomeric intermediates of [*PSI*$^+$] during prion assembly could explain why these amyloidogenic proteins are not toxic in this context (Serio *et al.*, 2000; Shorter & Lindquist, 2004; Krishnan *et al.*, 2012).

Sequence characteristics of prion domains

Many PrDs are striking in their unusual amino acid compositions, depleted in hydrophobic residues, and enriched in polar ones, principally asparagine (N) and glutamine (Q). Recent experimental evaluation of new yeast prion domains has suggested that N residues are far more amyloidogenic than Qs (Alberti *et al.*, 2009; Halfmann *et al.*, 2011); the ability of candidate PrDs to form amyloids *in vitro* correlated strongly with the N/Q ratio. Changing Qs to Ns increased amyloid formation in the yeast prions where this issue has been examined. In contrast, changing Ns to Qs lead to the production of toxic nonamyloid oligomers. Yeast PrDs also share sequence homology with intrinsically disordered, hydrogel-forming protein domains that have recently been characterized in mammalian cells (Kato *et al.*, 2012). Self-association is a key feature of each type of aggregate, but interaction stabilities vary. Prions with Q/N-rich domains form the most stable aggregates, followed by polyQ proteins, while hydrogels form the weakest assemblies (Fiumara *et al.*, 2010; Kato *et al.*, 2012).

Not all fungal prions contain domains with the biochemical features of the classic PrDs. For example, [*MOD*$^+$], formed by Mod5, a tRNA isopentenyl transferase, lacks any asparagine- or glutamine-rich sequences (Suzuki *et al.*, 2012). And Het-s from *Podospora anserina* is similarly devoid of such sequences, pointing to additional mechanisms of prion formation, and the possibility that other such prions remain to be discovered (Taneja *et al.*, 2007).

Interplay with chaperones

The cell employs a vast repertoire of molecular chaperones to contend with misfolded proteins. Many of these, like the widely conserved Hsp70 (heat-shock protein of 70 kDa), bind to and neutralize transiently (or aberrantly) exposed hydrophobic residues to promote proper folding. They also re-solubilize proteins that have already entered an aggregated state (Mayer & Bukau, 2005). This is achieved in concert with co-chaperones that help dictate specificity for particular substrates, or 'clients'. Chaperones are found throughout various cellular compartments, including the cytoplasm and the endoplasmic reticulum. Another type of chaperone is exemplified by Hsp104, a disaggregase that is essential for cell survival under proteotoxic stress, when great numbers of proteins aggregate (Doyle & Wickner, 2009). As many other chaperones, Hsp104 is constitutively expressed and induced further under conditions of stress (Sanchez *et al.*, 1992).

Prions partially resist chaperones' remodeling activities for correcting misfolded proteins, but they also rely on chaperones to be propagated within cells and from one generation to the next. In light of recent evidence supporting prions' adaptive value (discussed below), an appealing explanation for this relationship is that chaperones regulate prion formation, inheritance, and destruction to provide cells with a stress-regulated mechanism for the creation and maintenance of new, heritable traits.

Many, but not all, yeast prions require Hsp104 to promote disaggregation of their amyloid structures for seeding new aggregates and partitioning prions from mother cells to their daughters. Notably, this function of Hsp104 depends on Hsp70 (Shorter & Lindquist, 2008). One exception is found in [*ISP*$^+$], which is formed by the global transcriptional regulator Sfp1, and does not require Hsp104 for propagation (Volkov *et al.*, 2002; Rogoza *et al.*, 2010). Similarly, [*GAR*$^+$], which circumvents glucose repression, also does not require Hsp104 for its propagation, but does depend on the activity of Hsp70 (Brown & Lindquist, 2009). Several other prions also rely on Hsp70 function, pointing to a potentially broad role for this chaperone in driving self-perpetuating prion conformations (Sharma & Masison, 2009).

Physiological consequences and adaptive value

A large population of cells under acute stress or thrust into an unfamiliar environment could have little to lose if a minority sample prion states that modulate gene expression or alter metabolism, because these changes could be adaptive in principle (Fig. 2). Such changes would not require the lengthy process of a series of path-dependent genetic changes, and prions can arise in cells at frequencies far higher than genetic mutation (Lang & Murray, 2008; Brown & Lindquist, 2009; Lancaster *et al.*, 2010). Once arisen, prions could promote adaptation nearly instantaneously.

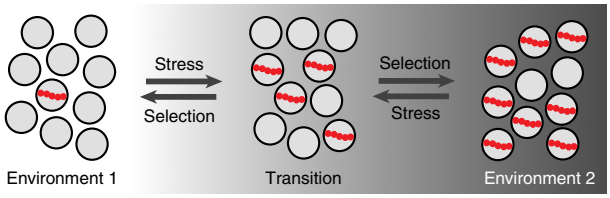

Fig. 2. A simple model of bet-hedging mediated by prions. In this example, [*prion*−] cells confer a fitness advantage in Environment 1, while [*PRION*$^+$] cells confer a similar advantage in Environment 2, leading to their respective selection. The stressful, reversible transitions between environments increase the rate of prion gain/loss, driving distinct steady-state distributions.

Beyond these theoretical musings, the abundance of PrDs in the yeast genome suggests that their ability to undergo conformational switching has been retained for biological purpose. These domains are enriched in proteins that lie at nodes of highly networked pathways – transcription factors, RNA binding proteins, and signal transducers (Alberti *et al.*, 2009). Thus, many proteins containing PrDs have the potential to drive phenotypic change when they convert to a prion form. In the case of [*PSI*$^+$], prion conversion sequesters most of Sup35's translation-termination activity away from the ribosome (Shorter & Lindquist, 2005). The ensuing read-through of stop codons creates novel phenotypes that depend on the genetic variation downstream of stop codons (i.e. in 3′ UTRs; True & Lindquist, 2000; True *et al.*, 2004). The partitioning of this element by Hsp104 is conserved between *S. cerevisiae* and the distantly related *Candida albicans* (Zenthon *et al.*, 2006), spanning an evolutionary distance of hundreds of millions of years. Because [*PSI*$^+$]-dependent phenotypes are sometimes beneficial, it has been suggested that prions could facilitate adaptation, allowing immediate access to novel phenotypes that could promote survival in fluctuating environments. This could lead to the rapid evolution of new prion-based traits that could be maintained epigenetically for indefinite time, or might be subsequently fixed by genetic mutation (True & Lindquist, 2000; True *et al.*, 2004; Halfmann *et al.*, 2012).

While the above ideas are appealing, the notion that prions might have adaptive value has been vigorously debated. Indeed, these elements have sometimes been categorized as 'diseases' of yeast or artifacts of laboratory culture (Nakayashiki *et al.*, 2005; McGlinchey *et al.*, 2011; Bateman & Wickner, 2012; Box 1). It is only very recently that we can begin to deconstruct this null view. In a large survey of *c.* 700 wild and industrial yeast strains, nearly one-third of the isolates displayed prion-dependent traits, many of them arising from elements that have yet to be characterized (Halfmann *et al.*, 2012). In this study, the [*PSI*$^+$] and [*MOT3*$^+$] prions conferred many valuable new phenotypes in the wild strains that naturally harbored them. Further strong evidence for the adaptive value of prions comes from the [*MOD*$^+$] prion (Suzuki *et al.*, 2012). This element is induced by and promotes resistance to antifungal agents, exemplifying prions' roles as Lamarckian elements.

[*MOT3*$^+$] provides an attractive framework for understanding how prions exert influence on such a broad array of phenotypes (Holmes *et al.*, 2013). The Mot3 transcription factor affects genes that regulate the composition of the cell wall, pheromone signaling, and a host of other biological properties. [*MOT3*$^+$] arises spontaneously at a high frequency, up to 1 in 10 000 cells, providing this subpopulation with diverse new lineage-specific phenotypes, including multicellularity and altered responses to environmental changes. This prion is induced by ethanol and eliminated by hypoxia. Thus, [*MOT3*$^+$] also links the appearance of new heritable traits to environmentally regulated stress responses.

Box 1. The opposing view: prions as diseases

A competing hypothesis suggests that prions are generally diseases and that any beneficial phenotypes that they may induce are simply side effects of infection (Nakayashiki *et al.*, 2005; McGlinchey *et al.*, 2011; Bateman & Wickner, 2012). Some of the evidence supporting this hypothesis includes (1) occasional generation of lethal [*PSI*$^+$] variants *in vivo* when the prion domain of Sup35 is expressed separately from the translation-termination domain (McGlinchey *et al.*, 2011), (2) in natural isolates, [*PIN*$^+$] variants exist at allele frequencies consistent with those expected for deleterious genes in outcrossing populations (Kelly *et al.*, 2012), (3) prions hypothesized to cause disease have variant strains, suggesting an absence of positive selection, while the fungal prion HET-s that is known to play an important role in heterokaryon incompatibility shows no such strain variation (Wickner *et al.*, 2007), (4) some prion-forming domains in proteins have alternate functions, which could also explain their evolutionary conservation (Wickner *et al.*, 2011), and (5) prion infection may lead to an elevated stress response (Wickner *et al.*, 2011). This hypothesis predicts that prions are deleterious at first acquisition and are quickly selected out of a population. Prions that are maintained in cells over time should be, on average, deleterious or neutral due to coevolution (similar to a virus becoming less virulent as it spreads in a population). If a prion is found to provide a benefit, this view holds that this is a rare exception and the relationship with the host did not begin that way.

[*GAR*$^+$] overcomes a quintessential feature of yeast biology: glucose repression. This trait was first discovered more than three decades ago, but its unusual, non-Mendelian inheritance patterns were mystifying at a time when prions were unknown (Ball *et al.*, 1976). Decades later, this element was shown to be a prion, known as [*GAR*$^+$] for its phenotype of suppressing glucose-associated repression (Brown & Lindquist, 2009). Most yeast in the wild do not find themselves with a constant supply of pure glucose to employ as an energy source, in sharp contrast to common laboratory conditions. Instead, they must compete with other microorganisms for mixtures of multiple sugars, with relative concentrations varying widely between different environmental niches. There is intuitive appeal in a metabolic switch that is under the epigenetic control of a prion-like element, especially one offering an immediate solution to unpredictable and fluctuating nutritional challenges. As for other prions, [*GAR*$^+$] could act as a bet-hedging mechanism, in this case for metabolic adaptation, bypassing an ancient genetic circuit to facilitate growth in the presence of a broad array of carbon sources.

All yeast prions studied to date (see Table 1) can produce beneficial traits. In contrast, the best-studied prion in animals, PrP^{Sc}, leads to a range of devastating pathologies in its self-perpetuating form. Although investigation of these disease states has led to many important insights into prions, they have also saturated our perception of the roles and consequences of these elements as being unequivocally negative. However, another prion found in animals, formed by the cytoplasmic polyadenylation element binding protein (CPEB), is changing this view. Originally discovered in the sea slug *Aplysia*, the prion-like conformation formed by CPEB has been linked to long-term synaptic facilitation (Si *et al.*, 2003, 2010). In *Drosophila*, the PrD in the CPEB homolog Orb2 and its ability to aggregate into amyloid oligomers are required for long-term memory (Keleman *et al.*, 2007; Majumdar *et al.*, 2012). This example, together with the observation of yeast prions being adaptive in many environments, points to the enticing likelihood that many more examples of beneficial prions remain to be discovered, broadening our understanding of the importance of epigenetic inheritance in nature.

A bet-hedging strategy for survival in fluctuating environments

The rates at which prions arise and revert make them an attractive mechanism for a reversible, epigenetic bet-hedging strategy (True & Lindquist, 2000; True *et al.*, 2004; Halfmann *et al.*, 2012). As a single yeast cell expands into a colony, a few [*PRION*$^+$] cells appear sporadically, and these cells express new, heritable traits. If those traits are detrimental, only the few individuals that harbor the prion will be lost. However, if the trait is advantageous,

Table 1. Fungal prions

Prion	Protein	Protein Function	Prion phenotype	References
[*PSI*$^+$]	Sup35	Translation-termination subunit	Nonsense suppression (increased readthrough of translation-termination stop codons)	Cox (1965) and Wickner (1994)
[URE3]	Ure2	Negative regulator of catabolism of poor nitrogen sources	Derepression of metabolic enzymes for poor nitrogen sources	Lacroute (1971) and Wickner (1994)
[*PIN*$^+$]/[*RNQ*$^+$]	Rnq1	Unknown	Acceleration of switch-on rates for other prions	Derkatch *et al.* (2001, 1997) and Sondheimer & Lindquist (2000)
[HET-s]	Het-s	Balance with expressed Het-S allele controls heterokaryon incompatibility	Increased heterokaryon incompatibility between normally incompatible strains	Coustou *et al.* (1997)
[*SWI*$^+$]	Swi1	Subunit of SWI/SNF chromatin remodeling complex	Reduced growth on carbon sources other than glucose	Alberti *et al.* (2009) and Du et al. (2008)
[*GAR*$^+$]	Pma1, Std1	Membrane proton pumps	Suppression of glucose repression	Brown & Lindquist (2009)
[*OCT*$^+$]	Cyc8	Subunit of Cyc8/Tup1 transcription repression complex	Derepression of Cyc8/Tup1 targets	Patel *et al.* (2009)
[*MOT3*$^+$]	Mot3	Transcription repressor	Derepression of Mot3 targets, environmentally responsive multicellularity	Alberti *et al.* (2009) and Holmes *et al.* (2013)
[*ISP*$^+$]	Sfp1	Transcription factor	Nonsense suppression, larger cell size, increased drug resistance	Rogoza *et al.* (2010) and Volkov *et al.* (2002)
[*NSI*$^+$]	Unknown	Unknown	Nonsense suppression	Saifitdinova *et al.* (2010)
[*MOD*$^+$]	Mod5	tRNA isopentenyl transferase	Reduced Mod5 activity, increased antifungal resistance	Suzuki *et al.* (2012)

those few individuals might ensure survival of the population when it might otherwise perish (Fig. 2). Prions are also lost sporadically, providing a complementary survival advantage should the environment revert to favor the [*prion*$^-$] state (Shorter & Lindquist, 2005). Importantly, the connection between environmental stress and protein homeostasis ensures that prions, and the phenotypes they create, will be gained and lost with greater frequency in stressful environments (Shorter & Lindquist, 2005; Tyedmers *et al.*, 2008). Therefore, cells may take chances on new protein-based heritable phenotypes more frequently when they are not ideally suited to their environments.

[*PSI*$^+$]-dependent traits are often genetically complex (True & Lindquist, 2000; True *et al.*, 2004; Halfmann *et al.*, 2012). Thus, in a single step, this prion offers a range of adaptive opportunities. Such [*PSI*$^+$]-dependent traits can also be assimilated in genetic crosses by re-assortment of underlying genetic variation (True & Lindquist, 2000; True *et al.*, 2004; Halfmann *et al.*, 2012), rendering them robust to environmental perturbation. This provides an avenue through which cells can maintain beneficial traits, but jettison the prion's cost of relaxed translational fidelity.

Widespread and wild

For many years, yeast prions were believed by some to be diseases or epigenetic oddities that were restricted to laboratory strains. Key to overturning these notions was finding how well conserved these elements are in nature. One early clue was the observation that while the N-terminal domain of Sup35 – which drives aggregation – is dispensable for the protein's translation-termination activity (Derkatch *et al.*, 1996; Paushkin *et al.*, 1996), it has nonetheless retained prion-forming capacity for hundreds of millions of years (Santoso *et al.*, 2000). Similar modular aggregation domains, or PrDs, were later observed in other yeast prion proteins. Using the shared sequence features of PrDs as a launching point, Lindquist and colleagues investigated the breadth of prion-like sequences within the yeast proteome (Alberti *et al.*, 2009). They scanned the genome for protein domains similar to the PrDs of previously characterized yeast prions, those enriched in Q and N residues. The top 100 scoring genes were subjected to a comprehensive series of genetic, cellular, and biochemical assays to test whether they might encode prions. Criteria examined included their ability to form foci indicative of aggregates in living cells, amyloid *in vitro* and *in vivo*, and their dependence on Hsp104 for transmission from mother cells to daughter cells. Twenty-four genes contained domains that rigorously passed every test. To demonstrate the success of this approach for *de novo* identification of endogenous prions, one of these candidates, the transcription factor Mot3, was investigated in detail and described for the first time to be a *bona fide* prion, [*MOT3*$^+$].

Contemporaneous directed studies reported that several other PrD-containing proteins (also identified in the above screen) also act as prions endogenously. [*SWI*$^+$], formed by the Swi1 protein, is a chromatin remodeling factor that transcriptionally regulates *c.* 6% of *S. cerevisiae* genes (Du *et al.*, 2008). [*OCT*$^+$], formed by the Cyc8 protein, is part of a transcriptional repressor complex controlling *c.* 7% of *S. cerevisiae* genes (Patel *et al.*, 2009). And [*MOD*$^+$], formed by the Mod5 protein, leads to upregulation of ergosterol biosynthesis and consequent increased resistance to the many first-line antifungal agents that inhibit this pathway (Suzuki *et al.*, 2012).

It remained uncertain for some time whether the beneficial traits observed in laboratory strains foretold any adaptive value for prions in nature. An initial survey of 70 wild yeast strains was not promising. Although around a dozen contained the [*RNQ*$^+$] prion (aka [*PIN*$^+$]), whose only known phenotype is increasing the frequency at which other prions arise (Derkatch *et al.*, 2001; Osherovich & Weissman, 2001), prions that confer more direct phenotypic consequences (e.g. [*PSI*$^+$] and [*URE3*]) were uniformly absent (Nakayashiki *et al.*, 2005).

Later, however, comprehensive examination of a much larger number of strains revealed a strikingly different picture. A large study tested close to 700 wild yeast strains from diverse ecotypes (e.g. soil, woodlands, wine, beer, fruit, infected human tissues) for the presence of prions, taking advantage of an unusual biochemical future of amyloid: insolubility in ionic detergents (Fig. 3a; Halfmann *et al.*, 2012). Semi-denaturing agarose gel electrophoresis and immunoblotting (Halfmann & Lindquist, 2008) revealed that many of these strains harbored [*RNQ*$^+$], as predicted by the previous study, but strikingly, many also contained [*PSI*$^+$] and [*MOT3*$^+$]. The inheritance of these elements critically depended on Hsp104, just as in laboratory strains. Each prion conferred beneficial traits in the wild strains where they were found. For example, [*PSI*$^+$] enabled one wine strain to penetrate the surface of agar, mimicking growth that would facilitate adhesion to grapes. This trait was lost when the prion was eliminated, or 'cured' (Fig. 3b). Likewise, [*MOT3*$^+$] enabled a Finnish soil isolate to survive in acidic growth medium, whereas it perished in these conditions without the prion (Fig. 3c; Halfmann *et al.*, 2012).

Because most prion-based traits strongly depend on Hsp104 for their propagation, they can be eliminated by transient passage of cells in media containing an Hsp104 inhibitor such as guanidinium hydrochloride (GdHCl) and do not return when Hsp104 function is restored.

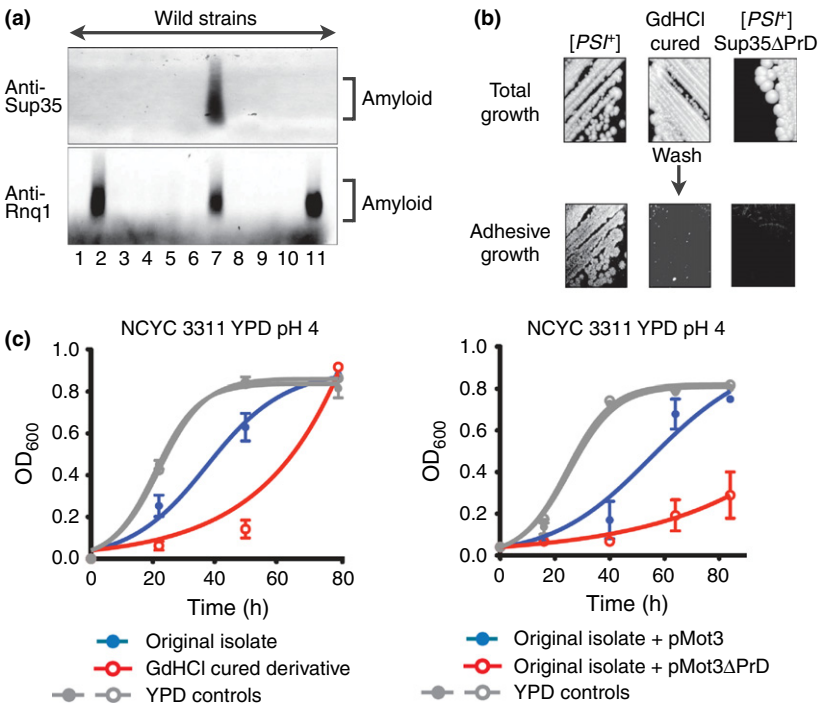

Fig. 3. Wild yeast strains harbor prions that can confer beneficial traits. (a) Wild yeast strains of diverse origins contain SDS-insoluble amyloid composed of Sup35 and Rnq1 protein (strains 2, 7, and 11). Amyloid runs as a high molecular weight species on semi-denaturing detergent-agarose gel electrophoresis (SDD-AGE) gels. (b) The [*PSI*$^+$] strain UCD978, isolated from Beaujolais wine, normally penetrated agar, but this trait was lost after curing with GdHCl or when expressing a mutant of Sup35 lacking its PrD. (c) The [*MOT3*$^+$] strain NCYC 3311 (filled blue circles, left panel) is resistant to acidic growth conditions. The phenotype is reversed by prion curing with GdHCl passage (open red circles, left panel), or expression of a nonaggregating version of Mot3, Mot3ΔPrD (right panel). Error bars represent the standard deviation of four independent biological replicates. Images reprinted with permission from Macmillan Publishers Ltd: Nature (Halfmann *et al.*, 2012).

Subjecting the wild strains to transient inhibition of Hsp104 eliminated at least one phenotype in one-third of them. For example, some clinical strains lost the ability to grow in the antifungal drug fluconazole, and some wine strains lost the ability grow in the acidic conditions typical of their niche. Many Hsp104-dependent traits that were examined in detail could be transmitted from wild strains to laboratory strains through cytoduction, or matings where cytoplasmic material is transferred from a donor cell to a recipient cell without exchange of nuclear material. Once transferred to the laboratory strains, these traits remained Hsp104-dependent.

The fraction of strains that were heritably affected by transient inhibition of Hsp104 vastly exceeded the number that harbored [*PSI*$^+$], [*RNQ*$^+$], or [*MOT3*$^+$]. This remarkable finding suggests that other prions are also abundant in wild yeasts, where they likewise confer a vast array of beneficial phenotypes. The ubiquity of PrDs in the yeast proteome (Alberti *et al.*, 2009) suggests many potential candidates for the molecular origin of these traits, promising much exciting work in the future to define the influence of these elements on biological function and to understand their importance in evolution.

Revealing cryptic genetic variation

Natural selection acts on phenotypes, not genotypes. Thus, any mechanism that increases the phenotypic output of genetic variation has the potential to amplify its adaptive potential. The [*PSI*$^+$] prion provides a compelling example. Translational read-through of stop codons in [*PSI*$^+$] cells leads to the appearance of new traits that depend on genetic variation residing in 3′ UTRs that is otherwise cryptic (True & Lindquist, 2000; True *et al.*, 2004). That is, the genetic variants that drive [*PSI*$^+$]-dependent traits are suppressed in the absence of the prion. The degree to which exposure of cryptic genetic variation might influence the evolution of new traits remains a topic of intense debate. [*PSI*$^+$] presents one amenable molecular mechanism for exposure of cryptic genetic variation. Another key example is found in the Hsp90 chaperone (Jarosz & Lindquist, 2010).

The aforementioned analysis of wild yeast strains suggests that exerting influence on cryptic genetic variation may be a common property of yeast prions. Curing prions through transient inhibition of Hsp104 weakened the correlation between similarity in genotype and similarity in phenotype in wild strains from many niches (Halfmann *et al.*, 2012). This relationship held true even after excluding strains harboring [*PSI*$^+$] and [*MOT3*$^+$], implying again that other prions were also at work in revealing cryptic genetic variation. Much work remains to identify and characterize the molecular players involved. What is clear, however, is that prions can broadly influence whether genetic polymorphisms are manifest in new biological traits.

Concluding remarks

Our understanding of prion biology has exploded in the past two decades. Many studies, principally in yeast, but increasingly in metazoans as well, suggest that prions are common and can do far more than simply cause disease (Coustou *et al.*, 1997; True & Lindquist, 2000; Si *et al.*, 2003; True *et al.*, 2004; Keleman *et al.*, 2007; Brown & Lindquist, 2009; Patel *et al.*, 2009; Halfmann *et al.*, 2012). The enrichment of PrDs in proteins that control gene regulation and signaling (Alberti *et al.*, 2009) means that prions can produce diverse traits, ranging from altered carbon source utilization to multicellularity (Brown & Lindquist, 2009; Holmes *et al.*, 2013). Moreover, the link between prion propagation, chaperone activities, and stress confers properties of Lamarckian inheritance to these trans-generationally stable epigenetic elements (Tyedmers *et al.*, 2008; Halfmann & Lindquist, 2010).

The recent discovery that prions are a common mechanism for phenotypic inheritance in wild yeasts establishes that these elements are not merely artifacts of laboratory cultivation (Halfmann *et al.*, 2012). Given the limited number of phenotypes examined in this study, it is not unreasonable to extrapolate that most yeast strains may harbor at least one trait that arises from a prion. Moreover, nearly half of all traits conferred by these elements were beneficial – a fraction that would be highly improbable to achieve through random mutation. The influence of these elements on the relationship between genotype and phenotype further suggests that many such traits arose from cryptic genetic variation. This last observation leads to an intriguing conclusion: Prions provide immediate phenotypic access to a silent reservoir of genetic variation that previously may have been subject to selection. That is, they provide a means through which cells can employ adaptive prediction to heritably diversify their phenotypes under stress in a manner that has previously been advantageous.

The burgeoning list of proteins that possess prion-like qualities has grown most rapidly in yeast, and is beginning to expand in other eukaryotes as well. It is not known whether prion-like elements are present in prokaryotes, although bacteria do generate amyloid and use it extensively in the production of biofilms (Barnhart & Chapman, 2006; Dueholm *et al.*, 2010). Theoretically, organisms that live in massive populations, including bacteria, would benefit extensively from a prion-based bet-hedging function. While PrDs can be found in bacterial proteomes – at varying frequencies, as in fungi – in contrast to fungi, they tend to reside in proteins that are strongly enriched in the cell wall as opposed to intracellular space (Espinosa Angarica *et al.*, 2013). Bacteria also lack an obvious prion partitioning activity. Despite these differences, the NM domain of Sup35 will generate [*PSI*$^+$]-like aggregates in *Escherichia coli* that can be used to infect yeast (Garrity *et al.*, 2010). And the fact that not all yeast prions have identical sequence or chaperone dependencies suggests that some of these perceived handicaps need not preclude protein-based inheritance in prokaryotes. Beyond amyloid, uncovering whether bacteria encode prion-like elements stands as a key objective for future investigation.

Although bet-hedging provides an attractive rationale for the maintenance of prions in unicellular organisms (True & Lindquist, 2000; Griswold & Masel, 2009), it is unlikely to motivate their retention in multicellular eukaryotes. Indeed, the discovery of beneficial prion-like aggregation in metazoans (e.g. CPEB multimers that enable long-term memory formation) has profoundly changed our understanding of such processes and may portend a much richer biology of protein aggregation. Another example is the human MAVS (mitochondrial antiviral signaling) protein, which forms prion-like aggregates on the surface of mitochondria in response to the presence of viral RNA. These induce a transcriptionally mediated antiviral interferon (Hou *et al.*, 2011). In these examples, prion-like aggregation is not a rare event that occurs by chance in a handful of cells. Rather, it is the basis for an adaptive, concerted change in cell physiology. These multicellular systems offer to further expand our understanding of how self-perpetuating protein folds serve as molecular memories in biology, influencing development, disease, and evolution.

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