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Minireview

MINIREVIEW

[*PIN*$^+$]ing down the mechanism of prion appearance

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One sentence summary: Prion appearance is the gateway to the creation of protein-only phenotypes, and recent studies highlight both the complexity of this process and the unanswered mechanistic questions remaining.
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ABSTRACT

Prions are conformationally flexible proteins capable of adopting a native state and a spectrum of alternative states associated with a change in the function of the protein. These alternative states are prone to assemble into amyloid aggregates, which provide a structure for self-replication and transmission of the underlying conformer and thereby the emergence of a new phenotype. Amyloid appearance is a rare event *in vivo*, regulated by both the aggregation propensity of prion proteins and their cellular environment. How these forces normally intersect to suppress amyloid appearance and the ways in which these restrictions can be bypassed to create protein-only phenotypes remain poorly understood. The most widely studied and perhaps most experimentally tractable system to explore the mechanisms regulating amyloid appearance is the [*PIN*$^+$] prion of *Saccharomyces cerevisiae*. [*PIN*$^+$] is required for the appearance of the amyloid state for both native yeast proteins and for human proteins expressed in yeast. These observations suggest that [*PIN*$^+$] facilitates the bypass of amyloid regulatory mechanisms by other proteins *in vivo*. Several models of prion appearance are compatible with current observations, highlighting the complexity of the process and the questions that must be resolved to gain greater insight into the mechanisms regulating these events.

Keywords: prion; chaperone; [*PSI*$^+$]; [*PIN*$^+$]; proteostasis; amyloid

INTRODUCTION

Protein-only traits underlie an increasing cross-section of biology. Outcomes as varied as the regulation of gene expression in budding yeast and the emergence and progression of neurodegenerative disease in humans have now been linked to a protein-only mechanism (Tuite and Serio 2010). A subset of these traits, which are determined by proteins known as prions (Prusiner 1982), are transmissible through either infection or heredity, shattering the long-held belief that information transfer had sole provenance in nucleic acids (Crick 1970). The breakthrough that led to this advance was the uncovering of the distinct nature of the information itself: while nucleic acid-based information is encoded by sequence, protein-based information is encoded in conformation. Thus, when a prion protein adopts a new conformation, its native activity is altered, and/or it acquires a new one, leading to a novel phenotype (Tuite and Serio 2010). Because the transmission of a protein-only trait requires the replication of its determinant in a new individual, prions represent the first *in vivo* example of an autonomous self-replicating shape (Penrose and Penrose 1957).

In the more than three decades that have elapsed since this breakthrough, much insight into the structure of the self-replicating conformation and the mechanism of self-replication has emerged (Knowles, Vendruscolo and Dobson 2014). Both prion proteins and prionoids, which determine protein-only but non-transmissible traits (Aguzzi 2009), can access an

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alternative protein folding trajectory, which competes with the pathway leading to the native state (Jahn and Radford 2008). Within this extended energy landscape, monomeric protein has the propensity to self-assemble into amyloid, a filamentous complex characterized by a cross-β structure, where the strands of a continuous β-sheet are arranged along the fiber length (Sunde *et al.* 1997). At each end of the fiber, an exposed strand acts as a templating surface, allowing the formation of hydrogen bonds between backbone residues and the packing of side chains into a steric zipper (Nelson *et al.* 2005; Sawaya *et al.* 2007). This configuration promotes bidirectional growth of the fiber (Goldsbury *et al.* 1999; Blackley *et al.* 2000; Scheibel *et al.* 2001) and concomitantly the depletion of alternative conformers of the same protein (Satpute-Krishnan and Serio 2005; Knowles *et al.* 2009).

Given the self-replicating nature of amyloid, its appearance is the primary gateway to the emergence of new traits associated with this state. For many proteins, the kinetic threshold for amyloidogenesis appears to be high, primarily due to the need for self-assembly (Gazit 2002; Baldwin *et al.* 2011). Amyloid formation proceeds via a nucleated process, in which monomers must assemble into an oligomer of defined size to become thermodynamically stable (i.e. the nucleus) (Jarrett and Lansbury 1993). Once this threshold is reached, amyloid accumulation increases both through continued assembly onto this nucleus and through the formation of secondary nuclei by fragmentation of existing fibers to create new ends or by *de novo* assembly stimulated along the lateral surfaces of fibers (Masel, Jansen and Nowak 1999; Masel and Jansen 2001; Knowles *et al.* 2009; Gaspar *et al.* 2017). The kinetic threshold for amyloidogenesis is easily overcome *in vitro*, where protein concentrations can be readily manipulated. However *in vivo*, amyloid appearance seems to be regulated beyond the intrinsic aggregation propensity of these proteins even at high concentration. For example, amyloid appearance increases during aging, with the decline of protein-quality control pathways known as the proteostasis network (Powers *et al.* 2009; Koga, Kaushik and Cuervo 2011) and in the presence of other misfolded proteins (Derkatch *et al.* 2001; Osherovich and Weissman 2001; Gidalevitz *et al.* 2006).

To understand the emergence of protein-only traits, we must then uncover not only how the complex energy landscape of protein folding is balanced by the intricate proteostasis network to suppress amyloidogenesis but also where the points of vulnerability in this intersection lie. In this review, I examine the literature on prion appearance in the yeast *Saccharomyces cerevisiae*, focusing on the interaction between the [*PSI*$^+$] and [*PIN*$^+$] prions, in pursuit of this insight.

[*PSI*$^+$] and [*PIN*$^+$]

[*PSI*$^+$] is the protein-only trait determined by the amyloid form of the prion protein Sup35 (Cox 1965; Doel *et al.* 1994; Ter-Avanesyan *et al.* 1994; Chernoff *et al.* 1995; Patino *et al.* 1996; Paushkin, Kushnirov and Smirnov 1996; Glover *et al.* 1997; King *et al.* 1997; Paushkin *et al.* 1997). [*PSI*$^+$] arises spontaneously in [*psi*$^-$] yeast, which have non-prion state Sup35, at a frequency of $\sim 10^{-8}$–10^{-7}/generation (Lancaster *et al.* 2010), but this ability to acquire [*PSI*$^+$] is specifically regulated *in vivo*. [*PIN*$^+$] yeast strains, which are inducible to [*PSI*$^+$] either spontaneously or by transient overexpression of Sup35, can be converted to a non-inducible state ([*pin*$^-$]) by treatment with millimolar concentrations of guanidium HCl (GdnHCl) (Lund and Cox 1981; Derkatch *et al.* 1997). The [*PIN*$^+$] trait segregates 4:0 in the meiotic progeny of a diploid strain formed by crossing [*PIN*$^+$] and [*pin*$^-$] yeast strains and is eliminated by deletion or overexpression of the molecular chaperone Hsp104 (Chernoff *et al.* 1995; Derkatch *et al.* 1997). Together, these observations suggested that the [*PIN*$^+$] determinant was an epigenetic factor (i.e. reversible) transmitted through the cytoplasm (i.e. inherited in a non-Mendelian pattern), and subsequent studies revealed that [*PIN*$^+$] was also a protein-only trait (Derkatch and Liebman 2007).

This [*PIN*$^+$]-dependent ability of Sup35 to bypass the forces that normally restrict its transition to the amyloid state provides a unique experimental tool to gain mechanistic insight into amyloid appearance *in vivo*. Indeed, the presence of [*PIN*$^+$] also promotes the accumulation of SDS-resistant aggregates, a hallmark of the amyloid state (Serio *et al.* 2000), of other proteins when they are overexpressed in the yeast cytosol, including the polyglutamine-expanded forms of the Machado-Joseph disease protein and exon 1 of huntingtin (Osherovich and Weissman 2001; Meriin *et al.* 2002; Alexandrov *et al.* 2008; Kochneva-Pervukhova, Alexandrov and Ter-Avanesyan 2012). This generality suggests that the [*PSI*$^+$]-[*PIN*$^+$] interplay is a representative example of a broader system of amyloid regulation *in vivo*.

Given its epigenetic nature, the determinant of the [*PIN*$^+$] trait was once proposed to be an intermediate conformation of the non-amyloid form of Sup35. According to this model, the [*PIN*$^+$], but not [*pin*$^-$], conformation of Sup35 was competent to self-assemble into amyloid (Derkatch *et al.* 1997). However, the authors also showed that the [*PIN*$^+$] phenotype is propagated in and transmissible through a yeast strain deleted for the prion-determining domain of the Sup35 protein (Derkatch *et al.* 1997), a glutamine and asparagine-rich N-terminal segment of the protein required for prion formation and propagation (Ter-Avanesyan *et al.* 1994). While these observations did not rule out a role for the non-prion domain of Sup35 in the propagation of [*PIN*$^+$], other factors were soon implicated. Overexpression of yeast proteins with prion-like QN-rich domains, including New1, Ure2, Lsm4, Ste18, Pin2, Yck1, Nup116 and Cyc8, was capable of inducing [*PIN*$^+$] in a [*pin*$^-$] strain, as expected for an amyloid-based trait (Wickner 1994; Derkatch *et al.* 2001; Osherovich and Weissman 2001). Moreover, deletion of the gene encoding Rnq1, the rich in asparagine (N) and glutamine (Q) protein determinant of the previously identified [*RNQ*$^+$] yeast prion (Sondheimer and Lindquist 2000), was sufficient to convert a strain from [*PIN*$^+$] to [*pin*$^-$] and to block transmission of [*PIN*$^+$] (Derkatch *et al.* 2001; Osherovich and Weissman 2001). Strikingly, the spontaneous appearance and GdnHCl-curing of [*PIN*$^+$] correlated with the appearance and disappearance, respectively, of Rnq1 aggregates (Derkatch *et al.* 2001). Moreover, *in vitro* assembled Rnq1 amyloid, but not non-aggregated Rnq1, induced the appearance of [*PIN*$^+$] when transformed into [*pin*$^-$] yeast (Patel and Liebman 2007). Thus, [*PIN*$^+$] is not conferred by an intermediate conformation of Sup35. Rather, it is primarily determined by the amyloid form of the Rnq1 protein in laboratory yeast strains, although the amyloid forms of multiple Q/N-rich proteins can serve in a similar, albeit reduced, capacity (Sondheimer and Lindquist 2000; Derkatch *et al.* 2001; Osherovich and Weissman 2001).

The genetic and physical interaction of the Sup35 and Rnq1 prion proteins in their amyloid, native and denatured states has been extensively analyzed *in vitro* and *in vivo*, with Susan Lindquist, her long-term collaborator Susan Liebman, and many of her former trainees (cited throughout) contributing to our mechanistic understanding of prion appearance *in vivo* through this body of work. These studies have been organized into multiple models (Fig. 1), which I present below along with a summary of experimental evidence and remaining open questions.

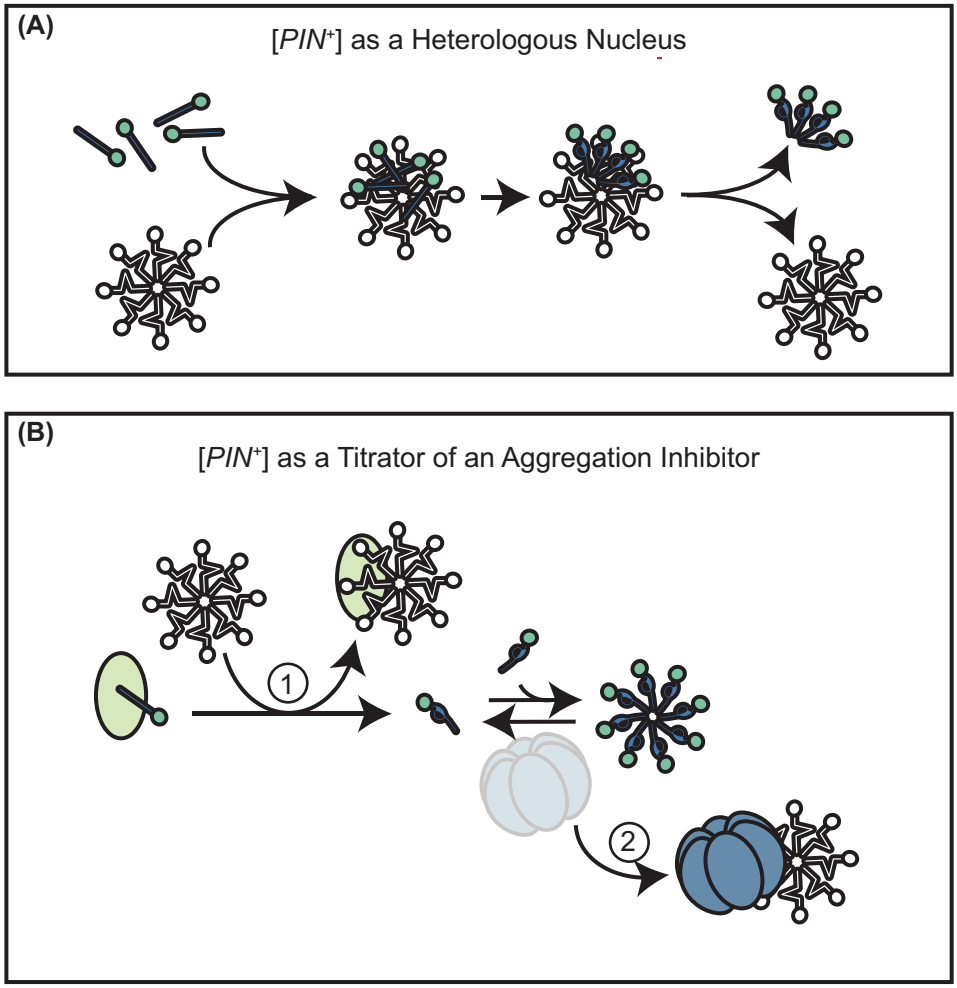

Figure 1. Models for the role of [*PIN*$^+$] in [*PSI*$^+$] appearance. Two models have been proposed to explain the requirement for [*PIN*$^+$] in the appearance of [*PSI*$^+$] *in vivo*. **(A)** The most widely accepted model for [*PIN*$^+$] is that of heterologous nucleus (unfilled pinwheel), stimulating the nucleation of Sup35 (green and blue) by direct interaction. Once nucleated, Sup35 and Rnq1 (the determinant of [*PIN*$^+$] in laboratory yeast strains) amyloids propagate as separate aggregates. (**B**) [*PIN*$^+$] may also promote [*PSI*$^+$] formation by titrating an inhibitor (green oval) that binds to non-prion state Sup35 and blocks its self-association (step 1). As a variation on the inhibitor model, [*PIN*$^+$] may titrate the fragmentation machinery (blue hexamer) away from spontaneously forming nascent Sup35 aggregates, allowing them to persist and amplify (step 2). See the text for details on each of the models.

Model 1: [*PIN*$^+$] acts as a heterologous nucleus for Sup35 amyloid formation

What is the role of Rnq1 amyloid in promoting the formation of a stably propagating [*PSI*$^+$] state? The most widely accepted possibility is the heterologous nucleation model. According to this idea, Rnq1 amyloid templates the formation of Sup35 amyloid through direct interaction, providing a pathway to overcome the kinetic barrier to amyloid appearance by promoting nucleation (Fig. 1A) (Derkatch *et al.* 1997, 2000; Osherovich and Weissman 2002). Indeed, fusing the Sup35 prion domain to Rnq1 promotes [*PSI*$^+$] formation in [*PIN*$^+$] yeast in the absence of Sup35 overexpression, suggesting that the act of artificially bringing together Sup35 monomers is sufficient to bypass a barrier to prion formation *in vivo* (Choe *et al.* 2009). The question is: Does [*PIN*$^+$] play the same role when it is not fused to Sup35? Many studies have been undertaken, both *in vitro* and *in vivo*, to directly test this model.

A factor acting as a heterologous nucleus for amyloid formation by another protein should accelerate the assembly of the latter both *in vitro* and *in vivo* in a manner that increases with the concentration of the former templates. While the prion-determining domain of Sup35 formed amyloid *in vitro* in the absence of Rnq1 amyloid (Glover *et al.* 1997; King *et al.* 1997), the addition of Rnq1 amyloid accelerated its formation, as assessed by thioflavin T fluorescence (Derkatch *et al.* 2004; Vitrenko *et al.* 2007; Sharma and Liebman 2013a). However, this stimulation did not recapitulate *in vivo* observations of conformation-specific genetic interactions between Rnq1 and Sup35 (Sharma and Liebman 2013a), was only weakly dependent on Rnq1 concentration (Derkatch *et al.* 2004) and required Rnq1 amyloid potentially in excess of the Rnq1:Sup35 ratios observed *in vivo* (Ghaemmaghami *et al.* 2003; Kulak *et al.* 2014). These points raise the possibility of non-specific effects. For example, Rnq1 amyloid at high concentration may increase molecular crowding and thereby Sup35 assembly (Lansbury 1999; Minton 2005; Huang *et al.* 2015). This alternative possibility also provides an explanation for the increased stimulation of Sup35 amyloid formation upon sonication of Rnq1 fibers (Sharma and Liebman 2013a), an effect that is smaller in magnitude than would be predicted for end-dependent polymerization (Serio *et al.* 2000) but consistent with an increased efficiency of crowding expected at lower viscosity and with a smaller crowder (Ellis and Minton 2006; Bokvist

and Gröbner 2007). Intriguingly, the stimulation of Sup35 assembly *in vitro* is not specific to Rnq1 fibers, which was cited as evidence of specificity (Derkatch *et al.* 2004). However, another possibility remains. The proteins that are capable of this activity (immunoglobulin, insulin and Rnq1) have isoelectric points close to neutrality, while those that are incapable of doing so (α-synuclein, lysozyme and transthyretin) have highly acidic or basic isoelectric points. If crowding is indeed the mechanism of stimulation *in vitro*, the charge of the crowder could be an important component of the effect, as has been previously suggested (Minton 1983).

In vivo, the relationship between [*PSI*$^+$] induction and Rnq1 amyloid concentration is similarly confounding in the context of the heterologous nucleation model. [*PIN*$^+$] can exist as a spectrum of variants, each of which corresponds to a different conformation of Rnq1 amyloid and varies in its frequency of [*PSI*$^+$] induction (Bradley *et al.* 2002; Sharma and Liebman 2013a; Stein and True 2014). Notably, the frequencies of [*PSI*$^+$] induction associated with these variants correspond neither to the number of heritable Rnq1 aggregates nor to the accumulation of aggregated Rnq1, as would be predicted for a heterologous nucleus (Bradley *et al.* 2002; Bardill and True 2009; Kalastavadi and True 2010; Sharma and Liebman 2013a, 2013b). Thus, studies of the effects of Rnq1 amyloid concentration on Sup35 amyloidogenesis *in vitro* and *in vivo* cannot currently provide strong support for the heterologous nucleation model.

The heterologous nucleation model also predicts a direct interaction between Rnq1 amyloid and Sup35 protein. Several lines of evidence support this prediction. First, some mutations in Rnq1 retain the ability to propagate [*PIN*$^+$], as assessed by Rnq1 aggregation and transmissibility, but have a reduced ability to support [*PSI*$^+$] induction (Bardill and True 2009; Stein and True 2014). The reduced [*PSI*$^+$]-inducibility of one mutant can be suppressed by a mutation in Sup35, providing support for molecular specificity in this process, although not necessarily through a direct Rnq1-Sup35 interaction (Keefer, Stein and True 2017). Second, overexpressed Sup35 and Rnq1 co-localize to cytoplasmic ring and dot structures, as detected by immunofluorescence or tagging with fluorescent proteins (Derkatch *et al.* 2004; Kimura *et al.* 2004; Tyedmers *et al.* 2010; Du and Li 2014; Arslan *et al.* 2015), and these structures have been previously linked to [*PSI*$^+$] appearance (Zhou, Derkatch and Liebman 2001). Third, Sup35 and Rnq1 have been demonstrated to physically interact by immunoprecipitation/immunocapture from yeast lysates, the capture of Rnq1 from yeast lysates on a Sup35-affinity resin and *in vitro* cross-linking of purified proteins (Salnikova *et al.* 2005; Tyedmers *et al.* 2010; Sharma and Liebman 2013a; Keefer, Stein and True 2017). Together, these observations provide support for an interaction between Rnq1 and Sup35, a necessary component of the heterologous nucleation model.

Conceptually, the idea of an unrelated protein serving as a heterologous nucleus can be seen as counter to the known specificity of amyloid copolymerization, which requires a high degree of sequence identity (Krebs *et al.* 2004). For example, the Sup35 homologs from the closely related species *S. paradoxus* and *S. bayanus* and from the more distantly related *Pichia methanolica* access a prion state, as assessed by loss of Sup35 native activity and GdnHCl reversibility, when overexpressed in the *S. cerevisiae* cytosol. However, these proteins are unable to support [*PSI*$^+$] propagation upon deletion of the *S. cerevisiae SUP35* gene, demonstrating the sequence specificity required for heterologous nucleation (Chernoff *et al.* 2000; Chen, Newnam and Chernoff 2007). Indeed, a single amino-acid change in Sup35 (S17R) disrupts the ability of preformed Sup35 amyloid fibers to accelerate the assembly of soluble wild-type Sup35 *in vitro*, although both wild-type and mutant Sup35s retain the ability to form amyloid on their own (DePace *et al.* 1998). More extensive studies have revealed that exact homology in short stretches of Sup35 is required for copolymerization, prion induction and prion propagation (Santoso *et al.* 2000; Resende *et al.* 2002), and these sequences mediate direct contacts between monomers that likely nucleate distinct Sup35 conformations (Chien *et al.* 2003; Krishnan and Lindquist 2005; Tessier and Lindquist 2007). Such examples of high-sequence specificity for amyloidogenesis among Sup35 proteins must necessarily lead to skepticism about [*PIN*$^+$] acting as a heterologous nucleus, especially given the large number of QN-rich proteins capable of promoting [*PSI*$^+$] appearance when overexpressed *in vivo* (Derkatch *et al.* 2001; Osherovich and Weissman 2001).

Nonetheless, the specificity described above reflects end-dependent copolymerization as a mechanism of nucleation (Derkatch and Liebman 2007), and there have been relatively few examples of such heteropolymeric amyloids identified to date (Sarell, Stockley and Radford 2013). Consistent with this reality, Sup35 and Rnq1 form separate SDS-resistant aggregates *in vivo* (Bagriantsev and Liebman 2004). Moreover, overexpression of Sup35 homologs promotes [*PSI*$^+$] appearance by *S. cerevisiae* Sup35 without efficiently adopting an SDS-resistant state, indicating that they have not formed amyloid themselves and would therefore be incapable of promoting [*PSI*$^+$] appearance by amyloid-based cross-seeding (Chen, Newnam and Chernoff 2007; Vishveshwara and Liebman 2009). Thus, other mechanisms must be rigorously considered in any attempt to understand a role for [*PIN*$^+$] as a heterologous nucleus.

An expanding repertoire of possibilities has been reported for other pairs of amyloidogenic proteins. For example, the sequence-specific binding of Aβ to tau promotes phosphorylation of the latter, which in turn reduces the affinity between the two proteins and potentially promotes their aggregation (Guo *et al.* 2006). New1 induces ATP-dependent fragmentation of Sup35 fibers *in vitro* to create new ends (Inoue *et al.* 2011), and an N-terminally truncated variant of β2-microglobulin induces a conformational change in the wild-type protein to promote amyloidogenesis (Eichner *et al.* 2011). Sickle hemoglobin polymerization is believed to include a heterogeneous nucleation step mediated along the lateral surface of the polymer through sequence-specific contacts (Ferrone *et al.* 1980; Ferrone, Hofrichter and Eaton 1985; Rotter *et al.* 2005), a mechanism proposed to explain the kinetics of amyloidogenesis *in vitro* for other proteins (Knowles *et al.* 2009; Gaspar *et al.* 2017) and the [*PIN*$^+$] activity of Lsm4 *in vivo* (Oishi *et al.* 2013).

Whether the mechanism of heterologous nucleation proceeds via end-dependent polymerization or another pathway, a significant gap in proof for the model remains: a definitive demonstration that the Sup35-Rnq1 interaction is required for [*PSI*$^+$] induction. For example, while the Sup35-Rnq1 interaction in yeast lysates is [*PIN*$^+$]-dependent, the extent of the interaction does not correlate with the distinct [*PSI*$^+$]-induction frequencies of [*PIN*$^+$] variants (Sharma and Liebman 2013a). Moreover, most mutations in Rnq1 that reduce [*PSI*$^+$]-inducibility, and the Sup35 mutation that suppresses this effect for one mutant, have not been assessed for corresponding changes in interaction (Bardill and True 2009; Stein and True 2014; Keefer, Stein and True 2017). In the one case where this analysis has been undertaken, both the [*PSI*$^+$]-induction defective Rnq1(N297S) (Bardill and True 2009) and wild-type Rnq1 are immunocaptured from yeast lysates with the Sup35 prion-determining domain to the same extent (Sharma and Liebman 2013a). In the absence of

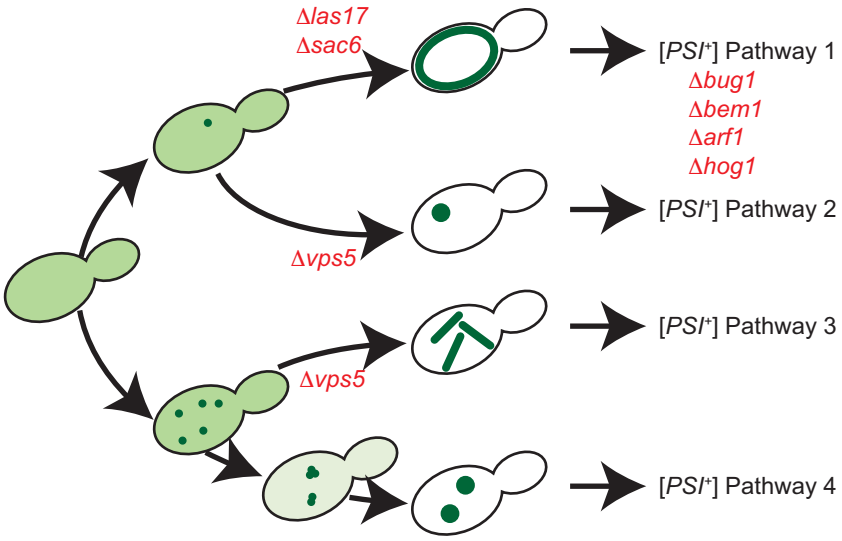

Figure 2. Genetic regulation of early steps of [*PSI*$^+$] appearance (adapted from Sharma *et al.* 2017). Overexpression of the prion-determining domain of Sup35 fused to GFP in [*PIN*$^+$] yeast cells leads to the appearance and evolution of microscopically visible protein aggregates of distinct types: rings (pathway 1), single dots (pathway 2), lines (pathway 3) and multiple dots (pathway 4), which all lead to [*PSI*$^+$] appearance (Sharma *et al.* 2017). Deletion mutants (red), which reduce [*PSI*$^+$] appearance, differentially impact the appearance and/or accumulation of these visible aggregates (e.g. Δ*las17*, Δ*vps5* or Δ*sac6*) or appear to act downstream of these events (e.g. Δ*bug1*, Δ*bem1*, Δ*arf1* or Δ*hog1*) (Manogaran *et al.* 2011; Sharma *et al.* 2017; Wisniewski *et al.* 2018).

evidence linking a reduction in [*PSI*$^+$] appearance to a decrease in Rnq1-Sup35 association, effects on unknown events downstream of the Rnq1-Sup35 association and/or indirect effects independent of this association cannot be eliminated from consideration (Sharma and Liebman 2013b).

Model 2: [*PIN*$^+$] titrates an aggregation inhibitor

[*PIN*$^+$] has also been proposed to function as a factor that titrates an inhibitor of aggregation (Fig. 1B—step 1) (Derkatch *et al.* 2001; Osherovich and Weissman 2001, 2002; Vitrenko *et al.* 2007). This model is not mutually exclusive with the heterologous template model because, by definition, it describes the *in vivo* regulation of protein misfolding. Consistent with this idea, growth under conditions of stress led to an increase in the spontaneous frequency of [*PSI*$^+$] appearance in a strain expressing a mutant form of Sup35 that more readily converts to the prion state (Liu and Lindquist 1999; Tyedmers, Madariaga and Lindquist 2008). Thus, the threshold for amyloidogenesis likely changes in distinct proteostatic niches, suggesting the potential for regulation.

As was the case for the heterologous nucleation model, a more nuanced consideration of potential mechanisms of inhibition is warranted. Several lines of evidence suggest that prion appearance *in vivo* is a multistep process. First, genes, whose deletion reduce [*PSI*$^+$] induction in [*PIN*$^+$] strains, fall into two classes: those that interfere with the formation of cytoplasmic rings of GFP fused to the Sup35 prion-determining domain, a hallmark of [*PSI*$^+$] induction in wild-type strains (Derkatch *et al.* 1997), and those that do not (Manogaran *et al.* 2011) (Fig. 2). Thus, there are at least two genetically separable events. Second, fluorescently detectible structures, once appearing, dynamically evolve into different forms, and this evolution appears to be genetically regulated (Sharma *et al.* 2017; Wisniewski *et al.* 2018) (Fig. 2). Third, SDS-resistant oligomers of the Sup35 prion-determining domain fused to GFP appear prior to microscopically visible structures (Sharma *et al.* 2017). Fourth, [*PSI*$^+$] induction frequencies increase with less stringent selection, suggesting that aggregate appearance occurs more commonly than standard [*PSI*$^+$]-selection conditions capture (Tyedmers, Madariaga and Lindquist 2008; McGlinchey, Kryndushkin and Wickner 2011; Gorkovskiy *et al.* 2017). Thus, the initial aggregation of Sup35 and the transition of these nascent aggregates into a stable [*PSI*$^+$] state appear to be separable events, raising the possibility that they are independently regulated (Manogaran *et al.* 2011).

Sup35 polymerization *in vivo*, as *in vitro*, is a nucleated event: GFP-tagged Sup35, which is newly synthesized or introduced by mating into a [*PSI*$^+$] strain expressing untagged Sup35, localized to cytoplasmic foci, reflecting conversion onto existing aggregates (Patino *et al.* 1996; Satpute-Krishnan and Serio 2005), and *in vitro* assembled Sup35 amyloid transformed into a [*psi*$^-$] strain induces [*PSI*$^+$] appearance (Tanaka *et al.* 2004). Thus, the barriers to [*PSI*$^+$] appearance likely involve the formation of an initial nucleus, its survival and its amplification. [*PIN*$^+$] is dispensable for [*PSI*$^+$] induction following transformation of Sup35 amyloid (Tanaka *et al.* 2004; King, Wang and Chang 2006) and for the propagation of existing [*PSI*$^+$] (Derkatch *et al.* 2000). Because each of these [*PIN*$^+$]-independent processes require continued amplification of Sup35 amyloid, [*PIN*$^+$] would most likely exert its effects on nucleus formation and/or survival in this model.

Factors interfering with nucleus formation would be expected to interact with non-prion state Sup35 or with a prion-competent intermediate form of the protein, and overexpression of Sup35 alone might be expected to overcome this mechanism of inhibition by exceeding the concentration of the inhibitor. While overexpression of Sup35 to a high level does not induce [*PSI*$^+$] in a [*pin*$^-$] strain (Derkatch *et al.* 1997), its inability to do so could simply reflect differential affinities of Rnq1 amyloid and Sup35 for the titration target. Indeed, the idea of an aggregation inhibitor is supported by the observation Sup35 amyloid formation *in vitro* is inhibited by the addition of yeast lysates (Uptain *et al.* 2001). Unfortunately, this inhibition was not assessed for dependence on [*PIN*$^+$]; thus, the relationship of this inhibitory activity to [*PIN*$^+$] remains an open question.

Nonetheless, if this model is correct, several predictions can be made. First, deletion or mutation of the inhibitor should promote [*PSI*$^+$] induction. Second, overexpression of the inhibitor should reduce [*PSI*$^+$] induction but not propagation, if its action is specific to nucleation or nucleus persistance. Third, binding of the inhibitor to Sup35 should be enriched in [*psi*$^-$] strains, if it is directly blocking conversion to the prion state. And fourth, [*PIN*$^+$] should play a role in titrating such an inhibitory factor away from its interaction with Sup35. To date, no candidate genes meeting these criteria have been identified despite the initial promise of some factors. For example, deletion of the cotranslationally acting Hsp70 homologs in yeast, *SSB1* and *SSB2*, promotes [*PSI*$^+$] appearance, both spontaneously and following transient overexpression of Sup35, by a factor of 10 (Chernoff *et al.* 1999). While the effect of Ssb1 overexpression on [*PSI*$^+$] appearance was not analyzed, overexpression of Ssb1 was subsequently shown to promote loss of existing [*PSI*$^+$] (Chernoff *et al.* 1999; Kushnirov *et al.* 2000; Chacinska *et al.* 2001; Allen *et al.* 2005), and Ssb1 binds to Sup35 in both [*psi*$^-$] and [*PSI*$^+$] strains by immunocapture (Allen *et al.* 2005; Holmes *et al.* 2014). Another once promising candidate is Sup45, the functional partner of Sup35 in translation termination (Dever and Green 2012). Overexpression of Sup45 reduces [*PSI*$^+$] induction by Sup35 overexpression and does not interfere with [*PSI*$^+$] propagation (Derkatch, Bradley and Liebman 1998), but Sup45 binding to Sup35 persists in [*PSI*$^+$] strains (Pezza *et al.* 2014; Arslan *et al.* 2015). Finally, deletion of either of the genes encoding the small heat shock proteins (sHsp) Hsp26 or Hsp42 mildly increases, while overexpression of either factor mildly reduces, [*PSI*$^+$] appearance following Sup35 overexpression, but the latter is likely due to an effect on existing aggregates, as overexpression of sHsps promotes loss of existing [*PSI*$^+$] *in vivo* (Duennwald, Echeverria and Shorter 2012). More generally, EMS mutagenesis, undertaken to inactivate the putative aggregation inhibitor, did not identify a factor capable of increasing the frequency of [*PSI*$^+$] appearance (Derkatch *et al.* 2001), but redundancy in and essentiality of genes necessarily complicate the search for putative aggregation inhibitors via this approach. Thus, inhibition of Sup35 nucleation and a role for [*PIN*$^+$] in promoting the bypass of this regulation remain theoretical possibilities to explore as new candidate genes are identified.

Whether a nucleus is formed spontaneously without regulation or following the bypass of inhibitory processes, it must persist and be amplified to establish a stable, transmissible prion state *in vivo* (Pezza and Serio 2007). Several lines of evidence suggest that persistence and amplification are related processes, reflecting a balance between growth and fragmentation of existing aggregates. For [*PSI*$^+$], amplification requires the fragmentation of existing Sup35 amyloid by the chaperone machinery, specifically the AAA + ATPase Hsp104 and its co-chaperones Hsp70 (Ssa1) and Hsp40 (Sis1) (Chernoff *et al.* 1995; Song *et al.* 2005; Satpute-Krishnan, Langseth and Serio 2007; Higurashi *et al.* 2008; Tipton, Verges and Weissman 2008). In a balanced system, where wild-type factors are expressed at native levels and growth occurs in the absence of stress, introduction of a single, preformed Sup35 aggregate is theoretically sufficient to induce a stable [*PSI*$^+$] state (Tanaka *et al.* 2004). However, a Sup35 mutant that reduces the kinetic stability of its amyloid state or the upregulation of molecular chaperones in response to a sublethal heat shock create proteostatic niches in which existing amyloid is cleared through the process of Hsp104-dependent fragmentation (DiSalvo *et al.* 2011; Klaips *et al.* 2014; Pei *et al.* 2017). Importantly, these niches occur in compartments characterized by an elevated chaperone:amyloid ratio due to the asymmetric inheritance of factors during yeast cell division (Derdowski *et al.* 2010; Klaips *et al.* 2014; Pei *et al.* 2017).

A similar situation likely exists during nucleation, where nascent amyloid appears at low abundance, and this reality raises the possibility of a different type of aggregation inhibitor—one that promotes disassembly of existing aggregates (Fig. 1B—step 2) (Derkatch *et al.* 2001; Osherovich and Weissman 2001, 2002; Vitrenko *et al.* 2007; Davis and Sindi 2016). Indeed, the importance of balance in the amplification and clearance pathways during [*PSI*$^+$] appearance is supported by studies of Sup35 and Hsp104 mutants. [*PSI*$^+$] can be induced in a strain expressing a fragmentation-defective deletion mutant of Sup35 (Δ22–69) only if Hsp104 levels are elevated (Borchsenius *et al.* 2001). Likewise, [*PSI*$^+$] variants induced in a strain overexpressing Hsp104 or in one expressing an Hsp104 mutant that is incapable of promoting [*PSI*$^+$] loss when overexpressed (T160M) are eliminated when Hsp104 is returned to its wild-type state (Borchsenius *et al.* 2006; Gorkovskiy *et al.* 2017).

Does [*PIN*$^+$] act to tip this balance in favor of amyloid persistence and amplification? The concept of titration of cellular factors by one amyloidogenic protein from another is supported by the surprising observation that the proteins that most efficiently confer the [*PIN*$^+$] phenotype when overexpressed (Pin4C, Cyc8C, Yck1, Ste18) also induce loss of existing [*PSI*$^+$] at the same elevated levels (Yang *et al.* 2013). In the case of Pin4C, overexpression leads to an increase in the size of Sup35 aggregates, as assessed by GFP-tagging/microscopy and by gel-based analysis of SDS-resistant aggregates from yeast lysates, and to a decrease in their mobility in some cells (Yang *et al.* 2013). These observations are consistent with a defect in Hsp104-mediated fragmentation and loss of [*PSI*$^+$] by the failure to transmit Sup35 aggregates during cell division (Satpute-Krishnan, Langseth and Serio 2007; Kawai-Noma *et al.* 2009). Consistent with this hypothesis, both Hsp104-GFP and Sis1-GFP co-localize with RFP-tagged Pin4C under these conditions, and [*PSI*$^+$] loss is ameliorated by overexpression of Hsp104 (T160M) or by overexpression of Sis1 (Hung and Masison 2006; Yang *et al.* 2013). However, the interplay between amyloid and the proteostasis network is complex, as the opposite scenario also appears to promote [*PSI*$^+$] formation: overexpression of Cyc8C elevates the levels of Hsp104 (significantly) and Sis1 (modestly) (Yang *et al.* 2013). Nonetheless, the impact of Pin4C overexpression provides clear support for the idea that overexpression of an amyloidogenic protein can titrate the fragmentation machinery away from nascent Sup35 aggregates, allowing them to persist (Yang *et al.* 2013).

The question then becomes, can the [*PIN*$^+$] phenotype, conferred by a self-replicating conformation of a prion protein expressed at its native level, be similarly linked to titration of the fragmentation machinery? Intriguingly, the presence of [*PIN*$^+$] can induce [*PSI*$^+$] loss in some cases (Bradley and Liebman 2003; Mathur, Hong and Liebman 2009; Westergard and True 2014), an observation that could reflect competition for cellular factors. However, the presence of [*PIN*$^+$] does not appear to alter the size of SDS-resistant aggregates of Sup35 (Bagriantsev and Liebman 2004), an observation that is at odds with a titration model or one that at a minimum suggests that titration is slight. Indeed, variants of [*PIN*$^+$] do not appear to be characterized by differences in their binding to chaperones (Sharma and Liebman 2013a; Stein and True 2014), despite their distinct [*PSI*$^+$]-induction frequencies (Bradley *et al.* 2002). Thus, strong support for the [*PIN*$^+$]-dependent titration of factors promoting the disassembly of nascent Sup35 aggregates is currently lacking.

Rigorous analysis of this model, moreover, is not a straightforward endeavor for a number of reasons. First, given the low

frequency of [*PSI*$^+$] appearance, the highest probability of detecting such an activity will occur in strains containing incompatible prions, which by definition induce each other's loss. Second, incompatibility between different prions is quite specific and is impacted not only by the constellation of prions present but also by their conformations, further restricting the experimental bandwidth in which to assess these effects (Bradley and Liebman 2003; Du and Li 2014). Third, the propagation of different prions and even different variants of the same prion are differentially sensitive to chaperone levels (Kushnirov *et al.* 2000; Wegrzyn *et al.* 2001; Kryndushkin *et al.* 2002; Fan *et al.* 2007; Tipton, Verges and Weissman 2008; Mathur, Hong and Liebman 2009; Hines *et al.* 2011a,b; DeSantis and Shorter 2012; Dulle and True 2013; Lancaster, Dobson and Rachubinski 2013; Dulle, Stein and True 2014; Harris *et al.* 2014; Stein and True 2014), raising the possibility of distinct titration targets. Fourth, the fragmentation machinery, if the target, would be required for both clearance and amplification of nascent Sup35 aggregates, and reductions in the expression of or mutations in these factors would be expected to lead to prion loss at additional points in the prion cycle beyond appearance. Fifth, chaperone-substrate interactions are notoriously transient. Given the constellation of intriguing observations compatible with this model, these challenges must be overcome to fully explore the pathway of prion appearance *in vivo*.

CONCLUSION

The predominant models for the role of [*PIN*$^+$] in [*PSI*$^+$] appearance are not mutually exclusive. Rather, the complexity and dynamics of the system, at the intersection between overlapping protein folding trajectories and proteostasis, and the large number of factors identified with the capacity to provide [*PIN*$^+$] activity in their amyloid state suggest a multitude of pathways to prion appearance *in vivo*. Indeed, the requirement for [*PIN*$^+$] can be completely bypassed if the prion-determining domain of Sup35 is fused to a random C-terminal extension (RVDLQACKLMIQYQRK), suggesting another route to overcome the *in vivo* barriers to prion appearance (Derkatch *et al.* 1997, 2000). Recent studies have embraced this possibility, and the extensive body of work contributed over the past two decades provides a strong foundation of genetic and physical interactions to guide future inquiry. To move forward, we must develop new approaches beyond the endpoint readout of assessing the appearance of a stable [*PSI*$^+$] state. The numerous questions remaining on prion appearance underscore the fact that mechanistic insight will only be gleaned through deconvolution of the interconnected processes of amyloid nucleation, persistence and amplification *in vivo*.

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REFERENCES

Aguzzi A. Beyond the prion principle. *Nature* 2009;**459**:924–5.

Alexandrov IM, Vishnevskaya AB, Ter-Avanesyan MD *et al.* Appearance and propagation of Polyglutamine-based amyloids in yeast. *J Biol Chem* 2008;**283**:15185–92.

Allen KD, Wegrzyn RD, Chernova TA *et al.* Hsp70 chaperones as modulators of prion life cycle. *Genetics* 2005;**169**:1227–42.

Arslan F, Hong JY, Kanneganti V *et al.* Heterologous aggregates promote de novo prion appearance via more than one mechanism. *PLoS Genet* 2015;**11**:e1004814.

Bagriantsev S, Liebman SW. Specificity of prion assembly *in vivo*. [*PSI*$^+$] and [*PIN*$^+$] form separate structures in yeast. *J Biol Chem* 2004;**279**:51042–8.

Baldwin AJ, Knowles TPJ, Tartaglia GG *et al.* Metastability of native proteins and the phenomenon of amyloid formation. *J Am Chem Soc* 2011;**133**:14160–3.

Bardill JP, True HL. Heterologous prion interactions are altered by mutations in the prion protein Rnq1p. *J Mol Biol* 2009;**388**:583–96.

Blackley HK, Sanders GH, Davies MC *et al.* In-situ atomic force microscopy study of beta-amyloid fibrillization. *J Mol Biol* 2000;**298**:833–40.

Bokvist M, Gröbner G. Misfolding of amyloidogenic proteins at membrane surfaces: the impact of macromolecular crowding. *J Am Chem Soc* 2007;**129**:14848–9.

Borchsenius AS, Muller S, Newnam GP *et al.* Prion variant maintained only at high levels of the Hsp104 disaggregase. *Curr Genet* 2006;**49**:21–29.

Borchsenius AS, Wegrzyn RD, Newnam GP *et al.* Yeast prion protein derivative defective in aggregate shearing and production of new seeds'. *EMBO J* 2001;**20**:6683–91.

Bradley ME, Edskes HK, Hong JY *et al.* Interactions among prions and prion "strains" in yeast. *P Natl Acad Sci USA* 2002;**99 Suppl 4**:16392–9.

Bradley ME, Liebman SW. Destabilizing interactions among [*PSI*$^+$] and [*PIN*$^+$] yeast prion variants. *Genetics* 2003;**165**:1675–85.

Chacinska A, Szczesniak B, Kochneva-Pervukhova NV *et al.* Ssb1 chaperone is a [*PSI*$^+$] prion-curing factor. *Curr Genet* 2001;**39**:62–67.

Chen B, Newnam GP, Chernoff YO. Prion species barrier between the closely related yeast proteins is detected despite coaggregation. *P Natl Acad Sci USA* 2007;**104**:2791–6.

Chernoff YO, Galkin AP, Lewitin E *et al.* Evolutionary conservation of prion-forming abilities of the yeast Sup35 protein. *Mol Microbiol* 2000;**35**:865–76.

Chernoff YO, Lindquist SL, Ono B *et al.* Role of the chaperone protein Hsp104 in propagation of the yeast prion-like factor [*PSI*$^+$]. *Science* 1995;**268**:880–4.

Chernoff YO, Newnam GP, Kumar J *et al.* Evidence for a protein mutator in yeast: role of the Hsp70-related chaperone ssb in formation, stability, and toxicity of the [*PSI*] prion. *Mol Cell Biol* 1999;**19**:8103–12.

Chien P, DePace AH, Collins SR *et al*. Generation of prion transmission barriers by mutational control of amyloid conformations. *Nature* 2003;**424**:948–51.

Choe Y-J, Ryu Y, Kim H-J *et al*. Increased [*PSI*$^+$] appearance by fusion of Rnq1 with the prion domain of Sup35 in *Saccharomyces cerevisiae*. *Eukaryot Cell* 2009;**8**:968–76.

Cox BS. [*PSI*], a cytoplasmic suppressor of super-suppression in yeast. *Heredity* 1965;**20**:505–21.

Crick F. Central dogma of molecular biology. *Nature* 1970;**227**:561–3.

Davis JK, Sindi SS. A mathematical model of the dynamics of prion aggregates with chaperone-mediated fragmentation. *J Math Biol* 2016;**72**:1555–78.

DePace AH, Santoso A, Hillner P *et al*. A critical role for amino-terminal glutamine/asparagine repeats in the formation and propagation of a yeast prion. *Cell* 1998;**93**:1241–52.

Derdowski A, Sindi SS, Klaips CL *et al*. A size threshold limits prion transmission and establishes phenotypic diversity. *Science* 2010;**330**:680–3.

Derkatch IL, Bradley ME, Hong JY *et al*. Prions affect the appearance of other prions. *Cell* 2001;**106**:171–82.

Derkatch IL, Bradley ME, Liebman SW. Overexpression of the SUP45 gene encoding a Sup35p-binding protein inhibits the induction of the de novo appearance of the [*PSI*$^+$] prion. *P Natl Acad Sci USA* 1998;**95**:2400–5.

Derkatch IL, Bradley ME, Masse SV *et al*. Dependence and independence of [*PSI*$^+$] and [*PIN*$^+$]: a two-prion system in yeast? *EMBO J* 2000;**19**:1942–52.

Derkatch IL, Bradley ME, Zhou P *et al*. Genetic and environmental factors affecting the *de novo* appearance of the [*PSI*$^+$] prion in *Saccharomyces cerevisiae*. *Genetics* 1997;**147**:507–19.

Derkatch IL, Liebman SW. Prion-prion interactions. *Prion* 2007;**1**:161–9.

Derkatch IL, Uptain SM, Outeiro TF *et al*. Effects of Q/N-rich, polyQ, and non-polyQ amyloids on the de novo formation of the [*PSI*$^+$] prion in yeast and aggregation of Sup35 *in vitro*. *P Natl Acad Sci USA* 2004;**101**:12934–9.

DeSantis ME, Shorter J. Hsp104 drives "protein-only" positive selection of Sup35 prion strains encoding strong [*PSI*$^+$]. *Chem Biol* 2012;**19**:1400–10.

Dever TE, Green R. The elongation, termination, and recycling phases of translation in eukaryotes. *CSH Persp Biol* 2012;**4**:a013706–6.

DiSalvo S, Derdowski A, Pezza JA *et al*. Dominant prion mutants induce curing through pathways that promote chaperone-mediated disaggregation. *Nat Struct Mol Biol* 2011;**18**:486–92.

Doel SM, McCready SJ, Nierras CR *et al*. The dominant PNM2- mutation which eliminates the psi factor of *Saccharomyces cerevisiae* is the result of a missense mutation in the *SUP35* gene. *Genetics* 1994;**137**:659–70.

Du Z, Li L. Investigating the Interactions of Yeast Prions: [*SWI*$^+$], [*PSI*$^+$], and [*PIN*$^+$]. *Genetics* 2014;**197**:685–700.

Duennwald ML, Echeverria A, Shorter J. Small heat shock proteins potentiate amyloid dissolution by protein disaggregases from yeast and humans. *PLoS Biol* 2012;**10**:e1001346.

Dulle JE, Stein KC, True HL. Regulation of the Hsp104 middle domain activity is critical for yeast prion propagation. *PLoS One* 2014;**9**:e87521.

Dulle JE, True HL. Low activity of select Hsp104 mutants is sufficient to propagate unstable prion variants. *Prion* 2013;**7**:394–403.

Eichner T, Kalverda AP, Thompson GS *et al*. Conformational conversion during amyloid formation at atomic resolution. *Mol Cell* 2011;**41**:161–72.

Ellis RJ, Minton AP. Protein aggregation in crowded environments. *Biol Chem* 2006;**387**:485–97.

Fan Q, Park K-W, Du Z *et al*. The role of Sse1 in the *de novo* formation and variant determination of the [*PSI*$^+$] prion. *Genetics* 2007;**177**:1583–93.

Ferrone FA, Hofrichter J, Eaton WA. Kinetics of sickle hemoglobin polymerization. *J Mol Biol* 1985;**183**:611–31.

Ferrone FA, Hofrichter J, Sunshine HR *et al*. Kinetic studies on photolysis-induced gelation of sickle cell hemoglobin suggest a new mechanism. *Biophys J* 1980;**32**:361–80.

Gaspar R, Meisl G, Buell AK *et al*. Secondary nucleation of monomers on fibril surface dominates α-synuclein aggregation and provides autocatalytic amyloid amplification. *Quart Rev Biophys* 2017;**50**:e6.

Gazit E. The "Correctly Folded" state of proteins: is it a metastable state? *Angew Chem Int Ed* 2002;**41**:257–9.

Ghaemmaghami S, Huh W-K, Bower K *et al*. Global analysis of protein expression in yeast. *Nature* 2003;**425**:737–41.

Gidalevitz T, Ben-Zvi A, Ho KH *et al*. Progressive disruption of cellular protein folding in models of polyglutamine diseases. *Science* 2006;**311**:1471–4.

Glover JR, Kowal AS, Schirmer EC *et al*. Self-seeded fibers formed by Sup35, the protein determinant of [*PSI*$^+$], a heritable prion-like factor of *S. cerevisiae*. *Cell* 1997;**89**:811–9.

Goldsbury C, Kistler J, Aebi U *et al*. Watching amyloid fibrils grow by time-lapse atomic force microscopy. *J Mol Biol* 1999;**285**:33–39.

Gorkovskiy A, Reidy M, Masison DC *et al*. Hsp104 disaggregase at normal levels cures many [*PSI*$^+$] prion variants in a process promoted by Sti1p, Hsp90, and Sis1p. *P Natl Acad Sci USA* 2017;**114**:E4193–202.

Guo J-P, Arai T, Miklossy J *et al*. Abeta and tau form soluble complexes that may promote self aggregation of both into the insoluble forms observed in Alzheimer's disease. *P Natl Acad Sci USA* 2006;**103**:1953–8.

Harris JM, Nguyen PP, Patel MJ *et al*. Functional diversification of hsp40: distinct j-protein functional requirements for two prions allow for chaperone-dependent prion selection. *PLoS Genet* 2014;**10**:e1004510.

Higurashi T, Hines JK, Sahi C *et al*. Specificity of the J-protein Sis1 in the propagation of 3 yeast prions. *P Natl Acad Sci USA* 2008;**105**:16596–601.

Hines JK, Higurashi T, Srinivasan M *et al*. Influence of prion variant and yeast strain variation on prion-molecular chaperone requirements. *Prion* 2011;**5**:238–44.

Hines JK, Li X, Du Z *et al*. [SWI], the prion formed by the chromatin remodeling factor Swi1, is highly sensitive to alterations in Hsp70 chaperone system activity. *PLoS Genet* 2011b;**7**:e1001309.

Holmes WM, Mannakee BK, Gutenkunst RN *et al*. Loss of amino-terminal acetylation suppresses a prion phenotype by modulating global protein folding. *Nat Commun* 2014;**5**:4383.

Huang L, Liu X, Cheng B *et al*. How our bodies fight amyloidosis: effects of physiological factors on pathogenic aggregation of amyloidogenic proteins. *Arch Biochem Biophys* 2015;**568**:46–55.

Hung G-C, Masison DC. N-terminal domain of yeast Hsp104 chaperone is dispensable for thermotolerance and prion propagation but necessary for curing prions by Hsp104 overexpression. *Genetics* 2006;**173**:611–20.

Inoue Y, Kawai-Noma S, Koike-Takeshita A *et al*. Yeast prion protein New1 can break Sup35 amyloid fibrils into fragments in an ATP-dependent manner. *Genes Cell* 2011;**16**:545–56.

Jahn TR, Radford SE. Folding versus aggregation: polypeptide conformations on competing pathways. *Arch Biochem Biophys* 2008;**469**:100–17.

Jarrett JT, Lansbury PT. Seeding "one-dimensional crystallization" of amyloid: a pathogenic mechanism in Alzheimer's disease and scrapie? *Cell* 1993;**73**:1055–8.

Kalastavadi T, True HL. Analysis of the [*RNQ*$^+$] prion reveals stability of amyloid fibers as the key determinant of yeast prion variant propagation. *J Biol Chem* 2010;**285**:20748–55.

Kawai-Noma S, Pack CG, Tsuji T *et al*. Single mother-daughter pair analysis to clarify the diffusion properties of yeast prion Sup35 in guanidine-HCl-treated [*PSI*] cells. *Gene Cells* 2009;**14**:1045–54.

Keefer KM, Stein KC, True HL. Heterologous prion-forming proteins interact to cross-seed aggregation in *Saccharomyces cerevisiae*. *Sci Rep* 2017;**7**:1–11.

Kimura Y, Koitabashi S, Kakizuka A *et al*. The role of pre-existing aggregates in Hsp104-dependent polyglutamine aggregate formation and epigenetic change of yeast prions. *Gene Cells* 2004;**9**:685–96.

King CY, Tittmann P, Gross H *et al*. Prion-inducing domain 2-114 of yeast Sup35 protein transforms in vitro into amyloid-like filaments. *P Natl Acad Sci USA* 1997;**94**:6618–22.

King C-Y, Wang H-L, Chang H-Y. Transformation of yeast by infectious prion particles. *Methods* 2006;**39**:68–71.

Klaips CL, Hochstrasser ML, Langlois CR *et al*. Spatial quality control bypasses cell-based limitations on proteostasis to promote prion curing. *Elife* 2014;**3**:1751.

Knowles TPJ, Vendruscolo M, Dobson CM. The amyloid state and its association with protein misfolding diseases. *Nat Rev Mol Cell Bio* 2014;**15**:384–96.

Knowles TPJ, Waudby CA, Devlin GL *et al*. An analytical solution to the kinetics of breakable filament assembly. *Science* 2009;**326**:1533–7.

Kochneva-Pervukhova NV, Alexandrov AI, Ter-Avanesyan MD. Amyloid-mediated sequestration of essential proteins contributes to mutant huntingtin toxicity in yeast. *PLoS One* 2012;**7**:e29832.

Koga H, Kaushik S, Cuervo AM. Protein homeostasis and aging: the importance of exquisite quality control. *Ageing Res Rev* 2011;**10**:205–15.

Krebs MRH, Morozova-Roche LA, Daniel K *et al*. Observation of sequence specificity in the seeding of protein amyloid fibrils. *Protein Sci* 2004;**13**:1933–8.

Krishnan R, Lindquist SL. Structural insights into a yeast prion illuminate nucleation and strain diversity. *Nature* 2005;**435**:765–72.

Kryndushkin DS, Smirnov VN, Ter-Avanesyan MD *et al*. Increased expression of Hsp40 chaperones, transcriptional factors, and ribosomal protein Rpp0 can cure yeast prions. *J Biol Chem* 2002;**277**:23702–8.

Kulak NA, Pichler G, Paron I *et al*. Minimal, encapsulated proteomic-sample processing applied to copy-number estimation in eukaryotic cells. *Nat Methods* 2014;**11**:319–24.

Kushnirov VV, Kryndushkin DS, Boguta M *et al*. Chaperones that cure yeast artificial [*PSI*$^+$] and their prion-specific effects. *Curr Biol* 2000;**10**:1443–6.

Lancaster AK, Bardill JP, True HL *et al*. The spontaneous appearance rate of the yeast prion [*PSI*$^+$] and its implications for the evolution of the evolvability properties of the [*PSI*$^+$] system. *Genetics* 2010;**184**:393–400.

Lancaster DL, Dobson CM, Rachubinski RA. Chaperone proteins select and maintain [*PIN*$^+$] prion conformations in *Saccharomyces cerevisiae*. *J Biol Chem* 2013;**288**:1266–76.

Lansbury PT. Evolution of amyloid: what normal protein folding may tell us about fibrillogenesis and disease. *P Natl Acad Sci USA* 1999;**96**:3342–4.

Liu JJ, Lindquist S. Oligopeptide-repeat expansions modulate "protein-only" inheritance in yeast. *Nature* 1999;**400**:573–6.

Lund PM, Cox BS. Reversion analysis of [*psi*$^-$] mutations in *Saccharomyces cerevisiae*. *Genet Res* 1981;**37**:173–82.

McGlinchey RP, Kryndushkin D, Wickner RB. Suicidal [*PSI*$^+$] is a lethal yeast prion. *P Natl Acad Sci USA* 2011;**108**:5337–41.

Manogaran AL, Hong JY, Hufana J *et al*. Prion formation and polyglutamine aggregation are controlled by two classes of genes. *PLoS Genet* 2011;**7**:e1001386.

Masel J, Jansen VA. The measured level of prion infectivity varies in a predictable way according to the aggregation state of the infectious agent. *Biochim Biophys Acta* 2001;**1535**:164–73.

Masel J, Jansen VA, Nowak MA. Quantifying the kinetic parameters of prion replication. *Biophys Chem* 1999;**77**:139–52.

Mathur V, Hong JY, Liebman SW. Ssa1 overexpression and [*PIN*$^+$] variants cure [*PSI*$^+$] by dilution of aggregates. *J Mol Biol* 2009;**390**:155–67.

Meriin AB, Zhang X, He X *et al*. Huntingtin toxicity in yeast model depends on polyglutamine aggregation mediated by a prion-like protein Rnq1. *J Cell Biol* 2002;**157**:997–1004.

Minton AP. The effect of volume occupancy upon the thermodynamic activity of proteins: some biochemical consequences. *Mol Cell Biochem* 1983;**55**:119–40.

Minton AP. Influence of macromolecular crowding upon the stability and state of association of proteins: predictions and observations. *J Pharm Sci* 2005;**94**:1668–75.

Nelson R, Sawaya MR, Balbirnie M *et al*. Structure of the cross-beta spine of amyloid-like fibrils. *Nature* 2005;**435**:773–8.

Oishi K, Kurahashi H, Pack C-G *et al*. A bipolar functionality of Q/N-rich proteins: Lsm4 amyloid causes clearance of yeast prions. *MicrobiologyOpen* 2013;**2**:415–30.

Osherovich LZ, Weissman JS. Multiple Gln/Asn-rich prion domains confer susceptibility to induction of the yeast [*PSI*$^+$] prion. *Cell* 2001;**106**:183–94.

Osherovich LZ, Weissman JS. The utility of prions. *Dev Cell* 2002;**2**:143–51.

Patel BK, Liebman SW. "Prion-proof" for [*PIN*$^+$]: infection with in vitro-made amyloid aggregates of Rnq1p-(132-405) induces [*PIN*$^+$]. *J Mol Biol* 2007;**365**:773–82.

Patino MM, Liu JJ, Glover JR *et al*. Support for the prion hypothesis for inheritance of a phenotypic trait in yeast. *Science* 1996;**273**:622–6.

Paushkin SV, Kushnirov VV, Smirnov VN. Propagation of the yeast prion-like [*PSI*$^+$] determinant is mediated by oligomerization of the SUP35-encoded polypeptide chain release factor. *EMBO J* 1996;**15**:3127–34.

Paushkin SV, Kushnirov VV, Smirnov VN *et al*. In vitro propagation of the prion-like state of yeast Sup35 protein. *Science NY* 1997;**277**:381–3.

Pei F, DiSalvo S, Sindi SS *et al*. A dominant-negative mutant inhibits multiple prion variants through a common mechanism. *PLoS Genet* 2017;**13**:e1007085.

Penrose LS, Penrose R. A Self-reproducing Analogue. *Nature* 1957;**179**:1183-.

Pezza JA, Serio TR. Prion propagation: the role of protein dynamics. *Prion* 2007;**1**:36–43.

Pezza JA, Villali J, Sindi SS *et al*. Amyloid-associated activity contributes to the severity and toxicity of a prion phenotype. *Nat Commun* 2014;**5**:4384.

Powers ET, Morimoto RI, Dillin A *et al.* Biological and chemical approaches to diseases of proteostasis deficiency. *Annu Rev Biochem* 2009;**78**:959–91.

Prusiner SB. Novel proteinaceous infectious particles cause scrapie. *Science* 1982;**216**:136–44.

Resende C, Parham SN, Tinsley C *et al.* The *Candida albicans* Sup35p protein (CaSup35p): function, prion-like behaviour and an associated polyglutamine length polymorphism. *Microbiology* 2002;**148**:1049–60.

Rotter MA, Kwong S, Briehl RW *et al.* Heterogeneous nucleation in sickle hemoglobin: experimental validation of a structural mechanism. *Biophys J* 2005;**89**:2677–84.

Salnikova AB, Kryndushkin DS, Smirnov VN *et al.* Nonsense suppression in yeast cells overproducing Sup35 (eRF3) is caused by its non-heritable amyloids. *J Biol Chem* 2005;**280**:8808–12.

Santoso A, Chien P, Osherovich LZ *et al.* Molecular basis of a yeast prion species barrier. *Cell* 2000;**100**:277–88.

Sarell CJ, Stockley PG, Radford SE. Assessing the causes and consequences of co-polymerization in amyloid formation. *Prion* 2013;**7**:359–68.

Satpute-Krishnan P, Langseth SX, Serio TR. Hsp104-dependent remodeling of prion complexes mediates protein-only inheritance. *PLoS Biol* 2007;**5**:e24.

Satpute-Krishnan P, Serio TR. Prion protein remodelling confers an immediate phenotypic switch. *Nature* 2005;**437**:262–5.

Sawaya MR, Sambashivan S, Nelson R *et al.* Atomic structures of amyloid cross-beta spines reveal varied steric zippers. *Nature* 2007;**447**:453–7.

Scheibel T, Kowal AS, Bloom JD *et al.* Bidirectional amyloid fiber growth for a yeast prion determinant. *Curr Biol* 2001;**11**:366–9.

Serio TR, Cashikar AG, Kowal AS *et al.* Nucleated conformational conversion and the replication of conformational information by a prion determinant. *Science* 2000;**289**:1317–21.

Sharma J, Liebman SW. Exploring the basis of [*PIN*$^+$] variant differences in [*PSI*$^+$] induction. *J Mol Biol* 2013a**425**:3046–59.

Sharma J, Liebman SW. Variant-specific prion interactions. *Cellular Logistics* 2013b;**3**:e25698.

Sharma J, Wisniewski BT, Paulson E *et al. De novo [PSI +] prion formation involves multiple pathways to form infectious oligomers*. *Sci Rep* 2017;**7**:76.

Sondheimer N, Lindquist S. Rnq1. *Mol Cell* 2000;**5**:163–72.

Song Y, Wu Y-X, Jung G *et al.* Role for Hsp70 chaperone in *Saccharomyces cerevisiae* prion seed replication. *Eukaryot Cell* 2005;**4**:289–97.

Stein KC, True HL. Extensive diversity of prion strains is defined by differential chaperone interactions and distinct amyloidogenic regions. *PLoS Genet* 2014;**10**:e1004337.

Sunde M, Serpell LC, Bartlam M *et al.* Common core structure of amyloid fibrils by synchrotron X-ray diffraction. *J Mol Biol* 1997;**273**:729–39.

Tanaka M, Chien P, Naber N *et al.* Conformational variations in an infectious protein determine prion strain differences. *Nature* 2004;**428**:323–8.

Ter-Avanesyan MD, Dagkesamanskaya AR, Kushnirov VV *et al.* The *SUP35* omnipotent suppressor gene is involved in the maintenance of the non-Mendelian determinant [*PSI*$^+$] in the yeast *Saccharomyces cerevisiae*. *Genetics* 1994;**137**:671–6.

Tessier PM, Lindquist S. Prion recognition elements govern nucleation, strain specificity and species barriers. *Nature* 2007;**447**:556–61.

Tipton KA, Verges KJ, Weissman JS. In vivo monitoring of the prion replication cycle reveals a critical role for Sis1 in delivering substrates to Hsp104. *Mol Cell* 2008;**32**:584–91.

Tuite MF, Serio TR. The prion hypothesis: from biological anomaly to basic regulatory mechanism. *Nat Rev Mol Cell Bio* 2010;**11**:823–33.

Tyedmers J, Madariaga ML, Lindquist S. Prion switching in response to environmental stress. *PLoS Biol* 2008;**6**:e294.

Tyedmers J, Treusch S, Dong J *et al.* Prion induction involves an ancient system for the sequestration of aggregated proteins and heritable changes in prion fragmentation. *P Natl Acad Sci USA* 2010;**107**:8633–8.

Uptain SM, Sawicki GJ, Caughey B *et al.* Strains of [*PSI*$^+$] are distinguished by their efficiencies of prion-mediated conformational conversion. *EMBO J* 2001;**20**:6236–45.

Vishveshwara N, Liebman SW. Heterologous cross-seeding mimics cross-species prion conversion in a yeast model. *BMC Biol* 2009;**7**:26.

Vitrenko YA, Gracheva EO, Richmond JE *et al.* Visualization of aggregation of the Rnq1 prion domain and cross-seeding interactions with Sup35NM. *J Biol Chem* 2007;**282**:1779–87.

Wegrzyn RD, Bapat K, Newnam GP *et al.* Mechanism of prion loss after Hsp104 inactivation in yeast. *Mol Cell Biol* 2001;**21**:4656–69.

Westergard L, True HL. Extracellular environment modulates the formation and propagation of particular amyloid structures. *Mol Microbiol* 2014;**92**:698–715.

Wickner RB. [*URE3*] as an altered URE2 protein: evidence for a prion analog in *Saccharomyces cerevisiae*. *Science* 1994;**264**:566–9.

Wisniewski BT, Sharma J, Legan ER *et al.* Toxicity and infectivity: insights from de novo prion formation. *Curr Genet* 2018;**64**:117–23.

Yang Z, Hong JY, Derkatch IL *et al.* Heterologous gln/asn-rich proteins impede the propagation of yeast prions by altering chaperone availability. *PLoS Genet* 2013;**9**:e1003236.

Zhou P, Derkatch IL, Liebman SW. The relationship between visible intracellular aggregates that appear after overexpression of Sup35 and the yeast prion-like elements [*PSI*$^+$] and [*PIN*$^+$]. *Mol Microbiol* 2001;**39**:37–46.