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Supporting Online Material for

A Size Threshold Limits Prion Transmission and Establishes Phenotypic Diversity

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Supporting Online Material

Materials and Methods

Strain and Plasmid Construction

All strains used in this study (Table S1) are derivatives of 74D-694 (S1). SY779 was constructed by crossing SY85 to a $[PSI^{\dagger}]^{Weak}$ strain (2600 in our strain collection) and isolating haploid meiotic progeny expressing Sup35-GFP from the resulting diploid. pRS304-P_{GPD}GST-UGA-GFP-PEST was constructed by substituting GFP-PEST, which was amplified by PCR from pSVA13 as an EcoRI-Xhol fragment using primers TRS406 and TRS407 (Table S2), into pRS304GST(UGA)DsRED for DsRED (S2, S3). SY1635 was constructed by transforming a [*PSI*⁺]^{Strong} strain (2606 in our strain collection) with pRS304-P_{GPD}GST-UGA-GFP-PEST that had been linearized with *Bsu36I* and selecting for tryptophan prototrophy. SY1637 was generated by guanidine HCI (GdnHCI) curing of SY1635 (*S4*), and SY1636 was generated by crossing SY1635 to a [*PSI*⁺]^{Weak} strain (1220 in our strain collection) and isolating haploid meiotic progeny expressing the SY1607 was constructed by transforming SY81 with a PCR product reporter. containing the P_{tet07} promoter, which was amplified from pCM225 with primers TRS218 and TRS219 (Table S1) (S5). G418-resistant colonies were screened for proper integration using primers TRS221/TRS270 and TRS217/TRS136 (Table S2).

[*PSI*⁺] loss and Propagon Counts

Exponentially growing cultures of the indicated strain were plated on YPD for single colonies, and the frequency of $[PSI^{+}]$ loss was determined by the number of red colonies arising as a function of the total. For age-controlled experiments, a newly budded daughter cell was isolated by micromanipulation, and each of its daughters was isolated by micromanipulation prior to propagon determination. The number of propagons per cell was determined by an *in vivo* dilution, colony-based method, as previously described (*S6*).

Centrifugal Elutriation

Exponentially growing cultures of SY1607 were treated with doxycycline for one generation and separated in a Beckman JE-5.0 elutriation rotor in a Beckman Avanti J-20 XP centrifuge at 4°C. Cells were loaded at a flow rate of 10 mL/min and at a centrifuge speed of 4000rpm until a clear cell boundary was observed. The centrifugal speed was then gradually decreased until cells began to exit the system as observed by spectrophotometry, and daughter-enriched fractions were collected. Daughter lysate was loaded by cell equivalents to correspond to the fraction of daughters in the total population.

Protein Analysis

Semi-denaturing agarose gel electrophoresis (SDD-AGE), SDS-PAGE, and quantitative immunoblotting, using anti-GFP antiserum (Santa Cruz Biotechnology) were performed as previously described (*S7*).

Fluorescence Imaging and Analysis

Studies analyzing Sup35-GFP fluorescence pattern were performed as previously described (*S3*). For analysis of fluorescence patterns, 500 cells were quantified for each independent experiment. FLIP experiments were performed on a Zeiss LSM510-META laser scanning confocal microscope equipped with a 63x objective (NA=1.4) and an Argon/Helium Neon laser. Frames (2x zoom, 100 x 100 pixels, 1 Airy unit) were collected using 488-nm excitation and 500-560nm emission wavelengths. Laser intensity was set to 5% for image acquisition and 100% for bleaching for 8 iterations. Cells with buds ~1/3 their size were chosen for analysis, and average fluorescence pixel intensity was monitored in the mother cell, the bud, and a nearby control cell, with bleaching of either the entire mother or bud following each image acquisition. For each data set, a minimum of 10 cells was analyzed. Image analysis was performed using Zeiss LSM Image Analysis software, where specific values were normalized to background levels of fluorescence loss and presented as time post-initial bleach. For flow cytometry, cells were analyzed using a FACSAria fluorescence-activated cell sorter (BD Biosciences, San Jose, CA). GFP expression in cell populations was analyzed

using 488 nm excitation and 515-545nm emission wavelengths, and the resulting data were used to generate histograms (FlowJo analysis software, Tree Star, Ashland, OR). For FACS, cells were separated and stained with 1mg/mL fluorescence brightener 28 (Sigma-Aldrich, St. Louis, MO), as previously described (*S8*). Bud scars were visualized by confocal microscopy using 405 nm excitation and >420nm emission wavelengths and optical sectioning (~8 μ m/slice). For each data set, a minimum of 50 cells was analyzed.

Statistical Analysis

Where indicated, statistical significance was determined using a Student's t-test.

Supporting Text

Introduction

A number of mathematical models have been developed to study the appearance and spread of prion disease in mammals (*S9-S12*). While the models differ in specific formulations, the rates of four biochemical events must be considered in an accurate assessment of accumulation of the prion conformation of the protein at steady state *in vivo*: 1) synthesis of the protein (α), 2) conversion to the prion conformation (β), 3) fragmentation of aggregates (γ), and 4) degradation of the protein (α). Within this framework, the concentrations of prion (aggregates; *Z*) and non-prion (soluble; *X*) conformations of the protein, as well as the number of aggregates per unit volume (*Y*), are modeled using a system of either ordinary or partial differential equations, for example:

$$\frac{dX}{dt} = \alpha - \beta XY - dX \frac{dY}{dt} = \gamma Z - dY \frac{dZ}{dt} = \beta XY - dZ.$$

A similar set of continuous time ordinary differential equations has been developed to describe the accumulation of the prion conformation in the yeast *Saccharomyces cerevisiae* (*S13*), but in this case, removal of existing protein from the system occurs through dilution by cell growth (R) rather than degradation of the protein:

$$\begin{aligned} \frac{dX}{dt} &= \alpha - \beta XY - RX\\ \frac{dY}{dt} &= \gamma Z - RY\\ \frac{dZ}{dt} &= \beta XY - RZ. \end{aligned}$$

These formulations settle to distinct equilibria between the soluble and aggregated forms of the protein that are defined by the rates of conversion (β) and fragmentation

(γ). Therefore, these models provide a relationship between the parameters that regulate prion aggregate dynamics (α , β , d or γ) for individual strains and the corresponding severity of the associated phenotype (*Z*). When parameters allow stability of the prion state and aggregates are initially present, the endpoint equilibria observed are reached regardless of the starting condition and are largely unperturbed by cell-to-cell variation introduced by a stochastic version of this model (*S13*). However, these continuous and stochastic models cannot be reconciled when stability of the phenotype (i.e. frequency of prion loss) is assessed, indicating that sources of heterogeneity must be considered to accurately model prion biology (*S14, S15*).

Dynamic Modeling of Prions in Growing Yeast Cultures

To explore the *in vivo* pathways by which conformation-based phenotypes of defined severity and stability are created and maintained, we developed a new stochastic model of Sup35 aggregate dynamics that integrates the prion protein misfolding pathway with its cellular environment for the first time. In this formulation, aspects of prion biology and of yeast cell biology contribute four potential sources of heterogeneity: 1) unequal cell division in *Saccharomyces cerevisiae*, 2) variation in aggregate size, 3) aggregate transmission, and 4) enzyme limitation of the fragmentation reaction. In this system-based formulation, in which each of these processes is integrated with one another, is necessary to accurately recapitulate the severity and stability of conformation-based phenotypes *in vivo*.

Preliminaries

Yeast Cell Growth:

Individual yeast cells exhibit asymmetric growth rates; the time to produce the first bud is substantially longer than that necessary to produce subsequent buds (*S16*). The standard model for the time, t, until cell division is a gamma distribution:

$$f(t;\rho,\lambda) = \frac{\rho^{-\rho\lambda}e^{-t\rho}t^{(\rho\lambda-1)}}{\Gamma(\rho\lambda)},$$

for $t \ge 0$ where $f(t;\rho,\lambda)$ has mean λ and variance λ/ρ . Following Byrne (*S14*), we use gamma distributions to model both the time for a daughter cell to mature to a mother cell, $f(t;\rho,\lambda_d)$, and the time for a mother cell to produce a new daughter $f(t;\rho,\lambda_m)$, where $\lambda_d = 0.21$ (hours), $\lambda_m = 1.16$ (hours) and $\rho = 31.03$. Using these conditions, our simulations reproduce the observed asymmetry in mother and daughter division times (Fig. S1), an exponential growth rate for the population (Fig. S2), and the observed population demographics (Fig. S3) (*17*).

Protein Transmission:

Transmission of protein from mother to daughter cells was modeled as occurring instantaneously when the time for cell division was reached. Because our model follows the number of molecules of each protein rather than their concentrations, we estimated the fraction of protein transmitted from mother to daughter during cell division. Based on the difference in cell volume between mother and daughter (*S16*) and the experimentally determined transmission of heritable prion species (propagons) (*S14*), it is likely that the transmissible Sup35 species freely diffuse between mother and daughter cells. Thus, we assume a 60:40 split between aggregates that are transmissible (see Stochastic Simulations).

Protein Synthesis:

The rates of protein synthesis of Sup35 and Hsp104, the catalyst for the fragmentation step, were determined from the number of molecules of each present at steady-state in a typical cell (*S18*). To attain population averages as a basis for comparison with empirical observations, we estimated that a cell generates 700 Sup35 monomers and 50 Hsp104 hexamers per minute. We assumed that all cells had the same rate of protein synthesis. With these rates of synthesis, the average number of Sup35 monomers in a cell is ~74,000 and the average number of Hsp104 hexamers is ~5,300 (Fig. S4), which is in excellent agreement with the experimentally determined values of 78,900 \pm 9,500 and 5,467, respectively (*S18*).

Minimum Aggregate Size:

Following a prior model, we impose a minimum size for an aggregate (*S13*). After fragmentation, aggregates with fewer than 6 monomers dissolve and rejoin the soluble pool.

Our stochastic model follows individual cells in a growing culture as well as the individual molecules within a cell.

Stochastic Model Formulation

Biochemical Equations:

Current understanding of prion biology indicates there are three processes necessary for the stable maintenance of prions: synthesis, conversion, and fragmentation (*S19*). These processes are modeled as biochemical equations with reaction rates α , synthesis (molecules/minute); β , conversion (molecules minute)⁻¹ and γ , fragmentation (1/minute) (*S15*). Depending on the form of these equations, different dynamics result. In our work, we explored two different systems of equations:

Hsp104-Independent Fragmentation Rate:

Hsp104-Limited Fragmentation Rate:

$$\begin{array}{cccc} \emptyset & \stackrel{\alpha_X}{\longrightarrow} & X \\ & \emptyset & \stackrel{\alpha_H}{\longrightarrow} & H \\ & & X + Y_i & \stackrel{\beta}{\longrightarrow} & Y_{i+1} \\ & & Y_i + H(\leftrightarrow [Y_iH]) & \stackrel{\gamma}{\longrightarrow} & Y_{i_1} + Y_{i_2} + H. \end{array}$$

Where in the biochemical equations: *X* represents a molecule of Sup35, *H* a hexamer of Hsp104, Y_i a prion aggregate of size *i*, and $[Y_iH]$ a complex of a prion aggregate of size *i* and a hexamer of Hsp104. (In the biochemical equations for fragmentation, we require that $i = i_1 + i_2$.)

Stochastic Simulations with the Gillespie Algorithm:

Protein dynamics were modeled following an approach described by Gillespie for exact stochastic simulations of biochemical equations (*S20*). The state of each cell consists of the number of individual molecules of Sup35, X, and of hexamers of Hsp104, H, and the length of each aggregate. The Gillespie Algorithm provides a method to sample a stochastic realization of the protein dynamics by computing successive reactions according to their respective rates and the current state of the cell. For example, conversion of soluble Sup35 onto existing aggregates occurs at a rate proportional to the product of the number of aggregates and the number of Sup35 monomers, with the mass action kinetics approximation of uniform mixing within cells.

To simplify the simulations when considering the model with Hsp104-limited fragmentation, we did not explicitly model the binding of Hsp104 to aggregates. That is, we modeled fragmentation using a Michaelis-Menten approximation with $K_{\text{max}} = \gamma H$ and $K_M = \frac{H}{2}$. Thus, as a function of the current number of Hsp104 hexamers, the rate of fragmentation is given by $\left(\frac{K_{\text{max}}Z}{K_M + Z}\right)$, where *Z* is the total number of available fragmentation sites. That is, when Y_i is the total number of aggregates of size *i*, $Z = \sum_i (i-1)Y_i$).

Stochastic Simulations

Each simulation began with a single cell having an identical initial prion configuration (29 aggregates of size 20, 59 aggregates of size 40 and 10 aggregates of size 50), Sup35 monomers (30,000) and Hsp104 hexamers (2000). Before the simulation began, this first cell underwent five division cycles, allowing the distribution of aggregates to

stabilize. The simulation then began with a daughter produced from this stabilized system. Because cells evolve independently from one another, we apply the model to each cell independently, allowing us to study large populations. We followed the resulting population for 1000 minutes at which point the culture consisted of ~ 7,000 cells. Empirically, we observed that, for the parameters we studied, prion loss was typically stabilized between 850 – 1000 minutes. In our analysis, we assumed the parameters related to cell division, protein synthesis, and protein transmission (discussed above) to be fixed for all scenarios. We then studied the behavior of prion dynamics under three different models.

Model 1: Aggregates of All Sizes Are Equally Transmissible

In this "abundance-based" model, aggregates of any size can be transmitted from mother to daughter, as previously described (*S13*). Because of differences in volume between a mother and her daughter, we expect an average of 40% of all prion aggregates to be transmitted (*S14, 16*). That is, during cell division there is a 40% chance for each aggregate to be transmitted from mother to daughter. To capture the physical differences between [*PSI*⁺]^{Weak} and [*PSI*⁺]^{Strong} strains, we evaluated a broad range of conversion ($4.5 \times 10^{-5} \le \beta \le 3.0 \times 10^{-4}$ (molecules min)⁻¹, with equal step size) and fragmentation rates ($1 \times 10^{-4} \le \gamma \le 1.3 \times 10^{-3}$ min⁻¹, with equal step size; Fig. S5). *In vivo*, the [*PSI*⁺]^{Weak} phenotype is three orders of magnitude less stable than the [*PSI*⁺]^{Strong} phenotype (*S21, 22*); however, our simulations were unable to recapitulate this difference, with the prion form stably persisting in the dividing population over nearly the entire range of rate parameters sampled (Fig. S5a). Under these conditions, we were also unable to model the steady-state differences in soluble Sup35 found in [*PSI*⁺]^{Weak} and [*PSI*⁺]^{Strong} strains (35% vs. 19%, respectively; Fig. S5b) (*S7*).

There is a clear explanation of why such a model will result in low levels of prion loss. If a mother cell has N aggregates, each of which can be independently inherited by the daughter with probability 0.4, the probability of a daughter inheriting no aggregates is $(0.6)^{N}$. Since mother cells in any of these simulations have, on average, many tens of aggregates, the probability of spontaneous loss will be far smaller than *in vivo*

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observations as previously suggested (*S13, S14*). This discrepancy in accurately depicting prion loss suggests that the transmission of prion aggregates from mother to daughter is restricted by factors in addition to their abundance and the volume differences between these cells. To identify these factors, we examined a size-dependent transmission of aggregates.

Model 2: Size-Dependent Transmission of Aggregates

One factor that has not been previously considered in mathematical analyses of prion strains is variation in the aggregate size distributions. Prion proteins assemble into SDS-resistant polymers, which are the major constituents of the larger aggregates present in vivo (S23, S24). These native complexes scale in size with the length of the polymers (typically 6-100 monomers) (S13, S23), but any given prion strain will accumulate aggregates only within a more narrow range of the potential size distribution (S23, S25). For example, the $[PSI^{\dagger}]^{Weak}$ conformation accumulates very few aggregates below 15-20 monomers in size, while nearly half of the size distribution of [PSI⁺]^{Strong} aggregates is found in this range (S23, S25). We therefore considered the possibility that conformation-based phenotypes are created and sustained by a subpopulation of native aggregates that are defined by the length of their SDS-resistant polymers. Because large increases in aggregate size brought about by inhibition of the fragmentation reaction decrease the mobility of these aggregates and the stability of the associated phenotypes in vivo (S23, S26, S27), we hypothesized that aggregates of different sizes, present even under normal conditions, would differ in their transmission efficiencies.

We incorporated this feature into our model by imposing a strict upper bound, T, on the size of aggregate that could be transmitted from mother to daughter. However, we do not rule out the possibility that all aggregates have the capacity to be transmitted but that only those below a certain size, which approximates our threshold, will do so with any biologically relevant probability. We allowed free diffusion of aggregates smaller than T, resulting in an expected 60:40 split between mother and daughter cells. Within

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each cell, prion dynamics evolved according to the biochemical equations with an Hsp104-independent fragmentation rate.

Unlike the "abundance-based" model, imposing a size limitation on aggregate transmission revealed variation in both the severity and stability of prion phenotypes. To objectively determine if our simulations could capture the [PSI⁺]^{Strong} and [PSI⁺]^{Weak} phenotypes, we used polynomial interpolation to determine the relationship of fragmentation and conversion rates to prion loss and to the fraction of soluble Sup35 using a continuous rather than a step-wise function, and these interpolations provided excellent fits for our simulation-derived data (Fig. S6). Using these interpolations, we determined the intersection of the frequencies of prion loss (0% and 2-5%) and soluble Sup35 (12-17% and 30-40%) to locate the parameter combinations consistent with the $[PSI^{\dagger}]^{\text{Strong}}$ and $[PSI^{\dagger}]^{\text{Weak}}$ states, respectively, for thresholds of 10, 15, and 20 monomers/aggregate (Fig. S7abc). However, this model does not recapitulate the known enhanced conversion rate (Fig. S7bc) or the increase in aggregate size (Fig. S7d) for $[PSI^{\dagger}]^{Weak}$ in comparison with $[PSI^{\dagger}]^{Strong}$ (S23, S28). With a transmission threshold of 10 monomers/complex, our simulations were unable to capture either the $[PSI^{\dagger}]^{Weak}$ or $[PSI^{\dagger}]^{Strong}$ states (Figs. S6ab, S7a). At higher transmission thresholds, the [*PSI*⁺]^{Weak} and [*PSI*⁺]^{Strong} phenotypes and their stabilities were recapitulated (Fig. S7bc); however, the outcomes of the simulations diverged from in vivo observations in other ways. Specifically, the [*PSI*⁺]^{Weak} phenotype appeared at a lower rate of conversion than did the [*PSI*⁺]^{Strong} phenotype (Fig. S7bc) although the opposite relationship has been experimentally determined (S13), and the aggregate size distributions predicted by the model were nearly identical for the two conformers despite their clear distinction in lysates (Fig. S7d) (S23). Thus, to accurately capture all experimental observations we must consider factors beyond size transmission alone.

Model 3: Hsp104 is Required for Aggregate Fragmentation

Hexamers of the yeast protein Hsp104 are essential for the fragmentation process (*S27, S29, S30*). We adapted our biochemical equations to limit fragmentation based on the availability of Hsp104 hexamers and of aggregates. As mentioned earlier, rather than

explicitly simulating the process of hexamer formation from monomers, we modeled directly the synthesis of Hsp104 hexamers. In addition, we modeled the fragmentation process using the Michaelis-Menten approximation for enzyme-substrate kinetics. As demonstrated in the main text, this "size-based" model captures the differences in soluble Sup35 concentration (Table 1) (S7), accounts for the differences in aggregate size distributions (Fig. S10) (S23), and recapitulates experimentally determined frequencies of prion loss for a [PSI⁺]^{Weak} strain (Table 1) (S21, 31), but only under a particular subset of conditions. If prion aggregates are instead transmitted based on their abundance and the volume differences between mother and daughter cells, we do not observe the variation in stability and severity of the prion phenotype that is necessary to explain the $[PSI^{\dagger}]^{Weak}$ and $[PSI^{\dagger}]^{Strong}$ states over a broad range of fragmentation (1 x $10^{-3} \le \gamma \le 1.3 \text{ x } 10^{-2} \text{ min}^{-1}$) and conversion (4.5 x $10^{-5} \le \beta \le 3.0 \text{ x } 10^{-4}$ (molecules min)⁻¹) rates (Fig. S8ab). However, if the transmission of prion aggregates is limited by their size, biologically observed conditions can be captured. [PSI⁺]^{Weak} strains do not significantly accumulate prion aggregates below a size of 15-20 monomers, in comparison with [PSI⁺]^{Strong} strains (S23). When we impose a size threshold for transmission within this range (Fig. S9abc), variability in the severity and stability of prion phenotypes can capture the two states. However, prions become stable and phenotypically strong at a threshold outside of this range (i.e. 40 monomers/aggregate; Fig. S9d), indicating that size-limitation is a necessary component of the model to accurately describe the diversity of conformation-based phenotypes.

Demonstrating Model Consistency

Model 3 described above considered both a size-limitation on aggregate transmission as well as an Hsp104-limited fragmentation of these complexes. To ascertain if the enzyme-limitation alone was sufficient to capture the [*PSI*⁺]^{Strong} and [*PSI*⁺]^{Weak} states, we returned to an "abundance-based" model, but in this case included Michaelis-Menten kinetics. In this model, we allow aggregates of any size to be transmitted with 40% probability. This model demonstrates greater phenotypic variation with changes in conversion and fragmentation rates, as assessed by the fraction of the soluble Sup35 (Fig. S8b), but the model cannot capture the experimentally determined relationship between phenotype and prion loss (Fig. S8ab), in contrast to our "size-based model" (Fig. S9). Moreover, this model does not recapitulate the difference in aggregate size distribution observed between mother and daughter cells (Fig. S8c, S10). These experimental observations are only recapitulated when both a size threshold for transmission and Hsp104-limited fragmentation are considered together (see main text).

In addition to recapitulating observations of spontaneous prion loss, our new formulation provides a physical explanation for the observed increased frequency of prion loss associated with higher rates of prion protein synthesis (Fig. 2D). As described in the main text, this loss is associated with a decrease in aggregate transmission efficiency that correlates with a shift in the size of these complexes. This behavior is unique to our new formulation. According to the continuous differential equation model (*S13*), the [*PSI*⁺] state will be asymptotically stable as long as

$$\beta\gamma > \left(\frac{R^{3}}{\alpha} \right),$$

where *R* is the rate of dilution due to cell growth, α the rate of Sup35 synthesis, β the rate of conversion and γ , the rate of fragmentation of aggregates. This condition predicts that prion stability will not be altered by increases in the rate of Sup35 synthesis (α) when the conversion and fragmentation rates are held constant. Thus, our model resolves an existing inconsistency between available mathematical models and empirical observations of prion loss (*S13, S14, S32-S34*).

Supporting Figures



Figure S1. Distribution of division times for mothers (black) and daughters (red) for a typical mathematical simulation.



Figure S2. Population growth over time for a typical mathematical simulation.



Figure S3. Population demographics for a simulated yeast culture. After an initial transience, the population becomes roughly 45% mothers (blue) and 55% daughters (red).



Figure S4. A typical distribution of Sup35 monomers (a) and Hsp104 hexamers (b) for a [*psi*] population of ~6700 yeast cells generated by our mathematical simulations.



Figure S5. Stochastic simulations for Model 1. We consider 400 different combinations of parameters for conversion, $4.5 \times 10^{-5} \le \beta \le 3.0 \times 10^{-4}$ (molecules min)⁻¹ and fragmentation, $1.0 \times 10^{-4} \le \gamma \le 1.3 \times 10^{-3}$ min⁻¹, of equal step size. At the conclusion of the simulation, we plot the fraction of cells with prion aggregates (a) and the fraction of Sup35 in the soluble state (b). With an abundance directed transmission of prion aggregates, we cannot recapitulate observations of prion phenotype severity or stability for the [*PSI*⁺]^{Strong} and [*PSI*⁺]^{Weak} states.



Figure S6. Polynomial interpolation of simulation results. We determined the relationship of fragmentation and conversion rates to prion loss (a) and the fraction of soluble Sup35 (b) with our stochastic simulations for a model in which prion aggregates are transmitted with a size threshold of 10 monomers/aggregate. We demonstrate the fit to the interpolation (red) and simulation results (blue) as a function of $log(\beta\gamma)$. To maximize confidence, we restricted our polynomial to lie within the range of 0 to 1 when conducting further analysis. Similar fits were obtained for thresholds of 15 and 20 monomers/aggregate (data not shown).



Figure S7. Identification of $[PSI^{\dagger}]^{Weak}$ and $[PSI^{\dagger}]^{Strong}$ states by intersection of the frequencies of prion loss and fraction of soluble Sup35 using polynomial interpolation of We consider ranges of parameter space stochastic simulation results for Model 2. consistent with the observed phenotypic stability and severity (i.e. fraction of soluble Sup35) for maximum transmission thresholds of (a) 10, (b) 15 and (c) 20. For the [PS/⁺]^{Strong} state, the purple contour represents 12-17% soluble Sup35 and the blue field represents 100% prion retention. For the [PSI⁺]^{Weak} state, the red contour represent 30-40% soluble Sup35 and the green contour represents 95-98% prion retention. The intersection of these parameters for each state identifies the unique combinations of conversion and fragmentation rates that are consistent with each state. However, this model, in which fragmentation occurs independently of Hsp104 concentration, does not recapitulate the known relationship between conversion and fragmentation rates for the two states (see main text for details). Moreover, parameter values that are consistent with the strains produce similar aggregate distributions (d) directly contradicting empirical observations.



Figure S8. Hsp104-limited fragmentation is not sufficient to recapitulate prion biology. (a) The fraction of cells with prion aggregates and (b) the fraction of soluble Sup35 were determined in stochastic simulations of prion dynamics over the range of conversion and fragmentation rates described in the text under the conditions of Hsp104-limitation and 40% transmission of prion aggregates of all sizes. While the fraction of soluble Sup35 (b) varies with conversion and fragmentation rates, the entire population has prions (a); therefore, these conditions do not maintain the link between phenotype and stability. (c) The size distribution of prion aggregates in the total (black), mother (red) and daughter (blue) populations is shown for a simulation matching the [*PSI*⁺]^{Strong} state in both stability and fraction of soluble Sup35. When there is no size threshold imposed on transmission the mother and daughter distributions will differ by only a slight change in magnitude, a result that is inconsistent with empirical observations (Fig. 3G).



Figure S9. We illustrate the fraction of the population maintaining prion aggregates (left) and the fraction of fraction of soluble Sup35 (right) under Hsp104-limited fragmentation and size-limited transmission from mother to daughter cells for thresholds of 10 (a), 20 (b), 30 (c), and 40 (d) monomers/aggregate. The $[PSI^+]^{\text{Strong}}$ and $[PSI^+]^{\text{Weak}}$ states are recapitulated by the solid white box and dotted white box, respectively.



Figure S10. The distribution of prion aggregate size for the total (solid blue), mother (dashed blue), and daughter (dotted blue) fractions of the $[PSI^{+}]^{\text{Strong}}$ and $[PSI^{+}]^{\text{Weak}}$ (red) conditions as determined by stochastic simulation of the model presented in Fig. S9b.



Figure S11: Relative Sup35GFP expression levels for $[PSI^+]^{\text{Strong}}$ strains expressing the protein from the endogenous Sup35 promoter (p35, SY81) or from a tetracycline regulated promoter (ptet, SY1607). N=4; error bars: SD.



Figure S12: Centrifugal elutriation enriches for daughter fraction of the population. A culture of a $[PSI^{+}]^{\text{Strong}}$ strain expressing Sup35-GFP from ptet (SY1607) were separated by centrifugal elutriation, and the number of bud scars on individual cells in the total and daughter fractions was determined on live cells using Fluorescence Brightener 28 (calcofluor). The proportion of cells containing either one bud scar (white) or multiple bud scars (black) is presented as a fraction of the total. A total of 35 cells were analyzed from each fraction in two separate experiments.

 Table S1. S. cerevisiae strains

Strain	Genotype	Reference
74D-694	MAT <u>a</u> [PSI ⁺] ade1-14 trp1-289 his3∆200 ura3-52	Chernoff <i>et al.</i> (1995)
	leu2-3,112	
SY81	MATα [<i>PSI</i> ⁺] ^{Strong} ade1-14 trp1-289 his3Δ200 ura3-	Satpute-Krishnan &
	52 leu2-3,112 SUP35-GFP	Serio (2005)
SY85	MATα [psi] ade1-14 trp1-289 his3∆200 ura3-52	Satpute-Krishnan &
	leu2-3,112 SUP35-GFP	Serio (2005)
SY779	MAT α [PSI ⁺] ^{Weak} ade1-14 trp1-289 his3 Δ 200 ura3-	This study
	52 leu2-3,112 SUP35-GFP	
SY1607	$MAT\alpha [PSI^{\dagger}]^{Strong}$ ade1-14 trp1-289 his3 Δ 200 ura3-	This study
	52 leu2-3,112 ptet ₀₇ SUP35-GFP	
SY1635	MATα [PSI ⁺] ^{Strong} ade1-14 trp1	This study
	289::TRP1::P _{GPD} GST(UGA)GFP-PEST his3∆200	
	ura3-52 leu2-3,112	
SY1636	MATα [PSI ⁺] ^{Weak} ade1-14 trp1-	This study
	289::TRP1::P _{GPD} GST(UGA)GFP-PEST his3∆200	
	ura3-52 leu2-3,112	
SY1637	MAT α [psi] ade1-14 trp1-	This study
	289::TRP1::P _{GPD} GST(UGA)GFP-PEST his3∆200	
	ura3-52 leu2-3,112	

Table S2. Oligonucl	leotide Primers
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Name	Sequence
TRS136	5' CTTGATATCCTTGCAAATTG 3'
TRS217	5' AGGTCAGGTTGCTTTCTCA 3'
TRS218	5' GGTAGTTTTGCTGATTGTTGCCTTGGTTTGAATCCGACATATAGGCCACTAGTGGATCTG 3'
TRS219	5' CTCGAGAAGATATCCATCATATTACCATTGTAATACTGCCCAGCTGAAGCTTCGTACGC 3'
TRS221	5' GAAGATATGGTGGGTGAC 3'
TRS270	5' GCACGTCAAGACTGTCAAGG 3'
TRS406	5' GAATTCATGTCTAAAGGTGAAGAATTA 3'
TRS407	5' CTCGAGTTATATTACTTGGGTATTGCCC 3'

Supporting References

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