Published in final edited form as:

Science. 2010 October 29; 330(6004): 680–683. doi:10.1126/science.1197785.

A Size Threshold Limits Prion Transmission and Establishes Phenotypic Diversity**

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Abstract

According to the prion hypothesis, atypical phenotypes arise when a prion protein adopts an alternative conformation and persist when that form assembles into self-replicating aggregates. Amyloid formation in vitro provides a model for this protein-misfolding pathway, but the mechanism by which this process interacts with the cellular environment to produce transmissible phenotypes is poorly understood. Using the yeast prion Sup35/[PSI⁺], we found that protein conformation determined the size distribution of aggregates through its interactions with a molecular chaperone. Shifts in this range created variations in aggregate abundance among cells due to a size threshold for transmission, and this heterogeneity, along with aggregate growth and fragmentation, induced age-dependent fluctuations in phenotype. Thus, prion conformations may specify phenotypes as population averages in a dynamic system.

Prion proteins adopt a spectrum of conformations or strains, which create phenotypes of distinct severity and stability in vivo (1-3). These phenotypes are linked to the assembly of the protein into aggregates that, at unique rates, template the conversion of newly-made prion protein to a similar state and are fragmented (4). But, how do these biochemical events translate into distinct phenotypes? One possibility is an “abundance-based” model, in which phenotypes are linked to an equilibrium between aggregated and soluble prion protein that determines protein activity and the number of heritable prions (propagons) (5,6). However, the conversion and fragmentation reactions also create heterogeneity in aggregate size, raising the possibility of a second, “size-based” model in which a subpopulation of aggregates establishes and propagates phenotypes (7).

To distinguish between these models, we focused on the [PSI⁺]^{Weak} and [PSI⁺]^{Strong} conformations of the yeast prion protein Sup35, which create phenotypes of different stabilities in vivo (8). To sustain these phenotypes in a dividing culture, Sup35 protein in the prion conformation must be inherited (7). To test whether conformational differences impact phenotypic stability by altering protein transmissibility, we monitored Sup35-GFP transfer to daughter cells. Using fluorescence loss in photobleaching (FLIP), a [PSI⁺]^{Weak} strain transferred half as much Sup35-GFP (~15% vs. ~30%; Fig. 1A) and contained ~50% fewer

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propagons than a $[PSI^+]^{\text{Strong}}$ strain (Fig. 1B). Thus, conformation-based differences in protein transmission correlate with phenotypic inheritance.

Following from the models, differences in aggregate abundance and/or size may create this variation in prion protein transmission (5,7), and indeed, $[PSI^+]^{\text{Weak}}$ strains differ from $[PSI^+]^{\text{Strong}}$ strains by the accumulation of fewer but larger aggregates (9-12). To distinguish between these possibilities, we simulated prion propagation via each transmission mechanism (13). For the “abundance-based” model, we were unable to recapitulate the severity and stability of $[PSI^+]^{\text{Weak}}$ and $[PSI^+]^{\text{Strong}}$ phenotypes (Fig. S5) (13). In contrast with a “size-based” model, we recapitulated all experimentally observed characteristics that distinguish these strains, including the relationship between the products of their conversion and fragmentation rates, the stabilities of their phenotypes, and their aggregate size distributions (see Table 1, Figs. S6-S10) (11-13). The latter model only captured the $[PSI^+]^{\text{Weak}}$ and $[PSI^+]^{\text{Strong}}$ states when the transmission size threshold was set within the range that distinguishes their aggregates (≤ 30 monomers/aggregate, Fig. S9) (11,12) and when fragmentation was modeled as an enzyme-limited process dependent on the catalyst for this reaction, the molecular chaperone Hsp104 (Figs. S8AB, S9) (14-16).

To experimentally test the hypothesis that aggregate size rather than abundance establishes conformation-based phenotypes, we altered Sup35 expression, which will impact phenotypic stability differently in the two models. For the “size-based” model, phenotypic stability decreases with Sup35 overexpression because aggregates increase in size (Fig. 2A) and decrease in transmissibility; however, for the “abundance-based” model, phenotypic stability increases because more aggregates are produced (5,13). Severe overexpression of prion proteins in yeast decreases phenotypic stability (17-19), which may reflect the assembly of alternative complexes (20-22). To circumvent this potential complication, we reversibly and modestly (~ 4 -6-fold) changed Sup35-GFP expression using a tetracycline-responsive promoter (Fig. S11). As predicted by the “size-based” model (Fig. 2A), larger aggregates appeared with Sup35-GFP overexpression (Fig. 2B), and this size shift induced a $\sim 50\%$ decrease in propagons (Fig. 2C), a ~ 70 -fold decrease in phenotypic stability (Fig. 2D), and a $\sim 50\%$ decrease in Sup35-GFP transmission (Fig. 2E). Conversely, transient repression of Sup35-GFP synthesis shifted aggregates to smaller sizes (Fig. 2B) and suppressed the inheritance defects, increasing propagons and Sup35-GFP transmission by 50% (Figs. 2C, E). These correlations between prion protein expression and heritability support the “size-based” model.

To further discriminate between the models, we characterized daughter-derived Sup35 directly. For the “abundance-based” model, daughters contain aggregates of similar size and abundance to their mothers (Fig. S8C). However, for the “size-based” model, daughters contain smaller and fewer aggregates than their mothers (Fig. S10 and Table 1), and the size of these aggregates is conformation-independent due to the selection imposed by transmission. Indeed, daughter-derived Sup35-GFP was transmitted with equal efficiency to mothers in $[PSI^+]^{\text{Strong}}$ and $[PSI^+]^{\text{Weak}}$ strains (Fig. 2F), and $[PSI^+]^{\text{Strong}}$ daughters, isolated by centrifugal elutriation (Fig. S12), contained only $\sim 17\%$ of the aggregates in the total population (Fig. 2G), again satisfying the “size-based” model predictions. Thus, aggregate size rather than abundance determines phenotypic stability in vivo.

How does this framework create phenotypes of different severity? Sup35 is a translation release factor whose activity is compromised by its conformational conversion and assembly into aggregates (23). Thus, cell-to-cell variation in aggregate abundance created by the “size-based” model (Figs. 2G, S10 and Table 1) raises the possibility that $[PSI^+]$ phenotypes reflect population averages of Sup35 activities. Consistent with this idea, individual $[PSI^+]$ cells accumulated Sup35-GFP in different physical states (Fig. 3A). This variation was more

severe for the less efficiently transmitted $[PSI^+]^{\text{Weak}}$ conformation, where only ~67% of cells accumulated Sup35-GFP in a primarily aggregated form (i.e. punctate, less efficiently transmitted fluorescence) in contrast with ~92% of $[PSI^+]^{\text{Strong}}$ cells (Figs. 1A, 3A). This heterogeneity in Sup35 physical state correlated with cell-to-cell variation in activity. While the range of translation termination activities in a population of $[PSI^+]^{\text{Strong}}$ cells expressing a stop codon readthrough reporter (GST-UGA-eGFP-PEST) overlapped only minimally with that of a non-prion $[psi^-]$ strain (~17%; Fig. 3B), $[PSI^+]^{\text{Weak}}$ activities overlapped significantly with those of $[psi^-]$ (~53%) and $[PSI^+]^{\text{Strong}}$ (~38%) (Fig. 3B). Thus, “size-based” transmission of aggregates creates phenotypic diversity within a population.

Despite these single-cell variations, $[PSI^+]$ strains form phenotypically homogeneous rather than sectorized colonies (24). To explore the molecular basis of this disconnect, we asked how single-cell variation is produced and maintained. According to our simulations (Table 1) and biochemical analysis (Fig. 2G), mother cells contain more aggregates than their daughters. To determine if cell-to-cell variations in Sup35 activity (Fig. 3B) similarly correspond to replicative age, we isolated subpopulations of a $[PSI^+]^{\text{Weak}}$ culture expressing GST-UGA-eGFP-PEST by fluorescence-activated cell sorting (FACS) and determined the number of bud scars on cells in each fraction. Young cells were strongly enriched in the fraction with the most accurate translation termination, where 90% of the cells had produced less than two daughters (Fig. 3C). In contrast, nearly half (~48%) of the cells in the fraction with the least accurate translation termination had produced three or more daughters (Fig. 3C).

This link between replicative age and phenotypic severity suggests that cells change during aging. Indeed, when young cells were isolated and cultured to regenerate a population, cells with reduced translation termination activity reappeared (Fig. 3D). What is the mechanism underlying this phenotypic transition? According to our simulations, the limited number of aggregates inherited by a daughter progressively amplifies to a steady-state plateau as that cell ages (Fig. 3E). Thus, early daughters will inherit fewer aggregates than later daughters. Indeed, propagons transmitted to successive daughters increased through the third generation and then remained relatively constant (Fig. 3F). Our observations provide a mechanistic explanation for previously observed cell-to-cell variability in propagons (6,25) and reveal age-dependent $[PSI^+]$ phenotypes at the single cell level.

Thus, the seemingly static phenotypes associated with prion protein conformations may actually reflect highly dynamic pathways of prion protein biogenesis in dividing cells. For any given cell, the complement of aggregates and the phenotype fluctuate in response to the interplay between the protein misfolding pathway and its cellular environment, creating a self-regenerating system that settles to a stable population average for each conformation. Thus, the cellular environment has profound effects on the phenotypic manifestations of prion protein conformations.

The dynamic “size-based” system that we have uncovered may contribute to the physiological consequences of protein misfolding in ways that are not possible for an “abundance-based” process. The phenotypic variation established and maintained through the events described here strengthens the argument that the prion mechanism, like other epigenetic processes, facilitates selection in new environments and thereby evolution (26). According to our model, access to an advantageous state may not require a $[prion^-]/[PRION^+]$ phenotypic switch (27) but, instead, may always be present within a population. In mammals, variation in aggregate size may similarly affect protein transmissibility between non-dividing cells and the spread of pathology in prion and perhaps other protein misfolding disorders, such as Parkinson’s, Alzheimer’s, and Huntington’s diseases (28-30). Indeed, prion protein conformation and expression, parameters that alter aggregate size, are

more reliable predictors of the clinical course of disease than the presence of protease resistant aggregates per se (1).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Propagons correlate with transmitted Sup35. (A) Sup35-GFP transmission to daughters was determined by FLIP (mean $\pm$ SEM). [*PSI*⁺]^{Strong} (open circles: SY81, *N*=11), [*psi*⁻] (black squares: SY85, *N*=7), and [*PSI*⁺]^{Weak} (gray diamonds: punctate SY779, *N*=7; open diamonds: diffuse SY779, *N*=7). (B) Propagons were quantified for [*PSI*⁺]^{Strong} (SY81) and [*PSI*⁺]^{Weak} (SY779). horizontal lines: 25th, 50th, and 75th percentiles; error bars: 10th and 90th percentiles; *N*=12 ([*PSI*⁺]^{Weak}) or 27 ([*PSI*⁺]^{Strong}); **p* = 0.0001.

Figure 2.

A size threshold limits aggregate transmission. (A) Aggregate size distributions for $[PSI^+]^{Strong}$ with wildtype (dotted) or 4X Sup35 (solid) or the latter following repression of Sup35 synthesis for one generation (gen; dashed) were determined by stochastic simulation (13). (B) Lysates from $[PSI^+]^{Strong}$ (+; p35: SY81 or ptet: SY1607) or $[psi^-]$ (-; SY85) in the presence (+) or absence (-) of doxycycline (Dox) for the indicated generations (gen) were analyzed by semi-denaturing agarose gel electrophoresis (SDD-AGE) and anti-GFP immunoblotting. (C) Propagons were quantified in $[PSI^+]^{Strong}$ cells (p35: SY81; ptet: SY1607) in the absence (-) or presence (+) of Dox for one gen. box plots as in Fig 1B. p35 ($N=27$); p35+Dox ($N=13$); ptet ($N=21$); ptet+dox ($N=16$); * $p=0.02$; ** $p=0.001$ (D) The frequency of prion loss was determined for $[PSI^+]^{Weak}$ (w, SY779) and $[PSI^+]^{Strong}$ (s) strains in B. (E) Sup35-GFP transmission to daughters was determined by FLIP (mean $\pm$ SEM; $N=3$) in $[PSI^+]^{Strong}$ (circles: SY81, squares: SY1607) in the absence (open) or presence (black) of Dox for one gen. (F) Sup35-GFP transmission to mothers was determined by FLIP (mean $\pm$ SEM; $N=3$) in $[PSI^+]^{Strong}$ (open circles: SY81) and $[PSI^+]^{Weak}$ (gray diamonds: SY779). (G) $[PSI^+]^{Strong}$ (SY1607) total (lane 1, solid) and daughter (lane 2, dashed) fractions were analyzed by SDD-AGE and anti-GFP immunoblotting. Densitometry traces are provided (right).

Figure 3.

“Size-based” transmission of aggregates creates population heterogeneity and fluctuations. (A) Sup35-GFP fluorescence pattern (left; punctate: white, diffuse: black) was quantified (right; mean $\pm$ SD; $N=3$) in $[PSI^+]^{Strong}$ (SY81), $[PSI^+]^{Weak}$ (SY779) and $[psi^-]$ (SY85). Scale bar = 5 μ m. (B) $[PSI^+]^{Strong}$ (solid black line, SY1635), $[PSI^+]^{Weak}$ (pink filled, SY1636), $[psi^-]$ (red line, SY1637; dotted black line, 74D-694) were analyzed by flow cytometry. vertical dotted lines: least and most fluorescent 20% (C) Bud scars were quantified in the fractions marked in B (SY1636; $N=3$). (D) $[PSI^+]^{Weak}$ (SY1636) cultures following FACS (dotted line) and regrowth (solid line) were analyzed by flow cytometry. (E) Aggregate size distributions were simulated for a mother cell prior to its 1st (red), 4th (orange), 7th (green), 10th (blue) and 12th (purple) divisions (13). (F) Propagons were quantified ($N \geq 3$) in successive daughters from a $[PSI^+]^{Strong}$ strain (74D-694). box plots as in Fig 1B; * $p=0.0081$.

Table 1
Comparison of $[PSI^{+}]^{\text{Weak}}$ and $[PSI^{+}]^{\text{Strong}}$ Characteristics From Simulated and Experimental Observations

Strain	Prion Loss		Relative Product of Conversion and Fragmentation Rates		Soluble Sup35		
					Total	Mother	Daughter
	Simulation	Experimental ^b (8,24)	Simulation ^a	Experimental ^b (5)	Simulation	Experimental ^b (31)	Simulation
$[PSI^{+}]^{\text{Strong}}$	0%	~0%	2.5	3	15%	19%	33.4%
$[PSI^{+}]^{\text{Weak}}$	1.6%	1-3%	1	1	31.3%	35%	87.9%

^a Simulated conversion rates for $[PSI^{+}]^{\text{Strong}}$ and $[PSI^{+}]^{\text{Weak}}$ are 0.0000245 and 0.0000645 (molecules min)⁻¹, respectively, and simulated fragmentation rates for $[PSI^{+}]^{\text{Strong}}$ and $[PSI^{+}]^{\text{Weak}}$ are 0.013 and 0.002 min⁻¹, respectively (13).

^b Experimental values are reported in the cited references.