Specificity of Prion Assembly in Vivo

[PSI⁺] AND [PIN⁺] FORM SEPARATE STRUCTURES IN YEAST*

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The yeast prions [PSI⁺] and [PIN⁺] are self-propagating amyloid aggregates of the Gln/Asn-rich proteins Sup35p and Rnq1p, respectively. Like the mammalian PrP prion “strains,” [PSI⁺] and [PIN⁺] exist in different conformations called variants. Here, [PSI⁺] and [PIN⁺] variants were used to model in vivo interactions between co-existing heterologous amyloid aggregates. Two levels of structural organization, like those previously described for [PSI⁺], were demonstrated for [PIN⁺]. In cells with both [PSI⁺] and [PIN⁺] the two prions formed separate structures at both levels. Also, the destabilization of [PSI⁺] by certain [PIN⁺] variants was shown not to involve alterations in the [PSI⁺] prion size. Finally, when two variants of the same prion that have aggregates with distinct biochemical characteristics were combined in a single cell, only one aggregate type was propagated. These studies demonstrate the intracellular organization of yeast prions and provide insight into the principles of in vivo amyloid assembly.

The formation of β-sheet-rich amyloid aggregates is a hallmark of many neurodegenerative disorders in mammals (1). Deposits of the self-propagating infectious prion protein PrP in the brain cause severe neuronal damage associated with transmissible spongiform encephalopathies (2–4). Expansion of polyglutamine stretches in huntingtin and MJD proteins leads to their aggregation as amyloids, which accompany Huntington’s and Machado-Joseph diseases, respectively (5). In Saccharomyces cerevisiae stably inherited epigenetic factors [PSI⁺] (6) and [PIN⁺] (7, 8) were established to be the prion forms of glutamine- and asparagine-rich (Gln/Asn-rich) proteins Sup35p and Rnq1p (9–11). [PSI⁺] and [PIN⁺] provide invaluable tools in the investigation of the principles of organization and interactions among amyloid aggregates in vivo.

Sup35p is an essential protein, and in the non-prionized soluble form, referred to as [psi−], it is involved in the termination of protein translation. In [PSI⁺] cells the pool of active Sup35p is severely depleted causing reduced translational termination efficiency (for reviews see Refs. 12–14). Intracellularly, [PSI⁺] is assembled into large aggregates containing SDS-stable Sup35p polymers (made of 9–50 monomers) and possibly other proteins (15). Rnq1p is not essential (16), and the role of the non-prionized Rnq1p, referred to as [pin−], is unknown. When in the [PIN⁺] state, Rnq1p dramatically enhances the rate of [PSI⁺] appearance, possibly by templating the prion conformation of Sup35p (11, 17).

Deletion of Hsp104p, a yeast chaperone involved in the refolding of aggregated or misfolded proteins, leads to loss of [PSI⁺] and [PIN⁺] (7, 10, 18, 19). Stable inheritance of the yeast prions requires each cell to contain discrete prion aggregates that can migrate to daughter cells during budding (20–22). Strong evidence supports the idea that Hsp104p maintains a sufficient number of prion aggregates by shearing them, thus securing successful prion transmission in the growing culture. Guanidine hydrochloride in non-denaturing concentrations blocks Hsp104p activity, which leads to an uncontrolled growth of the Sup35p polymers followed by prion loss (15, 20, 22–25). Interestingly, although guanidine often causes the simultaneous loss of [PSI⁺] and [PIN⁺] in two-prion [PSI⁺] [PIN⁺] cells, they can also be lost independently (7). This independence raises the question of whether two-prion cells contain homogeneous or heterogeneous prion bodies.

Like human amyloidogenic proteins, Sup35p and Rnq1p form self-seeding amyloid fibers in vitro (10, 26–29) and in vivo (30). It has also been shown recently that preformed Rnq1p fibers facilitate polymerization of the Gln/Asn-rich domain of Sup35p (17). Using fluorescently labeled Sup35p and Rnq1p, newly forming Sup35p aggregates that appeared during [PSI⁺] induction were found to co-localize with Rnq1p aggregates. However, once [PSI⁺] was established, the fluorescent aggregates co-localized less frequently (17). These observations are consistent with the hypothesis that [PIN⁺] aggregates interact with Sup35p and template [PSI⁺] induction. Interestingly, a number of other Gln/Asn-rich proteins can substitute for [PIN⁺], suggesting the importance of Gln/Asn-rich sequences in the physiology of amyloid formation (11, 31).

The [PSI⁺] and [PIN⁺] prions (19, 32) as well as the mammalian PrP prion (reviewed in Ref. 33), can exist as different “strains,” also called variants, determined by a specific conformation of the prion molecules (34–37). Weak [PSI⁺] variants are generally less mitotically stable than strong [PSI⁺] variants (19) and have more non-prionized (soluble) Sup35p molecules in the cytoplasm (29, 38). In a cross of strong and weak [PSI⁺] haploids the diploids and their progeny exhibit the strong [PSI⁺] phenotype, implying that strong [PSI⁺] variants are “dominant” over weak (19, 38). It is unclear, however, whether the prion variant dominance phenomenon is confined to a phenotypic dominance only (i.e. aggregates of both variants co-exist) or is accompanied by a complete elimination of the “recessive” variant aggregates.

[PIN⁺] variants have been distinguished by their fluorescent pattern of Rnq1p-green fluorescent protein fusion staining.
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(mostly single large fluorescent dots (s.d.)¹ versus multiple small (m.d.) fluorescent dots per cell) (39) and by the efficiency with which they facilitate the induction of [\(\text{PSI}^+\)]", ranging from low to very high (32). Variant dominance was shown to correlate inversely with the amount of non-prionized Rnq1p (32). Therefore it appears that the dominance of a prion variant is determined by the efficiency with which its aggregates recruit new molecules (32, 39). This is supported by the finding that protein extracts from cells carrying strong [\(\text{PSI}^+\)] variants were capable of converting non-prionized Sup35p into fibers more efficiently than the extracts from weak [\(\text{PSI}^+\)] (29, 40).

Interestingly, s.d. [\(\text{PIN}^+\)] variants known to facilitate [\(\text{PSI}^+\)] induction are also capable of making weak [\(\text{PSI}^+\)] highly mitotically unstable (39). It was unclear, however, whether [\(\text{PIN}^+\)] directly affects [\(\text{PSI}^+\)], for example by forming heterogeneous prion particles. Answering this question requires an understanding of the intracellular organization of the [\(\text{PSI}^+\)] and [\(\text{PIN}^+\)] aggregates in two-prion [\(\text{PSI}^+\)] [\(\text{PIN}^+\)] cells.

In this study we characterize [\(\text{PIN}^+\)] prion aggregates isolated from different [\(\text{PIN}^+\)] variants. We show that prions in two-prion cells, i.e. [\(\text{PSI}^+\)] [\(\text{PIN}^+\)], form isolated structures devoid of tight interactions at every organizational level. We provide biochemical data showing that prion variant dominance is indeed accompanied by the elimination of the “recessive” variant. Finally, we show that prion loss is not always accompanied by changes in the size of the prion particles.

**EXPERIMENTAL PROCEDURES**

**Yeast Strains—Derivatives of 74D-694 (MATa ade1-14 ura3-52 leu2-3,112 trp1-289 his3-200 [psi] [\(\text{PIN}^+\)] (L1751) (41) carry [\(\text{PIN}^+\)] variants with different [\(\text{PSI}^+\)] induction strength. “Original” high m.d. [\(\text{PIN}^+\)] (L1749), low s.d. [\(\text{PIN}^+\)] (L1943), medium s.d. [\(\text{PIN}^+\)] (L1945), high s.d. [\(\text{PIN}^+\)] (L17677–), and very high s.d. [\(\text{PIN}^+\)] (L1953) were used. [\(\text{PSI}^+\)] induction strength of L17677 was detected as described elsewhere (32). [\(\text{PIN}^+\)] variants were induced in L1749 by overexpressing Sup35p from pGAL::SUP35 resulting in weak [\(\text{PSI}^+\)] m.d. [\(\text{PIN}^+\)] (L1765) and strong [\(\text{PSI}^+\)] m.d. [\(\text{PIN}^+\)] (L1762). A weak [\(\text{PSI}^+\)] high s.d. [\(\text{PIN}^+\)] derivative was obtained by overexpressing Sup35p in L17677– resulting in L1767 (32). L1767 was used as a cytoplasm donor for kar1-1 d15 can5 cyh8 [\(\text{PIN}^+\)] [\(\text{rho}^+\)] derivatives of L1845 (MATa ade1-14 ura3-52 leu2-3,112 trp1-289 his3-200 bearing either the wild type Rnq1p or the rpn1::KANMX4 allele, resulting in weak [\(\text{PSI}^+\)] high m.d. and weak [\(\text{PSI}^+\)] low m.d. (L2668), respectively. A derivative of e10B-H49 (SUP5 ade2-1 lys1-1 his1-11,15 leu1 kar1-1 cyh8 [\(\text{rho}^-\)] [\(\text{PIN}^+\)] (L1575) was cytoduced with L1749, L1943, L1945, and L1953 resulting in [\(\text{PIN}^+\)] strains carrying m.d. (L1998), low s.d. (L1991), medium s.d. (L1992), and very high s.d. (L1996) [\(\text{PIN}^+\)] variants, respectively (39). These strains were crossed to L17677–. The diploids resulting from these crosses were selected on the media deficient in adenine.

**Preparation of Yeast Cell Lysates—** Yeast cultures were grown in liquid YPD media to A600 of 1.5–2.0. For investigations of the effect of guanidine hydrochloride, cells were grown in YPD to A600 of 1.5–2.0, and then cultures were divided in half, and the initial volume was reconstituted with YPD. Guanidine HCl was added to 7 mM final concentration to one of the cultures, and cells were grown until the appearance of the diluted cultures doubled. The cells were harvested, washed with water, resuspended, and lysed by vortexing with 0.5-mm glass beads in a protein extraction buffer containing antiproteases (PMB/AP; 25 mM Tris-HCl (pH 7.5), 50 mM KC1, 10 mM MgCl2, 1 mM EDTA-Na, 5% glycerol, 100 μM 1-chloro-3-ethylamido-7-amino-2-heptanone (TLCK), 100 μM 1-tosamido-2-phenylethyl chloromethyl ketone (TPCK), 10 mM 1,10-phenanthroline, 94 μM leupeptin, 1 μM pepstatin A, 5 μg/ml aprotinin, 2 mM phenylmethylsulfonyl fluoride, and “protease inhibitor mixture for yeast” (Sigma, 1:50)). Crude lysates were precleared by 1 min of centrifugation at 600 × g at 4 °C. Such a short preclaring step allows for efficient preservation of prion aggregates that may pellet along with the cell debris upon higher speed or longer duration of spin.

Protein concentration was determined as described (42) using the Bio-Rad protein assay.

**Analysis of Yeast Cell Lysates—** For sucrase gradient analyses, fresh lysates with or without the addition of SDS to 2% were incubated for 7 min at room temperature and fractionated at 4 °C in a swinging bucket rotor through a 20–60% continuous sucrose/PEG gradient for 40 min at 10,600 × g, unless indicated otherwise. Equal fractions were collected from top to bottom, diluted 1:2 in PBEAP, resolved in a 10% polyacryl-amide gel as described (43), and transferred to an immunoblot polyvi-nylidene difluoride membrane (Bio-Rad). Sup35p was detected using BE4 monoclonal antibodies against the Sup35p C-terminal domain. Rnq1p was detected by polyclonal antibodies (a kind gift from S. Lindquist). Signal was revealed using a Tropix kit (Applied Biosystems) as suggested by the manufacturer. To stain the membrane with different antibodies, it was stripped twice by incubation for 30 min in 0.2 M glycine (pH 2.2) containing 1% SDS and 1% Tween 20. Using this procedure we obtained the same images regardless of which antibodies were used first.

Semi-denaturing detergent agarose gel electrophoresis (SDD-AGE) was performed as described elsewhere (15) with the following changes. Lysates were incubated for 7 min in sample buffer (60 mM Tris-HCl (pH 6.8), 5% glycerol, 2% SDS, 0.05% bromphenol blue) at the indicated and 4 °C. Results show that in 1% and 2% SDS, the bands with the highest mobility correspond to aggregates that are less susceptible to SDS treatment or may be aggregates that were dragged down along with cell debris and thus rendered less accessible by SDS molecules. These results show that [\(\text{PIN}^+\)] cells contain prionized Rnq1p in the form of heavy SDS-unstable particles, henceforth referred to as prion aggregates.

¹The abbreviations used are: s.d., single dot; m.d., multiple dot; SDD-AGE, semi-denaturing agarose gel electrophoresis; TPCK, 1-tosamido-2-phenylethyl chloromethyl ketone; PEB, protein extraction buffer; AP, antiprotease; TLCK, 1-chloro-3-ethylamido-7-amino-2-heptanone.
grated at described under "Experimental Procedures." The bands shown migrated in a sucrose gradient and analyzed for the presence of Rnq1p as derivatives of 74D-694 with or without 2% SDS were separated by centrifugation. Interestingly, the size distributions of particles were evenly distributed and had a size range from ~600 to >3000 kDa, the size of high s.d. [PIN⁺] subparticles varied from ~400 to <3000 kDa. After probing the same gel with anti-Sup35p antibodies, we found Sup35p migrated as a monomer, indicating that most of the non-prionized Sup35p does not form stable interactions with subparticles from these [PIN⁺] variants (Fig. 2A).

The size distribution of subparticles from different s.d. [PIN⁺] variants that were previously characterized as possessing different [PSI⁺] induction strengths (low, medium, and very high) (32) are shown in Fig. 3A (lanes 1, 2, and 4). Subparticles from low, medium, and very high s.d. [PIN⁺] had size distributions similar to each other (but not exactly the same) and to m.d. [PIN⁺] (Fig. 3B), which had a high [PSI⁻]-inducing strength.

Because the [PIN⁺] prion is efficiently cured by growth in the presence of guanidine hydrochloride, we asked whether cultivation of [psi−] [PIN⁺] strains in the presence of guanidine would have an effect on [PIN⁺] subparticles. As expected, incubation of 74D-694 derivatives bearing m.d. or high s.d. [PIN⁺] variants in the presence of 7 mM guanidine for one generation increased the size distribution of the [PIN⁺] subparticles (Fig. 2A).

Several findings suggest that differences between prion variants are caused by the conformation that the prion molecule acquires (36, 37). If this were true then [PIN⁺] prion subparticles from different prion variants should have distinct physical properties. Indeed, although m.d. and s.d. [PIN⁺] subparticles were equally stable in 2 and 7.5% SDS (not shown) at room temperature and at 30 °C, when subjected to 60 °C their stability differed. Although all s.d. [PIN⁺] subparticles were stable at 60 °C (Fig. 3A), m.d. [PIN⁺] subparticles broke down into smaller species migrating as Rnq1p monomers (~46 kDa) and at ~200 and ~400 kDa (Fig. 3B). At 95 °C all [PIN⁺] subparticles disaggregated.

**FIG. 1.** Rnq1p is assembled into SDS-unstable aggregates in [PIN⁺] strains. Lysates of [psi−] [pin−] and [psi−] m.d. [PIN⁺] derivatives of 74D-694 with or without 2% SDS were separated by centrifugation in a sucrose gradient and analyzed for the presence of Rnq1p as described under "Experimental Procedures." The bands shown migrated at ~46 kDa. Similar results were obtained for a [psi−] high s.d. [PIN⁺] derivative of 74D-694 (not shown).

**FIG. 2.** [PIN⁺] aggregates are composed of SDS-stable variant-specific subparticles that increase in size upon guanidine treatment. A, [PIN⁺] subparticles are variant-specific and do not contain Sup35p. A preparation of chicken pectoralis extract (CPE) stained with Coomassie was used as a ladder. Lysates were treated with SDS at room temperature (RT) or at 30 °C, analyzed by SDD-AGE for the presence of Rnq1p (left panel), and then stripped and stained for Sup35p (right panel) as described under “Experimental Procedures.” Derivatives of [psi−] 74D-694 harboring [pin−] (−) or m.d. or high s.d. [PIN⁺] were used. B, guanidine leads to an increase in size of [PIN⁺] prion subparticles. m.d. [PIN⁺] and high s.d. [PIN⁺] derivatives of [psi−] 74D-694 were grown in YPD with (+) or without (−) the addition of 7 mM guanidine HCl (Gu) (see “Experimental Procedures”). Lysates were treated with 2% SDS at room temperature and analyzed for the presence of Rnq1p by SDD-AGE.
were undetectable in m.d. ants have different amounts of non-prionized Rnq1p, which lysates from cells carrying s.d. [PIN⁺] variants that promote induction (ind.) of [PSI⁺] with different efficiencies: low (L), medium (M), high (H), or very high (V). B, a lysate of 74D-694 derivative carrying m.d. [PIN⁺] variant.

into monomers (not shown). Thus, [PIN⁺] prion aggregates appear to be composed of SDS-stable prion subparticles possessing prion variant-specific characteristics.

When each of the [PIN⁺] variants was cytoduced (a process that involves cytoplasmic but not nuclear transfer) into a genetically distinct strain, neither the original size distribution of the [PIN⁺] subparticles nor the peculiar thermal susceptibility of m.d. [PIN⁺] was altered (compare Fig. 3A, lanes 1, 2, and 4 with Fig. 4A, lanes 2, 4, and 6, other data not shown). Thus, we showed that variant-specific properties of the [PIN⁺] subparticles are cytoducible.

“Dominant” [PIN⁺] Variant Leads to Elimination of the “Recessive” Variant—Previously we reported (32) that [PIN⁺] variants have different amounts of non-prionized Rnq1p, which were undetectable in m.d. [PIN⁺] and high s.d. [PIN⁺] (not shown) and increased in order in medium, low, and very high s.d. [PIN⁺] variants. Diploids made of crosses of two variants with different amounts of soluble Rnq1p exhibited the phenotype of the haploid parent, which had less soluble Rnq1p. When m.d. and high s.d. [PIN⁺] variants were crossed, the diploid exhibited the fluorescent staining phenotype of the m.d. variant. It was unclear, however, whether the presence of the “dominant” variant simply masks the recessive phenotype or leads to a complete elimination of the latter. To distinguish between these possibilities we assayed [PIN⁺] subparticles from the progeny of high s.d. [PIN⁺] crossed to other s.d. variants. The diploids possessed [PIN⁺] subparticles with the distinctive high s.d. [PIN⁺] distribution (Fig. 4A), supporting the hypothesis of takeover. Similarly, the diploids from the m.d. and high s.d. [PIN⁺] cross contained only m.d. [PIN⁺] subparticles (Fig. 4B). To eliminate the possibility that the presence of m.d. [PIN⁺] subparticles on the membrane simply overshadowed the s.d. [PIN⁺] subparticles, we pre-heated the diploid lysate to 60 °C. Even after the m.d. [PIN⁺] subparticles completely broke down, no s.d. [PIN⁺] subparticles were visible as opposed to the results obtained from a mechanical mix of the lysates of the parental cells. Thus, we found that “dominant” and “recessive” [PIN⁺] variants do not co-exist. Instead, only subparticles of the “dominant” variant can be observed in the diploids.

Rnq1p and Sup35p Form Separate Subparticles in [PSI⁺] [PIN⁺] Cells—Given that [PIN⁺] and [PSI⁺] can co-exist in one cell, we asked whether they might form mixed subparticles containing prionized molecules of both Rnq1p and Sup35p. However, the size distribution of neither m.d. (Fig. 5A) nor s.d. [PIN⁺] (Fig. 5C) prion subparticles was affected by the presence of [PSI⁺]. Furthermore, when [PSI⁺] m.d. [PIN⁺] lysates were heated to 60 °C, the m.d. [PIN⁺] subparticles broke down just as they did in the absence of [PSI⁺].

Likewise, the size distribution of [PSI⁺] subparticles was unaffected by the presence of [PIN⁺] as compared with its original size in a Δrnp1 derivative (Fig. 6). As a control to show that we could detect some size shifts, we cultivated a weak [PSI⁺] Δrnp1 strain in YPD containing 7 mM guanidine for one generation. Consistent with the results reported previously (15) we observed a significant increase in the size of the Sup35p subparticles (Fig. 6). Subparticles obtained from strong [PSI⁺] m.d. [PIN⁺] cells were smaller on average than [PSI⁺] subparticles from weak [PSI⁺] m.d. [PIN⁺] cells, and after being heated to 60 °C, [PSI⁺] subparticles (Fig. 5B) retained their original size distribution establishing that they are independent of m.d. [PIN⁺] subparticles that broke down under these conditions (Fig. 5A).

Sedimentation Profiles of Prion Aggregates Are Prion Variant-specific—We found that the prion variant-specific sedimentation profiles were different between m.d. and s.d. [PIN⁺] and between strong and weak [PSI⁺] aggregates (Fig. 7). Rnq1p from m.d. [PIN⁺] penetrated the gradient farther than s.d. [PIN⁺] and was found in virtually all fractions. Similarly, weak and strong [PSI⁺] aggregates had specific sedimentation pro-
files. Weak [ψI⁺] aggregates formed a “peak” in the third fraction; strong [ψI⁺] aggregates were distributed evenly throughout the gradient (Fig. 7).

Aggregates of [PIN⁺] and [PIN⁻] Form Independent Structures in [PSI⁺] / [PIN⁺] Cells—Because subparticles of [PIN⁺] were found to be [PIN⁻]-independent, we asked whether [ψI⁺] and [PIN⁺] aggregates containing Sup35p or Rnq1p subparticles, respectively, form tight interactions. To answer this question we analyzed centrifugation profiles of prion aggregates from strains bearing different combinations of prion variants. As shown in Fig. 7A, [ψI⁺⁺] aggregates were unable to drag m.d. or s.d. [PIN⁺] aggregates with them or alter the Rnq1p distribution in any way. Likewise, the sedimentation profile of [PIN⁻] aggregates was unchanged by the absence of [PIN⁺] (Fig. 7B, and not shown).

DISCUSSION

To dissect the intracellular organization of [PIN⁺] we used SDS treatment to disrupt non-amyloid interactions within the [PIN⁺] prion aggregates. SDS-stable subparticles that result from aggregate disassembly were reliably resolved in agarose gels (15), a technique that may prove useful in the investigation of amyloid aggregates and other SDS-stable heavy protein complexes. The two-level structural organization appears to be a general feature of yeast prions, having now been demonstrated for two prions, [ψI⁺⁺] (15) and [PIN⁻]. Interestingly, the [Het-s] prion of the fungus Podospora anserina was shown to be able to form elongated aggregates in vivo that grow by lateral association of shorter fibrillar aggregates (45). The finding that the subparticle size of both [ψI⁺⁺] (15) and [PIN⁻] increases immediately in response to growth in guanidine supports the current theory that guanidine inhibits the function of Hsp104p as a prion disaggregase, which leads to abrogation of prion aggregate generation via shearing (20, 22). We showed here that although all [PIN⁺] variants possessed SDS-stable subparticles, they were not uniform in size and thermal stability. All s.d. [PIN⁺] subparticles were heat-resistant in the presence of 2% SDS, whereas under the same conditions subparticles from the m.d. [PIN⁻] variant disassembled into complexes consistent with the size of tetramers and octamers of Rnq1p. Overall, the size distribution of [PIN⁺] subparticles was prion variant-specific and corresponded to 20–100 monomers of Rnq1p. This number is comparable with the 9–50 monomers of Sup35p reported for [PIN⁺] subparticles (15). Importantly, the specific size distribution of the [PIN⁺] subparticles from different variants and their susceptibility to heat treatment in the presence of SDS were cytoducible and hence not dependent on the genetic background but rather reflected variations in the physical properties of the prionized Rnq1p.
The size of the subparticles depends on the balance between the processes of new molecule recruitment on the one hand and Hsp104p-dependent subparticle shearing on the other. The essential factor in these processes is the conformation of the prion variants that probably determines the speed of recruitment and the affinity to Hsp104p. The outcome of the interplay between these processes should determine the overall efficacy with which the non-prionized molecules get recruited into the subparticles. Importantly, this efficacy should not necessarily correlate with the subparticle size, for high speed of recruitment may be compensated by low efficiency of shearing by Hsp104p, and vice versa. The low level of soluble protein is therefore a much more informative indicator of a “dominant” variant. The system we described precludes two prion variants with different conformational characteristics from co-existing, simply because one variant will prevail in a competition for the non-prionized molecules, and eventually it will exile the less effective variant from the culture. This is exactly what we found for the [PIN+] prion variants here; the diploids of the cross between different variants retained subparticles that were characteristic of the variant with less soluble Rnq1p.

We then challenged [PSI+] and [PIN+] selectivity and asked whether their subparticles can incorporate the molecules of each other when they co-exist. If this were true then the resulting mixed size distribution of the subparticles might be expected to be distinct from the original size distributions of [PSI+] and [PIN+] subparticles. In fact, size distribution of [PSI+] subparticles failed to undergo any detectable changes in the presence of different [PIN+] variants, and vice versa. Our findings that the heat treatment that triggered m.d. [PIN+] subparticle disaggregation had no effect on [PSI+] subparticles in the same cell unambiguously demonstrate their structural distinction, suggesting a highly selective method of amyloid assembly in vivo.

Even at the level of aggregates [PSI+] and [PIN+] remained structurally separated and did not form appreciably tight interactions with each other. This does not preclude the possibility that they co-localize in the cell. Neither can we rule out the hypothesis that these aggregates contain other, unidentified proteins. At the molecular level, two models of prion aggregate organization can be suggested. The first model postulates that a direct, SDS-sensitive interaction between subparticles is sufficient to assemble them into mature aggregates, which may or may not be decorated by additional proteins. The second model requires additional proteins to mediate aggregate assembly by forming SDS-sensitive interactions between the subparticles. Whichever model is correct, identification of the attendant proteins may shed light on the biological role of yeast prions and prion variants as well as provide insight into the mechanisms of protein sequestration by toxic prion aggregates that accompany neurodegenerative diseases in mammals (46).

We reported previously (39) that introduction of s.d. [PIN+] variants into [pin+] strains bearing weak [PSI+] rendered the latter mitotically unstable, suggesting that the presence of s.d. [PIN+] may cause an increase in weak [PSI+] aggregate or polymer size, possibly by direct co-aggregation (or via intermediates) followed by [PSI+] loss. Our finding that neither the size distribution of the weak [PSI+] subparticles nor the centrifugation profiles of its aggregates were changed in the presence of s.d. [PIN+] strongly suggests that although s.d. [PIN+] variants promote a rapid loss of weak [PSI+], unlike guanidine they do not alter the very structure of the [PSI+] prion. It is important to note, however, that we suppose that the dramatic influence of s.d. [PIN+] on weak [PSI+] must be accompanied by considerable alterations in prion-related processes. This implies therefore that s.d. [PIN+] intervenes in some other processes that define successful prion maintenance in the culture. Possibly, efficient transmission of weak [PSI+] particles to the daughter cell during budding is not carried out by a simple donation of prion aggregates along with part of the cytoplasm from the mother cell. Instead, there may be specific proteins that promote the transport of [PSI+] aggregates to the
daughter cells. The presence of the s.d. [PIN⁺] prion may alter the normal functioning of these proteins by directly sequestering them into s.d. [PIN⁺] aggregates or through an indirect mechanism.

Here we have presented biochemical evidence suggesting that heterologous prions [PSI⁺] and [PIN⁺] do not form “mixed” subparticles or aggregates. Intracellularly, these prions are highly selective and form rather separate structures, which may be in close proximity to each other but are devoid of tight interactions. Thus, the results presented in this study provided a deeper look into the structural organization of prion particles inside living cells.

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REFERENCES

1. Lansbury, P. T., Jr. (1999) Proc. Natl. Acad. Sci. U.S.A. 96, 3342–3344
2. Griffith, J. S. (1967) Nature 215, 1043–1044
3. Prusiner, S. B. (1982) Science 216, 136–144
4. Silveira, J. R., Caughey, B., and Baron, G. S. (2004) Curr. Top. Microbiol. Immunol. 284, 1–50
5. Perutz, M. F. (1999) Trends Biochem. Sci. 24, 58–63
6. Cox, B. S. (1965) Heredity 20, 505–521
7. Derkatch, I. L., Bradley, M. E., Zhou, P., Chernoff, Y. O., and Liebman, S. W. (1998) Proc. Natl. Acad. Sci. U.S.A. 95, 2400–2405
8. Derkatch, I. L., Bradley, M. E., and Liebman, S. W. (1997) Genetics 147, 507–519
9. Derkatch, I. L., Bradley, M. E., and Liebman, S. W. (1999) Proc. Natl. Acad. Sci. U.S.A. 96, 505–521
10. Sondheimer, N., and Lindquist, S. (2000) Mol. Cell 5, 163–172
11. Derkatch, I. L., Bradley, M. E., Hong, J. Y., and Liebman, S. W. (2001) Cell 106, 171–182
12. Wickner, R. B., Liebman, S. W., and Saupe, S. J. (2004) in Prion Biology and Diseases (Prusiner, S. B., ed) 2nd Ed., pp. 505–572, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
13. Uptain, S. M., and Lindquist, S. (2002) Annu. Rev. Microbiol. 56, 703–741
14. Tuite, M. F., and Cox, B. S. (2003) Nat. Rev. Mol. Cell. Biol. 4, 878–890
15. Kryndushkin, D. S., Alexandrov, I. M., Ter-Avanesyan, M. D., and Kushnirov, V. V. (2003) J. Biol. Chem. 278, 49636–49643
16. Giaever, G., Chu, A. M., Ni, L., Connelly, C., Riles, L., Winzeler, E. A., Yang, Y., Youngman, E., Ku, K., Bussey, H., Boone, J. D., Snyder, M., Philippsen, P., Davis, R. W., and Johnston, M. (2002) Nature 418, 387–391
17. Derkatch, I. L., Uptain, S. M., Outeiro, T. F., Krishnan, R., Lindquist, S. L., and Liebman, S. W. (2004) Proc. Natl. Acad. Sci. U.S.A. 101, 12934–12939
18. Chernoff, Y. O., Lindquist, S. L., Ono, B., Inge-Vechtomov, S. G., and Liebman, S. W. (1995) Science 268, 883–884
19. Derkatch, I. L., Chernoff, Y. O., Kushnirov, V. V., Inge-Vechtomov, S. G., and Liebman, S. W. (1996) Genetics 144, 1375–1386
20. Eaglestone, S. S., Roodick, L. W., Cox, B. S., and Tuite, M. F. (2000) Proc. Natl. Acad. Sci. U.S.A. 97, 249–244
21. Borchers, H. A., Weggren, R. D., Newnam, G. P., Inge-Vechtomov, S. G., and Chernoff, Y. O. (2001) EMBO J. 20, 6683–6691
22. Ness, F., Ferreira, P., Cox, B. S., and Tuite, M. F. (2002) Mol. Cell. Biol. 22, 5593–5605
23. Ferreira, P. C., Ness, F., Edwards, S. R., Cox, B. S., and Tuite, M. F. (2001) Mol. Microbiol. 40, 1357–1369
24. Grimminger, V., Richter, K., Imhof, A., Buchner, J., and Walter, S. (2004) J. Biol. Chem. 279, 7378–7383
25. Shorter, J., and Lindquist, S. (2004) Science 304, 1783–1797
26. King, C. Y., Tittmann, P., Gurse, H., Gebert, R., Aebi, M., and Wuthrich, K. (1997) Proc. Natl. Acad. Sci. U.S.A. 94, 6618–6622
27. Paushkin, S. V., Kushnirov, V. V., Smirnov, V. N., and Ter-Avanesyan, M. D. (1997) Science 277, 381–383
28. Glover, J. R., Kowal, A. S., Schirmer, E. C., Patino, M. M., Liu, J. J., and Lindquist, S. (1997) Cell 89, 111–115
29. Uptain, S. M., Sawicki, G. J., Caughey, B., and Lindquist, S. (2001) EMBO J. 20, 6236–6245
30. Kimura, Y., Koitabashi, S., and Fujita, T. (2003) Cell Struct. Funct. 28, 187–193
31. Osherovich, L. Z., and Weissman, J. S. (2001) Cell 106, 183–194
32. Bradley, M. E., Edske, H. K., Hong, J. Y., Wickner, R. B., and Liebman, S. W. (2002) Proc. Natl. Acad. Sci. U.S.A. 99 Suppl 4, 16392–16399
33. Bruce, M. E. (2003) Br. Med. Bull. 66, 99–108
34. Bessen, R. A., Kociako, D. A., Raymond, G. J., Nandan, S., Lansbury, P. T., and Caughey, B. (1995) Nature 375, 689–700
35. Safr, J., Wille, H., Itti, V., Göth, D., Serban, H., Törich, M., Cohen, F. E., and Prusiner, S. B. (1998) Nat. Med. 4, 1157–1165
36. Tanaka, M., Chien, P., Naber, N., Cooke, R., and Weissman, J. S. (2004) Nature 429, 323–328
37. King, C. Y., and Diaz-Avalos, R. (2004) Nature 428, 319–323
38. Zhou, P., Derkatch, I. L., Uptain, S. M., Patino, M. M., Lindquist, S., and Liebman, S. W. (1999) EMBO J. 18, 1182–1191
39. Bradley, M. E., and Liebman, S. W. (2003) Genetics 165, 1675–1685
40. Kochneva-Pervukhova, N. V., Chechenova, M. B., Valouev, I. A., Kushnirov, V. V., Smirnov, V. N., and Ter-Avanesyan, M. D. (2001) FEBS Lett. 489, 489–497
41. Chernoff, Y. O., Derkatch, I. L., and Inge-Vechtomov, S. G. (1993) Curr. Genet. 24, 268–270
42. Bradford, M. M. (1976) Anal. Biochem. 72, 248–254
43. Caughey, B. (1995) Nature 375, 689–700
44. Kim, K., and Keller, T. C., (2002) Annual Rev. Microbiol. 56, 703–741
45. Balguerie, A., Dos Reis, S., Coulary-Salin, B., Chaigneau, S., Sabourin, M., Schmitter, J. M., and Saupe, S. J. (2004) J. Cell Sci. 117, 2599–2610
46. McCammond, A., and Fischbeck, K. H. (2001) Nat. Med. 7, 528–530