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Variant-specific [*PSI*$^+$] Infection Is Transmitted by Sup35 Polymers within [*PSI*$^+$] Aggregates with Heterogeneous Protein Composition

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The [*PSI*$^+$] prion is the aggregated self-propagating form of the Sup35 protein from the yeast *Saccharomyces cerevisiae*. Aggregates of Sup35 in [*PSI*$^+$] cells exist in different heritable conformations, called "variants," and they are composed of detergent-resistant Sup35 polymers, which may be closely associated with themselves, other proteins, or both. Here, we report that disassembly of the aggregates into individual Sup35 polymers and non-Sup35 components increases their infectivity while retaining their variant specificity, showing that variant-specific [*PSI*$^+$] infection can be transmitted by Sup35 polymers alone. Morphological analysis revealed that Sup35 isolated from [*PSI*$^+$] yeast has the appearance of short barrels, and bundles, which seem to be composed of barrels. We show that the major components of two different variants of [*PSI*$^+$] are interacting infectious Sup35 polymers and Ssa1/2. Using a candidate approach, we detected Hsp104, Ssb1/2, Sis1, Sse1, Ydj1, and Sla2 among minor components of the aggregates. We demonstrate that Ssa1/2 efficiently binds to the prion domain of Sup35 in [*PSI*$^+$] cells, but that it interacts poorly with the nonaggregated Sup35 found in [*psi*$^-$] cells. Hsp104, Sis1, and Sse1 interact preferentially with the prion versus nonprion form of Sup35, whereas Sla2 and Ssb1/2 interact with both forms of Sup35 with similar efficiency.

INTRODUCTION

Proteins in amyloid conformations form highly stable β sheet-rich polymers. In mammals, amyloids may accumulate in the brain and cause various neurodegenerative diseases, such as transmissible spongiform encephalopathies (TSEs), characterized by deposits of the human prion protein (PrP) in the self-propagating amyloid form (PrP^{sc}). A large body of evidence suggests that the PrP^{sc} amyloids are infectious, and that they cause the TSEs, which are also referred to as "prion diseases" (reviewed in Harris and True, 2006). Variations in the conformation of the infectious PrP^{sc} amyloids are thought to underlie the existence of prion "strains" that cause different forms of prion diseases (for review, see Bruce, 2003). In the yeast *Saccharomyces cerevisiae*, a convenient model to study various amyloidoses (Vishnevskaia *et al*., 2007), the non-Mendelian genetic elements [*PSI*$^+$], [URE3], and [*PIN*$^+$] were shown to be self-propagating conformations (i.e., prions) of the Sup35, Ure2, and Rnq1 proteins, respectively (for review, see Wickner *et al*., 2007).

In [*psi*$^-$] cells, the essential Sup35 protein is monomeric, and it is required for termination of protein synthesis at stop (nonsense) codons (for review, see Inge-Vechtomov *et al*., 2003). In [*PSI*$^+$] cells that bear the aggregated, partially inactive prion form of Sup35, protein synthesis continues beyond some nonsense codons at an increased rate. Such nonsense codon readthrough is routinely assayed as nonsense suppression (see Chernoff *et al*., 2002). Analogously to the strains of the PrP^{sc} prion, the [*PSI*$^+$] prion has different "variants" (Derkatch *et al*., 1996), which result from differences in the amyloid conformation of Sup35 monomers, variations in the arrangement of the monomers within polymers, or both (King and Diaz-Avalos, 2004; Tanaka *et al*., 2004; Diaz-Avalos *et al*., 2005; Krishnan and Lindquist, 2005; Tessier and Lindquist, 2007; Toyama *et al*., 2007). The efficiency of [*PSI*$^+$] nonsense suppression is variant-specific and correlates with the degree of Sup35 aggregation. Cells that bear "strong" [*PSI*$^+$] variants contain mostly aggregated Sup35 and exhibit high levels of nonsense suppression. Cells that bear "weak" [*PSI*$^+$] variants contain more nonprionized Sup35, and exhibit lower levels of nonsense suppression (Derkatch *et al*., 1996; Zhou *et al*., 1999).

The prion properties of Sup35 are dictated by its 124-amino acid-long asparagine- and glutamine-rich N-terminal (N) domain (Ter-Avanesyan *et al*., 1994; DePace *et al*., 1998; Liu and Lindquist, 1999; Chernoff *et al*., 2000; Santoso *et al*., 2000; Parham *et al*., 2001; Bradley and Liebman, 2004; Ross *et al*., 2005). The N and the following M (amino acids 125–254) regions are dispensable for the activity of Sup35 in translational termination, which is carried out by its conserved C-terminal domain (Ter-Avanesyan *et al*., 1993). In vitro, the recombinant full-length Sup35 (Glover *et al*., 1997; Krzewska and Melki, 2006; Shorter and Lindquist, 2006) or its N-terminal prion domain alone (King *et al*., 1997; Paushkin *et al*., 1997; Serio *et al*., 2000; Krzewska *et al*., 2006; Shewmaker *et al*., 2006; Vitrenko *et al*., 2007) form amyloid fibers, which grow by recruiting Sup35 monomers (Scheibel *et al*., 2001; Collins *et al*., 2004) (but see Narayanan *et al*., 2006). Amyloid fibers of the N-terminal domain of Sup35 made in vitro are infectious and they induce [*PSI*$^+$] when delivered into [*psi*$^-$] cells (King and Diaz-Avalos, 2004; Tanaka *et al*., 2004).

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In vivo, [*PSI*$^+$] transmission occurs during budding when the daughter cell acquires a portion of mother's cytoplasm containing one or more "seeds" with Sup35 in the prion form. Seeds in the daughter cell then recruit nonprionized Sup35 molecules and convert them into the prion fold (Satpute-Krishnan and Serio, 2005). Experimental data support the idea that the Sup35-containing aggregates (Paushkin *et al*., 1996) that quickly sediment from yeast lysates upon centrifugation (see Serio *et al*., 1999) are the authentic [*PSI*$^+$] seeds (Satpute-Krishnan and Serio, 2005). When treated with SDS at room temperature, the aggregates decrease in size ~30-fold and yield smaller, SDS-stable polymers of Sup35 (Kryndushkin *et al*., 2003; Bagriantsev and Liebman, 2004). Based on these observations it was suggested that the aggregates are organized at two-levels: SDS-stable polymers of Sup35 binding to each other directly, or via other unknown proteins, through less stable SDS-sensitive interactions (Kryndushkin *et al*., 2003).

The de novo appearance and subsequent propagation of [*PSI*$^+$] (Park *et al*., 2006) (as well as [*PIN*$^+$] and [*URE3*]) are tightly controlled by chaperones (for review, see Jones and Tuite, 2005; Chernoff, 2007). The ribosome-associated proteins Ssb1 and Ssb2 (Ssb1/2) antagonize the de novo appearance of [*PSI*$^+$] (Chernoff *et al*., 1999; Allen *et al*., 2005), possibly by preventing the nascent Sup35 polypeptide from adopting the prion fold. Once [*PSI*$^+$] is established, it requires Hsp104 for propagation (Chernoff *et al*., 1995). Hsp104, with the help of other chaperones, disaggregates denatured proteins (Glover and Lindquist, 1998), and it is believed to use this disaggregating activity (with or without the help of other chaperones) to propagate [*PSI*$^+$] seeds (Inoue *et al*., 2004; Krzewska and Melki, 2006; Shorter and Lindquist, 2006). Ssa1 and Ssa2 (Ssa1/2), possibly with the help of its Hsp40 cochaperones (e.g., Sis1 and Sse1), seems to stabilize [*PSI*$^+$] propagation (for latest examples, see Jones *et al*., 2004; Allen *et al*., 2005; Loovers *et al*., 2006; Fan *et al*., 2007; Kryndushkin and Wickner, 2007). Several experiments have shown that the balance of different chaperones can affect [*PSI*$^+$] appearance, propagation, or both. For example, overexpression of Ssa1/2 antagonizes (Newnam *et al*., 1999) the [*PSI*$^+$]-curing effect of Hsp104 overexpression (Chernoff *et al*., 1995), whereas overexpression of Ssb potentiates it (Chernoff *et al*., 1999). Although many aspects of the prion–chaperone interactions remain unclear, evidence indicates that the yeast prions exploit the chaperone machinery for their own propagation. A recent study demonstrated that chaperones are associated with toxic polyglutamine aggregates in a yeast model of Huntington's disease (Wang *et al*., 2007). Chaperones are therefore the most obvious candidates as components of yeast prion aggregates, including [*PSI*$^+$].

Indeed, it was shown that Rnq1, the prion component of [*PIN*$^+$] aggregates, exists in the cytosol in an ~1:1 complex with Sis1, and it also interacts with Ssa1/2 and Ydj1 (Sondheimer *et al*., 2001; Lopez *et al*., 2003; Aron *et al*., 2007). Likewise, a physical association between Sup35 and the members of the yeast Hsp70 family Ssa1/2 and Ssb1 was shown in vitro and in lysates from yeast overexpressing Sup35 (Allen *et al*., 2005; Krzewska and Melki, 2006; Allen *et al*., 2007). Although some chaperones (e.g., Hsp104) may affect different yeast prions similarly (Chernoff *et al*., 1995; Moriyama *et al*., 2000; Sondheimer and Lindquist, 2000; Derkatch *et al*., 2001), the effects of others (e.g., Ssa1/2) are specific to a particular prion (Schwimmer and Masison, 2002; Kryndushkin and Wickner, 2007), suggesting that the same chaperones are not necessarily components of all the yeast prion aggregates.

Understanding the protein composition of prion aggregates may be important for uncovering the molecular mechanism by which prions propagate their variant-specific conformation from cell to cell. Although successful propagation of the same prion variant does not seem to require other proteins in vitro, the in vivo mechanism may involve chaperones, at least for some prions. For example, mutations in Sis1 alter the Rnq1–green fluorescent protein fluorescence pattern in [*PIN*$^+$] cells (Sondheimer *et al*., 2001; Lopez *et al*., 2003) in a way that resembles a switch from one [*PIN*$^+$] variant to another (Bradley and Liebman, 2003). Although a similar phenomenon has not been reported for [*PSI*$^+$], it was shown that different variants of [*PSI*$^+$] may respond differently to overproduced chaperones (Kushnirov *et al*., 2000b), suggesting that chaperones may control some [*PSI*$^+$] variants while having no effect on others, and that the protein composition of [*PSI*$^+$] aggregates may differ among [*PSI*$^+$] variants. In this study, we examined the morphology of different variants of [*PSI*$^+$] aggregates isolated from yeast, identified the protein components of these aggregates, and analyzed the requirement of their structural integrity for variant-specific [*PSI*$^+$] infection.

MATERIALS AND METHODS

Yeast Strains and Media

Standard yeast media, cultivation and transformation procedures were used as described previously (Sherman, 2002). Strains were grown either in complex medium (2% dextrose, 2% bacto peptone, and 1% yeast extract), or in complete synthetic medium (an artificial mix of 2% dextrose and all necessary amino acids and nucleobases) without the addition of adenine. [*PSI*$^+$] (a strong variant, L1763 in our collection) or [*psi*$^-$] (L1751) versions of yeast strain 74D-694 ([*pin*$^-$], *MATa ade1-14 ura3-52 leu2-3,112 trp1-289 his3-200*, Chernoff *et al*., 1993) were used. A *sup35*Δ::*LEU2* derivative of 74D-694 bearing a strong variant of [*PSI*$^+$] and expressing full-length Sup35 from a pRS313-based *CEN HIS3* plasmid under the natural *SUP35* promoter (L2979) was a gift from C. G. Crist and Y. Nakamura (Crist *et al*., 2003). A [*psi*$^-$] version of this strain (L2980) was obtained by two consecutive passages on complex medium supplemented with 5 mM [*PSI*$^+$]-curing agent guanidine hydrochloride (Tuite *et al*., 1981). The plasmid encoding Sup35 was first replaced in these strains with the pRS316-based *CEN URA3* plasmid encoding full-length Sup35 under its native promoter with a hemagglutinin (HA) tag between the M and RF domains (plasmid p1071 in our collection, a kind gift from J. Weissman, DePace *et al*., 1998), resulting in L2986. A [*psi*$^-$] version of L2986 was obtained by cultivating L2986 in the presence of guanidine (see above). Subsequently, p1071 in L2986 was replaced with a *CEN HIS3* plasmid encoding full-length Sup35 with a hexahistidine tag on the C terminus under the natural *SUP35* promoter (p1373), a gift from M. Tuite (pUKC1602; Ness *et al*., 2002), yielding the [*psi*$^-$] (L2983) and strong [*PSI*$^+$] (L2982) strains used in this study. Thus, L2979, L2986, and L2982 bear the same strong variant of [*PSI*$^+$]. A weak variant of [*PSI*$^+$] (L2988) was selected among the guanidine-curable weak nonsense suppressors induced in L2983 by overexpression of a pEMBL-ΔBal2 (p747) plasmid (a gift from Inge-Vechtomov; Ter-Avanesyan *et al*., 1993) bearing the prion domain of Sup35 with an amino acid extension that eliminated the requirement of [*PIN*$^+$] for [*PSI*$^+$] induction (Derkatch *et al*., 1997).

Strong [*PSI*$^+$] and [*psi*$^-$] *sup35ΔNM* strains bearing N(1-137) were constructed as described previously (Bradley and Liebman, 2004). Briefly, a [*psi*$^-$] [*rho*$^-$] *sup35Δ2-254* (*sup35ΔNM*) derivative of 74D-694 (L2275) bearing the N(1-137) domain of Sup35 under the natural *SUP35* promoter on a centromeric vector (p1328), was cytoduced with [*psi*$^-$] (L2198) or strong [*PSI*$^+$] (L2200) [*RHO*$^+$] [*pin*$^-$] derivatives of the yeast strain A3099 (*MATα ade2-1 SUQ5 lys1-1 his3-11,15 leu1 ura3::kanMX4 kar1-1*), and the [*RHO*$^+$] [*PSI*$^+$] and [*RHO*$^+$] [*psi*$^-$] cells bearing the genotype of L2275 were selected as described previously (Bradley and Liebman, 2004).

Isolation of [PSI$^+$] Aggregates

Cells were grown in 500 ml of complex medium to an optical density of ~3.0 at 600 nm, harvested (~2.5 g of wet pellet), washed in lysis buffer (LB: 25 mM Tris-HCl pH 7.6, 50 mM KCl, 5 mM $MgCl_2$, 10 mM imidazole, and 5% glycerol), harvested, resuspended in 15 ml of LB supplemented with antiproteases (10 mM phenylmethylsulfonyl fluoride plus 1.25% of the antiprotease cocktail for isolating histidine-tagged proteins; Sigma-Aldrich, St. Louis, MO), and lysed by violent agitation with 12 ml of 0.5-mm glass beads using a BeadBeater (BioSpec Products, Bartlesville, OK) for 40 s at 4°C. Crude lysate was centrifuged at 4500 × *g* (4°C) for 10 min followed by filtration through a

1-μm glass filter (GE Osmonics, Minnetonka, MN) to remove cellular debris. Five milliliters of lysate containing 5 mg of total protein (determined using the Bradford reagent from Bio-Rad, Hercules, CA) (Bradford, 1976) was subjected to centrifugation on top of a sucrose cushion (5 ml of 30% over 5 ml of 60%, wt/vol, sucrose in LB) for 1 h at 27,000 rpm (15°C) by using the SW27 rotor (Beckman Coulter, Fullerton, CA). The majority of the soluble proteins did not penetrate into the 30% sucrose layer (data not shown). The top 2.5 ml of the 30% sucrose layer containing [*PSI*$^+$] aggregates (determined by immunoblotting with a-Sup35C antibody, see below; data not shown) were incubated in a chromatography column with 1 ml of 1:1 LB slurry of the Co^{2+}-charged Talon resin (Clontech, Mountain View, CA) for 15 min at room temperature. The liquid phase was drained and the resin was washed with 3.5 ml of LB. The proteins were eluted with 600 μl of 30 mM Tris-HCl, pH 7.6, containing 50 mM KCl, 50 mM ethylenediamine tetraacetate-Na, and 1% of the antiprotease cocktail. For electron microscopy analysis and prion transformation, the resin was additionally washed with 7.5 ml of LB and 15 ml of PBSM buffer (1.5 mM KH_2PO_4, 2.7 mM Na_2HPO_4, pH 7.2, 155 mM NaCl, 10 mM imidazole, 5 mM $MgCl_2$, and 5% glycerol), and eluted with PBSM supplemented with 200 mM imidazole. The eluate was concentrated by centrifugation through a 100-kDa molecular weight cut-off filter (Biomax Ultrafree; Millipore, Billerica, MA). Analysis of the eluate by SDS-electrophoresis in agarose and immunoblotting (see below) showed that the sizes of the SDS-stable Sup35 polymers in the initial unfractionated lysate and final fraction (eluate) were the same (Supplemental Figure S2), indicating that we obtained a representative fraction of Sup35 molecules.

Immunocapture on Magnetic Beads

Yeast were grown in 50 ml of complex medium to an optical density of ~3.0 at 600 nm, harvested, and washed in protein extraction buffer (PEB: 40 mM Tris-HCl, pH 7.6, 150 mM KCl, 5 mM $MgCl_2$, and 5% glycerol). Cells were resuspended in 800 μl of PEB containing the Sigma anti-protease cocktail with phenylmethylsulfonyl (see above), and then they were disrupted by vortexing in a 1.5-ml tube with 750 μl of 0.5-mm glass beads. The crude lysate was precleared by centrifugation for 10 min at 10,000 × *g* at 4°C. KCl and Triton X-100 were added to precleared lysates to final concentrations of 350 mM and 1%, respectively. Five hundred to 800 μl of lysates (0.5–1.0 mg/ml) was mixed with 0.5 μl of α-Ssa1/2, or 4.0 μl of α-his6, or 5 μl of α-HA, or 10 μl of α-Hsp104 antibody (see below) and incubated for 2 h on ice. After incubation, 50 μl of magnetic beads with immobilized G protein (Miltenyi Biotec, Auburn, CA) were added, and the samples were further incubated on ice for 1 h. To remove nonspecifically bound proteins, the beads were washed with 1.0 ml of PEB, 150 mM KCl, 1% Triton X-100 (at 4°C), and with 1.0 ml of each of the following solutions and in the following order (at room temperature): PEB, 1% Triton X-100; PEB with 500 mM NaCl, 1% Triton X-100; PEB with 1% Triton X-100; and Tris-HCl, pH 7.6 (500 μl). Proteins were eluted with hot sample buffer (50 mM Tris-HCl, pH 6.8, 5% glycerol, and 0.05 and 2% β-mercaptoethanol), and then they were analyzed by electrophoresis and immunoblotting (see below). To check the stability of proteins during incubation, we incubated lysates (without antibodies) along with the experimental samples.

Electrophoresis and Immunoblotting

Samples were treated with sample buffer (50 mM Tris-HCl, pH 6.8, 5% glycerol, and 0.05% bromphenol blue for acrylamide, or with 25 mM Tris, 200 mM glycine, 5% glycerol, and 0.05% bromphenol blue for agarose gels) containing 2% SDS, for 7 min at 95°C or at room temperature and resolved by SDS-electrophoresis in polyacrylamide (Serio *et al*., 1999) or agarose (Bagriantsev *et al*., 2006) gels. Protein bands were detected by Coomassie G250 (Bio-Rad). Densitometry of Coomassie-stained protein bands was performed using an Alpha Imager 2200 (Alpha Innotech, San Leandro, CA) and processed on AlphaEaseFC imaging software. The same software was used to calculate the position of the 1500-kDa marker in Supplemental Figures S1B and S2. For immunoblotting, the proteins were electrophoretically transferred from the gels to an Immun-Blot polyvinylidene difluoride membrane (Bio-Rad) and detected with respective antibody (see below) by using a Western-Star chemiluminescence development kit (Applied Biosystems, Foster City, CA) as suggested by the manufacturer.

Antibodies

The following antibodies were used: α-Sup35C (BE4, mouse monoclonal against Sup35C (Bagriantsev and Liebman, 2006); α-Rnq1 (type II, rabbit polyclonal), α-Sup35N (Ab0332, rabbit polyclonal against amino acids 55-68 of Sup35), gifts from S. Lindquist; α-Ssa1/2 (SSA1/2 C1ΔB, rabbit polyclonal against the last 56 amino acids of Ssa1 (Lopez-Buesa *et al*., 1998), α-Ssb1 (rabbit polyclonal against the last 80 amino acids of Ssb1), α-Sis1 (rabbit polyclonal against full length Sis1; Yan and Craig, 1999), gifts from E. Craig; α-Sse1 (rabbit polyclonal against amino acids 663-684 of Sse1; Goeckeler *et al*., 2002), a gift from L. Brodsky; α-Sla2 (rabbit polyclonal against amino acids 664-968; Yang *et al*., 1999), a gift from D. Drubin; α-Hsp104 (SPA-1040, rabbit polyclonal against amino acids 894-908 of Hsp104; Assay Designs, Ann Arbor, MI); and α-polyhistidine (sc-803, rabbit polyclonal) and α-Ydj1 (sc-23749, goat polyclonal) were from Santa Cruz Biotechnology; α-hemagglutinin (HA-7, mouse monoclonal; Sigma-Aldrich).

Electron Microscopy

Samples (4–6 μl) were applied onto Formvar carbon-coated copper grids (FCF2010-Cu from Electron Microscopy Sciences), incubated for 3 min, washed with water three times, and stained with 2–2.5% aqueous uranyl acetate (Ted Pella, Redding, CA) for 90 s. Images were obtained using a JEOL JEM-1220 transmission electron microscope operating at 80 kV. Micrographs were collected using a Gatan digital camera. Data from three S [*PSI*$^+$], three control [*psi*$^-$], and one control S [*PSI*$^+$] wild-type (wt) isolations were analyzed. Each sample was applied onto several grids, and the whole visible area on at least two grids from each sample was examined. The structures shown in Figure 5 were found in S [*PSI*$^+$], but not in the control samples.

Prion Transformation

Transformation was performed as described previously (Tanaka *et al*., 2004) with modifications (Patel and Liebman, 2007). Briefly, a [*psi*$^-$] derivative of 74D-694 *sup35*Δ expressing Sup35 from a plasmid was grown to an optical density of 0.65 and transformed with a *URA3* plasmid (pRS316) mixed with 15 μl of a [*PSI*$^+$] aggregate preparation from L2988 or L2982, or mock aggregate preparation from L2983 or L2979. Ura$^+$ transformants were selected and transferred to complex and adenine-deficient medium to find nonsense suppressors ([*PSI*$^+$] candidates). Because the *ADE1* gene (required for adenine synthesis) in 74D-694 is represented by the *ade1-14* nonsense allele, [*psi*$^-$] cells are Ade$^-$ due to termination of Ade1 synthesis at the premature stop codon, and they are red on complex medium due to accumulation of a red intermediate of the adenine synthesis pathway. In [*PSI*$^+$] cells, the premature nonsense codon of *ade1-14* is suppressed, which makes the yeast Ade$^+$ and white (see Chernoff *et al*., 2002), as shown in Supplemental Figure S1A. To confirm that the nonsense suppressor phenotype was caused by [*PSI*$^+$], Ade$^+$ cells ([*PSI*$^+$] candidates) were transferred to complex medium supplemented with 5 mM guanidine hydrochloride. Cells that were Ade$^+$ and red on complex medium (lacking guanidine) after growth on guanidine were scored as [*PSI*$^+$]. The efficiency of prion induction was calculated as a proportion of [*PSI*$^+$] cells relative to the initial number of Ura$^+$ transformants.

Mass Spectrometry Analysis

Identification of the 70-kDa band was performed at the Proteomics and Informatics Services Facility (University of Illinois, Chicago, IL). The band was excised from the gel, digested with trypsin, and analyzed by capillary-liquid chromatography tandem mass spectrometry using the LTQ FT Ultra hybrid mass spectrometer (Thermo Electron, Waltham, MA). The database searches were performed using the Mascot (Matrix Science, Boston, MA) and Sequest (Thermo Electron) programs.

RESULTS

The Major Components of [PSI$^+$] Aggregates Are Sup35 and Ssa1/2

Previously, it was shown that [PSI$^+$] aggregates contain Sup35 in the form of polymers resistant to SDS treatment at room temperature, and possibly other proteins (Kryndushkin *et al*., 2003). To identify the major protein components of [*PSI*$^+$] aggregates, we used a [*pin*$^-$] yeast strain with a chromosomal deletion of the *SUP35* gene (*sup35*Δ) expressing a hexahistidine(his6)-tagged Sup35 from a centromeric vector under the natural *SUP35* promoter, with or without either a weak (W) or strong (S) [*PSI*$^+$] variant. As shown previously (Ness *et al*., 2002), the presence of the his6 tag on the C terminus of Sup35 does not influence the prion properties of the protein (Supplemental Figure S1). We partially purified [*PSI*$^+$] aggregates from yeast lysates by the sequential use of centrifugation on top of a sucrose cushion (to separate heavy protein aggregates from soluble proteins) and metal-chelating chromatography (Figure 1A). The procedure did not detectably affect the size of SDS-stable Sup35 polymers, which contained ~4–20 Sup35 monomers before and after purification (Supplemental Figure S2). The protein components of the partially purified aggregates were separated by SDS-polyacrylamide gel electrophoresis (PAGE) and visualized by Coomassie G250 staining after treatment with SDS at room temperature (to disassemble [*PSI*$^+$] aggregates but preserve Sup35 polymers), or at 95°C (to further monomerize Sup35 polymers). We hypothesized that the aggregates would contain SDS-stable Sup35 polymer(s) with

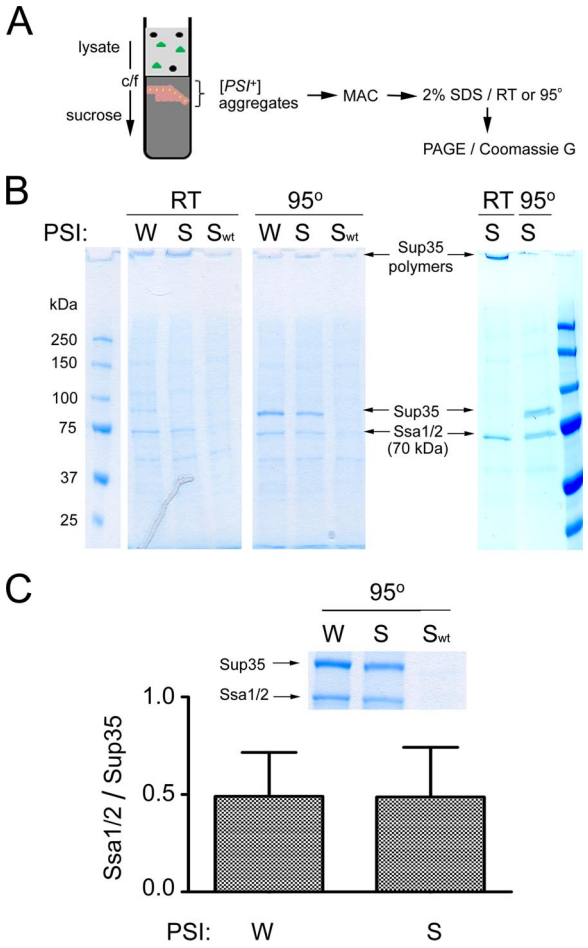

Figure 1. Sup35 and Ssa1/2 are the major components of weak and strong [*PSI*$^+$] aggregates. (A) Experimental scheme. Isolation of Sup35 (expressed from a centromeric vector under the *SUP35* promoter) and associated proteins from [*pin*$^-$] *sup35*Δ yeast was facilitated by a his6 tag on Sup35 (see *Materials and Methods*). Yeast lysates were fractionated by centrifugation (c/f) on a sucrose cushion, and the fraction containing heavy protein aggregates was collected and subjected to metal affinity chromatography (MAC). Proteins were eluted from the MAC column, treated with SDS at room temperature (RT) or at 95°C, and then analyzed by SDS-PAGE and Coomassie G250. (B) Ssa1/2 coisolated with his6-tagged Sup35 from W and S [*PSI*$^+$] lysates. The levels of proteins that were not specifically associated with Sup35, i.e., that also occurred in the no-his6 tag [*PSI*$^+$] control (S_{wt}), varied between isolations and sometimes were minimal (right panel). Sup35 polymers were resistant to SDS treatment at RT (seen in the bottom of the loading wells), but they disassembled into monomers at 95°C. Ssa1/2 was monomeric at both temperatures. (C) Ssa1/2 was on average two times less abundant than Sup35 in W and S [*PSI*$^+$] aggregates. The relative abundance of the Coomassie G-stained Sup35 and Ssa1/2 was measured by an in-gel densitometry. The assumption was made that the dye binds with equal efficacy to both proteins. The intensities of the corresponding areas in the control S_{wt} [*PSI*$^+$] eluate were subtracted from the initial values for Ssa and Sup35 in W and S [*PSI*$^+$] eluates before calculation. Data are presented as mean ± SD, n = 3. The strains used are [*pin*$^-$][*PSI*$^+$]: L2988 (W), L2982 (S), L2979 (S_{wt}).

other protein(s) bound to the polymers via regular (i.e., sensitive to SDS treatment at room temperature) protein–protein interactions.

As expected, the majority of Sup35 isolated from W and S [*PSI*$^+$] yeast was assembled into SDS-stable polymers, which monomerized only after SDS treatment at 95°C (Figure 1, B and C). We did not detect Sup35 in control isolations using S [*PSI*$^+$] yeast expressing wild-type untagged Sup35 (S_{wt}[*PSI*$^+$]). A comparison of the Coomassie-stained protein pattern of W and S [*PSI*$^+$] aggregate preparations against that of S_{wt}[*PSI*$^+$] from several isolations revealed a 70-kDa band that was the only major protein consistently associated with W and S [*PSI*$^+$] but not with S_{wt}[*PSI*$^+$]. The abundance of other, "contaminant" bands (i.e., bands that occurred in all lanes, including the untagged S_{wt}[*PSI*$^+$] control) varied and in some isolations was minimal (Figure 1B, right). This suggests that Sup35-his6 and the 70-kDa protein formed a complex. The 70-kDa band was excised from the gel and identified by mass spectrometry as a mixture of the homologous proteins Ssa1 and Ssa2 (68.8 and 69.5 kDa, respectively). Despite the 96% identity between the proteins, the mass spectrometry analysis identified peptides that were unique to Ssa1 or Ssa2 (Supplemental Table S1). Whether W and S [*PSI*$^+$] aggregates were treated with SDS at room temperature or at 95°C, Ssa1/2 migrated as a monomer. This suggests that Ssa1/2 was not a part of the SDS-stable Sup35 polymers, but it was bound to them via regular protein–protein interactions. Using an in-gel densitometry analysis of Coomassie-stained Sup35 and Ssa1/2, we determined that Sup35 was ~2 times more abundant than Ssa1/2 in W and S [*PSI*$^+$] aggregates (Figure 1C).

We confirmed the identities of the Coomassie-stained Sup35 and Ssa1/2 bands (Figure 1, B and C) by immunoblotting with respective antibodies (Figure 2A). To detect minor components of [*PSI*$^+$] aggregates, which were not revealed by Coomassie staining, we undertook a candidate approach and analyzed the aggregates by immunoblotting (Figure 2B). We found that Sla2 (Ganusova *et al.*, 2006), Hsp104 (Figures 2B and Supplemental Figure S3), as well as cochaperones Sis1, Sse1, and Ydj1 of Ssa1/2 were coisolated with Sup35 from W and S [*PSI*$^+$], but not from control S_{wt}[*PSI*$^+$] yeast (Figure 2B). Ssb1, which was previously shown to interact with Sup35 (Allen *et al.*, 2005), was also coisolated with Sup35, but we consistently detected some Ssb1/2 in control isolations. Sup35 aggregate preparations from our [*pin*$^-$] strains did not contain any detectable amounts of Rnq1, which is itself aggregated into a prion only in [*PIN*$^+$] strains. Thus, our data suggest that aggregates of different [*PSI*$^+$] variants have a similar protein composition and mostly contain Sup35 and Ssa1/2, plus some minor components, among which we found Hsp104, Sse1, Sis1, Ydj1, Sla2, and Ssb1/2, but not Rnq1.

Ssa1/2 Efficiently Binds to the Prion, but Not the Nonprion Form of Sup35

We asked whether Ssa1/2 binds to the nonprion Sup35 from [*psi*$^-$] yeast as efficiently as to the Sup35 prion from W or S [*PSI*$^+$]. Because the nonprion Sup35 is monomeric and therefore could not be isolated from fractionated lysates as described in Figure 1 (Supplemental Figure S4), we performed immunocapture experiments from whole unfractionated lysates by using α-his6 or α-Ssa1/2 antibodies. To avoid the bias toward isolating heavy protein aggregates due to the use of centrifugation in conventional immunoprecipitation technique, we instead pulled the antibodies from lysates via protein G-coupled magnetic beads. After washing the beads, we analyzed the coisolated proteins for the presence of Sup35 and Ssa1/2 by immunoblotting. Consistent with the data shown on Figure 1, Ssa1/2 was specifically coisolated with Sup35 from unfractionated W and S [*PSI*$^+$] lysates via both α-Ssa1/2 and α-his6 antibody (Figure 3). However, the interaction between Ssa1/2 and the nonprion form of Sup35 from [*psi*$^-$] lysates was very inefficient (yet detectable), al-

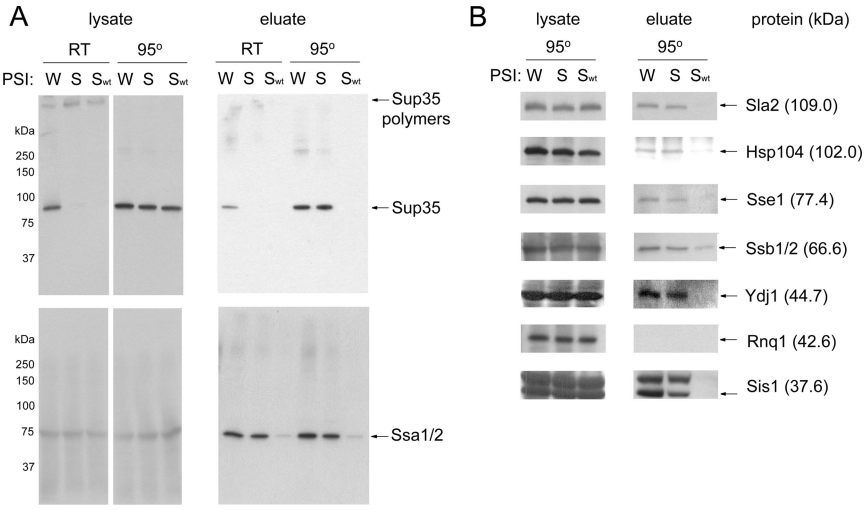

Figure 2. Immunoblot analysis of major and minor protein components of weak and strong [*PSI*$^+$] aggregates. [*PSI*$^+$] aggregates were obtained as described in Figure 1 (eluates), and then they were analyzed by immunoblotting with the indicated antibodies along with initial lysates after treatment with SDS at RT or at 95°C. (A) SDS treatment of W [*PSI*$^+$] lysates and eluates at room temperature revealed some Sup35 monomer, which was almost undetectable in S [*PSI*$^+$] samples. (B) In addition to Sup35 and Ssa1/2 (major components shown in A), [*PSI*$^+$] aggregates (eluates) contained Sla2, Hsp104, Sse1, Ssb1/2, Ydj1, and Sis1 (minor components). Rnq1 did not coisolate with Sup35.

though both α-his6 and α-Ssa1/2 pulled out their antigens. The cellular levels of Ssa1/2 and Sup35 were the same in [*psi*$^-$] and [*PSI*$^+$] yeast (Supplemental Figure S5), as well as immediately before immunocapture (Figure 3). Similarly, Ssa1/2 preferentially interacted with the prion, but not the nonprion form of Sup35 in wild-type *SUP35* yeast without plasmid (Figure 3B), ruling out the possibility that this preferential interaction of Ssa1/2 with the prion form of Sup35 was caused by the presence of the his6 tag or that it was dependent on the source of the *SUP35* gene (plasmid vs. genomic).

Because Ssa1/2 interacted preferentially with Sup35 in its prion form, it seemed likely that it would bind to the 137 amino acid long N-terminal region of Sup35. This region of Sup35 has different conformations when in the prion versus nonprion state (Glover *et al.*, 1997), and it is sufficient for [*PSI*$^+$] propagation in vivo (Bradley and Liebman, 2004). We checked whether Ssa1/2 interacts with N(1-137) expressed from a plasmid in [*psi*$^-$] and [*PSI*$^+$] yeast bearing a genomic deletion of the 2–252 region of Sup35. Indeed, Ssa1/2 interacted with the prion form of N(1-137), whereas its interaction with the nonprion form of N(1-137) or with Sup35C in [*psi*$^-$] and [*PSI*$^+$] was undetectable (Figure 3C).

Together, our data suggest that Ssa1/2 efficiently interacts with the prion, but not the nonprion form of the N-terminal domain of Sup35, and that it accounts for almost a third of total [*PSI*$^+$] aggregate weight. We note that Ssa1/2 is almost 8 times more abundant than Sup35 in yeast lysates (Ghaemmaghami *et al.*, 2003); thus, even in [*PSI*$^+$] cells, the majority of Ssa1/2 should be available for other interactions.

Some Minor Components of [PSI$^+$] Aggregates Interact Poorly with Nonprion Sup35

To check whether minor components of [*PSI*$^+$] aggregates interact with Sup35 in a prion-dependent manner, we performed immunocapture experiments from whole unfractionated lysates of *sup35*Δ yeast expressing a hemagglutinin-tagged Sup35 (Sup35-HA) from a centromeric vector under the *SUP35* promoter. As shown previously, the presence of the hemagglutinin tag does not affect the prion properties of Sup35 (DePace *et al.*, 1998). Consistent with our data presented here, Sup35-HA efficiently interacted with Ssa1/2 in [*PSI*$^+$], whereas the interaction was less efficient in [*psi*$^-$] (Figure 4). Interestingly, a similar pattern was detected for cochaperones Sse1 and Sis1 of Ssa1/2 and for Hsp104: these proteins efficiently interacted with Sup35 only in [*PSI*$^+$]. Although we cannot rule out the possibility that these proteins interact with Sup35 in [*psi*$^-$], they seem to do so less efficiently than in [*PSI*$^+$]. In contrast, Ssb1/2 and Sla2 interacted with Sup35 equally well in [*PSI*$^+$] and [*psi*$^-$]. Using this method of immunocapture, we did not detect interaction with Ydj1 in either of the strains, although Ydj1 did copurify with Sup35 via metal affinity chromatography (Figure 2B).

[PSI$^+$] Aggregates Contain Several Sup35 Polymers

Because SDS treatment of [*PSI*$^+$] aggregates at room temperature reduces their weight severalfold (Kryndushkin et al., 2003), it was hypothesized that the aggregates may contain more than one Sup35 polymer. If so, Sup35 polymers should be able to interact. To test this, we examined a preparation of Sup35 polymers isolated from [*PSI*$^+$] yeast by using electron microscopy (Figure 5). Polymers were obtained using the same protocol as in Figure 1, but with the use of stringent washing conditions in to obtain the purest preparation possible. The resulting sample contained ~90% pure Sup35 polymers without Ssa1/2 (Figure 5B). The sample was negatively stained with uranyl acetate and visualized by electron microscopy (Figure 5, C–E). Full-length Sup35 polymerized in vitro has the appearance of long ~20- to 50-nm-wide fibers (Glover *et al.*, 1997; Shorter and Lindquist, 2006). The size of the Sup35 molecule is estimated to be <10 nm (Kong *et al.*, 2004); hence, the width of the fibers is suggestive of a tight packing of Sup35 molecules within the fiber. Because Sup35 polymers in our [*PSI*$^+$] strain contained only ~4–20 Sup35 monomers (Supplemental Figure S1B), we expected them to have the appearance of short barrels rather than long fibers. Indeed, we observed ~20-nm-wide "barrels" (Figure 5E) and larger structures ("bundles"), which seem to be composed of these barrels (Figure 5D). These structures looked remarkably similar to what prion polymers made of full-length recombinant Sup35 look like when obtained in the presence of guanosine triphosphate and Hsp104 (see figure 5E in Shorter and Lindquist, 2006). We did not detect these species in control isolations from [*psi*$^-$] yeast expressing Sup35-his6 or [*PSI*$^+$] yeast expressing Sup35 without the tag. Our data are consistent with the hypothesis that Sup35 polymers, seen as barrels, interact in [*PSI*$^+$] aggregates to form bundles containing several Sup35 polymers each.

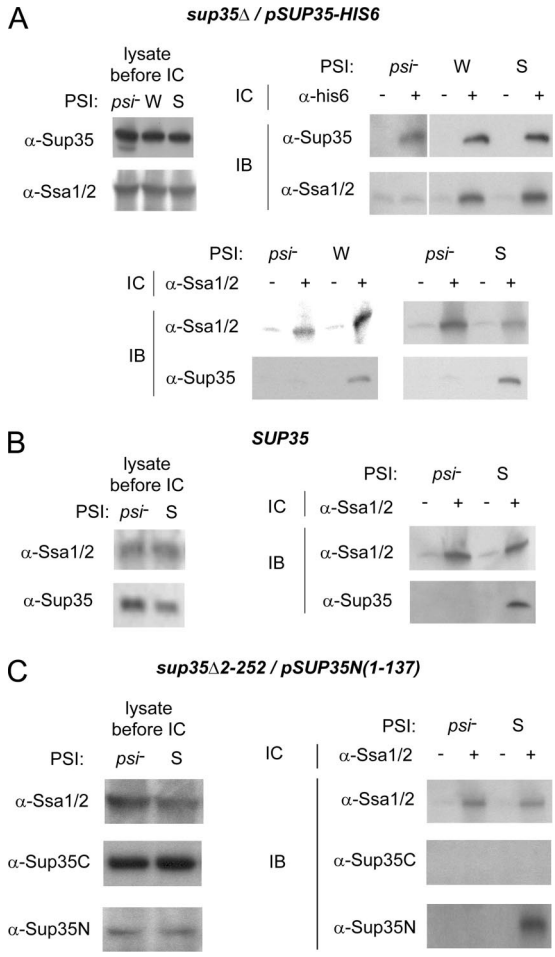

Figure 3. Ssa1/2 efficiently interacts with the prion but not the nonprion form of Sup35N(1-137). Interaction between Sup35 and Ssa1/2 was assayed in [*PSI*$^+$] (weak or strong variant) or [*psi*$^-$] strains by immunocapturing (IC) Sup35 or Ssa1/2 from whole unfractionated lysates (equalized by total protein) with α-his6 or α-Ssa1/2 antibody, respectively, immobilized on protein G-coupled magnetic beads (see *Materials and Methods*). After incubation, the beads were washed and the specifically bound proteins were eluted at 95°C in an SDS-containing buffer and analyzed by immunoblotting (IB) with indicated antibodies. Lysates of the same strains incubated along with the experimental samples without antibodies (lysates before IC) showed no degradation of Sup35 or Ssa1/2. The following strains were used: *sup35*Δ expressing Sup35-his6 (A) and *SUP35* yeast without plasmid (B). (C) *sup35*Δ2-252 yeast expressing the 137-amino acid-long N-terminal fragment of Sup35 (Sup35N1-137).

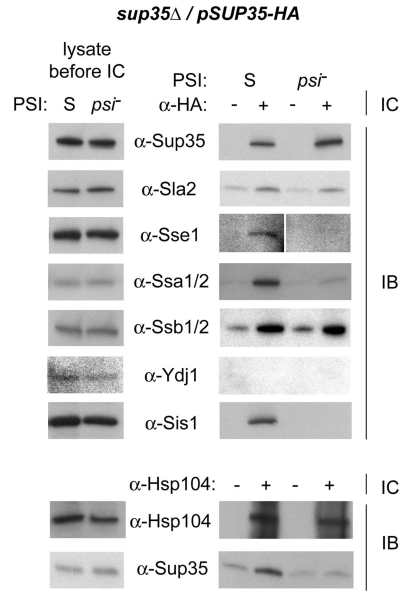

Figure 4. Some components of [*PSI*$^+$] aggregates interact poorly with Sup35 from [*psi*$^-$]. Interaction between Sup35 and minor components of [*PSI*$^+$] aggregates was assayed in S [*PSI*$^+$] or [*psi*$^-$] *sup35*Δ yeast expressing Sup35-HA, by immunocapture (IC) and imunoblotting (IB) as described in Figure 3. Sla2 and Ssb1/2 interacted with Sup35 in [*PSI*$^+$] and [*psi*$^-$] with similar efficiency. Hsp104, Sse1, Ssa1/2, and Sis1, preferentially interacted with Sup35 from [*PSI*$^+$]. Ydj1 association with Sup35 was not detected by immunocapture, although association of Ydj1 with [*PSI*$^+$] aggregates was shown by another method (see Figure 2B).

Sup35 Polymers within [PSI$^+$] Aggregates Bear the [PSI$^+$] Variant-specific Information

To determine whether the structural integrity of [*PSI*$^+$] aggregates is crucial for propagating [*PSI*$^+$] variant-specific information, we compared infectivity of W and S [*PSI*$^+$] aggregates treated or not treated with SDS. SDS treatment at room temperature disassembles [*PSI*$^+$] aggregates, leaving individual Sup35 polymers intact (Kryndushkin *et al*., 2003). We transformed [*psi*$^-$] *ura3*-52 cells with the preparations of W and S [*PSI*$^+$] aggregates mixed with a *URA3* plasmid, and we checked the [*PSI*$^+$] status of the Ura$^+$ transformants by using the standard *ade1-14*-based nonsense suppression assay (Supplemental Figure S1A), as described previously (Tanaka *et al*., 2004). As expected, W and S [*PSI*$^+$] aggregates were infectious and induced [*PSI*$^+$] when transformed into [*psi*$^-$] cells (Figure 6). Furthermore, SDS treatment increased the efficiency of [*PSI*$^+$] induction approximately twofold (Figure 6A). This is consistent with the idea that [*PSI*$^+$] aggregates contain several Sup35 polymers. Control preparations were never infectious regardless of SDS treatment.

Interestingly, when the aggregates were disassembled into polymers by SDS, the polymers retained the variant-specific information and caused infection with the original [*PSI*$^+$] variant (Figure 6B). To eliminate the possibility that rare aggregates that survived the SDS treatment served as seeds that induced the variant-specific reassembly of the aggregates from Sup35 polymers and Ssa1/2 during transfection, we performed a control experiment using SDS-treated aggregates plus 10% of the untreated aggregates from the opposite [*PSI*$^+$] variant that we have shown to be infectious. The presence of the untreated seed of a different [*PSI*$^+$] variant did not significantly change the variant-specific infectivity, as the majority of the transformants still bore the phenotype induced by the SDS-treated aggregates. Thus, the structural integrity of [*PSI*$^+$] aggregates is not required for the successful transmission of a [*PSI*$^+$] variant, and Sup35 polymers are the only variant-specific infectious agents within the aggregates.

DISCUSSION

In this study, we analyzed the protein composition and infectivity of partially purified [*PSI*$^+$] aggregates. It was shown previously that the aggregates contain Sup35 in the form of SDS-stable polymer(s) (Kryndushkin *et al*., 2003). We now show that the major components of [*PSI*$^+$] aggregates from different [*PSI*$^+$] variants are numerous SDS-stable

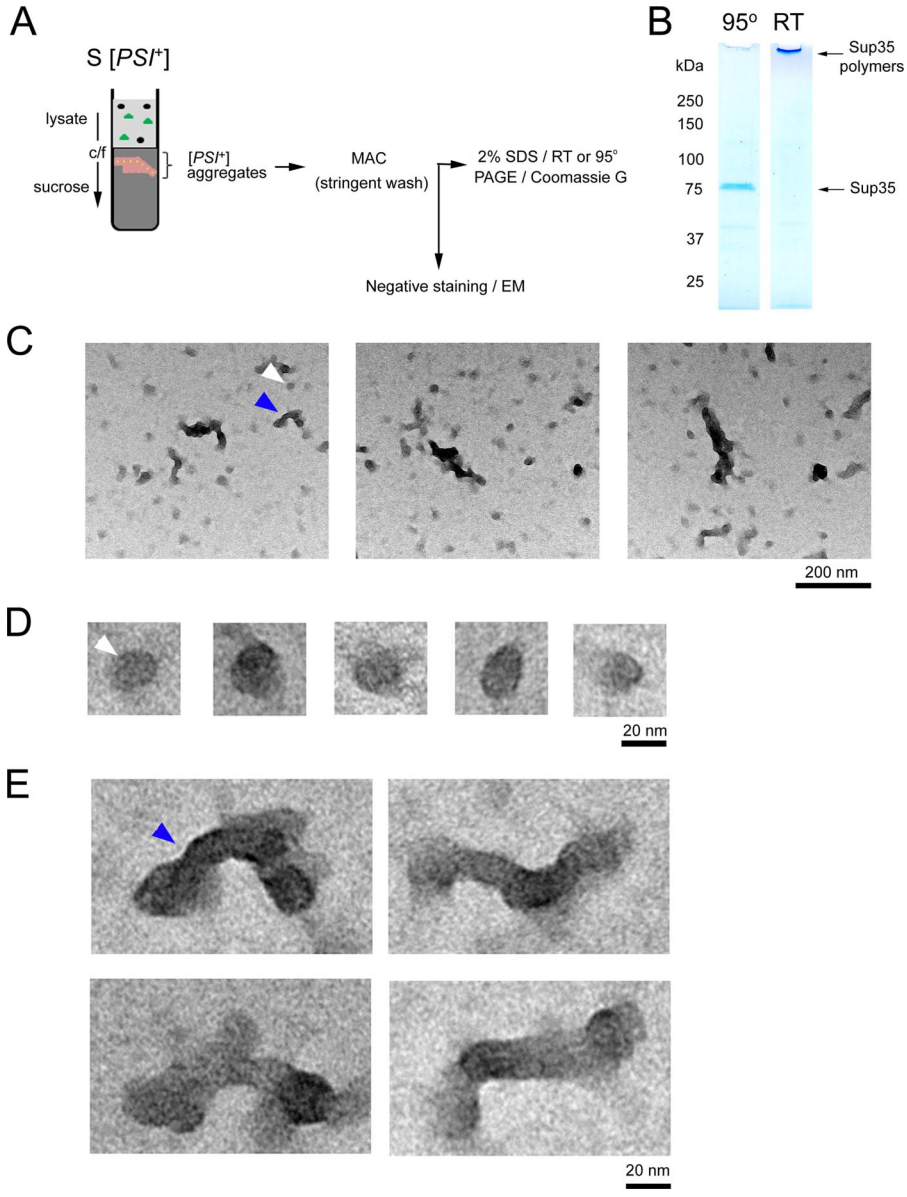

Figure 5. Morphological analysis of Sup35 isolated from [*PSI*$^+$] yeast. (A) Experimental scheme. Purification of Sup35 polymers was performed from an S [*PSI*$^+$] strain as in Figure 1, but with more stringent washing conditions (see *Materials and Methods*). (B) SDS-PAGE/Coomassie G250 analysis of Sup35 polymers isolated from S [*PSI*$^+$] yeast in A and treated with SDS at RT or at 95°C. (C–E) Electron micrographs of Sup35 polymers negatively stained with uranyl acetate (C). The sample contained numerous barrel-shaped structures (white arrowhead, D), which appeared to frequently form bundles (blue arrowhead, E). These structures were never detected in control isolations using [*psi*$^-$] yeast expressing Sup35-his6 or S [*PSI*$^+$] yeast expressing an untagged Sup35.

polymers of Sup35, and Ssa1/2. The polymers and Ssa1/2 interact via SDS-sensitive interactions. Our data support previous observations of a physical association between Sup35 and Ssa1/2 in vitro and in yeast overexpressing Sup35 (Allen *et al*., 2005, 2007; Krzewska and Melki, 2006). Here, using yeast expressing Sup35 at the normal cellular level we show that the Ssa1/2 interaction with the nonprion form of Sup35 is much weaker (yet detectable) than that with the Sup35 prion. We detected a similar pattern of interaction for cochaperones Sse1 and Sis1 of Ssa1/2. Like Ssa1/2, these proteins interacted with Sup35 in [*PSI*$^+$] more efficiently than in [*psi*$^-$]. Selective interaction between Ssa1/2 and Sup35 was not caused by an elevated level of Ssa1/2 in the presence of [*PSI*$^+$], even though [*PSI*$^+$] was previously shown to stimulate the expression of β-galactosidase placed under the control of the *SSA1* promoter (Jung *et al*., 2000; Schwimmer and Masison, 2002). Indeed, the level of Ssa1/2 and its cochaperones was the same in our [*PSI*$^+$] and [*psi*$^-$] cells. The enhanced ability of Ssa1/2 to bind to Sup35 in the prion versus nonprion form now explains why a deletion of *UBC4*, which causes an elevated rate of de novo [*PSI*$^+$] induction, also caused more Ssa1/2 to associate with overexpressed Sup35 (Allen *et al*., 2007).

We also show that Ssa1/2 efficiently interacts with the prion, but not the nonprion form of the Sup35N(1-137) domain produced in a *sup35*Δ*2-252* strain. Prionization profoundly refolds the N-terminal domain of Sup35 (Glover *et al*., 1997), which may explain why the efficiency of Ssa1/2 binding to Sup35 depends on the prion status of Sup35.

Genetic interactions between Sup35 and Ssa1/2 documented previously (for review, see (Jones and Tuite, 2005; True, 2006) support the finding that Ssa1/2 is associated with [*PSI*$^+$] aggregates. Inactivation of Ssa1/2 by mutations that promote substrate binding destabilizes [*PSI*$^+$] (see Jung *et al*., 2000; Jones and Masison, 2003; Jones *et al*., 2004; Hung and Masison, 2006; Loovers *et al*., 2006), whereas overexpression of Ssa1/2 1) causes Sup35 polymers to increase in length (Allen *et al*., 2005); 2) protects [*PSI*$^+$] from curing by overexpression of Hsp104 (Newnam *et al*., 1999; Wegrzyn *et al*., 2001), which presumably results in excessive shearing of

A

SEED	[*PSI*$^+$], % of total Ura$^+$		
exp.No.	1	2	3
buffer	0.0		
buffer SDS	0.0		
psi$^-$		0.0	
psi$^-$ SDS		0.0	
Swt	0.0		0.0
Swt SDS	0.0		0.0
W	36.6	8.0	21.9
W SDS	64.3	15.7	40.3
S	27.7	8.9	23.9
S SDS	62.0	26.5	49.2

B

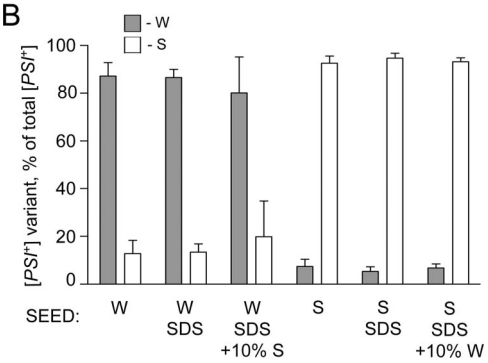

Figure 6. SDS-treated [*PSI*$^+$] aggregates induce [*PSI*$^+$] in a variant-specific manner. A [*psi*$^-$] *ade1-14 ura3-14* yeast strain was transformed with a *URA3* plasmid mixed with [*PSI*$^+$] aggregate preparations (SEED) obtained from the indicated strains as described in Figure 1 and treated or not with 1% SDS at room temperature. The presence of [*PSI*$^+$] was tested among Ura$^+$ transformants by using the *ade1-14* nonsense suppression assay (see *Materials and Methods* and Supplemental Figure S1A). At least 112 Ura$^+$ transformants were analyzed for each sample in each experiment. (A) SDS treatment increased the infectivity of [*PSI*$^+$] aggregates, as judged by the increase in the percentage of [*PSI*$^+$] colonies among Ura$^+$ transformants. Data from three independent experiments are shown. (B) [*PSI*$^+$] aggregates induced the same variant of [*PSI*$^+$], regardless of SDS treatment. Where indicated, 10% (vol/vol) of untreated aggregates from the opposite [*PSI*$^+$] variant was added to the transformation mixture. Data are presented as mean ± SD, n = 3.

Sup35 polymers (Shorter and Lindquist, 2006); and 3) destabilizes [*PSI*$^+$] variants with abnormally large aggregates (Borchsenius *et al*., 2001; Borchsenius *et al*., 2006). To explain these results, it was suggested (Allen *et al*., 2005; Song *et al*., 2005) that Ssa1/2 binds to the Sup35 prion and thus provides a steric hindrance to Hsp104, thereby inhibiting its shearing effect on Sup35 polymers, resulting in their increase in size. Here, we present support for this hypothesis by showing that [*PSI*$^+$] aggregates contain Sup35 and Ssa1/2 in an ~2:1 M ratio, and interact with Hsp104. The large amount of Ssa1/2 we found associated with Sup35 polymers makes it reasonable that Ssa1/2 could participate in prion propagation by shielding Sup35 polymers from the shearing activity of Hsp104, which seems not to require other components for this purpose, at least in vitro (Shorter and Lindquist, 2006, but see Inoue *et al*., 2004). Here, we provide the first evidence that Hsp104 indeed physically interacts with Sup35 in [*PSI*$^+$] lysates. We show that like Ssa1/2, Hsp104 interacts with Sup35 in the [*PSI*$^+$] form more efficiently than in the [*psi*$^-$] form. Hsp104 was previously shown to catalyze the conversion of preamyloid Sup35 oligomers into mature Sup35 prion polymers (Shorter and Lindquist, 2004). However, in our [*psi*$^-$] cells not overexpressing Sup35 the concentration of these oligomers is expected to be low, which may explain why we did not detect an interaction between Hsp104 and Sup35 in [*psi*$^-$].

Interaction between Sla2, a component of yeast cytoskeletal machinery (for review, see Toret and Drubin, 2006), with Sup35N(1-113) in a two-hybrid assay, or with immobilized Sup35N(1-251) in [*PSI*$^+$] lysate was shown previously (Ganusova *et al*., 2006). We now show that Sla2, in contrast to Ssa1/2, interacts with Sup35 from [*PSI*$^+$] and [*psi*$^-$] with similar efficiency. Because deletion of *SLA2* in [*PSI*$^+$] yeast is lethal, it was not possible to directly assay whether Sla2 participates in [*PSI*$^+$] induction in vivo (Ganusova *et al*., 2006). Our finding that Sla2 interacts with Sup35 in [*psi*$^-$] indicates that Sla2 may indeed play a role in [*PSI*$^+$] induction, which is yet to be clarified.

We also show that Ssb1/2, a yeast ribosome-associated chaperone that usually acts as a [*PSI*$^+$] antagonist (Chernoff *et al*., 1999; Kushnirov *et al*., 2000b; Chacinska *et al*., 2001; Allen *et al*., 2005, 2007), interacts with Sup35 equally in [*PSI*$^+$] and [*psi*$^-$] cells. This finding extends previous data showing a Sup35–Ssb1/2 interaction (Allen *et al*., 2005). Ssb1/2 could either recognize a site on Sup35 located outside the prion domain, or a motif within the prion domain regardless of the folding status of the domain. Regardless, our data suggest that the prion status of Sup35 does not significantly influence the Ssb1/2–Sup35 interaction.

The molecular composition of [*PSI*$^+$] aggregates has some similarity to the composition of the aggregates of another yeast prion, [*PIN*$^+$] (Sondheimer and Lindquist, 2000; Derkatch *et al*., 2001). Like [*PSI*$^+$], [*PIN*$^+$] aggregates are organized at two levels: SDS treatment of the aggregates dramatically reduces their weight and yields SDS-stable Rnq1-containing "subparticles" (probably, polymers of Rnq1) (Bagriantsev and Liebman, 2004). Rnq1 interacts with Ssa1/2 and its cochaperone Sis1 in [*PIN*$^+$], but not in [*pin*$^-$] cells. Although the stoichiometry of the Rnq1–Ssa1/2 interaction is unknown, Rnq1 and Sis1 are present in the aggregates in almost equimolar amounts (Sondheimer *et al*., 2001; Lopez *et al*., 2003). We detected Sis1 only among the minor components of [*PSI*$^+$] aggregates. Although we do not rule out the possibility that the difference in the abundance of Sis1 detected in association with [*PSI*$^+$] and [*PIN*$^+$] was due to dissimilarities in the experimental approaches, it seems likely that prion–chaperone interaction is specific and that it is defined by the prion-forming sequence and the adjacent region (e.g., the M domain of Sup35) of the protein. Changes in these sequences may trigger a functional recruitment of a specific chaperone. For example, overexpression of Sis1, which does not significantly affect propagation of a conventional [*PSI*$^+$], destabilizes the chimeric prion [*PSI*$^+_{ps}$] (Kryndushkin *et al*., 2002) formed in *S. cerevisiae* by a fusion of the C-terminal domain of *S. cerevisiae* Sup35 with the N-terminal prion domain of Sup35 from *Pichia methanolica* (Kushnirov *et al*., 2000a). Overexpression of Ssa1 cures [*URE3*], but it generally stabilizes [*PSI*$^+$] (Schwimmer and Masison, 2002). Overexpression of Hsp104 cures [*PSI*$^+$] (Chernoff *et al*., 1995), some variants of [*PSI*$^+_{ps}$] (Kushnirov *et al*., 2000a,b), but not [*URE3*] (Moriyama *et al*., 2000) or [*PIN*$^+$] (Derkatch *et al*., 1997). Thus, the presence of a chaperone in the prion aggregate is likely to reflect a specific functional interaction between the chaperone and the prion, rather than a nonspecific response of a protein quality control mechanism to the presence of a misfolded protein aggregate.

We show that an SDS-treated preparation of [*PSI*$^+$] aggregates isolated from yeast expressing a full-length Sup35 is infectious and that it induces variant-specific [*PSI*$^+$] when delivered to [*psi*$^-$] cells. This suggests that variant-specific [*PSI*$^+$] infection does not require the aggregates to be intact,

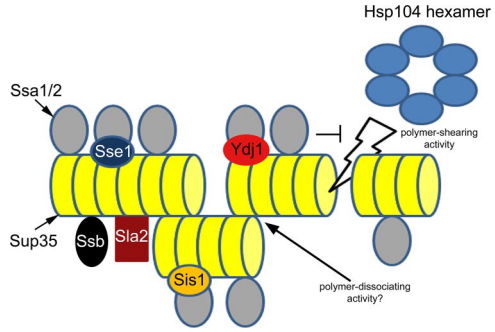

Figure 7. Model of the intracellular organization of [*PSI*$^+$] aggregates. [*PSI*$^+$] aggregates are proposed to be mainly composed of interacting infectious Sup35 polymers of various sizes, heavily decorated with Ssa1/2. The binding of Ssa1/2 to the polymers may be regulated via its cochaperones Sis1, Sse1, and Ydj1, which bind to Sup35 polymers independently or with Ssa1/2. Sup35 polymers also interact with Ssb1/2 and Sla2. Ssa1/2, which we find in an ~1:2 ratio with Sup35, is shown shielding Sup35 from Hsp104's shearing activity to explain the previous finding that excess Ssa1/2 in [*PSI*$^+$] causes an increase in Sup35 polymer size (Allen *et al*., 2005). The hypothetical polymer-dissociating activity, which separates individual polymers from each other, is indicated.

and infection is transmitted by individual Sup35 polymers within the aggregates. Although both SDS-treated and untreated aggregates were infectious, SDS treatment (followed by a dilution of SDS to 0.001% before contact with cells; see *Materials and Methods*) elevated the efficacy of the variant-specific [*PSI*$^+$] induction approximately twofold. The most plausible explanation of this observation is that each [*PSI*$^+$] aggregate contains several infectious Sup35 polymers, which were released after SDS treatment, thereby increasing the number of the infectious entities. This observation is supported by our electron microscopy analysis of a preparation of Sup35 aggregates, which revealed the presence of ~20-nm-wide barrel-shaped structures, as well as bundles, which seemed to be composed of these barrels. These structures are remarkably similar to the infectious polymers of Sup35 obtained in vitro in the presence of Hsp104 and GTP (i.e., in biologically relevant conditions; see figure 5E at 20′ in Shorter and Lindquist, 2006). As might be expected, because Sup35 in these in vitro experiments was polymerized for 8 h before the addition of Hsp104, the resulting Sup35 polymers looked longer and more branched, but otherwise similar to the Sup35 polymers we isolated directly from yeast. Our results suggest that Sup35 polymers interact in vivo and together with other proteins form [*PSI*$^+$] aggregates. This aspect is important, because in the literature there is no proper distinction between Sup35 polymers and aggregates. Here, we clarify this issue and emphasize the difference between Sup35 polymers and aggregates.

Based on the data presented here and obtained previously, we suggest a model where [*PSI*$^+$] aggregates are composed of interacting Sup35 polymers and other proteins, among which chaperones are the most abundant (Figure 7). This model hints at an intriguing possibility that [*PSI*$^+$] can be propagated not only by the frequently discussed cleavage of a single Sup35 polymer by Hsp104, but also via dissociation of separate polymers. Uncovering such activity will further elucidate the mechanism of prion propagation.

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