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Sequestration of essential proteins causes prion associated toxicity in yeast

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Summary

Prions are infectious, aggregated proteins that cause diseases in mammals but are not normally toxic in fungi. Excess Sup35p, an essential yeast protein that can exist as the $[PSI^+]$ prion, inhibits growth of $[PSI^+]$ but not $[psi^-]$ cells. This toxicity is rescued by expressing the Sup35Cp domain of Sup35p, which is sufficient for cell viability but not prion propagation. We now show that rescue requires Sup35Cp levels to be proportional to Sup35p overexpression. Overexpression of Sup35p appeared to cause pre-existing $[PSI^+]$ aggregates to coalesce into larger aggregates, but these were not toxic *per se* because they formed even when Sup35Cp rescued growth. Overexpression of Sup45p, but not other tested essential Sup35p binding partners caused rescue. Sup45-GFPp formed puncta that co-localized with large $[PSI^+]$ Sup35-RFPp aggregates in cells overexpressing Sup35p, and the frequency of the Sup45-GFPp puncta was reduced by rescuing levels of Sup35Cp. In contrast, $[PSI^+]$ toxicity caused by a high excess of the Sup35p prion domain (Sup35NMp) was rescued by a single copy of Sup35Cp, was not rescued by Sup45p overexpression and was not associated with the appearance of Sup45-GFPp puncta. This suggests $[PSI^+]$ toxicity caused by excess Sup35p versus Sup35NMp is respectively through sequestration/inactivation of Sup45p versus Sup35p.

Introduction

Protein misfolding and the presence of protein aggregates are associated with numerous neurodegenerative diseases (Ross and Poirier, 2004; Taylor *et al.*, 2002) that include aggregates of A β -42, mutant Huntingtin and PrP^{Sc} in Alzheimer's disease, Huntington's disease and prion diseases, respectively (Nussbaum and Ellis, 2003; Gil and Rego, 2008; Pruisner, 1982; Pruisner *et al.*, 1998). Since there is sometimes a poor correlation between the presence of aggregates and other markers of the disease, the role of the aggregate is now thought to be benign or even cytoprotective and toxicity has been attributed to a soluble oligomeric species (Haas and Selkoe, 2007; Arrasate *et al.*, 2004). Although not mutually exclusive, an alternate hypothesis is that the toxic species (either soluble or aggregated) causes death by interacting abnormally with or sequestering key factors in various pathways (Gil and Rego, 2008; Caughey and Baron, 2006).

Among these protein-misfolding diseases, prion diseases are unique as they are infectious. These diseases are associated with the accumulation of the prion form of the PrP protein, PrP^{Sc} (Pruisner, 1982). Prions were first discovered in mammals as the causative agent of some fatal neurodegenerative diseases, such as Scrapie in sheep and Creutzfeldt-Jakob disease in humans (Pruisner, 1998). PrP^{Sc} is in a β -sheet rich, aggregated, amyloid form that

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can attract normal monomers mainly in an α -helical soluble form, to join the amyloid aggregate in an autocatalytic process (Pan *et al.*, 1993). Interestingly, the same protein is able to cause different infectious forms (or strains) of the disease in inbred hosts with the same genotype (Bruce, 1996). These strains are distinguished by varying symptoms and are attributed to different structures of the amyloid form of PrP^{Sc} (Bessen *et al.*, 1995; Caughey, 2003).

Though prions were first discovered in mammals, the prion paradigm has been extended to yeast (Wickner *et al.*, 2004). The three best studied yeast prions [*PSI*⁺], [*PIN*⁺] and [*URE3*], are the prion forms of Sup35p, Rnq1p and Ure2p, respectively (Wickner, 1994; Sondheimer *et al.*, 2000; Derkatch *et al.*, 2001). Though none of these proteins are homologous to the mammalian prion protein PrP, they all share the properties of being β -sheet rich, aggregated, and infectious in their prion forms. In addition yeast prions also exist as different strains, called variants, that appear to differ in their amyloid conformations, which leads to phenotypic and biochemical variations (Derkatch *et al.*, 1996; Zhou *et al.*, 1999; Uptain *et al.*, 2001; Bradley *et al.*, 2002; Schlumpberger *et al.*, 2001; Tanaka *et al.*, 2004; King and Diaz-Avalos, 2004; Krishnan and Lindquist, 2005; Toyama *et al.*, 2007).

In this study we focus on the [*PSI*⁺] prion and its protein determinant Sup35p. Sup35p in its non-prion form associates with Sup45p to mediate translation termination (Stansfield *et al.*, 1995; Stansfield *et al.*, 1995). Sup35p and Sup45p, both encoded by essential genes, belong to the families of eukaryotic release factors, eRF3 and eRF1, respectively. Sup45p recognizes all three stop codons in the ribosome and facilitates the release of the nascent peptide (Bertram *et al.*, 2000; Song *et al.*, 2000), while Sup35p is the GTPase that promotes both these functions of Sup45p (Frolova *et al.*, 1996; Salas-Marco *et al.*, 2004; Alkalaeva *et al.*, 2006). The interaction between these two proteins mediates the release of newly formed peptide chains.

Sup35p can be divided into three domains. The C terminal globular domain associates with Sup45p, has a GTPase domain, and is necessary and sufficient for viability and the translation termination function (Ter-Avanesyan *et al.*, 1993). The N-terminal (N) and middle (M) domains are dispensable for viability but are necessary and sufficient for the induction and faithful maintenance of the prion variant state (Derkatch *et al.*, 1996; Patino *et al.*, 1996; Liu *et al.*, 2002; Bradley and Liebman, 2004). When Sup35p converts to its prion [*PSI*⁺] form, the activity of the C domain is compromised as most of the protein is in the aggregated form, leading to a decrease in translation termination efficiency (Patino *et al.*, 1996; Paushkin *et al.*, 1996; Cox, 1965). Variants of [*PSI*⁺] are distinguished by differences in their reduction in translation termination efficiency: strong [*PSI*⁺] variants have lower translation termination efficiency and more aggregated Sup35p compared to weak [*PSI*⁺] variants (Derkatch *et al.*, 1996; Zhou *et al.*, 1999; Uptain *et al.*, 2001).

[*PSI*⁺] can be induced by the overproduction of Sup35p or Sup35NMP. Presumably since there is more protein, there is a higher chance that some of the protein molecules will convert to the prion form, seeding the conversion of more monomers (Wickner, 1994; Derkatch *et al.*, 1996). Induction occurs efficiently only in the presence of other prions, such as [*PIN*⁺] ([*PSI*⁺] Inducible) (Derkatch *et al.*, 1997; 2001). This is explained by the cross-seeding model, which proposes that the [*PIN*⁺] prion behaves as an inefficient template to convert Sup35p into its prion form by a heterologous seeding mechanism (Derkatch *et al.*, 2004). [*PIN*⁺], like [*PSI*⁺], also exists as variants, which are characterized by different levels of aggregated Rnq1p and varying degrees of induction of [*PSI*⁺] (Bradley *et al.*, 2002).

Although neither the presence of $[PSI^+]$, nor the overexpression of Sup35p are toxic independently, it has been known for some time that overexpression of Sup35p (or to a lesser extent the *SUP35NM* domain) is growth inhibiting to $[PSI^+]$ yeast (Dagkesamanskaya *et al.*, 1991; Derkatch *et al.*, 1996). In cells lacking $[PSI^+]$, but that are $[PIN^+]$, increasing the level of Sup35p but not *SUP35NM* causes a growth defect (Derkatch *et al.*, 1996; Bradley *et al.*, 2002). However *SUP35NM* fused to green fluorescent protein (*GFP*) overexpressed in $[psi^-][PIN^+]$ cells leads to the formation of large ring and worm-like structures that are a precursor to $[PSI^+]$ and dramatically decreases the viability of just the cells with the ring/worm aggregates (Zhou *et al.*, 2001; Gausanova *et al.*, 2006). Apparently, only cells primed to form the $[PSI^+]$ prion are incompatible with overexpression of Sup35NM-GFPp.

The source of this lethality has been attributed to either the accumulation of Sup35p isoforms that might be toxic to the cell, or the depletion of essential factors (Derkatch *et al.*, 1996; 1998). A single copy of *SUP35C* or overexpression of *SUP45* or *SUP35C* relieves this inhibition, which suggests that these two essential factors might be sequestered (Derkatch *et al.*, 1998; Ter-Avanesyan *et al.*, 1993; Ter-Avanesyan *et al.*, 1994; Gausanova *et al.*, 2006). In this study we systematically examine the lethality caused by various levels of overexpression of *SUP35* and *SUP35NM* in different variants of $[PSI^+]$ and $[PIN^+]$. We show that at high levels of overexpressed Sup35NMp, the mechanism of $[PSI^+]$ mediated toxicity is through the loss of Sup35p function; whereas at high levels of Sup35p, the essential protein Sup45p is sequestered due to its association with Sup35p, and Sup35p activity is not limiting.

Results

Growth inhibition caused by overexpression of *SUP35* or its prion domain in $[PSI^+]$ yeast is alleviated by a proportional increase of Sup35Cp

We tested whether growth inhibition associated with various levels of overexpression of *SUP35* or its NM domains (*SUP35NM*), was due to sequestration of Sup35p itself. A wild-type strain or a Δ NM strain, containing only the chromosomally encoded C terminal domain of *SUP35*, were transformed with plasmids capable of overexpressing *SUP35* or *SUP35NM* at either a 10 fold (10X) or hundred fold (100X) level. When these two strains were mated to $[psi^-]$, weak and strong $[PSI^+]$ yeast, the $[psi^-]$ and weak $[PSI^+]$ diploids, homozygous for *SUP35* and with 10X *SUP35NM*, showed no growth defects while strong $[PSI^+]$ diploids were very sick (Figure 1A Panel A). This growth inhibition was alleviated in the cross with Δ NM (which contains one copy of *SUP35C*) (Figure 1A Panel B). When the level of *SUP35NM* was raised to 100X, even weak $[PSI^+]$ diploids showed a severe growth defect that was also relieved by one copy of *SUP35C* (Figure 1A Panels C, D). With 10X full length *SUP35*, strong $[PSI^+]$ diploids were inviable but could be rescued by one copy of *SUP35C* (Figure 1B Panels A, B). In contrast, the inviability caused by 100X *SUP35* in the presence of weak and strong $[PSI^+]$ was not rescued by one copy of Sup35Cp (Figure 1B Panels C and D).

Since one copy of *SUP35C* did not relieve toxicity caused by highly overexpressed *SUP35* in strains containing $[PSI^+]$, it suggests that at 100X *SUP35* either a Sup35p binding protein and not Sup35p itself is being sequestered, or a toxic species is formed at this level of overexpression. If a Sup35p binding partner is being sequestered, toxicity should be relieved by the simultaneous overexpression of *SUP35C*, as it does not join the $[PSI^+]$ aggregate. Sup35Cp would therefore compete for the sequestered protein, drawing it out of the prion aggregate. To test this, *GAL::SUP35C* plasmids were transformed into the Δ NM 100X *SUP35* strain and mated to $[psi^-]$, weak and strong $[PSI^+]$ yeast. Indeed, when *GAL::SUP35C* was expressed on a high copy plasmid (high level) the growth inhibition was

alleviated (Figure 1C Panel C). *GAL::SUP35C* expression on a centromeric plasmid (low level) was not enough to relieve the toxicity (Figure 1C Panel B).

To determine if the overexpression of *SUP35C* rescues growth inhibition by dissolving $[PSI^+]$ aggregates, lysates from $[PSI^+]$ and $[psi^-]$ diploids simultaneously overexpressing *SUP35* and *SUP35C* were subjected to high-speed centrifugation and analyzed on an SDS-PAGE. Since in $[psi^-]$ yeast, Sup35p can be found in the supernatant whereas in $[PSI^+]$ yeast Sup35p is absent from the supernatant fraction (Patino *et al.*, 1996; Paushkin *et al.*, 1996), we asked if simultaneous overexpression of Sup35Cp and Sup35p would cause Sup35p to appear in the supernatant in $[PSI^+]$ cells. However, Sup35p was not retained in the supernatant indicating that $[PSI^+]$ aggregates were not disrupted by Sup35Cp overexpression (Figure 1D).

***SUP35C* also rescues lethality caused by overexpression of *SUP35* in $[PIN^+]$ cells**

Growth inhibition was not observed in 10X *SUP35* $[PIN^+]$ diploids (data not shown). However as described previously (Bradley *et al.*, 2002) 100X *SUP35* caused inviability in high and very high $[PIN^+]$ diploids, although other $[PIN^+]$ variants and the $[pin^-]$ diploids were unaffected (Figure 2, Panel A). Expression of Sup35Cp at low levels did not relieve the toxicity, but at high levels of Sup35Cp, viability of high and very high $[PIN^+]$ diploids was rescued (Figure 2 Panels B, C). Lethality was observed only in cells overexpressing the full length Sup35p, not Sup35Nmp (Figure S1). This suggests that lethality is not due simply to the appearance of $[PSI^+]$, as overexpression of the *SUP35NM* domain leads to more induction of $[PSI^+]$ than the overexpression of the full-length protein (Derkatch *et al.*, 1996). Rather it suggests that toxicity is caused by the formation of $[PSI^+]$ aggregates by the overexpression of full length Sup35p that in turn sequesters an essential protein that normally binds to the C domain of Sup35p.

Sup35Cp alleviates growth inhibition of cells with large ring-like aggregates formed during the induction of $[PSI^+]$

During the induction of $[PSI^+]$ by overexpressed *SUP35NM-GFP* in $[PIN^+]$ cells, large ring and worm-like structures have been observed that are a hallmark of cells that grow into $[PSI^+]$ colonies (Figure 3A). These colonies have a markedly lower viability in comparison to their diffuse *SUP35NM-GFP* siblings (Zhou *et al.*, 2001; Ganusova *et al.*, 2006). We tested whether this reduced viability is due to the lack of Sup35Cp or one of its binding partners, by overexpressing *SUP35NM-GFP* in a high $[PIN^+]$ variant in the presence or absence of Sup35Cp. A single copy of Sup35Cp did not abolish the formation of these ring and worm-like structures (Figure 3A). Ring containing cells without Sup35Cp had low viability (20%) whereas the diffuse cells were much more viable (87%) (Figure 3B). Upon the addition of Sup35Cp, the viability of ring containing cells rose dramatically (73%).

Since cells with rings frequently give rise to $[PSI^+]$ colonies (Zhou *et al.*, 2001; Ganusova *et al.*, 2006) we determined whether Sup35Cp increased viability by decreasing the frequency of $[PSI^+]$ appearance. However, ring cells with Sup35Cp gave rise to a similar amount of $[PSI^+]$ colonies as ring cells without Sup35Cp (Figure 3B).

Overexpressed Sup35p appears to cause pre-existing $[PSI^+]$ aggregates to coalesce even in the presence of Sup35Cp

To determine whether Sup35Cp changes the morphology of pre-existing $[PSI^+]$ aggregates during overexpression of Sup35p, we took advantage of $[PSI^+]$ yeast containing an integrated copy of functional *SUP35-GFP* driven by a promoter repressed upon mating (*MFA::SUP35-GFP*) (Satpute-Krishnan *et al.*, 2005). $[PSI^+]$ is observed as numerous, tiny Sup35-GFP foci in this strain and since Sup35-GFP is not synthesized upon mating, it is

possible to monitor pre-existing $[PSI^+]$ aggregates in zygotes (Satpute-Krishnan *et al.*, 2005). 10X *SUP35* wild type and ΔNM strains were mated to the *MFA::SUP35-GFP* $[PSI^+]$ yeast and zygotes were monitored. As previously reported (Satpute-Krishnan *et al.*, 2005, Figure 2C), wild-type zygotes without overexpressed Sup35p retained the multitude of tiny foci (Figure 4A). In contrast, both wild type and ΔNM zygotes overexpressing Sup35p had large amorphous Sup35-GFP aggregates suggesting that pre-existing $[PSI^+]$ aggregates coalesce upon Sup35p overexpression (Figure 4A).

To determine if large aggregates are passed to daughter cells, we monitored the fate of zygotes with large Sup35-GFP aggregates. While 10X *SUP35/SUP35* zygotes (with *MFA::SUP35-GFP*) failed to divide, 10X *SUP35/ ΔNM* zygotes did give rise to daughter cells. The very large aggregates in these zygotes remained visible after several generations and were not passed on to daughter cells (Figure 4B). Though some tiny aggregates of $[PSI^+]$ are passed onto daughter cells where they are capable of forming aggregates larger than normal $[PSI^+]$ aggregates, none were comparable to the size of the initial large aggregates (Figure 4B).

We also monitored fluorescence in *SUP35-GFP/ ΔNM* diploids using endogenously tagged *SUP35-GFP* driven by the *SUP35* promoter (Satpute-Krishnan *et al.*, 2005) to enable us to observe $[PSI^+]$ aggregates in equilibrium with newly synthesized Sup35-GFP. Initial *SUP35-GFP/ ΔNM* zygotes overexpressing Sup35p also had large amorphous aggregates similar to zygotes with an extra copy of *MFA::SUP35-GFP* (data not shown). Daughters from such zygotes did contain some aggregates larger than normal $[PSI^+]$, though none were as large as in the initial zygote (Figure 4C) suggesting that very large aggregates are formed initially but are not inherited or formed in daughter cells.

To confirm the formation of larger $[PSI^+]$ aggregates biochemically, *SUP35-GFP* was transiently overexpressed in wild type $[PSI^+]$ yeast with untagged *SUP35*, in the presence or absence of Sup35Cp, and aggregates were analyzed by sucrose gradient centrifugation. In the absence of overexpression of *SUP35-GFP*, genomic Sup35p was primarily in the third fraction whereas upon the overexpression of *SUP35-GFP*, genomic Sup35p exhibited higher sedimentation with or without Sup35Cp, indicating that the average size of $[PSI^+]$ aggregates increases upon the overexpression of *SUP35-GFP* (Figure 4D).

***SUP45*, but not other Sup35p associating factors, relieves growth inhibition caused by overexpression of Sup35p but not Sup35NMp in $[PSI^+]$ cells**

To determine which Sup35p interacting protein might be sequestered, a strain containing $[PSI^+]$ capable of overexpressing *SUP35* was transformed with plasmids containing genes encoding essential proteins that interact with Sup35p. These genes included *NAB3*, *NRD1*, *PAB1*, *SPT15*, *ARP2*, *ACT1*, *SIS1* and *SUP45* (Gavin *et al.*, 2002; Cosson *et al.*, 2002; Sanders *et al.* 2002; Ganusova *et al.*, 2006; Bagriantsev *et al.*, 2008; Stansfield *et al.*, 1995). If Sup35p overexpression caused growth inhibition because Sup35p sequesters these essential proteins into the $[PSI^+]$ aggregate, then overexpression of these proteins should eliminate the growth inhibition. As previously reported (Derkatch *et al.*, 1998), Sup45p expressed on a high copy plasmid was able to rescue this inhibition (Figure 5A) whereas we now show that a lower expression of Sup45p on a centromeric vector is unable to rescue the growth inhibition (Figure 5A). All other genes failed to complement growth (data not shown).

As Sup45p was capable of rescuing the growth inhibition, three Sup45p interacting proteins were overexpressed to determine if they would restore growth. *TPA1*, *DBP5* and *MLC1* (Keeling *et al.*, 2006; Gross *et al.*, 2007; Valouev *et al.*, 2004) were chosen for their ability to interact with Sup45p and their involvement in translation termination or the ability to

complement a *SUP45* temperature sensitive mutation. However, none of these factors were able to rescue the growth inhibition (data not shown).

Since the growth inhibition was rescued by overexpression of Sup45p we asked if the toxicity was caused by a reduction in the level of Sup45p in the cell. Thus, we compared Sup45p levels in a [*PSI*⁺] strain transiently overexpressing Sup35-GFPp to [*PSI*⁺] cells that did not overexpress Sup35-GFPp (Figure S2). As the level of Sup45p is not reduced, toxicity is not caused by a decrease in Sup45p expression.

As overexpressed Sup45p rescued [*PSI*⁺] growth inhibition due to overexpressed Sup35p, we checked whether Sup45p could also rescue growth inhibition due to overexpressed Sup35NMp in [*PSI*⁺] strains. Wild-type strains simultaneously overexpressing *SUP35NM* at a 100X level and *SUP45* either on a low (low level) or high (high level) copy plasmid were crossed to [*psi*⁻], weak and strong [*PSI*⁺] strains. Weak and strong [*PSI*⁺] diploids did not show enhanced growth even in the presence of *SUP45* overexpression on a high copy plasmid (Figure 5B).

Sup45-GFPp co-aggregates with [*PSI*⁺] aggregates in the presence of *SUP35* but not *SUP35NM* overexpression

Since toxicity is not caused by reducing the absolute level of Sup45p in the cell, we asked if toxicity could be caused by inactivation of Sup45p via aggregation. Thus, we checked visually whether Sup45p exists as aggregates in the presence of [*PSI*⁺] and overexpressed *SUP35* or *SUP35NM*. A strain containing endogenously *GFP* tagged *SUP45* was transformed with plasmids overexpressing *SUP35*, *SUP35NM* or a control vector. These were mated to either [*psi*⁻] or [*PSI*⁺] yeast and Sup45-GFPp fluorescence was monitored. Approximately half the [*PSI*⁺] zygotes overexpressing *SUP35* showed bright Sup45-GFPp punctate dots whereas 98% of the [*psi*⁻] zygotes showed diffuse Sup45-GFPp (Figure 6A, Table 1, rows 1 and 2). [*PSI*⁺] diploids overexpressing *SUP35NM*, or not overexpressing either *SUP35* or *SUP35NM* (vector), also showed diffuse Sup45-GFPp (Table 1, rows 3 and 4).

Next we checked whether these Sup45-GFPp aggregates co-localized with coalesced [*PSI*⁺] aggregates. The *SUP45-GFP* strain overexpressing Sup35p was mated to a [*PSI*⁺] strain in which the endogenous Sup35 was tagged with *RFP* (kindly supplied by T. Serio. See Experimental Procedures) and fluorescence was monitored in the zygotes. Sup45-GFPp puncta were either completely co-localized (Figure 6B bottom), or partially overlapped with Sup35-RFPp puncta (Figure 6B top) suggesting that Sup45-GFPp is sequestered into Sup35-RFPp puncta.

As we hypothesized that Sup35Cp rescues [*PSI*⁺] mediated toxicity in the presence of high levels of Sup35p by drawing an essential protein out of the prion aggregate (Figure 1C) we asked if overexpressed Sup35Cp kept Sup45p out of the prion aggregate. The *SUP45-GFP* strain overexpressing Sup35p was mated to a [*PSI*⁺] strain overexpressing Sup35Cp and fluorescence was monitored. Indeed significantly fewer [*PSI*⁺] zygotes simultaneously overexpressing Sup35p and Sup35Cp had Sup45-GFPp puncta (~10%) (Table 1, row 5) compared to zygotes overexpressing only Sup35p (~55%).

Discussion

The presence of the [*PSI*⁺] or [*PIN*⁺] prion by themselves is not detrimental to yeast cells. However, the overexpression of *SUP35* in [*PSI*⁺] and [*PIN*⁺] cells leads to growth inhibition (Dagkesamanskaya *et al.*, 1991; Derkatch *et al.*, 1996). Two hypotheses have been proposed to explain the growth inhibition associated with overexpressed *SUP35* in [*PSI*⁺] and [*PIN*⁺]

cells (Derkatch *et al.*, 1996; 1998). One proposes that a toxic species of Sup35p is formed that is incompatible with the cell. It appears that this is the case in toxicity caused by overexpression of Rnq1p in $[PIN^+]$ cells, wherein a toxic oligomer is associated with cell death (Douglas *et al.*, 2008). The alternate, not mutually exclusive, hypothesis proposes that aggregated Sup35p sequesters, and thereby inactivates, an essential protein. Since Sup35Cp that does not join the $[PSI^+]$ aggregate, alleviates the growth inhibition, it seemed possible that Sup35p itself was the essential sequestered protein (Ter-Avanesyan *et al.*, 1993). Supporting this hypothesis, other groups and we have observed that one copy of *SUP35C* was enough to mitigate the growth inhibition of 10X *SUP35* in $[PSI^+]$ cells (Ter-Avanesyan *et al.*, 1994). However at higher levels of overexpressed Sup35p, the hypothesis was not supported by our finding that one copy of *SUP35C* is not enough to relieve the growth inhibition of 100X *SUP35* in $[PSI^+]$ cells. This is because if Sup35p were the essential factor being sequestered, even at a 100X level of overexpression one copy of *SUP35C* should be able to rescue growth inhibition, as Sup35Cp does not join the prior aggregate.

We show that at a 100X *SUP35* level, a proportional increase in Sup35Cp is able to rescue the growth defect. This suggests that at 100X *SUP35*, rather than Sup35p being sequestered, a Sup35p binding protein is sequestered into the $[PSI^+]$ aggregates. Because Sup35Cp competes with Sup35p for this binding partner, a proportional increase in Sup35Cp can alleviate the growth inhibition. Furthermore, weak $[PSI^+]$ variants, that have less aggregated Sup35p (Zhou *et al.*, 1999; Uptain *et al.*, 2001), are less growth inhibited indicating that the severity of growth inhibition decreases with an increase in soluble Sup35p.

Additionally, as growth inhibition in $[PIN^+]$ cells caused by 100X *SUP35* could be relieved not by one copy of *SUP35C*, but could be relieved by overexpression of Sup35Cp, it indicates that $[PIN^+]$ associated toxicity with overexpressed Sup35p also involves the loss of a Sup35p binding partner. Since growth inhibition occurs in very high $[PIN^+]$ but not in medium $[PIN^+]$, although very high $[PIN^+]$ has more soluble Rnq1p than medium $[PIN^+]$ (Bradley *et al.*, 2002), it appears that it is not the amount of aggregated Rnq1p but rather the Sup35p aggregate seeding potential that leads to toxicity. Since overexpression of Sup35Nmp did not cause growth inhibition in $[PIN^+]$ cells, although it leads to a higher frequency of $[PSI^+]$ induction than overexpression of full length Sup35p (Derkatch *et al.*, 1996), the formation of $[PSI^+]$ cannot be the sole cause of toxicity. Rather, a combination of the formation of $[PSI^+]$ and the ability of the aggregated, inducing fragment to directly sequester a Sup35Cp binding partner must lead to the growth defect.

To identify factors responsible for $[PSI^+]$ associated toxicity with overexpressed Sup35p, we overexpressed various essential genes whose products are known to associate with Sup35p. If overexpressed Sup35p were sequestering these factors into the $[PSI^+]$ aggregate, their overexpression should relieve the growth inhibition. Indeed, we have previously showed that excess Sup45p caused rescue (Derkatch *et al.*, 1998). While it is possible that factors that were not tested complement the growth defect, an overexpression screen (Tank and True, 2009, reported while this paper was under review) to identify factors that ameliorate the toxicity of excess Sup35p in $[PSI^+]$ cells revealed only Hsp104p (which cures cells of $[PSI^+]$; Chernoff *et al.*, 1995) and Sup45p. Also, we found that when factors known to associate with Sup35p and associated with a variety of processes from the actin-cytoskeleton machinery (*ACT1*) (Ganusova *et al.*, 2006) to RNA processing (*PAB1*) (Cosson *et al.*, 2002) were overexpressed, only *SUP45* was able to rescue the cells. Likewise, cells were not rescued by overexpression of a variety of proteins that associate with Sup45p, and are involved in translation termination (e.g. *DBP5*) (Gross *et al.*, 2007) that might have allowed Sup45p to remain soluble. This may reflect the fact that the association between Sup35p and Sup45p is stronger than other interactions, which would explain why only Sup35Cp and Sup45p were able to relieve to growth inhibition caused by overexpressed *SUP35*. Growth

inhibition caused by sequestration of Sup45p could be caused either by Sup45p monomers directly binding to the C terminal domain of Sup35p in the aggregates or by the transient formation of Sup35p:Sup45p complexes that then join the Sup35p aggregate via the Sup35p prion domain in the complex. In either case, we propose that Sup35Cp rescues growth inhibition due to overexpressed Sup35p by retaining Sup45p in the cytosol and that Sup45p overexpression rescues because there are then enough Sup45p molecules to carry on the function of translation termination.

We used microscopy to visualize [*PSI*⁺] aggregates when Sup35p is overexpressed and to show the accumulation of Sup45p in these aggregates. Previous work showed that when [*PSI*⁺] yeast with chromosomally tagged *SUP35-GFP* expressed under a promoter that is repressed upon mating, is crossed to an untagged [*psi*⁻] yeast strain, the approximate size of the *SUP35-GFP* aggregates is not altered in the zygotes. Furthermore, this was true even when prion fragmentation (Hsp104p) was inhibited in the zygote (see Figure 2C in Satpute *et al.*, 2005). In these zygotes, inhibition of fragmentation presumably causes the aggregates to enlarge, but this is invisible because the newly made Sup35p joining and enlarging the aggregates is untagged. Thus, we were surprised when our similar mating of a [*PSI*⁺] strain (with chromosomally tagged Sup35-GFPp expressed under a promoter that is repressed upon mating) to an untagged [*psi*⁻] strain that overexpressed untagged Sup35p, caused the immediate appearance of large fluorescent foci in the zygotes.

While it is expected that the large excess of aggregates that result from the Sup35p overexpression will overwhelm the fragmentation machinery, this by itself, is unlikely to explain the appearance of the large foci in the zygotes because, as described above, even inhibition of Hsp104p did not increase fluorescent foci size. Thus, we propose that when untagged Sup35p was overexpressed in *GFP* tagged [*PSI*⁺] zygotes, the overexpressed Sup35p “glues” the pre-existing fluorescent aggregates together into large, ill-defined foci. These large Sup35p aggregates are not toxic *per se*, as they are present even in the presence of Sup35Cp. However, only zygotes with Sup35Cp are able to propagate, suggesting Sup45p is sequestered in the [*PSI*⁺] aggregates. Indeed, we show that the essential protein Sup45p co-aggregates with these Sup35p foci and that Sup35Cp keeps some of the Sup45p out of the large aggregates.

The “gluing” of pre-existing [*PSI*⁺] aggregates might be due to the *SUP35NM* domain having a high glutamine/asparagine (Q/N) content, that has a high propensity to aggregate, a property shared by all known native yeast prions (DePace *et al.*, 1998; Osherovich *et al.*, 2004; Ross *et al.*, 2005). We propose that, initially, when [*PSI*⁺] aggregates and overexpressed Sup35p come together in the zygote, pre-existing aggregates coalesce and the prion shearing mechanism is overwhelmed, which leads to the formation of these large aggregates. In diploid cells continuously expressing chromosomally encoded *SUP35-GFP* and overexpressing untagged Sup35p, aggregates larger than normal [*PSI*⁺] aggregates are observed, although they are not as large as those in the zygote experiment. This suggests that either the prion shearing mechanism partially recovers in the diploid cultures, or that smaller aggregates are preferentially transferred to, and therefore propagated in, daughter cells.

One hypothesis explaining cell death in neurodegenerative diseases proposes that the aggregating proteins might interact aberrantly with several factors (Gil and Roge, 2008; Caughey and Baron, 2006). For example numerous transcription factors have been found to interact with the disease associated protein of Huntington’s disease. Overexpression of one such factor, Creb binding protein (CBP), showed relief of toxicity in cell culture suggesting that depletion of CBP function contributed to toxicity (Nucifora *et al.*, 2001; Jian *et al.*, 2006). Though Sup45p associates with Sup35p to perform the function of translation termination, the presence of Sup45p in [*PSI*⁺] aggregates is still unclear as one study found

Sup45p associated with $[PSI^+]$ aggregates whereas another did not (Paushkin *et al.*, 1997; Patino *et al.*, 1996). Though we do not see Sup45p in the $[PSI^+]$ pellet in wild type strains (data not shown), we do see Sup45-GFPp puncta co-localizing with $[PSI^+]$ aggregates in cells overexpressing Sup35p. We propose that upon overexpression of *SUP35*, Sup45p is abnormally sequestered resulting in cell death. Thus the association of Sup45p and Sup35p, which is required for translation termination, leads to toxicity during the overexpression of Sup35p in strains containing $[PSI^+]$.

In contrast to the toxicity caused by overexpression of the full-length Sup35p, toxicity due to overexpression of Sup35NMp in $[PSI^+]$ was not rescued by high levels of Sup45p. Furthermore Sup45-GFPp formed puncta only in $[PSI^+]$ cells overexpressing Sup35p but not Sup35NMp, suggesting overexpression of Sup35NMp does not sequester Sup45p. Rather, we propose, that overexpressed Sup35NMp forms an aggregate that captures a good deal of full-length Sup35p, whose depletion leads to growth inhibition. Similarly, during the *de novo* formation of $[PSI^+]$ by overexpression of *SUP35NM-GFP* in $[PIN^+]$ yeast, cells with large ring and worm-like *SUP35NM-GFP* aggregates are less viable (Zhou *et al.*, 2001; Ganusova *et al.*, 2006) but we find they can be rescued by Sup35Cp. Since these ring-like aggregates are a hallmark of newly appearing $[PSI^+]$, we propose that the large *SUP35NM-GFP* ring-like structures interact with and sequester Sup35p causing the observed toxicity.

Although Sup35p is in an aggregated form in $[PSI^+]$ cells, there must be enough functional Sup35p and Sup45p to carry on the essential function of translational termination. Thus, in normal $[PSI^+]$ cells there is a fine balance between the $[PSI^+]$ aggregates and functional translation termination complexes. Our results suggest that in $[PSI^+]$ cells highly overexpressing Sup35p or Sup35NMp, sequestration of Sup45p or Sup35p respectively into $[PSI^+]$ aggregates leads to growth inhibition.

Experimental procedures

Plasmids

SUP35 and *SUP35NM* overexpression plasmids (p743 and p749 respectively) are in a pEMBL vector under their own promoters (Ter-Avanesyan *et al.*, 1993). These 2 μ plasmids contain both the *URA3* and *LEU2-d* markers. While these plasmids are normally maintained at 10–20 copies per cell in *ura3 leu2* hosts, on media lacking uracil (SD-Ura), they can be amplified to around 100 copies by selecting on SD-Leu since the *LEU2-d* promoter is defective (Derkatch *et al.*, 1996).

GAL::SUP35 in pRS403 (Sikorski *et al.*, 1989) is an integrative vector with a *HIS3* marker (p1104). This was created by cutting the galactose inducible promoter (using *XhoI* and *BamHI*) from a pRS316 (Sikorski *et al.*, 1989) vector containing the promoter and ligating it into pRS403 (also cut with *XhoI* and *BamHI*) (Sikorski *et al.*, 1989). *SUP35* was cut from pVK71 (Derkatch *et al.*, 1996) (using *XbaI* and *SmaI*) and inserted into pRS403 containing the galactose inducible promoter.

p1160 is a centromeric plasmid with a *URA3* marker with *SUP35C* under its own promoter and p750 is a pEMBL vector with *SUP35C* under its own promoter (Ter-Avanesyan *et al.*, 1993). p1594 is a centromeric p*GAL::SUP35C* vector that was created by amplifying *SUP35C* by PCR and ligating it into a pRS414 (*TRP1* marker) (Sikorski *et al.*, 1989) vector containing a galactose inducible promoter. Expression from this plasmid is defined as a low level of Sup35Cp overexpression. *GAL::SUP35C* in pRS424 (p1598) is a 2 μ vector with a *TRP1* marker and was created by cutting p1594 with *XhoI* and *NotI* (removing the galactose promoter and *SUP35C*) and ligated at those sites in pRS424. Expression from this plasmid is defined as high level of Sup35Cp overexpression.

p1182 is a centromeric plasmid with a *LEU2* marker that contains Sup35NM-GFPp under the copper inducible promoter (Zhou *et al.*, 2001). To induce Sup35NM-GFPp, transformants were grown overnight in SD-Leu and reinoculated into copper containing media for 24–48 hours. To create pGAL::*SUP35-GFP*, the *SUP35-GFP* fusion was cut from pCup1::*SUP35-GFP* (p1083) (Zhou *et al.*, 2001) using *Bam*HI and *Sac*I and ligated into a pRS413 (Sikorski *et al.*, 1989) centromeric vector (*HIS3* marker) containing a galactose inducible promoter, cut with the same restriction sites.

Plasmids containing *SUP35* and *SUP45* interacting genes were kind gifts from various people: *PAB1* (pKF142, *URA3* 2 μ) from Curt Wittenberg; *ARP3* (pDW8, *URA3* CEN) from Rong Li; *SPT15* (M4480, *LEU2* 2 μ) from David Stillman; *NAB3* (425-NAB3, *LEU2* 2 μ) from Karen Arndt; *NRD1* (316-NRD1, *URA3* CEN) from David Brow; *ACT1* (pRB1454, *URA3* CEN) from Dan Burke; *TPA1* (pDB1005, *TRP1* CEN) from David Bedwell; *DBP5* (pCS833, *LEU2* 2 μ) from Charles Cole; *MLC1* (pRS290, *TRP1*, 2 μ) from Trisha Davis; pRS316Gal-*SSB1* from Yury Chernoff.

SUP45 with its promoter was cut from Yep13-*SUP45* (p757) (Derkatch *et al.*, 1998) using *Xho*I, *Bam*HI, blunt ended and ligated into either pRS314 or pRS324 (cut with *Bam*HI and blunt ended) to create *SUP45* with its promoter in a centromeric vector (p1672) or a 2 μ vector (p1673) with a *TRP1* marker. Expression from p1672 and p1673 are defined as low and high level respectively.

Yeast strains and Media

The following yeast strains are derivatives of 74-D694 (*MATa ade1-14 leu2-3,112 his3- Δ 200 trp1-289 ura3-52*) (Chernoff *et al.*, 1993): [*psi*⁻] (L1751); weak [*PSI*⁺] (L1759); strong [*PSI*⁺] (L1763) (Derkatch *et al.*, 1997); low [*PIN*⁺] (L1943); medium [*PIN*⁺] (L1945); high [*PIN*⁺] (L1749), very high [*PIN*⁺] (L1953) (Derkatch *et al.*, 2000; Bradley *et al.*, 2002). Wild type (L3059) and Δ NM (L3058) strains are derivatives of SL1010-1A (*MATa ade1-14 met8-1 leu2-1 his5-2 trp1-1 ura3-52*) (Zhou *et al.*, 1999). [*PSI*⁺] and [*psi*⁻] yeast with endogenous *SUP35* tagged with *GFP* (*MATa*) and strains containing an additional copy of *GFP* tagged *SUP35* under the *MFA* promoter (*MATa*) were kind gifts from Tricia Serio (Satpute-Krishnan and Serio, 2005). Unlike previous C terminal fusions of *GFP* to *SUP35* that are not functional, *SUP35* in these strains is tagged with *GFP* between the N and M domains and remains functional. *MATa* versions of the *MATa* strains were obtained by mating to an isogenic 74-D694 strain. [*PSI*⁺] yeast with endogenous *SUP35* similarly tagged with *RFP* (*MATa*) was also a kind gift from Tricia Serio (SY831). YBR143C (*MATa*) is a strain containing an endogenously tagged *SUP45-GFP* from Invitrogen. To create zygotes with this strain *MATa* versions of [*psi*⁻] (L1754: *MATa ade1-14 leu2-3,112 ura3-52 trp1-289 lys9- Δ 21*) (Derkatch *et al.*, 1997) and strong [*PSI*⁺] (L2717: made by cytoducing [*PSI*⁺] into L1754) were used. Mating type of YBR143C was switched to *MATa* by overexpressing the *HO* gene.

To make [*psi*⁻] (L3014) and strong [*PSI*⁺] (L3013) yeast with an integrated *GAL*::*SUP35*, an integrative plasmid *GAL*::*SUP35* in pRS403 (p1104, *HIS3*) was cut at the unique *Stu*I site in *SUP35*, transformed into L1751 ([*psi*⁻]) and L1763 ([*PSI*⁺]) and selected on SD-His.

Standard yeast media and cultivation procedures were used (Sherman *et al.*, 1986). Transformants were grown in media selective for the plasmids e.g. synthetic dextrose lacking tryptophan (SD-Trp). Diploids of 74-D694 and SL1010-1A were selected on SD-His with the appropriate selection for the various plasmids. For 10X overexpression of *SUP35* or *SUP35NM*, diploids were selected on SD-Ura-His and replica plated to SD-Ura-His-Leu to increase overexpression to 100X. Complex glucose media (YPD) was used to distinguish color of yeast strains.

To express *SUP35C* under the galactose inducible promoter, the media contained 2% raffinose along with 2% galactose.

To monitor fluorescence in zygotes, strains were mated on YPD for 2.5–4 hrs and fluorescence was observed using a Zeiss AxioScope2 with a 63X lens.

Analysis of viability and [*PSI*⁺] phenotype of ring bearing cells

To determine the viability of cells with the Sup35p rings, a high [*PIN*⁺] variant transformed with a copper inducible Sup35NM-GFPp plasmid (p1182), with or without *SUP35C*, was grown in plasmid selective media overnight and induced with 50μM copper for 24–48 hours. Individual cells with rings or diffuse Sup35NM-GFPp were micromanipulated onto agar patches while observing them under a fluorescence microscope at 20X. Patches were transferred to a YPD plate and colonies were grown and assayed for the [*PSI*⁺] phenotype.

To monitor the [*PSI*⁺] or [*psi*[−]] state of 74-D694 strains, the level of read through of the premature stop codon in the *ade1-14* allele was used (Chernoff *et al.*, 1995). [*PSI*⁺] cells read through the premature stop codon, as there is less functional Sup35p. Thus, they are able to grow on SD-Ade and are white (strong [*PSI*⁺]) or pink (weak [*PSI*⁺]) on YPD. [*psi*[−]] cells on the other hand, terminate efficiently at the stop-codon, and are thus unable to grow on SD-Ade and are red on YPD. White and pink colonies that grew on YPD from the micromanipulated cells were confirmed to be [*PSI*⁺] by mating to a [*psi*[−]][*pin*[−]] tester strain (L2174) bearing the copper inducible Sup35NM-GFPp plasmid. [*PSI*⁺] cells have Sup35NM-GFPp foci where as [*psi*[−]] cells show diffuse Sup35NM-GFPp fluorescence.

Preparation of yeast cell lysates and protein analysis

Crude cell extracts were prepared using glass beads (Biospec, 0.5 mm) in 750 μl of lysis buffer containing 50mM Tris/HCl, pH 7.5, 50 mM KCl, 10 mM MgCl₂ and 5% (w/v) glycerol supplemented with a protease inhibitor cocktail (P8215, Sigma) and 5 mM PMSF. Cells were lysed by vortexing (Vortex-Genie 2) at high speed, 3 times for 2 minutes with cooling on ice for 1 minute between each vortexing at 4°C. Crude lysates were precleared by centrifuging two times at 600g for 2 minutes at 4°C to remove unlysed cells and cell debris.

For high-speed centrifugation analysis, 500–750 μg of crude lysate in 250 μl was spun at 100,000g for 30 min at 4°C in a Sorvall TLA100.1 rotor. The supernatant was aspirated and run on a 10% Tris-HCl gel. Sup35p was detected using the monoclonal βe4 Sup35p antibody.

Sucrose gradient centrifugation was performed essentially as described previously (Bagriantsev *et al.*, 2004). Briefly, lysates were spun in a continuous 20–60% gradient in a swinging bucket rotor at 10,600 g for 40 minutes at 4°C. Fractions were diluted 1:2 in lysis buffer, resolved on a 10% acrylimide gel and transferred onto an immunoblot polyvinylidene difluoride membrane (Bio-Rad). This was then probed using the Sup35Cp β e-4 monoclonal antibody. Signal was detected with a Tropix kit (Applied Biosystems) using the manufacturer's specifications.

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Figure 1. *SUP35C* rescues growth inhibition caused by overexpression of *SUP35* and *SUP35NM* in [*PSI*⁺] cells

A. *Sup35Cp* rescues growth inhibition caused by overexpressed *SUP35NM* in [*PSI*⁺] strains. Diploids selected from different mating reactions with continued overexpression of *SUP35NM*: Wild type (w.t.) or a *SUP35C* containing strain (ΔNM) overexpressing *SUP35NM* ten fold (10X) (Panels A and B) or a hundred fold (100X) (Panels C and D) crossed to [*psi*⁻], weak or strong [*PSI*⁺] variants. The color variation on synthetic media is inconsistent and cannot be interpreted accurately.

B. *Sup35Cp* rescues growth inhibition caused by 10X but not 100X *SUP35* in [*PSI*⁺] strains. Diploids selected from different mating reactions with continued overexpression of *SUP35*:

Wild type (w.t.) or a Δ NM strain overexpressing full length *SUP35* 10X (Panels A and B) or 100X (Panels C and D) crossed to [*psi*⁻], weak or strong [*PSI*⁺] variants.

C. Overexpression of Sup35Cp rescues growth inhibition caused by 100X *SUP35* in [*PSI*⁺] strains. Diploids selected from different mating reactions with continued overexpression of 100X *SUP35*: Δ NM overexpressing full length 100X *SUP35* with either expression of a control vector (Panel A), a low level of *SUP35C* (Panel B) or a high level of *SUP35C* (Panel C) crossed to [*psi*⁻], weak or strong [*PSI*⁺] variants. Diploids were selected to maintain overexpression of vector or *SUP35C*

D. Overexpressed Sup35Cp does not dissolve [*PSI*⁺] aggregates. Lysates from [*PSI*⁺] or [*psi*⁻] *SUP35*/ Δ NM diploids, simultaneously overexpressing full length *SUP35* and *SUP35C*, subjected to high-speed centrifugation, resolved on an acrylamide gel and probed with an *anti-SUP35C* antibody. T: total lysate; S: supernatant after high-speed centrifugation P: Pellet after high-speed centrifugation.

Figure 2. *SUP35C* rescues growth inhibition caused by overexpression of 100X *SUP35* in cells with *[PIN⁺]* variants

Diploids selected from different mating reactions with continued overexpression of 100X *SUP35*. A ΔNM strain overexpressing full length 100X *SUP35* with expression of either a control vector (Panel A), a low level of *SUP35C* (Panel B) or a high level of *SUP35C* (Panel C) crossed to either a *[pin⁻]*, high, very high, low or medium *[PIN⁺]* variant.

Figure 3. *SUP35C* rescues the toxicity of cells with *SUP35NM-GFP* “rings” without inhibiting the formation of [PSI⁺]

A. *SUP35C* does not inhibit the formation of *NM-GFP* rings in a high [*PIN*⁺] variant. A high [*PIN*⁺] variant overexpressing *SUP35NM-GFP* under a copper inducible promoter, showing a ring-like structure, in the absence (vector) or presence of *SUP35C*. *SUP35C* is expressed on a centromeric plasmid under its own promoter. Arrow points to cells with a ring.

B. *SUP35C* increases the viability of [*PIN*⁺] cells with *NM-GFP* rings without decreasing *de novo* formation of [PSI⁺]. Viability and frequency of formation of [PSI⁺] in cells with diffuse *SUP35NM-GFP* and cells with *SUP35NM-GFP* “rings” in the presence or absence (vector) of *SUP35C*. (Total number of colonies tested per category: Minimum 80, Number of independent transformants: at least 4 per category). Presence of [PSI⁺] was determined by colony color and *SUP35NM-GFP* fluorescence (see Experimental procedures). Numbers indicate average percentages and error bars show standard error.

Figure 4. Pre-existing [PSI⁺] aggregates appear to coalesce in the presence of overexpressed Sup35p

A. Sup35Cp does not inhibit the formation of large Sup35-GFPp aggregates upon overexpression of Sup35p in [PSI⁺] cells. Zygotes from a strain containing [PSI⁺] and SUP35-GFP under the MFA promoter (MFA::SUP35-GFP, that is repressed upon mating) (*MATa*) crossed to a wild type strain overexpressing a control vector or SUP35, and Δ NM overexpressing SUP35 (*MATa*). The size of Sup35-GFPp aggregates in wild-type zygotes overexpressing Sup35p is not reproducibly larger than in the Δ NM zygote overexpressing Sup35p.

B. Large Sup35-GFPp aggregates formed in [PSI⁺] zygotes overexpressing Sup35p are not passed on to daughter cells. Zygote from a mating between a Δ NM strain overexpressing 10X SUP35 and strain containing [PSI⁺] and MFA::SUP35-GFP observed at different time points (Number of zygotes = 6). The growing zygote was placed on media that retained the plasmid overexpressing SUP35. The arrow points to a large aggregate in the original zygote. The arrowhead shows the formation of a new aggregate, larger than a normal [PSI⁺] aggregate. Due to decay of signal, images at 7 and 9 hours were brightened using photoshop to observe aggregates at those time points.

C. Large aggregates of Sup35-GFPp are formed in [PSI⁺] diploids with overexpressed Sup35p. SUP35-GFP/ Δ NM diploids overexpressing either a control vector or SUP35. Arrows point to enlarged [PSI⁺] aggregates.

D. Biochemical verification of formation of large Sup35-GFPp aggregates upon overexpression of Sup35p in [PSI⁺] cells, in the presence and absence of Sup35Cp. Non functional SUP35-GFP was transiently (2 hours) overexpressed or not overexpressed in a strain containing [PSI⁺] with a control vector or SUP35C, and lysates were subjected to sucrose gradient centrifugation, resolved on an acrylamide gel and probed with anti-

SUP35C antibody. The arrowheads point to the fraction in which genomic Sup35p is most abundant.

A

B.

Figure 5. Overexpressed SUP45 rescues growth inhibition caused by overexpressed SUP35 but not SUP35NM in [PSI⁺] cells

A. Overexpressed SUP45 rescues growth inhibition caused by overexpressed SUP35 in [PSI⁺] cells. Serial dilutions are of a strain containing strong [PSI⁺] with an integrated GAL::SUP35 transformed with a control vector, or SUP45 on a low copy or a high copy plasmid spotted on plasmid selective media where SUP35 is not (SD-Trp) or is (SGal-Trp) overexpressed.

B. Overexpressed SUP45 does not rescue growth inhibition caused by overexpressed SUP35NM in [PSI⁺] cells. Diploids were selected from different mating reactions with continued overexpression of 100X SUP35NM and selection for maintenance of vectors and overexpression of SUP45: a wild-type strain overexpressing 100X SUP35NM with either expression of a control vector (Panel A), a low level of SUP45 (Panel B) or a high level of SUP45 (Panel C) crossed to [psi⁻], weak or strong [PSI⁺] variants.

Figure 6. Sup45-GFPp is sequestered into [*PSI*⁺] aggregates in cells overexpressing *SUP35*

A. Sup45-GFPp forms detectable foci in [*PSI*⁺] cells overexpressing *SUP35*. A strain with a genomic copy of *SUP45-GFP*, overexpressing 10X *SUP35* (*MATα*) was mated to either a strain containing [*PSI*⁺] or [*psi*⁻] yeast (*MATα*) and fluorescence was monitored in zygotes. Two representative zygotes for the [*PSI*⁺] mating are shown.

B. Sup45-GFP aggregates co-localize with [*PSI*⁺] aggregates. A strain with a genomic copy of *SUP45-GFP*, overexpressing 10X *SUP35* (*MATα*) was mated to a [*PSI*⁺] strain (*MATα*) with a genomic copy of *SUP35* tagged with *RFP* and fluorescence was monitored in zygotes.

Table 1

Percentage of zygotes with punctate Sup45-GFP dots

Crosses			
Parent 1	X	Parent 2	% of zygotes with Sup45-GFP puncta
<i>SUP45-GFP</i> , 10X <i>SUP35</i>	X	[<i>PSI</i> ⁺]	55 ± 8.3
<i>SUP45-GFP</i> , 10X <i>SUP35</i>	X	[<i>psi</i> ⁻]	2.2 ± 0.44
<i>SUP45-GFP</i> , 100X <i>SUP35NM</i>	X	[<i>PSI</i> ⁺]	<1%
<i>SUP45-GFP</i> , 10X Vector	X	[<i>PSI</i> ⁺]	0
<i>SUP45-GFP</i> , 10X <i>SUP35</i>	X	[<i>PSI</i> ⁺], ↑ Sup35Cp	9.3 ± 1.47

Yeast strains with the indicated genotypes were mated for ~four hours and zygotes with Sup45-GFP puncta were counted using a fluorescence microscope. *SUP35C* was expressed from a high copy plasmid and overexpressed on SD-Leu (See Experimental Procedures). At least 5 independent mating reactions were performed and 100–150 zygotes were counted for each reaction. Standard deviation is shown.