Overexpression of the SUP45 gene encoding a Sup35p-binding protein inhibits the induction of the de novo appearance of the [PSI+] prion

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ABSTRACT [PSI+], a non-Mendelian element found in some strains of Saccharomyces cerevisiae, is presumed to be the manifestation of a self-propagating prion conformation of eRF3 (Sup35p). Translation termination factor eRF3 enhances the activity of release factor eRF1 (Sup45p). As predicted by the prion model, overproduction of Sup35p induces the de novo appearance of [PSI+]. However, another non-Mendelian determinant, [PIN+], is required for this induction. We now show that SUP45 overexpression inhibits the induction of [PSI+] by Sup35p overproduction in [PIN+] strains, but has no effect on the propagation of [PSI+] or on the [PIN] status of the cells. We also show that SUP45 overexpression counteracts the growth inhibition usually associated with overexpression of SUP35 in [PSI+] strains. We argue that excess Sup45p inhibits [PSI+] seed formation. Because Sup45p complexes with Sup35p, we hypothesize that excess Sup45p may sequester Sup35p, thereby reducing the opportunity for Sup35p conformational flips and/or self-interactions leading to prion formation. This in vitro yeast result is reminiscent of the in vitro finding by investigators of Alzheimer disease that apolipoprotein E inhibits amyloid nucleation, but does not reduce seeded growth of amyloid.

The [PSI+] factor is a non-Mendelian element present in some strains of Saccharomyces cerevisiae, which causes weak translational nonsense suppression and increases the efficiency of certain codon-specific translational suppressors (1–6). Despite intense study, [PSI+] was never linked to any extrachromosomal DNA or RNA (for review see ref. 5). Recently, Wickner (7) used the prion model, elaborated by the investigators of mammalian spongiform encephalopathies (8, 9), to explain [PSI+] phenomenon as well as the nature of [URE3], another yeast non-Mendelian element. Wickner proposed that [PSI+] is the manifestation of a self-propagating conformation of Sup35p, and numerous recent experiments continue to support this hypothesis. For reviews see refs. 10–17.

Sup35p belongs to a eukaryotic family of proteins with a C-terminal region that is homologous to the full-length proteins of the elongation factor EF-Tu/EF-1α family (18–24). The Sup35p homolog from Xenopus laevis was identified as the eukaryotic translation termination factor eRF3 (24) and shown to be a complex of ribosomes containing guanosine triphosphate (25). Sup35p probably performs the same function in yeast, because sup35 mutations cause the readthrough of stop codons (26–28) and the accumulation of ribosomes with bound peptidyl-tRNAs (29). In addition, X. laevis eRF3 complements the temperature sensitivity caused by a sup35 mutation in yeast (24). Genetic (26, 30–32) and biochemical (33, 34) data indicate that Sup35p interacts with Sup45p, the apparent yeast translation termination factor eRF1 (35–38). Simultaneous overexpression of SUP35 and SUP45 reduces readthrough of stop codons in nonsense alleles (antisuppression), presumably because of increased termination factor activity (33).

According to the prion model only [PSI+] but not [psi−], cells contain Sup35p in the prion (Sup35PSI+) conformation. Indeed, [PSI+] cells can be distinguished by the presence of aggregated and proteinase K-resistant Sup35p (39, 40). Protein molecules in the Sup35ΨPSI+ conformations are presumed to self-propagate by converting newly synthesized Sup35p molecules into the prion conformation. Recent in vitro observations (41, 42) support this prediction. Furthermore, the N-terminal region of Sup35p is sufficient for the biogenesis of [PSI+], suggesting that the [PSI+] prion determinant is located in the Sup35p N terminus (40, 41, 43–45).

One model (46) for prion propagation is that the normal and prion isoforms of the protein form a heterodimer, and that this interaction causes the normal isoform to take on the prion conformation. The newly created prion homodimer then dissociates and dimerizes with another normal protein molecule, and aggregation of prion molecules is a secondary process. An alternate model (47) hypothesizes that a seed composed of prion subunits induces normal protein molecules to join the prion aggregate and to change into the prion form upon binding. Some models predict that the conversion from a nonprion to a prion conformation proceeds through a metastable or partially unfolded intermediate (for reviews see refs. 12 and 48–52).

The de novo (sporadic) appearance of prions is proposed to occur through either spontaneous folding of a nonprion molecule into the prion shape or through the chance interaction of two nonprion protein molecules. Either process is presumed to be autocatalytic, but both are likely to depend on factors that affect the conformational liability of proteins and, if the seeded polymerization model is correct, the stability of prion aggregates. Indeed, a certain level of the chaperone protein Hsp104, known to facilitate protein conformational changes and the dissolution of protein aggregates formed during heat shock (11, 53), is required for successful [PSI+] propagation and the formation of Sup35ΨPSI+ aggregates (39, 40, 54).

Also, recent evidence suggests the existence of a non-Mendelian element, [PIN+], which is responsible for the ability of yeast [psi−] strains to be induced to the [PSI+] state by Sup35p overproduction. [PIN+] can be eliminated by incubation on media containing guanidine hydrochloride (GuHCl) or by transient Hsp104 inactivation (55). Unlike [PSI+], [PIN+] is not located in the N-terminal region of Sup35p and may either be a self-propagating determinant in the C-proximal part of Sup35p, or a prion domain in another protein that facilitates Sup35p conformational changes (55).

The finding that the frequency of the [PSI+] de novo appearance increases dramatically when Sup35p is overpro-

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duced (44, 56) supports the prediction of the prion model that either nonsporont folding of Sup35p into the Sup35p\textsuperscript{PSI}+ conformation or prion formation resulting from the interaction of two nonsporont Sup35p molecules would be more probable when the protein is present in excess. The induction of [PSI'] factors characterized by various efficiencies of suppression and mitotic stabilities in the same yeast strain suggests the existence of several Sup35p\textsuperscript{PSI}+ conformations (44).

Here we investigate the effect of SUP45 overexpression on the induction of the de novo appearance of [PSI'] by Sup35p overproduction. We find that an excess of SUP45 inhibits the phenotypes associated with excess Sup35p in both [PSI'] and [PSI'] strain derivatives: de novo induction of [PSI'] and growth inhibition, respectively. Although excess Sup35p inhibits [PSI'] seed formation, it has no effect on the propagation of [PSI'] and does not alter the [PIN] status of the cells. Because Sup35p complexes with Sup35p (33, 34), we hypothesize that excess Sup45p may sequester Sup35p, reducing the correlation (66). We previously have verified modulation of [PSI'] results of the same 4.9-kb [PSI']14–74-D694 derivatives of [PIN]74-D694 and are characterized by different efficiencies of suppression of the ade1–14 mutation (44). ade1–14 is efficiently suppressed in [PSI']1–74-D694, [PSI']7–74-D694, [PSI']8–74-D694, [PSI']15–74-D694, and [PSI']19–74-D694 derivatives (strong [PSI']), whereas its suppression in [PSI']13–74-D694, [PSI']14–74-D694, and [PSI']21–74-D694 derivatives is poor (weak [PSI']). [PSI'] derivatives, which cannot be induced to [PSI'] by Sup35p overproduction, were obtained by incubation of [PSI'] derivatives on medium containing 5 mM GuHCl (55).

Plasmids. Yep13-SUP45 and pJDB207-SUP45 are moderate- and high-copy number vectors, respectively, which contain the same 4.9-kb BamHI-HindIII insert bearing a functional SUP45 fragment under the original promoter cloned into Yep13 and pJDB207, respectively (58, 59). Yep13 (60) and pJDB207 (61) carry the LEU2 and leu2-d selective markers, respectively. The high copy number (up to 100 copies) of leu2-d-bearing vectors in cells grown on leucineless media is because of a promoter mutation that causes poor expression of this allele (62). pUKC802 is a Yep24-based moderate copy number vector (63) with a URA3 selective marker and the complete SUP45 gene within a 4.5-kb SalI–XhoI insert (64). pGAL::SUP35 (44) is a Yep50-based centromeric URA3 vector (65) that contains the SUP35 coding region under the control of the inducible CYC1–GAL1 (GAL) promoter. Plasmid pHCA/GAL4(1–93).ER.VP16 (66) contains HIS3 and a constitutively expressed fusion of the human estrogen receptor hormone-binding domain, the yeast GAL4 DNA-binding domain, and the VP16 viral transcriptional activator. When β-estradiol is added to the media, the fusion protein activates the GAL promoter in proportion to the β-estradiol concentration (66). We previously have verified modulation of GAL::SUP35 expression in 74-D694 by using this system (44).

Methods and Cultivation Procedures. Standard yeast media and cultivation procedures were used (67). Unless specifically mentioned, yeast were grown in organic complete medium (YPD). Transformants were grown in media selective for plasmid maintenance, e.g., SC-Ura and SC-Leu. Cotransformants were grown in media selective for maintenance of all the plasmids introduced, e.g., SC-Ura-Leu and SC-Ura-Leu,His. To eliminate plasmids bearing URA3, SC medium containing 1 mg/ml 5-fluoroorotic acid (+5-FOA; ref. 68) was used. The GAL promoter was induced on either synthetic complete medium containing 20 mg/ml galactose as a single carbon source (SGal) or, in the presence of pHCA/GAL4(1–93).ER.VP16, on synthetic complete glucose media containing 10, 20, 50, 100, or 200 nM β-estradiol.

Because ade1 mutations cause adenine auxotrophy and the accumulation of a red pigment, suppression of the ade1–14 nonsense mutation was estimated from growth at 20°C and 30°C on adenineless synthetic complete media, containing glucose or ethanol (2%) as a single carbon source (SC-Ad and SE-Ad, respectively), as well as from a color test on YPD. The better the growth on adenineless media and the lighter the color on YPD, the higher the efficiency of suppression. Tests for the induction of [PSI'] de novo appearance were performed essentially as described (44, 54). To analyze growth inhibition caused by SUP35 overexpression in [PSI'] derivatives with normal and increased SUP45 expression, yeast were cotransformed with pGAL::SUP35 or YCp50, and Yep13-SUP45 or Yep13. Transformants were grown in SC-Ura, where GAL::SUP35 was repressed, and 10-fold serial dilutions were spotted to media where GAL::SUP35 was induced (SGal-Ura-Leu) or repressed (SC-Ura-Leu).

To analyze the Pin phenotype (i.e., inducibility to [PSI']) following transient SUP45 overexpression, 74-D694 derivatives were transformed with Yep13-SUP45, pJDB207-SUP45, and control vectors. Following two replica platings on SC-Leu to allow for SUP45 overexpression, transformants were replica-plated twice on YPD to allow for plasmid loss and were then colony purified on YPD. Plasmidless Leu+ colonies were detected by replica plating. The [PIN] status of these Leu+ derivatives was determined by crossing them to [pin/+] or [PIN/] derivatives of tester strain 64-D697 transformed with pGAL::SUP35 or YCp50. Because [PIN+] is dominant (55), and the crosses of [pin/] derivatives of 74-D694 and 64-D697 result in [pin/] diploids (unpublished observations), the [PIN] status of the Leu+74-D694 derivatives determined the [PIN] status of diploids made with the [pin/] tester. [PSI'] induction in the latter diploids was analyzed on SC-Ad following transient overexpression of Sup35p on galactose medium. Alternatively, the effect of transient SUP45 overexpression on the Pin phenotype was tested by transforming yeast with pUKC802 (Yep24 in control experiments). Following two replica platings on SC-Ura, transformants were replica-plated twice on YPD, then twice on +5-FOA to select for plasmidless Ura− cells, and then on YPD medium containing the single carbon source, glycerol, to reduce the proportion of Pet− cells. The loss of plasmids was confirmed by replica plating to SC-Ura. All of the Foa− Pet+ progeny of each pUKC802 or Yep24 transformant grown on YPG plates was washed off and used as an inoculum for transformation with pGAL::SUP35 to allow for analysis of [PSI'] induction.

Transformations, DNA and RNA isolations, Northern blot hybridizations, and protein extractions were as described (44). Western blot analysis was according to Hulet et al. (69). The amount of protein loaded was normalized with L3 ribosomal protein and Coomassie brilliant blue staining. Antibodies against L3p and amino acids 137–151 of Sup35p (40) were gifts of J. Warner (Albert Einstein College of Medicine) and M. Patino and S. Lindquist (University of Chicago).

RESULTS

Efficiency of [PSI'] de Novo Induction by Sup35p Overproduction Is Reduced in SUP45 Overexpressing Transformants. Comparison of [PSI'] de novo induction in cells expressing different levels of SUP35 and SUP45. Because Sup35p and Sup45p interact in vivo (33, 34) we wanted to test whether SUP45 overexpression would affect the frequency of [PSI'] induction. To obtain different levels of SUP45 overexpression we used the moderate-
and high-copy number plasmids, YEpl3-SUP45 and pJDB207-SUP45, respectively (59). To transiently overexpress the SUP35 gene at different levels, pGAL::SUP35 was used. Although growth in galactose-containing media is sufficient to cause an approximately 6-fold GAL::SUP35 induction, the pHCA/GAL4(1–93)-ER,VP16/β-estradiol induction system (66) is required to obtain higher levels of SUP35 overexpression, up to a 100-fold (see ref. 44 and Materials and Methods). Northern and Western blot analyses were used to demonstrate that the presence of the high-copy SUP45-bearing plasmid does not reduce the levels of SUP35-encoded message (not shown) or protein (Fig. 1) when GAL::SUP35 is induced.

To induce SUP35 overexpression, cotransformants were spotted on synthetic media containing either galactose (SGal-Ura,Leu,His) or glucose plus different concentrations of β-estradiol (SC-Ura,Leu,His+β-estradiol). Two days later, the cultures were replica-plated to SC-Ade and SEt-Ade, where SUP35 was no longer overproduced and where growth required suppression of the ade1-14 nonsense mutation and was indicative of the de novo appearance of [PSI+]. We observed that such growth on adenineless media following Sup35p overproduction was slightly or severely reduced when SUP45 was amplified on a moderate- or a high-copy number plasmid, respectively, and galactose was used for GAL::SUP35 transient induction (Fig. 2A).

When the levels of SUP35 overexpression were increased by using the pHCA/GAL4(1–93)-ER,VP16/β-estradiol system, [PSI+] induction could be observed, although at reduced levels, even in the presence of the high-copy pJDB207-SUP45 plasmid (Fig. 2B). Moreover, the higher the β-estradiol concentrations used to induce GAL::SUP35 expression, the greater the fraction of cells that could suppress ade1-14 following the induction. Whereas the presence of pJDB207-SUP45 reduced the number of colonies observed on SC-Ade following [PSI+] induction, the large size of these colonies suggests that there was no reduction in suppression efficiency in those colonies that did become [PSI+]. The LEU2 marker, indicative of the presence of pJDB207-SUP45, was generally retained.

Although a correlation between the efficiency of [PSI+] induction and the levels of Sup35p overexpression was observed previously (44), the current data suggest that excess Sup35p interferes with the ability of excess Sup35p to induce [PSI+]. Alternatively, these results could also be explained if SUP45 overexpression reduced the efficiency of [PSI+]–associated suppression in major types of [PSI+]-variants or caused loss of [PSI+] or death of [PSI+]–containing cells. Below, we exclude these latter possibilities by using a collection of [PSI+]–derivatives with different phenotypes previously induced in the same strain by Sup35p overproduction (44).

SUP45 overexpression increases the efficiency of suppression caused by weak [PSI+]–variants and does not decrease the efficiency of suppression caused by strong [PSI+]–variants. Three weakly and five strongly suppressing 74-D694 [PSI+] derivatives were transformed with moderate- and high-copy SUP45-containing plasmids, YEpl3-SUP45 and pJDB207-SUP45, respectively. SUP45 overexpression in transformants with the high-copy number plasmid significantly increased the efficiency of suppression of ade1-14 in each of the weak [PSI+]–variants tested (Fig. 3). Because SUP45 overexpression didn’t cause detectable suppression of ade1-14 in a [psi+] 74-D694 derivative on either SC-Ade or SEt-Ade, the observed increase of suppression in [PSI+]–strains is because of an enhancement of the [PSI+]–suppressor phenotype (allosuppression). The allosuppressor effect of the moderate-copy number plasmid, YEpl3-SUP45, was only observed in one of the weak [PSI+]–derivatives, [PSI+]21-D694, and was a weak effect (data not shown).

Likewise, SUP45 overexpression did not decrease the level of nonsense suppression in the 74-D694 derivatives containing

<ref>Fig. 1. The presence of a high-copy SUP45-containing plasmid does not reduce the level of Sup35p overexpression. [PSI+]–growth of 74-D694 cotransformants bearing pGAL::SUP35 and pJDB207-SUP45, or control vectors YCp50 and pJDB207, respectively, were grown in galactose medium to induce GAL::SUP35. –, presence of control vectors; +, presence of SUP35– and SUP45-bearing plasmids. Arrows indicate positions of Sup35p and L3p (loading control).</ref>

<ref>Fig. 2. Induction of the de novo appearance of [PSI+] in [psi+]–[PIN+] 74-D694 transformants with SUP35– and SUP45–bearing plasmids. (A) Spots show the growth of cotransformants with the plasmid pairs indicated on galactose (SGal-Ura,Leu,His) and on glucose (SC-Ura,Leu,His) media where GAL::SUP35 is induced or repressed, respectively, and on repressing SC-Ade medium for suppression analysis. Arrows indicate replica plating. Growth on SC-Ade following the induction of GAL::SUP35 is indicative of [PSI+]. Growth of transformants bearing the pJDB207 control vector (not shown) was essentially the same as the growth of YEpl3-bearing transformants. (B) Spots show the growth of cotransformants on the media listed. Growth on SC-Ade in lanes A–F follows replica plating from SC-Ura,Leu,His containing 0, 10, 20, 50, 100, and 200 nM β-estradiol, respectively. +, Presence of the SUP45– and SUP35–bearing plasmids pJDB207-SUP45 and pGAL::SUP35, respectively; –, presence of control vectors not bearing SUP45 or SUP35, pJDB207 and YCp50, respectively. Plasmid pHCA/GAL4(1–93)-ER,VP16 was also present in all cotransformants. pJDB207-SUP45 caused a growth reduction on adenineless media following induction of the GAL::SUP35 construct by galactose regardless of the presence of the pHCA/GAL4(1–93)-ER,VP16 plasmid (data not shown).</ref>
SUP45 overexpression increases the efficiency of suppression of ade1–14 caused by weak [PSI+] variants and does not decrease the efficiency of suppression caused by strong [PSI+] variants. Spots show the growth of transformants of [PSI+] [21–74–D694 (weak [PSI+]), [PSI+]7–74–D694 (strong [PSI+]), and [psi–][PIN–]74–D694 (psi–)] with the indicated plasmids on the media listed.

SUP45 Overexpression Reverses the Growth Inhibition Caused by Sup35p Overproduction in [PSI+] Derivatives. Growth of strong and weak [PSI+] derivatives of 74–D694, bearing pGAL::SUP35, was analyzed on GAL::SUP35-inducing medium in the presence and absence of the moderate-copy SUP45-containing plasmid. In both [PSI+] derivatives tested, growth was better when SUP35 and SUP45 were overexpressed simultaneously, than when SUP35 was overexpressed alone (Fig. 4). This shows that growth inhibition caused by excess Sup35p, rather than being enhanced, is reversed by excess SUP45p.

Does Transient SUP45 Overexpression Affect the Ability of [psi–] Derivatives to Be Induced to the [PSI+] State? SUP45 overexpression does not convert [PIN+] derivatives into [pin–] derivatives. To test whether transient SUP45 overexpression permanently reduces or eliminates the ability of [psi–] derivatives to be induced to [PSI+] derivatives, plasmids were selected against and plasmidless Ura– cells were transformed with pGAL::SUP35 to directly test for the Pin phenotype. The data (see below) show that transient SUP45 overexpression does not affect the ability of yeast strains to become [PSI+], because most of the progeny retained the Pin+ phenotype following SUP45 overexpression. Some derivatives with an exaggerated or reduced Pin+ phenotype were observed in the mitotic progeny of transformants with either SUP45-containing plasmid or with control vector. Because the Pin+ phenotype has been shown to be mitotically stable (ref. 55 and unpublished results), we attribute the appearance of such derivatives to the effects of the transformation procedure. Cases where transformants with the SUP45-containing or control vector predominantly gave rise to progeny with reduced Pin+ phenotypes probably reflect modifications of the Pin+ phenotype that occur as a result of this first transformation. Cases where derivatives differ in the expressivity of their Pin phenotype from most of the sibling progeny of a given pUKC802 and YEp24 transformant must reflect later events.

In the mitotic progeny of 9 of the 12 independent pUKC802 transformants tested, [PSI+] could be induced in 337 of 339 Pet+ pGAL::SUP35 transformants. The efficiency of [PSI+] induction was approximately the same as prior to SUP45 overexpression in 326 of these pGAL::SUP35 transformants and was increased or reduced, in 6 and 5 transformants, respectively. Most derivatives characterized by increased or reduced efficiency of [PSI+] induction appeared independently because they were found in the progeny of different pUKC802 transformants. The two Pin− derivatives were from the same pUKC802 transformant and thus may not have been independent. Mitotic progeny of the remaining three pUKC802 transformants tested were also Pin+; however, the Pin− phenotype was slightly or significantly reduced in 109 of 114 pGAL::SUP35 transformants. Twelve YEp24 transformants were involved in the control experiment. [PSI+] could be induced by Sup35p overproduction in all the progeny from 10 of 12 YEp24 transformants with usually the same and occasionally reduced or increased efficiency (128, 8, and 9 pGAL::SUP35 transformants, respectively). In the mitotic progeny of two other YEp24 transformants, the Pin− phenotype was generally slightly reduced (19 of 24 pGAL::SUP35 transformants).

SUP45 overexpression does not convert [pin–] derivatives into [PIN+]. To test whether transient SUP45 overexpression permanently restores the ability of [pin–] derivatives to be induced to [PSI+], [psi–][PIN+][74–D694] was transformed with YEp13–SUP45, pJDB207–SUP45, or control vectors. Following transient SUP45 overexpression, the Pin phenotype was analyzed in diploids made with a [pin–] tester. All resulting diploids were inducible to the [PSI+] state (Pin+). Forty-six, 20, 21, and 9 plasmidless derivatives from 12, 6, 8, and 4 YEp13–SUP45, YEp13, pJDB207–SUP45, and pJDB207 transformants were analyzed, respectively. These results indicate that transient SUP45 overexpression does not cause cells to lose the ability to complement the previously described recessive [pin–].

To exclude the possibility that overexpression of SUP45 does induce a recessive Pin− phenotype, but that this phenotype is not caused by the loss of the bona fide [PIN+] element, [psi–][PIN+] [74–D694] was transformed with pUKC802 or YEp24. Following a period of selective maintenance to allow for SUP45 overexpression, plasmids were selected against and plasmidless Ura− cells were transformed with pGAL::SUP35 to directly test for the Pin phenotype. The data (see below) show that transient SUP45 overexpression does not affect the ability of yeast strains to become [PSI+], because most of the progeny retained the Pin+ phenotype following SUP45 overexpression. Some derivatives with an exaggerated or reduced Pin+ phenotype were observed in the mitotic progeny of transformants with either SUP45-containing plasmid or with control vector. Because the Pin+ phenotype has been shown to be mitotically stable (ref. 55 and unpublished results), we attribute the appearance of such derivatives to the effects of the transformation procedure. Cases where transformants with the SUP45-containing or control vector predominantly gave rise to progeny with reduced Pin+ phenotypes probably reflect modifications of the Pin+ phenotype that occur as a result of this first transformation. Cases where derivatives differ in the expressivity of their Pin phenotype from most of the sibling progeny of a given pUKC802 and YEp24 transformant must reflect later events.
Our finding that excess Sup45p rescues the lethality of excess Sup35p in [PSI+] strains can be explained by the model proposed by Paushkin et al. (34) that Sup35p overproduction causes growth inhibition because too much Sup45p is sequestered in [PSI+] aggregates. However, because Sup45p is reported to be in [PSI+] aggregates in some (34) but not the other (40) studies, this model is unproven. Excess of Sup45p might also overcome a loss of termination activity caused by sequestration of a protein(s) other than Sup45p. Indeed, the fact that the mammalian analogs of Sup45p can alone promote translation translation in vitro (35, 74), and that overexpression of human eRF1 alone has an antisuppressor effect in human cells (75), can be interpreted to mean that eRF1 (Sup45p) is the major component of the translational termination machinery possessing intrinsic polypeptide chain release activity that is only improved by other factors (75). The presence of such factors can be less crucial when Sup45p is in excess.

The fact that Sup45p overexpression in [PSI+] strains has an allo-suppressor rather than an antisuppressor effect is not consistent with the Sup45p sequestration hypothesis (34). We attribute [PSI+] -associated suppression to the lack of functional Sup35p and propose that Sup45p overexpression might increase the level of translational readthrough by further unbalancing the translational termination machinery.

Because Sup45p overdose inhibits [PSI+] induction but not [PSI+] propagation or stability, it must uniquely affect the step of [PSI+] seed formation. The same step is apparently affected by [PIN+], another factor involved in [PSI+] biogenesis. [PIN+] is a non-Mendelian element that determines whether [PSI+] can be induced de novo by the overproduction of Sup35p (55). The molecular basis of the [PIN+] factor is unknown and could involve a prion form of a general molecular chaperone, a prion protein that exclusively affects Sup35p conformational liability, or a new Sup35p prion variant distinct from [PSI+] and determined by the conformation of a region in the C-proximal part of Sup35p (55). Transient SUP45 overexpression did not cause any detectable loss or induction of [PIN+]. Thus, excess Sup45p and Sup45p/Sup35p binding are unlikely to induce a permanent conformational change in either Sup35p or Sup45p affecting the [PIN] status of the cell. Furthermore, the [PIN+] determinant is unlikely to be a prion form of Sup45p, because in that case an excess of Sup45p would be expected (12) to induce [PIN+].

The finding that SUP45 overexpression does not inhibit the propagation of existing [PSI+] is consistent with the report (34) that Sup35p domains capable of binding Sup45p are not located in the N-terminal [PSI+] domain, because this means that Sup35p bound to Sup45p is presumably still able to join existing [PSI+] aggregates via the N-terminal domain. In contrast, de novo formation of [PSI+] seeds may require a rare spontaneous Sup35p conformational switch or Sup35p/Sup35p intermolecular interactions. Either process would be more efficient when Sup35p is in excess. However, the situation apparently changes when the excess in Sup35p is balanced by an excess in Sup45p. If seed formation is preceded by a conformational change in a Sup35p molecule, Sup45p might inhibit this event by stabilizing the Sup35p+[psi] -conformation. Alternatively, Sup45p binding may inhibit Sup35p from interacting with other soluble Sup35p molecules, thereby inhibiting seed formation.

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