Proc. Natl. Acad. Sci. USA
Vol. 95, pp. 2400–2405, March 1998
Genetics

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

IRINA L. DERKATCH, MICHAEL E. BRADLEY, AND SUSAN W. LIEBMAN*

Laboratory for Molecular Biology, Department of Biological Sciences, University of Illinois at Chicago, Chicago, IL 60607

Communicated by Emanuel Margoliash, University of Illinois, Chicago, IL, December 16, 1997 (received for review August 19, 1997)

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 vivo* 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 intensive 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 variable N-terminal regions and conserved C-proximal regions that are 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 translational termination factor eRF3 (24) and was shown to be an eRF1- and ribosome-dependent guanosine triphosphatase (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 (Sup35^{Psi+}) conformation. Indeed, *[PSI$^+$]* cells can be distinguished by the presence of aggregated and proteinase K-resistant Sup35p (39, 40). Protein molecules in the Sup35^{Psi+} 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 Sup35^{Psi+} 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-

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

*To whom reprint requests should be addressed at: University of Illinois at Chicago, Laboratory for Molecular Biology, Molecular Biology Research Facility, 900 South Ashland Avenue, Chicago, IL 60607. e-mail: suel@uic.edu.

duced (44, 56) supports the prediction of the prion model that either spontaneous folding of Sup35p into the $Sup35p^{Psi+}$ conformation or prion formation resulting from the interaction of two nonprion 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 $Sup35^{Psi+}$ 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 Sup45p 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 Sup45p inhibits *[PSI$^+$]* seed formation, it has no effect on the propagation of *[PSI$^+$]* and does not alter the *[PIN]* status of the cells. Because Sup45p complexes with Sup35p (33, 34), we hypothesize that excess Sup45p may sequester Sup35p, reducing the opportunity for Sup35p conformational flips and/or self-interactions leading to prion formation. This *in vivo* result is reminiscent of the *in vitro* finding by Alzheimer disease investigators that apolipoprotein E inhibits amyloid nucleation, but does not reduce seeded growth of amyloid (57).

MATERIALS AND METHODS

Strains. *[PSI$^+$]*, *[psi$^-$][PIN$^+$]* and *[psi$^-$][pin$^-$]* derivatives of the yeast strains 74-D694, *MAT***a** *ade1–14*(UGA) *trp1–289*(UAG) *his3-*Δ*200 leu2–3,112 ura3–52* and 64-D697, *MAT*α *ade1–14*(UGA) *trp1–289*(UAG) *lys9-A21(UAA) leu2–3,112 ura3–52* were used. *[PSI$^+$]* derivatives were obtained by overproducing wild-type Sup35p in [psi$^-$][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$^-$][pin$^-$]* derivatives, which cannot be induced to *[PSI$^+$]* by Sup35p overproduction, were obtained by incubation of [psi$^-$][PIN$^+$] 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 *Bam*HI–*Hin*dIII 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 *Sal*I–*Xho*I insert (64).

pGAL::SUP35 (44) is a YCp50-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-fluoroortic 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-Ade and SEt-Ade, 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,Leu 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., inducibilty 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$^-$]* and *[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-Ade 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 +5FOA to select for plasmidless Ura$^-$ cells, and then on YPG 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 Hulett *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, YEp13-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 Sup35p 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. 2*A*).

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. 2*B*). 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 Sup45p 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, YEp13-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, YEp13-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

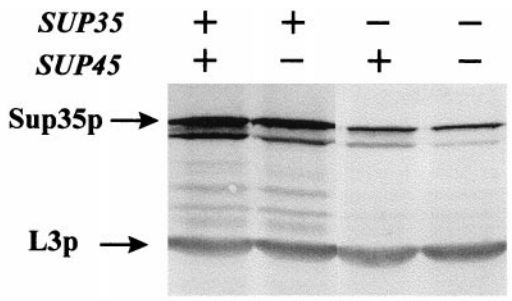

FIG. 1. The presence of a high-copy *SUP45*-containing plasmid does not reduce the level of Sup35p overproduction. [psi$^-$][PIN$^+$]74-D694 cotransformants bearing pGAL::SUP35 and pJDB207-SUP45, or control vectors YCp50 and pJDB207, respectively, were grown in galactose medium to induce *GAL*::*SUP35*. −, presence of control vectors; +, presence of *SUP35*- and *SUP45*-bearing plasmids. Arrows indicate positions of Sup35p and L3p (loading control).

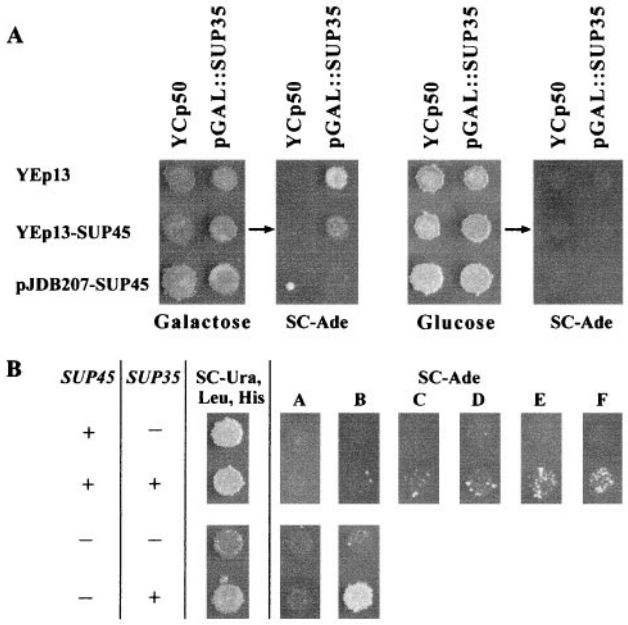

FIG. 2. Induction of the *de novo* appearance of *[PSI$^+$]* in [psi$^-$][PIN$^+$]74-D694 transformants with *SUP35*- and *SUP45*-bearing plasmids. (*A*) Spots show the growth of cotransformants with the plasmid pairs indicated on galactose (SGal-Ura,Leu,His) and on glucose (SC-Ura,Leu,His) media where *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 YEp13-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 severe 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).

†The same correlation between the β-estradiol concentration used to induce *GAL*::*SUP35* and the efficiency of *[PSI$^+$]* induction was observed in pGAL::SUP35, pHCA/GAL4(1–93).ER.VP16 transformants also bearing YEp13-SUP45 or YEp13, but differences were hard to score because of the overall high level of suppression of *ade1–14*. The inhibitory effect of moderate *SUP45* overexpression on the efficiency of *[PSI$^+$]* induction was weak at all levels of *SUP35* overexpression tested (data not shown).

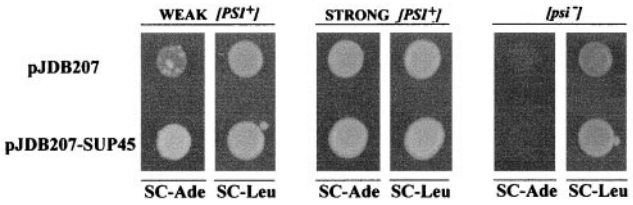

FIG. 3. *SUP45* overexpression increases the efficiency of suppression of *ade1–14* caused by weak *[PSI$^+$]* variants and does not decrease the efficiency of suppression caused by strong *[PSI$^+$]* variants. Spots show the growth of transformants of [PSI$^+$]21–74-D694 (weak *[PSI$^+$]*), [PSI$^+$]7–74-D694 (strong *[PSI$^+$]*), and [psi$^-$][PIN$^+$]74-D694 (*[psi$^-$]*) with the indicated plasmids on the media listed.

strong *[PSI$^+$]* variants (Fig. 3). Indeed, weak allosuppression was observed in pJDB207-SUP45 transformants after the first 2 days of incubation on adenineless media. However, the high level of *ade1–14* suppression in derivatives containing strong *[PSI$^+$]* variants complicated allosuppression scoring.

[PSI$^+$] Stability Is Not Reduced by Excess Sup45p. *[PSI$^+$]* stability was analyzed in the transformants described above on YPD, where the appearance of red sectors or colonies indicates the loss of *[PSI$^+$]*. When derivatives containing strong *[PSI$^+$]* variants were transformed with *SUP45*-bearing plasmids they remained white, and no red sectors indicative of *[PSI$^+$]* elimination were observed. Derivatives containing weak *[PSI$^+$]* variants are less stable mitotically (44); however, their stability was the same whether *SUP45* was overexpressed or not. About 4,000 colonies, with and without excess *SUP45*, were examined for each type of *[PSI$^+$]* variant (data not shown).

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.

The results described in this section show that *SUP45* overexpression indeed interferes with the ability of excess Sup35p to induce *[PSI$^+$]*.

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$^-$].* To test whether transient *SUP45* overexpression permanently reduces or eliminates the ability of *[psi$^-$]* derivatives to be induced

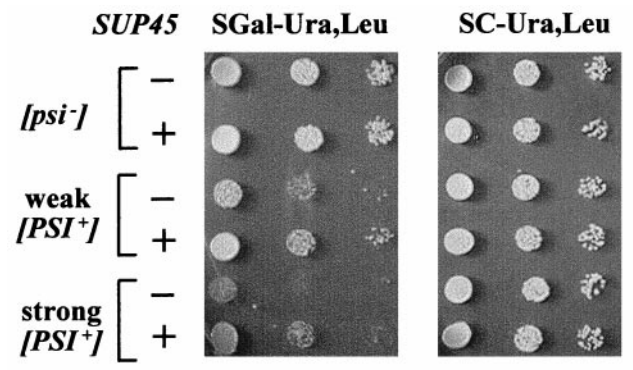

FIG. 4. *SUP45* overexpression reverses the growth inhibition caused by Sup35p overproduction in *[PSI$^+$]*-containing strains. Spots of 10-fold serial dilutions show growth of cotransformants of [psi$^-$][PIN$^+$]74-D694 (*[psi$^-$]*), [PSI$^+$]21–74-D694 (weak *[PSI$^+$]*), and [PSI$^+$]8–74-D694 (strong *[PSI$^+$]*) bearing pGAL::SUP35 and either YEp13-SUP45 (+) or YEp13 (−) on the media listed.

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.

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$^-$]64-D697 was transformed with pUKC802 and four transformants were selected and incubated on SC-Ura for approximately 14 cell generations to allow for *SUP45* overexpression. Plasmidless Ura$^-$ cells were then selected and transformed with pGAL::SUP35 to directly test for the Pin phenotype. Because *[PSI$^+$]* could not be induced in any of the 593 pGAL::SUP35 transformants tested, we concluded that transient *SUP45* overexpression does not efficiently convert *[pin$^-$]* derivatives into *[PIN$^+$]*.

To test whether simultaneous *SUP45* and *SUP35* overexpression would allow for *[PSI$^+$]* induction in genotypically *[pin$^-$]* cells, [psi$^-$][pin$^-$]74-D694 was cotransformed with

pGAL::SUP35 or YCp50 and YEp13-SUP45 or YEp13. To allow for *SUP35* and/or *SUP45* overexpression, transformants were replica-plated twice on SGal-Ura,Leu or, if plasmid pHCA/GAL4(1–93).ER.VP16 was also present, on SC plus different concentrations of β-estradiol. Transformants were then replica-plated to SC-Ade and SEt-Ade to score for *[PSI$^+$]* induction. No *ade1–14* suppression indicative of *[PSI$^+$]* was observed. Thus, *SUP45* overexpression does not affect the *[PIN]* status of *[psi$^-$]* derivatives.

DISCUSSION

Prions are generally viewed as self-propagating conformational protein variants that interact with and direct other molecules with the same amino acid sequence to fold into their prion conformation. Recent experimental data strongly supporting this view include the demonstration of *in vitro* and *in situ* conversion of PrP^C into a proteinase K-resistant conformation in the presence of PrP^{Sc} (70, 71) and the *in vitro* demonstration that protein extracts from *[PSI$^+$]* yeast strains stimulate Sup35p conversion into an aggregated proteinase K-resistant form in *[psi$^-$]* extracts and induce fiber formation by purified Sup35p (41, 42). The macromolecular PrP^{Sc} and $Sup35p^{Psi+}$ aggregates in these and similar studies are suggested to be the exclusive carriers of such *in vitro* converting activity (41, 72). Furthermore, when in concentrated solutions, Sup35p or its N-terminal fragment form fibers *de novo* even in the absence of other yeast proteins or $Sup35p^{Psi+}$ seeds (42, 45). However, although these *in vitro* reactions appear to reflect the *in vivo* genesis and propagation of prions, it remains likely that both the genesis and propagation of prions *in vivo* are strongly influenced by other cellular factors. One can imagine two classes of these factors. General factors, such as molecular chaperones or proteins that degrade abnormal proteins, are likely to interfere with the appearance and maintenance of a wide range of self-propagating protein conformations. One such factor, essential for the maintenance of both *[PSI$^+$]* and *[PIN$^+$]* (40, 54, 55), is the heat shock protein Hsp104, which is known to promote resolubilization of protein aggregates following heat shock (53). Specific factors, which interact with either normal or prion conformational variants of a particular prion protein, are likely to affect the genesis and propagation only of a single prion. We argue that Sup45p, a protein known to complex with Sup35p (33, 34), is such a specific factor for *[PSI$^+$]*, because our data indicate that the overexpression of *SUP45* interferes with the *de novo* induction of *[PSI$^+$]*.

The major observation is that transformation with high-copy *SUP45*-containing plasmids causes a considerable reduction in the ability of transiently overproduced Sup35p to induce the appearance of Ade$^+$ colonies, indicative of *[PSI$^+$]*. Although this is likely to be because of a negative effect of excess Sup45p on *[PSI$^+$]* induction, it could be also explained by a Sup45p-stimulated reduction in the *[PSI$^+$]* suppression phenotype or by an incompatibility of *[PSI$^+$]* and excess Sup45p. We eliminate the latter possibilities by showing that *SUP45* overexpression does not inhibit growth of *[PSI$^+$]* cells with normal or elevated Sup35p levels, does not reduce the efficiency of readthrough of an *ade1–14* nonsense allele, and does not cause the elimination of *[PSI$^+$]*. In contrast, *SUP45* overexpression rescues the inhibition of growth caused by Sup35p overproduction in *[PSI$^+$]* derivatives and increases the nonsense suppression in derivatives containing weak *[PSI$^+$]* variants. These phenotypes are likely to be typical of major inducible *[PSI$^+$]* types, because in our experiments we used different *[PSI$^+$]* variants induced by Sup35p overproduction. Furthermore, overexpression of *SUP45* in unrelated *[PSI$^+$]* strains (where *[PSI$^+$]* was not induced by Sup35p overproduction) also caused an increase in suppression efficiency (59), appeared to be compatible with *[PSI$^+$]* (59, 73), and allowed for *SUP35* overexpression (33). Thus, we are left with the conclusion that *SUP45* overexpression reduces the efficiency with which Sup35p overproduction can induce *[PSI$^+$]*.

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 termination *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 allosuppressor 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 *SUP45* 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.

We thank J. Warner (Albert Einstein College of Medicine), and M. Patino and S. Lindquist (University of Chicago) for antibodies; N. Kochneva-Pervukhova and M. Ter-Avanesyan (Institute of Experimental Cardiology, Russia) for sharing their unpublished data; Y. Chernoff (Georgia Institute of Technology) and P. Zhou for helpful discussions; and P. Zhou and M. Nelson for technical assistance. This work was partially supported by grants from the National Institutes of Health (GM56350) and the Alzheimer's Association.

1. Cox, B. S. (1965) *Heredity* **20,** 505–521.
2. Liebman, S. W., Stewart, J. W. & Sherman, F. (1975) *J. Mol. Biol.* **94,** 595–610.

3. Liebman, S. W. & Sherman, F. (1979) *J. Bacteriol.* **139,** 1068–1071.
4. Ono, B., Ishino-Arao, Y., Tanaka, M., Awano, I. & Shinoda, S. (1986) *Genetics* **114,** 363–374.
5. Cox, B. S., Tuite, M. F. & McLaughlin, C. S. (1988) *Yeast* **4,** 159–178.
6. Tikhodeev, O. N., Getmanova, E. V., Tikhomirova, V. L. & Inge-Vechtomov, S. G. (1990) in *Molecular Mechanisms of Genetic Processes*, eds. Shestakov, S. V. & Tarasov, V. A. (USSR Academy of Sciences, Nauka, Moscow), pp. 218–228.
7. Wickner, R. B. (1994) *Science* **264,** 566–569.
8. Griffith, J. S. (1967) *Nature (London)* **215,** 1043–1044.
9. Prusiner, S. B. (1982) *Science* **216,** 136–144.
10. Cox, B. (1994) *Curr. Biol.* **4,** 744–748.
11. Lindquist, S., Patino, M. M., Chernoff, Y. O., Kowal, A. S., Singer, M. A., Liebman, S. W., Lee, K. H. & Blake, T. (1995) *Cold Spring Harbor Symp. Quant. Biol.* **60,** 451–460.
12. Wickner, R. B., Masison, D. C. & Edskes, H. K. (1995) *Yeast* **11,** 1671–1685.
13. Wickner, R. B. (1996) *Annu. Rev. Genet.* **30,** 109–139.
14. Wickner, R. B. & Masison, D. C. (1996) *Curr. Top. Microbiol. Immunol.* **207,** 147–160.
15. Tuite, M. F. & Lindquist, S. L. (1996) *Trends Genet.* **12,** 467–471.
16. Lindquist, S. (1996) *Mol. Psychiatry* **1,** 376–379.
17. Lindquist, S. (1997) *Cell* **89,** 495–498.
18. Kushnirov, V. V., Ter-Avanesyan, M. D., Telckov, M. V., Surguchov, A. P., Smirnov, V. N. & Inge-Vechtomov, S. G. (1988) *Gene* **66,** 45–54.
19. Kushnirov, V. V., Ter-Avanesyan, M. D., Didichenko, S. A., Smirnov, V. N., Chernoff, Y. O., Derkach, I. L., Novikova, O. N., Inge-Vechtomov, S. G., Neistat, M. A. & Tolstorukov, I. I. (1990) *Yeast* **6,** 461–472.
20. Wilson, P. G. & Culbertson, M. R. (1988) *J. Mol. Biol.* **199,** 559–573.
21. Hoshino, S., Miyazawa, H., Enomoto, T., Hanaoka, F., Kikuchi, Y., Kikuchi, A. & Ui, M. (1989) *EMBO J.* **8,** 3807–3814.
22. Tassan, J. P., Le Guellec, K., Kress, M., Faure, M., Camonis, J., Jacquet, M. & Philippe, M. (1993) *Mol. Cell. Biol.* **13,** 2815–2821.
23. Samsonova, M. G., Inge-Vechtomov, S. G. & Taylor, P. (1991) *Genetica* **85,** 35–44.
24. Zhouravleva, G., Frolova, L., Le Goff, X., Le Guellec, R., Inge-Vechtomov, S., Kisselev, L. & Philippe, M. (1995) *EMBO J.* **14,** 4065–4072.
25. Frolova, L., Le Goff, X., Zhouravleva, G., Davydova, E., Philippe, M. & Kisselev, L. (1996) *RNA* **2,** 334–341.
26. Inge-Vechtomov, S. G. & Andrianova, V. M. (1970) *Genetica* **6,** 103–115.
27. Hawthorne, D. C. & Leupold, U. (1974) *Curr. Top. Microbiol. Immunol.* **64,** 1–47.
28. Gerlach, W. L. (1975) *Mol. Gen. Genet.* **138,** 53–63.
29. Surguchov, A. P., Fominykch, E. S., Berestetskaya, Y. V., Smirnov, V. N. & Inge-Vechtomov, S. G. (1980) *Mol. Gen. Genet.* **177,** 675–680.
30. Cox, B. S. (1977) *Genet. Res.* **30,** 187–205.
31. Ter-Avanesyan, M. D., Inge-Vechtomov, S. G., Surguchov, A. P. & Smirnov, V. N. (1984) *Dokl. Akad. Nauk SSSR* **277,** 226–231.
32. Ter-Avanesyan, M. D., Didichenko, S. A., Kushnirov, V. V. & Dagkesamanskaya, A. R. (1993) in *Protein Synthesis and Targeting in Yeast*, eds. Brown, A. J. P., Tuite, M. F. & McCarthy, J. E. G. (Springer, Heidelberg, Germany), Vol. 71, pp. 81–90.
33. Stansfield, I., Jones, K. M., Kushnirov, V. V., Dagkesamanskaya, A. R., Poznyakovski, A. I., Paushkin, S. V., Nierras, C. R., Cox, B. S., Ter-Avanesyan, M. D. & Tuite, M. F. (1995) *EMBO J.* **14,** 4365–4373.
34. Paushkin, S. V., Kushnirov, V. V., Smirnov, V. N. & Ter-Avanesyan, M. D. (1997) *Mol. Cell. Biol.* **17,** 2798–2805.
35. Frolova, L., Le Goff, X., Rasmussen, H. H., Cheperegin, S., Drugeon, G., Kress, M., Arman, I., Haenni, A. L., Celis, J. E., Philippe, M. & *et al.* (1994) *Nature (London)* **372,** 701–703.
36. Stansfield, I., Eurwilaichitr, L., Akhmaloka & Tuite, M. F. (1996) *Mol. Microbiol.* **20,** 1135–1143.
37. Stansfield, I., Kushnirov, V. V., Jones, K. M. & Tuite, M. F. (1997) *Eur. J. Biochem.* **245,** 557–563.
38. Urbero, B., Eurwilaichitr, L., Stansfield, I., Tassan, J. P., Le Goff, X., Kress, M. & Tuite, M. F. (1997) *Biochimie* **79,** 27–36.
39. Paushkin, S. V., Kushnirov, V. V., Smirnov, V. N. & Ter-Avanesyan, M. D. (1996) *EMBO J.* **15,** 3127–3134.
40. Patino, M. M., Liu, J. J., Glover, J. R. & Lindquist, S. (1996) *Science* **273,** 622–626.
41. Paushkin, S. V., Kushnirov, V. V., Smirnov, V. N. & Ter-Avanesyan, M. D. (1997) *Science* **277,** 381–383.
42. Glover, J. R., Kowal, A. S., Schirmer, E. C., Patino, M. M., Liu, J. J. & Lindquist, S. (1997) *Cell* **89,** 811–819.
43. Ter-Avanesyan, M. D., Dagkesamanskaya, A. R., Kushnirov, V. V. & Smirnov, V. N. (1994) *Genetics* **137,** 671–676.
44. Derkatch, I. L., Chernoff, Y. O., Kushnirov, V. V., Inge-Vechtomov, S. G. & Liebman, S. W. (1996) *Genetics* **144,** 1375–1386.
45. King, C. Y., Tittmann, P., Gross, H., Gebert, R., Aebi, M. & Wuthrich, K. (1997) *Proc. Natl. Acad. Sci. USA* **94,** 6618–6622.
46. Cohen, F. E., Pan, K. M., Huang, Z., Baldwin, M., Fletterick, R. J. & Prusiner, S. B. (1994) *Science* **264,** 530–531.
47. Jarrett, J. T. & Lansbury, P. T., Jr. (1993) *Cell* **73,** 1055–1058.
48. Prusiner, S. B. (1994) *Annu. Rev. Microbiol.* **48,** 655–686.
49. Prusiner, S. B. (1996) *Trends Biochem. Sci.* **21,** 482–487.
50. Weissmann, C. (1996) *FEBS Lett.* **389,** 3–11.
51. Caughey, B. & Chesebro, B. (1997) *Trends Cell Biol.* **7,** 56–62.
52. Wickner, R. B. (1997) *Prion Diseases of Mammals and Yeast: Molecular Mechanisms and Genetic Features* (Landes, Austin, TX).
53. Parsell, D. A., Kowal, A. S., Singer, M. A. & Lindquist, S. (1994) *Nature (London)* **372,** 475–478.
54. Chernoff, Y. O., Lindquist, S. L., Ono, B., Inge-Vechtomov, S. G. & Liebman, S. W. (1995) *Science* **268,** 880–884.
55. Derkatch, I. L., Bradley, M. E., Zhou, P., Chernoff, Y. O. & Liebman, S. W. (1997) *Genetics* **147,** 507–519.
56. Chernoff, Y. O., Derkach, I. L. & Inge-Vechtomov, S. G. (1993) *Curr. Genet.* **24,** 268–270.
57. Evans, K. C., Berger, E. P., Cho, C. G., Weisgraber, K. H. & Lansbury, P. T., Jr. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 763–767.
58. Inge-Vechtomov, S. G., Karpova, T. S., Tikhodeev, O. N., Kashkin, P. K. & Trofimova, M. V. (1987) in *Abstracts of the 5th Congress of USSR Genetic Society*, ed. Strunnikov, V. A. (U.S.S.R. Academy of Sciences, Moscow), Vol. 5, p. 38.
59. Chernoff, Y. O., Inge-Vechtomov, S. G., Derkatch, I. L., Ptyushkina, M. V., Tarunina, O. V., Dagkesamanskaya, A. R. & Ter-Avanesyan, M. D. (1992) *Yeast* **8,** 489–499.
60. Broach, J. R. & Hicks, J. B. (1980) *Cell* **21,** 501–508.
61. Beggs, J. D. (1981) in *Alfred Benzon Symposium*, eds. Wettstein, D., Friis, J., Kielland-Brandt, M. & Stenderup, A. (Munksgaard, Copenhagen), Vol. 16, pp. 383–390.
62. Erhart, E. & Hollenberg, C. P. (1983) *J. Bacteriol.* **156,** 625–635.
63. Botstein, D., Falco, S. C., Stewart, S. E., Brennan, M., Scherer, S., Stinchcomb, D. T., Struhl, K. & Davis, R. W. (1979) *Gene* **8,** 17–24.
64. Stansfield, I., Grant, G. M., Akhmaloka & Tuite, M. F. (1992) *Mol. Microbiol.* **6,** 3469–3478.
65. Rose, M. D., Novick, P., Thomas, J. H., Botstein, D. & Fink, G. R. (1987) *Gene* **60,** 237–243.
66. Louvion, J. F., Havaux-Copf, B. & Picard, D. (1993) *Gene* **131,** 129–134.
67. Sherman, F., Fink, G. R. & Hicks, J. B. (1986) *Methods in Yeast Genetics* (Cold Spring Harbor Lab. Press, Plainview, NY).
68. Boeke, J. D., LaCroute, F. & Fink, G. R. (1984) *Mol. Gen. Genet.* **197,** 345–346.
69. Hulett, F. M., Bookstein, C. & Jensen, K. (1990) *J. Bacteriol.* **172,** 735–740.
70. Kocisko, D. A., Come, J. H., Priola, S. A., Chesebro, B., Raymond, G. J., Lansbury, P. T. & Caughey, B. (1994) *Nature (London)* **370,** 471–474.
71. Bessen, R. A., Raymond, G. J. & Caughey, B. (1997) *J. Biol. Chem.* **272,** 15227–15231.
72. Caughey, B., Kocisko, D. A., Raymond, G. J. & Lansbury, P. T. (1995) *Chem. Biol.* **2,** 807–817.
73. Dagkesamanskaya, A. R. & Ter-Avanesyan, M. D. (1991) *Genetics* **128,** 513–520.
74. Drugeon, G., Jean-Jean, O., Frolova, L., Le Goff, X., Philippe, M., Kisselev, L. & Haenni, A. L. (1997) *Nucleic Acids Res.* **25,** 2254–2258.
75. Le Goff, X., Philippe, M. & Jean-Jean, O. (1997) *Mol. Cell. Biol.* **17,** 3164–3172.