

The Yeast $[PSI^+]$ Prion: Making Sense of Nonsense*

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The $[PSI^+]$ element was first described by Brian Cox in 1965 (1) in the course of his studies of a Mendelian nonsense suppressor. The efficiency with which this suppressor could misread UAA stop codons as sense was dependent upon the presence of a non-Mendelian factor, which Cox named $[PSI^+]$. Nearly 30 years of intriguing investigations followed, but the molecular nature of $[PSI^+]$ remained unclear (see Refs. 2 and 3 for excellent reviews of this period). Recent reviews (e.g. Refs. 4–9) incorporate these data with current results in the context of the startling hypothesis (Ref. 10 and see below) that $[PSI^+]$ is a prion.

Discovery of $[PSI^+]$

Although the original suppressor mutation acted on by $[PSI^+]$ was in a tRNA gene and was specific for UAA codons, $[PSI^+]$ was later shown to enhance the efficiency of suppression caused by other nonsense and frameshift tRNA suppressors or by aminoglycoside antibiotics (allosuppression). Eventually $[PSI^+]$ was shown to be an “omnipotent” suppressor because it could cause readthrough of certain UAA, UAG, and UGA codons in the absence of other suppressors or drugs (11). Similar allosuppressor and omnipotent suppressor phenotypes were also associated with different *sup35* and *sup45* mutant alleles. However, whereas the *sup35* and *sup45* mutations were recessive and showed the 2:2 segregation pattern expected of Mendelian genes, the $[PSI^+]$ factor was dominant and segregated 4:0, i.e. to all meiotic progeny. Consistent with this non-Mendelian segregation, the $[PSI^+]$ factor appeared to be located in the cytoplasm because it was transmitted by cytoduction when the cytoplasm of a $[PSI^+]$ donor haploid was transferred to a $[psi^-]$ recipient haploid without altering the recipient's nucleus (2). Thus it was possible that a cytoplasmic nucleic acid encoded $[PSI^+]$, although it was shown not to depend upon mitochondrial DNA, 2 μ DNA, killer viruses, or 20 S RNA (2, 12, 13).

The findings that “mutations” of $[PSI^+]$ to $[psi^-]$ were induced by conventional mutagens with single-hit kinetics and that UV mutagenesis was dependent upon DNA repair genes and was reduced by photoreactivation suggested that $[PSI^+]$ was encoded by single-copy DNA (14). However, growth in the presence of the mild protein denaturant guanidine hydrochloride or various stress-inducing agents, none of which cause the mutation of nuclear genes, caused the efficient loss of $[PSI^+]$. The kinetics of loss suggested that $[PSI^+]$ was encoded by a multicopy element. Another important $[PSI^+]$ paradox was the observation that although $[PSI^+]$ was dominant *in vivo*, it was recessive *in vitro*, because mixtures of $[PSI^+]$ and $[psi^-]$ lysates used in a cell-free translation system did not exhibit the high level of readthrough of nonsense codons characteristic of $[PSI^+]$ lysates (15).

Prion Model for $[PSI^+]$

In 1994, Reed Wickner (10) presented evidence that $[URE3]$ is a prion form of the Ure2 protein (see accompanying review by Wickner *et al.* (58)) and at the same time suggested that $[PSI^+]$ is a prion form of the Sup35 protein (Sup35p). This revolutionary hypothesis (Table I) explained much of the old $[PSI^+]$ data as well as two newer results that directly linked *SUP35* to $[PSI^+]$ (16, 17). The

idea was not that Sup35p (or Ure2p) had any functional similarity with the mammalian PrP^{Sc} prion but rather that all three had a similar novel mode of inheritance involving the self-propagation of alternate protein conformations.

It was proposed (10) that in $[psi^-]$ cells the conformation of Sup35p is fully functional (Sup35p^{psi⁻}) and promotes efficient termination at stop codons. Indeed, we now know that Sup35p is translational release factor eRF3 (see below). In $[PSI^+]$ cells some or all of the Sup35p was proposed to take on an alternate conformation (Sup35p^{PSI⁺}) leading to less efficient termination and thus nonsense suppression. The hypothesis also predicts that Sup35p^{PSI⁺} catalyzes the conversion of Sup35p^{psi⁻} molecules into Sup35p^{PSI⁺} (Fig. 1). The *in vivo* dominance and non-Mendelian nature of $[PSI^+]$ and the fact that the impairment of Sup35p either by Mendelian mutations in *SUP35* or by the presence of $[PSI^+]$ causes similar phenotypes is consistent with this model. The prion hypothesis also explains the paradox (see above) that $[PSI^+]$ is recessive *in vitro* because a mixture of $[PSI^+]$ and $[psi^-]$ lysates would be expected to contain enough fully functional Sup35p^{psi⁻} for efficient termination unless the Sup35p^{psi⁻} was converted to Sup35p^{PSI⁺} in these *in vitro* mixtures, which was not the case (18).

In 1993 the curious finding was reported that a multicopy plasmid carrying *SUP35* efficiently induced the *de novo* appearance of $[PSI^+]$ (16). This result was interpreted (10) as evidence for the prion model because the *SUP35* overexpression increased the probability that a Sup35p^{psi⁻} molecule would take on a prion shape by chance. It was also shown that $[PSI^+]$ could reappear after curing (16, 19), arguing against the possibility that curing was because of the loss of a cytoplasmic nucleic acid with no nuclear master gene.

The other crucial result connecting *SUP35* with $[PSI^+]$ that influenced the prion hypothesis was that a dominant mutation, which caused the loss of $[PSI^+]$, *PNM2* (Psi-No-More) (20), was an allele of *SUP35* with a missense mutation that altered the N-terminal region of Sup35p (3, 17). At about the same time another paper showed that cells bearing a deletion of the N-terminal coding region of *SUP35* were unable to maintain $[PSI^+]$ (21). These results are analogous to the requirements for the *Prn-p* and *URE2* genes for susceptibility to prion infection and maintenance of $[URE3]$, respectively. The host genes encoding PrP, Ure2p, and Sup35p must be present to provide a continuous supply of protein that can be converted to the prion form (see accompanying reviews by Wickner *et al.* (58) and Weissmann (59)).

Additional experiments testing and exploring the prion hypothesis for $[PSI^+]$ then followed at a fast pace. The finding that the chaperone protein Hsp104 is required for the propagation of $[PSI^+]$ (22) provided dramatic support for the prion model, because the only known function of Hsp104 is to facilitate the folding of proteins (see below). Also in support of the “protein only” hypothesis, the demonstration that the induction of $[PSI^+]$ by overexpression of *SUP35* was because of an excess of Sup35 protein and not an excess of *SUP35* DNA or mRNA established that Sup35p is not only necessary, but is also sufficient, to cause $[PSI^+]$ (23).

There is now direct evidence that Sup35p exists in different structural states in $[PSI^+]$ and $[psi^-]$ cells. Sup35p in lysates of $[PSI^+]$ but not $[psi^-]$ strains showed increased protease resistance and aggregation (24, 25), two characteristics typical of mammalian prions. $[PSI^+]$ aggregates were detected by sedimentation and were visualized *in vivo* using fusions of Sup35p with green fluorescent protein (25). Also, Sup35p isolated from $[PSI^+]$ but not $[psi^-]$ strains could bind the N-terminal fragment of Sup35p (24).

Another important achievement has been the demonstration that Sup35p^{PSI⁺} can efficiently stimulate the aggregation of soluble Sup35p^{psi⁻} from $[psi^-]$ lysates (18). Because the only cellular fraction that promotes this *in vitro* conversion is that containing the Sup35p^{PSI⁺} aggregates, it appears that soluble Sup35p^{psi⁻} molecules either do not exist or are unable to initiate the conversion. This finding supports the seeded polymerization model that

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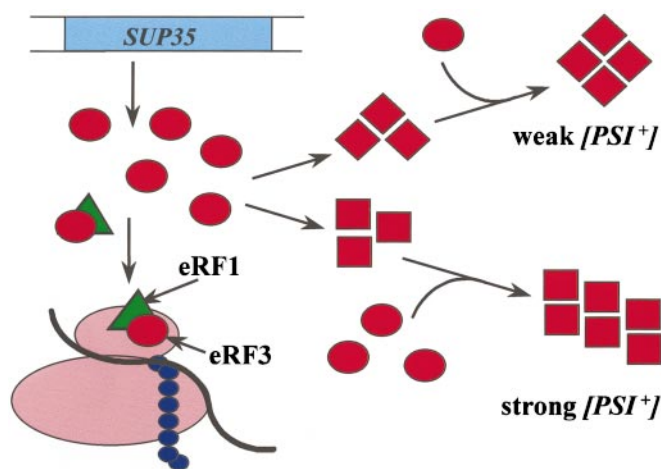


FIG. 1. Schematic illustration of the prion model for $[PSI^+]$. The *SUP35* gene encodes release factor 3 (eRF3), which, in its normal $Sup35p^{psi-}$ conformation (circles), functions together with eRF1 at the ribosome to release polypeptide chains. The frequency with which $Sup35p^{psi-}$ can spontaneously change into the $Sup35p^{psi+}$ conformation (squares) is proportional to the level of $Sup35p^{psi-}$ available in the cell. The crystal seed model is depicted showing that once a seed of $Sup35p^{psi+}$ is established additional $Sup35p^{psi-}$ can join the seed rapidly leading to the formation of $[PSI^+]$ aggregates. In $[PSI^+]$ cells less non-aggregated eRF3 is available to function in translation, leading to inefficient termination at stop codons and the phenotype of nonsense suppression. Shown are two types of $[PSI^+]$ aggregates hypothesized to grow at different rates and to correspond to $[PSI^+]$ elements that cause mild (weak) or more severe (strong) degrees of impairment of the termination function.

TABLE I
Key evidence supporting the prion hypothesis for $[PSI^+]$

Inheritance of $[PSI^+]$ is non-Mendelian
Propagation of $[PSI^+]$ requires the presence of the <i>SUP35</i> gene
$[PSI^+]$ and mutations in <i>SUP35</i> have the same phenotypes
Overproduction of Sup35p efficiently induces $[PSI^+]$
$[PSI^+]$ can reappear after it is cured
Propagation of $[PSI^+]$ requires an intermediate level of Hsp104p
Sup35p forms protease-resistant aggregates in $[PSI^+]$ but not $[psi^-]$ strains
Sup35p forms fibers <i>in vitro</i>
Sup35p ^{PSI+} efficiently converts Sup35p ^{psi-} to an aggregated protease-resistant form <i>in vitro</i>

predicts that conversion to the prion conformation occurs upon aggregation. Finally, purified Sup35p has been shown to form ordered fibers *in vitro*, and the rate of fiber formation is stimulated by the addition of small amounts of preformed fiber (26, 27). Sup35p fibers are similar to amyloid fibers associated with certain human diseases because they bind Congo red and are rich in β -sheet structure. Although such fibers have not yet been found *in vivo*, these results suggest that $Sup35p^{psi+}$ is an amyloid-like fiber and that the *in vitro* change in conformation represents the mechanism for propagation of $[PSI^+]$. These results can be interpreted in terms of the seeded nucleation model; however, the initial rate of unseeded fiber nucleation is not as dependent upon the concentration of soluble Sup35p monomers as predicted by the original polymerization model (26, 28).

The $[PSI^+]$ Prion Domain Is in the Sup35p N Terminus

Sup35p can be roughly divided into three domains: N, M, and C (Fig. 2). The C domain is essential, apparently because of its function in translation termination (see below). The N region and the highly charged M region are not essential (29), but, as mentioned above, strains lacking the N or NM regions cannot maintain $[PSI^+]$ (21). The inability to maintain $[PSI^+]$ (Pnm phenotype) in such deletion-bearing mutants is recessive. Furthermore, the overexpression of just the N-terminal fragments of Sup35p (as small as 113 amino acids) is sufficient to induce the *de novo* appearance of $[PSI^+]$ visualized as $[PSI^+]$ -specific aggregates with the green fluorescent protein fusion constructs and/or detected genetically (23, 25, 30). These data suggest that the $[PSI^+]$ prion domain in Sup35p

is limited to the N part of the protein. Moreover, most of the *in vitro* experiments described above as proof of the prion model for $[PSI^+]$ /Sup35p were successfully reproduced using the Sup35p N part alone (Fig. 2).

The N region of Sup35p contains two structurally unusual regions involved in $[PSI^+]$ biogenesis. All *sup35* point mutations that inhibit $[PSI^+]$ propagation (*PNM*) or reduce $[PSI^+]$ -associated suppression (*ASU*, antisuppression) are located within or very close to these regions. The first region (amino acids 8–26) is rich in glutamine and asparagine. Changes there lead to both Pnm and Asu phenotypes (28). Generally, the presence of plasmids bearing *PNM* mutant alleles led to a reduction in aggregation of both the mutant and wild-type Sup35 proteins, whereas *ASU* plasmids reduced the aggregation of just the mutant protein. The second region (amino acids 56–97) consists of four and a half nanopeptide repeats that are structurally similar to the octapeptide repeats found in the mammalian PrP prion (see Ref. 4). A G58D substitution in the repeat region was originally described as a dominant *PNM* mutation (17). It now appears that this mutation, called *PNM2*, only causes $[PSI^+]$ loss in some genetic backgrounds¹ and can cause the *de novo* induction of $[PSI^+]$ when overexpressed (30).¹ Even in backgrounds where *PNM2* does not cure $[PSI^+]$ it affects the $[PSI^+]$ phenotype in an unusual manner (see below).

Heritable Differences between $[PSI^+]$ Factors

Although differences between $[PSI^+]$ and $[psi^-]$ strains have been known for some time, it has only recently become clear that not all $[PSI^+]$ strains contain the same $[PSI^+]$ factors (23). Indeed, $[PSI^+]$ derivatives with different characteristics, called weak and strong $[PSI^+]$ variants, can be obtained in the same strain when the same *SUP35* gene is overproduced (Fig. 1). Weak $[PSI^+]$ derivatives suppress nonsense mutations poorly and are lost in 1–3% of mitotic progeny. Strong $[PSI^+]$ variants cause higher levels of suppression and are very stable. These phenotypic differences appear to be associated with the $[PSI^+]$ elements and are not the result of nuclear mutations. Although Sup35p is aggregated in both weak and strong $[PSI^+]$ derivatives, the level and speed of aggregation are greater in strong $[PSI^+]$ derivatives.² Strong $[PSI^+]$ is dominant over weak $[PSI^+]$,¹ but it remains unclear if $Sup35p^{weakPSI+}$ is lost in the presence of $Sup35p^{strongPSI+}$ or if the different $[PSI^+]$ forms co-exist.

The different $[PSI^+]$ elements are analogous to the mysterious scrapie “strains” causing distinct pathologies that have posed one of the biggest challenges for the prion hypothesis (see accompanying review by Weissmann (59)). If scrapie were caused by a virus, strains could reflect mutations in the viral nucleic acid. Alternatively, because PrP proteins associated with different disease strains are cleaved at different sites by proteinase K, it has been proposed that a single PrP^{Sc} protein can compose different types of prion aggregates (32). The finding in yeast of different $[PSI^+]$ variants arising from overexpression of Sup35p is incompatible with the viral hypothesis and by analogy with the mammalian results suggests that there is more than one type of $Sup35p^{psi+}$ conformation or more than one type of $[PSI^+]$ aggregate. Indeed, the Sup35p fibers formed *in vitro* have been shown to exist in distinct structural forms, “wavy” or “straight,” and transitions between these forms are not observed within the same fiber, indicating that they may represent a structural basis for $[PSI^+]$ variants (26). Finally, $[PSI^+]$ variants can also be distinguished by their opposite phenotypic responses to overexpression of the *PNM2* allele of *SUP35*; weak $[PSI^+]$ variants are allosuppressed and stabilized whereas strong $[PSI^+]$ variants are antisuppressed.¹ These data suggest that Sup35p encoded by *PNM2* interacts differently with different $Sup35p^{psi+}$ conformations or aggregate types.

The $[PSI^+]$ strains found in various yeast collections appear to bear only weak $[PSI^+]$ factors. For example, $[ETA^+]$, originally described as a meiotically unstable non-Mendelian factor incompatible with *sup35-2* or *sup45-2* mutations (33), is clearly a weak $[PSI^+]$, because it has now been shown to have a weak suppressor phenotype and to require an intermediate level of Hsp104 as well

¹ Derkatch, I. L., Bradley, M. E., Zhou, P., and Liebman, S. W. (1999) *Curr. Genet.*, in press.

² Zhou, I. L., Derkatch, S. M., Uptain, M. M., Patino, S., Lindquist, and S. W. Liebman, submitted for publication.

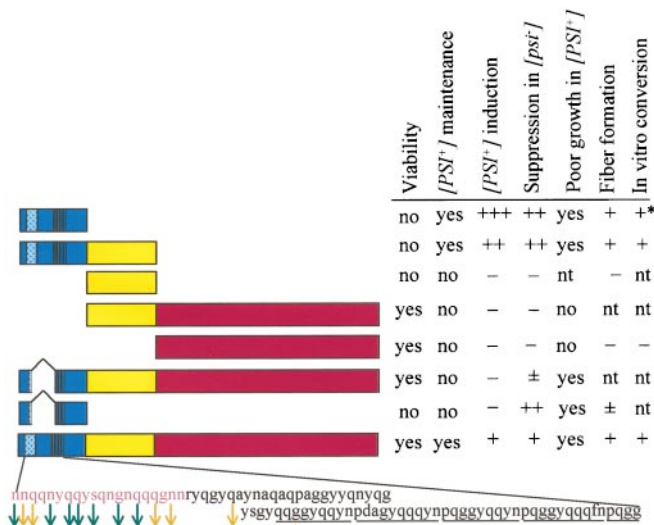


FIG. 2. Identification of the $[PSI^+]$ prion domain within Sup35p. The N domain of Sup35p (amino acids 1–123) is shown in blue, the highly charged M domain (amino acids 124–253) in yellow, and the C domain (amino acids 254–685) in red. Within the N domain the black vertical lines indicate the nanopeptide repeats and the patterned region shows the Gln/Asn-rich region. The sequence of amino acids 8–97 is shown. The Gln/Asn rich stretch is in pink; nanopeptide repeats are underlined; green and orange arrows mark positions of PNM and ASU mutations, respectively. The fragments of Sup35p are scored for the properties indicated. The ability to maintain $[PSI^+]$ is defined either genetically or as the *in vivo* aggregation of the fragment. Induction of $[PSI^+]$, suppression, and growth inhibition are determined when the fragment is overproduced. Fiber formation used purified fragments. Conversion indicates the ability of the fragments to become protease-resistant and/or aggregated in the presence of $[PSI^+]$ lysates. * indicates a fragment 30 amino acids longer was used. Relative levels within a column are indicated with +++, ++, +, ±, -, nt means not tested. Data are from Refs. 18, 21, 23–29, 31, and 34.

as the Sup35p N terminus for maintenance and because $[PSI^+]$ factors induced by Sup35p overproduction are lethal with *sup35-2* and *sup45-2*.²

The Effect of Chaperones on the Maintenance of $[PSI^+]$

Because protein conformational switches and the formation of ordered protein aggregates are central to the prion hypothesis, the finding that chaperones are involved in prion maintenance constitutes strong support for the prion hypothesis. Indeed, an intermediate level of the chaperone Hsp104 has been shown to be required for the maintenance of $[PSI^+]$ (22). Deletion of *HSP104* cured $[PSI^+]$, whereas overexpression of *HSP104* reduced the $[PSI^+]$ phenotype or, at a higher dose, caused the permanent loss of the $[PSI^+]$. Similarly, cells cured of $[PSI^+]$ by deletion or overexpression of *HSP104* lack $[PSI^+]$ aggregates, and even under conditions when $[PSI^+]$ is not cured, overexpression of Hsp104 causes partial release of Sup35p from the pellet fraction (24, 25, 34).

The ability of overexpressed Hsp104 to cure $[PSI^+]$ or inhibit its phenotype has recently been shown to be interfered with by the simultaneous overexpression of *SSA1*, a member of the Hsp70 family. This may explain why conditions that normally induce Hsp104 together with Hsp70, such as heat shock, stationary phase growth, and sporulation, do not efficiently cure $[PSI^+]$ (60).

Hypotheses that explain the paradox that either the lack or excess of Hsp104 causes the loss of $[PSI^+]$ in the context of the general role of Hsp104 in the rescue of proteins from aggregates have recently been reviewed in detail (6) and will not be discussed here. It will now be important to determine whether *HSP104* is involved in the *in vivo* propagation of other prions. The yeast $[URE3]$ prion is not affected by deletion of *HSP104*, although an extra copy of *HSP104* did inhibit the $[URE3]$ phenotype.³ Although the *in vitro* formation of $[PSI^+]$ fibers proceeds in the absence of Hsp104 (26, 27), Hsp104 has been shown to specifically interact with both Sup35p and PrP *in vitro* (35). Be-

cause an Hsp70 protein has been proposed to be an integral component of translating ribosomes (36), it remains possible that the interaction between Hsp104 and Sup35p results from an unknown role for Hsp104 in translation.

Does Induction of $[PSI^+]$ Require Another Prion, $[PIN^+]$ (37)?

The prion model postulates that $[PSI^+]$ should be reversibly curable. Indeed, when $[PSI^+]$ is cured by transient overexpression of Hsp104, $[PSI^+]$ can be re-induced by the overproduction of Sup35p. However, when $[PSI^+]$ is cured by transient inactivation of *HSP104* none of the resulting $[psi^-]$ derivatives can be re-induced to become $[PSI^+]$ by overexpression of the complete Sup35p. The designations $[PIN^+]$ and $[pin^-]$ (P*si* I*N*ducible) have been used to describe $[psi^-]$ strains that can and cannot be induced to become $[PSI^+]$ by overexpression of Sup35p, respectively. Surprisingly, overexpression of certain Sup35p fragments induced $[PSI^+]$ even in the absence of $[PIN^+]$, showing that Sup35p can assume the Sup35p^{PSI+} conformation in $[pin^-]$ strains under certain conditions.

$[PIN^+]$ has three properties characteristic of prions. 1) Maintenance of $[PIN^+]$ requires Hsp104. 2) Growth on medium containing guanidine hydrochloride can cure $[PIN^+][psi^-]$ strains of $[PIN^+]$ and can cure $[PIN^+]$ $[PSI^+]$ strains of either or both elements.⁴ 3) $[PIN^+]$ is dominant over $[pin^-]$, and the meiotic segregation of $[PIN^+]$ is non-Mendelian. One possibility is that $[PIN^+]$, like $[PSI^+]$, is caused by prion conformations of Sup35p. However, $[PIN^+]$ does not require the Sup35 N terminus for its propagation, so the prion domain that determines $[PIN^+]$ would have to be distinct from the prion domain that determines $[PSI^+]$. Alternatively, the Pin prion protein is encoded by another gene that is unlikely to correspond to *SUP45* or *HSP104* because their overexpression does not induce $[PIN^+]$ (37, 38) or to *UPF1* or *SAL6* because their disruption does not cure $[PIN^+]$.⁴

Sup35p Is an eRF3 Translational Termination Factor

Translational termination in prokaryotes involves class I release factors (RF1 and RF2), which are molecular mimics of tRNA, and a class II release factor (RF3), which is a structural analog of EF-Tu and EF-G. Recent evidence (39, 40) suggests that when the ribosome encounters a stop codon, a class I RF binds and catalyzes peptidyl-tRNA hydrolysis. RF3-GTP then binds and promotes the release of the class I RF in a translocation-like event accompanied by GTP hydrolysis. Termination factors with analogous *in vitro* activities are also known in eukaryotes (41); the yeast Sup45p is an eRF1 whereas Sup35p is an eRF3 (42–44). Unlike their prokaryotic analogs, eRF1 and eRF3 have been shown to form a complex off the ribosome, suggesting that eRF3 may escort eRF1 to the ribosome just as EF-Tu escorts tRNA within a ternary complex (44, 45). This difference may explain why eRF3 is essential in eukaryotes whereas RF3 is not essential in prokaryotes.

Proteins That May Interact with Sup35p

SUP45—Interaction between Sup35p and Sup45p has been demonstrated with the two-hybrid system, by immunoprecipitation in cell lysates (45), and with purified Sup35C and glutathione *S*-transferase-Sup45p components (34). Two Sup45p binding sites were localized within Sup35p, one at the NM border and another in the first half of C (34), and Sup45p has been shown to sediment with Sup35p $[PSI^+]$ aggregates in some (34) but not other (25) experiments.

Although overexpression of Sup45p inhibits the *de novo* induction of $[PSI^+]$ by excess Sup35p, it has no effect on the propagation of $[PSI^+]$ (38). The excess Sup45p may bind to Sup35p^{psi-}, thereby inhibiting a *de novo* conformational change to Sup35p^{PSI+}, but may fail to compete with established Sup35p^{PSI+} aggregates for the binding of Sup35p^{psi-}. Simultaneous overexpression of Sup45p and Sup35p causes antisuppression and does not cause growth inhibition even in the presence of strong $[PSI^+]$, whereas overexpression of Sup45p alone causes allosuppression of weak $[PSI^+]$, and overexpression of Sup35p alone severely inhibits growth of strong $[PSI^+]$ derivatives (38, 45, 46). Possibly the unbalanced excess of one of the release factors allows it to deplete the termination complex of an essential protein.

SAL—Mutations in the *SAL* genes were isolated as allosuppres-

³ Y. O. Chernoff and S. W. Liebman, unpublished results.

⁴ I. L. Derkatch, M. E. Bradley, V. Prapapanich, and S. W. Liebman, manuscript in preparation.

sors, which enhanced the efficiency of suppressors, and were shown to be at unlinked loci (2). Surprisingly, certain recessive *sal6* alleles failed to complement recessive allosuppressor mutations in the *SAL1*, *SAL2* (allelic to *UPF1*),⁵ *SUP35*, *SUP45*, and *SAL5* loci (47). This unusual complementation pattern and the fact that *sal6* and *sup45* mutants had a synergistic interaction leading to a cold-sensitive phenotype suggested that the proteins encoded by all these genes interact. Indeed, we now know that Sup35p, Sup45p, and Upf1p do interact in a complex (see below), and it is possible that Sal6p, a PP1-serine threonine phosphatase with a long Ser-Asp-rich N-terminal extension (48), is also part of or may modify proteins in this complex. *SAL1* and *SAL5* remain to be cloned.

UPF—Purified eRF1 and eRF3 bind to purified Upf1p, a member of the group I family of helicases (49). Upf2p and Upf3p also appear to complex with Upf1p (50). It was proposed that these proteins form a “surveillance complex” that functions first in translational termination, and then, after the dissociation of the RF factors, in the decay of mRNA containing premature nonsense codons (49). Upf1p is not required for the maintenance of [PSI⁺] but was found associated with [PSI⁺] aggregates (49). It is thus possible that strong [PSI⁺] elements may stabilize mRNA with premature stops by removing Upf1p from the “surveillance complex” into the [PSI⁺] aggregate. Although a strong [PSI⁺] strain has not yet been examined, no difference in mRNA stability was observed when an isogenic weak [PSI⁺] and [psi⁻] strain were compared.⁵

ASU—Antisuppressor mutations in *ASU9* reduce the efficiency of *sup45* and *sup35* suppressors but have no effect on other suppressors, whereas mutations in *ASU10* act only on *sup35* (51, 52). Furthermore, the fact that the *asu9-1* mutation reduces the paromomycin sensitivity of *sup45-2* even though it causes sensitivity to paromomycin in the absence of *sup45-2* suggests a physical interaction between Asu9p and Sup45p.

Does [PSI⁺] Exist in Other Organisms and Are There Other Roles for Sup35p?

The analysis of Sup35p homologs from humans (53), *Xenopus laevis* (44), *Podospora anserina* (54), and *Pichia pinus* (55) suggests that the C-terminal region of Sup35p is highly conserved (approximately 60% similarity between different species), and it is generally assumed to function as eRF3 in all eukaryotes. The N termini of the Sup35 proteins are not well conserved (approximately 40% similarity), and only the amino acid composition is generally similar. It is still possible that the N-terminal extensions each confer prion properties despite the lack of sequence similarity, although this has not yet been demonstrated in any organism except *Saccharomyces cerevisiae*. If it turns out that the prion nature of Sup35p is generally conserved, it would suggest that the prion conversion is associated with a cellular function either in translation termination or in another process in which Sup35p participates. It will be of great interest to define this function.

Sup35p may have functions distinct from its role in the termination of translation. The potential role of Sup35p and [PSI⁺] in nonsense-mediated mRNA decay was discussed above. It has also been suggested that Sup35p may be involved in the control of the cell cycle (56) and processes involving microtubules (57). Finally, deletion of the Sup35p N-terminal region in *P. anserina* alters the sexual cycle of this organism (54).

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⁵ A. Jacobson, unpublished results.