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Translation termination efficiency can be regulated in *Saccharomyces cerevisiae* by environmental stress through a prion-mediated mechanism

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[PSI$^+$] **is a protein-based heritable phenotype of the yeast *Saccharomyces cerevisiae* which reflects the prion-like behaviour of the endogenous Sup35p protein release factor. *[PSI$^+$]* strains exhibit a marked decrease in translation termination efficiency, which permits decoding of translation termination signals and, presumably, the production of abnormally extended polypeptides. We have examined whether the *[PSI$^+$]*-induced expression of such an altered proteome might confer some selective growth advantage over *[psi$^-$]* strains. Although otherwise isogenic *[PSI$^+$]* and *[psi$^-$]* strains show no difference in growth rates under normal laboratory conditions, we demonstrate that *[PSI$^+$]* strains do exhibit enhanced tolerance to heat and chemical stress, compared with *[psi$^-$]* strains. Moreover, we also show that the prion-like determinant *[PSI$^+$]* is able to regulate translation termination efficiency in response to environmental stress, since growth in the presence of ethanol results in a transient increase in the efficiency of translation termination and a loss of the *[PSI$^+$]* phenotype. We present a model to describe the prion-mediated regulation of translation termination efficiency and discuss its implications in relation to the potential physiological role of prions in *S.cerevisiae* and other fungi.**

Keywords: environmental stress/phenotype/prion/yeast (*Saccharomyces cerevisiae*)

Introduction

It is some 30 years since the extrachromosomal determinant *[PSI$^+$]* was first described in the yeast *Saccharomyces cerevisiae* as a modifier of nonsense suppression (Cox, 1965). The *[PSI$^+$]* determinant confers a dominant allosuppressor phenotype, with *[PSI$^+$]* strains exhibiting a marked increase in the readthrough of mutant translation termination codons (Cox *et al.*, 1988). In 1994, Wickner proposed that *[PSI$^+$]* may represent an example of protein-based inheritance, whereby the heritable element behaves like an endogenous 'prion-like' determinant (Wickner, 1994). The term prion was first used to describe the proteinacious agent thought to be solely responsible for the family of neurodegenerative diseases, known as the transmissible spongiform encephalopathies (Griffith, 1967; Prusiner, 1982; Weissman, 1996; Horwich and Weissman, 1997).

In yeast, the *[PSI$^+$]* factor is a product of the *SUP35* gene (Chernoff *et al.*, 1993; Doel *et al.*, 1994; Ter-Avanesyan *et al.*, 1994) which encodes eRF3 (Sup35p), an essential eukaryotic polypeptide release factor. Eukaryote translation termination is mediated by a soluble cytoplasmic complex, which encompasses eRF3 and at least one other factor, namely eRF1 [Sup45p] (Stansfield *et al.*, 1995a; Zhouravleva *et al.*, 1995). As well as folding into its native structure, Sup35p is believed to be capable of adopting a second aberrant conformation, which manifests as the prion-associated phenotype (Chernoff *et al.*, 1995; Paushkin *et al.*, 1996; Tuite and Lindquist, 1996). In *[PSI$^+$]* strains, Sup35p is present both as a soluble factor and as large intracellular aggregates, resulting from the propensity of the prion conformer to coalesce (Patino *et al.*, 1996; Paushkin *et al.*, 1996). The resulting intracellular depletion of soluble termination factors facilitates the decoding of termination signals by mutant nonsense suppressor tRNAs, or by near-cognate aminoacyl-tRNAs, giving rise to the prion-associated allosuppressor phenotype. Intriguingly, both Sup35p and the mammalian prion protein (PrP) possess a conserved N-terminal domain, comprising several imperfect glutamine-rich repeats (Oesch *et al.*, 1985; Kushnirov *et al.*, 1988). Whilst this motif is not a prerequisite for the aetiology of the PrP-associated diseases (Fischer *et al.*, 1996), the N-terminal domain of Sup35p is essential for the maintenance of *[PSI$^+$]* (Ter-Avanesyan *et al.*, 1994). Although the prion-inducing domain of Sup35p is essential for neither translation termination nor viability, it is nonetheless conserved and expressed in *S.cerevisiae* (Ter-Avanesyan *et al.*, 1993; Doel *et al.*, 1994).

Whilst no detrimental phenotype has been associated with *[PSI$^+$]*, neither has a benefit been ascribed to the presence of the prion-inducing domain of Sup35p and its prion-associated phenotype. One possible scenario is that the conservation of this prion-associated domain may represent a novel means of environmental adaptation, whereby *[PSI$^+$]*-induced allosuppression permits decoding of translation termination signals, resulting in an altered pattern of gene expression and the 'profitable' generation of abnormally extended polypeptides (Lindquist, 1997). In an effort to identify such a phenotypic advantage, the stress tolerance of otherwise isogenic *[PSI$^+$]* and *[psi$^-$]* strains was compared. A strain bearing a nuclear allosuppresor mutation of the *SUP35* gene (*sal3-4*) was also studied, since this mutation elicits a similar translation termination deficiency as the *[PSI$^+$]* element, without the associated polymerization of eRF3. Using the *ade2-1/SUQ5* (Cox, 1965) and *lacZ*-'readthrough' reporter systems (Firoozan *et al.*, 1991; Stansfield *et al.*, 1995b), it was possible to study allosuppression in both *[PSI$^+$]* and *sal3-4* strains, when grown under both 'resting' and 'stressed' conditions. Our findings suggest that *[PSI$^+$]*

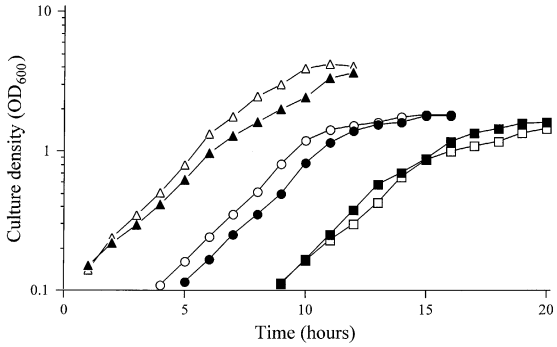

Fig. 1. *[PSI$^+$]* and *[psi$^-$]* variants of *S.cerevisiae* strains show no difference in growth under resting conditions. The exponential growth of the *[PSI$^+$]* (filled symbols) and *[psi$^-$]* (empty symbols) variants of three strains was compared. Strains (●○) BSC 783/4a, (▲△) MT 766/12a and (■□) BSC 772/9d were grown in minimal medium at 30°C.

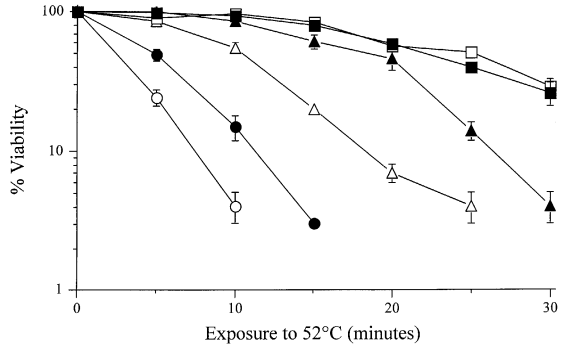

Fig. 2. *[PSI$^+$]* strains exhibit an enhanced tolerance to thermal stress. The viability of *[PSI$^+$]* (filled symbols) and *[psi$^-$]* (empty symbols) variants of three strains (●○) BSC 783/4a, (▲△) MT 766/12a and (■□) BSC 772/9d was monitored, upon exposure to a lethal temperature of 52°C, following a 1 h pre-treatment at 37°C. At regular intervals, aliquots were removed from the cultures at 52°C and put on ice. Samples were diluted and then spread in triplicate onto solid YEPD medium to generate a viable count. Each sample gave rise to ~400 cells per plate, and the error bars illustrate the variation between three viable counts per sample.

strains do exhibit a phenotypic difference from *[psi$^-$]* strains, namely enhanced tolerance to physical and chemical stresses, and we conclude that the prion-like behaviour of Sup35p may facilitate the adaptation of yeast to new growth environments.

Results

[PSI$^+$] does not influence growth rate

The only phenotypic difference between *[PSI$^+$]* and *[psi$^-$]* strains so far described is the enhanced efficiency of nonsense suppression (Cox, 1965) and the elevation of stop codon readthrough in quantitative assays (Firoozan *et al*., 1991) in *[PSI$^+$]* cells. Given that >90% of the underlying Sup35p protein—an essential translation termination factor—is present as non-functional aggregates in *[PSI$^+$]* strains (Patino *et al*., 1996; Paushkin *et al*., 1996), then one might expect *[PSI$^+$]* strains to have an associated slow growth phenotype. Three *[PSI$^+$]* strains were selected on the basis of their difference in genetic background and for the presence of the *ade2-1/SUQ5* reporter system (Cox, 1965). The *[psi$^-$]* variant of each strain was induced by growth in the presence of 2.5 mM guanidine hydrochloride, a compound which induces a *[PSI$^+$]* to *[psi$^-$]* reversion with near 100% efficiency (Tuite *et al*., 1981). The exponential growth of the three pairs of *[PSI$^+$]* and *[psi$^-$]* strains was compared in minimal medium at 30°C (Figure 1). As demonstrated by the identical growth profiles of the *[PSI$^+$]* and *[psi$^-$]* variant of each of three strains, it is clear that the prion-like determinant *[PSI$^+$]* does not influence the exponential propagation of yeast in typical laboratory culture conditions, with the doubling times of each pair being essentially identical.

[PSI$^+$] strains exhibit an enhanced thermotolerance

Whilst *[PSI$^+$]* does not elicit any obvious change in growth rate, it was considered that the prion-associated termination deficiency might manifest as an activation of the cellular stress response. The stress response machinery acts to protect cells against the detrimental effects of changes in the extracellular environment, such as an increase in temperature or ethanol concentration. Stresses that induce the synthesis of aberrant proteins also lead to the overexpression of a particular subset of proteins, namely the heat shock or stress proteins. For example, in yeast, the antibiotic paromomycin, which stimulates mistranslation on cytoplasmic ribosomes, also induces the heat shock response (Grant *et al*., 1989). In a termination-deficient *[PSI$^+$]* background, the production of abnormal C-terminally extended proteins might also be expected to trigger such a response. Alternatively, the presence of aggregated Sup35p itself might trigger the stress response.

Thermotolerance assays were used, therefore, in order to quantify any stress response conferred by the *[PSI$^+$]* determinant. Strains subjected to a short period of stress or experiencing a constant stimulus of the stress response will display an enhanced viability upon exposure to a lethal temperature, reflecting an elevation of the intracellular concentration of heat shock proteins (Sanchez and Lindquist, 1990; Mager and Ferreira, 1993). Thermotolerance analysis of the three pairs of strains demonstrated that, for two of the three pairs, the *[PSI$^+$]* strains exhibited a significant increase in thermotolerance, in comparison with the corresponding *[psi$^-$]* variants (Figure 2). However, in the third strain examined (BSC 772/9d), no difference in thermotolerance between the *[PSI$^+$]* and *[psi$^-$]* variants was observed. This particular strain has a 'naturally' high degree of thermotolerance (LD_{50} of 25 min at 52°C; Figure 2) which we assume masks any effect *[PSI$^+$]* may have had on thermotolerance. Given the integral relationship between *[PSI$^+$]* and Hsp104p (Chernoff *et al*., 1995), strain MT 766/12a was employed to address the role, if any, of Hsp26p in the maintenance of *[PSI$^+$]* or the stress-induced prion-mediated regulation of translation termination efficiency. Clearly, this *HSP26*-disruptant strain can support *[PSI$^+$]*, thereby demonstrating that Hsp26p is not essential for *[PSI$^+$]* maintenance. Our data support previous studies which demonstrate that Hsp26p has little, if any, role in thermotolerance (Figure 2) (Tuite *et al*., 1990). Whilst *[PSI$^+$]* strains exhibited an enhanced thermotolerance, Western blot analyses failed to reveal any difference in the endogenous level of the heat shock proteins Hsp104, Hsp70 and Hsp26 between the *[PSI$^+$]*

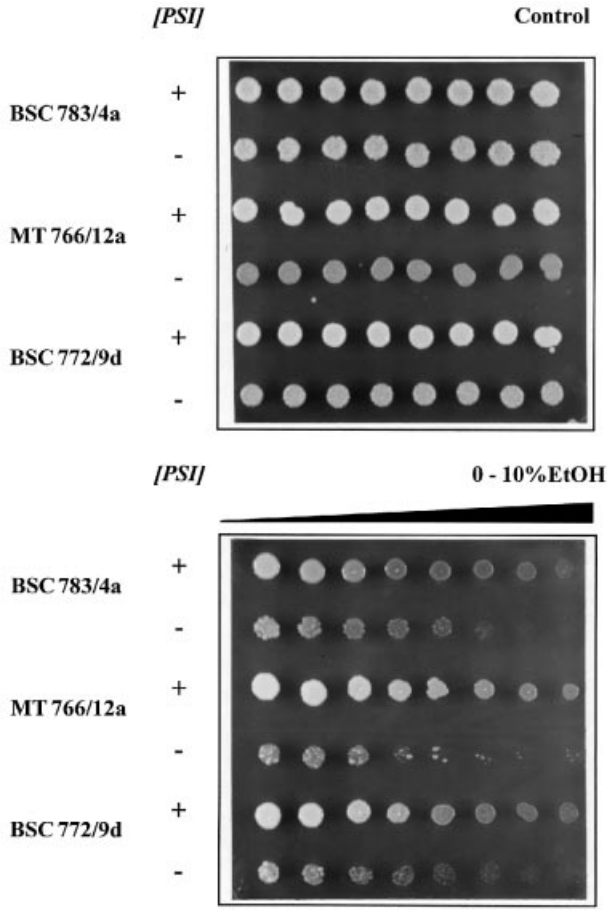

Fig. 3. *[PSI$^+$]* strains exhibit an enhanced tolerance to ethanol. The stress tolerance of the *[PSI$^+$]* and *[psi$^-$]* variants of each of the three test strains was compared by growth upon solid YEPD medium, containing a gradient of 0–10% (v/v) ethanol. Strains suspensions were normalized with respect to cell density, and equal volumes (i.e. numbers) of cells were spotted either onto solid 1/4YEPD medium (control) or onto a plate containing a gradient of 0–10% ethanol. Plates were then incubated at 30°C for 5 days.

and *[psi$^-$]* variants when grown at 25°C. Similarly, whilst an elevation of Hsp104p expression was detected upon heat shock at 37°C, no significant difference was observed between *[PSI$^+$]* and *[psi$^-$]* strains (unpublished data).

[PSI$^+$] strains exhibit enhanced chemotolerance

Having demonstrated that *[PSI$^+$]* enhances thermotolerance, we next asked whether *[PSI$^+$]* enhanced tolerance to other forms of stress. *In vivo*, some components of the stress response machinery do confer tolerance to more than one form of stress. For example, Hsp104p is known to be the principal agent that confers tolerance to both heat and ethanol (Sanchez and Lindquist, 1990; Sanchez *et al*., 1992; Piper, 1995). The growth of the *[PSI$^+$]* and *[psi$^-$]* variants was therefore compared on gradient agar plates which contain an increasing concentration of stress reagent in a rich growth medium. As with thermotolerance, the *[PSI$^+$]* strains exhibited a greater degree of tolerance to 'chemical stress' than the *[psi$^-$]* variants. The most marked enhancement of chemotolerance was observed when the strains were grown on gradients of ethanol (Figure 3). In contrast, no difference in growth was observed between the *[PSI$^+$]* and *[psi$^-$]* variants when grown on gradients of glycerol or salts such as sodium, potassium or magnesium chloride (unpublished data). Therefore, *[PSI$^+$]* strains appear to show a higher degree of resistance to potential environmental abuses than the corresponding *[psi$^-$]* strains.

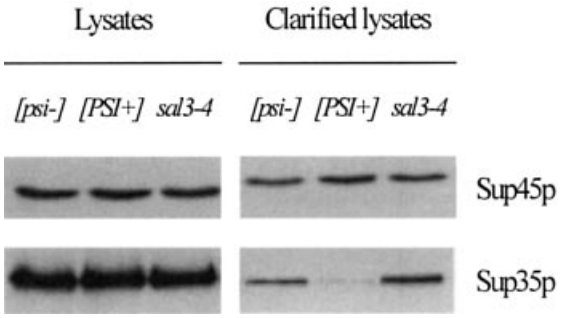

Fig. 4. The *sal3-4* mutant encodes a soluble part-functional component of the eukaryote release factor. The sedimentation properties of the two eukaryotic release factors, Sup35p and Sup45p, were determined in the *[PSI$^+$]* and *[psi$^-$]* variants of strain BSC 783/4c and in a mutant strain bearing the nuclear allosuppressor allele, *sal3-4*. Proteins were detected by Western blot analyses of whole-cell lysates and the soluble fraction of total cell extracts, following the clarification of lysates by centrifugation.

Table I. Quantification of termination efficiency in strains bearing the *[PSI$^+$]* and *sal3-4* allosuppressor determinants, upon growth in a stress-inducing medium

	% Readthrough		
	[psi$^-$]	*[PSI$^+$]*	*[psi$^-$] sal3-4*
0% Ethanol	4.7 ± 1.0	23.8 ± 5.7	18.5 ± 3.8
7% Ethanol	3.9 ± 0.8	7.7 ± 1.5	15.6 ± 3.0

The level of termination signal readthrough was measured in strain BSC 783/4c, using a β-galactosidase reporter system (described by Stansfield *et al*., 1995b). Readthrough of termination codons was quantified using the plasmid pUKC817 (which carries the *lacZ* gene that bears a premature termination codon) and expressed as a proportion of control β-galactosidase levels, measured in transformants carrying the control plasmid pUKC815 (which carries the wild-type *lacZ* gene).

Allosuppression enhances stress tolerance

To determine how the *[PSI$^+$]* determinant elicits an enhanced stress tolerance, the thermo- and chemotolerant properties of a [*PSI$^+$*] strain and a [*psi$^-$*] strain bearing the *sal3-4* mutation were compared. The *sal3-4* mutation lies in the *SUP35* gene (Doel *et al*., 1994), presumably within the functional C-terminal domain of the encoded eRF3, thereby leading to a termination defect not through Sup35p aggregation, but from biochemical malfunction. Western blot analysis of the *sal3-4* mutant strain, using polyclonal antiserum raised against Sup35p, confirmed that unlike the *[PSI$^+$]* strains under examination, the *sal3-4* allele encodes primarily soluble Sup35p (Figure 4). The *[PSI$^+$]* strain BSC 783/4c and the *sal3-4* allosuppressor mutant elicited a similar degree of termination codon readthrough (Table I) and thus are presumed to elicit the same degree of production of C-terminally extended polypeptides. Like the *[PSI$^+$]* strains, the *sal3-4* mutant did not exhibit an impairment of exponential growth (unpublished data). The *sal3-4* mutant also showed

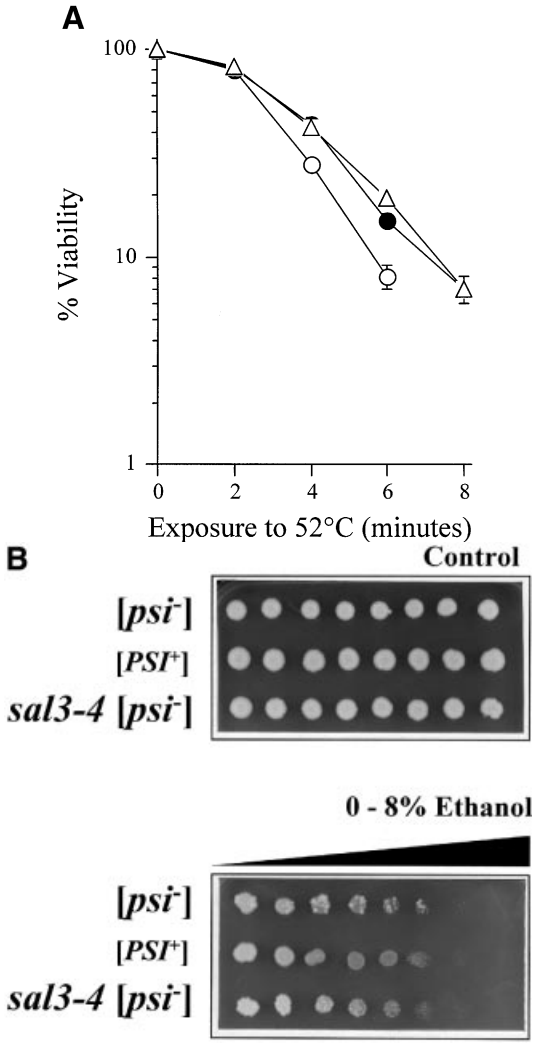

Fig. 5. The prion-like determinant *[PSI⁺]* and the nuclear allosuppressor mutation *sal3-4* both elicit enhanced tolerance to stress. (**A**) The thermotolerance of the *[PSI⁺]* (●) and the *[psi⁻]* (○) variants of BSC 783/4c was compared with that of a *[psi⁻]* variant of BSC 783/4c that bears the nuclear allosuppressor allele *sal3-4* (△). Cultures were exposed to a lethal temperature (52°C), following a 1 h pre-treatment at 37°C. At regular intervals, culture aliquots were removed from 52°C and put on ice. Samples were then diluted and spread in triplicate onto solid YEPD medium to generate a viable count. Each sample gave rise to ~400 cells per plate, and the error bars represent the variation between three viable counts per sample. (**B**) The ethanol tolerance of the BSC 783/4c variants was also compared by studying their growth on solid rich medium supplemented with ethanol. Strain suspensions were normalized with respect to cell density, and equal volumes (i.e. numbers) of cells were spotted either onto solid 1/4YEPD medium (control) or onto a plate containing a gradient of 0–8% ethanol. Plates were incubated for 5 days at 30°C.

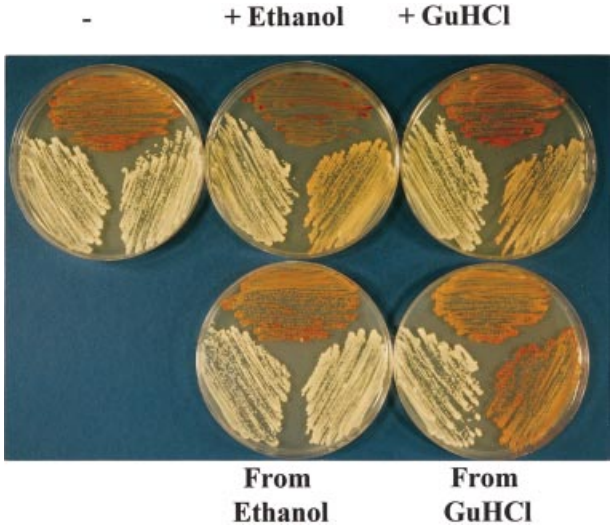

Fig. 6. Stress induces a reversible antisuppression in *[PSI⁺]* strains, but not in strains bearing the nuclear allosuppressor mutation *sal3-4*. Top row: the variants of strain BSC 783/4c were grown on control 1/4YEPD medium (left) and medium supplemented with either 5% ethanol (middle) or 2.5 mM guanidine hydrochloride (right). Each plate: strain BSC 783/4c, clockwise from the top *[psi⁻]*, *[PSI⁺]* and *[psi⁻] sal3-4*. Bottom row: variants were then re-streaked from stress-inducing media (top row) onto control 1/4YEPD medium. By virtue of the *ade2-1/SUQ5* genetic markers, variants that exhibit a translation termination deficiency (i.e. nonsense suppression) develop as white colonies, whereas strains efficient for translation termination accumulate a red pigment.

enhanced stress tolerance (Figure 5), thereby demonstrating that it is a deficiency of translation termination that is the principal trigger for an elevated intracellular stress response in *[PSI⁺]* strains, rather than the presence of Sup35p aggregates.

[PSI⁺] strains exhibit antisuppression when stressed

Intriguingly, *[PSI⁺]* strains exhibited a transient loss of their allosuppressor phenotype when grown on certain 'chemical' media. That is to say, *[PSI⁺]* strains bearing the *SUQ5/ade2-1* reporter genes gave rise to pink or red colonies (reflecting an enhanced efficiency of translation termination) when grown on stress-inducing, ethanol-containing media (Figure 6). This stress-induced 'antisuppression' was not simply 'curing' of the *[PSI⁺]* prion (as is induced by guanidine hydrochloride; Tuite *et al*., 1981), since the stressed *[PSI⁺]* strains reverted to an allosuppressor phenotype upon transfer to ethanol-free media (Figure 6). Growth in the presence of the stress-inducing agent did not result in the heritable loss of the *[PSI⁺]* determinant, despite the transient loss of the *[PSI⁺]* phenotype. The degree of stress-induced antisuppression varied between the various *[PSI⁺]* strains, but antisuppression was invoked readily in *[PSI⁺]* strains by growth in the presence of ethanol (Table I). It is noteworthy that strain MT 766/12a also exhibited a transient antisuppressor phenotype when grown at 37°C (unpublished data). In contrast, a strain bearing the nuclear allosuppressor mutation *sal3-4* failed to exhibit antisuppression in response to stress (Table I, Figure 6). Thus, strains bearing the prion-like determinant *[PSI⁺]* exhibit a reversible decrease in termination codon readthrough (i.e. antisuppression) when grown under stress which, unlike growth on guanidine hydrochloride, does not result from the elimination of the *[PSI⁺]* determinant. Western blot analysis of Sup35p in *[PSI⁺]* cells exposed to ethanol did not reveal a significant increase in the levels of soluble Sup35p (data not shown). This suggests that the *[PSI⁺]* phenotype can be transiently reversed by the low level of newly synthesized Sup35p molecules which are unable to enter aggregates or, alternatively, by the release of previously synthesized Sup35p molecules from the pre-formed *[PSI⁺]* aggregates.

Discussion

The two yeast prion elements so far described, namely *[PSI$^+$]* and *[URE3]*, may both serve as regulators of different cellular physiological processes, namely translation termination and nitrogen catabolism, respectively (Cox, 1994; Wickner and Masison, 1996). Since this realization, there has been considerable speculation as to the much wider role of prions in biological systems (Patino *et al.*, 1996; Tuite and Lindquist, 1996; Wickner and Masison, 1996; Lindquist, 1997). Indeed, this notion appears to be supported by the recent description of a prion-like element, which confers heterokaryon incompatability, in the filamentous fungus *Podospora anserina* (Coustou *et al.*, 1998). If prion elements and their associated heritable traits are prevalent throughout different species, they should presumably confer some benefit to the organism carrying them. By studying the growth of *[PSI$^+$]* and *[psi$^-$]* yeast strains under adverse environmental conditions, we have demonstrated that the yeast prion *[PSI$^+$]* does indeed confer a beneficial phenotypic difference, namely that of enhanced tolerance to environmental stress.

Both the prion-like determinant *[PSI$^+$]* and the nuclear allosuppressor mutation *sal3-4* are derived from the *SUP35* gene and confer a partial translation termination defect to yeast (Table I). In this study, we have shown that the *sal3-4* mutation elicits an increase in translation termination codon readthrough, similar to that conferred by the *[PSI$^+$]* determinant, and that the *sal3-4* allosuppressor mutation results in the expression of a soluble, but partially non-functional polypeptide release factor. This is different from *[PSI$^+$]*-induced allosuppression that arises from the prion-like propensity of a conformer of Sup35p to aggregate, which results in the intracellular depletion of functional soluble termination factor (Paushkin *et al.*, 1996, 1997; Tuite and Lindquist, 1996). Both prion- and nuclear mutation-induced allosuppression produce an enhancement of thermo- and chemotolerance. Plausibly, the readthrough of translation termination codons elicits an enhancement of stress tolerance via a constitutive stress response, which is invoked by the presence of proteins that have misfolded C-terminal extensions. This notion has also been applied to account for the evolution of a tRNA^{Ser} of *Candida albicans* which inserts serine at the leucine-encoding codon CUG (Santos *et al.*, 1996). Transformation of *S.cerevisiae* with a gene encoding this recoded tRNA generates an alternative 'mistranslated' proteome that also results in an enhancement of stress tolerance.

Rather than increasing stress tolerance by triggering a constitutive stress response, it might be suggested that *[PSI$^+$]*-induced allosuppression (and indeed the same would be true of the *sal3-4* mutation) permits the production of one or more novel protein(s) which bear an extra C-terminal domain. The modified biochemical properties of such elongated polypeptides might directly enhance stress tolerance; for example, if these extended polypeptides stabilized the cell membrane. Whilst *[PSI$^+$]* leads to an increased tolerance of *S.cerevisiae* to adverse environmental conditions, *[PSI$^+$]* is not a regulator of the stress response. The potentially beneficial phenotype of enhanced stress tolerance is a consequence of *[PSI$^+$]*-induced allosuppression. Whereas strains bearing the *sal3-4* allosuppressor mutation do not exhibit a significant change in termination efficiency in response to increased environmental stress, the prion-like element *[PSI$^+$]* mediates a reversible, stress-induced increase in translation termination efficiency (Figure 6; Table I). Paradoxically, the elevated intracellular stress response which results from the prion-induced production of extended polypeptides (and manifests as an enhancement in thermotolerance) is selected against in an adverse environment since the generation of misfolded proteins is disfavoured by the prion-mediated decrease in the extent of translation termination codon readthrough.

Deletion analysis has revealed that whilst the N-terminal domain of Sup35p is essential for the maintenance of *[PSI$^+$]*, it is not essential for cell viability, and thus appears to play no direct functional role in translation termination (Ter-Avanesyan *et al.*, 1993, 1994). However, this glutamine-rich domain of Sup35p has been conserved in *S.cerevisiae*, either as an indirect result of the *[PSI$^+$]* phenotype or because of some as yet unidentified function. Whilst *[PSI$^+$]* recently has been described as a 'laboratory-confined disease' of *S.cerevisiae* (Chernoff *et al.*, 1998), we have demonstrated that the prion element *[PSI$^+$]* does not infleunce exponential growth, but does actually confer a beneficial phenotype to yeast, namely enhanced stress tolerance. Intriguingly, there is evidence to suggest that Sup35p does serve a role in eukaryotes other than that of a polypeptide release factor. For example, Sup35p has been identified as a putative cell cycle factor of *S.cerevisiae* (Kikuchi *et al.*, 1988), and recently the *Drosophila* homologue of yeast Sup35p has been shown to mediate meiotic spindle assembly (Basu *et al.*, 1998).

In *S.cerevisiae*, *[PSI$^+$]* represents a novel mechanism for the inheritence of a regulated translation termination deficiency. Clearly, the lethal consequences of a nonsense mutation in an essential gene would be overcome in an allosuppressor background. *[PSI$^+$]* is an omnipotent allosuppressor determinant, in that it enhances nonsense suppression of all three termination codons (Firoozan *et al.*, 1991), thus *[PSI$^+$]* would be expected to offer protection against most potentially lethal nonsense mutations. One such example has been described in which a strain exhibits a *[PSI$^+$]* dependence, since it bears a nonsense mutation within the essential gene *HSF1*, which encodes the heat shock transcription factor (Lindquist and Kim, 1996). Alternatively, *[PSI$^+$]* might permit the regulated expression of abnormally extended polypeptides, which results from the extended decoding of open reading frames (ORFs) by by-passing ORF-defining termination codons. Genome analysis has revealed the existance of several potentially 'readthrough-regulated' genes (Lindquist, 1997) but, as a caution, it should be noted that translation termination is not only regulated by the interaction of release factors with termination codons. In particular, the efficiency of nonsense suppression, and hence the expression of any abnormally extended polypeptide, will also be governed by the presence and properties of endogenous nonsense suppressor tRNA and by the 'context' of the stop signal within the mRNA (Fearon *et al.*, 1994; Bonetti *et al.*, 1995). Our data suggest that *[PSI$^+$]* does not promote the alternative expression of genes whose products are absolutely required for

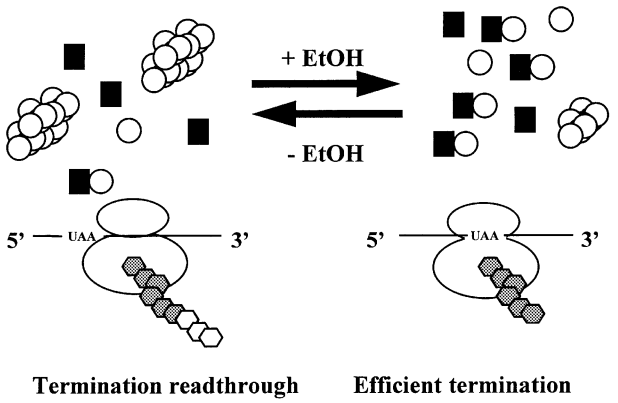

Fig. 7. A model for the prion-mediated regulation of translation termination. In a *[PSI$^+$]* background, efficient translation termination is prevented by the intracellular polymerization of Sup35p and depletion of a functional eRF3 (Sup35p) (○)–eRF1 (Sup45p) (■) termination factor complex. An increase in environmental stress, such as an increase in ethanol concentration, transiently elevates the production of stress proteins (e.g. Hsp104), which mediates the partial resolubilization of Sup35p aggregates. The emergence of soluble Sup35p elicits an increase in translation termination efficiency. Upon restoration of the cell to a normal environment, the expression of stress proteins subsides and allosuppression is restored by the sequestration of Sup35p into 'aggregates' by residual *[PSI$^+$]* seeds.

growth under adverse conditions. Indeed, the reversible antisuppression induced by stress may serve to reduce termination codon readthrough, thereby preventing the harmful production of extended polypeptides. The *FLO8* gene has been identified as a likely 'regulated' template in the strain S288C (Liu *et al.*, 1996; Lindquist, 1997). This gene product is essential for filamentous growth, and yet this strain bears an apparently 'internal' termination codon. Whilst *[PSI$^+$]*-induced allosuppression would be expected to elicit the expression of 'full-length' Flo8p and permit filamentous growth of S288C, we predict that the environmental conditions that normally induce filamentous growth would also invoke the transient shift of the *[PSI$^+$]* strain to an antisuppressor state, thereby reducing nonsense suppression of termination codons and precluding pseudohyphae formation.

As proposed by Lindquist (1997), the interplay between stress proteins (Hsp104p) and prions does provide a plausible molecular mechanism for a cell to respond to its environment with a heritable change in phenotype. We would expand this concept to suggest that the yeast prion *[PSI$^+$]* actually represents a mechanism for the regulation of translation termination efficiency in response to changes in enviromental conditions, rather than a simple switching between a *[PRION$^+$]* and *[prion$^-$]* heritable phenotype. Cells possess specific mechanisms for tolerating adverse environmental conditions and for the recovery and elimination of stress-denatured proteins. One such mechanism in *S.cerevisiae* involves the action of the stress protein Hsp104p, which is known to be important for protecting yeast against the detrimental effects of heat and alcohol (Sanchez and Lindquist, 1990; Sanchez *et al.*, 1992; Piper, 1995). Moreover, Hsp104p is a prerequisite for the maintenance and propagation of *[PSI$^+$]* (Chernoff *et al.*, 1995). We propose that the stress-mediated regulation of translation termination efficiency in *[PSI$^+$]* strains arises from the increased levels of stress proteins which mediate the partial regeneration of functional Sup35p and hence a transient increase in translation termination efficiency. Thermal and ethanol stress induce the elevated expression of Hsp104p and Ssa1p (Hsp70p) (Mager and Ferreira, 1993; Piper, 1995), which is known to result in the loss of the loss of *[PSI$^+$]*-induced allosuppression (Chernoff *et al.*, 1995; Paushkin *et al.*, 1996). We postulate that Hsp104p is the primary factor in *[PSI$^+$]*-meditated regulation of translation termination efficiency, in response to environmental stress. Clearly, not all stress-induced proteins have a role in this phenomenon, e.g. Hsp26p (this study). As a regulatory mechanism, the interplay between the stress proteins and the prion protein is such that the *[PSI$^+$]* determinant is not eliminated from the cell. Upon removal of the environmental stress and the subsequent decrease in the intracellular level of stress proteins, Sup35p is sequestered by residual 'seeds' into large intracellular polymers. The corresponding depletion of soluble Sup35p results in a decrease in translation termination efficiency and the restoration of the *[PSI$^+$]* allosuppressor phenotype (Figure 7).

Materials and methods

Strains

Three *[PSI$^+$]* strains were selected on the basis of their difference in genetic background and for the presence of two specific genetic elements: (i) the ochre suppressor serine-inserting tRNA encoded by the *SUQ5* gene; and (ii) the *ade2-1* mutation, which permit a direct visualization of allosuppression status by colony colour (Cox, 1965). Strains exhibiting efficient translation termination give rise to red colonies, whereas those strains displaying an allosuppressor phenotype (i.e. termination inefficient) grow white. The three strains were rendered prion-deficient (i.e. *[psi$^-$]*) on the basis of a stable white to red colony colour change that occurs upon growth on media containing 2.5 mM guanidine hydrochloride (Tuite *et al.*, 1981).

The genotypes of the four strains used in these studies were: BSC 783/4a, *SUQ5, ade2-1, ura3-1, his3-11, his3-15, leu2-3, leu2-112, MAT*a; BSC 783/4c, *SUQ5, ade2-1, ura3-1, his3-11, his3-15, leu2-3, leu2-112, MAT*a; BSC772/9d, *SUQ5, ade2-1, ura3-1, his4-166, leu2-2, lys1-1, can1-100, MAT*a; and MT 766/12a, *SUQ5, ade2-1, ura3-1, hsp26::HIS3, MAT*a

Growth media

Yeast strains were grown either in liquid YEPD complete medium [2% (w/v) glucose, 1% (w/v) Bacto-peptone, 1% (w/v) yeast extract] or defined minimal medium (0.67% Difco defined minimal medium without amino acids, 2% glucose) supplemented with the appropriate amino acids and cofactors (20 mg/l, except adenine which was added at 200 mg/l). For colony colour enhancement, yeast strains were grown on 1/4YEPD solid medium [4% (w/v) glucose, 1% (w/v) Bacto-peptone, 0.25% (w/v) yeast extract, 2% (w/v) agar]. The reduced content of yeast extract in 1/4YEPD ensures the maximum accumulation of the chromogenic adenine biosynthetic precursor. For studies using ethanol-supplemented media, strains were grown in flasks sealed with rubber bungs to reduce evaporation of the alcohol from the medium.

Sedimentation analysis of polypeptide release factors

Yeast strains were grown to an OD_{600} of 0.5, harvested and lysed with glass beads at 4°C, in buffer [25 mM Tris–HCl pH 8, 150 mM NaCl, 5 mM EDTA, 5 mM phenylmethylsulfonyl fluoride (PMSF), complete protease inhibitor mix (Boehringer Mannheim, one tablet per 5 ml of buffer)]. Lysates were then clarified by centrifugation for 10 min at 13 000 *g* and stored on ice. Samples of lysate and clarified lysate were boiled for 10 min, after the addition of 5× SDS–PAGE sample buffer [2.5 ml of glycerol, 1.25 ml of 20% (v/v) SDS, 0.25 ml of 2-mercaptoethanol, 1.25 ml of 0.5 M Tris–HCl pH 6.8]. Samples were cooled on ice and then spun for 5 min at 13 000 *g*. Protein samples were analysed using 10% SDS–polyacrylamide gels and electrophoretically transferred to nitrocellulose for Western blot analysis, employing polyclonal sera raised against yeast Sup35p and Sup45p expressed in *Escherichia coli*, as described previously (Stansfield *et al.*, 1995a).

Thermotolerance assays

Strains were grown at 25°C to mid-exponential phase in YEPD (OD_{600} 0.4) and then transferred to 37°C. Following a 1 h pre-treatment, strains were diluted to a density of ~3.5×10^3 cells/ml. Strains were then transferred to a 52°C shaking water bath, whereupon aliquots were removed at regular intervals and stored on ice. Aliquots of 150 µl (~500 cells) were plated in triplicate onto solid YEPD agar, and viable counts were determined after 5 days growth at 30°C.

Chemotolerance assays

A fresh colony of each strain was taken up from a 1/4YEPD plate, resuspended in 500 µl of sterile water and diluted to ~1×10^6 cells/ml. Then, 8×2 µl strain suspensions were spotted onto a chemical gradient plate. Each plate comprised 50 ml of 1/4YEPD agar supplemented with a stress reagent, which was poured 24 h prior to use. The stress agar was allowed to set with the plate tilted, so that there was no stress agar at one end of the plate. The plates were used following the addition of 50 ml of 'top' 1/4YEPD agar as soon as the top agar had set. Following the spotting of the strain suspensions, all gradient plates were sealed to prevent evaporation of stress-inducing agents and then incubated for 5 days at 30°C.

Quantifying allosuppression levels in vivo

Yeast strains were transformed with one of the two 'readthrough' vectors pUKC815 or pUKC817 (Stansfield *et al.*, 1995b). Essentially, the single-copy control vector pUKC815 carries the *lacZ* gene under the control of the constitutive *PGK* promotor. The readthrough vector pUKC817 has an in-frame premature ochre (TAA) termination signal. Strains were grown in appropriate minimal media to an OD_{600} of 0.45, whereupon the cells from 10 ml culture aliquots were harvested and stored at –20°C. Strains were grown in the presence of 7% (v/v) ethanol for at least 24 h, to ensure a true reflection of readthrough levels (i.e. stress exposure was prolonged to permit the 'turnover' of any residual β-galactosidase produced during growth in normal media). The degree of allosuppression was determined as a function of the β-galactosidase levels of the strains bearing the readthrough vectors. The cell pellets from the culture aliquots were resuspended in 600 µl of buffer Z (60 mM Na_2HPO_4, 40 mM NaH_2PO_4, 10 mM KCl, 1 mM $MgSO_4$, pH 7.0) supplemented with EDTA (5 mM final), 2-mercaptoethanol (50 mM final) and PMSF (5 mM final). Cells were lysed at 4°C by vortexing three times for 30 s (with 30 s intervals on ice) in the presence of an equal volume of glass beads. β-Galactosidase and Bradford assays were then performed on lysates clarified by centrifugation (10 min, 13 000 *g*, 4°C) as described previously (Stansfield *et al.*, 1995b).

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