A yeast prion provides a mechanism for genetic variation and phenotypic diversity

Heather L. True & Susan L. Lindquist

Department of Molecular Genetics and Cell Biology, Howard Hughes Medical Institute, The University of Chicago, Chicago, Illinois 60637, USA

A major enigma in evolutionary biology is that new forms or functions often require the concerted effects of several independent genetic changes. It is unclear how such changes might accumulate when they are likely to be deleterious individually and be lost by selective pressure. The *Saccharomyces cerevisiae* prion [*PSI*$^+$] is an epigenetic modifier of the fidelity of translation termination, but its impact on yeast biology has been unclear. Here we show that [*PSI*$^+$] provides the means to uncover hidden genetic variation and produce new heritable phenotypes. Moreover, in each of the seven genetic backgrounds tested, the constellation of phenotypes produced was unique. We propose that the epigenetic and metastable nature of [*PSI*$^+$] inheritance allows yeast cells to exploit pre-existing genetic variation to thrive in fluctuating environments. Further, the capacity of [*PSI*$^+$] to convert previously neutral genetic variation to a non-neutral state may facilitate the evolution of new traits.

How might several independent genetic changes occur in concert to produce a new form or function? One solution is to posit the existence of mechanisms that allow changes to accumulate in a temporarily neutral fashion. Such a mechanism was proposed in 1972 when Koch[1] extended Ohno's recognition of the importance of gene duplications in the evolution of new functions[2] to include inactivation, modification and reactivation. A duplicated gene that is retained in an inactive state would be free to accumulate variations that might compromise fitness, and beneficial combinatorial change could occur upon reactivation[1]. Gene duplications are common and these genes frequently acquire divergent functions[3–5], indicating that such a mechanism may have operated widely in evolution. However, the known mechanisms for the reactivation of inactive genes work sporadically, act infrequently and provide no obvious means for sampling coding changes in several genes simultaneously[6].

Here we report that a protein-based element of inheritance (a prion) in the yeast *Saccharomyces cerevisiae* provides a means to expose this and other types of silent protein-coding information. The prion [*PSI*$^+$] reduces the fidelity with which ribosomes terminate translation at stop codons in a metastable, heritable manner[7–9]. The reduction in translational fidelity caused by [*PSI*$^+$] is routinely monitored by the production of active products from genetic markers containing stop-codon mutations[7]. Presumably, [*PSI*$^+$] also causes ribosomes to read through some naturally occurring stop-codons, but its biological significance is a mystery.

Despite this uncertainty, a great deal of information has accumulated on the mechanism of [*PSI*$^+$] inheritance because it represents a new class of genetic elements that produce heritable changes in phenotype with no underlying changes in nucleic acids[10]. [*PSI*$^+$] propagates through a self-perpetuating change in the state of Sup35, a translation termination factor[11–14]. In its prion state, Sup35 forms inactive complexes that have the capacity to capture newly made Sup35 and convert it to the same form, perpetuating the loss-of-function phenotype[13].

Sup35 has three distinct regions[15–17]. The carboxyl-terminal region (C) is responsible for the translation-termination activity and is essential for viability[18]. The amino-terminal (N) and middle (M) regions allow Sup35 to acquire a stable prion conformation and switch between [*PSI*$^+$] and [*psi*$^-$] states[13,14,18,19]. The N and M regions can be deleted in cells that do not carry the prion, [*psi*$^-$] cells, with no apparent effect[18]. Deletion of N and M in [*PSI*$^+$] cells results in the loss of the prion and restoration of translational fidelity[18]. The prion-determining region of Sup35 has been retained in distantly related yeast[20–23], indicating that the unusual ability of Sup35 to produce a heritable conformational and phenotypic switch may provide a selective advantage.

Diverse growth phenotypes produced by [*PSI*$^+$]

We compared the growth characteristics of [*PSI*$^+$] and [*psi*$^-$] cells in more than 150 phenotypic assays (Table 1, Supplementary Information, and data not shown) including growth on fermentable and non-fermentable carbon sources (Figs 1, 2a and 3), on simple and complex nitrogen sources in the presence of salts and metals (Fig. 2b), in the presence of inhibitors of diverse cellular processes, such as DNA replication, signalling, protein glycosylation, and microtubule dynamics (Fig. 2c), under general stress conditions, and at different temperatures (Fig. 4). We examined cells from seven different genetic backgrounds to distinguish traits that might be a universal feature of acquiring the [*PSI*$^+$] state from those that might be specific to individual genomes. In each genetic background we created fresh, stable isogenic [*PSI*$^+$] and [*psi*$^-$] pairs by guanidine hydrochloride curing[24]. This procedure ensured that phenotypic differences detected would be due to the direct effects of [*PSI*$^+$] on gene expression and physiology, rather than to secondary genetic changes that might have occurred during long-term culture. Each strain had a different intrinsic level of [*PSI*$^+$]-mediated nonsense suppression (data not shown), as measured with a series of β-galactosidase test constructs[9]. Suppression levels ranged from a low of 0.2, 0.6 and 0.8% (in strain D1142-1A) to a high of 1.7, 4.5 and 16% (in strain 5V-H19) for the UAA, UAG and UGA codons, respectively in the context of this reporter gene.

We also examined NM deletion strains in two backgrounds (74D-694 and 33G-D373). These strains are deleted for the entire N and M regions of Sup35. The NM region of Sup35 might have a function other than prion maintenance, and this function might be inactivated by structural occlusion when the protein acquires the [*PSI*$^+$] state. NM deletion mutants (ΔNM) would exhibit phenotypes associated with the loss of this putative function but not phenotypes due to [*PSI*$^+$], because they are obligately [*psi*$^-$][18].

Yeast cells undergo many physiological changes in the transition between growth stages. Extreme care was taken to ensure that each strain pair was examined at the same growth stage. Mid-log phase cells grown in rich medium (YPD; ref. 25) were serially diluted in

Table 1 **Phenotypic testing of [*PSI*$^+$] / [*psi*$^-$] isogenic strain pairs**

	74D+	74D-	33G+	33G-	SL+	SL -	D11+	D11-	5V+	5V -	10B+	10B-	Bsc+	Bsc-
Carbon sources														
Fermentable														
Dextrose 2% (YPD) pH 6.8	5R	5R	5R	5R	5R	5R	5R	5R	5R	5R	5R	5R	5R	5R
Dextrose (YPD) pH 6	5R	5R	4M	4M	5R	5R	1S	4M	5R	4M	5R	5R	5R	5R
Dextrose (YPD) pH 4.9	4S	4S^c	1S	1S^c	4R	4R	NG	NG	5M	5M	5M	5R	4M	4M
Galactose 2%	5M	5M^c	4S	4S^c	5M	5M	5R	5R	5S	NG	5R	5R	5R	5R
Lactate 2%	5M	5M	2M	4M	5R	5R	NG	NG	5R	5R	5M	5M	5R	5R
Nonfermentable														
K-Acetate 3%	5R	5R	5S	5M^a	5R	5R	4S	4S	5R	5R	5M	5R	5R	5R
Ethanol 3%	5M	5M	2M	4M^c	5R	5R	5R	5S	5R	5R	5M	5M	5R	5R
Nitrogen sources														
allantoin 1 mg ml^{-1}	NG	NG	NG	NG	2S	1S	NG	NG	2S	2S	1V	3S	NG	NG
ammonia 1 mg ml^{-1}	NG	NG	NG	NG	2S	1S	NG	NG	1S	2S	1S	3S	NG	NG
glutamate 1 mg ml^{-1}	4V	4V^c	NG	NG	5V	5V	NG	NG	1S	5S	2S	5S	NG	NG
glutamine 1 mg ml^{-1}	1V	1V^c	NG	NG	4S	4S	NG	NG	NG	5V	5V	5S	NG	NG
ornithine 1 mg ml^{-1}	4S	3S^c	NG	NG	5S	5S	NG	NG	1V	5S	5V	5S	NG	NG
proline 1 mg ml^{-1}	2S	1S^c	NG	NG	4S	4S	NG	NG	NG	5S	2V	5S	NG	NG
serine 1 mg ml^{-1}	2V	1V^c	NG	NG	3S	2S	NG	NG	NG	2S	2V	5S	NG	NG
threonine 1 mg ml^{-1}	2S	1S^c	NG	NG	3S	3S	NG	NG	NG	5S	5V	5S	NG	NG
Salts and metals														
$BaCl_2$ 50 mM	5R	5R	NG	NG	5R	5R	5M	5R	5M	5R	5R	5R	5R	5R
$CaCl_2$ 0.5 M	5M	5M	5M	5M	5M	5M	NG	NG	5V	NG	5M	5R	5M	5M
$CdCl_2$ 20 µM	2S	2M^c	4S	4M^a	2M	5M	5M	3S	5M	5R	2V	4S	NG	NG
$CoCl_2$ 750 µM	5R	5R	5R	5R	5R	5R	5R	5R	5R	5R	5R	5R	5R	5R
CsCl 25 mM	5R	5R	4M	4M^c	5M	5M	3V	4M	5M	5R	5R	5R	5R	5R
CsCl 0.1 M	NG	NG	NG	NG	5R	5R	NG	NG	NG	NG	NG	NG	5R	2R
$CuSO_4$ 2.5 mM	3M	3M	5M	5R	4M	4M	4M	4R	4M	5R	5M	5R	4M	5R
$CuSO_4$ 5 mM	4M	4M^c	3S	3S^c	5M	5M	3S	5R	5S	5R	5M	5R	5R	5R
CuSO4 10 mM	NG	NG	NG	NG	NG	NG	NG	NG	NG	1S	2S	5M	3M	5M
LiCl 50 mM	5R	5R	2V	1V^c	5R	5R	5M	5M	5S	2V	5R	5R	5R	5R
LiCl 0.15 M	3S	4R^a	NG	NG	4M	4R	NG	NG	NG	NG	3S	2V	5M	4M
$MgCl_2$ 0.5 M	4R	4R	5R	5R	4R	4R	4R	4R	5M	5R	5R	5R	5R	5R
$MnCl_2$ 4 mM	5R	5R	NG	NG	5M	5R	1M	3M	5M	5M	5M	5M	4S	4S
NaCl 0.3 M	5R	5R	2S	NG^a	4M	4M	4M	4M	4M	4V	5R	5R	5R	5R
NaCl 0.7 M	4M	4M	NG	NG	5M	5M	3M	3S	3S	3V	5M	5M	5M	5M
$ZnCl_2$ 2.5 mM	5R	5R	5R	5R	5R	5R	5M	5R	5M	5R	5M	5R	5M	5R
$ZnCl_2$ 5 mM	5M	5R^a	4S^b	4M	5M	5R	4V	5M	2V	5R	5S	5M	5M	5R
Inhibitors														
anisomycin 20 µg ml^{-1}	1S	1S^c	5M	2S^c	2M	5R	NG	NG	2V	2R	3S	5M	5R	5R
benomyl 1 µg ml^{-1} (37 °C)	5M	5M	5M	5M	5R	5R	5R	5R	4S	3S	5M	5R	5S	5R
benomyl 20 µg ml^{-1}	5M	5M	5M	5M	5M	5M	4S	4M	5R	5M	5M	5M	4M	4M
benomyl 20 µg ml^{-1} (37 °C)	5R	5R	5R	5R	5R	5R	5S	5S	4M	4S	2V	3S	1V	3V
benomyl 40 µg ml^{-1}	4S	4S^c	3V	5S^c	4M	5M	1S	3S	4S	3V	3S	5M	3S	4M
bleomycin 10 µg ml^{-1}	3S	4M^c	3M	NG^a	3S	4M	NG	NG	NG	3S	NG	4M	5M	5M
caffeine 10 mM	4M	4M	4R	4M	4R	4M	5R	5R	5M	3V	5R	5R	NG	NG
calcofluor white 1 mg ml^{-1}	5S	5M^c	4V	4M^c	3S	4M	2M	4R	5M	5R	5M	5R	NG	NG
canavanine 30 µM (SD-arg)	5R	5R	5V	5V^c	5R	5R	NG	NG	5M	5R	NG	NG	5S	5S
camptothecin 5 µg ml^{-1}	4M	4M	3S	3S	4M	4M	2S	4S	5M	5M	5M	5M	5S	5S
cycloheximide 0.2 µg ml^{-1}	2V	2V	5R	5R	1V	4S	NG	NG	NG	NG	5R	4V	3V	3S
cycloheximide 3 µg ml^{-1}	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	4M	NG	NG	NG
diamide 1 mM	5M	5M	4M	5M^a	5M	5M	4M	4M	4M	4M	4M	4M	4M	4M
diamide 1.5 mM	NG	NG^c	3S	4M^c	4M	3M	4S	4M	4S	4M	5M	5M	2S	NG
ethanol 6%	5R	5R^c	4M	4M^c	5R	5R	5R	3M	5R	5R	4R	4R	4R	4R
ethanol 10%	5R	5M^a	3S	1S^a	4R	4R	5R	5R	5M	2S	3M	3M	4R	3R
D-his 0.5 mM (L-pro)	2S	2S^c	5S	5S^c	5R	5R	5S	2V	5R	5R	NG	NG	4M	4M
hydroxyurea 0.1 M	5M	5M^c	4S	4S^c	4M	4M	4S	4S	5V	5R	5M	5R	5R	5R
neomycin 5 mg ml^{-1}	5R	5R	4S	4S	5R	5R	3S	4M	5S	4V	5R	5R	5R	5R
4-NQO 2.5 µg ml^{-1}	1S	1S	5M	5M	2M	3M	NG	NG	3M	4M	1V	2S	NG	NG
nystatin 2 µg ml^{-1}	5M	5M	5S	5S	5M	5M	4V	4V	5R	5R	5R	5R	5V	5V
oligomycin 1 µg ml^{-1} (YPGE)	5R	5R	5R	5R	5R	5R	5R	5R	5R	5S	5R	5R	5R	5R
oligomycin 2.5 µg ml^{-1} (YPGE)	5V	5V	5M	5M	3V	3V	5S	5V	5M	5S	4R	4R	5M	5M
oligomycin 5 µg ml^{-1} (YPGE)	3V	3V	5V	5M^a	3V	3V	5V	5V	5V	5V	4R	4R	5M	5M
paraquat 1 mM	5S	5S	5R	5M^a	1V	4S	NG	NG	5S	5S	5S	5M	5M	5M
paraquat 5 mM	4S	1S^a	3S	3S^c	NG	NG	NG	NG	NG	NG	NG	4S	2S	2S
paromomycin 100 µg ml^{-1}	5M	5M	4S	4S^c	5M	5M	4S	4M	4S	5M	5M	5M	5M	5M
paromomycin 200 µg ml^{-1}	5M	5M	3S	3S	5M	5M	3S	4M	4S	5M	5M	5M	5M	5M
rapamycin 0.1 µg ml^{-1}	5M	5S^a	1V	1V	4M	4M	NG	NG	4M	5R	5M	5R	NG	NG
staurosporine 1 µg ml^{-1} (37 °C)	5S	4M^c	4V	4V^c	5M	5M	5R	5R	5S	5S	5M	5M	1V	4S
vanadate (no KCl)	NG	NG	NG	NG	NG	NG	NG	NG	1V	2M	NG	NG	NG	NG
Miscellaneous														
K-Acetate + PB	5R	5R	3S	4M	5R	5R	5R	5R	5R	5R	5V	5R	5R	5R
Osmotic stress														
KCl 1.3 M	4M	4M	5S	5S	5S	5S	4M	4M	2S	1S	3M	4M	3M	2M
Temperature														
13 °C (YPD)	5R	5R^c	1S	1S	4S	5R	3S	5R	5S	5R	5S	5R	5S	5R
24 °C (YPD)	5R	5R	4R	5R^a	5R	5R	5M	5R	5R	5R	5M	5R	5M	5R
37 °C (YPD)	5R	5R	5R	5R	5R	5R	5R	5R	5R	4S	4M	5R	4R	5R
13 °C (SD)	4R	4R^c	NG	NG	4S	5R	3S	5R	5S	5R	5S	5R	5S	5R
24 °C (SD)	5R	5R	4R	4R	5R	5R	4R	5R	5R	5R	4M	5R	5R	5R
37 °C (SD)	5R	5R	5R	5R	4R	4R	5R	5R	1M	NG	1M	2R	5R	5R
Thermotolerance	M+	M	M	M	L	L	H	H+	M	M	H	H	M	M
Ethanol gradient	H	H	M	M	H	H	M+	M	L	L	M	M	M	M

5-fold increments and spotted onto test plates. Amino-acid supplements, added in parallel experiments, did not alter growth patterns (not shown), establishing that phenotypic differences between isogenic [*PSI*$^+$] and [*psi*$^-$] cells were not owing to secondary effects of suppressing auxotrophic markers these strains contained. Most of the strains carried adenine mutations, some of which were suppressed by [*PSI*$^+$]. These produced the expected differences in red/white colony colour on YPD medium[7]. However, on this medium the growth rates of the [*PSI*$^+$] derivatives in each of our isogenic pairs were always very similar to those of the corresponding [*psi*$^-$] derivatives (Fig. 1, left).

In contrast, marked growth differences were observed between isogenic [*PSI*$^+$] and [*psi*$^-$] derivatives on many test plates. For example, when the pH of the YPD medium was changed from 6.8 to 6.0 by the addition of sodium acetate, the [*psi*$^-$] derivative grew better than the [*PSI*$^+$] strain in the D1142-1A background (Fig. 1, right), but in the 5V-H19 background [*PSI*$^+$] cells grew better than [*psi*$^-$] cells. Differences in the growth properties of isogenic [*PSI*$^+$] and [*psi*$^-$] cells under our test conditions are highlighted in Table 1. Representative raw data are shown in Figs 1–5. These growth differences were highly reliable. Within experiments, YPD control plates (periodically interspersed with test plates) confirmed that cells were consistently spotted at the same densities with the same efficiencies, and the same phenotypic distinctions appeared in repeat experiments (data not shown).

In nearly half of the conditions tested, [*PSI*$^+$] exerted a substantial effect in at least some strains and in more than 25% of these tests, its effect was positive. The changes in growth were highly idiosyncratic (Table 1 and Fig. 2). Each strain exhibited a unique constellation of phenotypes. In some cases very modest changes in conditions produced large growth differences between isogenic [*PSI*$^+$] and [*psi*$^-$] cells. In others, conditions that inhibited growth affected [*PSI*$^+$] and [*psi*$^-$] cells similarly. Simple patterns were not readily discernible. For example, in both the 10B-H49 and 5V-H19 backgrounds, [*PSI*$^+$] inhibited growth on several nitrogen sources. However, under many other test conditions the phenotypic changes induced by [*PSI*$^+$] in 10B-H49 diverged from those in 5V-H19. Moreover, in the SL1010-1A and 74D-694 backgrounds, [*PSI*$^+$] enhanced growth on some nitrogen sources.

In the presence of the alkali metal caesium (25 mM; Table 1, Fig. 2b), [*PSI*$^+$] had little effect on most strains, but strongly inhibited growth in the D1142-1A background. In the BSC783/4c background, [*PSI*$^+$] strongly enhanced growth, but only when the concentration of caesium was raised to 100 mM. In the presence of the alkali metal lithium, [*PSI*$^+$] inhibited growth in 74D-694, but enhanced growth in a concentration-dependent manner in both the 5V-H19 and 10B-H49 backgrounds. In the presence of inhibitors that affect a wide variety of cellular processes[26,27] (many of which are naturally occurring antimicrobial agents[28]), [*PSI*$^+$] produced an equally divergent range of phenotypes (Fig. 2c, Table 1).

In some cases [*PSI*$^+$] changed the rate of colony growth: on YPD, pH 6.0 (strain 5V-H19; Fig. 1) or 3% ethanol (strain D1142-1A; Fig. 2a), [*PSI*$^+$] and [*psi*$^-$] cells yielded similar numbers of colonies, but [*psi*$^-$] colonies grew more slowly. In other cases, [*PSI*$^+$] altered the extent to which cultures could be diluted and still yield viable colonies: on lactate (33G-D373) or anisomycin (SL1010-1A), [*PSI*$^+$] and [*psi*$^-$] colonies were almost the same size, but [*psi*$^-$] cells could be diluted further and still grow. In many cases, both growth parameters were affected (Table 1 and Fig. 2). Under some conditions, [*PSI*$^+$] completely inhibited growth (on bleomycin, strains 5V-H19 and 10B-H49; Fig. 2c) or enabled growth when it was otherwise inhibited (33G-D373 on bleomycin, Fig. 2c, or 5V-H19 on galactose, Fig. 2a).

Morphological changes in [*PSI*$^+$] colonies

[*PSI*$^+$] also produced marked changes in colony morphology. When cells were plated onto media with 3% acetate as a sole carbon source (Fig. 3) 5V-H19 [*PSI*$^+$] cells produced unusually large colonies with a puckered, dimpled surface (Fig. 3a). The colony morphologies of

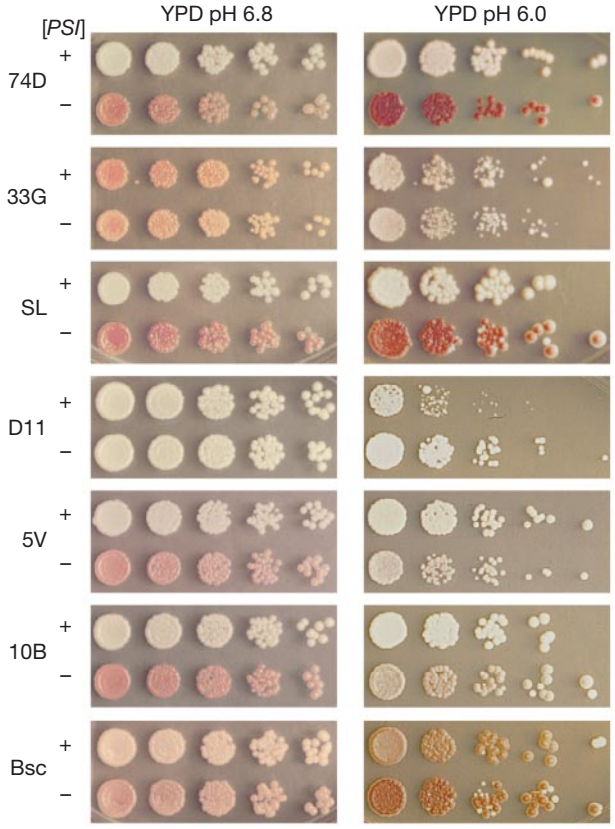

Figure 1 [*PSI*$^+$] does not alter growth on standard rich media (YPD, pH 6.8), but causes strain-specific changes at pH 6.0. Exponentially growing [*PSI*$^+$] and [*psi*$^-$] derivatives (30 °C in YPD) of different strain backgrounds were serially diluted 5-fold, spotted on the indicated media and incubated at 30 °C. Strains: 74D, 74-D694 (*MAT* a, *ade1-14, trp1-289, his3-Δ200, ura3-52, leu2-3,112*; from Y. Chernoff[44]); 33G, 33G-D373 (*MAT* a, *pheA10, his7-1, lys9-A21, trp1-289, ade 2-144,717, ura3-52, leu2-3,112*; from Y. Chernoff[45]); SL, SL1010-1A (*MAT* α, *ade1-14, met 8-1, leu2-1, his5-2, trp1-1, ura 3-52*; from S. W. Liebman[46]); D11, D1142-1A (*MAT* a, *aro7, cyc1, his4, leu2, lys2-187, met8-1, trp5-48, ura3*; from F. Sherman[47,48]); 5V, 5V-H19 (*MAT* a, *ade2-1, can1-100, leu2-3,112, ura3-52, SUQ5*; from M. D. Ter-Avanesyan[49]); 10B, 10B-H49u (*MAT* a, *ade2-1, lys1-1, his3-11,15, leu2-3,112, kar1-1, ura3::kanMX4*; from M. D. Ter-Avanesyan[18]; and BSC, BSC783/4c (*MAT* a, *ade2-1, SUQ5, leu2-3,112, ura3-1, his3-11, his3-15*; from M. Tuite[32]). Strains 5V-H19, 10B-H49, and BSC783/4c share common ancestry as do the strains 74D-694, 33G-D373, and SL1010-1A.

◀ **Table 1** Phenotypic testing of [*PSI*$^+$]/[*psi*$^-$] isogenic strain pairs. The unique pattern of phenotypes produced by [*PSI*$^+$] in each strain background are designated by colour. Blue, change in colony morphology; green, enhanced growth in the [*psi*$^-$] derivative; gold, enhanced growth in [*PSI*$^+$], with more intense colours showing a stronger effect. Exponentially growing cells in YPD were serially diluted (5-fold from 1×10^6 cells ml^{-1}), plated and scored for growth in two ways: numbers designate the highest dilution that produced significant growth in repeat experiments, and letters indicate growth rates (R, rapid; M, medium; S, slow; V, very slow; and NG, no growth). Superscripts are used in cases where a change in growth was evident in a corresponding ΔNM strain: a indicates growth similar to [*psi*$^-$]; b indicates similar to [*PSI*$^+$]; and c indicates different from either [*PSI*$^+$] or [*psi*$^-$]. For stress assays H, M and L are high, medium, and low growth rates, respectively, and plus indicates slightly increased tolerance. Additional conditions tested can be found in the Supplementary Information. The base medium for carbon and nitrogen source plates was prepared as described[27]. Metals and inhibitors[26,27] were added to YPD medium, after it was autoclaved and cooled to 55 °C. Abbreviations and additives: AntA, antimycin A; +KCl, 0.5M KCl, PB, phloxine B. The inhibitors used target the several cellular processes[26,28] (see also Supplementary Information). The solvents used were tested separately in YPD plating assays and had no effect on growth (data not shown).

5V-H19 diploids were less extreme than those of haploids (not shown), but neither haploids nor diploids of the [*psi*$^-$] derivative were affected (Fig. 3b and data not shown). Notably, in this case both [*PSI*$^+$] and [*psi*$^-$] cells grew at similar rates with similar plating efficiencies.

Individual cells from puckered colonies had normal morphologies by light and electron microscopy and revealed normal bud-scar distributions by calcofluor white staining[29] (H.L.T., T. Kowal and S.L.L., data not shown). Perhaps related to this difference in colony morphology, 5V-H19 [*PSI*$^+$] cells were more flocculant than [*psi*$^-$] cells, forming clumps when growing in liquid media. This colony morphology phenotype provided a vivid illustration of the tendency of yeast cells to switch spontaneously between [*PSI*$^+$] states during normal growth[7,30]. Figure 3c displays a large, puckered colony that arose in a group of 5V-H19 [*psi*$^-$] cells on acetate medium. Further analysis (for example, guanidine hydrochloride curing[24]) established that this colony phenotype was due to a spontaneous conversion to the [*PSI*$^+$] state.

Interaction with agents affecting translation

[*PSI*$^+$] is a prion form of the translation termination factor that changes the fidelity of protein synthesis. As translational fidelity is already compromized in [*PSI*$^+$] cells, other modulators of protein synthesis might be expected to show a common response to [*PSI*$^+$]. Indeed, growth at lower temperatures is known to increase nonsense suppression[31] and in five of the seven genetic backgrounds, on both rich (YPD) and minimal (SC) media[25], [*psi*$^-$] derivatives grew better than [*PSI*$^+$] derivatives at 13 °C. However, at the standard temperature (30 °C) antibiotics that specifically affect translational fidelity (anisomycin, neomycin and paromomycin), [*PSI*$^+$] sometimes increased and sometimes decreased growth (Fig. 2c and Table 1). Apparently, the strain-specific effects of [*PSI*$^+$] on growth supersede the direct action of the translation inhibitors.

Are there common biological effects of [*PSI*$^+$]?

In examining reasons for the conservation of this prion, one possibility is that [*PSI*$^+$] might enhance stress tolerance by increasing heat-shock protein (Hsp) synthesis[32]. Hsps increase survival during exposure to heat, ethanol and other environmental stresses, with the general signal for their induction being an increase in the cellular concentration of misfolded protein[33,34]. [*PSI*$^+$]-mediated read-through of stop codons might produce misfolded proteins in sufficient quantity to trigger this induction. Supporting this hypothesis, all three previously tested [*PSI*$^+$] strains exhibited greater ethanol tolerance than their isogenic [*psi*$^-$] derivatives and two of the three showed greater heat tolerance[32].

We examined ethanol tolerance in our seven freshly created isogenic pairs, by growing them on agar plates containing 4% dextrose as a carbon source and a gradient of ethanol (from 0 to 10%) (Fig. 4a)[32]. Each genetic background had a different intrinsic tolerance to ethanol, but it made little difference whether the strains were [*PSI*$^+$] or [*psi*$^-$]. We examined heat tolerance by treating cells at 37 °C, exposing them to 50 °C for various periods, and plating to determine the number of colony forming units remaining (Fig. 4b). Again, each genetic background exhibited a different intrinsic level of tolerance. In most cases [*PSI*$^+$] had no effect, but it slightly increased survival in the 74D-694 background (20 min and 30 min time points) and slightly decreased survival in the D1142-1A background (30 min and 45 min time points). Finally, the presence or absence of [*PSI*$^+$] did not correlate with the rate of growth at high temperatures (37 °C, Table 1). [*PSI*$^+$] might be involved in stress tolerance under natural growth conditions not mimicked by these experiments, but its effects in standard laboratory tests were neither

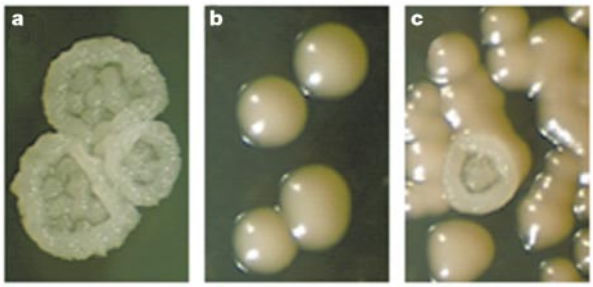

Figure 3 Colony morphology changes produced by [*PSI*$^+$] in strain 5V-H19. Cells were grown in YPD medium, diluted, spotted onto medium containing potassium acetate as the sole carbon source and grown at 30 °C. **a**, [*PSI*$^+$] colonies; **b**, [*psi*$^-$] colonies. **c**, Spontaneous appearance of a [*PSI*$^+$] colony within a group of [*psi*$^-$] cells.

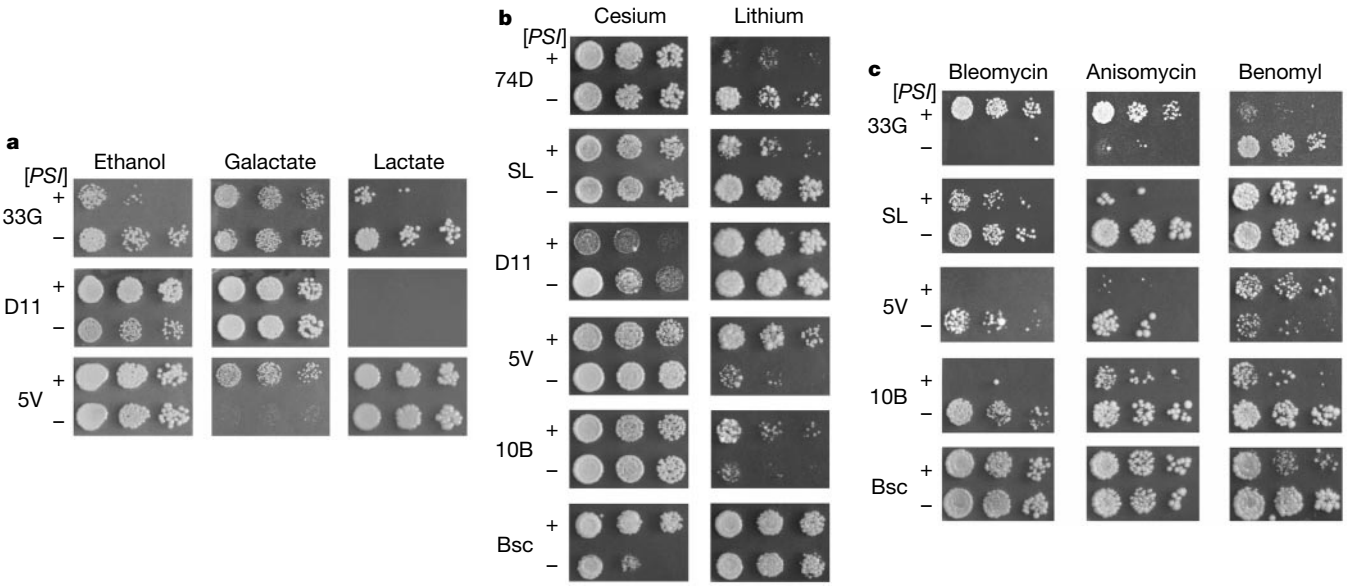

Figure 2 [*PSI*$^+$] affects growth in different ways in different genetic backgrounds. **a**, Representative growth differences on media containing ethanol, galactose or lactate as the sole carbon source. **b**, Growth on media containing caesium (left) at 25 mM (100 mM for the strain BSC783/4c) or lithium (right) at 150 mM (50 mM for the strains D1142-1A and 5V-H19). **c**, Growth on media containing the antibiotics bleomycin (10 μg ml^{-1}), anisomycin (20 μg ml^{-1}) and benomyl (40 μg ml^{-1}). Cells were grown and plated as described in Fig. 1 and Table 1.

strong nor consistent.

Of the many effects on growth we observed in response to [*PSI*$^+$], in only one case did it affect all backgrounds. In all seven strain pairs, [*PSI*$^+$] derivatives grew more slowly than their isogenic [*psi*$^-$] derivative on media containing 5 mM $ZnCl_2$ (Fig. 5). [*PSI*$^+$] also affected all six of the strains that were capable of growth in the presence of calcofluor white in the same way (Table 1). Given the number and variety of phenotypes examined, such effects might occur by chance, but it is possible that zinc metabolism or cell-wall biosynthesis reflect common physiological effects of [*PSI*$^+$].

NM-specific phenotypes

Another explanation for the conservation of the prion-determining region is that it has a function separate from its role in [*PSI*$^+$] formation[35]. This possibility is supported by the fact that NM deletions produced distinct phenotypes in our assays. The most dramatic effect occurred during growth in the presence of benomyl, a microtubule-destabilizing agent. We examined benomyl toxicity extensively (Table 1 and Fig. 2c) because certain mutations in *SUP35* increase sensitivity to benomyl[36]. Under conditions where benomyl reduced cell growth, [*PSI*$^+$] generally reduced it further, but in the 5V-H19 background [*PSI*$^+$] increased growth. Thus, benomyl does not reveal a common biological effect of [*PSI*$^+$]. However, in the two backgrounds tested, ΔNM mutants grew better in the presence of benomyl than did either of their isogenic [*PSI*$^+$] or [*psi*$^-$] variants. When considered with the previous data on the *SUP35* mutants[36], these results indicate that benomyl may perturb a function of the NM region.

Other effects of NM deletions are shown in Table 1. NM-deletion phenotypes were occasionally similar to those of the [*psi*$^-$] derivative or the [*PSI*$^+$] derivative, but most commonly were distinct from either. These observations support the notion that NM might play a role in yeast biology that is distinct from its role in [*PSI*$^+$] formation.

Discussion

We have demonstrated that the [*PSI*$^+$] element of the yeast *S. cerevisiae* provides a means to unveil silent genetic information to produce new heritable phenotypes. In the context of an individual cell, [*PSI*$^+$] allows alternative heritable phenotypes to be encoded by a single genome. In the context of diverging populations it provides a vast array of new phenotypic states with unique growth advantages and disadvantages. We propose that the epigenetic and metastable nature of [*PSI*$^+$], associated with the fundamental process of translation, potentiates survival in a fluctuating environment and provides a conduit for the evolution of new traits.

Yeast cells spontaneously switch from the [*psi*$^-$] to the [*PSI*$^+$] state[30], with rates generally varying between 1 in 10^5 to 1 in 10^7. Thus, once a population has reached an appreciable size, some of its genetically identical members will have acquired a new, heritable phenotype. If the environment does not favour this phenotype, the loss of these cells will have a minimal impact on the fitness of that genotype. If the environment does favour it, the original genotype

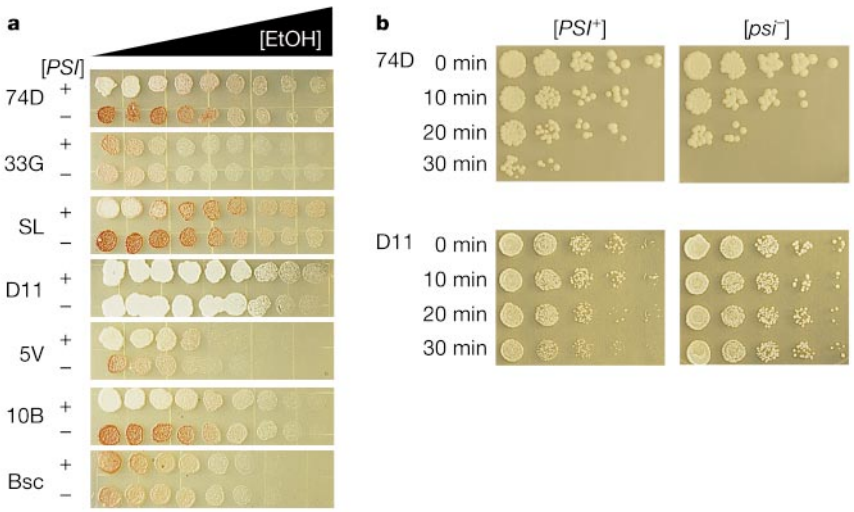

Figure 4 Stress tolerance in [*PSI*$^+$] and [*psi*$^-$] derivatives. **a**, For ethanol tolerance, cells were spotted at equal densities (1×10^6 cells ml^{-1}) on plates containing a gradient of ethanol from 0 to 10% in 0.25× YEPD media[32] **b**, For induced thermotolerance, cells in log phase (4×10^6 cells ml^{-1}) were incubated at 37 °C for 30 min and transferred to 50 °C for the indicated times. Cells were serially diluted 5-fold in YPD, plated on YPD, and grown at 30 °C. Heat-shock inhibited colour development in 74D-694, but simultaneous plating to media lacking adenine demonstrated that most cells remained in the [*PSI*$^+$] state.

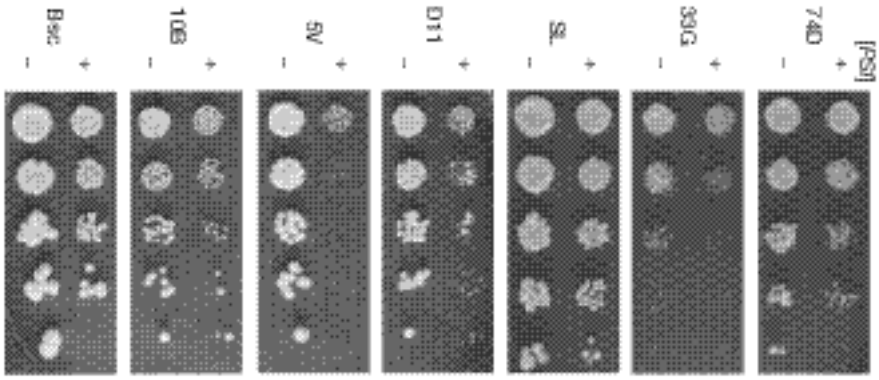

Figure 5 [*PSI*$^+$] negatively affects the growth of all seven strain backgrounds in the presence of 5mM $ZnCl_2$. Cells were grown and plated as described in Table 1.

can thrive even if the original phenotype is disfavoured. Once progeny with the new phenotype have reached sufficient densities, the spontaneous appearance of [*psi*$^-$] derivatives will ensure that the original phenotype is available should the environment change.

[*PSI*$^+$] allows cells to occupy a new niche without foregoing their capacity to occupy the old. If the new phenotype remains advantageous, the size of the growing population increases the likelihood that mutations will arise to eliminate stop codons that are relevant to the phenotype and require [*PSI*$^+$] for read-through. These would make that phenotype more robust and fix the trait; cells would retain the phenotype upon reversion to the [*psi*$^-$] state (presumably normal translation fidelity will generally be favoured in nature). By this mechanism, [*PSI*$^+$] could facilitate the evolution of new traits.

How might such a system evolve and be maintained? We suggest three different, not exclusive possibilities. First, the self-perpetuating conformational change that that drives [*PSI*$^+$] inheritance might have arisen as an inadvertent consequence of some aspect of the NM sequence that is required for another function of this region. Our data are consistent with a separate NM function, as are recent findings that NM interacts with Sla1, an actin-binding protein[35]. Second, yeast might so commonly occupy fluctuating environments (eg. warm, nutrient-replete summers versus cool, nutrient-poor winters) that [*PSI*$^+$] could be maintained as a means of alternating between heritable phenotypic states adapted to these fluctuations. Finally, the natural environment occupied by *S. cerevisiae* during its evolution may have been sufficiently erratic to provide the pressure required to maintain a global mechanism for exploiting genome-wide variation to produce new phenotypes.

[*PSI*$^+$] is a suppressor of nonsense codon mutations in genetic markers[37]. Presumably, the wealth of new phenotypes we observe in [*PSI*$^+$] strains results from the read-through of two classes of naturally occurring nonsense codons: those located in open reading frames (ORFs) that have acquired inactivating stop-codon mutations (ISCMs) and those employed as natural translation-termination signals at the 3′ ends of active genes. ORFs might accumulate ISCMs when gene duplications eliminate selective constraints (~16% of the *S. cerevisiae* proteome is present in duplicate[38]), or when growth conditions alter selective pressures[39]. The phenotypic diversity generated from these ORFs by [*PSI*$^+$] would depend upon which ORFs had acquired ISCMs, the frequency of their read-through, and the presence of additional mutations they may have acquired while in the inactive state. Any phenotypic diversity generated from the 3′ untranslated regions would depend upon the context of the stop codon and the coding potential of the downstream sequence. The 3′ untranslated regions of most genes are naturally under less constraint than coding sequences and are highly polymorphic[40]. [*PSI*$^+$]-mediated read-through might alter the stability of the messenger RNA, its capacity to be translated, and the function or stability of the encoded protein.

Many read-through events may have little phenotypic consequence, and others may have strong effects. In either case, the interplay between nonsense-mediated decay and translation termination[41] might increase accumulation of the read-through transcript. Feedback regulation by the gene product might reduce it. Each primary event may produce many secondary and tertiary changes in gene expression and some, but not all, of these may contribute to phenotypic change. Preliminary DNA microarray analysis of [*PSI*$^+$]-induced changes in the mRNA populations of the seven strain pairs (H.L.T., V. Iyer, P. Brown and S.L.L., unpublished observations) together with the existence of many potential ORFs with single nonsense codons (H.L.T., J. Henikoff, S. Henikoff and S.L.L., unpublished observations) in the only *S. cerevisiae* strain whose genome has been sequenced (http://genome-www.stanford.edu/Saccharomyces/)[42] indicate it will not be trivial to pinpoint the causes of [*PSI*$^+$]-induced phenotypic variation. However, continuing advances in genomics and proteomics should make such an understanding attainable.

It may also be possible directly to test the effects of [*PSI*$^+$] on the evolution of phenotypic and genetic diversity. We obtained [*PSI*$^+$] and [*psi*$^-$] strain pairs from several laboratories, and they were assumed to be isogenic. However, when the [*PSI*$^+$] derivatives were cured of the prion (converted to [*psi*$^-$]) they were phenotypically distinct from the original [*psi*$^-$] derivatives. It may be that independent errors in strain husbandry occurred in each laboratory. It seems more likely that selective pressures from [*PSI*$^+$]-induced changes in gene expression led, during the years the strains were cultured, to the selection of genetic variants.

We previously proposed another mechanism for uncovering hidden genetic variation and facilitating the process of evolutionary change in *Drosophila* and other eukaryotes[43]. That mechanism involves environmentally induced changes in the capacity of the protein chaperone Hsp90 to maintain the activity of metastable regulatory proteins. There are many differences in the manner that Hsp90 and [*PSI*$^+$] uncover variation. The former employs mendelian re-assortment of pre-existing variation and several rounds of selection for traits to become heritable and fixed. The latter involves an epigenetic change that produces heritable phenotypes in a single, readily reversible step, with additional mutations required for fixation. Both provide mechanisms for unveiling pre-existing variation in a combinatorial, genome-wide manner that converts neutral, or near-neutral, mutations to a non-neutral state. Such mechanisms may be present more broadly than previously suspected and exert an important influence on the rates and mechanisms of evolutionary change. □

Received 31 May; accepted 24 July 2000.

1. Koch, A. L. Enzyme evolution. I. The importance of untranslatable intermediates. *Genetics* **72,** 297–316 (1972).
2. Ohno, S. *Evolution by Gene Duplication* (Springer, New York, 1970).
3. Ohta, T. Simulating evolution by gene duplication. *Genetics* **115,** 207–213 (1987).
4. Walsh, J. B. How often do duplicated genes evolve new functions? *Genetics* **139,** 421–428 (1995).
5. Nadeau, J. H. & Sankoff, D. Comparable rates of gene loss and functional divergence after genome duplications early in vertebrate evolution. *Genetics* **147,** 1259–1266 (1997).
6. Hall, B. G., Yokoyama, S. & Calhoun, D. H. Role of cryptic genes in microbial evolution. *Mol. Biol. Evol.* **1,** 109–124 (1983).
7. Cox, B. [PSI], a cytoplasmic suppressor of super-suppression in yeast. *Heredity* **20,** 505–521 (1965).
8. Liebman, S. W. & Sherman, F. Extrachromosomal psi+ determinant suppresses nonsense mutations in yeast. *J. Bact.* **139,** 1068–1071 (1979).
9. Firoozan, M., Grant, C. M., Duarte, J. A. & Tuite, M. F. Quantitation of readthrough of termination codons in yeast using a novel gene fusion assay. *Yeast* **7,** 173–183 (1991).
10. Serio, T. R. & Lindquist, S. L. [PSI+]: an epigenetic modulator of translation termination efficiency. *Annu. Rev. Cell Dev. Biol.* **15,** 661–703 (1999).
11. Stansfield, I. *et al.* The products of the SUP45 (eRF1) and SUP35 genes interact to mediate translation termination in *Saccharomyces cerevisiae*. *EMBO J.* **14,** 4365–4373 (1995).
12. Zhouravleva, G. *et al.* Termination of translation in eukaryotes is governed by two interacting polypeptide chain release factors, eRF1 and eRF3. *EMBO J.* **14,** 4065–4072 (1995).
13. Patino, M. M., Liu, J. J., Glover, J. R. & Lindquist, S. Support for the prion hypothesis for inheritance of a phenotypic trait in yeast. *Science* **273,** 622–626 (1996).
14. Paushkin, S. V., Kushnirov, V. V., Smirnov, V. N. & Ter-Avanesyan, M. D. Propagation of the yeast prion-like [psi+] determinant is mediated by oligomerization of the SUP35-encoded polypeptide chain release factor. *EMBO J.* **15,** 3127–3134 (1996).
15. Kikuchi, Y. & Kikuchi, A. in *Gene Expression and Regulation: the Legacy of Luigi Gorini* (eds Bissell, M., Deho, G., Sironi, G. & Torriani, A.) 257–264 (Elsevier, Amsterdam, 1988).
16. Kushnirov, V. V. *et al.* Nucleotide sequence of the SUP2 (SUP35) gene of *Saccharomyces cerevisiae*. *Gene* **66,** 45–54 (1988).
17. Wilson, P. G. & Culbertson, M. R. SUF12 suppressor protein of yeast. A fusion protein related to the EF-1 family of elongation factors. *J. Mol. Biol.* **199,** 559–573 (1988).
18. Ter-Avanesyan, M. D., Dagkesamanskaya, A. R., Kushnirov, V. V. & Smirnov, V. N. The SUP35 omnipotent suppressor gene is involved in the maintenance of the non-Mendelian determinant [psi+] in the yeast *Saccharomyces cerevisiae*. *Genetics* **137,** 671–676 (1994).
19. Liu, J. J. & Lindquist, S. Oligopeptide-repeat expansions modulate 'protein-only' inheritance in yeast. *Nature* **400,** 573–576 (1999).
20. Kushnirov, V. V. *et al.* Divergence and conservation of SUP2 (SUP35) gene of yeast *Pichia pinus* and *Saccharomyces cerevisiae*. *Yeast* **6,** 461–472 (1990).
21. Chernoff, Y. *et al.* Evolutionary conservation of prion-forming abilities of the yeast Sup35 protein. *Mol. Microbiol.* **35,** 865–876 (2000).
22. Santoso, A., Chien, P., Osherovich, L. Z. & Weissman, J. S. Molecular basis of a yeast prion species barrier. *Cell* **100,** 277–288 (2000).
23. Kushnirov, V. V., Kochneva-Pervukhova, N. V., Chechenova, M. B., Frolova, N. S. & Ter-Avanesyan, M. D. Prion properties of the Sup35 protein of yeast *Pichia methanolica*. *EMBO J.* **19,** 324–331 (2000).
24. Tuite, M. F., Mundy, C. R. & Cox, B. S. Agents that cause a high frequency of genetic change from [psi+] to [psi-] in *Saccharomyces cerevisiae*. *Genetics* **98,** 691–711 (1981).
25. Adams, A., Gottschling, D. E., Kaiser, C. A., Stearns, T. *Methods in Yeast Genetics* (Cold Spring Harbor Laboratory Press, Plainview, 1997).

26. Hampsey, M. A review of phenotypes in *Saccharomyces cerevisiae*. *Yeast* **13,** 1099–1133 (1997).
27. Rieger, K. J. *et al.* Large-scale phenotypic analysis—the pilot project on yeast chromosome III. *Yeast* **13,** 1547–1562 (1997).
28. Budavari, S. (ed.) *The Merck Index* (Merck and Company, Rahway, New Jersey, 1989).
29. Roncero, C., Valdivieso, M. H., Ribas, J. C. & Duran, A. Isolation and characterization of *Saccharomyces cerevisiae* mutants resistant to Calcofluor white. *J. Bacteriol.* **170,** 1950–1954 (1988).
30. Lund, P. M. & Cox, B. S. Reversion analysis of [psi-] mutations in *Saccharomyces cerevisiae*. *Genet. Res.* **37,** 173–182 (1981).
31. Derkatch, I. L., Bradley, M. E., Zhou, P., Chernoff, Y. O. & Liebman, S. W. Genetic and environmental factors affecting the de novo appearance of the [PSI+] prion in *Saccharomyces cerevisiae*. *Genetics* **147,** 507–519 (1997).
32. Eaglestone, S. S., Cox, B. S. & Tuite, M. F. Translation termination efficiency can be regulated in *Saccharomyces cerevisiae* by environmental stress through a prion-mediated mechanism. *EMBO J.* **18,** 1974–1981 (1999).
33. Grant, C. M., Firoozan, M. & Tuite, M. F. Mistranslation induces the heat-shock response in the yeast *Saccharomyces cerevisiae*. *Mol. Microbiol.* **3,** 215–220 (1989).
34. Parsell, D. A. & Lindquist, S. The function of heat-shock proteins in stress tolerance: degradation and reactivation of damaged proteins. *Annu. Rev. Genet.* **27,** 437–496 (1993).
35. Bailleul, P. A., Newnam, G. P., Steenbergen, J. N. & Chernoff, Y. O. Genetic study of interactions between the cytoskeletal assembly protein sla1 and prion-forming domain of the release factor sup35 (eRF3) in *Saccharomyces cerevisiae*. *Genetics* **153,** 81–94 (1999).
36. Tikhomirova, V. L. & Inge-Vechtomov, S. G. Sensitivity of sup35 and sup45 suppressor mutants in *Saccharomyces cerevisiae* to the anti-microtubule drug benomyl. *Curr. Genet.* **30,** 44–49 (1996).
37. Liebman, S. W. & Derkatch, I. L. The yeast [PSI+] prion: making sense of nonsense. *J. Biol. Chem.* **274,** 1181–1184 (1999).
38. Seoighe, C. & Wolfe, K. H. Updated map of duplicated regions in the yeast genome. *Gene* **238,** 253–261 (1999).
39. Olson, M. V. When less is more: gene loss as an engine of evolutionary change. *Am. J. Hum. Genet.* **64,** 18–23 (1999).
40. Levitt, R. C. Polymorphisms in the transcribed 3′ untranslated region of eukaryotic genes. *Genomics* **11,** 484–489 (1991).
41. Czaplinski, K. *et al.* The surveillance complex interacts with the translation release factors to enhance termination and degrade aberrant mRNAs. *Genes Dev.* **12,** 1665–1677 (1998).
42. Mewes, H. W. *et al.* Overview of the yeast genome. *Nature* **387,** 7–65 (1997).
43. Rutherford, S. L. & Lindquist, S. Hsp90 as a capacitor for morphological evolution. *Nature* **396,** 336–342 (1998).
44. Chernoff, Y. O., Lindquist, S. L., Ono, B., Inge-Vechtomov, S. G. & Liebman, S. W. Role of the chaperone protein Hsp104 in propagation of the yeast prion-like factor [psi+]. *Science* **268,** 880–884 (1995).
45. Chernoff, Y. O. *et al.* Conservative system for dosage-dependent modulation of translational fidelity in eukaryotes. *Biochimie* **74,** 455–461 (1992).
46. Zhou, P. *et al.* The yeast non-Mendelian factor [ETA+] is a variant of [PSI+], a prion- like form of release factor eRF3. *EMBO J.* **18,** 1182–1191 (1999).
47. Eustice, D. C., Wakem, L. P., Wilhelm, J. M. & Sherman, F. Altered 40S ribosomal subunits in omnipotent suppressors of yeast. *J. Mol. Biol.* **188,** 207–214 (1986).
48. Wakem, L. P. & Sherman, F. Isolation and characterization of omnipotent suppressors in the yeast *Saccharomyces cerevisiae*. *Genetics* **124,** 515–522 (1990).
49. Ter-Avanesyan, M. D. *et al.* Deletion analysis of the SUP35 gene of the yeast *Saccharomyces cerevisiae* reveals two non-overlapping functional regions in the encoded protein. *Mol. Microbiol.* **7,** 683–692 (1993).

Supplementary information is available on *Nature*'s World-Wide Web site (http://www.nature.com) or as paper copy from the London editorial office of *Nature*.

Acknowledgements

We thank members of the Lindquist lab and M. Jensen for discussion and comments on the manuscript, and V. Iyer, P.O. Brown, J. Henikoff and S. Henikoff for collaborative efforts and for allowing us to cite unpublished work. This research was supported by the Jane Coffin Childs Memorial Fund for Medical Research (H.L.T.), the National Institutes of Health and the Howard Hughes Medical Institute.

Correspondence and requests for materials should be addressed to S.L.L. (e-mail: s-lindquist@uchicago.edu).