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Prions are a common mechanism for phenotypic inheritance in wild yeasts

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The self-templating conformations of yeast prion proteins act as epigenetic elements of inheritance. Yeast prions might provide a mechanism for generating heritable phenotypic diversity that promotes survival in fluctuating environments and the evolution of new traits. However, this hypothesis is highly controversial. Prions that create new traits have not been found in wild strains, leading to the perception that they are rare 'diseases' of laboratory cultivation. Here we biochemically test approximately 700 wild strains of *Saccharomyces* for [*PSI*$^+$] or [*MOT3*$^+$], and find these prions in many. They conferred diverse phenotypes that were frequently beneficial under selective conditions. Simple meiotic re-assortment of the variation harboured within a strain readily fixed one such trait, making it robust and prion-independent. Finally, we genetically screened for unknown prion elements. Fully one-third of wild strains harboured them. These, too, created diverse, often beneficial phenotypes. Thus, prions broadly govern heritable traits in nature, in a manner that could profoundly expand adaptive opportunities.

The heritable variation that drives new forms and functions is generally ascribed to mutations in the genetic code. We previously proposed an entirely different pathway for creating heritable phenotypic diversity[1], through which the inheritance of new traits can precede the genetic changes that ultimately hardwire them. The mechanism for this seemingly paradoxical flow of information resides in epigenetic switches encoded entirely by self-perpetuating changes in protein structure, known as prions.

The best studied prion is the yeast translation-termination factor Sup35. Like other prions, Sup35 carries a prion-determining domain (PrD) that is dispensable for the protein's normal function. This PrD occasionally adopts an amyloid conformation. When it does, that amyloid perpetuates itself by templating the same conformation to the PrDs of other Sup35 molecules. This sequesters most Sup35 into insoluble fibres[2]. The ensuing reduction in translation–termination activity increases stop codon read-through, producing a variety of new traits that depend upon previously cryptic genetic variation.

Just as the mitotic apparatus ensures inheritance of chromosomally determined traits, the prion-partitioning function provided by Hsp104 (refs 3, 4) ensures inheritance of prion phenotypes. Hsp104 is a molecular machine that severs prion fibres, allowing replicating prion templates to be faithfully inherited by daughter cells. The prion formed by Sup35 is known as [*PSI*$^+$], brackets denoting its cytoplasmic inheritance and capital letters its dominant phenotypes.

Cells expressing Sup35 in the non-prion [*psi*$^-$] state spontaneously switch to [*PSI*$^+$] at a frequency of about 1 in 10^6 (refs 5,6). We have proposed that [*PSI*$^+$] provides a beneficial 'bet-hedging' mechanism to enhance survival in the face of fluctuating environments: by the time a yeast colony has reached appreciable size, a few [*PSI*$^+$] cells will have appeared, expressing heritable new traits. If the trait is detrimental, only a few individuals in a large population will be lost. However, if it is advantageous, those few cells might ensure survival under conditions when the population would otherwise perish. [*PSI*$^+$] is also lost sporadically. This guarantees that [*psi*$^-$] cells will arise in [*PSI*$^+$] colonies, providing a complementary survival advantage.

A particularly attractive feature of this mechanism is that it provides immediate access to genetically complex traits[1,7]. Regions downstream of stop codons frequently accumulate genetic variation. [*PSI*$^+$]-mediated read-through allows this previously cryptic variation to have biological consequences at multiple loci simultaneously. The complex traits produced by this prion would be less likely to evolve if the individually contributing mutations had to be selected for as they arose. In the long run, reduced translational fidelity should be detrimental. However, advantageous phenotypes initially dependent on [*PSI*$^+$] might be assimilated by various means[7], allowing the prion to be lost and the trait maintained.

Several lines of evidence support this hypothesis. First, mathematically, even an infrequent selective advantage for [*PSI*$^+$] would be sufficient to maintain Sup35's prion switching capacity[8,9]. Second, the sequence of Sup35's PrD is highly divergent but has retained, for at least 500 million years, two unusual features that regulate bi-stable inheritance of prion and non-prion phenotypes. An extreme amino acid bias in one segment drives the PrD into a self-templating prion amyloid, whereas an immediately adjacent charged segment stabilizes it in the soluble non-prion state. Third, the rates at which cells switch into and out of the prion state increases when cells are not well-suited to their environments and new phenotypes have a better chance of being beneficial[10]. Increased switching is a direct consequence of the effects that diverse environmental stresses have on protein folding and homeostasis[11,12] and also fulfils a critical theoretical prediction for such an evolvability function[6,13].

In addition to Sup35, at least two dozen other proteins can form prions that are transmitted through the prion-partitioning activity of Hsp104 (refs 14, 15) in laboratory yeast. These prions are strikingly enriched in transcription factors and RNA-processing proteins that are well situated to transduce genetic variation into phenotypic effect. They too, therefore, might enable the inheritance of diverse biological

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traits, enhancing survival in fluctuating environments. However, as attractive as such ideas may be[16], and as intensely studied as yeast prions have been, their proposed adaptive value remains highly controversial[17–19]. Indeed, prions are often categorized as rare 'diseases' of yeast or mere artefacts of laboratory cultivation. A key argument is that [*PSI*$^+$] and other prions with phenotypic consequences have not been found in wild strains, despite attempts to find them. Here we establish the natural biological importance of prions through biochemical and biological analyses of hundreds of wild strains.

[*PSI*$^+$] occurs in wild strains

To search for [*PSI*$^+$] in wild strains we took advantage of the unusual stability of prion amyloid assemblies in ionic detergents, which enables their identification by semi-denaturing detergent–agarose gel electrophoresis (SDD–AGE)[4]. We modified the technique to enable high-throughput detection. Ultimately we analysed 690 yeast strains from diverse ecological niches (Supplementary Table 1). Amyloid polymers of Sup35 were present in ten (Fig. 1a, Supplementary Table 1 and Supplementary Fig. 1).

To ensure that these strains were not simply derived from a recent, prion-containing common ancestor, we sequenced the genomes of two. Over 25,000 single nucleotide polymorphisms established their independent origins (Supplementary Fig. 2). We also sequenced the *SUP35* gene in several of the strains, which established that they, too, had independent origins (Supplementary Table 2).

Do the Sup35 amyloids in these strains represent true prions? Owing to its central role in the inheritance of prion templates, even transient inhibition of Hsp104's protein remodelling activity heritably 'cures' cells of their prion elements. We inhibited Hsp104 function by growth on medium containing low concentrations of guanidine hydrochloride (GdHCl), which selectively inhibits its ATPase activity, and then plated cells back to media without GdHCl. In all cases this eliminated the amyloid (black arrows, Fig. 1b and Supplementary Fig. 1B). To ensure that curing was not due to an off-target effect of GdHCl we used a genetic approach — transiently expressing a dominant negative Hsp104 variant[3], Hsp104^{DN}, on a plasmid marked with antibiotic resistance. (Wild strains contain no auxotrophies.) This also cured cells of the amyloid, confirming their prion-based inheritance (black arrows, Fig. 1b and Supplementary Fig. 1b, c).

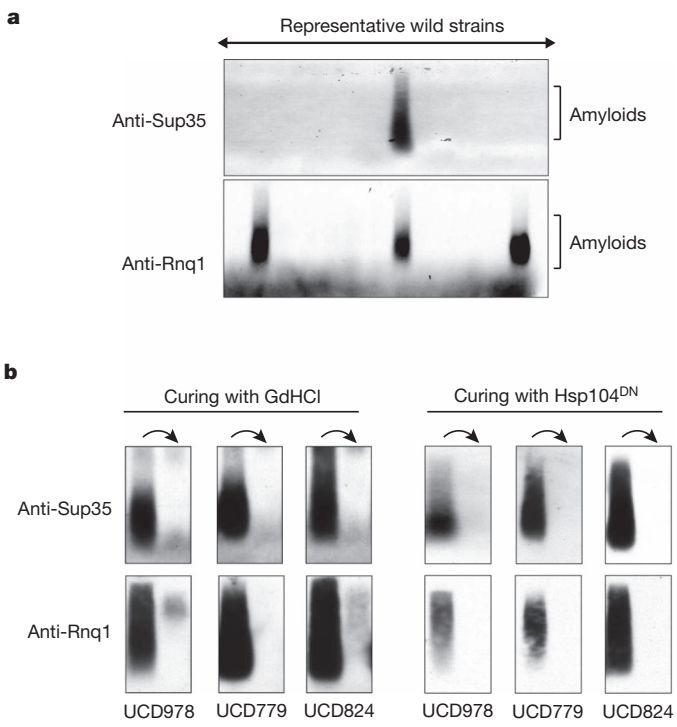

Figure 1 | Identification and verification of prions in wild yeast. **a**, Representative SDD–AGE blot of wild yeast isolates probed with antibodies against Sup35 and Rnq1. Amyloid polymers produce characteristic smears. SDD–AGE does not reliably detect monomeric proteins. **b**, Transient inactivation of Hsp104 by GdHCl or expression of a dominant negative mutant of Hsp104 eliminates these amyloids, indicating that they are [*PSI*$^+$] and [*RNQ*$^+$] prions.

Laboratory culture is not responsible

Might these prions have arisen simply as an artefact of laboratory culture conditions? In archiving wild strains, great care is taken to maintain their wild character (personal communication, L. Bisson). To determine directly if the conditions used might have inadvertently selected for the *de novo* appearance of [*PSI*$^+$], we compared growth of the archived strains with their cured derivatives on all of the relevant media. No growth advantage was found for [*PSI*$^+$] on any of these media (yeast potato dextrose, YPD, YM broth, FM broth, wort agar or Wallerstein nutrient agar) in any of the strains, and it was sometimes detrimental (Supplementary Table 3). However, in strain 5672 [*PSI*$^+$] produced an extreme selective advantage on synthetic grape must, a medium that recapitulates conditions of early wine fermentation[20]. This suggests the prion was advantageous in the strain's natural ecological niche (Supplementary Fig. 3). In any case, this and several other experiments (Supplementary Information), indicated that prions harboured by the wild strains almost certainly originated in the yeasts' natural environments.

Wild [*PSI*$^+$] is associated with [*RNQ*$^+$]

In the laboratory, Sup35's switch to the prion state strongly depends on the prion-enabling factor [*RNQ*$^+$][21]. [*RNQ*$^+$] is itself a prion formed by the Rnq1 protein[22]. [*RNQ*$^+$] is the only prion previously known to exist in wild strains[18,23,24]. We screened our collection for [*RNQ*$^+$] amyloids, finding them in 43 strains (Fig. 1b). These, too, depended on the prion-partitioning factor Hsp104 (arrows, Fig. 1b and Supplementary Fig. 1). The correlation between [*RNQ*$^+$] and [*PSI*$^+$] ($P < 0.0001$, Fisher's exact test) was striking: all the [*PSI*$^+$] strains contained [*RNQ*$^+$]. This strongly indicates that [*RNQ*$^+$] acts as a prion-inducing factor for [*PSI*$^+$] in nature.

[*PSI*$^+$] transforms natural genetic variation

Do wild prions generate phenotypic diversity from otherwise-cryptic natural variation? We compared growth of the wild [*PSI*$^+$] strains with that of their cured derivatives in four carbon sources, under osmotic, oxidative, pH or ethanol stresses, and in the presence of antifungal drugs or DNA damaging agents. We also assessed their ability to invade the growth substratum.

Prion curing produced many phenotypic changes that varied with the genetic background (Fig 2a). For example, strain UCD824, isolated from white wine, was resistant to acidic conditions and to fluconazole. UCD939, isolated from Lambrusco grapes, was resistant to the DNA-damaging agent 4-nitroquinoline 1-oxide (4-NQO). These beneficial phenotypes were greatly reduced by prion curing. UCD978, isolated from Beaujolais wine, was sensitive to the oxidative stressor tBOOH and became more resistant on curing. This same strain normally penetrated the agar surface, but this ability was lost after prion curing (Fig. 3a). Thus, in UCD978 the prion produced a trade-off, creating traits that were potentially detrimental or beneficial, depending on the circumstances.

GdHCl and Hsp104^{DN} cures cells of other Hsp104-dependent prions in addition to [*PSI*$^+$][14,15]. To determine if such curable phenotypes arose from [*PSI*$^+$] itself, we transformed the ten strains with a plasmid expressing a Sup35 variant lacking the PrD (Sup35ΔPrD). This protein is immune to [*PSI*$^+$]-mediated sequestration and restores normal translation termination in [*PSI*$^+$] cells without altering other prions[14]. In most cases Sup35ΔPrD produced the same changes as curing with GdHCl and Hsp104^{DN} (Fig. 2b, Fig. 3b, see Supplementary

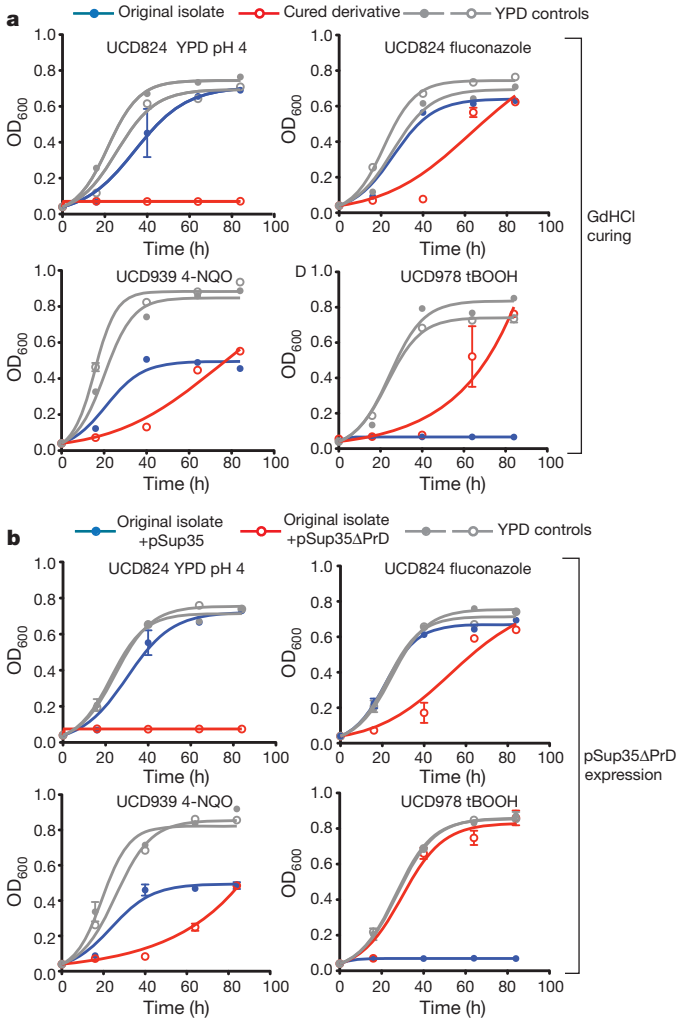

Figure 2 | Prion-contingent phenotypes of [*PSI*$^+$] isolates. **a**, Wild [*PSI*$^+$] strains show diverse phenotypes that are eliminated by transient Hsp104 inhibition. Growth curves for wild strains and cured derivatives in the indicated selective condition are in closed blue circles and open red circles, respectively. Growth in YPD is presented for each wild strain (closed grey circles) and its cured derivative (open grey circles) as a control. OD_{600}, optical density measured at 600 nm. **b**, Phenotypes of the wild [*PSI*$^+$] strains were also eliminated by expression of Sup35ΔPrD. Growth curves for wild strains expressing Sup35 or Sup35ΔPrD in the indicated selective condition are in closed blue circles and open red circles, respectively. Growth in YPD for the indicated wild strain expressing Sup35 (closed grey circles) or Sup35ΔPrD (open grey circles) is presented as a control. Error bars are present on all points and represent the standard deviation from four independent biological replicates.

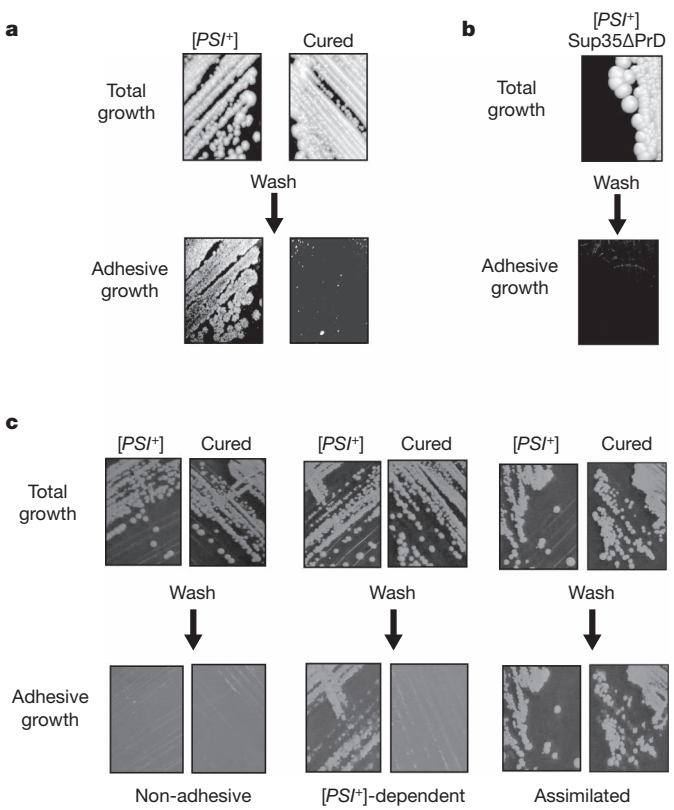

Figure 3 | Genetic assimilation of the [*PSI*$^+$]-dependent adhesive phenotype in meiotic progeny of UCD978. **a**, [*PSI*$^+$] allows the wine yeast UCD978 to adhere to agar surfaces. **a**, **b**, Adhesion is eliminated by GdHCl (**a**) or by expression of a non-aggregating version of Sup35, Sup35ΔPrD (**b**). **c**, Meiotic progeny of strain UCD978 show a diversity of phenotypes. In some spores the adhesive phenotype was assimilated and remained even after the [*PSI*$^+$] prion was cured. In others the adhesive phenotype retained [*PSI*$^+$] dependence. Finally, some lost the phenotype altogether, irrespective of [*PSI*$^+$] status.

Information for discussion). Thus, most of the original traits were due to [*PSI*$^+$].

Fixation of a [*PSI*$^+$]-dependent phenotype

When laboratory strains of diverse backgrounds are crossed and sporulated, meiotic re-assortment of the genetic variants they contain can lead to the fixation of a prion trait[1,7]. That is, whereas the trait initially depends on the prion, it can become prion-independent. Might this mechanism allow wild strains to drive prion-dependent traits to fixation? Wild yeasts frequently harbour considerable heterozygosity, and sequencing has revealed that the [*PSI*$^+$] strain UCD978 was highly polymorphic. We asked if simple re-assortment of these polymorphisms could fix a [*PSI*$^+$]-dependent trait.

Thirty random haploid progeny of UCD978 were tested for agar adhesion before and after curing. Five retained [*PSI*$^+$]-dependent adhesion; twenty were no longer adhesive, with or without [*PSI*$^+$]; five remained adhesive even after [*PSI*$^+$] curing (Fig. 3c). Given the frequency of fixation, it probably required the re-assortment of a few different polymorphisms. But clearly, the naturally occurring genetic variation present in this strain was alone sufficient to fix this trait.

[*MOT3*$^+$] occurs in wild strains

Nearly two-dozen proteins with prion-forming capacity have recently been discovered in yeast (reviewed in ref. 15). Serendipitously, an endogenous hexahistidine motif in one, the transcriptional repressor Mot3, permits detection on SDD–AGE immunoblots[14]. Sixteen yeast proteins contain a hexahistidine motif, but only Mot3 has a prion-like sequence[14]. We found [*MOT3*$^+$] amyloids in six of the 96 diverse strains we tested (Supplementary Table 1).

To determine if wild [*MOT3*$^+$] prions produced potentially adaptive phenotypes, we first took advantage of Mot3's known function as a transcriptional repressor of genes involved in cell wall production. We tested wild [*MOT3*$^+$] strains for resistance to the cell wall toxin calcofluor white. Strain Y-35, isolated from holly berries, was highly resistant to calcofluor. Resistance was heritably reduced by GdHCl treatment, and this treatment also eliminated [*MOT3*$^+$] amyloids (Fig. 4a).

As a transcriptional repressor, when Mot3 switches into or out of its prion form it has the potential to broadly transform information flow. We next screened wild [*MOT3*$^+$] strains and their cured derivatives against the same growth conditions used for the wild [*PSI*$^+$] strains. Many phenotypes were altered by prion curing. For example, [*MOT3*$^+$] NCYC 3311, a Finnish soil isolate, was resistant to acidic conditions. [*MOT3*$^+$] Y-1537, isolated from grape must, was resistant

to fluconazole. Both traits were virtually eliminated by curing with GdHCl (Fig. 4b).

To determine if the traits were [*MOT3*$^{+}$]-dependent, we expressed a Mot3 protein lacking the PrD (Mot3ΔPrD) that is immune to prion sequestration but retains normal transcriptional function (R.H., personal communication). Analogous to Sup35ΔPrD, this eliminates [*MOT3*$^{+}$] phenotypes without affecting other prions. NCYC 3311 lost acid resistance and Y-1537 lost fluconazole resistance with this plasmid, but not with plasmids expressing the full-length protein (Fig. 4b). These phenotypes were, therefore, [*MOT3*$^{+}$]-dependent. More broadly, the divergent consequences of this prion in different strains establish that, like [*PSI*$^{+}$], [*MOT3*$^{+}$] provides phenotypic diversity by altering the manifestation of natural genetic variation.

Wild strains harbour additional prions

How commonly do wild strains harbour prions that can create such heritable phenotypic diversity? Lacking means of detecting them by SDD–AGE, we used a phenotypic approach: we measured the growth of wild strains before and after GdHCl curing, across the same conditions used for [*PSI*$^{+}$] and [*MOT3*$^{+}$] (Supplementary Fig. 4). To ensure that any such phenotypes did not arise from *de novo* mutations, we compared four colonies of each wild strain with four cured derivatives (in total testing 5,520 isolates across 12 conditions).

Remarkably, over a third of the original wild strains (255) had phenotypes that differed in the same way between all four parental wild strains and all four cured derivatives. Moreover, nearly half of the growth properties conferred by these GdHCl-curable heritable elements were beneficial (Supplementary Table 1). The wild strain collection was biased towards wine isolates derived from natural fermentations. But it also contained many samples from beer, soil, fruit, infected human patients and commercial strains recently subject to man-made selective pressures to enhance properties for baking and brewing. Curable phenotypes, both beneficial and detrimental, occurred in yeasts from all of these niches. Even among the limited number of conditions tested here, prion curing had mixed phenotypic consequences in 15% of the strains. Thus, like [*PSI*$^{+}$] and [*MOT3*$^{+}$],

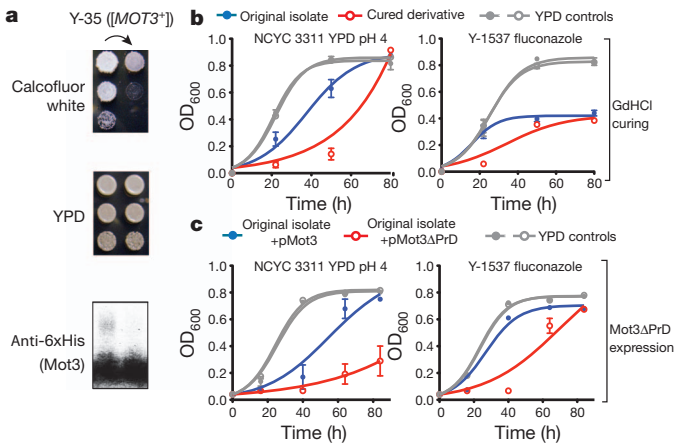

Figure 4 | Prions of the cell wall-remodelling transcription factor, Mot3, have diverse phenotypic consequences in wild strains. **a**, Strain Y-35 is resistant to the cell-wall-targeting drug, calcofluor white, but resistance is strongly reduced by passage on GdHCl. Probing for Mot3′s endogenous hexahistidine motif reveals that Mot3 amyloids are retained in the uncured, but not the cured isolates. Apparent monomeric signal results from cross-reactivity to other yeast proteins. **b**, The [*MOT3*$^{+}$] strains NCYC 3311 and Y-1537 (each shown in filled blue circles) are resistant to acidic growth conditions and fluconazole, respectively. Each phenotype is reversed by prion curing with GdHCl passage (open red circles). **c**, These phenotypes are also reversed by expression of a non-aggregating version of Mot3, Mot3ΔPrD (open red circles). Expression of Mot3 itself (closed blue circles) did not affect the phenotype of either strain. Error bars represent the standard deviation of four independent biological replicates.

these prions created different trade-offs — traits that were beneficial or detrimental depending on circumstances.

To test whether the altered phenotypes arose from prion-mediated protein templating or from some unknown (yet somehow heritable and reproducible) effect of transient GdHCl exposure, we investigated 25 randomly chosen strains more rigorously. Transient expression of Hsp104^{DN} phenocopied the effects of GdHCl curing in 22 of the 25 strains (Supplementary Table 1), establishing their dependence on this prion-partitioning factor.

Another signature of prions is transmission to other cells via cytoplasmic transfer (cytoduction). Do the curable phenotypic elements of wild yeasts share this property? Because of the complexities in working with wild yeast, we used a derivative of W303, a common laboratory strain, as a universal 'recipient' for cytoplasmic transfer. Because prion-dependent traits can differ with genetic background, we chose wild strains with multiple curable traits as donors for these unknown prions, to increase the likelihood that transfer could be scored phenotypically. The South African wine strain WE372 had two traits that were heritably lost by prion curing (Fig. 5a, arrows): unusually robust growth on rich medium and poor growth at pH 9. The clinical isolate YJM428 had three such traits (Fig. 5a, arrows): robust growth in sodium chloride and 4-NQO, but poor growth on maltose.

After crossing donor and recipient strains to produce heterokaryons, we selected buds that bore only the nucleus of the W303 recipient but had received cytoplasm from the wild donor (Fig. 5b top; see Methods for details). We tested 12 such cytoductants from each mating to determine if they had inherited stable new traits from the cytoplasmic transfer.

Poor growth at pH 9 was not transferred, but robust growth on rich medium was transferred from WE372 donors to all 12 W303 recipients (Fig. 5c, red arrows). NaCl resistance was not transferred, but both enhanced growth on 4-NQO and poor growth on YP-maltose was transferred from YJM428 donors to all 12 W303 recipients (Fig. 5c, red arrows). The fidelity of the transferred traits established that they were not due to rare chromosome transfers that can occur in such crosses. The lack of transfer for some traits suggests that, as for [*PSI*$^{+}$] and [*MOT3*$^{+}$], traits produced by these unknown heritable cytoplasmic factors depend upon the genetic background. All transferred traits were curable by passage on GdHCl (Fig. 5c, black arrows), strongly indicating they were due to prions. Excluding the possibility that these traits were due to mitochondrial DNA transfer, we repeated the entire experiment with WE372 and YJM428 donors that had been cured of prions before heterokaryon formation (Fig. 5b, bottom). None acquired the new phenotypes (Fig. 5d).

Prions alter the relationship between genotype and phenotype in wild strains

How significantly do the prions of wild yeasts alter the phenotypic manifestation of genetic diversity? We examined the relationship between genotype and phenotype in the 21 strains in our collection whose genomes had been fully sequenced. As previously reported[25,26], the Spearman's correlation (rho) between genotypes and phenotypes is typically on the order of 0.3 to 0.4 for wild yeast (in our strains and conditions, rho = 0.39; $P = 3.5 \times 10^{-15}$). Prion loss made the correlation between genotype and phenotype weaker (Spearman's rho = 0.27; $P = 1.5 \times 10^{-7}$). This finding was robust to random permutations of the data ($P = 0.0001$) and was clear even when [*PSI*$^{+}$] and [*MOT3*$^{+}$] strains were removed from the analysis. Thus, the prions these wild strains harbour broadly interface with polymorphisms in their genomes to influence the relationship between genotype and phenotype.

Discussion

The stable inheritance and complex phenotypes that prions produce arise from changes in protein conformation rather than from changes

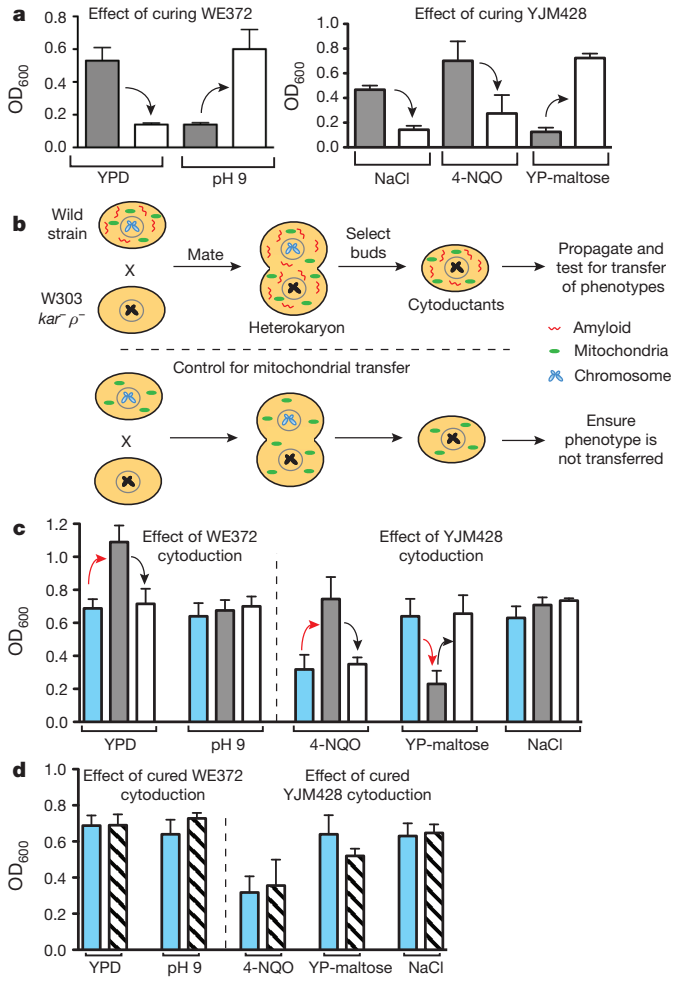

Figure 5 | The curable Hsp104-dependent epigenetic elements in wild yeast can be cytoplasmically transferred. **a**, Growth of wild strains WE372, YJM428, and their cured derivatives in selective conditions. Original isolates of each strain are shown in grey bars and their cured derivatives (indicated by black arrows) are shown in open bars. Error bars are one standard deviation from six biological replicates. **b**, Schematic for cytoduction experiments and control to ensure that phenotypes are due to prions, rather than transfer of mitochondrial DNA. **c**, Growth measurements of the laboratory recipient (blue bars), cytoductants (grey bars; red arrows denote cytoplasmic transfer of phenotypes), and cured derivatives of those cytoductants (open bars; black arrows denote curability of phenotypes). Error bars are the standard deviation of growth measurements from 12 cytoductants, the 12 cured derivatives of those cytoductants, or 6 biological replicates of the recipient strain. **d**, Cytoductants that received cytoplasm from cured derivatives of the original wild isolates (hashed bars) did not show an equivalent change in phenotype. Error bars represent the standard deviation of growth measurements from 12 cytoductants or 6 biological replicates of the recipient strain.

in nucleic acids. This non-canonical mode of inheritance has sparked considerable excitement and provoked intense mechanistic study (for review see ref. 27). But doubts about whether [*PSI*$^+$] and other prions exist in nature have fuelled deep controversy over their relevance[11,28]. We find that prions and prion-dependent phenotypes are widespread in nature, establishing their biological importance.

Saccharomyces cerevisiae is perhaps the most thoroughly characterized organism in experimental science. How, then, could this pervasive influence on the inheritance of biological traits have been missed for so long? The frequency of [*PSI*$^+$] in wild strains suggests that previous efforts to find it simply did not examine enough strains (see Supplementary Information for further discussion). But we suspect that standard practices in yeast genetics provide a far more general explanation. Phenotypic analysis of new traits typically begins by testing for 2:2 segregation in crosses and discarding variants that do not follow this Mendelian pattern. It is equally common to discard variants that prove to be restricted to individual strains. Thus, prion-based phenotypes may largely have been ignored because investigations were strongly biased by the known rules of nucleic-acid based inheritance and because of a pragmatism that neglected the biological significance of strain-to-strain variation.

We find that in wild strains a prion-dependent trait can readily be fixed by the meiotic re-assortment of endogenous genetic variation. (They can also probably be fixed by new mutations, a phenomenon not yet explored.) Thus, prions provide a robust mechanism by which yeast can increase their phenotypic diversity epigenetically, in a manner that readily allows that diversity to become hard-wired in subsequent generations. Evidence for an uncannily similar transition — from an epigenetically inherited trait to a genetically hard-wired trait — has recently surfaced for drug resistance in cancer cells[29,30]. The underlying epigenetic mechanism in that case is chromatin-based rather than protein-based. But together, these observations point to a new view of the importance of epigenetics in evolutionary processes.

Under stressful conditions, cells increase the rate at which they switch into and out of the [*PSI*$^+$] state[10]. This link between heritable phenotypic diversity and environmental contingency is a natural consequence of stress-induced disruptions in protein homeostasis[31–34]. Such stress-regulated factors interface with protein homeostasis to drive increased prion switching in several ways (see Supplementary Information for discussion). Beyond these more general homeostatic mechanisms, it seems likely that conformational switches in individual prion proteins will prove to be regulated by additional protein-specific interactions and post-translational protein modifications.

Surprisingly, 40% of the traits produced by the wild prions we observed were beneficial to growth under the 12 conditions we tested. This contrasts with the consequences of mutational variants, the vast majority of which are either silent or detrimental. It seems probable, therefore, that the underlying, cryptic variation that creates prion-based traits in wild yeast, as well as the prions themselves, have been subject to previous selective events[35]. In any case, the gain and loss of prions seems to constitute a sophisticated bet-hedging mechanism that allows cells to explore heritable new phenotypes more frequently in circumstances where they are not well suited to their environments.

Most of the 25 prions discovered to date are RNA-binding proteins, DNA-binding proteins and signal transducers — proteins that play key roles in governing the flow of information in cellular networks. Prion-mediated alterations in such functions allow access to complex traits in a single step. Together, the interface between prions, environmental stress, cryptic genetic variation and fixation provide a means to transition from a Lamarckian[36] mode of inheritance to a Darwinian framework of mutation and natural selection. As for Hsp90 (ref. 26), prions provide a robust route to the inheritance of environmentally induced traits that has moved from being merely plausible to demonstrable.

METHODS SUMMARY

SDD–AGE. Cells were grown for 18–24 h in 1 ml YPD followed by lysis in 96-well blocks. Lysates were treated with 2% SDS at room temperature and then clarified by centrifugation. Electrophoresis and blotting were performed as described[37].

Molecular cloning and yeast techniques. Standard cloning procedures[14] were used for the construction of yeast plasmids marked with drug resistance cassettes. Yeast strains were obtained from the sources indicated in the Supplementary Information. Yeast handling, propagation and prion curing were as described[14]. Cytoductions used donor strains created by disrupting the *HO* locus with an antibiotic-resistance marker[38] and then sporulating. The recipient strain was a respiration deficient *KAR1-15* derivative of W303. High throughput phenotyping and data analyses were performed as described[26].

Sequence analysis of wild strains. Strains UCD978 and 5672 were sequenced to 100-fold coverage using the Illumina HiSeq platform. After quality control filtering, each genome was aligned against the *S. cerevisiae* reference sequence using

the BWA aligner[39]. Correlation between similarity in genotype and similarity in phenotype was calculated as in ref. 26.

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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METHODS

Sequencing of wild strains. We sequenced two wild [*PSI*$^+$] strains (UCD978 and 5672), a clinical isolate (YJM653), a wine strain (I14) and two lab isolates (W303 and YDJ25, a strain almost completely isogenic to the laboratory reference S288C strain). Using an Illumina HiSeq platform we obtained two lanes of 76 base pair paired-end reads and one lane of 101 base pair paired-end reads, resulting in an average coverage of 100-fold (all reads will be available at NCBI under accession number PRJNA81619). After quality control filtering, each genome was aligned against the *S. cerevisiae* reference sequence (sacCer2, June 2008 assembly, downloaded from UCSC on April 1, 2011: http://hgdownload.cse.ucsc.edu/goldenPath/sacCer2/chromosomes/) using the BWA aligner[39] followed by SNP and indel calling with respect to the reference using mpileup from the samtools[40] package.

We then estimated the genetic distance between each unique pair of genomes using a combination of custom code and the Genome Analysis Toolkit (GATK)[41]. For each genome pair we considered the superset of SNPs in both genomes: if a SNP was present only in one genome, but not the other, it was retained. SNPs that were present in both genomes were discarded. We constructed a neighbour-joining tree using the APE R package[42] by using the genetic distance matrix from the counts of retained SNPs with quality score of at least 150 as called by mpileup. The horizontal scale bar in Supplementary Fig. 2 represents a genetic distance of 10,000 SNPs. To ensure that the tree was not sensitive to choice of the SNP quality cutoff, we performed the analysis at different quality thresholds and obtained essentially identical results.

We also separately sequenced the prion-determining region of *SUP35*. Only one had a non-synonymous change in the PrD, and this change was unlikely to influence Sup35's inherent prion propensity. All polymorphisms in the region spanning nucleotides −338 to 1102 of *SUP35* are indicated in Supplementary Table 2.

Molecular cloning. Standard cloning procedures were used[14]. Gateway destination vectors were constructed as follows. The *URA3* ORF in pAG416-GPD-ccdB, pAG416-TEF-ccdB and pAG416-SUP35-ccdB[14,43] was replaced with cassettes conferring G418 or hygromycin B resistance from plasmids pUG6 and pAG32, respectively, to generate pAG41NEO-GPD-ccdB, pAG41NEO-SUP35-ccdB and pAG41HPH-TEF-ccdB. These plasmids contain the *GPD1*, *SUP35* and *TEF2* promoters, respectively, for driving the expression of exogenous genes. Gateway entry clones bearing the coding sequences for *SUP35*, *SUP35*ΔPrD, *HSP104*, *HSP104*^{KTKT} and *MOT3* were constructed as described[14] using S288C genomic DNA and a plasmid bearing *HSP104* K218T K620T (ref. 3) as PCR templates. Site-directed mutagenesis was used to delete the PrD (amino acids 8–157) from the *MOT3* entry clone (R.H., personal communication). Entry clones and destination vectors were recombined in Gateway reactions to yield pAG41NEO-GPD-HSP104, pAG41NEO-GPD-HSP104^{KTKT}, pAG41NEO-SUP35-SUP35, pAG41NEO-SUP35-SUP35ΔPrD, pAG41HPH-TEF-MOT3 and pAG41HPH-TEF-MOT3ΔPrD.

Yeast techniques. Yeast strains (Supplementary Table 1) were obtained from stock centres or generously provided by the sources indicated. All strains were stored as glycerol stocks at −80 °C and revived on YPD before testing. Strains that were [*PSI*$^+$] in the original screen were re-ordered individually from the Department of Viticulture and Enology collection (University of California Davis) and the prion status was verified on a second SDD–AGE. Yeast were grown in YPD at 30 °C unless indicated otherwise. The following media supplements were included where relevant: 3 mM GdHCl, 200 μg ml^{-1} G418, or 250 μg ml^{-1} hygromycin B. Yeast were transformed with a standard lithium–acetate protocol as described[44].

To eliminate prions chemically, strains were passaged four times on rich medium containing 3 mM GdHCl. To eliminate prions by overexpression of Hsp104, cells were transformed with plasmids expressing Hsp104 (wild type or K218T, K620T) from a strong constitutive promoter (GPD). Transformants were passaged three times on selective media, followed by four passages on YPD to allow plasmid loss, which was confirmed by the absence of growth on selective media. Finally, as GdHCl is known to increase the frequencies of petites, all GdHCl- and Hsp104^{DN}-cured isolates used in the phenotyping experiments for [*PSI*+], [*MOT3*+], and the analyses of 25 strains containing unknown prions, were first checked for respiration competence on glycerol. Cured and pre-cured isolates grew comparably well on glycerol.

A PCR-based deletion strategy[45] was used to replace one genomic copy of *URA3* in strain UCD978 with an hphMX4 module from pAG32. The resulting strain was then sporulated. Random sporulants recovered on YPD containing hygromycin B were then sporulated again. Hygromycin-resistant sporulants from the second round of sporulation were tested for ploidy by growth on media containing 5-fluoroorotic acid (5-FOA), inability to grow on media lacking uracil, and ability to mate with haploid tester strains. Mating type was observed to be stable, indicating that UCD978 is heterothallic. Genomic DNA content was determined by SYTOX Green staining as described[46], using a BD LSR II flow cytometer. BY4741 and BY4743 were used as haploid and diploid references, respectively.

For cytoduction experiments, we used a derivative of the common lab strain, W303, as a recipient for cytoplasmic transfer (Fig. 5b). The strain carried a dominant *KAR1-15* allele, which prevents nuclear fusion during mating but allows cytoplasmic transfer. The strain also carried multiple auxotrophic markers and a mitochondrial DNA defect. This allowed cytoplasmic transfer to be scored through the restoration of mitochondrial respiration, in the absence of transfer of auxotrophic markers. Haploid, mating-competent derivatives of the wild 'donor' strains were created using an antibiotic-resistance marker to knock out the *HO* locus[38]. (HO would otherwise preclude mating by causing haploid cells to self-mate). The recipient and donor strains were patched together on rich media, followed by selection of heterokaryons on dropout media containing glycerol as a carbon source.

[*RNQ*+] strain UCD664 was verified to be *Saccharomyces uvarum* by colony PCR amplification and sequencing of the rDNA internal transcribed spacer region using oligonucleotides ITS1 and ITS4, as described[47]. Another prion-containing strain not annotated as *S. cerevisiae*, UCD587, was originally annotated "*S. cerevisiae* race *bayanus*". We ordered this strain two independent times from the stock centre and found it to be *S. cerevisiae* based on *ITS1* sequence and growth characteristics.

Phenotypic assays. For agar adhesion and invasion analyses, colonies were allowed to grow for 5–7 days at 30 °C. Plates were then gently rinsed under running water to remove non-adherent cells; then photographed. The agar surface was then gently rubbed with a gloved finger under running water to remove all remaining non-invasive cells.

For growth measurements, wild yeast strains were inoculated in quadruplicate into 384-well plates containing 40 μl YPD per well and grown to saturation at 30 °C (typically 48 h) in a humidified chamber. After complete re-suspension, QRep polypropylene 384-well pin replicators (Genetix) were used to transfer cells (200–500 nl) to new 384-well plates in duplicate. These new plates contained 40 μl per well of selective media: with alternative carbon sources at 2% final concentration (YP-maltose, YP-galactose, YP-glycerol or YP-raffinose), compounds dissolved in YPD (0.5 M NaCl, 5% ethanol, 0.4 mg ml^{-1} 4-NQO, 1 mM tBOOH, 32 mg ml^{-1} fluconazole or 50 mM hydroxyurea), acidic and basic conditions (YPD at pH 4 or pH 9), and finally in YPD alone (as a control). Concentrated stocks of 4-NQO (4 mg ml^{-1}) were made in molecular biology grade dimethyl sulphoxide (DMSO) and stocks of fluconazole (64 mg ml^{-1}) were made in ethanol. All plates were incubated at 30 °C, covered and in a humidified chamber. Growth was measured approximately every 20 h by OD_{600} with a Tecan Sapphire2 plate reader (after complete re-suspension by gentle agitation). Hits were chosen if all four replicates showed a significant change in growth ($P < 0.01$ determined by *t*-test) for at least two consecutive time points after curing of Hsp104-dependent prions.

Growth rates of [*PSI*$^+$]-containing wild strains (and their cured derivatives) in conditions used for laboratory propagation (YPD, YM broth, FM broth, wort agar or Wallerstein nutrient agar) was measured in quadruplicate, diluting 10^5 exponentially-growing cells of each strain in humidified 96-well plates containing 150 μl of medium. OD_{600} of each well was measured every 15 min after re-suspension by agitation (15 s) in a plate reader (Multiskan Go, Thermo Scientific). Plates were incubated at 30 °C. After 4 days no appreciable loss in volume was observed in the exterior wells of the plates, ensuring that the growth rates we measured were not an artefact of evaporation. The exponential phase of plots of OD_{600} versus time were fit to determine growth rates.

Correlation between similarity in genotype and similarity in phenotype was calculated in the R statistical computing software package using published genotypic correlation among sequenced strains and linear regression across the conditions in Supplementary Table 1. Significance was established by random perturbation of the phenotype data. Ten thousand permutations of the data did not reveal a similarly large change in genotype phenotype correlation occurring by chance.

SDD–AGE. Except where indicated below, SDD–AGE was performed as follows. Yeast were inoculated into 1 ml YPD in 96-well round-bottom blocks (Nunc P8241) with each well containing a single 3-mm glass bead. The blocks were incubated 18–24 h at 30 °C with 220 r.p.m. agitation. Cells were collected by centrifugation at 3,000 r.c.f. for 2 min, resuspended in 200 μl water, and then centrifuged again. Approximately 100 μl of acid-washed glass beads were then added to each well followed by 80 μl lysis buffer (100 mM Tris pH 8, 1% Triton X-100, 50 mM β-mercaptoethanol, 3% HALT protease inhibitor cocktail, 30 mM N-ethylmaleimide, and 12.5 U ml^{-1} Benzonase nuclease). Blocks were then sealed with a rubber mat (Nunc 276002) and shaken at max speed twice for 3 min on a Qiagen TissueLyser II. To each well was then added 35 μl 4× sample buffer (2× TAE, 20% glycerol, 8% SDS, 0.01% bromophenol blue). The blocks were then vortexed briefly and allowed to incubate at room temperature for three

minutes, followed by centrifugation for 2 min at 3,000 r.c.f. to remove cell debris. Electrophoresis and capillary blotting to Hybond ECL nitrocellulose were performed as described[37].

Sup35 and Rnq1 were detected using well-characterized antibodies[14]. Detection of amyloids using the Sup35 antibody was markedly improved by treating the blots with Re-Blot Plus stripping solution (Millipore) before probing. Available antibodies to yeast prion proteins Ure2, Swi1 and Cyc8 did not satisfactorily distinguish lab strains containing amyloids of these proteins from those that did not. Consequently, we did not attempt to identify these prions in wild strains. Horseradish-peroxidase-conjugated secondary antibodies were detected with Lumigen TMA-6 chemiluminescent substrate (GE).

For the detection of Mot3 amyloids, cells and lysates were prepared as described[37]. Blots were probed with a hexahistidine antibody (GE Biosciences), which recognizes an endogenous hexahistidine motif in Mot3. From multiple experiments with a well characterized [*MOT3*+] isolate in the S288C genetic background (ref. 14 and R.H., personal communication), we estimated false-negative and false-positive rates with this antibody to be approximately 40% and 5%, respectively, under the conditions used here. We occasionally observed the appearance of [*mot3*$^{-}$] colonies (as determined by SDD–AGE and phenotype) upon restreaking of [*MOT3*$^{+}$] isolates. Polymorphisms in *MOT3* (ref. 25) as well as differences in the growth and spheroplasting efficiencies of wild strains may influence the validity of SDD–AGE for assessing Mot3's prion status. Nevertheless, phenotypic differences verified our designations of [*MOT3*+] for three of six strains identified by SDD–AGE.

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