Prion Variants and Species Barriers among

Saccharomyces Ure2 proteins

Herman K. Edskes, Lindsay McCann, Andrea Hebert and Reed B. Wickner

Laboratory of Biochemistry and Genetics, National Institute of Diabetes Digestive and
Kidney Diseases, National Institutes of Health, Bethesda, MD 20892-0830

Contact information:

Bldg. 8, Room 225, NIH

8 Center Drive MSC 0830

Bethesda, MD 20892-0830

Phone: 301-496-3452

Fax: 301-402-0240

wickner@helix.nih.gov
Summary

As hamster scrapie cannot infect mice, due to sequence differences in their PrP proteins, we find ‘species barriers’ to transmission of the [URE3] prion in S. cerevisiae among Ure2 proteins of Saccharomyces cerevisiae, paradoxus, bayanus, cariocanus and mikatae based on differences among their Ure2p prion domain sequences. The rapid variation of the N-terminal Ure2p prion domains results in protection against the detrimental effects of infection by a prion, just as the PrP residue 129 Met/Val polymorphism may have arisen to protect humans from the effects of cannibalism. Just as spread of bovine spongiform encephalopathy prion variant is less impaired by species barriers than is sheep scrapie, we find that some [URE3] prion variants are infectious to another yeast species while other variants (with the identical amino acid sequence) are not. The species barrier is thus prion variant-dependent as in mammals. [URE3] prion variant characteristics are maintained even on passage through the Ure2p of another species. Ure2p of Saccharomyces castelli has a N-terminal Q/N rich ‘prion domain’ but does not form prions (in S. cerevisiae) and is not infected with [URE3] from Ure2p of other Saccharomyces. This implies that conservation of its ‘prion domain’ is not for the purpose of forming prions. Indeed the Ure2p prion domain has been shown to be important, though not essential, for the nitrogen catabolism regulatory role of the protein.

Introduction

Studies of the transmissible spongiform encephalopathies (TSEs) gave rise to the concept of a ‘prion’ meaning an ‘infectious protein’, without the need for an
accompanying nucleic acid to transmit the corresponding disease state (GRIFFITH 1967; PRUSINER 1982). The PrP protein, product of the Sinc gene, is believed to be the protein whose amyloid form is infectious (AGUZZI et al. 2008; BOLTON et al. 1982; CAUGHEY and BARON 2006; DICKINSON et al. 1968). Infection of sheep TSE (scrapie) to goats requires a longer incubation than from sheep to sheep or from goat to goat (CUILLE and CHELLE 1939), a phenomenon found to be general and now called the ‘species barrier’ (COLLINGE and CLARKE 2007). The species barrier is largely due to sequence differences in the PrP protein (PRUSINER et al. 1990).

Different scrapie isolates can have clearly distinct incubation times, disease symptoms, and distribution of brain lesions in a single mouse line. This phenomenon, called prion ‘strains’ or ‘variants’, implies differences in the infectious agent, and the finding of differences between prion strains in the protease resistant part of PrP supported PrP’s role as the infectious agent (BESSEN and MARSH 1992). The properties of a prion variant are (with some exceptions) maintained even though the prion is passed through different species with significantly different PrP sequences (BRUCE et al. 1994).

The species barrier and prion strain/variant phenomena are connected in that the extent of the species barrier depends critically on the prion strain. Most dramatically, although centuries of human exposure to sheep scrapie has not produced detectable infection, the bovine spongiform encephalopathy (BSE) epidemic led to >200 cases of a variant of Creutzfeldt-Jakob disease (vCJD). That this difference is due to prion variant differences is shown by studies with transgenic mice (reviewed in (COLLINGE and CLARKE 2007)).

Saccharomyces cerevisiae prions include [URE3], [PSI+], [PIN+] and [SWI+] and
[β], which are prions of Ure2p, Sup35p, Rnq1p, Swi1p and Prb1p, respectively (DERKATCH et al. 2001; DU et al. 2008; ROBERTS and WICKNER 2003; WICKNER 1994). Ure2p is a regulator of nitrogen catabolism, repressing the genes encoding enzymes and transporters needed for the utilization of poor nitrogen sources (e.g. allantoate) specifically when a good nitrogen source (e.g. ammonia) is available (COOPER 2002). Dal5p is the allantoate transporter and ureidosuccinate (USA), the product of aspartate transcarbamylase (Ura2p), is structurally similar to allantoate (TUROSCY and COOPER 1987). The presence of the [URE3] prion is thus assayed by the uptake by the Ure2-controlled Dal5p transporter of USA or by activity of ADE2 driven by the DAL5 promoter (LACROUTE 1971; SCHLUMPBERGER et al. 2001).

The [URE3], [PSI+] and [PIN+] prions are based on self-propagating amyloids of their respective proteins (BRACHMANN et al. 2005; KING and DIAZ-AVALOS 2004; PATEL and LIEBMAN 2007; TANAKA et al. 2004) reviewed in (WICKNER et al. 2008b). Species barriers have been described for [PSI+] (CHEN et al. 2007; CHERNOFF et al. 2000; KUSHNIROV et al. 2000; SANTOSO et al. 2000) and [URE3] (BAUDIN-BAILLIEU et al. 2003; EDSKES and WICKNER 2002) and prion variants have been recognized in yeast prions [PSI+], [URE3] and [PIN+] (BRACHMANN et al. 2005; BRADLEY et al. 2002; DERKATCH et al. 1996; SCHLUMPBERGER et al. 2001). The [URE3], [PSI+] and [PIN+] prion amyloids are each parallel in-register β-sheet structures (BAXA et al. 2007; SHEWMAKER et al. 2006; WICKNER et al. 2008a), and different prion variants of [PSI+] differ at least in the extent of the β-sheet structure (CHANG et al. 2008; TOYAMA et al. 2007).

Here we examine Ure2 proteins in the genus Saccharomyces, finding that one
does not form prions, that there are species barriers between different Ure2 proteins and that the extent of the species barrier depends on the individual prion variant (presumably amyloid structure) that is being transmitted. Moreover, prion variant properties can be maintained through passage in a prion protein of a different species.

RESULTS

Divergence of URE2 sequences of Saccharomyces species. The genus Saccharomyces includes a number of species that are significantly diverged but that nonetheless can mate with each other (SIPICZKI 2008). The Ure2 proteins of S. cerevisiae, mikatae, cariocanus, paradoxus, bayanus and castelli are shown in Fig. 1. As previously noted for a wider range of yeasts (EDSKES and WICKNER 2002), the N-terminal ~40 residues are largely conserved, but there are a number of differences in the following ~50 residues of the prion domain, and the C-terminal parts of the molecule are nearly invariant.

The URE2 open reading frames of S. cerevisiae, mikatae, cariocanus, paradoxus, bayanus, and castellii were used to replace the kanMX cassette at the URE2 locus in cerevisiae strain LM9 (MATα,ura2 leu2 his3 ure2::kanMX) (see Methods).

Nomenclature. We indicate a [URE3] prion variant originating in cerevisiae and propagating in cells expressing the Ure2p of mikatae by the symbol [URE3cer]mik. A prion isolate (variant) number can be added, e.g. [URE3cer4]mik.

Prion formation by Ure2p of Saccharomyces species in cerevisiae.

To determine if the different Ure2 proteins could form [URE3], the homologous Ure2p or the cerevisiae Ure2p was overexpressed from a GAL1 promoter on a plasmid
and prion formation was detected on USA or –Ade plates containing dextrose (Table 1). The chromosomal URE2 from each species was driven by the constitutive *cerevisiae* URE2 promoter. The *cerevisiae, mikatae, cariocanus, paradoxus* and *bayanus* Ure2p’s could each form [URE3], and, as expected, the frequency with which [URE3] arose was greater when the over-expressed Ure2p was the same as that encoded on the chromosome.

Except for *URE2paradoxus*, each of the URE2s used here had its native stop codon: *cerevisiae* had TGA, *bayanus* TAA, *castellii* TAG, *cariocanus* TGA and *mikatae* TAA. *URE2paradoxus* with a TGA stop codon, allows some read-through (TALAREK et al. 2005), and so we also constructed *URE2paradoxus* with a TAA stop sequence, the same as in the native *URE2paradoxus* gene. In this case as well, we observed that overexpression of the protein induced the high frequency *de novo* appearance of the prion (Table 2).

**S. castellii Ure2p does not form a prion.** Because the *castellii* URE2 gene was toxic to *E. coli*, all constructs were made using PCR directly in yeast (see Methods). Moreover, the Ade- phenotype was somewhat leaky and not useful so all experiments were done using USA uptake to indicate Ure2p activity. Over expression of Ure2*castellii* did not induce the appearance of USA+ colonies (Table 1). Cytoduction of [URE3] from several [URE3cer]cer, into cells expressing Ure2*castellii* produced only a few USA+ clones among >250 cytoductants. Each of these was further tested for cytoduction into another Ure2*castellii* strain and none transferred the USA+ phenotype to cytoductants. A meiotic cross of YHE1236 (Ure2*castellii*) and YHE1233 ([URE3cer109]cer) produced uniform 2 USA+:2USA- segregation in each of 24 four-spored tetrads. In each of the 12 tetrads
checked by PCR with primers specific for the castellii and cerevisiae URE2 genes, the USA+ segregants all had the cerevisiae gene and the USA- segregants all had the castellii gene. Thus, cells with Ure2castellii do not develop their own [URE3] at a detectable frequency, nor can they be infected with [URE3cer]cer, the best one at crossing species barriers (see next section).

Species barriers among Saccharomyces [URE3]s. A single [URE3] variant derived by over expression of each Ure2p was used as cytoduction donor to [ure-o] strains of opposite mating type carrying each of the URE2 genes (Table 3). In each case the transmission was best when the URE2 of the donor (and origin of the [URE3] isolate) was the same as the URE2 of the recipient. Most cases in which the donor and recipient had different URE2s resulted in lower transmission frequency – a species barrier.

[URE3cer109]cer (i.e. the [URE3] in strain LM109 generated from the cerevisiae Ure2p and in a strain whose Ure2p is from cerevisiae) was efficiently transmitted to each of the other species’ Ure2p (Table 3). In many cases, having crossed the species barrier, a prion is less stable than with its Ure2p of origin. For example, although [URE3cer3687]cer propagates with Ure2ps of five other species, it is more unstable in those strains than with Ure2cerevisiae (data not shown). We also compared ability of Ure2paradoxus terminated with TGA and TAA to be a recipient of [URE3cer3687]cer and found them similar (Supplemental Table 1).

Species barriers are not symmetrical (Table 3). For example, [URE3cer109]cer efficiently infects Ure2bayanus, but [URE3bay121]bay does not infect Ure2cerevisiae at all. However, these results are very much dependent on which variant of each is considered.
[URE3\textsubscript{cer}] maintains ability to propagate on Ure2p\textsubscript{cer} in hosts with other Ure2ps.

[URE3\textsubscript{cer3687}]\textsubscript{cer} (WICKNER 1994) was cytoduced into cells expressing \textit{bayanus}, \textit{mikatae}, \textit{paradoxus} or \textit{cariocanus} Ure2p, and stable [URE3\textsubscript{cer3687}]\textsubscript{XYZ} derivatives of each were obtained. These were grown several times to single colonies and then cytoduced back into LM60 (MAT\textsubscript{a} leu2 trp1 kar1 URE2\textsubscript{cerevisiae} \textit{PDAL5ADE2} \textit{PDAL5CAN1} [ure-o]) (Table 4). Although transmission of [URE3]s native to other species’ Ure2s are often poorly transmitted to Ure2\textsubscript{cerevisiae} (Table 3), transmission of this [URE3\textsubscript{cer3687}] (originating in \textit{cerevisiae}) from each of the other species to Ure2\textsubscript{cerevisiae} was quite efficient (Table 4). This indicates that the strain characteristics of [URE3\textsubscript{cer3687}] were maintained during passage in cells expressing only the Ure2p of another species.

Species barriers vary with [URE3] prion variant. We examined the ability of several [URE3\textsubscript{car}]\textsubscript{car} isolates to be transmitted to Ure2\textsubscript{mikatae} (Table 5). As with transmission of [URE3\textsubscript{cer3687}]\textsubscript{cer} to Ure2\textsubscript{mikatae}, [URE3\textsubscript{cer3687}]\textsubscript{car} was transmitted well to Ure2\textsubscript{mikatae}. While [URE3\textsubscript{car146}]\textsubscript{car} and [URE3\textsubscript{car147}]\textsubscript{car} were likewise transmitted as well to Ure2\textsubscript{mikatae} as they were to Ure2\textsubscript{cariocanus}, [URE3\textsubscript{car145}]\textsubscript{car} and [URE3\textsubscript{car151}]\textsubscript{car} were well transmitted to Ure2\textsubscript{cariocanus}, but only poorly to Ure2\textsubscript{mikatae} (Table 5).

Dependence of species barrier on prion variant can also be seen in a contrast of strains whose [URE3] arose from the same Ure2p compared with prions arising from the Ure2p of another species. For example, each of six [URE3\textsubscript{mik}]\textsubscript{mik} transmitted poorly to cells expressing Ure2\textsubscript{cariocanus}, but [URE3\textsubscript{cer3687}]\textsubscript{mik} transmitted readily to the same
cells (Supplemental Table 2), as does [URE3cer109]cer (Table 3). Similarly, each of three [URE3bay]bay isolates transmit poorly to each other species, but a [URE3cer3687]bay transmits well to all (Supplemental Table 3). Thus, the species barrier depends on the particular prion variant, and, as before, the species range is preserved with transmission through another species.

**Species barrier does not mean absence of interaction.** All [URE3bay]bay prions tested are poorly transmitted to any of the other Ure2s (Supplemental Table 3). However, we find that expression of each of the other Ure2s as Ure2-GFP fusion proteins interferes with propagation of [URE3bay121]bay, curing it quite efficiently (Supplemental Table 4).

**DISCUSSION**

**Usa and Ade phenotypes are not always parallel.** The Ure2 proteins from other Saccharomyces species generally function in cerevisiae, but not uniformly so. For example, the castellii Ure2p does not completely repress the PDAL5ADE2 gene. Repression of USA uptake apparently requires less active Ure2p than does repression of ADE2 in the PDAL5ADE2 gene. This may reflect either differences in the turnover numbers of Dal5p and of Ade2p or that Ure2p regulates both transcription and protein maturation of Dal5p, but only transcription of the PDAL5ADE2 gene. Although the C-terminal glutathione-S-transferase-like domains of Ure2p are highly conserved among the Saccharomyces species, Gln3p, with which it interacts, is poorly conserved. The cerevisiae Ure2p is in ~20-fold excess over Gln3p (Fig. S1) and is a dimer evenly distributed in the cytoplasm in [ure-o] cells, whereas Gln3p is present in a large complex
(Fig. S2) and appears in localized sites in the cell (Tate & Cooper, 2007). Thus, the relation of \([\text{URE3}]\) variants to their phenotypes is certainly not simple and is not yet understood.

**Why do URE2 prion domain - encoding sequences vary so rapidly?** The Ure2p prion domain is important for the nitrogen regulation function of the molecule, protecting it from degradation and facilitating interaction with other proteins related to nitrogen regulation (SHEWMAKER \textit{et al.} 2007). The part of the prion domain that performs this function has not been determined, but it is possible that the 40-90 region is simply under no functional constraint and so varies rapidly. Both parts of the prion domain are unstructured (PIERCE \textit{et al.} 2005) indicating that maintaining a stable protein fold does not determine the difference between the relatively conserved region (1-40) and the rapidly varying region (40-90).

Alternatively, the variation may be selected for in order to acquire the ‘species barrier’ that we observe. Collinge has suggested that the human PrP M/V polymorphism at residue 129 may have been selected because heterozygotes are immune to infectious CJD in an era when cannibalism was more common than it is now (MEAD \textit{et al.} 2003). Similarly, \([\text{URE3}]\) is a substantial detriment to its host (NAKAYASHIKI \textit{et al.} 2005) and the rapid variation in the 40-90 region of the prion domain may be a consequence of selection for immunity to the \([\text{URE3}]\) infectious disease.

**Why is there a conserved region?** The conservation of sequence in the 1-40 region might be interpreted as important for prion formation if the \([\text{URE3}]\) prion were not known to be detrimental. However, beyond this, it has been shown that sequence of the Ure2p prion domain is of minimal importance for prion formation and that only amino
acid composition is important (ROSS et al. 2004), so prion formation cannot explain the conservation of sequence. It is likely that the conserved region is important for its role in facilitating nitrogen regulation (SHEWMAKER et al. 2007).

Prion domains that cannot be a prion. We find that the Ure2p of S. castellii will not form [URE3] itself, nor can it be infected with [URE3] from Saccharomyces cerevisiae. This suggests that the Ure2p ‘prion domain’ is not conserved in this organism for the purpose of prion formation, but it remains possible that the S. castellii Ure2p can form a prion in S. castellii. Although we had observed [URE3] formation by the S. paradoxus Ure2p (EDSKES and WICKNER 2002), it was reported that this was a result of our construct having a TGA termination codon resulting in significant read-through (TALAREK et al. 2005). Moreover, it was found that the S. paradoxus Ure2p (identical in sequence to that we used) could not become a prion in either cerevisiae (BAUDIN-BAILLIEU et al. 2003) or in paradoxus itself (TALAREK et al. 2005). We have constructed a strain with the TAA termination codon and find that in cerevisiae it can be infected with [URE3] from the cerevisiae Ure2p. The discrepancy between these results may be the result of some other strain background difference.

Species barriers among Saccharomyces Ure2 proteins. For each pair of species forming [URE3] prions, there is some degree of incompatibility. Generally the cerevisiae [URE3] isolates showed the smallest species barriers. Whether this is a result of the experiments having been carried out in S. cerevisiae or is an inherent feature of the sequences is not yet clear. The differences in amino acid sequence among the Ure2 prion domains of Saccharomyces species are comparable to that among the PrP proteins of different mammals. Collinge has proposed that each sequence has a range of
conformations that it can assume in the prion polymers, and that the height of the species barrier is generally inversely proportional to the overlap of the conformational ranges of the two sequences (COLLINGE 1999; COLLINGE and CLARKE 2007). This model doubtless applies to *Saccharomyces* prions as well as it does to those of mammals.

But how does a minor difference in amino acid sequence result in a substantial species barrier in spite of the fact that randomizing the sequence does not prevent prion formation (ROSS *et al.* 2004; ROSS *et al.* 2005a)? We proposed that only a parallel in-register β-sheet structure can explain this finding (ROSS *et al.* 2005b), and indeed infectious amyloids of the prion domains of Ure2p, Sup35p and Rnq1p have this architecture (BAXA *et al.* 2007; SHEWMAKER *et al.* 2006; WICKNER *et al.* 2008a). This structure is stabilized by ‘polar-zipper’ H-bonds between aligned glutamine and asparagine side chains (CHAN *et al.* 2005; NELSON *et al.* 2005; PERUTZ *et al.* 1994) and hydrophobic interactions both of which would be decreased by having a mixture of sequences. This structure can also be viewed as a linear crystal, and, like a 3D crystal, the introduction of a non-identical molecule is expected to disrupt the structure beyond its actual location.

**Species barriers vary with prion variants.** Unlike sheep scrapie, the BSE epidemic spread to humans and other animals. This was not only a consequence of the sequence of bovine PrP, but of the conformation of the BSE prion variant (reviewed in (COLLINGE and CLARKE 2007)). We observe a clear dependence of species barrier on prion variant. For example, the [*URE3cer3687*]bayan is well transmitted to all Ure2 sequences, but four other *bayanus* [*URE3*]s strongly prefer *Ure2*<sup>bayanus</sup>. Applying the Collinge model, one would say that, unlike the others, [*URE3 cer3687*]<sup>bay</sup> is a conformation easily adopted by
the other Ure2ps.

In yeast prions, variants have been defined by strength of the prion phenotype, stability of the prion, and the effects of various chaperones (e.g., (BORCHSENIUS et al. 2006; DERKATCH et al. 1996; KRYNDUSHKIN and WICKNER 2007). As shown here for yeast, and long known in mammalian systems, the host range can also serve as a means to distinguish different prion variants. Since two variants based on different structures could easily have the same phenotype intensity and stability, it is important to have as many distinguishing characteristics as possible.

**[Het-s] has only one variant – because it is adaptive.** Only one variant of the [Het-s] prion has been described (BENKEMOUN and SAUPE 2006), and the very sharp lines seen in 2D solid-state NMR studies of amyloid of HETs218-289 (the prion domain) (RITTER et al. 2005) indicate that it adopts a very specific single conformation, while the multiple variants (BRACHMANN et al. 2005; BRADLEY et al. 2002; DERKATCH et al. 1996) and wider 2D solid-state NMR peaks for the prion domains of Ure2p, Sup35p and Rnq1p (BAXA et al. 2007; SHEWMAKER et al. 2006; WICKNER et al. 2008a) indicate that they spontaneously form a mixture of distinct amyloid structures. This may reflect the fact that [Het-s] carries out a function - either the host function, heterokaryon incompatibility, or the 'spore-killer' meiotic drive function. In contrast, [URE3] and [PSI+] are diseases of yeast in which the amyloids do not have an evolved structure-function relationship (NAKAYASHIKI et al. 2005). A knee bends in a very specific way, but a leg may be broken in many ways.

**METHODS**
Media. Rich medium (YPAD = yeast extract peptone adenine dextrose), minimal medium (SD = synthetic dextrose), glycerol rich medium (YPG = yeast extract peptone glycerol) and sporulation medium were prepared as described by Sherman (SHERMAN 1991). YES medium is 0.5% yeast extract, 3% glucose, 30 mg/l tryptophan.

Strains. YHE256 is MATα kar1 ura2 arg-[URE3cer3687]cer (EDSKES and WICKNER 2000)

LM9 (MATα ura2 leu2::hisG his3::hisG ure2::kanMX P_DAL5ADE2 P_DAL5CAN1) was constructed in the Σ1278b background (a) integrating CAN1 with the DAL5 promoter amplified from strain BY108 (BRACHMANN et al. 2005) selected for canavanine resistance using ammonia as a nitrogen source, (b) replacing 500 bp upstream of the ADE2 ORF with 561 bp of sequence upstream of the DAL5 ORF using strain BY241 (BRACHMANN et al. 2005) as described (SCHLUMPBERGER et al. 2001) and (c) replacing the URE2 ORF with the kanMX4 cassette with a PCR fragment obtained using a ure2::kanMX4 strain from the Saccharomyces Genome Deletion Project.

Transformation of LM9 with DNA fragments containing the URE2 ORFs from several Saccharomyces species flanked by upstream and downstream sequences of S. cerevisiae URE2 resulted in strains: LM11 (S. bayanus URE2), LM16 (S. mikatae URE2), LM18 (S. paradoxus URE2-TGA), LM27 (S. castellii URE2), LM41 (S. cariocanus URE2), and LM45 (S. cerevisiae URE2). Transformants were selected through their resistance to canavanine. Those which had become G418-sensitive were checked for acquisition of the corresponding URE2 gene by PCR amplification from genomic DNA and sequencing. A tentative tree of Ure2p sequences is shown in Supplemental Fig. 3.
YHE1254 containing URE2 from *S. paradoxus* terminating with a TAA codon was created using URE2 tagged with a 3’ located HIS3 gene flanked by loxP sites as a marker. HIS3 was removed by expression of CRE from plasmid YEp351-cre-cyh (DELNERI et al. 2000).

**Adding kar1, trp1 and changing mating type in LM9 derived strains.**

Kar1Δ15 (VALLEN et al. 1992) containing a deletion in the ORF between bp 106 and 192 was amplified by PCR from strain L2598 (gift from S. Liebman). The PCR product started 80 bp upstream of the *KAR1* start codon and terminated 30 pb downstream of its stop codon. The hygromycin-resistance expression cassette from pAG32 (GOLDSTEIN and MCCUSKER 1999) was amplified by PCR using primers containing *KAR1* sequences downstream of the stop codon (5’ primer nt 11-30 and 3’ primer nt 37-57). Fusion PCR created a fragment containing kar1Δ15-hygromycin-KAR1 (nt 57-347 downstream of the stop codon). Transformants were selected through their resistance to hygromycin.

The mating type was switched by expressing of HO from pJH132 (JENSEN and HERSKOWITZ 1984) upon galactose induction.

Strains were made tryptophan prototrophs and histidine auxotrophs by replacing the *TRP1* ORF with the *HIS3* ORF from pRS423 (CHRISTIANSON et al. 1992). The resulting strains have the genotype MATa ura2 leu2::hisG his3::hisG trp1::HIS3

*P*$_{DAL5ADE2}$ *P*$_{DAL5CAN1}$ kar1::kar1Δ15-hphMX4 URE2*. URE2* represents URE2 from the different *Saccharomyces* species with LM105 containing *S. cerevisiae* URE2, LM107 containing *S. mikatae* URE2, LM108 containing *S. cariocanus* URE2, LM117 containing *S. paradoxus* URE2 terminating with a TGA stop codon, LM194 containing S.
bayanus URE2, and LM191 containing S. castellii URE2.

**Strains containing S. castellii URE2.** LM191 (see above) forms white colonies on Yes medium. Several attempts to remake LM191 always resulted in strains that formed white colonies on Yes plates. LM191 and LM27 (see above) were crossed and sporulation of the diploids resulted in irregular segregation of red and white spore clones. Mating and sporulation of two red spore clones resulted in tetrads giving nearly 100% red spore clones. One of these, YHE1242 (MATα ura2 leu2::hisG his3::hisG URE2castellii PDAL5ADE2 PDAL5CAN1 kar1::kar1Δ15-hphMX4) was used for [URE3] induction experiments. Upon propagation, most URE2castellii cells become light red and easily revert further to an Ade+ phenotype. Growth on medium containing 3 mM guanidine does not restore the red/Ade- phenotype. These cells remain clearly USA- throughout.

LM51 (MATα ura2 leu2 trp1 kar1 ure2::kanMX4 PDAL5CAN1 PDAL5ADE2) was a meiotic segregant of LM9 x BY304 (gift from A. Brachmann). The URE2 gene from S. cerevisiae was introduced into LM51 as described above resulting in strain LM60 (MATα ura2 leu2 trp1 PDAL5CAN1 PDAL5ADE2 kar1).

**Plasmid construction.** pH125 (2μ LEU2 PADH1- TADH1) (EDSKES and WICKNER 2000). The NheI-BamHI bordered ADH1 promoter was replaced by the 179 bp URE2cerevisiae 5’ UTR creating pH795. Next the XhoI-BglII bordered ADH1 terminator was replaced with the 400 bp URE2cerevisiae 3’ UTR creating pH795. Finally the NsiI-BbeI fragment of pH795 containing most of the 2μ origin of replication and LEU2 was replaced with a StuI site.

URE2 was amplified by PCR from yeast strains S. cariocanus 50791, S. castellii
NRRL Y-12630, and S. mikatae 1815 (gifts from Jim Dover wustl) using primers based
on the sequences obtained by Cliften et al. (CLIFTEN et al. 2001). Yeast cells were
heated to 95°C for 3 minutes in 0.25% SDS and the cleared lysate was used for PCR.
Cloning of \textit{URE2} from \textit{S. cerevisiae}, \textit{S. bayanus} (YJM562), and \textit{S. paradoxus} (YJM498)
has been described (EDSKES and WICKNER 2002). All \textit{URE2} sequences contained a
BamHI site upstream of the start codon and a XhoI site downstream of the stop codon.
PCR products were cloned into the BamHI/XhoI window of pH317 (2 micron replicon
\textit{LEU2} \textit{P}_{\text{GAL1}} \text{(EDSKES and WICKNER 2000)}, creating pH376 (\textit{S. cerevisiae URE2}
(BRADLEY et al. 2002)), pLM100 (\textit{S. bayanus URE2}), pLM99 (\textit{S. paradoxus URE2}),
pLM96 (\textit{S. cariocanus URE2}), and pLM98 (\textit{S. mikatae URE2}). These same PCR
products were also cloned into pTIF2 (\textit{P}_{\text{URE2}}\textit{cerevisiae}-\textit{T}_{\text{URE2}}\textit{cerevisiae}) creating pTIF5
(\textit{S. cerevisiae URE2}), pLM93 (\textit{S. bayanus URE2}), pLM92 (\textit{S. paradoxus URE2}), pLM89
(\textit{S. cariocanus URE2}), and pLM91 (\textit{S. mikatae URE2}).

The \textit{URE2} ORF present in pLM99 and pLM92 terminates with a TGA stop
codon. However, the native \textit{S. paradoxus URE2} stop codon is TAA. Cloning of a PCR
amplified \textit{S. paradoxus URE2} containing a TAA-A stop sequence into the BamHI/XhoI
window of pH317 created pH1010. This same TAA-A containing the \textit{S. paradoxus URE2}
sequence was cloned into the BamHI/XhoI window of pTIF2 creating pH1001. \textit{HIS3}
was amplified by PCR from pRS423 (nt 201-1163) using oligos containing loxP sites (5’-
ATAACTTCGTATAGCATACATTATACGAAGTTAT-3’). LoxP - bordered \textit{HIS3} was
ligated as a XhoI fragment into the XhoI site of pH1001 creating pH1002.

We used homologous recombination cloning in yeast to make a 2 micron DNA
\textit{LEU2} - based plasmid over-expressing the \textit{S. castellii URE2} using a \textit{GAL1} promoter. A
632 bp \textit{GAL1} promoter fragment and a 316 bp \textit{ADH1} terminator fragment, identical to those present in pH317, were fused by PCR to the \textit{S. castelli URE2} ORF. This PCR product together with BamHI/XhoI digested pH317 was transformed into yeast.

pH327 ((EDSKES et al. 1999) expresses \textit{S. cerevisiae URE2} fused to GFP from a centromeric \textit{LEU2} containing plasmid using a URE2 promoter. The unique NdeI site was removed by changing it from CATATG into CACATG using the QuickChange II site-directed mutagenesis kit (Stratagene) creating pLM147. The \textit{URE2} ORFs from \textit{S. cerevisiae}, \textit{S. paradoxus}, \textit{S. bayanus}, \textit{S. cariocanus} and \textit{S. mikatae} all contain an unique NdeI site 45 bp before the stop codon. In this region all five Ure2 proteins have identical amino acid sequences. Replacement of the \textit{URE2} - containing BamHI/NdeI fragment from pLM147 with similar fragments from pLM99 (\textit{S. paradoxus}), pLM100 (\textit{S. bayanus}), pLM96 (\textit{S. cariocanus}), and pLM98 (\textit{S. mikatae}) created respectively pLM152, pLM153, pLM150, and pLM151.

\textbf{Acknowledgements.} This work was supported by the Intramural Program of the National Institute of Diabetes Digestive and Kidney Diseases. We would like to thank Tiffany Weinkopff for the construction of pTIF2 and pTIF5, and Jim Dover of Washington University at St. Louis for the gift of \textit{Saccharomyces} strains. We are grateful to Anthony Furano for protein sequence analysis.
References:


CLIFTEN, P. F., L. W. HILLIER, L. FULTON, T. GRAVES, T. MINER et al., 2001
Surveying Saccharomyces genomes to identify functional elements by
comparative DNA sequence analysis. Genome Res. 11: 1175 - 1186.


COLLINGE, J., and A. R. CLARKE, 2007 A general model of prion strains and their

COOPER, T. G., 2002 Transmitting the signal of excess nitrogen in Saccharomyces
cerevisiae from the Tor proteins to th GATA factors: connecting the dots. FEMS
Microbiol. Revs. 26: 223-238.

CUILLE, J., and P. L. CHELLE, 1939 Experimental transmission of trembling to the

DELNERI, D., G. C. TOMLIN, J. L. WIXON, A. HUTTER, M. SEFTON et al., 2000
Exploring redundancy in the yeast genome: an improved strategy for use of the

DERKATCH, I. L., M. E. BRADLEY, J. Y. HONG and S. W. LIEBMAN, 2001 Prions
affect the appearance of other prions: the story of [PIN]. Cell 106: 171 - 182.

DERKATCH, I. L., Y. O. CHERNOFF, V. V. KUSHNIROV, S. G. INGE-VECHTOMOV and S. W. LIEBMAN, 1996 Genesis and variability of [PSI]

which controls the incubation period of some strains of scrapie in mice. J. Comp.
Path. 78: 293 - 299.


PRUSINER, S. B., M. SCOTT, D. FOSTER, K.-M. PAN, D. GROTH et al., 1990

RITTER, C., M. L. MADDELEIN, A. B. SIEMER, T. LUHRS, M. ERNST et al., 2005


SIPICZKI, M., 2008 Interspecies hybridization and recombination in *Saccharomyces wine yeasts*. FEMS Yeast Res. 8: 996-1007.


WICKNER, R. B., F. DYDA and R. TYCKO, 2008a Amyloid of Rnq1p, the basis of the PIN prion, has a parallel in-register β-sheet structure. Proc Natl Acad Sci U S A 105: 2403 - 2408.


**Figure Legends:**

Fig. 1. Ure2 protein sequences of *Saccharomyces* species. "-" means a residue deletion and "•" means a residue identical to the *S. cerevisiae* sequence.
<table>
<thead>
<tr>
<th>Source of URE2 (strain)</th>
<th>overexpress</th>
<th>overexpress</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>cerevisiae</td>
<td>homol.</td>
</tr>
<tr>
<td></td>
<td>USA+ / 10^6 cells</td>
<td>ADE+ / 10^6 cells</td>
</tr>
<tr>
<td>cerevisiae (LM45)</td>
<td>3</td>
<td>27,000</td>
</tr>
<tr>
<td>mikatae (LM16)</td>
<td>6</td>
<td>2,675</td>
</tr>
<tr>
<td>cariocanus (LM41)</td>
<td>4</td>
<td>4,175</td>
</tr>
<tr>
<td>paradoxus (LM18) TGAC.</td>
<td>15</td>
<td>3,775</td>
</tr>
<tr>
<td>bayanus (LM11)</td>
<td>6</td>
<td>325</td>
</tr>
<tr>
<td>castellii</td>
<td>4</td>
<td>5</td>
</tr>
</tbody>
</table>

Table 1. Induction of prion formation in *S. cerevisiae* strains (MATα ura2 leu2::hisG his3::hisG *P_{DAL5}CAN1 P_{DAL5}ADE2* [ure-o]) with chromosomal URE2 from various *Saccharomyces* species. Ure2p of *S. cerevisiae* or of the same Ure2p as encoded on the chromosome (homol.) was transiently overproduced from a plasmid with a *GAL1* promoter. Numbers are averages of four independently assayed transformants (except for *castellii* where pooled red/Ade minus clones were used in two independent assays). ND = not done.
<table>
<thead>
<tr>
<th>Strain</th>
<th>Plasmid</th>
<th>USA+/10⁶</th>
<th>Ade+/10⁶</th>
</tr>
</thead>
<tbody>
<tr>
<td>URE2paradoxus TGA</td>
<td>vector</td>
<td>14</td>
<td>19</td>
</tr>
<tr>
<td>URE2paradoxus TGA</td>
<td>PGAL1 URE2par TGA</td>
<td>1230</td>
<td>110,300</td>
</tr>
<tr>
<td>URE2paradoxus TGA</td>
<td>PGAL1 URE2par TAAA</td>
<td>1630</td>
<td>104,300</td>
</tr>
<tr>
<td>URE2paradoxus TAA</td>
<td>vector</td>
<td>119</td>
<td>76</td>
</tr>
<tr>
<td>URE2paradoxus TAA</td>
<td>PGAL1 URE2par TGA</td>
<td>4870</td>
<td>67,500</td>
</tr>
<tr>
<td>URE2paradoxus TAA</td>
<td>PGAL1 URE2par TAAA</td>
<td>3800</td>
<td>49,300</td>
</tr>
</tbody>
</table>

Table 2. The ability to induce \([URE3]\) upon overexpression of Ure2p was compared in strains LM18 (URE2paradoxus TGA) and YHE1254 (URE2paradoxus TAAA), each having genotype $\text{MAT}^a\text{ura}2\text{leu2::hisG} \text{his}3::\text{hisG} \ P_{\text{DAL5}}\text{CAN1} \ P_{\text{DAL5ADE2}}$ [ure-o]. Plasmids were: vector, pH317 = 2 micron DNA - \textit{LEU2} - PGAL1; PGAL1 URE2paradoxus TGA, pLM99 = 2 micron DNA - \textit{LEU2} - PGAL1

URE2paradoxus TGA; PGAL1 URE2par TAAA, pH1010 = 2 micron DNA - \textit{LEU2}
Table 3. Species barriers among Ure2p’s of *Saccharomyces cerevisiae*, *mikatae*, *cariocanus*, *paradoxus* and *bayanus*. Donors and recipients are all isogenic *S. cerevisiae*. From 44 to 107 cytoductants were scored and % USA+ and % ADE+ are shown. The *cariocanus* and *paradoxus* donors were >98% mitotically stable. Donor genotypes: MATα *ura2 leu2::hisG his3::hisG P*<sub>DAL5</sub>*CAN1 P*<sub>DAL5</sub>*ADE2*. Recipient genotypes: MATα *ura2 leu2::hisG his3::hisG trp1::HIS3 kar1Δ15-hphMX4 P*<sub>DAL5</sub>*CAN1 P*<sub>DAL5</sub>*ADE2*.
<table>
<thead>
<tr>
<th>Donors</th>
<th>Species</th>
<th>Recipient</th>
<th>Strain</th>
<th>Cytoductants</th>
<th>USA+</th>
<th>ADE+</th>
</tr>
</thead>
</table>
| [URE3cer3687]
| cer             | *cerevisiae*     | LM69   | 115          | 114  | 114  |
| [URE3cer3687]
| bay              |         | LM29   | 127          | 106  | 111  |
| [URE3cer3687]
| mik              |         | LM34   | 45           | 44   | 44   |
| [URE3cer3687]
| par              |         | LM36   | 83           | 82   | 71   |
| [URE3cer3687]
| car              |         | LM68   | 82           | 73   | 76   |

**Table 4.** Prion variant characteristics are maintained on passage through another species.

[URE3cer3687]cer was cytoduced into strains expressing each of the indicated Ure2s, and then, after extensive growth on YES plates, cytoduced into LM60 expressing the *cerevisiae* Ure2p. Donor genotypes: MATα ura2 leu2::hisG his3::hisG P<sub>DAL5</sub>CAN1 P<sub>DAL5</sub>ADE2. Recipient genotypes: MATα ura2 leu2 trp1 kar1 P<sub>DAL5</sub>CAN1 P<sub>DAL5</sub>ADE2.
Table 5. Prion variants of the same molecule may differ in their degree of species barrier.

One [URE3cer][car] and four independent [URE3car][car] variants were each cytoduced into strains expressing the either Ure2p[cariocanus] or Ure2p[mikatae], and cytoductants were scored for USA uptake. Donor genotypes: MATα ura2 leu2::hisG his3::hisG P-DAL5CAN1 P-DAL5ADE2. Recipients LM108 with URE2[cariocanus] and LM107 with URE2[mikatae] have genotype MATa ura2 leu2::hisG his3::hisG trp1::HIS3 kar1Δ15-hphMX4 P-DAL5CAN1 P-DAL5ADE2.