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Prion Variants and Species Barriers Among Saccharomyces Ure2 Proteins

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ABSTRACT

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 *Saccharomyces cerevisiae* among Ure2 proteins of *S. cerevisiae*, *paradoxus*, *bayanus*, *cariocanus*, and *mikatae* on the basis of 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 an 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.

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 (Dickinson *et al.* 1968; Bolton *et al.* 1982; Caughey and Baron 2006; Aguzzi *et al.* 2008). 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*$^+$], [*SWI*$^+$], and [β], which are prions of Ure2p, Sup35p, Rnq1p, Swi1p, and Prb1p, respectively (Wickner 1994; Derkatch *et al.* 2001; Roberts and Wickner 2003; Du *et al.* 2008). 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

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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 (King and Diaz-Avalos 2004; Tanaka *et al.* 2004; Brachmann *et al.* 2005; Patel and Liebman 2007; reviewed in Wickner *et al.* 2008b). Species barriers have been described for [*PSI*$^+$] (Chernoff *et al.* 2000; Kushnirov *et al.* 2000; Santoso *et al.* 2000; Chen *et al.* 2007) and [URE3] (Edskes and Wickner 2002; Baudin-Baillieu *et al.* 2003) and prion variants have been recognized in yeast prions [*PSI*$^+$], [URE3], and [*PIN*$^+$] (Derkatch *et al.* 1996; Schlumpberger *et al.* 2001; Bradley *et al.* 2002; Brachmann *et al.* 2005). The [URE3], [*PSI*$^+$], and [*PIN*$^+$] prion amyloids are each parallel in-register β-sheet structures (Shewmaker *et al.* 2006; Baxa *et al.* 2007; Wickner *et al.* 2008a), and different prion variants of [*PSI*$^+$] differ at least in the extent of the β-sheet structure (Toyama *et al.* 2007; Chang *et al.* 2008).

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.

MATERIALS AND 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 (1991). YES medium is 0.5% yeast extract, 3% glucose, 30 mg/liter tryptophan.

Strains: YHE256 is *MAT***a** *kar1 ura2 arg-* [URE3cer3687]^{cer} (Edskes and Wickner 2000). LM9 (*MAT*α *ura2 leu2::hisG his3::hisG ure2::kanMX* P_{DAL5}*ADE2* P_{DAL5}*CAN1*) 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 usptream 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 Saccahromyces species flanked by upstream and downstream seqeuences 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 that 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 Figure 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 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 *MAT***a** *ura2 leu2::hisG his3::hisG trp1::HIS3* P_{DAL5}*ADE2* P_{DAL5}*CAN1 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 ~100% red spore clones. One of these, YHE1242 (*MAT*α *ura2 leu2::hisG his3::hisG URE2castellii* P_{DAL5}*ADE2* P_{DAL5}*CAN1 kar1::kar1Δ15-hphMX4*) was used for [URE3] induction experiments. Upon propagation, most URE2 castellii 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 (*MAT*α *ura2 leu2 trp1 kar1 ure2::kanMX4* P_{DAL5}*CAN1* P_{DAL5}*ADE2*) was a meiotic segregant of LM9 × BY304 (gift from A. Brachmann). The *URE2* gene from *S. cerevisiae* was introduced into LM51 as described above resulting in strain LM60 (*MAT*α *ura2 leu2 trp1* P_{DAL5}*CAN1* P_{DAL5}*ADE2 kar1*).

Plasmid construction: pTIF2 was created starting from pH125 (2μ *LEU2* P_{ADH1}-T_{ADH1}) (Edskes and Wickner 2000). The *Nhe*I–*Bam*HI bordered *ADH1* promoter was replaced by the 179 bp $URE2^{cerevisiae}$ 5′-UTR creating pH795. Next the *Xho*I–*Bgl*II bordered *ADH1* terminator was replaced with the 400 bp $URE2^{cerevisiae}$ 3′-UTR creating pH795. Finally the *Nsi*I–*Bbe*I fragment of pH795 containing most of the 2μ origin of replication and *LEU2* was replaced with a *Stu*I 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.* (2001). Yeast cells were heated to 95° for 3 min in 0.25% SDS and the cleared lysate was used for PCR. Cloning of *URE2* from *S. cerevisiae*, *S. bayanus* (YJM562), and *S. paradoxus* (YJM498) has been described (Edskes and Wickner 2002). All *URE2* sequences contained a *Bam*HI site

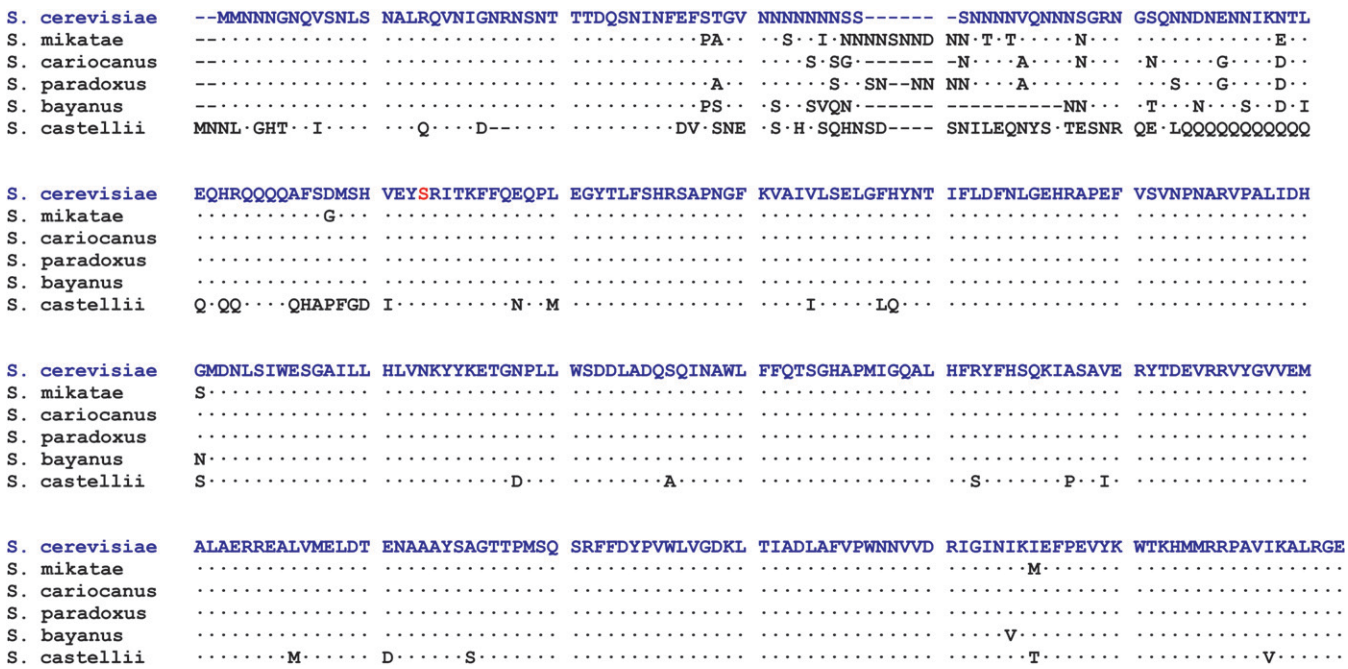

FIGURE 1.—Ure2 protein sequences of Saccharomyces species. "–" means a residue deletion and "·" means a residue identical to the *S. cerevisiae* sequence.

upstream of the start codon and a *Xho*I site downstream of the stop codon. PCR products were cloned into the *Bam*HI/*Xho*I window of pH317 [2μ replicon *LEU2* P_{GAL1} (EDSKES and WICKNER 2000), creating pH376 (*S. cerevisiae URE2*) (BRADLEY *et al.* 2002)], pLM100 (*S. bayanus URE2*), pLM99 (*S. paradoxus URE2*), pLM96 (*S. cariocanus URE2*), and pLM98 (*S. mikatae URE2*). These same PCR products were also cloned into pTIF2 (P_{URE2}*cerevisiae*-T_{URE2}*cerevisiae*) creating pTIF5 (*S. cerevisiae URE2*), pLM93 (*S. bayanus URE2*), pLM92 (*S. paradoxus URE2*), pLM89 (*S. cariocanus URE2*), and pLM91 (*S. mikatae URE2*).

The *URE2* ORF present in pLM99 and pLM92 terminates with a TGA stop codon. However, the native *S. paradoxus URE2* stop codon is TAA. Cloning of a PCR amplified *S. paradoxus* URE2 containing a TAA-A stop sequence into the *Bam*HI/*Xho*I window of pH317 created pH1010. This same *S. paradoxus URE2* containing the TAA-A sequence was cloned into the *Bam*HI/*Xho*I window of pTIF2 creating pH1001. *HIS3* was amplified by PCR from pRS423 (nt 201–1163) using oligos containing loxP sites (5′-ATAACTTCGTATAGCATACATTAT ACGAAGTTAT-3′). LoxP-bordered *HIS3* was ligated as a *Xho*I fragment into the *Xho*I site of pH1001 creating pH1002.

We used homologous recombination cloning in yeast to make a 2μ DNA *LEU2*- based plasmid overexpressing the *S. castellii URE2* using a *GAL1* promoter. A 632-bp *GAL1* promoter fragment and a 316-bp *ADH1* terminator fragment, identical to those present in pH317, were fused by PCR to the *S. castelli URE2* ORF. This PCR product together with BamHI/XhoI-digested pH 317 was transformed into yeast.

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

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 protein sequences of *S. cerevisiae*, *mikatae*, *cariocanus*, *paradoxus*, *bayanus*, and *castelli* are shown in Figure 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 (*MAT*α *ura2 leu2 his3 ure2::kanMX*) (see MATERIALS AND METHODS).

Nomenclature: We indicate a [URE3] prion variant originating in *cerevisiae* and propagating in cells expressing the Ure2p of *mikatae* by the symbol [URE3*cer*]^{mik}. A prion isolate (variant) number can be added, *e.g.*, [URE3*cer*4]^{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

TABLE 1

Induction of prion formation in *S. cerevisiae* strains (*MAT*α *ura2 leu2::hisG his3::hisG* P_{DAL5}*CAN1* P_{DAL5}*ADE2* [ure-o]) with chromosomal *URE2* from various Saccharomyces species

Source of URE2 (strain)	Overexpress Vector	Overexpress *cerevisiae*	Overexpress Homol.	Overexpress Vector	Overexpress *cerevisiae*	Overexpress Homol.
	USA+/10^6 cells			ADE+/10^6 cells		
cerevisiae (LM45)	3	27,000	27,000	10	357,500	357,000
mikatae (LM16)	6	2,675	14,200	28	1,200	74,500
cariocanus (LM41)	4	4,175	6,050	11	5,825	86,750
paradoxus (LM18) TGAC..	15	3,775	12,800	4	5,050	8067
bayanus (LM11)	6	325	5,600	4	341	8,400
castellii	4	5	0		ND	ND

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.

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 overexpressed Ure2p was the same as that encoded on the chromosome.

Except for $URE2^{paradoxus}$, each of the *URE2*s used here had its native stop codon: *cerevisiae* had TGA, *bayanus* TAA, *castellii* TAG, *cariocanus* TGA, and *mikatae* TAA. $URE2^{paradoxus}$ with a TGA stop codon, allows some readthrough (Talarek *et al.* 2005), and so we also constructed $URE2^{paradoxus}$ with a TAA stop sequence, the same as in the native $URE2^{paradoxus}$ 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 *Escherichia coli*, all constructs were made using PCR directly in yeast (see materials and methods). Moreover, the Ade− phenotype was somewhat leaky and not useful so all experiments were done using USA uptake to indicate Ure2p activity. Overexpression 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, *cerevisiae* cells with $Ure2^{castellii}$ 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

TABLE 2

The ability to induce [URE3] upon overexpression of Ure2p was compared in strains LM18 ($URE2^{paradoxus}$TGA) and YHE1254 ($URE2^{paradoxus}$TAAA), each having genotype *MAT*α *ura2 leu2::hisG his3::hisG* P_{DAL5}*CAN1* P_{DAL5}*ADE2* [ure-o]

Strain	Plasmid	USA+/10^6	Ade+/10^6
$URE2^{paradoxus}$TGA	Vector	14	19
$URE2^{paradoxus}$TGA	$P_{GAL1}URE2^{par}$TGA	1,230	110,300
$URE2^{paradoxus}$TGA	$P_{GAL1}URE2^{par}$TAAA	1,630	104,300
$URE2^{paradoxus}$TAAA	Vector	119	76
$URE2^{paradoxus}$TAAA	$P_{GAL1}URE2^{par}$TGA	4,870	67,500
$URE2^{paradoxus}$TAAA	$P_{GAL1}URE2^{par}$TAAA	3,800	49,300

Plasmids were: vector, pH317 = 2μ DNA - *LEU2* - P_{GAL1}; $P_{GAL1}URE2^{paradoxus}$TGA, pLM99 = 2μ DNA - *LEU2* - P_{GAL1} $URE2^{paradoxus}$TGA; $P_{GAL1}URE2^{par}$TAAA, pH1010 = 2μ DNA - *LEU2* P_{GAL1} $URE2^{paradoxus}$TAAA.

TABLE 3

Species barriers among Ure2p's of *Saccharomyces cerevisiae*, *mikatae*, *cariocanus*, *paradoxus*, and *bayanus*

		Recipient				
Donor	Strain	*cerevisiae* LM105	*mikatae* LM107	*cariocanus* LM108	*paradoxus* LM117	*bayanus* LM194
		%USA+/%ADE+				
[URE3cer][cer]	LM109	100/98	98/93	98/96	97/97	84/88
[URE3mik][mik]	LM112	44/52	100/97	7/9	11/3	47/94
[URE3car][car]	LM150	59/75	21/38	60/71	75/80	12/33
[URE3par][par]	LM156	17/38	8/34	63/77	75/80	2/10
[URE3bay][bay]	LM121	0/0	6/6	0/0	2/0	100/100

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: *MAT*α *ura2 leu2::hisG his3::hisG* P_{DAL5}*CAN1* P_{DAL5}*ADE2*. Recipient genotypes: *MAT***a** *ura2 leu2::hisG his3::hisG trp1::HIS3 kar1*Δ*15-hphMX4* P_{DAL5}*CAN1* P_{DAL5}*ADE2*.

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 *URE2*s 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 Ure2[cerevisiae] (data not shown). We also compared ability of Ure2[paradoxus] 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 Ure2[bayanus], but [URE3bay121][bay] does not infect Ure2[cerevisiae] at all. However, these results are very much dependent on which variant of each is considered.

[URE3cer] maintains ability to propagate on Ure2p[cer] in hosts with other Ure2ps: [URE3cer3687][cer] (Wickner 1994) was cytoduced into cells expressing *bayanus*, *mikatae*, *paradoxus*, *or cariocanus* Ure2p, and stable [URE3cer3687][xyz] derivatives of each were obtained. These were grown several times to single colonies and then cytoduced back into LM60 (*MAT***a** *leu2 trp1 kar1* $URE2^{cerevisiae}$ P_{DAL5}*ADE2* P_{DAL5}*CAN1* [ure-o]) (Table 4). Although transmission of [URE3]s native to other species' Ure2s are often poorly transmitted to Ure2[cerevisiae] (Table 3), transmission of this [URE3cer3687] (originating in *cerevisiae*) from each of the other species to Ure2[cerevisiae] was quite efficient (Table 4). This indicates that the strain characteristics of [URE3cer3687] 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 [URE3car][car] isolates to be transmitted to Ure2[mikatae] (Table 5). As with transmission of [URE3cer3687][cer] to Ure2[mikatae], [URE3cer3687][car] was transmitted well to Ure2[mikatae]. While [URE3car146][car] and [URE3car147][car] were likewise transmitted as well to Ure2[mikatae] as they were to Ure2[cariocanus], [URE3car145][car] and [URE3car151][car] were well transmitted to Ure2[cariocanus], but only poorly to Ure2[mikatae] (Table 5).

TABLE 4

Prion variant characteristics are maintained on passage through another species

Donor		Recipient			
[URE3]	Strain	Species strain	Cytoductants	USA+	ADE+
[URE3cer3687][cer]	LM69	*cerevisiae* LM60	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

[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: *MAT*α *ura2 leu2::hisG his3::hisG* P_{DAL5}*CAN1* P_{DAL5}*ADE2*. Recipient genotypes: *MAT***a** *ura2 leu2 trp1 kar1* P_{DAL5}*CAN1* P_{DAL5}*ADE2*.

TABLE 5

Prion variants of the same molecule may differ in their degree of species barrier

		Recipients	
		USA+/total cytoductants	
Donor	Strain	*cariocanus*	*mikatae*
[ure-o][car]	LM41	0/48	0/47
[URE3cer3687][car]	LM68	39/43	32/36
[URE3car145][car]	LM145	43/48	2/47
[URE3car151][car]	LM151	43/48	3/45
[URE3car146][car]	LM146	28/45	26/42
[URE3car147][car]	LM147	22/40	32/44

One [URE3cer][car] and four independent [URE3car] [car] variants were each cytoduced into strains expressing either the Ure2p[cariocanus] or Ure2p[mikatae], and cytoductants were scored for USA uptake. Donor genotypes: *MAT*α *ura2 leu2::hisG his3::hisG* P_{DAL5}*CAN1* P_{DAL5}*ADE2*. Recipients LM108 with *URE2*[*cariocanus*] and LM107 with *URE2*[*mikatae*] have genotype *MAT***a** *ura2 leu2::hisG his3::hisG trp1::HIS3 kar1*Δ*15-hphMX4* P_{DAL5}*CAN1* P_{DAL5}*ADE2*.

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 [URE3mik][mik] transmitted poorly to cells expressing Ure2[cariocanus], but [URE3cer3687][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 P_{DAL5}*ADE2* gene. Repression of USA uptake apparently requires less active Ure2p than does repression of *ADE2* in the P_{DAL5}*ADE2* 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 P_{DAL5}*ADE2* 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 (supplemental Figure S1) and is a dimer evenly distributed in the cytoplasm in [ure-o] cells, whereas Gln3p is present in a large complex (supplemental Figure S2) and appears in localized sites in the cell (Tate and Cooper 2007). Thus, the relation of [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 *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 *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 to acquire the species barrier that we observe. John 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 *et al.* 2003). Similarly, [URE3] is a substantial detriment to its host (Nakayashiki *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 [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 [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 *S. 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 readthrough (Talarek *et al.* 2005). Moreover, it was found that the *S. paradoxus* Ure2p (identical in sequence to what we used) could not become a prion in either *cerevisiae* (Baudin-Baillieu *et al.* 2003) or *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, 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 (Shewmaker *et al.* 2006; Baxa *et al.* 2007; Wickner *et al.* 2008a). This structure is stabilized by "polar-zipper" H-bonds between aligned glutamine and asparagine side chains (Perutz *et al.* 1994; Chan *et al.* 2005; Nelson *et al.* 2005) 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 nonidentical 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]^{bay} is well transmitted to all Ure2 sequences, but four other *bayanus* [URE3]s strongly prefer Ure2^{bayanus}. Applying the Collinge model, one would say that, unlike the others, [URE3 cer3687]^{bay} 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.*, Derkatch *et al.* 1996; Borchsenius *et al.* 2006; 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 HETs$^{218–289}$ (the prion domain) (Ritter *et al.* 2005) indicate that it adopts a very specific single conformation, while the multiple variants (Derkatch *et al.* 1996; Bradley *et al.* 2002; Brachmann *et al.* 2005) and wider 2D solid-state NMR peaks for the prion domains of Ure2p, Sup35p, and Rnq1p (Shewmaker *et al.* 2006; Baxa *et al.* 2007; 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.

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