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Prion-Forming Ability of Ure2 of Yeasts Is Not Evolutionarily Conserved

Herman K. Edskes,* Abbi Engel,* Lindsay M. McCann,* Andreas Brachmann,*,[1] Huei-Fung Tsai† and Reed B. Wickner*,[2]

*Laboratory of Biochemistry and Genetics, National Institute of Diabetes, Digestive, and Kidney Diseases †Clinical Mycology Section, Laboratory of Clinical Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20892

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ABSTRACT

[URE3] is a prion (infectious protein) of the *Saccharomyces cerevisiae* Ure2p, a regulator of nitrogen catabolism. We show that wild *S. paradoxus* can be infected with a [URE3] prion, supporting the use of *S. cerevisiae* as a prion test bed. We find that the Ure2p of *Candida albicans* and *C. glabrata* also regulate nitrogen catabolism. Conservation of amino acid sequence within the prion domain of Ure2p has been proposed as evidence that the [URE3] prion helps its host. We show that the *C. albicans* Ure2p, which does not conserve this sequence, can nonetheless form a [URE3] prion in *S. cerevisiae*, but the *C. glabrata* Ure2p, which does have the conserved sequence, cannot form [URE3] as judged by its performance in *S. cerevisiae*. These results suggest that the sequence is not conserved to preserve prion forming ability.

THE *Saccharomyces cerevisiae* Ure2 protein is central in nitrogen catabolite repression—the ability to repress the uptake and utilization systems for poor nitrogen sources when a good nitrogen source is present (Cooper 2002). The C-terminal domain of Ure2p (amino acids 94–354) is sufficient for its regulatory function when overproduced (Masison and Wickner 1995), although the first 93 amino acids are important to stabilize the protein against degradation (Shewmaker *et al.* 2007). However, these proximal 93 amino acids allow Ure2p to form a prion, an infectious inactive form of the protein (Masison and Wickner 1995; Wickner 1994). The first 65 amino acids have been shown sufficient to propagate the prion form of Ure2p (Masison *et al.* 1997), and form infectious amyloid with a parallel in-register β-sheet structure (Brachmann *et al.* 2005; Baxa *et al.* 2007; reviewed in Wickner *et al.* 2008).

The C-terminal domains of the Ure2 proteins of ascomycete yeasts, starting at amino acid 100 of *S. cerevisiae*, show strong conservation (Edskes and Wickner 2002; Baudin-Baillieu *et al.* 2003; Harrison *et al.* 2007). For instance the C-terminal domains of the Ure2 proteins of the human pathogenic yeasts *Candida albicans* and *C. glabrata* share, respectively, 80 and 92% sequence identity with the *S. cerevisiae* protein, and each can functionally substitute for *S. cerevisiae* Ure2p (Edskes and Wickner 2002).

In contrast to the conservation in the C-terminal domains the N-terminal domains of these three proteins are poorly conserved, although they all contain a substantial amount of asparagine residues (Edskes and Wickner 2002; Baudin-Baillieu *et al.* 2003; Harrison *et al.* 2007). In addition, the *S. cerevisiae* and *C. glabrata* proteins share a 30-amino-acid domain found in some but not all Ure2 proteins (residues 10–39 of the *S. cerevisiae* Ure2p) (Edskes and Wickner 2002). This domain, in isolation, has a strong propensity to form amyloid (Baxa *et al.* 2005; Chan *et al.* 2005), but that amyloid is not infectious (Brachmann *et al.* 2005).

Ure2p is thought to transmit information about the nitrogen state of the environment through an interaction with the transcription factor Gln3p (reviewed in Cooper 2002; Magasanik and Kaiser 2002). Curiously, in contrast to Ure2 proteins of different ascomycete yeasts, the Gln3 proteins show limited sequence conservation. Identity is limited to ~52 centrally located amino acids comprising a zinc finger domain. This zinc finger domain is characteristic of fungal transcription factors involved in nitrogen catabolite repression. In addition, the three Gln3 proteins share a 7- or 8-residue C-terminal sequence. Overall identity between *S. cerevisiae* and *C. glabrata* Gln3 proteins is 34%, between

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Microarray data have been uploaded to the NCBI GEO microarray repository with accession no. GSE26620, with subseries nos. GSE26612 for the Agilent *C. glabrata* array, GSE26613 for *C. albicans*, and GSE26611 for *S. cerevisiae*.

[1]*Present address:* Biozentrum der LMU Munchen, Bereich Genetik, Grosshaderner Str. 2-4, 82152 Martinsried, Germany.

[2]*Corresponding author:* Bldg. 8, Room 225, National Institutes of Health, 8 Center Dr., MSC 0830, Bethesda, MD 20892-0830.
E-mail: wickner@helix.nih.gov

S. cerevisiae and *C. albicans* Gln3 proteins is 24%, and between *C. glabrata* and *C. albicans* Gln3 proteins is also 24%. While the Gln3p of *C. albicans* is known to regulate nitrogen catabolism (Liao *et al.* 2008), it is not clear that the *albicans* Ure2p does so, or does so through Gln3p.

C. albicans and *C. glabrata* are predominantly found as commensals of warm-blooded animals. Both species can proliferate in healthy people and, when the immune system is weakened, can cause mucosal and bloodstream infections (Kaur *et al.* 2005; Bialkova and Subik 2006; Noble and Johnson 2007; Brisse *et al.* 2009; Lewis 2009). *C. albicans* causes 45–60% of invasive candidiasis while *C. glabrata* contributes 20–22% of the cases. Bloodstream infections of either organism have a death rate of 40–50% (Lewis 2009). These organisms are distantly related, with *C. glabrata* closer to *S. cerevisiae*. *C. glabrata* is a haploid yeast, whereas *C. albicans*, like *S. cerevisiae*, is found in the wild as a diploid. Although an incomplete sexual cycle has been described for *C. albicans*, no mating has been observed in *C. glabrata* (Nielsen and Heitman 2007). Nonetheless, loci with sequence similarity to the *S. cerevisiae MAT***a** and *MAT*α are found in *C. glabrata* populations.

Prions (infectious proteins) of yeast and fungi include [URE3], [*PSI*$^+$], [*PIN*$^+$], [*SWI*$^+$], [MCA], [*OCT*$^+$], [*MOT*$^+$], and [*ISP*$^+$] of *S. cerevisiae* and [Het-s] of *Podospora anserina*, which are amyloids of Ure2p, Sup35p, Rnq1p, Swi1p, Mca1p, Cyc8p, Mot3p, Sfp1p, and HETs, respectively (Wickner 1994; Coustou *et al.* 1997; Derkatch *et al.* 2001; Du *et al.* 2008; Alberti *et al.* 2009; Nemecek *et al.* 2009; Patel *et al.* 2009; Rogoza *et al.* 2010). Sup35p is a subunit of the translation termination factor; Rnq1p has no known function; Swi1p, Cyc8p, Mot3p, and Sfp1 are transcription factors; and Mca1p is a metacaspase (putative protease). HETs, in its prion form, is involved in heterokaryon incompatibility in *P. anserina*. In yeast and fungi, prions are both infectious proteins (the definition of a prion), spreading horizontally by cytoplasmic mixing (cytoduction), and heritable as genes, passing vertically to mitotic offspring.

The first suggestion that prions might be beneficial was the discovery of a prion underlying a heterokaryon incompatibility phenomenon in *P. anserina* (Coustou *et al.* 1997; comment by Wickner 1997), but this prion also induces a meiotic drive system, calling that interpretation into question (Dalstra *et al.* 2003). It was then proposed that [*PSI*$^+$] had a beneficial effect on yeast, enabling it to resist stress (Eaglestone *et al.* 1999) and thereby promoting evolvability (True and Lindquist 2000). It has likewise been suggested that [URE3] is beneficial (Shorter and Lindquist 2005), and the conservation, in some yeasts, of a 30-residue region in the Ure2p prion domain (see above) was cited as evidence for the usefulness of prion formation (Harrison *et al.* 2007). Since the Ure2p of *C. glabrata* has this conserved region, but it is missing from that of *C. albicans*, we undertook a study of their prion forming abilities in part as a test of these hypotheses.

MATERIALS AND METHODS

Strains and media: Media are as described by Sherman (1991), except YES medium, which contains 5 g/liter yeast extract, 30 g/liter dextrose, and 30 mg/liter tryptophan. Strains are listed in Table 1. All *C. albicans* strains were derived from SN148 (Noble and Johnson 2005). Disruption of genes and marker exchange were performed according to Dennison *et al.* (2005). Transformation of *C. albicans* was performed using the Li acetate method at 44° (Ramon and Fonzi 2009). The *URA2* gene was disrupted with LAL and LHL (Dennison *et al.* 2005). Subsequently, *IRO1* and *URA3* were restored through transformation with a PCR product originating 418 bp upstream of *IRO1* and terminating 408 bp downstream of *URA3*. As template, genomic DNA from the Darlington strain of *C. albicans* was used (Kakeya *et al.* 2000). After *URA3* was restored, *CRE*-mediated recombination was initiated [to remove the arginine (LAL) and histidine (LHL) markers] using a *LEU2*-marked *CRE* gene controlled by the *MET3* promoter and directed to the *ARG4* locus. This produced strain HCA17. Sequences containing 5′ *ARG4* and the *MET3* promoter as well as *ARG4* 3′ sequences were amplified from pCAD (Dennison *et al.* 2005). *LEU2* was amplified from SN100 genomic DNA (Noble and Johnson 2005). The whole *CRE* cassette was cloned into the *Eco*RV site of pBC KS$^+$ (Stratagene) resulting in pH948. The *URE2* gene was then disrupted with LAL and LHL and the markers again removed through CRE-mediated recombination giving strains HCA37 and HCA40, which both grew slightly slower than the parental strain HCA17.

URE2 was disrupted in *C. glabrata* strain BG88b (Cormack and Falkow 1999) using a PCR product obtained from genomic DNA of *C. glabrata* strain 37A (Miyazaki *et al.* 1998) that contained the *HIS3* gene (bordered by loxP sites), starting 443 bp upstream of the start codon and terminating at the *HIS3* stop codon. *C. glabrata* strains BG14 and BG88b are both derived from clinical isolate BG2 (Fidel *et al.* 1996). *C. glabrata* was transformed by the Li acetate method (Geitz and Woods 2002). Both HCg1 and HCg7 grew slightly slower than the parental strain BG14.

URE2 of YHE711 was replaced with *kanMX4* by transformation with a PCR- amplified *ure2::kanMX4* from the corresponding *S. cerevisiae* Genome Deletion Project strain. *S. cerevisiae* was transformed by the Li acetate method (Geitz and Woods 2002). TIFY3 grew slightly slower than parental strain YHE711.

We integrated *URE2*^{albicans} and *URE2*^{glabrata} into the *URE2* locus of strain BY256 replacing the *kanMX* gene present there (*ure2::kanMX*). The ORF of *URE2*^{albicans} or *URE2*^{glabrata} flanked by 179 bp 5′-UTR and 463 bp 3′-UTR from the *S. cerevisiae URE2* was transformed into strain BY256. Transformants containing *URE2*^{albicans} (BY302) or *URE2*^{glabrata} (BY304) at the *S. cerevisiae URE2* locus were selected using their resistance to canavanine, sensitivity to G418, and inability to grow on medium lacking adenine. There are no CTG codons in *URE2*^{albicans}.

Assay of [URE3]: *DAL5*, encoding alantoate permease, is strongly repressed by Ure2p. Dal5p also recognizes and takes up ureidosuccinate (USA), an intermediate in uracil biosynthesis. As a result, inactivity of Ure2p may be detected by ability of *ura2* cells to grow on USA (33 μg/ml) in the

TABLE 1

Strains of *S. cerevisiae* and *S. paradoxus*

Strain	Genotype	Reference
	Strains used for *S. paradoxus* [URE3] study	
MA544	*S.p. MAT*α *ade1 leu1 trp5 lys1 ho::kanMX*	Talarek *et al.* (2005)
MA578	*S.p. MAT***a** *ade2 his4 ura3 leu2 ho::kanMX* P_{DAL5}*:ADE2*	Talarek *et al.* (2005)
LM156	*S.c. MAT*α *ura2 leu2 his3* P_{DAL5}*:ADE2 URE2*^{paradoxus} [URE3para156]	Edskes *et al.* (2009)
4884	*S.c. MAT***a** *trp1 ura2 his3 leu2 kar1Δ15hyg*^{r} P_{DAL5}*:ADE2 URE2*^{paradoxus} [ure-o]	This work
4891	*S.c. MAT***a** *trp1 ura2 his3 leu2 kar1Δ15hyg*^{r} P_{DAL5}*:ADE2 URE2*^{paradoxus} [URE3para156]	This work
4888	*S.c. MAT*α *ura2 his3leu2 kar1-1* P_{DAL5}*:ADE2 URE2*^{paradoxus} [URE3para156]	This work
4899	*S.p. MAT*α *ura3 leu1 trp5 ade2-1* P_{DAL5}*:ADE2 ho::kanMX* [ure-o]	Meiotic segregant of MA544 and MA578
YJM498	Wild-type *S.p.* isolated from a patient	J. McCusker; Nakayashiki *et al.* (2005)
Y-1548	Wild-type *S.p.* isolated from oak exudate in The Netherlands	C. Kurtzman; Nakayashiki *et al.* (2005)
	Strains of *Candida albicans*	
HCA17 (wt)	*MAT***a**/*MAT*α *arg4Δ/arg4Δ leu2Δ/leu2Δ his1Δ/his1Δ ura2::loxP/ura2::loxP ura3::imm*^{434}*/URA3 iro1::imm*^{434}*/IRO1*	This work
HCA37, HCA40 (HCA17 *ure2*Δ)	*MAT***a**/*MAT*α *arg4Δ/arg4Δ leu2Δ/leu2Δ his1Δ/his1Δ ura2::loxP/ura2::loxP ura3::imm*^{434}*/URA3 iro1Δ::imm*^{434}*/IRO1 ure2::loxP/ure2::loxP*	
	Strains of *C. glabrata*	
BG14 (wt)	ura3Δ::TN903NeoR	Cormack and Falkow (1999)
BG88b = BG14*his3*Δ	*ura3*Δ::TN903NeoR *his3*Δ	Cormack and Falkow (1999)
HCg1 = BG88b *ure2*Δ	*ura3*Δ::TN903NeoR *his3*Δ *ure2::HIS3*	
HCg7 = BG88b *ure2*Δ	*ura3*Δ::TN903NeoR *his3*Δ *ure2::HIS3*	
	Strains of *S. cerevisiae*	
YHE711 (wt)	*MAT*α *ura2 leu2::hisG*	Edskes *et al.* (1999b)
TIFY3	*MAT*α *ura2 leu2::hisG ure2::G418*	Tiffany Weinkopff
BY256	*MAT***a** *his3 leu2 trp1 ure2::kanMX* P_{DAL5}*:ADE2* P_{DAL5}*:CAN1 kar1*	Brachmann *et al.* (2005)
BY302	*MAT***a** *his3 leu2 trp1 URE2*^{albicans} P_{DAL5}*:ADE2* P_{DAL5}*:CAN1 kar1*	
BY304	*MAT***a** *his3 leu2 trp1 URE2*^{glabrata} P_{DAL5}*:ADE2* P_{DAL5}*:CAN1 kar1*	
YHE1178	*MAT*α *ura2 leu2 URE2*^{albicans} P_{DAL5}*:ADE2*	BY302 × YHE887
YHE1174	*MAT*α *ura2 leu2 kar1 URE2*^{glabrata} P_{DAL5}*:ADE2*	BY304 × YHE887
YHE887	*MAT*α *ura2 leu2 ure2::kanMX* (sigma background)	
YHE1265	BY304 with *URE2*^{glabrata} replaced with *URE2*^{cerevisiae}	
YHE1207	BY302 [URE3alb]	
YHE1271	YHE1265 [URE3cer]	

HCA37 and HCA40 share the first disrupted *URE2* allele but were independently disrupted in the second URE2 allele. The "156" in [URE3para156] identifies a particular isolate.

presence of ammonia as nitrogen source (*e.g.*, Difco yeast nitrogen base without amino acids). Alternatively, the *DAL5* promoter may be fused to *ADE2* and adenine prototrophy, and a change from red to white colony color on adenine-limiting media indicates Ure2p inactivity.

Cytoduction: Cytoplasm is transferred from one strain to another using the *kar1* mutation defective in nuclear fusion (Conde and Fink 1976). The donor of cytoplasm is ρ^+ and the recipient, of opposite mating type, is made ρ^o by growth on ethidium bromide. Cells are mixed in water and incubated on rich medium for ~7 hr. The mixture is then streaked for single colonies on plates selecting against the donor strain. Donor or recipient must have a *kar1* mutation to largely prevent nuclear fusion. Mating occurs and the unfused nuclei separate at the next cell division. Clones with the nuclear markers of the recipient (but not diploid) and ρ^+ are cytoductants and are scored for the prion phenotype.

Microarray protocols: Total RNA was isolated from log phase cultures of *S. cerevisiae* (strains YHE711 and TIFY3), *C. albicans* (strains HCA17, HCA37, and HCA40) and *C. glabrata* (strains BG88b, HCg1, and HCg7) grown in YPAD at 30° using Trizol (Invitrogen) and purified using the RNeasy MiniElute cleanup kit (Qiagen). *S. cerevisiae* microarrays were purchased from Agilent. For *C. glabrata*, Agilent custom microarrays were used as described (Tsai *et al.* 2010) *C. albicans* microarrys (Brown *et al.* 2006) were purchased from the Genome

Sequencing Center at Washington University, St. Louis, MO. Microarray hybridization was performed by the Genomic Technologies Section, Research Technologies Branch, National Institute of Allergy and Infectious Diseases, National Institutes of Health (NIH), Bethesda, MD (Tim Myers) as described by Tsai *et al.* (2010). Statistical calculations were performed on the processed signal data by using the mAdb analysis system provided by the BIMAS group at the Center for Information Technology, NIH. Data were filtered with the parameters that included genes present in three or more arrays per group and each array with 80% or more genes present. Genes not present in both *URE2* deletion strains of *C. albicans* and *C. glabrata* were discarded. Signals of genes scoring consistently <100 pixels on all arrays were discarded as background. Genes for which reciprocal labeling showed an inconsistent trend were discarded as well as genes positive in three or more arrays per group but not positive in the majority of arrays.

RESULTS

Nomenclature: As previously described (Edskes *et al.* 2009), we indicate a [URE3] prion originating in cells expressing the *C. albicans* Ure2p and propagating in cells expressing the *S. cerevisiae* Ure2p by the symbol [URE3alb]^{cer} (if there were such a strain). In this work, [URE3alb]^{alb} will usually be abbreviated to [URE3alb]. When we refer to [URE3] of the *S. paradoxus* Ure2p in *S. paradoxus* cells, we will be explicit.

Wild *S. paradoxus* strains can propagate [URE3]: Because of the difficulties of Candida genetics, we chose to examine the prion-forming abilities of *C. albicans* and *C. glabrata* Ure2p in *S. cerevisiae*, an approach often used by others as well (Chernoff *et al.* 2000; Kushnirov *et al.* 2000; Santoso *et al.* 2000; Nakayashiki *et al.* 2001). However, while the Ure2p of *S. paradoxus* forms a prion in *S. cerevisiae* (Edskes and Wickner 2002; Edskes *et al.* 2009), it was found to not form [URE3] in *S. paradoxus* itself (Talarek *et al.* 2005), casting doubt on this approach.

As reported by Talarek *et al.* (2005), we found that we could not select [URE3para]^{para} in *S. paradoxus* strain MA578, even on overproduction of Ure2p^{para}. We tested whether the [URE3para]^{para}, generated in *S. cerevisiae* carrying the *paradoxus URE2* gene in place of that of *cerevisiae*, could be transmitted to *S. paradoxus* by cytoduction (cytoplasmic mixing) to strain MA578, and found that none of several [URE3para] variants were transmitted (Table 2 and data not shown). However, using genetic crosses, we constructed several other *S. paradoxus* strains with the P_{DAL5}*:ADE2* construct used for scoring [URE3] as Ade$^+$, and found that, for example, *S. paradoxus* strain 4899 could be made [URE3para] by cytoduction from *S. cerevisiae* [URE3para] donors (Table 2). Each of these Ade$^+$ cytoductants was cured by growth on 3 mm guanidine, an inhibitor of the disaggregating chaperone Hsp104, whose activity is necessary for propagating the *S. cerevisiae* [URE3]. Further, these cytoductants could transmit their Ade$^+$ trait back to *S. cerevisiae* strain 4884 by cytoduction. Thus, *S. paradoxus* strain 4899 can propagate [URE3para].

To determine whether wild *S. paradoxus* can propagate [URE3para], we carried out meiotic crosses between *S.p.*4899 and germinating meiotic spores from the wild *S.p.* strains YJM498 and Y-1548. From the former cross we chose 20 and from the latter 8 suitably marked (*ade2-1* P_{DAL5}*:ADE2 ho::kanMX*) segregants, and used each as a cytoduction recipient from a *S.c.* [URE3para] donor. We found that each of the 28 segregants from the two crosses could stably maintain [URE3para], indicating that there is at least no single gene defect in either wild strain preventing [URE3para] propagation (Table 2). These results indicate that wild *S. paradoxus* can be [URE3] and suggest that the use of *S. cerevisiae* as a test bed for potential prion proteins appears to be valid.

***C. albicans* and *C. glabrata* Ure2p regulate N-catabolism genes:** *GLN3* is known to have an important role in nitrogen regulation in *C. albicans* (Dabas and Morschhauser 2007; Dabas and Morschhauser 2008; Liao *et al.* 2008). As the Ure2 proteins from *C. glabrata* and *C. albicans* fully restore nitrogen regulation in a *S. cerevisiae ure2*Δ strain (Edskes and Wickner 2002), it seems likely that nitrogen regulation in these two Candida species is also directed by Ure2p. However, the limited sequence identity found among the Gln3 proteins from *S. cerevisiae, C. albicans*, and *C. glabrata* (supporting information, Figure S1) brings into question whether the response of these organisms to Ure2p inactivation parallel each other. To record the transcriptional response of these three organisms to Ure2p inactivation, we disrupted *URE2* in *S. cerevisiae, C. albicans*, and *C. glabrata*.

We find that among the 182 genes of *S. cerevisiae* whose transcription is repressed twofold or more by Ure2p in rich medium, 73 are involved in nitrogen utilization and amino acid metabolism (File S1). Of these 182 genes, 47 were reported by Godard *et al.* (2007) to be nitrogen catabolite repression (NCR) target genes. None of the 22 genes that we found twofold or more upregulated by Ure2p were NCR targets. These results are similar to previous work in *S. cerevisiae* (Cox *et al.* 1999; Shamji *et al.* 2000; Ross and Wickner 2004).

Of the 46 *C. albicans* genes derepressed by *ure2*Δ, 16 are homologous to Godard *et al.*'s (2007) NCR target genes. Of the 209 genes upregulated in *ure2*Δ *C. glabrata*, 19 are NCR targets, but 5 genes listed by Godard *et al.* (2007) as NCR targets are downregulated in *C. glabrata* (File S1).

Overall, the roles of Ure2p in the three yeasts seem to be quite similar, but must differ in some details. All three organisms respond to deletion of URE2 by changing uptake and metabolism pathways of amino acids. They also enhance uptake and metabolism of nucleotides. In addition, all three organisms promote the uptake and utilization of urea as a nitrogen source. These shared responses are highlighted by the eight genes whose expression we

TABLE 2

***S. paradoxus* can propagate [URE3paradoxus]**

Donors	Recipients	Cytoductants
*S.c.*4888[URE3para]	*S.p.*MA544	25 all Ade$^-$
*S.c.*4888[URE3para]	*S.p.*4899	7 Ade$^+$, 5 Ade$^-$
*S.c.*4891[URE3para156], *S.c.*4888[URE3para156]	20 segregants of *S.p.* YJM498 spores X *S.p.* 4899	>20 Ade$^+$ in each cytoduction, rare Ade$^-$
*S.c.*4891[URE3para156], *S.c.*4888[URE3para156]	8 segregants of *S.p.* Y-1548 spores X *S.p.* 4899	>20 Ade$^+$ in each cytoduction, rare Ade$^-$

S.c., Saccharomyces cerevisiae; *S.p., Saccharomyces paradoxus*. An interspecies cytoduction of [URE3para] from *S.c. URE2*$^{\text{paradoxus}}$ [URE3para] strains to *S.p.* strains was carried out as for *S. cerevisiae*. The P_{DAL5}:*ADE2*, with the *DAL5* promoter controlling the *ADE2* gene, was used as a reporter of Ure2p activity. [ure-o] cells are Ade$^-$, while [URE3] cells are Ade$^+$ because active Ure2p prevents transcription from the *DAL5* promoter (Rai *et al.* 1987).

find changes in the same direction in *S. cerevisiae*, *C. albicans*, and *C. glabrata*: *CAR1* (arginase), *DIP5* (dicarboxylic amino acid permease), *DUR1* (urea amidolyase), *GAP1* (general amino acid permease), *GLN1* (glutamine synthase), *UGA1* (γ-aminobutyrate transaminase), *FCY2* (purine-cytosine permease), and *YPS1* (aspartyl protease) (File S1, Figure S2).

***C. albicans* Ure2p can form a prion in *S. cerevisiae*:** Overproduction of a prion protein increases the frequency of *de novo* formation of the prion form (Wickner 1994), thus providing both a means of obtaining cells with the prion and evidence that it is indeed a prion. Neither *C. albicans* nor *C. glabrata* Ure2p overproduction induces the appearance of *S. cerevisiae* [URE3] (Edskes and Wickner 2002). Further, when *C. albicans* or *C. glabrata* Ure2p expression was directed from a plasmid in a *S. cerevisiae* strain carrying a chromosomal *ure2* deletion neither protein could propagate [URE3] derived from *S. cerervisiae* Ure2p introduced by cytoduction. These results indicate that there is at least a substantial species barrier between *S. cerevisiae* Ure2p and the two Candida Ure2ps.

Using pH563 (pP_{GAL1}*URE2*^{albicans}) in BY302 (*URE2*^{albicans}), we transiently overproduced Ure2p^{albicans} by growing cells in galactose-containing medium. [URE3]-containing cells were detected using a P_{DAL5}:*ADE2* fusion. *DAL5*, encoding allantoate permease, is repressed by Ure2p when a rich nitrogen source, such as ammonia, is present. Dal5p can also transport USA, the product of Ur**a**2p (aspartate transcarbamylase). Thus, deficiency of Ure2p (as when [URE3] is present) derepresses *DAL5* transcription, allowing a *ura2* mutant to grow on USA in place of uracil. Alternatively, placing the *ADE2* gene under the control of the *DAL5* promoter results in normal cells being Ade$^-$ and [URE3] cells being Ade$^+$. After growth on galactose, cells were plated on −Ade plates with glucose as the carbon source to shut off the overproduction of Ure2p. The frequency of Ade$^+$ cells was increased >100-fold by overproduction of Ure2p^{albicans}, indicating that [URE3alb] had formed (Table 3).

Many of the Ade$^+$ clones induced by Ure2^{albicans} overexpression were stable on growth under nonselective conditions (YPAD), but were then cured by growth on YPAD containing 3 mm guanidine HCl (Figure 1), a known feature of *S. cerevisiae* [URE3] (Moriyama *et al.* 2000).

Prions are, in most cases, readily transmitted by cytoduction. Seven stably Ade$^+$ guanidine-curable putative [URE3alb] clones were used as cytoduction donors to a rho^o [ure-o] recipient. In four cases, all (20) or nearly all (19 or 20) of the cytoductants had become USA$^+$, confirming that these isolates were [URE3alb].

Two variants of [URE3alb]: A single protein can adopt different amyloid structures and thus show distinct heritable and infectious biological properties (reviewed by Collinge and Clarke 2007). Cytoductants of [URE3-alb] isolates YHE1161 and YHE1171 into the common recipient strain YHE1178 were slow growing and only weakly Ade$^+$ (red on adenine-limiting media), while cytoductants of isolates YHE1162 and YHE1169 into the same recipient were fast-growing and strongly Ade$^+$ (Figure 2). Thus, this prion, as other yeast prions, can display variants.

***C. glabrata* Ure2p cannot form a [URE3] prion:** While transient overexpression of Ure2p^{albicans} induced the formation of [URE3alb], the same was not true of overexpression of Ure2p^{glabrata} in cells expressing only Ure2p^{glabrata} (Table 3), although Ure2p^{glabrata} was indeed strongly overexpressed (Figure S2). The frequency of Ade$^+$ clones did not increase, and none of the stably Ade$^+$ clones were cured by growth on guanidine, so they were not prions. Six Ade$^+$ clones were used as cytoduction donors with strain YHE1174 [*MAT*α *ura2 leu2 kar1 URE2*^{glabrata} P_{DAL5}:*ADE2*] as recipient. Not a single case of transfer of the ADE$^+$ phenotype from donor cells to recipient cells was observed (total of 79 cytoductants with 8–17 cytoductants per BY304 ADE$^+$ isolate).

It has been shown that the prion forming domain of *S. cerevisiae* Ure2p is far more efficient in inducing [URE3] when transiently overexpressed than the full-length protein (Masison and Wickner 1995). We thus

TABLE 3

Prion induction by transient overexpression of full-length Ure2p^{albicans} or Ure2p^{glabrata}

Host	Plasmid	ADE$^+$ per 10^6 cells plated
BY302 *albicans URE2*	pH317 (vector)	11 ± 8
	pH563 (*albicans*)	1680 ± 490
BY304 *glabrata URE2*	pH317 (vector)	20 ± 15
	pH659 (*glabrata*)	16 ± 16

BY302 (or BY304) with a chromosomal copy of *URE2^{albicans}* (or *URE2^{glabrata}*) with the *S. cerevisiae URE2* promoter was transformed with a copy of the same *URE2* controlled by the *GAL1* promoter or the vector control. Transformants were grown in leucine dropout media containing 2% galactose and 1% rafinose as carbon source. Cells were washed, diluted, and plated onto adenine dropout plates containing glucose as the carbon source. Colonies were counted after 5 days at 30°. pH317 2μ, *LEU2* P_{GAL1} (Edskes and Wickner 2002); pH563 pH317 with *URE2^{albicans}* (Edskes and Wickner 2002); pH659 pH317 with *URE2^{albicans}* (Edskes and Wickner 2002).

transiently overexpressed the N-terminal domains from the *S. cerevisiae, C. albicans*, and *C. glabrata* Ure2 proteins and measured their ability to induce [URE3] (Table 4). While the *cerevisiae* and *albicans* N-terminal domains' overproduction dramatically increased Ade$^+$ colony formation, only a small increase was seen for *glabrata*. Guanidine curing experiments confirmed that all of the *albicans* Ade$^+$ colonies contained prions (12 of 12 tested cured by guanidine). Tests of 40 of the *glabrata* Ade$^+$ clones showed that none were guanidine curable, although some were completely converted to Ade$^-$ on growth in rich medium.

In a further attempt to induce [URE3gla] formation, we introduced cytoplasm with [URE3cerevisiae] or [URE3alb] by cytoplasmic mixing. Neither prion was successfully transferred to cells expressing Ure2p^{glabrata}, although each was efficiently transmitted to a recipient expressing a Ure2p identical to the donor (Table 5). [URE3alb] was not transmitted to cells expressing Ure2p from several Saccharomyces species.

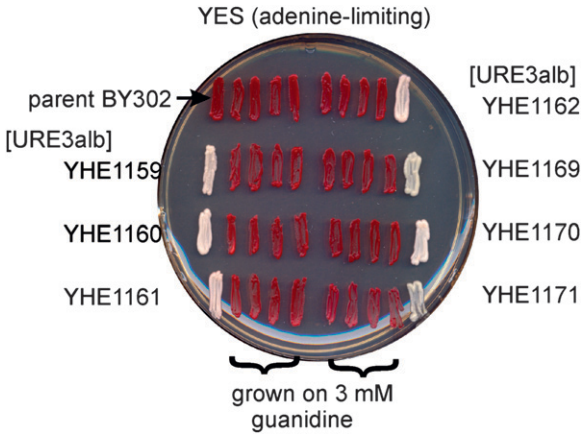

Figure 1.—Curing of [URE3alb] by growth on 3 mM guanidine. The parent strain BY302 and seven Ade$^+$ derivatives were streaked for single colonies on YPAD with 3 mM guanidine HCl. Four single colonies from each were streaked on adenine-limiting medium (YES). Red clones are Ade$^-$. The white clones on the left and right are the Ade$^+$ derivatives of BY302 not exposed to guanidine.

We considered the possibility that in the process of constructing BY304 some other change had been inadvertently introduced that made the cells unable to propagate a prion. We therefore, replaced *URE2^{glabrata}* with *URE2^{cerevisiae}* forming YHE1265. Overexpression of Ure2$^{cerevisiae1\text{-}65}$ or $^{1\text{-}99}$ each stimulated prion formation (Table 4), indicating that no such mutation was present.

Aggregate formation *in vivo*: Ure2-GFP fusion proteins that include the prion domain can be incorporated into the prion filaments *in vivo* and allow visualization of the prion aggregates (Edskes *et al.* 1999a) (Figure 3). The fusion protein expression also may cure the prion (Edskes *et al.* 1999a). We found that when Ure2$^{albicans1\text{-}65}$-GFP (Figure 3) was expressed from the *S. cerevisiae URE2* promoter in a [URE3alb] cell, and cells were grown selecting for the prion, dot-like aggregates were observed in a large fraction of cells. Unexpectedly, Ure2$^{cerevisiae1\text{-}65}$-GFP or Ure2$^{cerevisiae1\text{-}99}$-GFP (also expressed from the *S. cerevisiae URE2* promoter) also showed the dot appearance in [URE3alb] strains (Figure 3). No foci were observed in isogenic [ure-o] cells.

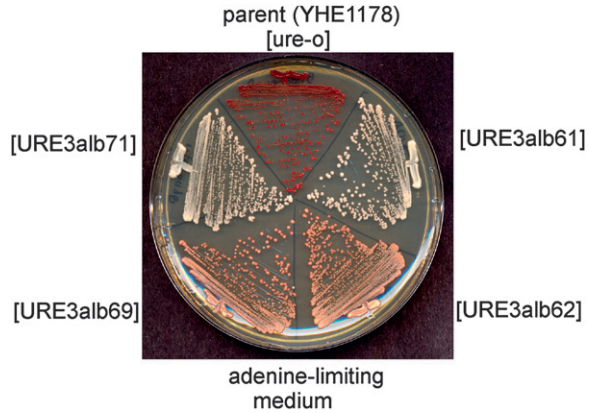

Figure 2.—Two [URE3alb] prion variants. [URE3alb] in strains YHE1171, YHE1169, YHE1161, and YHE1162 was cytoduced into YHE1178. Strain YHE1178 and cytoductants from each were streaked on adenine-limiting medium (YES). Two strains may be distinguished by colony color.

TABLE 4

[URE3] induction assays using N-terminal domains

Host	Plasmid	ADE$^+$/per million cells plated ± SD
BY302 *albicans URE2*	Vector (pH317)	3 ± 3
	cerevisiae 1–65 (pH382)	3 ± 3
	cerevisiae 1–99 (pH565)	1 ± 1
	albicans 1–89 (pH564)	224,000 ± 39,000
	glabrata 1–100 (pH1026)	2 ± 4
BY304 *glabrata URE2*	Vector	13 ± 5
	cerevisiae 1–65	18 ± 4
	cerevisiae 1–99	11 ± 3
	albicans 1–89	15 ± 14
	glabrata 1–100	85 ± 25
YHE1265 *cerevisiae URE2*	Vector	36 ± 15
	cerevisiae 1–65	29,900 ± 6000
	cerevisiae 1–99	23,700 ± 14,000
	albicans 1–89	1070 ± 500
	glabrata 1–100	32 ± 9

Cells were grown to saturation in leucine dropout medium containing 2% galactose and 1% rafinose as carbon sources. Tenfold dilutions were plated onto dextrose medium lacking adenine and incubated for 5 days at 30°. Viable cells were measured by plating of dilutions on YPAD. YHE1265 was derived from BY304 by replacing $URE2^{glabrata}$ with $URE2^{cerevisiae}$.

Overexpression of many proteins, including prion proteins, often produce an aggregated appearance of protein-GFP fusions even without prion formation. Overexpressing $Ure2^{cerevisiae1\text{-}99}$ from a *GAL1* promoter produces aggregates that are revealed by $Ure2^{cerevisiae1\text{-}65}$-GFP expressed at a low level from the *URE2* promoter. However, $Ure2^{glabrata1\text{-}100}$-GFP or $Ure2^{glabrata1\text{-}65}$-GFP did not detect any aggregates, when $Ure2^{glabrata1\text{-}100}$ was overexpressed from a *GAL1* promoter, indicating that $Ure2^{glabrata}$ had little tendency to aggregate.

URE2-GFP curing: We found that expression of $URE2^{albicans1\text{-}65}$ from the *URE2* promoter cured [URE3-alb] (in strain YHE1207), but not [URE3cer] (in strain YHE1271) (Table S1).

DISCUSSION

S. cerevisiae and *S. paradoxus* are separated by ~5–20 million years of evolution (Kellis *et al.* 2003). We find that the *S. paradoxus* Ure2p can form a prion in *S. paradoxus* itself, thus resolving an apparent disparity with results in *S. cerevisiae*. While it may be reassuring that *S. cerevisiae* turns out to be a good test bed for prion formation in this case, there are numerous chromosomal genes affecting prion generation and propagation, so that this assumption may not always prove to be valid in the many studies which have used this approach. The Ure2p of *S. castellii* is unable to form a prion in *S. cerevisiae* (Edskes *et al.* 2009), but it has not yet been tested in *S. castellii* itself. Engineering other organisms to detect prions is a daunting task. We found that the Ure2p of *C. albicans* and *C. glabrata* have nitrogen regulation functions, like that of *S. cerevisiae*. However, when we constructed *ura2*Δ*/ura2*Δ *ure2*Δ*/ure2*Δ homozygous knockout mutants of *C. albicans*, we found that they did not utilize ureidosuccinate in place of uridine, so we could not use this approach to test for [URE3] in *C. albicans*. *C. albicans* has a *DAL5* homolog, but *C. glabrata* does not, so we did not attempt this approach.

A protein domain that can substitute for the Sup35 prion domain in prion formation may not be able to do so in its native context. Since prion formation by prion domains of Ure2p or Sup35p are hundreds or thousands of times more likely to form prions than the full-length proteins expressed at similar levels (Masison and Wickner 1995; Kochneva-Pervukhova *et al.* 1998), it is possible that some domains that can be prions in isolation or a foreign environment may be completely stabilized in their native context.

The Ure2p of *C. albicans* forms a [URE3alb] prion with properties similar to those of the [URE3] of *S. cerevisiae*. We observed elevation of prion generation frequency by overproducing the full-length $Ure2^{albicans}$ and even greater induction by overexpressing just the prion domain. The prion is cured by guanidine, transmitted by cytoduction, and shows at least two prion variants.

In contrast, the Ure2p of *C. glabrata* does not detectably form a prion under the conditions tested. Even with overproduction of $Ure2^{glabrata}$ or its prion domain, we could not detect [URE3gla] formation. Attempts to observe aggregated $Ure2^{glabrata}$ using fusions with GFP were likewise unsuccessful. Because of the substantial dependence of prion propagation on chaperones and other cellular components, it remains possible that $Ure2^{glabrata}$ can form a prion in *C. glabrata* itself.

It has been suggested that the conservation of sequence in the prion domains of Ure2p and Sup35p are indications that the prion must have some function beneficial to the cell that has been selected, thus resulting in the conserved prion domain sequence

TABLE 5

Species barriers among [URE3] prions

Donor [URE3]/URE2	Recipient/URE2	Cytoductants	
		Total	USA$^+$
LM69 *S. cerevisiae*	YHE1202 *C. glabrata*	26	0
LM69 *S. cerevisiae*	YHE1177 *C. albicans*	26	0
LM69 *S. cerevisiae*	LM60 *S. cerevisiae*	19	19
YHE1211 *C. albicans*	LM11 *S. bayanus*	8	0
YHE1211 *C. albicans*	LM14 *S. kudriavzevii*	10	0
YHE1211 *C. albicans*	LM16 *S. mikatae*	7	0
YHE1211 *C. albicans*	LM18 *S. paradoxus*	8	0
YHE1211 *C. albicans*	LM27 *S. castellii*	7	0
YHE1211 *C. albicans*	LM41 *S. cariocanus*	13	0
YHE1211 *C. albicans*	LM45 *S. cerevisiae*	16	0
YHE1211 *C. albicans*	YHE1203 *S. glabrata*	45	0
YHE1211 *C. albicans*	YHE1181 *C. albicans*	85	85

All strains are *S. cerevisiae* with *URE2* from the indicated species. Donor strains have the corresponding [URE3cer] or [URE3alb].

(Shorter and Lindquist 2005; Harrison *et al.* 2007). However, we find that the *C. glabrata* Ure2p, which has the conserved prion domain sequence (residues 10–39 in *S. cerevisiae)*, cannot form a prion. Similarly,

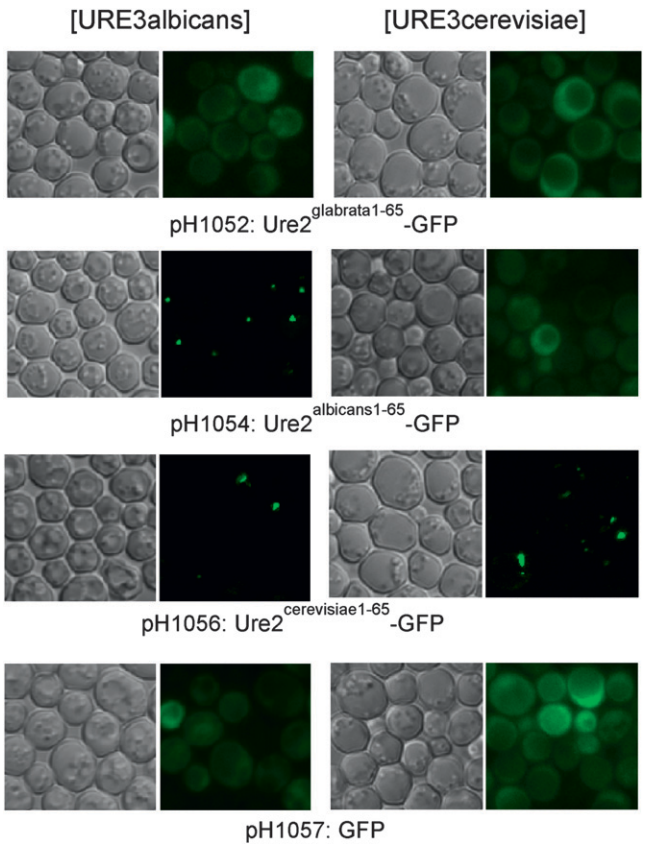

Figure 3.—Ure2p^{albicans}-GFP fusion protein shows aggregation in [URE3alb] strains. YHE1207 (*URE2^{albicans}* [URE3alb]) or YHE1272 (*URE2^{cerevisiae}* [URE3cer]) were transformed with the indicated plasmids expressing Ure2-GFP fusion proteins and observed after for 3 days on medium without adenine (selective for retention of the [URE3alb] prion).

the *Kluyveromyces lactis* Ure2p, which also has the conserved prion domain sequence, cannot form a [URE3] prion as tested in *K. lactis* itself (Safadi *et al.* 2011). In contrast, the Ure2p of *C. albicans*, which lacks the conserved sequence, forms a prion much like that of *S. cerevisiae*. Thus the sequence conservation is not a sign of conservation of prion-forming ability. In fact, prion-forming ability is not even conserved within Saccharomyces. The Ure2p of *S. castellii* cannot form [URE3] in *S. cerevisiae* (Edskes *et al.* 2009), and one-fourth of *S. cerevisiae* strains tested in one study had a large deletion in the prion domain of Sup35p, making them unable to propagate [*PSI*$^+$] (Resende *et al.* 2003).

Beyond these facts, it should be noted that this argument that prions must be adaptive assumes that the conservation of sequence in the prion domain would be necessary for, or at least favor, prion formation. In fact, the amino acid sequences of the Ure2p or Sup35p prion domains may be randomly shuffled and each of the five shuffled domains of each domain can form prions (Ross *et al.* 2004, 2005). Thus, the conservation of sequence is not an argument for conservation of prion forming ability.

Why are there conserved sequences in the prion domains? The Sup35p prion domain is important for the normal process of mRNA turnover in yeast and humans (Hoshino *et al.* 1999; Hosoda *et al.* 2003; Funakoshi *et al.* 2007), while the Ure2p prion domain is important for the stability against degradation of the full-length protein (Shewmaker *et al.* 2007). It is likely that these important functions are the basis for the conservation of sequence of these prion domains.

Does prion function benefit the yeast cells or are prions diseases? Since [*PSI*$^+$] and [URE3] can be quite stable, if they were beneficial, they would spread rapidly in wild populations and, like mitochondrial DNA, be found in most wild strains. In fact, the prion state of Ure2p or Sup35p is rare in nature, as none of the many wild strains examined carry the [URE3] or [*PSI*$^+$] prions

(Nakayashiki *et al.* 2005). The [*PIN*$^+$] prion is found at a frequency comparable to that of the mildly detrimental RNA viruses and 2-μm DNA plasmid (Nakayashiki *et al.* 2005). Since deletion of *RNQ1* has no phenotype (Sondheimer and Lindquist 2000), it is possible that there is not enough of a detriment of carrying [*PIN*$^+$] to eliminate it from the population.

Another test of whether or not cells are happy to be [*PSI*$^+$] or [URE3] is the response of their stress reaction system, the heat shock proteins. Transcription of *HSP104* and *SSA1* are stimulated by a variety of stresses, including heat, high ethanol, high salt, high osmolarity, heavy metals, dessication, or starvation (among other conditions). Masison and coworkers have found that Hsp104 and Ssa1 protein levels are consistently elevated by the presence of the [*PSI*$^+$] or [URE3] prions (Jung *et al.* 2000; Schwimmer and Masison 2002), suggesting that these prions are recognized by the cell as stress conditions. The occasional broken limb trait is widely conserved among vertebrates, but one would not argue that the limb structures are conserved for this purpose.

On the basis of the very sparse evidence so far available, [URE3] and [*PSI*$^+$] prion-forming ability appears to be sporadically distributed among species, as its occurence in the wild is, at most, sporadic. Further work will be necessary to explore the properties of prions in other organisms.

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GENETICS

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Prion-Forming Ability of Ure2 of Yeasts Is Not Evolutionarily Conserved

Herman K. Edskes, Abbi Engel, Lindsay M. McCann, Andreas Brachmann, Huei-Fung Tsai and Reed B. Wickner

DOI: 10.1534/genetics.111.127217

File S1

Genes affected by deletion of *URE2* in *S. cerevisiae, C. albicans* and *C. glabrata*.

File S1 is available for download as a PDF at http://www.genetics.org/cgi/content/full/genetics.111.127217/DC1.

Gln3 proteins ClustalW

```
cerevisiae      MQDDPENSKLYDLLNSHLDVHGRSNEEPRQTGDSRSQSSGNTGENEEDIAFASGLNGGTF 60
glabrata        MEGFQDDLDGMGVLNSFLKDG--SGES--KSGAEKSSTTKPQAEN--VVPFVN----DNY 50
albicans        ------------------------------------MTTSNSQSKQ--------------- 10
                                                     ::   .::

cerevisiae      DSMLEALPDDL--YFTDFVSPFTAAATTS---VTTKTVKDTTPATN-----------HMD 104
glabrata        DSMLEALPDELNLDFTSLLSPFPAPGQGNDDDITVNVNPYIGQSNNDFYKIDEVPEEDLQ 110
albicans        ---FETVPSIVDLLYSAKKLLNLQPRIDN---------------------------LHLR 40
                   :*::*. :   ::        .   .                            .:

cerevisiae      DDIAMFDSLATTQPIDIAAS-------------NQQNGEIAQLWDFNVDQFNMTPSNSSG 151
glabrata        QGSEIINSTTQLQPITINNSKQQNYNQINVDSSNQQNGEIAQFWDFNVDTLNITPSNSSG 170
albicans        RSNSRTQLVMQSQALSMNNR-------------NTASNGKDDNQSFSISPVNNQALGATH 87
                 .    :     *.: :                *  ..   :  .*.:. .*  . .::

cerevisiae      SATISAPNSFTSDIPQYNHGSLGNSVSKSSLFPYNSSTSNSNINQP-SINNNSNTNAQSH 210
glabrata        SATISAPNSYNSEIPLGVTTGFNNNNN-NHLFAHGVLGGGSSIGNSNIINNTSLTYSNQQ 229
albicans        DKVLDLLSPLSIDDLKNNTITTTTTTTTSYHQNKSNQRLNGNVNTN-PTHNTGNNSDTRS 146
                . .:.  .. . :          .. . .     .    ...:.     :*.. .

cerevisiae      HSFNIYKLQNNNSSSSAMNITNNN--NSNNSNIQHPFLKKSDSIG--------------- 253
glabrata        NSFPGSSLHSKPTTATTVTYTNTPPIKENQSIIANSSLKASQFTGKRPISVVGSNNSYSE 289
albicans        TLFKPRSPHSENTSPKNILSSSSS--SATTTVVAVPKVNN-------------------- 184
                  *   . :.: ::.. :  :..   . . : :  . ::

cerevisiae      ----LSSSNTTNSVRKNSLIKPMSSTSLANFKRAASVSSSISNMEPSGQNKKPLIQCFNC 309
glabrata        NPYILSAANTSNSVRKNSLPRQLSSTSLNNYR--KSVSSSERPPDPD------AVHCDNC 341
albicans        ----LNHNTTTNNDGGNSEFK-SSNSSLPSKK---------------------VTKCYNC 218
                    *.  .*:*.   **  :  *.:** . :                     :* **

cerevisiae      KTFKTPLWRRSPEGNTLCNACGLFQKLHGTMRPLSLKSDVIKKRISKKRAKQTDPNIAQN 369
glabrata        KTYKTPLWRRSPEGKVLCNACGLFQKLHGTMRPLSLKTDVIRKRNSKKRTKIQMN--PQQ 399
albicans        NTTATPLWRRDAEGNTLCNACGLFLKLHGTCRPLSLKSDVIKKRNSRKTSTSSKVGTSTN 278
                :*  ******..**:.******** ***** ******:***:** *:* :.      . :

cerevisiae      TPSAPATASTSVTTTNAKPIRSRKKSLQQNSLSRVIP--EEIIRDNIGNTNNILNVNRGG 427
glabrata        TQSQQRQHIQYQTQQNSMQKLLSNHSSPTDTFSSGKS--RRKLSSTRLNQD--MTTIRYS 455
albicans        QFINTSVRGNDVRMKQTPIAIAATSSSTSLSGSGGVSGANSLPSSQRFKNVLILPKPPSG 338
                              ::      . *    : *   .  .    .   :    :     .

cerevisiae      YNFNSVPSPVLMNSQSYNSSNANFNGASNANLNSNNLMRHNSNTVTPNFRRSSRRSSTSS 487
glabrata        REGGSLTKQGFTTTPTYNSRSPNYS---------------NSAAASNTRKSKSRRSSTSS 500
albicans        TNLTGSARTKSIPIPANNAPSPDGP-------------FSPSLKRKKSEVDVGPRTPTSL 385
                 :  . .        : *: ..:                 *   . .    . *:.**

cerevisiae      NTSSSSKSSSRSVVPILPKPSPNSANSQQFNMNMNLMN------TTNNVS-------AGN 534
glabrata        VNSNSSRSSSRSNVPILPKISGHSS-SGNSPMNIQSTTPGGGPQTSNSVGNFLAMGAYSN 559
albicans        SASASASFSMRNRVPSSSSLTGTSFTNSIKRNNSFSNRKSSLTSLMQRKNTLGTTPTTTN 445
                  * *:  * *. **  .. :  *  .     *             :  .         *

cerevisiae      SVASSPRIISSANFNSNSPLQQNLLSNSFQRQGMNIPRRKMSRNASYSSSFMAASLQQLH 594
glabrata        SLTSSPRNAS--IYGTNAPSRN--LSSSASKYGVSMPGRKLSRNASYSSSFINTNPQQTQ 615
albicans        SLTSSNINILNQRFPQPTYFEN-FGNNNSGPQSPAISRHNSTTTLMNSNQVILENVNTPG 504
                *::**        :   :  .:   ...    .  :. :: : .   *...:  . :

cerevisiae      EQQQVDVNSNTNTNSNRQNWNSSNSVSTNSRSSNFVSQKPNFDIF----------NTPVD 644
glabrata        DIG--NFNDDQSIQGNGTGVNSQNIRRIN---SNYDSPQPNFDLFRL----DSNKDSPEN 666
albicans        SYNSTNSFPSYVPHETPNSIPETPLNVNDLLPSSFNRRSSQMSTRQERVLEEYRASKPPA 564
                .    :   .   : .  .  .      :   *.:   ..::.           ..*

cerevisiae      SPSVSRPSSRKS------HTSLLSQQLQNSE---SNSFIS--NHKFNNR----------L 683
glabrata        IPDVLRSDSRLSQKSQVSHTSLLSQQIQNQQRMRGEKSISQENLQFKQTPTMQGQTERNI 726
albicans        MPHKGIDDEMMILDALNDFPGLEAIDMGNPNGMSTDNYNNEYNDLFRKFTSLENDPFVDQ 624
                 *     ..         ...* : :: * :    :.  .  *  *.:

cerevisiae      SSDSTSPIKYEADVSAGGKISEDNS---------------------------------- 708
glabrata        PSRTTSSASYSESVFQQRGMQPNNSNSQFFENQHQANSNTDQRQFLEGMSYDLHNGDNTI 786
```

```
albicans        TANSNQNNNFGFQQIPTGNNFYKVGNNGNIG----------------------------- 655
                .: :..  .:  .         . .

cerevisiae      ---------------TKGSSKESSAIADELDWLKFGI 730
glabrata        KTVILGQEQNFANPEANGNEAKKSALEQDLDWLKFGI 823
albicans        ----------SANKNNSSNVSNNSGDYKDLDWLKFDI 682
                           ...  :.*.  .:******.*
```

FIGURE S1.—Limited homology among Gln3s of *S. cerevisiae*, *C. albicans* and *C. glabrata*.

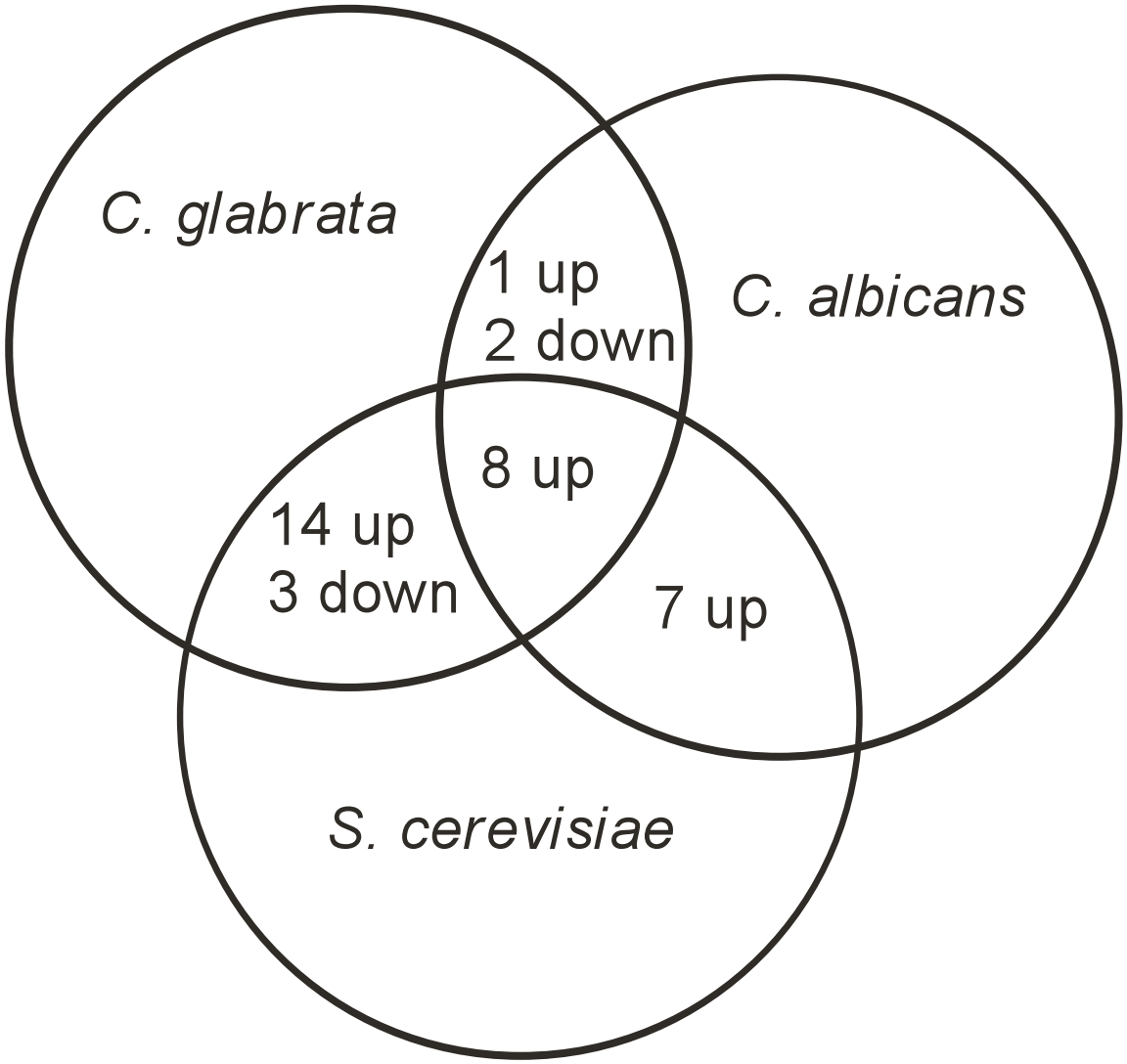

FIGURE S2.—Venn diagram of genes regulated by *URE2* in *S. cerevisiae*, *C. albicans* and *C. glabrata*. "up" means up-regulated in *ure2*Δ strain.

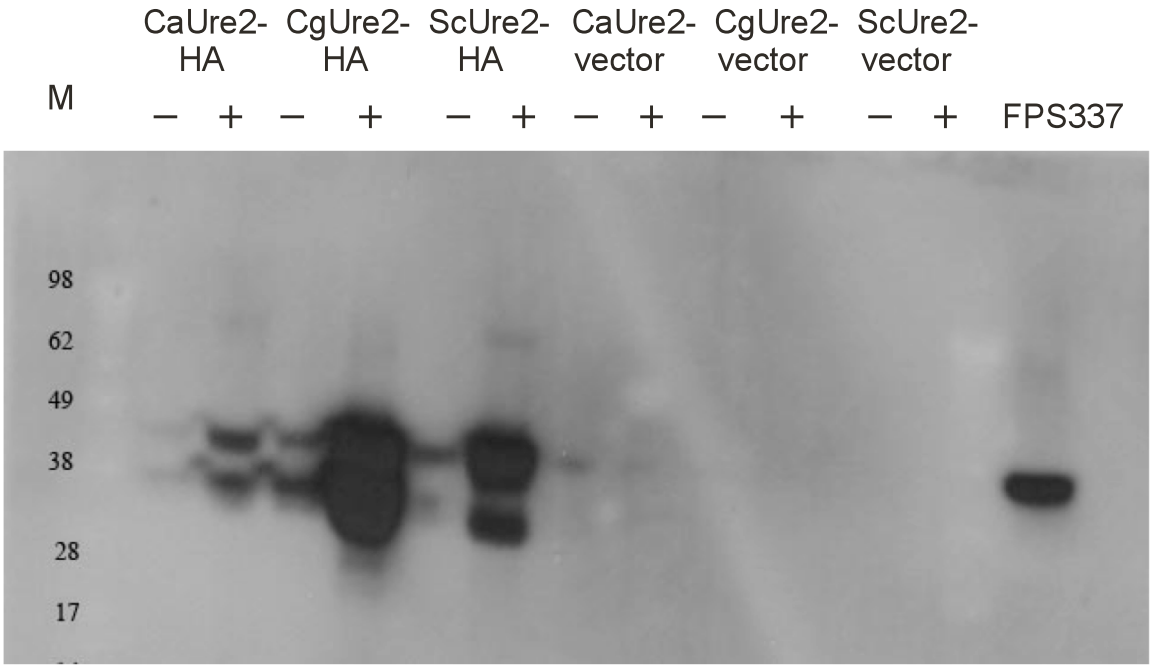

FIGURE S3.—*C. glabrata* Ure2p is overproduced on galactose induction. Minus indicates no induction (glucose media), plus indicates induction (2% galactose/1% raffinose media), M = markers (kDa) (SeeBlue Plus2, Invitrogen), FPS337 = lysates of strain FPS 337 with chromosomal *URE2*^{cerevisiae}-3HA (Table 1). CaUre2-HA = BY302 carrying pH1017 (2μ *LEU2* PGAL1*URE2*^{albicans}-*HA*), CgUre2-HA = BY304 carrying pH1018 (2μ *LEU2* PGAL1*URE2*^{glabrata}-*HA*), ScUre2-HA = YHE1265 carrying pH1016 (2μ *LEU2* PGAL1*URE2*^{cerevisiae}-*HA*). The vector was pH317 (2μ *LEU2* PGAL1).

TABLE S1

Curing of [URE3alb] by Ure2 $^{albicans\ 1\text{-}65}$-GFP expression

plasmid		Red on YES	Total colonies checked
pH1051	URE2$^{1\text{-}100}$glabrata-GFP	0	48
pH1052	URE2$^{1\text{-}65}$glabrata-GFP	0	27
pH1054	URE2$^{1\text{-}65}$albicans-GFP	48	48
pH1055	URE2$^{1\text{-}99}$cerevisiae-GFP	0	48
pH1056	URE2$^{1\text{-}65}$cerevisiae-GFP	0	48
pH1057	GFP	0	48

Strain YHE1207 (BY302 [URE3alb]) was transformed with the indicated plasmids, each with the *URE2*^{cerevisiae} promoter driving expression of the protein shown. Colony color was checked on YES (adenine-limiting) medium.