Ure2p, normally a regulator of nitrogen catabolism in *Saccharomyces cerevisiae*, can be a prion (infectious protein) by forming a folded in-register parallel amyloid called [URE3]. Using *S. cerevisiae* as a test bed, we previously showed that Ure2p of *Candida albicans* (CaUre2p) can also form a prion, but that Ure2p of *C. glabrata* (CgUre2p) cannot. Here, we constructed *C. glabrata* strains to test whether CgUre2p can form a prion in its native environment. We find that while CaUre2p can form a [URE3] in *C. glabrata*, CgUre2p cannot, although the latter has a prion domain sequence more similar to that of ScUre2p than that of CaUre2p. This supports the notion that prion formation is not a conserved property of Ure2p but is a pathology arising sporadically. We find that some [URE3*albicans*] variants are restricted in their transmissibility to certain recipient strains. In addition, we show that the *C. glabrata* HO can induce switching of the *C. glabrata* mating type locus.

**Materials and Methods**

*C. glabrata* strains for assessing Ure2p function. We set up a Ure2p activity system in *C. glabrata* based on a similar system developed by Schlumberger et al. (23). *C. glabrata* does not contain a DAL5 gene, which is often used in *S. cerevisiae* to study nitrogen regulation and to monitor the presence of the [URE3] prion. However, *C. glabrata* Ure2p (CgUre2p) represses CgDUR3 transcription (14), so the CgDUR3 promoter was fused to CgADE2 in *C. glabrata* to measure Ure2p activity. Yeasts were transformed by the Li acetate method (24), and media were as previously described (14).

The parental strain was BG88b ([MATa ura3::C418 his3::URA3 (5FOA)] 25). CgADE2 was replaced with FRT-HIS3-FRT, producing strain HCg8, and then CgHIS3 was deleted by recombination, making strain HCG12 [MATa ura3::C418 his3::URA3 (5FOA) ade2::FRT]. Specifically, the CgADE2 5′ untranslated region (UTR) (nucleotides [nt] −510 to +20 relative to the start ATG of ADE2) was amplified from BG88b using primers HE776 and HE782. The CgADE2 3′ UTR (nt 1714 to 2152 relative to the start ATG; the ADE2 open reading frame [ORF] ends at nt 1713) was amplified from BG88b using primers HE780 and HE781. CgHIS3 flanked...
by FRT sites was amplified from strain 37A (26) using primers HE783 and HE775 was amplified from strain 37A (26) using primers HE783 and HE792. PCR products were cloned into plasmid pBC KS(+) (Amersham Pharmacia) using T4 DNA ligase. The resulting fusion product was transformed into BG88b, selecting for His+ colonies. His+ colonies unable to grow without adenine were identified.

CgHis3 was deleted from the HcG8 ADE2 locus by expression of the Flp recombinase using plasmid pRD16 (27), forming strain HcG12. HcG12 could not grow on medium without adenine and formed red colonies on medium containing 30 mg/liter adenine. Thus, the ade2 mutant phenotype in C. glabrata is similar to that in S. cerevisiae, suggesting that CgADE2 is useful as a marker in C. glabrata to assay for Ure2p activity. CgDUR3 (CAGL0K031507g), the homologue of S. cerevisiae YHL016c, is a plasma membrane transporter for both urea and polyamines (28, 29). DUR3 expression is derepressed more than 100-fold upon deletion of URE2 in C. glabrata. CgDAP1 (CAGL0I03267g), the homologue of S. cerevisiae (CgTTR039w) encoding the general amino acid permease, is the only other gene derepressed 100-fold, but CgGAP1 has a higher basal expression level than CgDUR3 (14).

HcG16. For the strain HcG16 [MATa ura3::G418 his3::ura3(5FOA) dur3::ADE2-FRT-HIS3-FRT] (the single colon refers to a promoter-ORF fusion) the CgDUR3 5' UTR (nt −685 to −1 of DUR3) was amplified from BG88b with a 5' NotI site and a 3' PstI site using oligonucleotides HE784 and HE788. The CgADE2 ORF was amplified from BG88b with a 5' PstI site and a 3' HindIII site using oligonucleotides HE786 and HE787. CgHis3 flanked by FRT sites and containing a 5' HindIII site and a 3' SalI site was amplified from strain 37A using oligonucleotides HE789 and HE790. The CgDUR3 3' UTR (nt 2153 to 2278 of DUR3; DUR3 ends at nt 3175) was amplified from BG88b with a 5' SalI site and a 3' ApaI site using oligonucleotides HE791 and HE792. PCR products were cloned into pBC KS(+) (Agilent Technologies) in the following order: DUR3 5' UTR→ADE2 ORF→FRT-HIS3-FRT→DUR3 3' UTR, creating pH1104. All PCR products were checked by sequencing. The cassette was released from pH1104 by digestion with NotI and Xhol (which cleaves in the 5' UTR of DUR3 at nt 2696) and transformed into BG88b, selecting for cells capable of growing without histidine, producing HcG16.

HcG18. For the HcG18 [MATa ura3::G418 his3::ura3(5FOA) dur3::ADE2-FRT] strain, the CgHis3 marker was removed from HcG16 through Flp-mediated recombination using plasmid pRD16 (as described for HcG12).

HcG19 and HcG20. For the HcG19 and HcG20 [MATa ura3::G418 his3::ura3(5FOA) dur3::ADE2-FRT ade2::FRT-HIS3-FRT] strains, the CgADE2 ORF was replaced with FRT-HIS3-FRT as described for HcG12. Transformants were selected on plates lacking histidine and containing either arginine (1 g/liter) or uracil (10 mM) as a nitrogen source (arginine is converted to urea and ornithine). Although the DUR3 urea permease is disrupted, HcG18 can still grow on plates containing urea as the sole nitrogen source, although very slowly. Growth on medium containing urea as the sole nitrogen source should activate the DUR3 promoter and result in the production of adenine.

HcG23 and HcG25. For HcG23 and HcG25 [MATa ura3::G418 his3::ura3(5FOA) dur3::ADE2-FRT ade2::FRT] strains, the HIS3 marker was removed from HcG19 (creating HcG23) and from HcG20 (creating HcG25) through Flp-mediated recombination using plasmid pRD16.

Deleting CgURE2 to create HcG27. For HcG27 [MATa ura3::G418 his3::ura3(5FOA) dur3::ADE2-FRT ade2::FRT ura2::FRT-HIS3-FRT], the CgURE2 5' UTR (nt −501 to −1 of URE2) was amplified from BG88b with a 5' NotI site and a 3' BamHI site using oligonucleotides HE815 and HE816. CgHis3 flanked by FRT sites and containing a 5' BamHI site and a 3' PstI site was amplified from strain 37A using oligonucleotides HE819 and HE820. The CgURE2 3' UTR (nt 1074 to 1565 of URE2; the URE2 ORF ends at nt 1608) was amplified from BG88b with a 5' Xhol site and a 3' Apal site using oligonucleotides HE821 and HE822. The PCR products were cloned into pBc KS(+) (Agilent Technologies) in the following order: URE2 5' UTR→FRT-HIS3-FRT→URE2 3' UTR, creating pH1111. All PCR products were checked by sequencing. The cassette was released from pH1111 by digestion with NotI and Apal and transformed into HcG23, selecting for cells capable of growing without histidine.

HcG29. For HcG29 [MATa ura3::G418 his3::ura3(5FOA) dur3::ADE2-FRT ade2::FRT ura2::FRT], the HIS3 marker was removed from HcG27 through Flp-mediated recombination using plasmid pRD16.

Replacing the CgURE2 ORF with the CaURE2 ORF. For HcG30 [MATa ura3::G418 his3::ura3(5FOA) dur3::ADE2-FRT ade2::FRT Cgure2::CaURE2::FRT-HIS3-FRT], the CaURE2 ORF from the Darlington strain (30) was amplified with a 5' BamHI site and a 3' PstI site using oligonucleotides HE817 and HE818. This CaURE2 PCR product was cloned between the CaURE2 5' UTR and FRT-HIS3 in pH1111 (see HcG27), creating pH1113. The cassette was released by digestion with NotI and Apal and transformed into HcG29, selecting for cells capable of growing without histidine.

HcG32. For HcG32 [MATa ura3::G418 his3::ura3(5FOA) dur3::ADE2-FRT ade2::FRT ura2::URE2(C. albicans)-FRT], the HIS3 marker was removed from HcG30 through Flp-mediated recombination using plasmid pRD16.

C. glabrata expression plasmids. The centromeric S. cerevisiae plasmid pRS316 (31) is capable of replicating in C. glabrata (32, 33) with a copy number of 10 to 30. The ADH1 promoter cassette was amplified from pVT103 (34) (using oligonucleotides HE66 and HE67) and cloned in the Pavll window of pRS316, replacing the multiple cloning region. In the resulting plasmid, pH130, the ADH1 and URA3 promoters are facing each other. By site-directed mutagenesis using oligonucleotide HE127, a PstI site was removed from URA3, resulting in pH393.

No inducible promoter system is available in C. glabrata. The TPI1 constitutive strong promoter (nt −959 to −1 of TPI1; CAGL0H08327g, triosephosphate isomerase) was amplified from C. glabrata CBS138 genomic DNA using oligonucleotides HE892 and HE893. The ScADH1 promoter from pH393 was replaced with the CgTPI1 promoter using flanking Nhel and BamHI sites, resulting in pH132. In order to check if pH132 could be used as an expression vector in C. glabrata, green fluorescent protein (GFP) was inserted behind the promoter as a BamHI-Xhol fragment from pH119 (35), resulting in plasmid pH1231. Transformation of this plasmid into C. glabrata BG88b resulted in very bright GFP fluorescence in all of the cells, while the vector pRS316 showed only very faint background fluorescence. Thus, pH132 can be used as an expression plasmid in C. glabrata.

CgURE2 was amplified from BG88b with primers HE297 and HE299, introducing a BamHI site upstream of the start codon and an Xhol site downstream of the stop codon, and inserted as a BamHI/Xhol fragment into expression vector pH1232, resulting in pH1259. CgURE2 was transferred as a BamHI/Xhol fragment from pH563 (12) into pH132, resulting in pH1258. CgURE2 lacking the 5' prion domain (starting at nt 292; M98) was amplified with primers HE941 and HE299 and cloned as a BamHI/Xhol fragment into pH1322, resulting in pH1274. CgURE2 lacking the 5' prion domain (starting at nt 262; Q88) was amplified with primers HE942 and HE188 and cloned as a BamHI/Xhol fragment into pH1322, resulting in pH1272.

[URE3] induction constructs. The centromeric S. cerevisiae plasmid pRS313 (31) containing HIS3 was modified by site-directed mutagenesis to remove 2 BgIII sites [oligonucleotides HE123 and HE124, 1 PstI site (oligonucleotides HE125), 1 Nhel site (oligonucleotide HE125), and 2 HindIII sites (oligonucleotides HE122 and HE125), resulting in pH339. An ADH1 promoter cassette was amplified from pVT103 (34) using oligonucleotides HE66 and HE67 and cloned in the Pavll window of pH339, replacing the multiple cloning region. In the resulting plasmid, pH403, the ADH1 and HIS5 promoters are facing each other.

The Nhel/BamHI-bordered ADH1 promoter in pH403 was replaced with the CgTPI1 promoter from pH1323, creating pH1286. The CgTPI1

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promoter and a fragment of CgURE2 containing the first 100 amino acids was transferred as an Nhel/Xhol fragment into pH403, creating pH1287. The CgTPPI promoter and a fragment of CaURE2 containing a fragment of the 89th amino acids was transferred as an Nhel/Xhol fragment into pH403, creating pH1288.

Switching C. glabrata mating type loci. The HO ORF (CAGL0G5423) was amplified from C. glabrata BG88b using oligonucleotides HE894 and HE895 and cloned as a BamHI/XhoI fragment into pJH232, resulting in pH1254. pH1254 was transformed into HCg25, and transformants capable of growing without uracil were streaked as single colonies on YPAD (14). Nearly all of the YPAD colonies had lost plasmid pH1254. The MAT locus was scored using two sets of primers described by Butler et al. (36), as well as BG88b (MATa) and CBS138 (MATa) as controls. Among 20 clones in which HO had been expressed using pH1254, four isolates were identified that had become MATa. The same procedure, starting with strain HCg32, produced 7 MATa clones of 20 examined. Thus, CgHO has the ability to change the mating type locus. HCg25 MATa was named HCg58.

The 5′ UTR of CgLEU2 (CAGL0H03795g; nt −399 to +17 of LEU2) was amplified from BG88b using oligonucleotides HE831 and HE833 with a 3′ PstI site, and containing a 5′ BamHI site and a 3′ PstI site, was amplified from strain 37A using oligonucleotides HE819 and HE820 and cloned into pBC KS+, creating pH1107. The 3′ UTR of CgLEU2 (nt 1099 to 1511 of LEU2 with the LEU2 ORF terminating at nt 1098) was amplified with oligonucleotides HE834 and HE832 with a 5′ XhoI site and a 3′ ApaI site. PCR products were cloned into pBS KS+ (Aigent Technologies) in the order LEU2 5′ UTR→FRT-HIS3-FRT→LEU2 3′ UTR, creating pH1125. The cassette was released from pH1125 by digestion with BamHI and ApaI and transformed into HCg58, selecting for cells capable of growing without histidine, producing the letuc C. glabrata strain HCg62.

Transfection of [URE3alianicans] from C. glabrata extracts into S. cerevisiae cells. Six Ade+ C. glabrata isolates expressing CaUre2p (HCg32+pH1254+pH1288) were grown in SD medium to saturation, diluted into 50 ml YPAD, and grown for 2 to 3 doublings. As a positive control, S. cerevisiae YHE1161 (BY302 [URE3] MATa his3 leu2 trp1 [CagURE2] and HCg29 [CgURE2]) was transformed with the opposite mating type and different markers. TRPI was amplified from S. cerevisiae S288c using oligonucleotides HE899 and HE900, and BY302 cells that had become Ade+ after receiving protein extracts of HCg32 expressing CaURE2 and CaURE2N were restored to Trp+ by transformation with the PCR product. The mating type of BY302 was changed from MATa to MATa by transfection of ORF (CAGL0H03795g; nt 1099 to 1511 of LEU2 with the LEU2 ORF terminating at nt 1098) was amplified with oligonucleotides HE831 and HE833 with a 3′ PstI site, and containing a 5′ BamHI site and a 3′ PstI site, was amplified from strain 37A using oligonucleotides HE819 and HE820 and cloned into pBC KS+, creating pH1107. The 3′ UTR of CgLEU2 (nt 1099 to 1511 of LEU2 with the LEU2 ORF terminating at nt 1098) was amplified with oligonucleotides HE834 and HE832 with a 5′ XhoI site and a 3′ ApaI site. PCR products were cloned into pBS KS+ (Aigent Technologies) in the order LEU2 5′ UTR→FRT-HIS3-FRT→LEU2 3′ UTR, creating pH1125. The cassette was released from pH1125 by digestion with BamHI and ApaI and transformed into HCg58, selecting for cells capable of growing without histidine, producing the letuc C. glabrata strain HCg62.

RESULTS

Ure2p activity in C. glabrata. Candida glabrata strains HCg25 (CgURE2), HCg32 (CaURE2; genomic replacement), and HCg29 (ure2Δ) have the Ure2p-regulated DUR3 promoter fused to ADE2, making growth on medium without adenine a measure of Ure2p activity (Table 1). We tested nitrogen sources for Ure2p activation (making cells Ade−) (Fig. 1). The ure2Δ strain HCg29 grew equally well on all nitrogen sources, producing single colonies within 2 days, except on media containing urea, where the absence of the Dur3 urea permease slowed growth.

Ammonium, glutamine, asparagine, urea, or the combination of ammonium and glutamate activated C. glabrata Ure2p, but arginine, proline, or glutamate did not, a pattern similar to that observed in S. cerevisiae. CaUre2p showed the same pattern, except that urea did not activate CaUre2p and there was a bit more growth on ammonium and glutamine medium than for CgUre2p. When plates were incubated beyond the 2 days used to assess the effects of the various nitrogen sources described above, all strains formed single colonies on all media tested. This indicates that background expression of the DUR3 promoter in C. glabrata is higher than that of the promoter routinely used in S. cerevisiae, DAL5. It is nevertheless remarkable that CaURE2 functions so well in both S. cerevisiae and C. glabrata given the limited homology between the Gln3 proteins of these three organisms and the fact that Ure2p largely acts through Gln3p in S. cerevisiae (1, 38). Homology is limited to the zinc finger domain in the middle of the protein and the last 9 amino acids (14). However, it is not known whether CgUre2p or CaUre2p acts largely through Gln3p in their native species.

Expressing CgUre2p or CaUre2p from plasmids in the ure2Δ strain HCg29 resulted in a tight Ade+ phenotype on medium containing asparagine plus glutamine as nitrogen sources (1 g/liter each) for some transformants, but a substantial number of transformants were Ade+ on the same medium.

[URE3] induction in C. glabrata. Because strains HCg25 (CgURE2) and HCg32 (CaURE2) with genomic URE2 genes grew without adenine on all media tested (including the combination of asparagine and glutamine) when incubated for more than 2 days, we combined overexpression of each full-length Ure2p from plasmids with chromosomal expression, resulting in a tight Ade− phenotype and overexpressed N-terminal fragments (known to be the prion domain in the case of CaUre2p) in attempts to induce prion formation. Essentially all double transformants were Ade− in the presence of ammonium or asparagine plus glutamine even after 5 days of incubation. This was sufficient to allow selection of rare Ade− putative [URE3] clones (Table 2).

Confirming [URE3] generation in C. glabrata. (i) Mating in C. glabrata. If the C. glabrata Ade+ colonies generated in strain
TABLE 1 Yeast strains and plasmids

| Strain | Description | Source or reference |
|--------|-------------|---------------------|
| **Saccharomyces cerevisiae** | | |
| BY302  | MATa his3 leu2 trp1 CaURE2 P\_DAL5\_ADE2 P\_DAL5\_CAN1 kar1 | 14 |
| YHE1160 | BY302 [URE3albican01160] | 14 |
| YHE1161 | BY302 [URE3albican01161] | 14 |
| YHE1162 | BY302 [URE3albican01162] | 14 |
| YHE1170 | BY302 [URE3albican01170] | 14 |
| YHE1181 | MATa ura2 leu2 kar1 CaURE2 P\_DAL5\_ADE2 P\_DAL5\_CAN1 [rho\_+\+] | This work |
| YHE1364 | BY302 changed to MATa HIS3 [rho\_+] | This work |
| **Candida glabrata** | | |
| BG88b  | MATa ura3::G418 his3::URA3 (5FOA) | 25 |
| HCG8   | MATa ura3::G418 his3::URA3 (5FOA) ade2::FRT-HIS3-FRT | This work |
| 37A    | Clinical isolate | 26 |
| HCG12  | MATa ura3::G418 his3::URA3 (5FOA) ade2::FRT | This work |
| HCG16  | MATa ura3::G418 his3::URA3 (5FOA) dur3::ADE2-FRT-HIS3-FRT | This work |
| HCG18  | MATa ura3::G418 his3::URA3 (5FOA) dur3::ADE2-FRT | This work |
| HCG19, HCG20 | ura3::G418 his3::URA3 (5FOA) dur3::ADE2-FRT ade2::FRT-HIS3-FRT | This work |
| HCG23, HCG25 | MATa ura3::G418 his3::URA3 (5FOA) ade2::FRT | This work |
| HCG27  | MATa ura3::G418 his3::URA3 (5FOA) dur3::ADE2-FRT ade2::FRT ADH1 promoter CgTPI1 | This work |
| HCG29  | MATa ura3::G418 his3::URA3 (5FOA) dur3::ADE2-FRT ade2::FRT dur3:ADE2-FRT ade2::FRT | This work |
| HCG30  | MATa ura3::G418 his3::URA3 (5FOA) dur3::ADE2-FRT ade2::FRT | This work |
| HCG32  | ura3::G418 his3::URA3 (5FOA) dur3::ADE2-FRT ade2::FRT ura2::CaURE2-FRT | This work |
| HCG62  | MATa ura3::G418 his3::URA3 (5FOA) dur3::ADE2-FRT ade2::FRT ura2::FRT-HIS3-FRT | This work |
| **Plasmids** | | |
| pRS316 | CEN ARS URA3 A’ | 31 |
| pVT103 | ADH1 promoter with multiple cloning site | 34 |
| pH1232 | pRS316 with ADH1 promoter, MCS from VT103 CEN ARS URA3 A’ ADH1 promoter | This work |
| pH1259 | pH1232 with CgURE2 | This work |
| pH1258 | pH1232 with CaURE2 | This work |
| pH1274 | pH1232 with CgURE2NΔ (lacks aa 1–97) | This work |
| pH1272 | pH1232 with CgURE2NΔ (lacks aa 1–87) | This work |
| pH1286 | CEN ARS HIS3 CgTPI1 promoter | This work |
| pH1287 | CEN ARS HIS3 CgTPI1 promoter CgURE2N (aa 1–100) | This work |
| pH1288 | CEN ARS HIS3 TPI1 promoter CaURE2 (aa 1–89) | This work |
| pH1254 | CEN ARS URA3 A’ CgTPI1 promoter CgHO | This work |

HCG32 (CaUERE2) are indeed [URE3], the Ade\_+\_ phenotype has to be transferable. However, no mating has been observed in C. glabrata (39). Three mating type-like loci, similar to those found in S. cerevisiae, are present in C. glabrata (40). Furthermore, C. glabrata has an HO endonuclease which is essential in S. cerevisiae for mating type switching (36). No mating type switching has been observed in C. glabrata grown in culture (41), but both MATa and MATa cells are found in the same clade, suggesting the occurrence of mating type switching and mating in the wild (42).

Overexpression of CgHO changed the mating type locus of HCG25 (or HCG32) from MATa to MATa, indicating that CgHO is active. HCG25 (MATa his3) was crossed with the switched strain, HCG62 (MATa leu2), on plates containing various nitrogen sources or on plates containing acetate as a carbon source (S. cerevisiae sporulation medium). Plates were incubated at several temperatures. Single colonies emerged on plates containing glutamate as the sole nitrogen source that had been placed for 4 days at 37°C and then left at room temperature for 3 weeks. These colonies propagated when transferred to a fresh plate or to a plate containing ammonium as the nitrogen source, although the growth rate remained similarly low. However, when streaked on YPAD, all single colonies showed only the parental marker combinations. Thus, no mating was observed, indicating that C. glabrata cannot be used for prion infectivity studies.

(ii) Curing of the Ade\_+\_ phenotype. C. glabrata grows well on YPAD with 120 mM guanidine HCl (GuHCl), but no curing of the Ade\_+\_ phenotype was observed. A few colonies showed white to red sectoring, but these had to be purified several times on YPAD with 120 mM GuHCl in order to obtain Ade\_−\_ colonies. Thus, guanidine curing does not seem to work in C. glabrata. It is unknown if CgHsp104p can be inhibited by guanidine (43).

(iii) Protein transformation into S. cerevisiae. When spheroplasts of S. cerevisiae are exposed to ScUre2p amyloid or to a protein extract from a [URE3] cell, the recipient cells can acquire the prion (6, 44). [URE3] based on CaUre2p but propagating in S. cerevisiae can be transferred to recipient S. cerevisiae cells by cytoduction or by exposure to a protein extract from infected cells (14, 15). If C. glabrata propagates [URE3], based on CaUre2p the prion should be transferable from a protein extract of these cells into recipient S. cerevisiae similarly expressing CaUre2p. Using the genetic tools available for S. cerevisiae, the prion then can be characterized.
Extracts of six Ade⁺ *C. glabrata* clones (HCg32 + pH1258 + pH1288) expressing CaUre2p were transformed into spheroplasts of *S. cerevisiae* BY302 (Ca⁺URE2), selecting first for clones which had been transformed with a LEU2 plasmid included in the transformation mix and then checking Leu⁺ clones for Ade⁺, a sign that the same cell had been infected with the [URE3 *albicans*] prion (Table 3).

Since prion-infected cells should be guanidine curable, BY302 cells that received protein extracts from *C. glabrata* HCg32 colonies 1, 2, and 6 appear to contain [URE3 *albicans*] (Table 3). This indicates that [URE3 *albicans*] was present in these *C. glabrata* clones. All of the [URE3 *albicans*] variants are weak, as colonies turn red on adenine-limiting (YES/W) medium or on minimal medium with no adenine (SD/H+W/L).

(iv) Cytoduction. A suitable cytoduction recipient was constructed from BY302 by changing its mating type and markers (see Materials and Methods). Each of several *S. cerevisiae* strains expressing CaUre2p and transformed to guanidine-curable Ade⁺ by extracts of *C. glabrata* (Ca⁺URE2) (Ade⁺ clones 1, 2, and 6) could transmit [URE3 *albicans*] by cytoplasmic mixing to the constructed recipient (Table 4).

Thus, transformation of a protein extract from *C. glabrata* cells selected to be Ade⁺ into *S. cerevisiae* resulted in the recipient cells becoming infected with [URE3 *albicans*].

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**TABLE 3 Infection of *S. cerevisiae* with [URE3 *albicans*] from extracts of *C. glabrata* strainsa**

| Protein extract source | Ade⁺ ScBY302 of 48 tested | Ade⁺ clone no. | No. Ade⁺/color on YES after growth on: |  |
|------------------------|---------------------------|----------------|----------------------------------------|---|
| YHE1161                |                           |                | YPAD                                   |  |
|                        |                           | 19             | 12/white                               | 0/red  |
|                        |                           | 20             | 12/white                               | 0/red  |
|                        |                           | 21             | 12/white                               | 0/red  |
|                        |                           | 22             | 12/white                               | 0/red  |
| H₂O                    |                           | 11             | 11/red                                 | 0/red  |
| HCg32                  |                           | 12             | 12/red                                 | 0/red  |
|                        |                           | 13             | 12/red                                 | 0/red  |
|                        |                           | 14             | 12/red                                 | 0/red  |
|                        |                           | 15             | 12/red                                 | 0/red  |
|                        |                           | 16             | 12/red                                 | 0/red  |
|                        |                           | 17             | 12/red                                 | 0/red  |
|                        |                           | 18             | 12/red                                 | 0/red  |
|                        |                           | 2              | 8                                       | 10/red  |
|                        |                           | 3              | 10                                      | 0/red  |
|                        |                           | 4              | 7/white                                 | 7/white |
|                        |                           | 5              | 7                                       | 12/white |
|                        |                           | 6              | 1                                       | 12/white |

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**TABLE 2 Induction of [URE3] in *Candida glabrata* a**

| Strain     | URA3 plasmid       | HIS3 plasmid     | No. Ade⁺/10⁶ cells in expt: |
|------------|--------------------|------------------|-----------------------------|
|            |                    |                  | 1  | 2  | 3  | 4  | 5  |
| HCg25 CgURE2 | CgURE2 (pH1259) | Vector (pH1286) |   | <1 | <1 | <1 | <1 |
|            | CgURE2 (pH1259) | CgURE2 aa 1–100 (pH1287) |   | <1 | <1 | <1 | <1 |
| HCg32 URE2albicans | CgURE2 (pH1258) | Vector (pH1286) |   | <1 | <1 | 12 | 1  |
|            | CgURE2 (pH1258) | CgURE2 aa 1–89 (pH1288) | 36 | 15 | 15 | 24 | 95 |

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*a* Cells were grown on medium without adenine, adjusted to pH 5.6, and contained both ammonium and glutamate as nitrogen sources. Colonies were counted after 5 days at 30°C. No histidine was added, as this seemed to increase the background.
which can transfer their prion to YHE1181, and YHE1160 and YHE1170. Those cytoductants could readily transfer their prion to YHE1181 (Table 5).

Transmission variants of [URE3]albicans. Transient overexpression of CaUre2p in a S. cerevisiae strain in which ScUre2 has been replaced with CaUre2 results in an increase of cells containing [URE3]albicans (14). Each of four [URE3]albicans-containing isolates from strain BY302 (MATa CaUre2 PDALS:ADE2 kar1) could be cured of [URE3]albicans by growth on medium containing 3 mM guanidine hydrochloride (14), and, when crossed with strain YHE1181 (MATα CaUre2 PDALS:ADE2 kar1), diploids remained capable of propagating [URE3]. However, whereas [URE3]albicans was readily cytoduced from two of the [URE3] isolates, YHE1161 and YHE1162, into strain YHE1181, no [URE3]albicans cytoductants could be identified when the other two isolates, YHE1160 and YHE1170, were used as donors (Table 5).

Strains YHE1160 and YHE1170 were cured of [URE3]albicans by growth on guanidine, and [URE3]albicans originating in YHE1161 or YHE1162 was introduced into each by cytoduction (see Table S2 in the supplemental material). Those cytoductants could readily transfer [URE3]albicans or [URE3]albicans by cytoduction to YHE1181 (see Table S3, middle column). Thus, there is a difference in the [URE3]albicans variants between YHE1161 and YHE1162, which can transfer their prion to YHE1181, and YHE1160 and YHE1170, which cannot.

| Protein extract | S. cerevisiae BY302 Ade+ clone no. and color on YES | No. of Ade+ cytoductants/total |
|-----------------|----------------------------------------------------|---------------------------------|
| YHE1161        | 19; white                                           | 28/28                           |
|                 | 20; white                                           | 30/30                           |
| HcG32           | 1                                                  | 26/28                           |
|                 | 2                                                  | 18/18                           |
|                 | 4                                                  | 24/44                           |
|                 | 6                                                  | 41/42                           |

The cytoduction recipient was YHE1364, isogenic with BY302, but with different markers. Cytoductants were tested for ability to grow without adenine. Numbers are expressed as no. of Ade+ cytoductants out of the total number of cytoductants assayed.

becoming Ade+. The Ade+ phenotype was curable by growth on YPAD containing 3 mM guanidine HCl and was transferrable by cytoduction. This indicates that the Ade+ phenotype in the recipient S. cerevisiae cells was due to the presence of [URE3]albicans. We conclude that the C. glabrata donor cells contain [URE3]albicans.

A combination of the [URE3]albicans variant and the host background must block transfer of [URE3]albicans from YHE1160 and YHE1170 to YHE1181. Note that [URE3]albicans originating from YHE1161 changed during the transfers between the hosts BY302 and YHE1181, so that during its second time in the latter host it no longer supported growth on adenine dropout medium. The difference in results between the two adenine-lacking media was also displayed by the [URE3]albicans variants derived from C. glabrata when introduced into S. cerevisiae BY302.

It is possible that the failure to find prion formation by PrP in C. glabrata and C. albicans is the result of differences in the host background, particularly the endosymbiotic system. The endosomal system has not been properly set up in the attempts to make PrP a prion in yeast.

DISCUSSION

Here, we show that Ure2p of C. glabrata cannot form a prion at detectable frequency even in its own environment. As a control, we show that Ure2p of C. albicans can do so. Like previous results on K. lactis Sup35p and Ure2p (19, 20) and S. paradoxus Ure2p (14), this result supports the use of S. cerevisiae as a test bed for potential prion proteins (PrP). The one noted exception is the failure to find prion formation by PrP in S. cerevisiae. It is possible that the cell surface location of the normal form, PrP1, and the formation of the prion form, PrPSc, either on the cell surface or in the endosomal system has not been properly set up in the attempts to make PrP a prion in yeast.

Our work confirms that prion-forming ability is not generally conserved. While the CgUre2p prion domain sequence is much closer to that of S. cerevisiae than is that of CaUre2p, only CaUre2p can form prions in S. cerevisiae or C. glabrata. Indeed, CgUre2p also does not form amyloid in vitro, while the C. albicans protein readily does so (15). Aigle's group has likewise found that the Ure2p of Kluyveromyces lactis is incapable of forming [URE3] in

### Table 4 Cytoduction tests of S. cerevisiae transformed with extracts of C. glabrata Ade+ clones

| Protein extract | S. cerevisiae BY302 Ade+ clone no. and color on YES | No. of Ade+ cytoductants/total |
|-----------------|----------------------------------------------------|---------------------------------|
| YHE1161         | 19; white                                           | 28/28                           |
|                 | 20; white                                           | 30/30                           |
| HcG32           | 1                                                  | 26/28                           |
|                 | 2                                                  | 18/18                           |
|                 | 4                                                  | 24/44                           |
|                 | 6                                                  | 41/42                           |

The cytoduction recipient was YHE1364, isogenic with BY302, but with different markers. Cytoductants were tested for ability to grow without adenine. Numbers are expressed as no. of Ade+ cytoductants out of the total number of cytoductants assayed.

### Table 5 Variants of [URE3]albicans differ

| [URE3]albicans donor | [ure-α] recipient | Cytoductants | Diploids | No. of Ade+ mated cytoductants |
|----------------------|-------------------|--------------|----------|-------------------------------|
| YHE1160              | YHE1181           | 0            | 86/35    | 48/86                         |
| YHE1161              | YHE1181           | 100          | 88/72    | 47/86/88                      |
| YHE1162              | YHE1181           | 100          | 89/54    | 48/89/89                      |
| YHE1170              | YHE1181           | 0            | 91/38    | 38/91                         |

Cells were tested for the presence of [URE3]albicans by growth on adenine dropout media. When SD medium was used, lacking adenine and only containing the amino acids needed for growth, the same result was obtained. Cytoductants were mated to strain YHE1186 (BY302 [rho+]), and the diploid phenotype was tested similarly.

### Table 6 All [URE3]albicans variants are transmitted to a recipient isogenic to donors

| [URE3]albicans donor | Recipient        | SD+ U+L | − Ade |
|----------------------|------------------|---------|-------|
| AH1160 TRP+          | YHE1181          | 3/26    | 1/26  |
| AH1161 TRP+          | YHE1181          | 48/48   | 48/48 |
| AH1162 TRP+          | YHE1181          | 48/48   | 48/48 |
| AH1170 TRP+          | YHE1181          | 0/42    | 0/42  |
| AH1160 TRP+          | YHE1364          | 93/93   | 6/96  |
| AH1162 TRP+          | YHE1364          | 48/48   | 48/48 |
| AH1162 TRP+          | YHE1364          | 48/48   | 48/48 |
| AH1170 TRP+          | YHE1364          | 94/94   | 32/96 |

YHE1364 is isogenic to BY302, the parent of the [URE3]albicans donors. Cytoductants are listed as Ade+ colonies per number of cytoductants tested. Adenine prototrophy was assessed on minimal medium plus the other required amino acids (SD+ U+L) or on complete defined medium lacking only adenine (− Ade).
K. lactis itself (20). The fact that prion formation by Ure2p or Sup35p is not restricted to S. cerevisiae has been used as an argument that it is a conserved trait, and that prion formation therefore must have a function for yeast (and those other species) (45, 46). Our results indicate that the ability of a protein from each of two species to form prions does not imply that that ability is conserved; it may well be a sporadically occurring trait. The same point is inferred from the finding that sequence is not important for prion-forming ability by the Ure2p and Sup35p prion domains (47–49). Our results parallel the fact, long known for mammalian prion proteins, that the ability of mammalian PrP to assume the prion form varies dramatically from one species to another and even within the same species (for examples, see reference 50).

In the course of preparing strains for this study, we have verified that, through Ure2p, C. glabrata selectively activates or represses transcription of genes in response to the availability of nitrogen sources in the growth environment. We showed previously that C. glabrata Ure2p regulates a set of genes similar to that of S. cerevisiae; however, we did not show that glabrata reacts to the nitrogen state of the environment, as does S. cerevisiae. As in S. cerevisiae, ammonia, asparagine, and glutamate activate Ure2p, whereas proline does not (1, 51, 52). Glutamate derepresses some genes, notably the general amino acid permease GAP1, are transcribed when glutamate is present as the sole nitrogen source but the protein is channeled to the vacuole for degradation (54, 55). Medium containing both ammonium and glutamate was used to isolate the first [URE3] prion (56). In C. glabrata the combination of ammonium and glutamate is among the strongest repressors of the nitrogen state of the environment, as does S. cerevisiae. Gene 290:1–18.

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