nature genetics

Passage through the mammalian gut triggers a phenotypic switch that promotes *Candida albicans* commensalism

Kalyan Pande[1], Changbin Chen[1] & Suzanne M Noble[1,2]

Among ~5,000,000 fungal species[1], *C. albicans* is exceptional in its lifelong association with humans, either within the gastrointestinal microbiome or as an invasive pathogen[2]. Opportunistic infections are generally ascribed to defective host immunity[3] but may require specific microbial programs. Here we report that exposure of *C. albicans* to the mammalian gut triggers a developmental switch, driven by the Wor1 transcription factor, to a commensal cell type. Wor1 expression was previously observed only in rare genetic backgrounds[4–6], where it controls a white-opaque switch in mating[4–7]. We show that passage of wild-type cells through the mouse gastrointestinal tract triggers *WOR1* expression and a novel phenotypic switch. The resulting GUT (gastrointestinally induced transition) cells differ morphologically and functionally from previously defined cell types, including opaque cells, and express a transcriptome that is optimized for the digestive tract. The white-GUT switch illuminates how a microorganism can use distinct genetic programs to transition between commensalism and invasive pathogenesis.

The yeast *C. albicans* is well known as the most common agent of symptomatic fungal disease[8,9], but its more typical role is as a permanent resident of the healthy gastrointestinal microbiome[2]. Longitudinal molecular typing studies indicate that disseminated *C. albicans* infections originate from individuals' own commensal strains[10], and the transition to virulence is generally thought to reflect impaired host immunity[3]. Nevertheless, the ability of this commensal pathogen to thrive in radically different host niches speaks to the existence of functional specializations for commensalism and disease. To investigate the *C. albicans* commensal lifestyle, we developed a mouse model of stable gastrointestinal candidiasis in which the animals remain healthy, despite persistent infection with high titers of yeast[11]. Using this model, we found that a *C. albicans* mutant lacking the Efg1 transcriptional regulator had enhanced commensalism, such that mutant cells strongly outcompeted wild-type cells in mixed infections (**Fig. 1a**); similar findings were recently reported elsewhere[12].

Efg1 has diverse cellular functions, including the inhibition of *WOR1* (ref. 13), the master regulator of a white-opaque epigenetic switch that controls *C. albicans* sexual competency[4–6] (**Fig. 1b**). We asked whether *WOR1* also regulates commensalism by testing a *wor1*ΔΔ deletion mutant in the same mouse model. Indeed, *wor1*ΔΔ cells were rapidly depleted from the gastrointestinal tract (**Fig. 1c**), indicating that *WOR1* is required for normal commensal fitness. Similar defects were observed with two additional *wor1*ΔΔ mutants (**Supplementary Fig. 1a**,**b**), confirming genetic linkage between *WOR1* and fitness. Moreover, the substantial defect in commensalism of a heterozygous knockout mutant (*wor1*Δ/*WOR1*; **Supplementary Fig. 1c**) indicates that *WOR1* dosage is also key. Notably, a *his1*ΔΔ, *leu2*ΔΔ auxotroph exhibited wild-type fitness in the same assay (**Supplementary Fig. 1d**), indicating that the gene disruption markers used to construct the *wor1* mutants are neutral for commensalism, as they are for virulence[14].

These *in vivo* results were notable in light of multiple reports in the *C. albicans* literature suggesting that only rare cell types are competent for *WOR1* expression *in vitro*[4–6]. Under laboratory conditions, *WOR1* is repressed in most isolates of this diploid species by a potent transcriptional repressor, **a**1-α2, whose subunits are encoded by the distinct **a** and α alleles, respectively, of the *MTL* mating type–like locus[4–6]. Therefore, only strains that had undergone loss of either *MTL***a** or *MTL*α were considered capable of expressing *WOR1* (refs. 4–6). However, our analysis was performed with *MTL***a**/α strains.

We hypothesized that signals present in the mammalian gastrointestinal tract might elicit *WOR1* expression in *MTL***a**/α cells. To test this idea, we used a technique that permanently marks cells when a promoter is activated *in vivo*[15,16] (**Supplementary Fig. 2a**). We created a *WOR1*$_{promoter}$-*FLP* strain containing an endogenous *WOR1* promoter fused to the gene for Flp recombinase, as well as a copy of *URA3* (conferring sensitivity to 5-fluoroorotic acid, 5-FOA) flanked by FRT recombination sites. Activation of the *WOR1* promoter results in expression of *FLP*, deletion of *URA3* and resistance to 5-FOA. Using this strain, we determined the frequency of *WOR1* expression in *MTL***a**/α *C. albicans* cells propagated in the laboratory or in the mouse commensal model. After eight generations of mid-log growth at 37 °C, only 0.0002% of cells propagated *in vitro* exhibited Flp-mediated excision of *URA3* (**Fig. 1d** and **Supplementary Fig. 2b**,**c**). In contrast, after 3 d of growth in the host, 1.9% of cells showed evidence of this event (**Fig. 1d** and **Supplementary Fig. 2b**,**c**). These figures likely underestimate the true frequency of *WOR1* expression, as Flp is reportedly unstable at mammalian body temperature[17]. Nevertheless, comparison of the rates suggests that propagation of *MTL***a**/α cells in the mammalian gastrointestinal tract increases the probability of *WOR1* expression by roughly 10,000-fold.

[1]Department of Microbiology and Immunology, University of California at San Francisco, San Francisco, California, USA. [2]Division of Infectious Diseases, Department of Medicine, University of California at San Francisco, San Francisco, California, USA. Correspondence should be addressed to S.M.N. (suzanne.noble@ucsf.edu).

Received 2 November 2012; accepted 28 June 2013; published online 28 July 2013; doi:10.1038/ng.2710

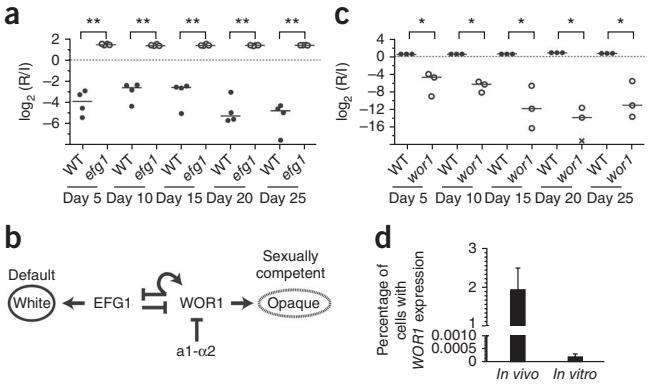
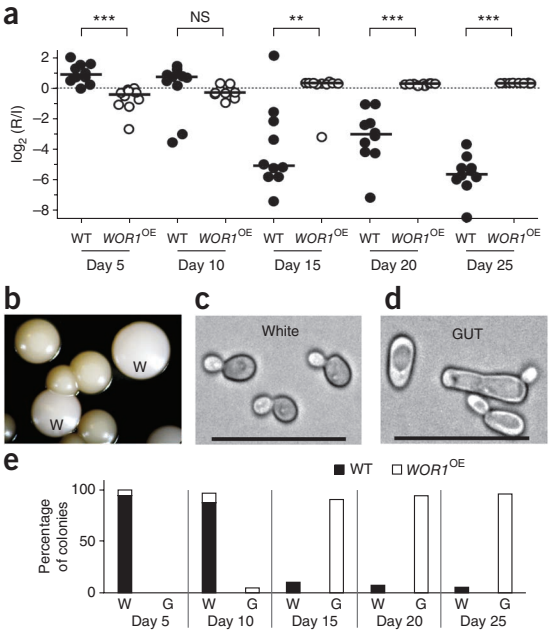


Figure 1 *EFG1* inhibits and *WOR1* promotes *C. albicans* fitness in the commensal milieu. (**a**) Competition between wild-type (WT, SN250) and *efg1*ΔΔ mutant (*efg1*; SN1011) strains in four mice. Relative abundance of the strains was determined by quantitative PCR using strain-specific primers, $\log_2$ (R/I) represents the log ratio of the abundance of each strain after recovery from the feces of a given animal (R) to its abundance in the infecting inoculum (I). $^{**}P < 0.001$ by *t* test (two-tailed, unpaired samples). Horizontal black bars denote the median value for each group. Similar results were obtained when the *efg1*ΔΔ mutant was competed in a pool of 48 strains (data not shown). (**b**) Schematic of the transcriptional regulation of the white-opaque switch. (**c**) Competition between wild-type (SN250) and *wor1*ΔΔ (*wor1*; SN881) in three mice as described in **a**. $^{*}P < 0.05$. Similar results were obtained in tests of two additional *wor1*ΔΔ strains (**Supplementary Fig. 1a**,**b**). (**d**) Comparison of *WOR1* expression in *MTL***a**/α cells grown in the commensal model (*in vivo*) or *in vitro*. The $WOR1_{promoter}$-*FLP* strain (SN1020) was propagated for 3 d in the mouse model or for eight generations in liquid culture medium, and 5-FOA selection and PCR of 5-FOA–resistant colonies were performed to verify Flp-mediated deletion of *URA3*. Mean percentages of cells meeting these criteria are plotted on the *y* axis. Error bars reflect s.d. from six (*in vivo*) and four (*in vitro*) biological replicates.

To determine whether overexpression of *WOR1* in *MTL***a**/α cells would confer a gain-of-function phenotype, we constructed a strain (*WOR1*^{OE}) in which a single copy of *WOR1* is driven by the strong, constitutively expressed *TDH3* promoter (**Supplementary Fig. 3a–c**). *WOR1*^{OE} cells exhibited a transient competitive deficit in the mouse commensal model (day 5), followed by the predicted competitive advantage over wild-type cells (days 10–25) (**Fig. 2a**).

Inspection of colonies recovered from animals showed an unexpected consequence of *WOR1* overexpression. *C. albicans* usually exist in the white phase, characterized by round-to-oval yeast cell morphology and white, domed colonies[17]. Whereas white-phase strains were used to infect the commensal model, yeast cells recovered 10 d later produced two types of colonies (**Fig. 2b**). One colony type was white, similar to colonies generated by the infecting strains (**Fig. 2b**), but the second colony type was dark and flattened, with an appearance resembling that of sexually competent opaque colonies[18]. Using light microscopy, cells from white colonies were observed to be round to oval in shape (**Fig. 2c**), but cells from dark colonies were found to be elongated with prominent vacuoles (**Fig. 2d**), a morphology resembling



that of opaque cells. Unlike cells from true opaque colonies, however, cells from both colony morphotypes generally retained heterozygosity at *MTL* (**Supplementary Table 1**). An analysis of colony morphology and strain identity over the experimental time course (**Fig. 2e**) showed that dark colonies were formed exclusively by *WOR1*^{OE} cells and that, once apparent, these colonies rapidly dominated the population.

These results established that *WOR1* overexpression in the mammalian gut produces two profound changes in *C. albicans* biology. First, *WOR1*^{OE} cells acquire enhanced commensal fitness, such that they outcompete wild-type cells in mixed infections. Second, although the *WOR1*^{OE} strain remains white indefinitely when propagated strictly *in vitro*, exposure to the mammalian digestive tract triggers a heritable switch to a dark phenotype. We hypothesized that this phenotype might signify a larger program of changes that are adaptive in the gut and that fixed expression of *WOR1* somehow stabilizes the program, even after host cues are removed. We tested the first part of this hypothesis by performing competitive infections with wild-type *C. albicans* and *WOR1*^{OE} yeast cells recovered from dark colonies. Dark-phase *WOR1*^{OE} cells were almost immediately hypercompetitive (**Supplementary Fig. 4a**), unlike the original white-phase strain (**Fig. 2a**). The specificity of this fitness advantage was determined by competing dark and white cells in two other systems. Notably, the fitness of dark cells was significantly attenuated in both liquid culture medium (**Supplementary Fig. 4b**) and in a mouse bloodstream model of virulence (**Supplementary Fig. 4c**). These results support a specific role in commensalism for the dark-phase developmental program, hereafter termed GUT for 'gastrointestinally induced transition'.

We explored the relationship between *MTL***a**/α GUT cells and morphologically similar *MTL***a** opaque cells by assaying each cell type for the characteristics of the other. Apart from loss of heterozygosity at *MTL*, opaque cells are characterized by (i) heat sensitivity, such that they rapidly convert to white cells at temperatures >25 °C; (ii) cell surface 'pimple' structures, detectable by scanning electron microscopy; (iii) production of mating filaments in response to

Figure 2 Wor1 promotes a white-GUT transition that confers enhanced fitness in the mammalian gastrointestinal tract. (**a**) Competition between wild-type (SN425) and *WOR1*^{OE} (SN928) *MTL***a**/α strains in the mouse commensal model ($n = 10$ mice). The *t* test (two-tailed, unpaired samples) was used to determine significance. $^{**}P < 0.001$; $^{***}P < 0.0001$; NS, not significant. Horizontal black bars denote the median value for each group. A replicate of this experiment yielded similar results (data not shown). (**b**) Appearance of distinct colony morphologies after recovery from the mouse. W signifies colonies with typical white morphology. Colonies with GUT morphology are unmarked. (**c**,**d**) Appearance in light microscopy of cells recovered from white (**c**) and GUT (**d**) colonies. Scale bars, 20 μm. (**e**) Analysis of colonies recovered from the wild-type versus *WOR1*^{OE} commensal competition experiment. White (W) versus GUT (G) phenotypes were visually assessed, and strain identities were determined (wild type versus *WOR1*^{OE}) by colony PCR.

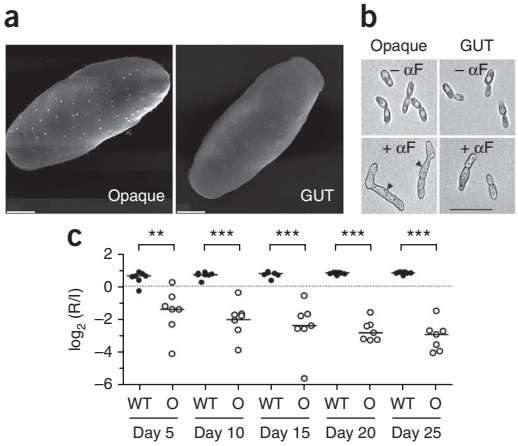
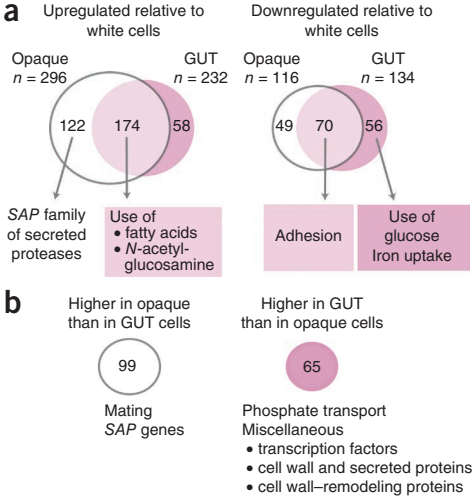


Figure 3 GUT cells are distinct from previously identified opaque cells. (**a**) Scanning electron micrographs showing pimples on the surface of opaque (SN967) but not GUT (SN1045) cells. Scale bars, 1 μm. (**b**) Opaque but not GUT cells form mating filaments in response to mating pheromone (αF). Arrowheads indicate mating projections. Scale bar, 20 μm; all images were obtained at the same magnification. (**c**) Opaque cells (SN967) are significantly outcompeted by wild-type (SN425) cells in the mouse commensal model ($n = 7$ mice). $^{**}P < 0.005$, $^{***}P < 0.001$ by t test. Horizontal black bars denote the median value for each group. A replicate of this experiment yielded similar results (data not shown).

mating pheromone; (iv) capacity for mating with a partner of the opposite sex; and (v) an opaque-specific transcriptome, defined by comparison with the transcriptome of white-phase cells[19]. The first indication that *WOR1*^{OE} GUT cells are not the same as opaque cells came from observations of their stability when incubated at 30 °C (the temperature at which they were initially recovered) and at 37 °C (**Supplementary Fig. 5a**). Visualization of GUT cells by scanning electron microscopy identified few if any pimple structures (**Fig. 3a**; see **Supplementary Fig. 5b** for images of white-phase controls), and GUT cells proved unresponsive to mating pheromone (**Fig. 3b**; see **Supplementary Fig. 5c** for responses by white-phase controls). Similarly, quantitative mating assays showed that GUT cells mated with approximately 2 million–fold lower efficiency than opaque cells ($<3 \times 10^{-7}$ versus 8.0×10^{-1}, respectively, after 5 d; **Supplementary Table 2**). Using these criteria, GUT cells lack the defining characteristics of opaque cells.

The major characteristic of GUT cells, identified in this study, is enhanced fitness in the mammalian gastrointestinal tract. We therefore tested opaque cells in the same commensal model, using the same white-phase competitor as in previous tests of GUT cells (**Fig. 2a** and **Supplementary Fig. 4a**). The opaque and white strains were isogenic, apart from allelism at *MTL*. Opaque cells were severely attenuated for commensalism (**Fig. 3c**). Taken together with the inability of GUT cells to exhibit the characteristics of opaque cells, the inability of opaque cells to establish robust commensal infections indicates that the two cell types are distinct.

The mammalian gastrointestinal tract differs from the bloodstream and from standard *in vitro* conditions in well-described nutritional and physical parameters[11,20]. We hypothesized that an analysis of the transcriptome in GUT cells, in light of these differences, might yield insights into the functional specializations of these cells. GUT cells, opaque cells and isogenic white cells were propagated in glucose-containing medium, were maintained at room temperature to preserve the phenotype of opaque cells and were profiled using custom *C. albicans* ORF microarrays. The results are schematized in **Figure 4a**,



with the full data set and results for genes of interest appearing in **Supplementary Tables 3** and **4**, respectively. Compared to white-phase controls, both GUT and opaque cells demonstrated 2-fold or greater upregulation of a common set of 174 genes. These genes were enriched for gene ontology (GO) terms associated with the catabolism of fatty acids ($P = 1.11 \times 10^{-9}$) and *N*-acetylglucosamine ($P = 5.69 \times 10^{-3}$) compared to the genome as a whole. Opaque cells but not GUT cells also upregulated six members of the *SAP* gene family, which encode secreted aspartylproteases with previously demonstrated roles in nutrient acquisition[21] and virulence[22]. GUT and opaque cells downregulated a common set of 70 genes, including 6 genes associated with biological adhesion ($P = 3.95 \times 10^{-2}$). Notably, GUT cells but not opaque cells also downregulated genes associated with iron acquisition ($P = 4.96 \times 10^{-5}$) and glucose catabolism ($P = 7.40 \times 10^{-4}$).

Consistent with our prediction, GUT cells exhibited a significant reorientation of cellular metabolism toward the nutrients available in the distal mammalian gastrointestinal tract. Because mammals digest and absorb dietary starch and oligosaccharides in the proximal small intestine[23], glucose is relatively depleted more distally. The large intestine is instead replete with short-chain fatty acids, which are produced by microbial fermentation of indigestible carbohydrates[23], and *N*-acetylglucosamine, which is a component of host mucin[24] and bacterial peptidoglycan[25]. Iron is abundant throughout the gastrointestinal tract[26] but is predicted to be more bioavailable in the anaerobic atmosphere of the large intestine, where microbial limitation of iron uptake may defend against iron-related toxicity[11,27]. This optimization of GUT cell metabolism for the conditions encountered in the mammalian digestive tract may contribute to its success as a commensal organism, although additional factors are likely important, given that opaque cells, which are attenuated for commensalism (**Fig. 3c**), share several of these metabolic features (**Fig. 4a** and ref. 28).

Direct comparison of the transcriptomes of GUT and opaque cells highlights key differences between the two cell types. In addition to higher expression of secreted aspartylproteases, opaque cells also have

Figure 4 GUT and opaque cells exhibit overlapping but distinct patterns of gene expression. RNA from *WOR1*^{OE} *MTL***a**/α GUT cells (SN1045; $n = 4$ biological replicates), *MTL***a** opaque cells (SN967; $n = 2$ biological replicates) and isogenic white-phase controls ($n = 2$–4 biological replicates) was profiled using custom Agilent *C. albicans* ORF microarrays and a pooled reference. Significant changes in gene expression were defined as ones with BAGEL P value < 0.05 after implementation of a Bonferroni correction for the comparison of multiple variables. (**a**) Venn diagram of genes that are significantly upregulated or downregulated in both opaque and GUT cells relative to white-phase cells. (**b**) Genes that are differentially expressed by GUT versus opaque cells.

significantly higher expression of genes required for mating (*STE2*, *STE4*, *STE18* and *CAG1*; **Fig. 4b** and **Supplementary Table 4**). Conversely, GUT cells express higher levels of genes for phosphate uptake ($P = 3.00 \times 10^{-2}$), as well as of certain predicted transcription factors, cell surface or secreted proteins, and cell wall–remodeling enzymes (**Supplementary Table 4**) that could potentially modulate cell identity.

Our results indicate that the ability of a commensal organism to produce disease is not merely a consequence of impaired host immunity. In the case of *C. albicans*, we propose that a wholesale change of cell identity underlies its transition from commensal to pathogen. Pathogenic *C. albicans* consist of white-phase budding yeast cells, pseudohyphae and hyphae[29]. On the basis of the results describe here, we propose that cues from the mammalian gastrointestinal tract trigger *WOR1* expression and the white-GUT switch in at least a fraction of host-associated yeast cells (**Supplementary Fig. 6**). GUT-phase *MTL***a**/α cells thrive in the gastrointestinal tract because of metabolic adaptations to the locally available nutrients, as well as because of other, undefined specializations. Continuous exposure to gastrointestinal tract–specific signals is probably important to maintain the GUT state, as wild-type cells are recovered in the white phase after exit from the host. Such signals may include carbon dioxide and *N*-acetylglucosamine, which have very recently been reported to trigger morphological elongation and *WOR1* RNA accumulation in a subset of *MTL***a**/α clinical isolates[30]. Yet, our recovery from animals of *WOR1*^{OE} isolates that retain the GUT phenotype over generations of growth in the laboratory suggests that *WOR1* expression is sufficient to maintain the phenotype. It is also possible that, under particular conditions, GUT cells naturally give rise to sexually competent opaque cells via loss of one allele of *MTL*; however, the observation that 95% of clinical isolates remain heterozygous at *MTL*[31,32] argues that this loss may be rare in humans. In conclusion, we describe a new cell type, programmed by Wor1, that drives the commensal lifestyle of *C. albicans*. The identification of specialized states for *C. albicans* commensalism and virulence offers opportunities for the prevention as well as the treatment of clinical diseases produced by this important human pathogen.

URL. *Candida* Genome Database, http://www.candidagenome.org/.

METHODS

Methods and any associated references are available in the online version of the paper.

Accession code. Transcriptional profiling data are available at the Gene Expression Omnibus (GEO) under accession GSE43972.

Note: Any Supplementary Information and Source Data files are available in the online version of the paper.

ACKNOWLEDGMENTS

We are grateful to R. Bennett for providing protocols for *C. albicans* scanning electron microscopy and response to mating pheromone and to A. Johnson (University of California at San Francisco) for strains and antibodies to α-Wor1. We thank S. Beyhan and M. Voorhies for guidance with BAGEL software. M. Mwangi assisted with the preparation of images of GUT and opaque cells and with colony PCR. J. Cox, H. Madhani, Q. Mitrovich and A. Sil provided helpful comments on the manuscript. This work was supported by US National Institutes of Health (NIH) grant R21AI099659-01, a University of California at San Francisco (UCSF) Program for Breakthrough Biomedical Research award, a Burroughs Wellcome Fund CABS (Career Awards in the Biomedical Sciences) award and a Pew Foundation scholarship.

AUTHOR CONTRIBUTIONS

K.P. identified *C. albicans* mutants with altered commensal fitness, characterized the white-GUT switch and analyzed mating and pheromone response. C.C. performed strain construction, expression profiling and scanning electron microscopy. S.M.N. oversaw the work and wrote the manuscript.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

1. Blackwell, M. The fungi: 1, 2, 3 … 5.1 million species? *Am. J. Bot.* **98**, 426–438 (2011).
2. Odds, F.C. *Candida and Candidosis, a Review and Bibliography* (W.B. Saunders, London, 1988).
3. Casadevall, A. & Pirofski, L.A. Accidental virulence, cryptic pathogenesis, martians, lost hosts, and the pathogenicity of environmental microbes. *Eukaryot. Cell* **6**, 2169–2174 (2007).
4. Huang, G. *et al.* Bistable expression of *WOR1*, a master regulator of white-opaque switching in *Candida albicans*. *Proc. Natl. Acad. Sci. USA* **103**, 12813–12818 (2006).
5. Srikantha, T. *et al. TOS9* regulates white-opaque switching in *Candida albicans*. *Eukaryot. Cell* **5**, 1674–1687 (2006).
6. Zordan, R.E., Galgoczy, D.J. & Johnson, A.D. Epigenetic properties of white-opaque switching in *Candida albicans* are based on a self-sustaining transcriptional feedback loop. *Proc. Natl. Acad. Sci. USA* **103**, 12807–12812 (2006).
7. Miller, M.G. & Johnson, A.D. White-opaque switching in *Candida albicans* is controlled by mating-type locus homeodomain proteins and allows efficient mating. *Cell* **110**, 293–302 (2002).
8. Edmond, M.B. *et al.* Nosocomial bloodstream infections in United States hospitals: a three-year analysis. *Clin. Infect. Dis.* **29**, 239–244 (1999).
9. Zaoutis, T.E. *et al.* The epidemiology and attributable outcomes of candidemia in adults and children hospitalized in the United States: a propensity analysis. *Clin. Infect. Dis.* **41**, 1232–1239 (2005).
10. Odds, F.C. *et al. Candida albicans* strain maintenance, replacement, and microvariation demonstrated by multilocus sequence typing. *J. Clin. Microbiol.* **44**, 3647–3658 (2006).
11. Chen, C., Pande, K., French, S.D., Tuch, B.B. & Noble, S.M. An iron homeostasis regulatory circuit with reciprocal roles in *Candida albicans* commensalism and pathogenesis. *Cell Host Microbe* **10**, 118–135 (2011).
12. Pierce, J.V. & Kumamoto, C.A. Variation in *Candida albicans EFG1* expression enables host-dependent changes in colonizing fungal populations. *MBio* **3**, e00117–12 (2012).
13. Zordan, R.E., Miller, M.G., Galgoczy, D.J., Tuch, B.B. & Johnson, A.D. Interlocking transcriptional feedback loops control white-opaque switching in *Candida albicans*. *PLoS Biol.* **5**, e256 (2007).
14. Noble, S.M. & Johnson, A.D. Strains and strategies for large-scale gene deletion studies of the diploid human fungal pathogen *Candida albicans*. *Eukaryot. Cell* **4**, 298–309 (2005).
15. Camilli, A., Beattie, D.T. & Mekalanos, J.J. Use of genetic recombination as a reporter of gene expression. *Proc. Natl. Acad. Sci. USA* **91**, 2634–2638 (1994).
16. Staib, P. *et al.* Host-induced, stage-specific virulence gene activation in *Candida albicans* during infection. *Mol. Microbiol.* **32**, 533–546 (1999).
17. Buchholz, F., Ringrose, L., Angrand, P.O., Rossi, F. & Stewart, A.F. Different thermostabilities of FLP and Cre recombinases: implications for applied site-specific recombination. *Nucleic Acids Res.* **24**, 4256–4262 (1996).
18. Slutsky, B. *et al.* "White-opaque transition": a second high-frequency switching system in *Candida albicans*. *J. Bacteriol.* **169**, 189–197 (1987).
19. Bennett, R.J. & Johnson, A.D. Mating in *Candida albicans* and the search for a sexual cycle. *Annu. Rev. Microbiol.* **59**, 233–255 (2005).
20. Wilson, M. The gastrointestinal tract and its indigenous microbiota. in *Microbial Inhabitants of Humans* 251–317 (University of Cambridge Press, Cambridge, 2005).
21. Naglik, J.R., Challacombe, S.J. & Hube, B. *Candida albicans* secreted aspartyl proteinases in virulence and pathogenesis. *Microbiol. Mol. Biol. Rev.* **67**, 400–428 (2003).
22. Jackson, B.E., Wilhelmus, K.R. & Hube, B. The role of secreted aspartyl proteinases in *Candida albicans* keratitis. *Invest. Ophthalmol. Vis. Sci.* **48**, 3559–3565 (2007).
23. Wong, J.M. & Jenkins, D.J. Carbohydrate digestibility and metabolic effects. *J. Nutr.* **137**, 2539S–2546S (2007).
24. Goodman, M.J., Kent, P.W. & Truelove, S.C. Glucosamine synthetase activity of the colonic mucosa in ulcerative colitis and Crohn's disease. *Gut* **18**, 219–228 (1977).
25. Hill, D.A. & Artis, D. Intestinal bacteria and the regulation of immune cell homeostasis. *Annu. Rev. Immunol.* **28**, 623–667 (2010).
26. Miret, S., Simpson, R.J. & McKie, A.T. Physiology and molecular biology of dietary iron absorption. *Annu. Rev. Nutr.* **23**, 283–301 (2003).
27. Pierre, J.L., Fontecave, M. & Crichton, R.R. Chemistry for an essential biological process: the reduction of ferric iron. *Biometals* **15**, 341–346 (2002).
28. Lan, C.Y. *et al.* Metabolic specialization associated with phenotypic switching in *Candida albicans*. *Proc. Natl. Acad. Sci. USA* **99**, 14907–14912 (2002).
29. Lockhart, S.R., Wu, W., Radke, J.B., Zhao, R. & Soll, D.R. Increased virulence and competitive advantage of **a**/α over **a**/**a** or α/α offspring conserves the mating system of *Candida albicans*. *Genetics* **169**, 1883–1890 (2005).
30. Xie, J. *et al.* White-opaque switching in natural *MTL***a**/α isolates of *Candida albicans*: evolutionary implications for roles in host adaptation, pathogenesis, and sex. *PLoS Biol.* **11**, e1001525 (2013).
31. Legrand, M. *et al.* Homozygosity at the *MTL* locus in clinical strains of *Candida albicans*: karyotypic rearrangements and tetraploid formation. *Mol. Microbiol.* **52**, 1451–1462 (2004).
32. Lockhart, S.R. *et al.* In *Candida albicans*, white-opaque switchers are homozygous for mating type. *Genetics* **162**, 737–745 (2002).

ONLINE METHODS

Strain construction. All strains used in this study are derivatives of the clinical isolate SC5314 (ref. 33). Strains are listed in **Supplementary Table 5**, plasmids are listed in **Supplementary Table 6** and primers are listed in **Supplementary Table 7**. The *wor1*ΔΔ mutant (SN881) was constructed by fusion PCR as previously described[14].

The *WOR1*^{OE}/*wor1*Δ strain (SN928) used for animal experiments was created in two steps. PCR and homologous recombination in *Saccharomyces cerevisiae*[34] were used to engineer a plasmid (pSN209) containing, from 5′ to 3′, a PmeI restriction site, a *WOR1* promoter fragment ending ~300 bp upstream of the ORF, *SAT1* (nourseothricin resistance gene), the *C. albicans TDH3* promoter, the first ~300 bp of the *WOR1* ORF and a PmeI restriction site. PmeI-digested pSN209 was transformed into Arg$^-$ reference strain SN250 to generate *WOR1*^{OE}/*WOR1*. The wild-type allele of *WOR1* was next disrupted using *Candida dubliniensis ARG4* as described[14]. In these and subsequent strains, colony PCR was used to verify the 5′ and 3′ junctions of DNA integration events.

The *MTL***a**/**a** opaque strain (SN967) used for transcriptome analysis was kindly provided by the laboratory of A. Johnson[35]. The white version of this strain (SN966) was isolated after incubation of freshly plated opaque cells at 37 °C. The His$^-$Arg$^+$ *MTL***a** opaque strain (SN1038) used for mating assays was created by replacing ~9.2 kb of *MTL*α in reference strain SN152 with *C. dubliniensis ARG4*, and colonies were visually inspected for opaque sectors. The His$^+$Arg$^-$ *MTL*α opaque strain (SN1008) was constructed by replacing ~10 kb of the *MTL***a** locus with *C. dubliniensis HIS1*.

The His$^+$Arg$^-$ *WOR1*^{OE}/*wor1*Δ white strain (SN1001) was constructed via transformation of PmeI-digested pSN209 into SN999 (His$^+$Arg$^-$ *WOR1*/*wor1*Δ). The corresponding GUT-phase isolate (SN1046) was obtained after passage of SN1001 through the mouse commensal model and visual inspection of colonies for the GUT phenotype.

The *WOR1*$_{promoter}$-*FLP* (SN1020) strain was created in two steps. To exchange *FLP* for the *WOR1* ORF, plasmid pSN288 was engineered to contain, from 5′ to 3′, a PmeI restriction site, the terminal ~500 bp of the *WOR1* promoter, the *FLP* ORF, *SAT1*, ~500 bp of *WOR1* downstream sequence and a PmeI restriction site. The source of *FLP* and *SAT1* was pSFS2A[36]. PmeI-digested pSN288 was transformed into Ura$^-$ reference strain SN78 to create SN1013. To introduce a copy of *URA3* flanked by FRT recombination sites into the *LEU2* locus, plasmid pSN290 was engineered to contain, from 5′ to 3′, a PmeI restriction site, the terminal ~440 bp of the *LEU2* promoter, FRT, *URA3*, FRT, ~550 bp of *LEU2* downstream sequence and a PmeI restriction site. Finally, PmeI-digested pSN290 was transformed into SN1013.

Competitive infections. All procedures involving animals were approved by the University of California at San Francisco Institutional Animal Care and Use Committee, which enforces the ethical and humane use of animals. Each experiment used the (estimated) minimum number of animals required for the detection of a significant biological effect. Blinding and randomization were not necessary because, for a given competition experiment, pooled *C. albicans* strains were assessed in the same animals. In commensalism experiments, 6- to 10-week-old female BALB/c mice were infected by gavage with a 1:1 mixture of competing *C. albicans* strains as previously reported[11]. Relative abundances of strains in the infecting inoculum and after recovery from mouse fecal pellets were determined by quantitative PCR[11] using strain-specific primers (**Supplementary Table 7**). In virulence experiments, 6- to 10-week-old female BALB/c mice were infected via the lateral tail vein as described[11]. Relative abundances of strains in the infecting inoculum and after recovery from mouse kidneys were determined by quantitative PCR using the same strain-specific primers. The results of competition experiments have also been plotted as the percent abundance of the less fit strain in **Supplementary Figure 7**.

Estimation of *WOR1* expression by *MTL*a/α cells. For *in vitro* experiments, four independent cultures of the *WOR1*$_{promoter}$-*FLP* strain (SN1020) were diluted to an optical density at 600 nm (OD_{600}) of 0.1 in liquid SC medium[37] and were incubated with rolling at 37 °C. To maintain cells in mid-log phase, cultures were diluted 1:20 with prewarmed SC medium when they reached OD_{600} of 2–3. After ~8 doublings, aliquots of cells were plated on nonselective Sabouraud medium (Difco) and on 5-FOA–containing medium[37] (with 25 μg/ml uridine), which selects for uracil auxotrophs. 5-FOA–resistant colonies were further analyzed by colony PCR to determine their genotype at *MTL* and to verify Flp-mediated deletion of *URA3*. In the latter assay (depicted in **Supplementary Fig. 2**), primers SNO509 and SNO840 were used to amplify a 346-bp PCR product from strains that had undergone Flp-mediated recombination. The frequency of cells expressing *WOR1* was estimated as the ratio of 5-FOA–resistant, PCR-positive colonies to the total number of colonies. For *in vivo* experiments, SN1020 was used in commensal infections of six BALB/c mice. After 3 d, *C. albicans* was recovered from fecal pellets and analyzed as above.

RNA extraction and quantitative RT-PCR analysis. Total RNA was prepared using a hot-phenol method[7] and treated with DNase I using the Turbo DNA-free kit (Ambion). RNA (10 μg) was used in standard reverse transcriptase reactions using oligo$(dT)_{20}$ primers. cDNA was quantified by quantitative PCR with the primers listed in **Supplementary Table 7**, and expression levels were normalized against those of *ACT1*.

Protein extraction and immunoblotting. *C. albicans* protein extracts were prepared as described[11]. Lysates corresponding to cells with OD_{600} of 1 were analyzed by SDS-PAGE and immunoblotted using polyclonal antibodies to Wor1 (kindly provided by the laboratory of A. Johnson; 1:10,000 dilution) and antibody to α-tubulin (Novus Biologicals, NB100-1639; 1:1,000 dilution).

Expression profiling. Saturated overnight cultures of wild-type *MTL***a**/α white cells (SN425), *MTL***a** white cells (SN966), *MTL***a** opaque cells (SN967), *WOR1*^{OE} *MTL***a**/α white cells (SN1044) and *WOR1*^{OE} *MTL***a**/α GUT cells (SN1045) were inoculated into SC medium[37] (with 100 μg/ml uridine) at OD_{600} of 0.1 and incubated with shaking at room temperature for 6–8 h before harvesting at OD_{600} of 0.8–1.0. Two to four biological replicates were performed per strain. RNA isolation, cDNA labeling and hybridization to custom Agilent *C. albicans* ORF microarrays were performed as described[11].

Equal amounts of cDNA from each sample were pooled to prepare a mixed reference. Cy5-labeled cDNA from each strain was directly hybridized against the pooled Cy3-labeled reference. Arrays were scanned using a GenePix 4000A Axon scanner, and spots were filtered using GenePix Pro software. Data were normalized using Goulphar[38] (LOWESS normalization) and subjected to Bayesian Analysis of Gene Expression Levels (BAGEL)[39]. Significant changes in expression were defined as ones with BAGEL P value < 0.05 after implementing a correction for the comparison of multiple variables (multiplication by the number of ORFs on the microarray; n = 6,168).

Mating filament assays. Saturated overnight cultures of *MTL***a** opaque (SN967), *MTL***a** white (SN966), *WOR1*^{OE} *MTL***a**/α white (SN1044) and *WOR1*^{OE} *MTL***a**/α GUT (SN1045) cells were tested as described[40].

Quantitative mating assays. SN1038 (His$^-$Arg$^+$ *MTL***a**; opaque) was used as a common partner for the following His$^+$Arg$^-$ strains: SN1008 (*MTL*α; opaque), SN235 (*MTL***a**/α; white), SN1001 (*WOR1*^{OE} *MTL***a**/α; white) and SN1046 (*WOR1*^{OE} *MTL***a**/α; GUT). Mating assays were performed as described[7], except that mating mixtures were spotted onto sterile filter paper rather than using a vacuum manifold.

Scanning electron microscopy. Wild-type *MTL***a**/α white cells (SN425), *MTL***a** opaque cells (SN967), *WOR1*^{OE} *MTL***a**/α white cells (SN928) and *WOR1*^{OE} *MTL***a**/α GUT cells (SN1045) were plated from frozen glycerol stocks onto SC medium and incubated for 48 h at room temperature. Cells were applied to poly-L-lysine–coated silicon wafers and fixed overnight at 4 °C with 2.5% gluteraldehyde in 0.1 M sodium cacodylate, pH 7.4. Washed cells were post-fixed in the dark in 1% aqueous osmium tetroxide in 0.1 M sodium cacodylate, pH 7.4, for 90 min, progressively dehydrated in ethanol (33–66% to 95–100%) and dried in a Tousimis autoSamdri 815 critical-point dryer. Samples were mounted on stubs and coated with gold/palladium alloy in a Tousimis sputter coater before scanning using a Hitachi S-5000 scanning electron microscope.

doi:10.1038/ng.2710

Statistical analysis. We used *t* tests (two-tailed, comparison of unpaired samples) for the evaluation of competitive fitness, with significance defined as $P < 0.05$. We used BAGEL[39] to analyze *C. albicans* transcriptomes. The Bonferroni correction for multiple-hypothesis testing (multiplication by 6,168 or the total number of ORFs) was applied, and significance was defined as $P < 0.05$. The GO Term Finder tool on the *Candida* Genome Database website[41] was used to identify functional groups with altered gene expression. This algorithm uses a hypergeometric distribution with Bonferroni correction; significance was defined as $P < 0.05$.

33. Gillum, A.M., Tsay, E.Y. & Kirsch, D.R. Isolation of the *Candida albicans* gene for orotidine-5′-phosphate decarboxylase by complementation of *S. cerevisiae ura3* and *E. coli pyrF* mutations. *Mol. Gen. Genet.* **198**, 179–182 (1984).
34. Oldenburg, K.R., Vo, K.T., Michaelis, S. & Paddon, C. Recombination-mediated PCR-directed plasmid construction *in vivo* in yeast. *Nucleic Acids Res.* **25**, 451–452 (1997).
35. Mitrovich, Q.M., Tuch, B.B., Guthrie, C. & Johnson, A.D. Computational and experimental approaches double the number of known introns in the pathogenic yeast *Candida albicans*. *Genome Res.* **17**, 492–502 (2007).
36. Reuss, O., Vik, A., Kolter, R. & Morschhauser, J. The SAT1 flipper, an optimized tool for gene disruption in *Candida albicans*. *Gene* **341**, 119–127 (2004).
37. Guthrie, C. & Fink, C.G. *Guide to Yeast Genetics and Molecular and Cell Biology* (Academic Press, San Diego, 2002).
38. Lemoine, S., Combes, F., Servant, N. & Le Crom, S. Goulphar: rapid access and expertise for standard two-color microarray normalization methods. *BMC Bioinformatics* **7**, 467 (2006).
39. Townsend, J.P. & Hartl, D.L. Bayesian analysis of gene expression levels: statistical quantification of relative mRNA level across multiple strains or treatments. *Genome Biol.* **3**, RESEARCH0071 (2002).
40. Lin, C.H., Choi, A. & Bennett, R.J. Defining pheromone-receptor signaling in *Candida albicans* and related asexual *Candida* species. *Mol. Biol. Cell* **22**, 4918–4930 (2011).
41. Inglis, D.O. *et al.* The *Candida* genome database incorporates multiple *Candida* species: multispecies search and analysis tools with curated gene and protein information for *Candida albicans* and *Candida glabrata*. *Nucleic Acids Res.* **40**, D667–D674 (2012).