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

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Among ~5,000,000 fungal species1, *C. albicans* is exceptional in its lifelong association with humans, either within the gastrointestinal microbiome or as an invasive pathogen2. Opportunistic infections are generally ascribed to defective host immunity3 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 backgrounds4–6, where it controls a white-opaque switch in mating4–7.

We show that passage of wild-type cells through the mouse gastrointestinal tract triggers Wor1 expression and a novel phenotypic switch. The resulting Gut/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 disease8,9, but its more typical role is as a permanent resident of the healthy gastrointestinal microbiome2. Longitudinal molecular typing studies indicate that disseminated *C. albicans* infections originate from individuals’ own commensal strains10, and the transition to virulence is generally thought to reflect impaired host immunity3. 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 yeast11. 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 elsewhere12.

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 competency4–6 (Fig. 1b). We asked whether Wor1 also regulates commensalism by testing a wor1ΔΔ deletion mutant in the same mouse model. Indeed, wor1ΔΔ 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 wor1ΔΔ mutants (Supplementary Fig. 1a,b), confirming genetic linkage between Wor1 and fitness. Moreover, the substantial defect in commensalism of a heterozygous knockout mutant (wor1Δ/wor1Δ; Supplementary Fig. 1c) indicates that Wor1 dosage is also key. Notably, a his1ΔΔ, leu2ΔΔ 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 virulence14.

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 vitro4–9. Under laboratory conditions, Wor1 is repressed in most isolates of this diploid species by a potent transcriptional repressor, a1–a2, whose subunits are encoded by the distinct a and α alleles, respectively, of the MTL mating type–like locus4–6. Therefore, only strains that had undergone loss of either MTLa or MTLa were considered capable of expressing Wor1 (refs. 4–6). However, our analysis was performed with MTLa/α strains.

We hypothesized that signals present in the mammalian gastrointestinal tract might elicit Wor1 expression in MTLa/α cells. To test this idea, we used a technique that permanently marks cells when a promoter is activated in vivo15,16 (Supplementary Fig. 2a). We created a Wor1promoter–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 that Wor1 expression in MTLa/α 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 temperature17. Nevertheless, comparison of the rates suggests that propagation of MTLa/α cells in the mammalian gastrointestinal tract increases the probability of Wor1 expression by roughly 10,000-fold.

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To determine whether overexpression of WOR1 in MTLα/α cells would confer a gain-of-function phenotype, we constructed a strain (WOR1OE) in which a single copy of WOR1 is driven by the strong, constitutively expressed TDK3 promoter (Supplementary Fig. 3a–c). WOR1OE cells exhibited a transient competitive deficit in the mouse commensal model (day 3), 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. 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. 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 WOR1OE 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, WOR1OE cells acquire enhanced commensal fitness, such that they outcompete wild-type cells in mixed infections. Second, although the WOR1OE 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 WOR1OE yeast cells recovered from dark colonies. Dark-phase WOR1OE 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 ‘gastrointestinal induced transition’.

We explored the relationship between MTLα/α GUT cells and morphologically similar MTLα opaque cells by assessing 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
A replicate of this experiment yielded similar results (data not shown).

Opaque (SN967) but not GUT (SN1045) cells 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. The first indication that WOR1OE 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 × 10⁻³ 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 characteristic features 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. 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 × 10⁻⁷) and N-acetylglucosamine (P = 5.69 × 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 and virulence. GUT and opaque cells downregulated a common set of 70 genes, including 6 genes associated with biological adhesion (P = 3.95 × 10⁻²). Notably, GUT cells but not opaque cells also downregulated genes associated with iron acquisition (P = 4.96 × 10⁻⁵) and glucose catabolism (P = 7.40 × 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, 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 and N-acetylglucosamine, which is a component of host mucin and bacterial peptidoglycan. Iron is abundant throughout the gastrointestinal tract 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. 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.

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...
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 pathogenic. Pathogenic \(C.\) albicans consist of white-phage budding yeast cells, pseudohyphae and hyphae. On the basis of the results described 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 \(MTLA/\alpha\) 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 \(MTLA/\alpha\) clinical isolates. Yet, our recovery from animals of WOR1\(^{\text{D}}\) 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 \(MTL\)\(^{\text{31,32}}\) 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.

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AUTHOR CONTRIBUTIONS

K.P. identified \(C.\) albicans mutants with altered communal fitness, characterized the white-GUT switch and analyzed mating and pheromone response. C.G. 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.

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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 worΔα mutant (SN881) was constructed by fusion PCR as previously described\(^4\).

The WOR\(^{1OE}\)/worΔα strain (SN928) used for animal experiments was created in two steps. PCR and homologous recombination in Saccharomyces cerevisiae\(^\text{34}\) were used to engineer a plasmid (pSN209) containing, from 5’ to 3’, a Pmel restriction site, a WOR1 promoter fragment ending –300 bp upstream of the ORF, SAT1 (nourseothricin resistance gene), the C. albicans TDI3 promoter, the first ~300 bp of the WOR1 ORF and a Pmel restriction site. Pmel-digested pSN209 was transformed into SN250 to generate WOR\(^{1OE}/\text{WOR1}\). The wild-type allele of WOR1 was next disrupted using Candida dubliniensis ARG4 as described\(^4\). In these and subsequent strains, colony PCR was used to verify the 5’ and 3’ junctions of DNA integration events.

The MTL\(^{a}\) opaque strain (SN967) used for transcriptome analysis was kindly provided by the laboratory of A. Johnson\(^\text{35}\). The white version of this strain (SN966) was isolated after incubation of freshly plated opaque cells at 37 °C. The His\(^{\text{a}}\) MTL\(^{a}\) opaque strain (SN1038) used for mating assays was created by replacing ~9.2 kb of C. dubliniensis ARG4 via transformation of Pmel-digested pSN209 into SN999 (His\(^{+}\)Arg\(^{-}\) promoter, the MTL\(^{a}\) gene). The wild-type Arg\(^{+}\) strain (SN1001) was constructed by replacing ~10 kb of the MTL\(^{a}\) locus with C. dubliniensis HIS1.

The His\(^{\text{a}}\) WOR1\(^{1OE}/\text{worΔα}\) white strain (SN1000) was constructed via transformation of Pmel-digested pSN209 into SN999 (His\(^{\text{a}}\)Arg\(^{+}\) WOR1/\text{worΔα}). 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\(_{\text{promoter}}\)/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 Pmel restriction site, the terminal ~500 bp of the WOR1 promoter, the FLP ORF, SAT1, ~500 bp of WOR1 downstream sequence and a Pmel restriction site. The source of FLP and SAT1 was pSF2S2A\(^\text{36}\). Pmel-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 Pmel restriction site, the terminal ~440 bp of the LEU2 promoter, FRT, URA3, FRT, ~550 bp of LEU2 downstream sequence and a Pmel restriction site. Finally, Pmel-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 enforced by the Institutional Animal Care and Use Committee, which enforces the ethical and humane use of animals. Each procedure was approved by the University of California at San Francisco Institutional Animal Care and Use Committee, which enforces the ethical and humane use of animals.

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 °C) with 25 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 array. Arrays were scanned using a GenePix 4000A Axon scanner, and spots were filtered using GenePix Pro software. Data were normalized using Goulphar\(^\text{38}\) (LOWESS normalization) and subjected to Bayesian Analysis of Gene Expression Levels (BAGEL)\(^39\). Significant changes in expression were defined as ones with BAGEL\(^{\text{39}}\) p < 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\(^{a}\)/α 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\(^{\text{a}}\)Arg\(^{+}\)/MTL\(^{a}\)/α opaque) was used as a common partner for the following His\(^{\text{a}}\)Arg\(^{+}\) strains: SN1008 (MTL\(^{a}\)/α opaque), SN235 (MTL\(^{a}\)/μ/α; white), SN1001 (WOR1\(^{OE}\)/MTL\(^{a}\)/α; white) and SN1046 (WOR1\(^{OE}\)/MTL\(^{a}\)/α; GUT). Mating assays were performed as described\(^2\), except that mating sporulating cells 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 polyl-l-lysine–coated silicon wafers and fixed overnight at 4 °C with 2.5% glutaraldehyde 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.
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 $P < 0.05$.

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