Environmental Induction of White–Opaque Switching in *Candida albicans*

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**Abstract**

*Candida albicans* strains that are homozygous at the mating type locus (*MTLa* or *MTLx*) can spontaneously switch at a low frequency from the normal yeast cell morphology (white) to an elongated cell type (opaque), which is the mating-competent form of the fungus. The ability to switch reversibly between these two cell types also contributes to the pathogenicity of *C. albicans*, as white and opaque cells are differently adapted to specific host niches. We found that in strain WO-1, a strain in which genomic alterations have occurred, but not in other tested strains, switching from the white to the opaque phase can also be induced by environmental conditions. Transient incubation of white cells under anaerobic conditions programmed the cells to switch en masse to the opaque phase. The anaerobic induction of white–opaque switching was controlled by the transcription factor *CZF1*, which in heterozygous *MTLa/x* cells regulates filamentous growth under embedded, hypoxic conditions. Intriguingly, passage of white cells of strain WO-1 through the mouse intestine, a host niche in which the cells are likely to be exposed to anaerobic conditions, resulted in a strongly increased frequency of switching to the opaque phase. These results demonstrate that white–opaque switching is not only a spontaneous process but, in combination with genomic alterations, can also be induced by environmental signals, suggesting that switching and mating of *C. albicans* may occur with high efficiency in appropriate niches within its human host.

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**Introduction**

The yeast *Candida albicans* is a member of the microbial flora of the gastrointestinal and urogenital tract in many healthy people, but it can also cause serious infections when host defenses are compromised. In severely immunosuppressed patients, *C. albicans* can disseminate and infect virtually all body locations, indicating that *C. albicans* is a versatile opportunist that can adapt to many different environmental conditions within its host. The great morphological variability of *C. albicans* contributes to its capacity to spread to and establish itself within new host niches [1]. In response to environmental cues, e.g., the presence of serum, an increase in pH and temperature, or starvation conditions, *C. albicans* transitions from the budding yeast form to filamentous growth forms (true hyphae and pseudohyphae). This yeast-hyphal dimorphism, which includes dramatic alterations in the gene expression pattern, is important for the pathogenicity of *C. albicans* and mutants that are locked in the yeast or the filamentous form are attenuated for virulence [2,3].

In addition to the yeast-hyphal transition, *C. albicans* can also switch from the normal, round-to-oval yeast cell morphology (white) to an elongated cell type with an altered surface structure, which has been termed opaque because of the appearance of the colonies produced by these cells on agar plates [4]. It was recently discovered that opaque cells are the mating-competent form of *C. albicans* and only strains that are homozygous at the mating type locus (*MTLa* or *MTLx*) can switch from the white to the opaque form and mate with opaque cells of the opposite mating type [5,6]. The majority of *C. albicans* strains are *MTLa/x* heterozygous and produce the a1–a2 repressor, which is encoded by the two mating type loci and inhibits white-opaque switching. However, these strains can become *MTL* homozygous and switching-competent by loss of one homologue of the *MTL* carrying chromosome 5 and duplication of the remaining homologue or by mitotic recombination [7].

White-opaque switching also contributes to a better adaptation of *C. albicans* to new host niches. While white cells are much more virulent than opaque cells after intravenous infection, opaque cells are better able than white cells to infect skin [8,9]. Switching between the white and opaque phases occurs spontaneously at a relatively low frequency, such that white or opaque cell populations usually contain about 0.1% of cells of the opposite phase. This frequency of switching allows a semi-stable maintenance of the white and opaque phases once they are established and at the same time ensures that some cells in the population are preadapted to altered environmental conditions upon encountering new host niches [10]. Since opaque cells are unstable at 37°C, it is believed that switching from the white to the opaque form may be relevant especially in host niches with lower temperatures, like skin, which also facilitates mating [11].

The molecular basis of the control of white-opaque switching has recently been elucidated [12–14]. In *MTL* heterozygous strains, the a1–a2 repressor inhibits expression of the *WOR1* gene, which encodes a transcription factor that induces switching of white cells to the opaque phase and also upregulates its own expression. White cells of *MTL* homozygous strains express *WOR1* at very low levels, but stochastic expression of *WOR1* above a
encompassed within its host. have major implications for our understanding of the control of the anaerobically induced white-opaque switching also occurred at factor Czf1p and depended on additional genomic alterations. As to the opaque phase, which was mediated by the transcription white cells under anaerobic conditions resulted in mass switching spontaneously at low frequency but can also be induced by explain the stochastic nature of white-opaque switching.

Results

Anaerobic conditions induce white-opaque switching in strain WO-1

In experiments aimed at elucidating the function of phase-specific genes we incubated strain WO-1, the MIT1a strain in which white-opaque switching was originally discovered, and mutants derived from it on Lee’s agar plates at 25°C under anaerobic conditions [see Materials and Methods]. Under these conditions the cells soon ceased to grow and formed only microcolonies consisting of few cells. When the plates were then transferred to aerobic conditions to allow growth and formation of visible colonies, we made the striking observation that virtually all white cells of strain WO-1 had switched to the opaque phase whereas they remained in the white phase when the plates were kept under aerobic conditions without the transient anaerobic incubation (Fig. 1A). Microscopic examination of the cells recovered from the plates directly after the anaerobic incubation showed that they did not exhibit the elongated opaque morphology but formed enlarged round cells (Fig. 1B). However, when these cells were plated on Lee’s agar plates and incubated at room temperature under aerobic conditions, most of them produced opaque colonies consisting of cells with the typical opaque morphology. The opaque cells expressed the opaque-phase-specific OP4 and SAPI genes and had downregulated expression of the white-phase-specific WH11 gene (Fig. 1C, left panels). In contrast, cells that were kept under aerobic conditions exhibited the white cell morphology and did not detectably express the OP4 and SAPI genes, but continued to express the WH11 gene (Fig. 1C, right panels). These results indicated that a transient incubation under anaerobic conditions programs white cells to switch to the opaque phase. In control experiments we tested if the heterogeneous MIT1a/α strain SC5314 would also switch to the opaque phase when incubated under anaerobic conditions. However, no opaque colonies were produced by this strain, suggesting that, as for spontaneous white-opaque switching, MIT1 homozygosity is a prerequisite for the anaerobically induced switching of white cells to the opaque phase.

Anaerobic conditions are likely to be encountered in host niches like the intestinal tract, i.e., at the body temperature of 37°C at which opaque cells are unstable. We therefore tested whether anaerobic conditions would also induce white-opaque switching at 37°C. Unexpectedly, switching of white cells to the opaque phase was even accelerated at 37°C. While at 25°C an anaerobic incubation for 2 days was required to induce most cells to switch to the opaque phase, nearly complete switching was observed already after 24 h of incubation under anaerobic conditions at 37°C. Shorter anaerobic incubation times resulted in little (at 37°C) or no significant (at 25°C) switching (Fig. 1D). The more efficient switching at 37°C presumably does not reflect a specific temperature signal but may rather be due to the increased metabolic activity at the higher temperature.

As the cells could not grow on Lee’s agar plates under anaerobic conditions, we considered the possibility that switching to the opaque phase might have been induced by growth inhibition and not by the anaerobic conditions per se. Therefore, we also incubated white cells in distilled water for 48 h and then plated them on Lee’s medium for colony formation. Under these conditions the cells remained in the white phase and showed only the normal spontaneous switching frequency. As an alternative means to generate anaerobic conditions, we cultured the cells in liquid Lee’s medium treated for 10 min with nitrogen gas to remove the oxygen. After 2 days of incubation at 37°C, about 9% of the cells formed opaque colonies or mixed white/opaque colonies, whereas no opaque colonies were observed when the cells were incubated in the same way in medium not treated with nitrogen. While switching was not as efficiently induced under these conditions as during incubation on solid medium in an anaerobic jar, presumably because treatment of the liquid medium with nitrogen gas did not generate completely anaerobic conditions, these experiments nevertheless confirmed that anaerobic or hypoxic conditions induced switching of strain WO-1 from the white to the opaque phase.

Anaerobic conditions result in ergosterol depletion and accumulation of precursor molecules, as several enzymes in the ergosterol biosynthesis pathway require oxygen for their activity, and yeast cells may sense anaerobic conditions by measuring the levels of sterols and biosynthetic intermediates [15,16]. To investigate whether, similar to anaerobic conditions, inhibition of ergosterol biosynthesis would also induce white-opaque switching, white cells of strain WO-1 were incubated for 2 days at 30°C in

Author Summary

Some strains of the opportunistic fungal pathogen Candida albicans can reversibly switch from the normal yeast morphology (white) to an elongated cell type termed opaque, which is the mating-competent form of C. albicans. C. albicans strains first have to become homozygous at the mating type locus to be able to switch to the usually less virulent opaque phase. As white-opaque switching occurs spontaneously only at a low frequency, the encounter of opaque cells of different strains and mating types is expected to be an extremely rare event and little recombination between strains occurs in nature. We found that genomic alterations may render white-opaque switching environmentally inducible in certain C. albicans strains. A transient incubation of such a strain under anaerobic conditions induced mass switching of white cells to the opaque phase, and this induction was mediated by the transcription factor Czf1p. Most strikingly, passage of white cells through the mammalian intestine, an environment in which the cells encounter anaerobic conditions, also stimulated switching to the opaque phase. Therefore, some strains of C. albicans may be induced to switch to the mating-competent opaque phase in response to environmental signals, giving them an opportunity for efficient genetic exchange in appropriate host niches.
Figure 1. Anaerobic conditions induce white-opaque switching in *C. albicans* strain WO-1. (A) White-phase cells were spread on Lee’s agar plates with phloxine B, incubated for 2 days at 25°C under anaerobic conditions, and then grown for 7 days under aerobic conditions (left) or grown for 7 days under aerobic conditions without a previous anaerobic incubation (right). (B) Microscopic appearance of white-phase cells that had been incubated for 2 days at 25°C on Lee’s agar plates under anaerobic (left) or aerobic conditions (right). Cells in both panels are shown at the same magnification. (C) Expression of the opaque-phase-specific OP4 and SAP1 genes and the white-phase-specific WH11 gene after anaerobic induction of white-opaque switching. Derivatives of strain WO-1 expressing the GFP reporter gene under control of the OP4, SAP1, or WH11 promoter were incubated under anaerobic (left) or aerobic (right panels) conditions as described in (A). Cells from the resulting opaque colonies after the anaerobic incubation and from white colonies on the control plates were grown to log phase in liquid Lee’s medium at 25°C and observed by microscopy. Shown are corresponding differential interference contrast (top panels) and epifluorescence micrographs (bottom panels) of the cells. (D) Percentage
of white (white bars), opaque (black bars), and mixed white/opaque colonies (grey bars) after incubation of white-phase cells on Lee's agar plates for 12 h, 24 h, and 48 h under anaerobic conditions at 25°C (left) or 37°C (right). (E) Percentage of white, opaque, and mixed white/opaque colonies after incubation of white-phase cells for 48 h in liquid Lee's medium containing the indicated amounts of lovastatin or ketoconazole and subsequent growth of the cells on Lee's agar plates. (F) Percentage of white, opaque, and mixed white/opaque colonies after incubation of white-phase cells on agar plates with or without 5 μg ml⁻¹ ketoconazole for 24 h or 48 h at 25°C under anaerobic conditions. Control plates were incubated under aerobic conditions.

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Lee's medium containing lovastatin or ketoconazole. As can be seen in Fig. 1E, treatment with these ergosterol biosynthesis inhibitors induced white-opaque switching in a dose-dependent manner, with ketoconazole exhibiting the strongest effect (about 80% opaque cells at 5 μg ml⁻¹ ketoconazole). The presence of ketoconazole also accelerated the anaerobically induced switching when the cells were incubated on agar plates at 25°C (Fig. 1F).

These experiments supported the hypothesis that anaerobic conditions induce white-opaque switching by causing the depletion of ergosterol and/or the accumulation of sterol biosynthesis intermediates in the cells.

The transcription factor CZF1 controls anaerobically induced white-opaque switching

The finding that white cells of strain WO-1 can be efficiently induced to switch to the opaque phase by anaerobic conditions suggested that transcription factors that regulate aerobic/anaerobic gene expression might also be involved in the control of white-opaque switching. We therefore tested if expression of such transcription factors from an inducible promoter would cause white cells of strain WO-1 to switch to the opaque phase. Several candidate transcription factors were chosen for this analysis. UPC2 encodes a transcription factor that is required for sterol uptake and for the upregulation of ERG genes under anaerobic/hypoxic conditions and in the presence of sterol biosynthesis inhibitors [17,18]. The HAP41, HAP42 and HAP43 genes are the C. albicans homologues of S. cerevisiae HAP4, which encodes a subunit of the Hap2/3/4/5p complex involved in aerobic gene regulation and whose expression levels control the activity of this transcription factor [19,20]. In C. albicans, the Hap2/3/4/5p complex is also involved in the regulation of filamentous growth [20]. RFG1 is the C. albicans homologue of S. cerevisiae REX1, which is a transcription factor that is itself regulated by Hap1p to mediate repression of hypoxic genes. Although RFG1 is not involved in the regulation of aerobic/anaerobic gene expression in C. albicans, it is an important regulator of filamentous growth in MTLa/α cells [21,22]. Similarly, CZF1 encodes a transcription factor that is required for filamentous growth of C. albicans under embedded/hypoxic conditions [23], and we hypothesized that it might also be involved in the regulation of another morphogenetic program, white-opaque switching, in MTLa homozygous strains under anaerobic conditions. The coding regions of these transcription factors were placed under the control of a tetracycline-inducible promoter [24] and the expression cassettes integrated into the genome of strain WO-1. A control construct contained the GFP reporter gene instead of a transcription factor. Two independent transformants containing a single copy of the inducible expression cassette were kept in each case (see Table 1 for a description of all strains used in this study). White cells of the parental strain WO-1 and its derivatives were grown for 24 h at room temperature in Lee's medium in the presence or absence of 50 μg ml⁻¹ doxycycline and then plated on Lee's agar plates to determine the percentage of white and opaque colonies. In the absence of doxycycline all strains remained in the white phase and showed only the normal, low-frequency switching to the opaque phase. In contrast, doxycycline-induced expression of the CZF1 gene resulted in almost complete switching of the cells to the opaque phase, whereas expression of the other genes had no effect (Table 2). These results implicated CZF1 in the regulation of white-opaque switching, a finding that has recently been reported independently by two other groups [25,26].

To determine whether CZF1 is also required for anaerobically induced white-opaque switching, we deleted the gene from strain WO-1. Two independent series of heterozygous and homozygous czf1Δ mutants as well as complemented strains in which a functional CZF1 copy was reintegrated were constructed using the SAT1-flipping strategy [27]. Deletion of CZF1 did not abolish white-opaque switching, as all strains could spontaneously switch to the opaque phase (data not shown). However, the anaerobically induced switching of white cells to the opaque phase was drastically decreased, albeit not eliminated, in mutants lacking CZF1, and the heterozygous and complemented strains also exhibited a slightly reduced efficiency of induced switching (Fig. 2).

These results demonstrated that CZF1 plays an important role in the anaerobic induction of white-opaque switching.

WOR1 encodes the master regulator of white-opaque switching and wor1Δ cells can not switch to or maintain the opaque phenotype [12–14]. To elucidate the relationship between CZF1 and WOR1, we investigated whether WOR1 was also required for anaerobically induced white-opaque switching, whether CZF1 required the presence of WOR1 to induce switching and, vice versa, whether WOR1 required an intact CZF1 gene to induce switching. For this purpose, we constructed two independent wor1Δ mutants of strain WO-1 using the SAT1-flipping strategy and expressed the CZF1 and WOR1 genes from the Tet-inducible promoter in the wild type and in the czf1Δ and wor1Δ mutants. In line with previously reported results [12–14], no spontaneous switching to the opaque phase was observed in the wor1Δ mutants, and neither anaerobic conditions (see below) nor transient expression of CZF1 or WOR1 from the Tet-inducible promoter could induce white-opaque switching in the absence of the endogenous WOR1 gene (Table 3). These results demonstrated that CZF1-mediated, anaerobically induced switching to the opaque phase requires the master regulator WOR1. In contrast, doxycycline-induced expression of WOR1 efficiently induced white-opaque switching in czf1Δ mutants (Table 3). Similarly, a transient expression of CZF1 from the Tet-inducible promoter was sufficient to induce white-opaque switching even in a czf1Δ background, indicating that in contrast to WOR1 no further expression of CZF1 is required to maintain the opaque phenotype once switching to the opaque phase has been induced. To investigate whether Czf1p induces WOR1 expression, we compared the WOR1 mRNA levels in strains expressing CZF1 under control of the Tet-inducible promoter as well as in strains containing a control construct with GFP instead of CZF1. As can be seen in Fig. 3, WOR1 was expressed at very low levels in the control strains and in the strains containing the P_czf1-CZF1 fusion in the absence of doxycycline (between 0.03 and 0.05% of ACT1 mRNA levels). However, doxycycline-induced CZF1 expression resulted in high WOR1 expression levels (13–14% of ACT1 mRNA levels, i.e., a more than 300-fold induction). Altogether, these results indicated that the role of CZF1 in the environmentally
Table 1. C. albicans strains used in this study.

| Strain                  | Parent | Relevant genotype or characteristics | Reference |
|-------------------------|--------|--------------------------------------|-----------|
| Wild-type strains       |        |                                      |           |
| WO-1                    | MTLα   |                                      | [4]       |
| 12C                     | MTLα   |                                      | [47]      |
| 19F                     | MTLα   |                                      | [47]      |
| L26                     | MTLα   |                                      | [6]       |
| P37005                  | MTLα   |                                      | [6]       |
| P57072                  | MTLα   |                                      | [48]      |
| P78048                  | MTLα   |                                      | [48]      |
| Reporter strains expressing GFP from phase-specific promoters |        |                                      |           |
| WUGO4A and -B           | WO-1   | ura3Δ::FRT/ura3Δ::FRT                | [49]      |
| WUGS1A and -B           | WO-1   | ura3Δ::FRT/ura3Δ::FRT                | [50]      |
| WUGW11A and -B          | WO-1   | ura3Δ::FRT/ura3Δ::FRT                | [50]      |
| czf1Δ mutants           |        |                                      |           |
| WCZF1M1A and -B         | WO-1   | czf1Δ::SAT1-FLIP                     | this study|
| WCZF1M2A                | WCZF1M1A | czf1Δ::FRT                          | this study|
| WCZF1M2B                | WCZF1M1B | czf1Δ::FRT                          | this study|
| WCZF1M3A                | WCZF1M2A | czf1Δ::FRT/czf1Δ::SAT1-FLIP          | this study|
| WCZF1M3B                | WCZF1M2B | czf1Δ::FRT/czf1Δ::SAT1-FLIP          | this study|
| WCZF1M4A                | WCZF1M3A | czf1Δ::FRT/czf1Δ::FRT               | this study|
| WCZF1M4B                | WCZF1M3B | czf1Δ::FRT/czf1Δ::FRT               | this study|
| WCZF1MK1A               | WCZF1M4A | czf1Δ::FRT/CZF1::SAT1-FLIP           | this study|
| WCZF1MK1B               | WCZF1M4B | czf1Δ::FRT/CZF1::SAT1-FLIP           | this study|
| WCZF1MK2A               | WCZF1MK1A | czf1Δ::FRT/CZF1::FRT               | this study|
| WCZF1MK2B               | WCZF1MK1B | czf1Δ::FRT/CZF1::FRT               | this study|
| wor1Δ mutants           |        |                                      |           |
| WWOR1M1A and -B         | WO-1   | WOR1/WOR1/wor1Δ::SAT1-FLIP           | this study|
| WWOR1M2A                | WWOR1M1A | WOR1/WOR1/wor1Δ::FRT               | this study|
| WWOR1M2B                | WWOR1M1B | WOR1/WOR1/wor1Δ::FRT               | this study|
| WWOR1M3A                | WWOR1M2A | WOR1/wor1Δ::FRT/wor1Δ::SAT1-FLIP    | this study|
| WWOR1M3B                | WWOR1M2B | WOR1/wor1Δ::FRT/wor1Δ::SAT1-FLIP    | this study|
| WWOR1M4A                | WWOR1M3A | WOR1/wor1Δ::FRT/wor1Δ::SAT1-FLIP    | this study|
| WWOR1M4B                | WWOR1M3B | WOR1/wor1Δ::FRT/wor1Δ::SAT1-FLIP    | this study|
| WWOR1M5A                | WWOR1M4A | wor1Δ::FRT/wor1Δ::FRT/wor1Δ::SAT1-FLIP | this study|
| WWOR1M5B                | WWOR1M4A | wor1Δ::FRT/wor1Δ::FRT/wor1Δ::SAT1-FLIP | this study|
| WWOR1M6A                | WWOR1M5A | wor1Δ::FRT/wor1Δ::FRT/wor1Δ::FRT    | this study|
| WWOR1M6B                | WWOR1M5B | wor1Δ::FRT/wor1Δ::FRT/wor1Δ::FRT    | this study|
| Strains expressing transcription factors or GFP from the Tet-inducible promoter |        |                                      |           |
| WTET1-CZF1A and -B      | WO-1   | ADH1/ADH1::Ptet-CZF1                | this study|
| WTET1-HAP41A and -B     | WO-1   | ADH1/ADH1::Ptet-HAP41               | this study|
| WTET6-HAP42A and -B     | WO-1   | ADH1/ADH1::Ptet-HAP42               | this study|
| WTET1-HAP43A and -B     | WO-1   | ADH1/ADH1::Ptet-HAP43               | this study|
| WTET6-RFG1A and -B      | WO-1   | ADH1/ADH1::Ptet-RFG1                | this study|
| WTET6-UPC2A and -B      | WO-1   | ADH1/ADH1::Ptet-UPC2                | this study|
| WTET6-WOR1A and -B      | WO-1   | ADH1/ADH1::Ptet-WOR1                | this study|
| WNIM1A and -B           | WO-1   | ADH1/ADH1::Ptet-GFP                 | this study|
| WCZF1MTET1-CZF1A        | WCZF1M4A | czf1Δ::FRT/czf1Δ::FRT               | this study|
|                           |        |                                      | ADH1/ADH1::Ptet-CZF1 |
induced white-opaque switching is the activation of \textit{WOR1} expression in response to anaerobic conditions, which then allows switching to and maintenance of the opaque phase.

**Genomic alterations place white-opaque switching under environmental control**

Since all experiments described above had been performed with the \textit{MTL}a strain WO-1, we investigated if white-opaque switching would also be induced under anaerobic conditions in \textit{MTL}a and in other \textit{MTL}a strains. Therefore, we tested a set of three \textit{MTL}a strains (19F, L26, P37005) and three \textit{MTL}a strains (19F, P57072, P78048) provided by the Soll laboratory. However, none of these strains could be induced to switch from the white to the opaque phase under anaerobic conditions or in the presence of ketokonazole (Table 4 and data not shown), indicating that strain WO-1 exhibits unique characteristics that allow white-opaque switching to become inducible by environmental conditions. During the construction of the \textit{wor1A} mutants we noted that three rounds of allelic replacement were required to generate null mutants (Fig. 4A, lanes 1–4), suggesting that the possession of an additional copy of the master regulator \textit{WOR1}, whose expression levels determine whether cells adopt the white or the opaque phase [12–14], might have placed white-opaque switching under environmental control. Indeed, deletion of one \textit{WOR1} allele decreased white-opaque switching under anaerobic conditions, although the mutants containing two instead of three \textit{WOR1} alleles could still be induced to switch to the opaque phase (Fig. 4B). Deletion of two of the three alleles abolished the inducibility of white-opaque switching.

To investigate whether the \textit{WOR1} duplication was a peculiarity of the WO-1 stock maintained in our laboratory, we obtained strain WO-1 from the Soll lab. When this strain was transformed with the \textit{WOR1} deletion cassette, Southern hybridization analysis of the resulting transformants showed that the strain also contained three \textit{WOR1} alleles, as judged from the relative signal intensities of the bands representing intact \textit{WOR1} and mutated.
war1Δ alleles (Fig. 4A, lane 5). Comparative genome hybridization (CGH) experiments performed in the Berman lab demonstrated that the WO-1 stock that we obtained from the Soll lab (here referred to as Soll 1) as well as a WO-1 stock that was sequenced by the Broad institute (WO-1 Broad) were trisomic for chromosome 1 on which WOR1 is located (A. Forche and J. Berman, personal communication). In contrast, another WO-1 stock obtained by the Berman lab from the Soll lab (Soll 2) contained only two chromosome 1 copies. Southern hybridization analysis of transformants of strains WO-1 Broad and WO-1 Soll 2 in which one WOR1 allele was replaced by our deletion cassette confirmed that they contained three and two WOR1 copies, respectively (Fig. 4A, lanes 6 and 7).

To investigate whether the acquisition of an additional WOR1 copy was sufficient to place white-opaque switching under environmental control, we tested the anaerobic induction of switching in the other WO-1 stocks and their mutant derivatives in which one WOR1 allele was deleted. As shown in Fig. 4C, switching was efficiently induced in strains WO-1 Soll 1 and WO-1 Broad. Interestingly, deletion of one of the three WOR1 alleles had no effect on the inducibility of switching in WO-1 Soll 1 and only slightly reduced switching in WO-1 Broad. Switching was also efficiently induced in WO-1 Soll 2, which contains only two WOR1 copies, although not to the same levels as in the other strains. However, deletion of one WOR1 allele in this strain abolished the induction of switching by anaerobic conditions. These results demonstrated that the copy number of the master regulator WOR1 affects the inducibility of white-opaque switching in a strain-dependent fashion, but trisomy for chromosome 1 and WOR1 does not explain the environmental induction of switching in strain WO-1. This conclusion was further corroborated when we isolated a derivative of strain WO-1 Soll 1 in which the anaerobic induction of white-opaque switching was strongly reduced after prolonged passaging on agar plates (strain WO-1mut). We suspected that this strain might have lost one WOR1 copy. However, Southern hybridization analysis of a transformant in which one WOR1 allele was replaced by our deletion cassette demonstrated that the strain still retained three WOR1 copies (Fig. 4A, lane 8) and the deletion of one copy did not significantly

Table 2. Frequency of white-opaque switching in strains expressing transcription factors under control of a Tet-inducible promoter.

| Strain          | Gene expressed from Ptet | no. of white, opaque, and mixed white/opaque (w/o) colonies* |
|-----------------|--------------------------|-------------------------------------------------------------|
|                 |                          | -Dox white opaque w/o                                        | -Dox white opaque w/o |
| WO-1            | -                        | 6,185 2 7 8,650 0 12                                         | 8,650 0 12 |
| WINM1A          | GFP                      | 6,170 3 10 6,990 3 14                                        | 6,990 3 14 |
| WINM1B          | GFP                      | 6,875 3 6 6,145 1 10                                        | 6,145 1 10 |
| WTET1-UPC2A     | UPC2                     | 9,235 4 19 15,533 2 15                                       | 15,533 2 15 |
| WTET1-UPC2B     | UPC2                     | 11,165 2 31 13,675 3 33                                       | 13,675 3 33 |
| WTET1-HAP41A    | HAP41                    | 12,570 4 31 14,533 5 64                                       | 14,533 5 64 |
| WTET1-HAP41B    | HAP41                    | 9120 11 20 12,410 9 50                                       | 12,410 9 50 |
| WTET1-HAP42A    | HAP42                    | 12,070 3 29 13,124 4 44                                       | 13,124 4 44 |
| WTET1-HAP42B    | HAP42                    | 13,400 3 40 13,505 2 47                                       | 13,505 2 47 |
| WTET1-HAP43A    | HAP43                    | 6,430 5 89 11,220 8 162                                       | 11,220 8 162 |
| WTET1-HAP43B    | HAP43                    | 7,175 2 49 10,565 6 42                                       | 10,565 6 42 |
| WTET1-RFG1A     | RFG1                     | 12,030 2 43 14,505 9 73                                       | 14,505 9 73 |
| WTET1-RFG1B     | RFG1                     | 10,290 1 20 10,160 4 47                                       | 10,160 4 47 |
| WTET1-CZF1A     | CZF1                     | 120 6,435 23 8,055 4 50                                        | 8,055 4 50 |
| WTET1-CZF1B     | CZF1                     | 96 4,825 5 8,100 3 29                                        | 8,100 3 29 |

*Results are from 4–5 independent experiments performed on separate days.

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Figure 2. The transcription factor Czf1p is required for anaerobically induced white-opaque switching. White-phase cells of the wild-type strain WO-1 (CZF1/CZF1) and two independently constructed heterozygous (CZF1/czf1Δ) and homozygous (czf1Δ/czf1Δ) czf1 mutants and complemented strains (czf1Δ/czf1Δ+czf1Δ) were incubated for 2 days under anaerobic conditions at 25 °C on Lee's agar plates and subsequently grown for one week under aerobic conditions to determine the percentage of opaque (black bars) and mixed white/opaque colonies (grey bars).

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affect the anaerobically induced switching frequency of this mutant (Fig. 4C).

To study the effect of increasing the \( WOR1 \) copy number on switching in other \( MTL \) homozygous \( C. albicans \) strains, we integrated an additional \( WOR1 \) allele under control of its own regulatory sequences at an ectopic site into the genome of several of the non-inducible \( MTLa \) and \( MTLa \) strains mentioned above (see Materials and Methods). The results shown in Table 4 demonstrate that the effect of the additional \( WOR1 \) copy on white-opaque switching depended on the strain background. Most strains still remained uninducible, while in two independent transformants of one strain (L26) the switching frequency under anaerobic conditions was significantly increased, albeit not to the levels observed in strain WO-1. Altogether, these results demonstrate that unstable genomic alterations that have occurred in strain WO-1 allow white-opaque switching to be inducible by environmental conditions.

White-opaque switching is induced in the mammalian gastrointestinal tract

Our finding that white-opaque switching can be induced in certain \( C. albicans \) strains under anaerobic conditions even at 37°C suggested that appropriate host niches in which such conditions are encountered, like the mammalian intestine, might promote white-opaque switching. To address this hypothesis, we infected

**Table 3.** Frequency of white-opaque switching in strains expressing \( CZF1 \) or \( WOR1 \) in wild-type, \( czf1\Delta \), and \( wor1\Delta \) backgrounds.

| Strain | Relevant genotype | no. of white, opaque, and mixed white/opaque (w/o) colonies* |
|--------|-------------------|----------------------------------------------------------|
|        |                   | +Dox | −Dox | +Dox | −Dox | +Dox | −Dox |
|        |                   | white | opaque | w/o | white | opaque | w/o |
| WTE1-CZF1A | P\(_{tet}\)CZF1 | 39 | 612 | 25 | 779 | 1 | 10 |
| WTE1-CZF1B | P\(_{tet}\)CZF1 | 27 | 538 | 11 | 681 | 1 | 5 |
| WTE6-WOR1A | P\(_{tet}\)WOR1 | 110 | 150 | 13 | 567 | 0 | 19 |
| WTE6-WOR1B | P\(_{tet}\)WOR1 | 198 | 92 | 13 | 559 | 0 | 18 |
| WCZF1MAA | czf1Δ/czf1Δ | 328 | 0 | 0 | 454 | 0 | 0 |
| WCZF1MAB | czf1Δ/czf1Δ | 399 | 0 | 0 | 542 | 0 | 0 |
| WCZF1M4TET1-CZF1A | czf1Δ/czf1Δ | 3 | 360 | 0 | 450 | 0 | 0 |
| WCZF1M4TET1-CZF1B | czf1Δ/czf1Δ | 15 | 351 | 3 | 347 | 0 | 0 |
| WCZF1M4TET6-WOR1A | czf1Δ/czf1Δ | 4 | 118 | 0 | 423 | 0 | 0 |
| WCZF1M4TET6-WOR1B | czf1Δ/czf1Δ | 6 | 156 | 1 | 356 | 0 | 0 |
| WWOR1M6A | wor1Δ/wor1Δ | 378 | 0 | 0 | 410 | 0 | 0 |
| WWOR1M6B | wor1Δ/wor1Δ | 406 | 0 | 0 | 563 | 0 | 0 |
| WWOR1M6TET1-CZF1A | wor1Δ/wor1Δ | 370 | 0 | 0 | 275 | 0 | 0 |
| WWOR1M6TET1-CZF1B | wor1Δ/wor1Δ | 351 | 0 | 0 | 159 | 0 | 0 |
| WWOR1M6TET6-WOR1A | wor1Δ/wor1Δ | 204 | 0 | 0 | 329 | 0 | 0 |
| WWOR1M6TET6-WOR1B | wor1Δ/wor1Δ | 237 | 0 | 0 | 163 | 0 | 0 |

*Results are from two or three experiments performed in parallel for each strain.

**Figure 3.** Tetracycline-induced \( CZF1 \) expression results in \( WOR1 \) induction. Strains expressing \( CZF1 \) (P\(_{tet}\)CZF1) or GFP (control) from the Tet-inducible promoter were grown for 18 h at 30°C in liquid Lee’s medium with (+) or without (−) 50 \( \mu \)g ml\(^{-1}\) doxycycline (Dox). RNA was isolated from the cultures and \( WOR1 \) transcript levels were quantified by real-time RT-PCR and normalized to \( ACT1 \) transcript levels. Two independently constructed strains (A and B) were used in each case. doi:10.1371/journal.ppat.1000089.g003
mice intragastrically with white cells of strain WO-1 and collected the cells during the following days from the feces of the animals, i.e., after passage through the intestine. As can be seen in Fig. 5A, in a first set of experiments between 4 and 10% of the cells recovered after one day from the feces had switched to the opaque phase, i.e., the frequency of switching was increased by up to two orders of magnitude as compared with the spontaneous switching rate. No cells were recovered from the feces after 48 h and on the following days, indicating that the mice had efficiently eliminated the C. albicans cells from the intestine. In order to achieve a prolonged colonization of the intestine, we performed a second set of experiments using antibiotic-treated mice and a higher inoculum of C. albicans cells. As can be seen in Fig. 5B, white-opaque switching was further induced under these conditions, with the percentage of opaque and sectored colonies reaching more than 40% in some animals during the 3 days on which the cells were recovered from the feces. These results demonstrated that the conditions encountered in the mammalian intestine induced white-opaque switching in a large proportion of the cell population. We also tested two of the strains that did not switch to the opaque phase in response to anaerobic conditions, the MTLα strain 19F and the MTLα strain L26, under the same in vivo conditions. However, no significant induction of white-opaque switching was observed in 10 antibiotic-treated mice (5 infected mice for each of the two strains), indicating that the ability to switch to the opaque phase in the mammalian gastrointestinal tract is linked to the ability of C. albicans to switch in response to anaerobic conditions.

**Discussion**

Since the discovery of white-opaque switching in C. albicans more than 20 years ago [4], it has been known that environmental conditions can influence the frequency of switching between the two phases. Opaque cells are stable only at lower temperatures and revert to the white phase at 37°C, and the switching frequency from the white to the opaque phase can also be modulated by the growth conditions [10]. Nevertheless, white-opaque switching was thought to be a stochastic process that occurs spontaneously at a relatively low frequency, which allows the two phases to be stably maintained and yet ensures that a population also contains cells of the opposite phase that may be better adapted to new host niches encountered during an infection [10].

Our observation that anaerobic conditions induce white cells to switch en masse to the opaque phase came as a surprise for several reasons. In a mammalian host anaerobic niches should be encountered within the body, i.e., at 37°C, a temperature at which opaque cells are unstable. In contrast, it has been suggested that a preferred host niche for opaque cells is skin, where the temperature is lower and which should represent a largely aerobic environment [9,11]. In addition, a comparison of the gene expression patterns of white and opaque cells indicated that opaque cells exhibit an oxidative metabolism whereas white cells express a fermentative metabolism, pointing to a metabolic specialization of the two phases to the nutrients available in different anatomical sites [28]. Anaerobic conditions would not favor an oxidative metabolism and were therefore not expected to represent a suitable environment for opaque cells. However, our findings are in line with a recent report that anaerobic conditions stabilize the opaque phase at 37°C and that opaque cells can even mate at 37°C under anaerobic conditions [29].

Only a minority of C. albicans strains can switch to the opaque phase, as most strains are heterozygous at the mating type locus and first have to become MTL homozgyous to become MTL homzygous. Only mutants of the type 1-2 repressor. Interestingly, deletion of one allele of the hemoglobin response gene HBRI in a heterozygous MTLα/α strain has been found to result in repression of MTLα1 and MTLα2 expression and allowed white-opaque switching to occur with the same low frequency as in MTL homzygous strains [30]. Chromosomal alterations are therefore a prerequisite for cells to become switching-competent, either by mitotic recombination, chromosome loss and duplication of the homologous chromosome, or loss of one allele of a regulatory gene [5,7,30,31]. It is intriguing that additional genomic alterations allow white-opaque switching to become inducible by environmental conditions. We found that different stocks of strain WO-1 contain three WOR1 copies due to

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**Table 4. Anaerobically induced white-opaque switching in MTLα and MTLα strains and derivatives carrying an additional WOR1 copy.**

| Strain      | anaerobic |   | aerobic |   |
|-------------|-----------|---|---------|---|
|             | white     | opaque | w/o     | white | opaque | w/o |
| WO-1        | 2         | 84    | 0       | 91    | 0      | 0   |
| 19F         | 141       | 0      | 0       | 92    | 0      | 0   |
| 19F-WOR1    | 135       | 0      | 0       | 171   | 0      | 0   |
| L26         | 126       | 1      | 5       | 169   | 0      | 0   |
| L26-WOR1A   | 69        | 11     | 35      | 155   | 0      | 0   |
| L26-WOR1B   | 34        | 8      | 28      | 75    | 0      | 0   |
| P37005      | 120       | 0      | 0       | 130   | 0      | 0   |
| P37005-WOR1A| 151       | 3      | 6       | 156   | 1      | 0   |
| P37005-WOR1B| 132       | 1      | 1       | 128   | 0      | 0   |
| P78048      | 138       | 0      | 0       | 101   | 0      | 0   |
| P78048-WOR1A| 102       | 2      | 2       | 103   | 0      | 0   |
| P78048-WOR1B| 165       | 0      | 1       | 130   | 0      | 0   |

*Results from two independently tested colonies were combined in each experiment. doi:10.1371/journal.ppat.1000089.t004
Figure 4. WOR1 copy number and inducibility of white-opaque switching in different stocks of strain WO-1 and mutant derivatives. (A) Southern hybridization of EcoRI/XhoI-digested genomic DNA of various strains with a probe from the WOR1 upstream region. Lanes 1–4 show the hybridization pattern of the wild-type strain WO-1 (WOR1/WOR1/WOR1) and mutants in which one (WOR1/WOR1/wor1Δ), two (WOR1/wor1Δ/wor1Δ), or all three WOR1 alleles (wor1Δ/wor1Δ/wor1Δ) were deleted. Lanes 5–8 show the hybridization pattern of mutants of additional WO-1 stocks in which one WOR1 allele was replaced by the SAT1 flipper cassette. The identity of the hybridizing DNA fragments is indicated. The size of the fragment representing the wor1Δ allele remains the same after excision of the SAT1 flipper cassette due to an introduced XhoI site. (B) White-phase cells of the wild-type strain WO-1 and two independently constructed series of mutants lacking one, two, or all three WOR1 alleles were incubated for two days under anaerobic conditions at 25 °C on Lee’s agar plates and subsequently grown for one week under aerobic conditions to determine the percentage of opaque (black bars) and mixed white/opaque colonies (grey bars). Stars (*) indicate that no opaque colonies were observed in these strains. (C) White-phase cells of different WO-1 stocks and mutant derivatives were incubated for two days under anaerobic conditions at 37 °C on Lee’s agar plates and the percentage of opaque and mixed white/opaque colonies was determined as described in (B). Results are from two experiments performed in parallel for each strain. WT: wild-type parent, M1: mutant in which one WOR1 allele was deleted.

The WOR1 regulatory region is very large and it is likely to be subject to multiple regulatory inputs [12]. In addition to the Czf1p-mediated anaerobic induction of white-opaque switching, it is therefore possible that additional environmental signals also induce WOR1 expression and switching of white cells to the opaque phase, possibly after other genomic alterations have occurred. Genomic alterations may alter the balance between the levels of environmentally sensitive positive and negative regulators of white-opaque switching, explaining the differences in the efficiency of switching induction and its dependence on WOR1 copy number in various derivatives of strain WO-1.

The findings described in this study also shed new light on our view of the C. albicans life cycle. As stated above, only a minority of C. albicans strains are MTL homozygous and can switch to the opaque phase. As white-opaque switching itself occurs only at a relatively low frequency, opaque cells of opposite mating type from different strains would be expected to encounter one another very rarely in nature to allow mating and genetic exchange, in line with the observation that C. albicans populations are largely clonal [40]. For rare opaque cells in mixed MTLa and MTLα populations to come into contact, the surrounding white cells form a biofilm that is induced by the pheromone secreted from the opaque cells and allows stabilization of the pheromone gradient along which the shmoo of the opaque cells grow towards each other [41]. Our observation that specific environmental conditions can induce mass switching of white cells to the opaque phase in some strains suggests that in appropriate host niches the formation of such biofilms may not be required to allow mating, as a frequent contact of opaque cells of the two populations would be ensured. Such strains seem to be rare, and it will be important to identify an MTLa strain that, like the MTLα strain WO-1, switches to the opaque phase in response to anaerobic conditions or other signals encountered in the host to test this hypothesis. If more such inducible strains exist, they might represent a subpopulation of C. albicans in which genetic exchange occurs more frequently.
Growth on sorbose selects for strains that can utilize sorbose, which occurs after loss of one chromosome 5 homolog, and then results in strains that are \textit{MTL} homozygous and switching-competent \cite{5,37}. One might speculate that certain environmental conditions could select for or even induce the chromosomal alterations that are necessary for white-opaque switching to become inducible and thus allow efficient mating when two strains occupy such a niche. The mammalian gastrointestinal tract, in which mating has been shown to be highly facilitated \cite{29} and which we have shown here to induce white-opaque switching, might represent such a niche, as it is the normal habitat of \textit{C. albicans} in its commensal state.

\section*{Materials and Methods}

\textbf{Strains and growth conditions}

\textit{C. albicans} strains used in this study are listed in Table 1. All strains were stored as frozen stocks with 15\% glycerol at \(-80\)\degree C. The strains were subcultured separately in the white and opaque phases at room temperature on agar plates containing Lee’s medium, pH 6.8 \cite{42}, and 5 \(\mu\)g ml\(^{-1}\) phloxine B, which selectively stains opaque colonies pink \cite{43}.

Incubation under anaerobic conditions was performed by plating appropriate dilutions of white-phase overnight cultures on Lee’s agar plates and incubating the plates for different periods of time at 25\degree C or 37\degree C in an anaerobic jar (Anaerocult, Merck KGaA, Darmstadt, Germany) that generates an oxygen-free milieu in a CO\(_2\) atmosphere (18\% CO\(_2\)) within one hour. The plates were then transferred to aerobic conditions and further incubated at 25\degree C or at room temperature for 5–7 days to allow the development of visible colonies. Alternatively, cells were recovered from the plates after the anaerobic incubation period and used for microscopy or plated on fresh agar plates that were incubated under aerobic conditions for colony formation. For growth under anaerobic conditions in liquid medium, a 50 ml plastic tube with Lee’s medium and the oxygen indicator...
resazurine was inoculated with white-phase cells of strain WO-1, flushed for 10 min with nitrogen gas to remove oxygen, sealed, and incubated for 2 days at 37°C. The cells were then appropriately diluted, spread on Lee’s agar plates, and incubated for one week at room temperature to determine the percentage of white and opaque colonies. Treatment with ergosterol biosynthesis inhibitors was performed by diluting an overnight culture of white-phase cells 10^-3 into Lee’s medium containing the indicated concentrations of lovastatin or ketoconazole (stock solutions were 10 mg ml^-1 lovastatin or ketoconazole in 15% [vol/vol] ethanol, 0.25% [wt/vol] NaOH; 2 mg ml^-1 ketoconazole in DMSO) or the corresponding solvent without drug. The cultures were grown for two days at 30°C, plated at an appropriate density on Lee’s agar plates and incubated for one week at 25°C to allow colony formation. To test for a synergistic effect of ketoconazole and anaerobic conditions, white cells of strain WO-1 grown overnight in Lee’s medium were spread on Lee’s agar plates containing 5 μg ml^-1 ketoconazole or DMSO only and incubated at 25°C in an aerobic jar. The cells were recovered from the plates after 24 h and 48 h, spread at an appropriate density on Lee’s agar plates, and incubated for one week at room temperature under aerobic conditions to allow colony formation. For determining the spontaneous switching frequency of the strains, several thousand colonies (100–300 colonies per plate) were counted. To test the effect of gene deletions on the inducibility of white-opaque switching of strain WO-1, the effect of ergosterol biosynthesis inhibitors on switching, and the induction of switching by anaerobic conditions in other strains, only a few hundred colonies were usually counted. In all experiments several independent white colonies of each strain were tested.

Plasmid constructions

To express CZF1, HAP41, HAP42, HAP43, RFG1, UPC2, and WOR1 from the Tet-inducible promoter [24], the coding sequences of these genes were amplified by polymerase chain reaction (PCR) with the primer pairs CZF1-1/CZF1-2, HAP41/ HAP42, HAP41/HAP42, HAP43/HAP43, RFG1/RFG3, UPC2-1/UPC2-2, and WOR1-1/WOR1-2, respectively (primer sequences are given in Table S1). The PCR products were digested at the introduced SalI or XhoI and BglII sites and cloned into the SalI/BglII-digested pNIM1 [24] or a derivative, pNIM6, in which the TEF3 transcription termination sequence was substituted for the ACT1 transcription termination sequence, resulting in plasmids pTET1-CZF1, pTET1-HAP41, pTET6- HAP42, pTET1-HAP43, pTET6-RFG1, pTET6-UPC2, and pTET6-WOR1. To generate the CZF1 deletion construct, the CZF1 upstream and downstream regions were amplified with the primer pairs CZF1-3/CZF1-4 and CZF1-5/CZF1-6, respectively, digested at the introduced ApaI/XhoI and SalI/SacI sites, and cloned on both sides of the SAT1 flipper cassette in plasmid pSSF2 [27] to generate pCZF1M2. To obtain a WOR1 deletion construct, the WOR1 upstream and downstream regions were amplified with the primer pairs WOR1-3/WOR1-4 and WOR1-5/WOR1-6, respectively, digested at the introduced ApaI/XhoI and SalI/SacI sites, and substituted for the CZF1 flanking sequences in pCZF1M2 to generate pWOR1M2. To introduce an additional WOR1 copy into the ACT1 locus of MTLa and MTLa strains, a 1 kb-Clad fragment from pTET6-WOR1 containing the WOR1 coding sequence, the TEF3 transcription termination sequence, and part of the csnAT1a selection marker was first cloned into the KpnI/Clad-digested pMPG25 [44] to result in pWOR1-1. A part of the WOR1 coding region and upstream sequences were then amplified with the primers WOR1-9 and WOR1-10 and the PCR product digested with NolI/ EcoRI and cloned between the same sites in pWOR1-1 to generate pWOR1-2. Additional WOR1 upstream sequences were amplified with the primers WOR1-11 and WOR1-12, digested at the introduced SalI site and at a naturally occurring NolI site, and cloned together with an ACT1 fragment that was amplified with the primers ACT39 and ACT40 and digested with ApaI/SalI into the ApaI/NolI-digested pWOR1-2. The resulting plasmid pWOR1-3 contains 9 kb of WOR1 upstream sequences and the WOR1 coding region fused to the TEF3 transcription termination sequence, the csnAT1a selection marker, and flanking ACT1 sequences for genomic integration.

C. albicans transformation

C. albicans strains were transformed by electroporation [45] with gel-purified inserts from the plasmids described above. Nourseothricin-resistant transformants were selected on YPD agar plates containing 200 μg ml^-1 nourseothricin (Werner Bioagents, Jena, Germany) as described previously [27]. Single-copy integration of all constructs was confirmed by Southern hybridization.

Southern hybridization

Genomic DNA from C. albicans strains was isolated as described previously [46]. 10 μg of DNA was digested with appropriate restriction enzymes, separated on a 1% agarose gel, and, after ethidium bromide staining, transferred by vacuum blotting onto a nylon membrane and fixed by UV crosslinking. Southern hybridization with enhanced chemiluminescence-labeled probes was performed with the Amersham ECL™ Direct Nucleic Acid Labelling and Detection System (GE Healthcare, Braunschweig, Germany) according to the instructions of the manufacturer.

Fluorescence microscopy

Cells from white and opaque colonies of the strains carrying GFP reporter fusions were grown for 18 h at 25°C in liquid Lee’s medium and aliquots were spotted on microscope slides. Fluorescence microscopy was performed with a Zeiss Axioplan microscope equipped for epifluorescence microscopy with a 50 W mercury high pressure bulb and a Zeiss fluorescence-specific filter.

RNA isolation and real-time RT-PCR

Overnight cultures of strains WTET1-CZF1A and -B and WNNIMA and -B were diluted 10^-2 in liquid Lee’s medium with or without 50 μg ml^-1 doxycycline and grown for 18 h at 30°C. Total RNA was isolated using the RNeasy Mini Kit (Qiagen, Hilden, Germany) and treated with Turbo DNase (Ambion, Austin, TX). Total RNA from each sample was linearly reverse-transcribed using Superscript III Super Mix (Invitrogen, Karlsruhe, Germany), and cDNA was amplified by quantitative PCR with the primers WOR1LRT and WOR1KRT (for WOR1) and ACT1LRT and ACT2RT (for ACT1), as monitored by Sybr Green fluorescence in a MyQ real-time PCR detection system (Bio-Rad Laboratories, Hercules, CA). The signals for WOR1 were normalized to the ACT1 transcript level for each strain and culture condition.

In vivo experiments

White-phase cells of strain WO-1 were grown overnight in Lee’s medium, washed two times in phosphate-buffered saline (PBS), and adjusted to a density of 10^9 cells per ml. In a first experiment, two male Balb/c mice were intragastrically inoculated with 10^6 cells. After 24 h and on the following days, the feces of the mice were collected, homogenized in sterile water, and a dilution series was spread on Lee’s agar plates containing 10 μg ml^-1 gentamycin and incubated for one week at room temperature to determine the percentage of white and opaque colonies. The
experiment was repeated one week later with the same two mice and two additional mice. A third experiment was performed with antibiotic-treated mice. For this purpose, five Balb/c mice were fed with 1 mg ml\(^{-1}\) tetracycline, 2 mg ml\(^{-1}\) streptomycin, and 0.1 mg ml\(^{-1}\) gentamycin in their drinking water, starting from day 4 prior to infection. The mice were infected as described above with 5 \(\times\) 10\(^5\) white-phase cells of strain WO-1 and the fungal cells were recovered from the feces of the animals on the following three days and plated on Lee’s agar plates containing 50 \(\mu\)g ml\(^{-1}\) chloramphenicol to determine the percentage of white and opaque colonies. The latter experiment was repeated in an identical fashion with strains 19F and L26.

Sequence data

Sequence data for \textit{Candida albicans} was obtained from the Candida Genome Database (http://www.candidagenome.org/).

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Supporting Information

Table S1 Primers Used in This Study.

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Author Contributions

Conceived and designed the experiments: BR OR YP KO JM. Performed the experiments: BR OR YP KO. Analyzed the data: BR OR YP JM. Wrote the paper: JM.
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