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Candida albicans cell type switches and functional plasticity in the mammalian host

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Abstract

Candida albicans is a ubiquitous commensal of the mammalian microbiome as well as the most prevalent fungal pathogen of humans. A cell type transition between *C. albicans* yeast and hyphal morphologies was thought to underlie much of the variation in virulence in different host tissues. However, novel yeast-like cell morphotypes, including opaque$^{a/\alpha}$, gray, and GUT cell types, were recently reported that exhibit pronounced differences *in vitro* and in animal models of commensalism and disease. In this Review, we explore the characteristics of the classic cell types yeast, hyphae, pseudohyphae and chlamydospores as well as the newly identified yeast-like morphotypes. We highlight emerging knowledge about the associations of these different morphotypes with different host niches, virulence potential as well as the environmental cues and signalling pathways involved in the morphological transitions.

First described ~150 years ago, *Candida albicans* is now recognized as the most prominent fungal commensal and pathogen of humans. As a commensal, *C. albicans* colonizes the gastrointestinal tract[1], mouth[2], skin[3, 4], and female reproductive tract[5, 6] of at least 70% of healthy adults.[7] Human hosts are usually colonized in infancy[8] and longitudinal molecular typing studies indicate that strains persist clonally for many years, with little evidence for strain replacement.[9] These observations, coupled with the failure to identify an environmental reservoir, suggest that *C. albicans* is exquisitely adapted to healthy mammalian hosts. However, benign commensal colonization can become pathogenic if hosts develop immune deficits, epithelial damage, or microbial dysbiosis (Text Box 1).[10] Ironically, the pool of vulnerable patients has increased with the availability of modern medical treatments such as antibiotics, cancer chemotherapy, and solid organ transplantation, and *Candida* species now rank as the 3rd or 4th most common cause of invasive bloodstream infections in hospitals in the United States.[11, 12, 13] In this context, it is notable that fundamental questions regarding the mechanisms by which *C. albicans* thrives during its commensal and pathogenic lifestyles remain to be answered. For example, how is

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commensal colonization first established, and how does *C. albicans* persist for extended periods despite host immunity and bacterial competition? What controls the transition from commensalism to pathogenesis in vulnerable hosts? How does *C. albicans* succeed in the wide diversity of niches it encounters as a commensal and a pathogen? Some insights into these questions have been provided by a series of reports that link newly described *C. albicans* cell types to niche-specific functional adaptations.[14, 15, 16]

Text Box 1

Candida albicans occupies many niches in health and disease

The ability of *C. albicans* to thrive on and in human tissues cannot be overstated. As a commensal, *C. albicans* colonizes mucocutaneous surfaces of the mouth, skin, female reproductive tract and gastrointestinal tract of most healthy humans.[7] In addition, patients with specific risk factors are vulnerable to *C. albicans* disease syndromes involving virtually any organ.[10, 12, 147] For example, wet diapers and athletic socks are associated with superficial *C. albicans* skin and nail infections. Prosthetic devices put patients at risk for *C. albicans* biofilm formation and infection of surrounding tissues. Patients with AIDS and others with defective T cell immunity frequently suffer from oral thrush and invasive esophagitis. Gastrointestinal surgery can be complicated by leakage of gut commensals, putting patients at risk for postoperative infections such as intra-abdominal abscess. Prematurity is a strong risk factor for *Candida* meningitis. Antibiotic treatment is the most common risk factor for a broad range of candidiasis syndromes, presumably because antibiotics deplete bacterial competitors. Moreover, rarer defects in cell-mediated immunity, owing to hematologic malignancy, organ transplant, or cytotoxic chemotherapy, confer the highest risk for invasive disease. Immunocompromised patients as well as immunocompetent patients with multiple risk factors (for example, hospitalized individuals, treatment with antibiotics, catheters, surgery, and other invasive procedures) are highly vulnerable to bloodstream candidiasis, which carries a mortality of ~40%[12] and creates the opportunity for secondary infections of the eye (*Candida* endophthalmitis), heart (*Candida* endocarditis), bone (*Candida* osteomyelitis), liver and spleen (hepatosplenic candidiasis), and many other tissues.

The fungal kingdom is characterized by vast morphological plasticity. Fungi range in scale from the micron-sized microsporidia family of obligate intracellular pathogens [17] to *Armillaria ostoyae,* a tree pathogen whose 9.6 km^2 mycelial clone in Northern Oregon is considered the world's largest living organism.[18] Furthermore, many species undergo morphological transformations in response to specific environmental cues. For example, 'thermally dimorphic' fungal pathogens propagate as multicellular, branching, filamentous structures known as mycelia in environmental niches such as soil, and transition into unicellular, budding yeasts (or spherules, in the case of *Coccidioides immitis*) within warm-blooded hosts.[19, 20, 21] Given that the entire known lifecycle of *C. albicans* occurs in mammalian hosts, one might expect less morphological plasticity from this species; however, the opposite is true, and nine distinct cell shapes have already been described. In this Review, we discuss the characteristics of the classic cell types yeasts, hyphae, pseudohyphae and chlamydospores as well as yeast-like morphotypes, including opaque$^{\mathbf{a/\alpha}}$,

gray and GUT cells. We highlight emerging knowledge about the associations of these different morphotypes with different host niches and propensities towards virulence vs. commensalism. Finally, we discuss the environmental cues, signalling pathways, and transcriptional regulatory circuits that control the morphological transitions.

Classic cell types

Yeasts, hyphae, pseudohyphae and chlamydospores were the first *C. albicans* cell types to be described. They differ in morphology, mode of division, occurrence and virulence potential.

Yeasts, hyphae, pseudohyphae and chlamydospores

Among the four classic *C. albicans* cells types, yeasts and hyphae are the best characterized (Table 1 and Figure 1A; reviewed in[22, 23, 24]), whereas pseudohyphae and chlamydospores are less well understood (Table 1 and Figure 1A and 1B; reviewed in[22, 23, 24, 25]). Standard yeasts, also known as 'white' cells, have a round-to-oval cell morphology, similar to that of *Saccharomyces cerevisiae*. Yeasts reproduce by budding, and nuclear division occurs at the junction between mother and daughter cells. Because progeny cells detach completely from their mothers after cytokinesis, yeasts are considered to be unicellular (reviewed in[22]; see also[26]). By contrast, hyphal cells are thin, tube-shaped cells that resemble segments of a garden hose (Figure 1A). Nuclear division occurs within hyphal daughter cells, followed by migration of one progeny nucleus back into the mother cells. Hyphal cells remain firmly attached end-to-end following cytokinesis, such that iterative rounds of cell division produce multicellular, sparsely branched, filamentous structures called mycelia. Ellipsoid-shaped pseudohyphal cells share features of both yeasts and hyphae (Figure 1A), and there remains some controversy over whether they represent a *bona fide* terminal cell type or an intermediate between these other, better characterized cell types.[27] Unlike for yeasts and hyphae, there are no known *in vitro* conditions to induce pure, stable populations of pseudohyphae. Like hyphae, pseudohyphal cells remain attached following cytokinesis and generate mycelia after multiple rounds of cell division. As in yeasts, nuclear division in pseudohyphae occurs at mother-daughter junctions; in contrast to hyphae, these junctions are demarcated by visible indentations. Finally, chlamydospores are large, spherical, thick-walled cells observed *in vitro* under certain harsh conditions, such as starvation and hypoxia[28] (Figure 1B; reviewed in[29]). Chlamydospores are generated by suspensor cells, which are cells at the distal ends of mycelial filaments. Nuclear division occurs within the suspensor cell parent, followed by migration of a progeny nucleus to the nascent chlamydospore, which remains attached to its mother.[30]

Virulence in yeasts, hyphae and pseudohyphae

Yeasts, hyphae, and pseudohyphae can either propagate stably as the same cell type or give rise to other cell types in a process known as morphogenesis (Figure 1A), depending on cues from the local environment (see below). Morphogenesis has long been a central focus of *C. albicans* research because of links between each of these cell types and important host-fungal interactions. Traditionally, the filamentous forms (hyphae and pseudohyphae) were considered pathogenic, whereas yeasts were primarily viewed as commensals. Hyphae are intrinsically invasive on solid media and hyphal tip cells exhibit thigmotropism, or the

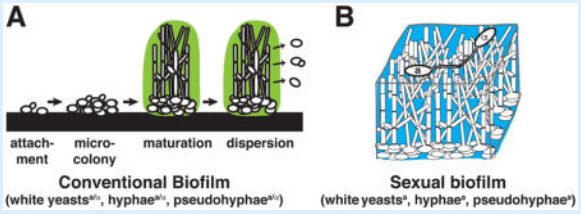
unusual ability to 'track' along substrate surface irregularities[31] (reviewed in[32]). Moreover, hyphae express numerous cell type-specific virulence factors such as adhesins (for example, Hwp1, Als3, Als10, Fav2 and Pga55), tissue-degrading enzymes (for example, Sap4, Sap5 and Sap6), antioxidant defense proteins (for example, Sod5), and even a recently described cytolytic peptide toxin (Ece1).[27, 33, 34, 35, 36] The increased virulence potential of hyphae compared to other cell types has been conclusively shown in superficial candidiasis models, such as models of oropharyngeal[37, 38] and vulvovaginal[39] infection (Figure 1C and 1D). For example, hyphae, but not yeasts, induce their endocytic uptake by cultured human oral epithelial cells via a specific interaction between the hyphal adhesin, Als3, and host E-cadherin; internalized hyphae then proceed to damage the host cells.[38] Hyphae can also actively penetrate into oral epithelial cells, possibly via physical pressure and secreted enzymes.[40, 41] Thus, in a reconstituted model of human oral epithelial tissue, invading hyphae trigger multiple pro-inflammatory host signaling pathways, whereas yeasts, which merely colonize the surface of the tissue without causing damage, produce a more muted inflammatory response.[37]

However, the simple dichotomy between virulent hyphae vs. commensal yeasts does not account for observations of disseminated candidiasis, where both cell types seem to contribute to disease. For example, yeasts, hyphae and pseudohyphae are all present in infected tissues that were recovered from human patients and animals with disseminated candidiasis (Figure 1E).[42, 43, 44] Moreover, *C. albicans* mutants trapped as either yeasts or filaments are both defective in bloodstream infection models, suggesting that the ability to interconvert among different cell types is required for virulence (see, for example, REFS[45, 46, 47]). Traditionally, yeasts, being smaller and unicellular, were hypothesized to disseminate through the bloodstream, whereas hyphae, being naturally invasive, were thought to escape the vasculature, penetrate into internal organs, and damage the host. Therefore, it came as a surprise when a study using a tetracycline-regulatable strain that can be propagated indefinitely as either yeasts or hyphae showed that yeast-locked *C. albicans* is as capable of egress from blood vessels, penetration into internal organs, and propagation within host tissues as a wild-type strain that can transition into hyphae.[48] Nevertheless, unlike wild-type *C. albicans,* the yeast-locked strain failed to kill its host, supporting previous observations that the yeast-to-hypha transition is required for virulence in disseminated infections. Similar to the requirement for all three cell types for virulence in the bloodstream infection model, they are also required for biofilm formation (Text Box 2; reviewed in[49, 50]), a *C. albicans* attribute of substantial clinical importance. Together, these observations in localized vs. disseminated infection models support a central role for yeast-hypha-pseudohypha morphogenesis in *C. albicans*-host interactions, but also suggest that yeasts may have different roles in different host niches. In contrast to the other cell types, chlamydospores, which are readily induced *in vitro* [51, 52], have rarely been observed in clinical specimens[53] or animal models of disease[54], and their biological role remains undefined.[25]

Text Box 2

Yeasts, hyphae, and pseudohyphae are required for biofilm formation

Biofilms are communities of microorganisms that often form on solid surfaces in the environment or within mammalian hosts. Medical device-associated biofilms are of enormous clinical importance because of their high prevalence and intrinsic resistance to antibiotics and the mammalian immune system. *C. albicans MTL***a**/*MTL*α white$^{a/\alpha}$ cells form **conventional biofilms** in a stereotyped fashion (reviewed in [148, 149], see the figure, part a). Biofilms are initiated when white$^{a/\alpha}$ phase yeasts attach to a solid substrate. Yeasts proliferate to form microcolonies, followed by the appearance and proliferation of hyphae and pseudohyphae, which constitute the bulk of the mature biofilm, together with an extracellular matrix composed of proteins, polysaccharides, and nucleic acids. Biofilm dispersion is thought to occur when white$^{a/\alpha}$ yeasts detach from a mature biofilm only to reattach at a second site. *MTL***a** and *MTL*α white^{a} cells have recently been shown to form **sexual biofilms** (see the figure, part b). These biofilms differ from conventional biofilms by multiple criteria, including increased permeability, decreased resistance to antibiotics and host immune cells, and promotion of chemotropism between opaque^{a} *MTL***a** and *MTL*α cells.[76, 77, 149] It has been proposed that a primary function of white^{a} cell biofilms is to facilitate mating between sexually competent *MTL***a** and *MTL*α opaque^{a} cells.[77]



Yeast-like morphotypes

In addition to standard ‘white’, round-to-oval yeast morphology, described above, *C. albicans* transitions into several more elongated yeast-like cell types (opaque, gray, and GUT) that exhibit distinct *in vitro* properties and interactions with the host. Moreover, a minority of white and opaque cells that have lost genetic material at the Mating Type-Like Locus *(MTL)* exhibit further alterations in their propensities for mating, filamentation, virulence, commensalism, and/or biofilm formation, as described below and in Text Box 2. The different types of white and opaque cells are not generally distinguished by genotype in the *C. albicans* literature. To clarify cell identity in this Review, however, we will introduce the convention of appending a superscript ‘**a**/α’ to white or opaque cells with the standard genotype of *MTL***a**/*MTL*α. A superscript ‘α’ will designate cells containing only the *MTL*α allele, whereas a superscript ‘**a**’ will be used either as a general term for cells containing a single allele of the *MTL* (*MTL***a** or *MTL*α) or as a specific term for ones containing only the *MTL***a** allele.

White^{a} and opaque^{a} cells

White^{a} and opaque^{a} cells were first described in a particular *C. albicans* clinical isolate, WO-1, based on *in vitro* observations of rare but heritable changes in cell and colony morphology (Figure 1F and Table 1).[55] White^{a} WO-1 yeasts have an identical appearance to standard white$^{a/\alpha}$ yeasts, described above, and form similar, creamy white, shiny, domed colonies on solid media. On glucose-containing media maintained at room temperature, however, white^{a} colonies occasionally (~1/10,000 cell divisions) give rise to slower growing sectors of opaque^{a} cells. Opaque^{a} colony sectors appear slightly darker, matte and flattened compared to white^{a} colonies. For undetermined reasons, opaque^{a} cells also take up a dye, phloxine B, which allows for rapid visualization of opaque$^{\alpha}$ colonies and colony sectors that are stained bright pink on media containing this dye. Microscopically, opaque^{a} cells are elongated compared to white^{a} cells and ~3 times larger (by volume), with more pronounced vacuoles.[56] Additional opaque^{a}-specific features include cell surface 'pimples' (that is, protuberances with an unknown biological role that are detected by scanning electron microscopy),[57] relative resistance to phagocytosis by host macrophages and neutrophils,[58, 59] sensitivity to distinct filamentation-inducing cues,[6061] and changes in the expression of >1000 genes, including genes important for mating and respiration.[62, 63, 64] Similarly to yeast-hypha-pseudohypha morphogenesis (Figure 2), switching between the white^{a} and opaque^{a} phenotypes is highly sensitive to environmental conditions: N-acetylglucosamine, ≥5% CO_2, and acidic pH all favor switching to the opaque^{a} state,[65, 66, 67] whereas glucose, low CO_2 levels, alkaline pH, and mammalian body temperature promote the reverse switch back to the white^{a} state.[55]

Morphogenesis and mating type

The functional significance of the white^{a} -to-opaque^{a} switch was revealed with the discovery that *C. albicans* opaque^{a} cells are specialized for mating.[68] Fungal mating has been well described in the model yeast, *S. cerevisiae,* in which haploid cells are the sexually-competent cell type (reviewed in[69]). Haploid '**a**' cells express the *MAT***a** allele of the Mating Type Locus, whereas haploid 'α' cells express the *MAT*α allele. *MAT***a** and *MAT*α encode different transcription factors that activate key mating genes in the respective haploid cell types. When **a** and α cells occur in proximity, pheromones secreted by mating partners of opposite mating type induce mutual cell cycle arrest, production of polarized mating projections, and cell and nuclear fusion to produce diploid **a**/α cells. Wild *S. cerevisiae* exists in the diploid form except under nutrient starvation conditions, which triggers meiosis and the formation of hardy haploid spores. These spores germinate when nutrients become available, and the mating cycle resumes.

In contrast to *S. cerevisiae, C. albicans* has never been observed to undergo meiosis or sporulation and was long considered to be an asexual species. However, in 2000, two groups reported low frequency mating between *C. albicans* **a** and α cells.[70, 71] Most *C. albicans* strains carry single copies of two different alleles of the Mating Type-Like Locus, *MTL***a** and *MTL*α, one apiece on two copies of Chromosome 5; these *MTL* alleles are orthologous to *S. cerevisiae MAT***a** and *MAT*α.[72] Researchers generated '**a**' and 'α' cells by deleting *MAT*α or *MAT***a** from **a**/α strains via targeted gene disruption[70] or selection for loss of one copy of Chromosome 5.[71] Remarkably, mixtures of these engineered **a** and α cells *in vitro*[71]

or in a mouse bloodstream infection model[70] produced a small number of tetraploid cells containing markers of both parental strains. One group subsequently determined the relationship between allelism at *MTL,* opaque$^{\mathbf{a}}$ or opaque$^{\alpha}$ cell formation, and mating: unlike typical **a**/α cells, **a** and α cells (including the natural α strain, WO-1) can switch to the opaque$^{\mathbf{a}}$ or opaque$^{\alpha}$ states, respectively, and opaque$^{\mathbf{a}}$ and opaque$^{\alpha}$ cells are the mating-competent cell types in *C. albicans* (Figure 1G).[68] The molecular mechanism preventing the white$^{\mathbf{a}}$-to-opaque$^{\mathbf{a}}$ switch in **a**/α cells is mediated by direct transcriptional repression of genes required for the switch by a1/α2*,* which is a heterodimeric transcription factor encoded by the combination of *MTL***a** with *MTL*α.[63, 73, 74, 75] More recently, another study reported that white$^{\mathbf{a}}$ and white$^{\alpha}$ cells may also play a role in mating via formation of specialized 'sexual' biofilms that constrain mating-competent opaque$^{\mathbf{a}}$ and opaque$^{\alpha}$ cells in space (Text Box 2).[76, 77]

Despite these advancements in our understanding of the relationship between *MTL* genotype, white$^{\mathbf{a}}$-to-opaque$^{\mathbf{a}}$ switching and mating competency, the larger contribution of sex to *C. albicans* biology remains uncertain. Analysis of *C. albicans* population structures has revealed a primarily clonal mode of reproduction, with little evidence for sexual recombination among naturally circulating strains.[78, 79] The rarity of sexual recombination is consistent with the observation that more than 90% of clinical isolates are heterozygous at the *MTL* locus and therefore incapable of switching or mating.[80, 81] Similarly, it remains unknown why *C. albicans,* along with its close relatives, *C. dubliniensis* and *C. tropicalis,* introduced a baroque requirement for a white$^{\mathbf{a}}$-to-opaque$^{\mathbf{a}}$ phenotypic switch into its mating program, given that *S. cerevisiae* and the vast majority of fungi mate efficiently without such a system. Some insights into the latter question are suggested by the recent discovery of three additional cell morphologies with some features of opaque$^{\mathbf{a}}$ cells in the *MTL* **a**/α genetic background**,** discussed below.

Opaque$^{\mathbf{a/\alpha}}$, gray and GUT cells

The opaque$^{\mathbf{a/\alpha}}$, gray, and GUT cell types exhibit physical similarities to opaque$^{\mathbf{a}}$ cells, but are functionally and genotypically distinct.

Opaque$^{\mathbf{a/\alpha}}$ and gray cells

A recent study discovered opaque$^{\mathbf{a/\alpha}}$ cells in a screen of 94 *C. albicans* clinical isolates for morphological responses to opaque$^{\mathbf{a}}$-inducing signals.[14] This group had previously shown that exposure of white$^{\mathbf{a}}$ cells to 1% N-acetylglucosamide (as a sole carbon source) and 5% CO_2 induces 100% full-colony switching to the opaque$^{\mathbf{a}}$ phenotype.[66] Using the same conditions, they found that ~1/3 of their **a**/α isolates developed opaque$^{\mathbf{a}}$-like (that is, bright pink-staining with phloxine B) colony sectors. Moreover, opaque cells recovered from pink sectors were elongated, contained cell surface pimples, and expressed several opaque$^{\mathbf{a}}$-specific genes (Figure 3A and Table 1), like traditional opaque$^{\mathbf{a}}$ cells. However, unlike opaque$^{\mathbf{a}}$ cells, opaque$^{\mathbf{a/\alpha}}$ cells were incapable of mating.[14] The same group subsequently discovered additional **a**/α isolates that switch among white$^{\mathbf{a/\alpha}}$, opaque$^{\mathbf{a/\alpha}}$, and a novel 'gray' phenotype (Figure 3A and Table 1).[15] Gray cells are smaller than conventional yeasts, lack pimples, stain only moderately with phloxine B and mate with very low efficiency.[15] In strains that are capable of white$^{\mathbf{a/\alpha}}$-opaque$^{\mathbf{a/\alpha}}$-gray switching, the transition to gray cell

morphology is induced by exposure to nutrient-rich growth medium (YEPD), whereas exposure to nutrient-poor medium (Lee's), N-acetylglucosamine, and elevated CO_2 favour the opaque$^{\mathbf{a/\alpha}}$ phenotype.[15]

Interestingly, initial studies in mammalian infection models suggest that opaque$^{\mathbf{a/\alpha}}$, gray, and opaque$^{\mathbf{a}}$ cells may have increased fitness on host epithelial surfaces (Figure 3B).[14, 15, 82] For example, opaque$^{\mathbf{a/\alpha}}$ and opaque$^{\mathbf{a}}$ cells have each been reported to colonize skin more effectively than isogenic white$^{\mathbf{a/\alpha}}$ or white$^{\mathbf{a}}$ strains in a neonatal mouse skin infection model.[14, 82] Likewise, in an *ex vivo* tongue infection model, gray cells have the fastest doubling time, followed by opaque$^{\mathbf{a/\alpha}}$ cells, with white$^{\mathbf{a/\alpha}}$ cells proliferating most slowly.[15] By contrast, white$^{\mathbf{a/\alpha}}$ cells are consistently most virulent in mouse bloodstream infection models.[14, 15, 82, 83] The mechanisms underlying these functional differences have not yet been defined but, as described below, cell type-specific differences in metabolism and/or enzyme secretion appear likely to play a role.[14, 15, 63, 64]

GUT cells

C. albicans "GUT" (gastrointestinally induced transition) cells were discovered by means of a genetic screen for fungal mediators of commensalism within the mammalian digestive tract.[16] Pools of **a**/α gene deletion mutants were competed in a mouse model of persistent gastrointestinal colonization, in which the host remains healthy despite high levels of commensally growing *C. albicans,* and the fitness of each fungal strain was calculated as a ratio of the relative abundance in mouse feces to that in the infecting inoculum. Two mutants affecting a pair of mutually inhibitory transcription factors emerged because of their striking and opposite effects on commensal fitness: the *efg1* knockout mutant was hyperfit, outcompeting all other mutants and wild-type *C. albicans*, whereas *wor1* was strongly attenuated in this model. Consistent with a positive role for Wor1 in promoting commensal fitness, it was shown that expression of the *WOR1* gene was induced 10,000-fold when wild-type yeasts were propagated within the host digestive tract compared to standard laboratory conditions. Furthermore, forced expression of *WOR1* (*WOR1*$^{\mathrm{OE}}$) via a strong, heterologous promoter *WOR1*$^{\mathrm{OE}}$) induced a hypercompetitive phenotype. Unexpectedly, after ~10 days of exposure to the mammalian model, a subset of the *WOR1*$^{\mathrm{OE}}$ yeasts recovered from animals exhibited altered cell and colony morphology. Moreover, these GUT cells rapidly dominated the recovered yeast population for the remainder of the 25-day time course. Similar to opaque$^{\mathbf{a}}$ cells (and opaque$^{\mathbf{a/\alpha}}$ cells), GUT cells are elongated relative to isogenic white$^{\mathbf{a/\alpha}}$ cells and generate darker, flattened colonies that stain (weakly) with phloxine B (Figure 3C and Table 1;[16] and Gianetti and Noble, unpublished data). Intriguingly, the initial appearance of the GUT phenotype coincided with a sharp gain in fitness of the *WOR1*$^{\mathrm{OE}}$ strain, suggesting that the two phenotypes might be linked. Indeed, when GUT cells are introduced into naive animals, they are immediately hypercompetitive, unlike white$^{\mathbf{a/\alpha}}$ isolates of the same strain.

After demonstrating that GUT cells lack the class features of white$^{\mathbf{a/\alpha}}$ and opaque$^{\mathbf{a}}$ cells, it was hypothesized that this novel cell type might be specialized for commensalism within the mammalian digestive tract. In support of this hypothesis, it was shown that GUT cells are substantially more fit than both white$^{\mathbf{a/\alpha}}$ and opaque$^{\mathbf{a}}$ cells in the gastrointestinal

commensalism model, with a relative fitness of GUT≫white$^{a/\alpha}$≫opaque^{a}. This fitness advantage seems to be specific to gastrointestinal commensalism, as GUT cells proliferate more slowly than white$^{a/\alpha}$ cells under standard laboratory conditions and are less virulent in a mouse bloodstream infection model. Furthermore, unlike opaque^{a} cells, GUT cells lack surface pimples and are unable to mate. Taken together, these data support a model in which signals from the mammalian gastrointestinal tract induce *C. albicans* yeasts to express *WOR1* and switch from white$^{a/\alpha}$ to GUT (Figure 3C). Whereas GUT cells thrive within the digestive tract, wild-type *C. albicans* strains rapidly revert to the white$^{a/\alpha}$ phenotype upon exit from animals, when signals required to maintain the GUT phenotype are removed. Thus, the detection of GUT cells outside of the host reflected the serendipitous use of a *WOR1*^{OE} strain, as continuous expression of Wor1 presumably stabilizes the phenotype. Future investigations will be required to define the host signals and fungal machinery that affect the white$^{a/\alpha}$-to-GUT switch.

Fitness and metabolism of yeast morphotypes

Comparative transcriptomics of white$^{a/\alpha}$ [15, 16], opaque$^{a/\alpha}$ [15], gray [15], GUT [16], white^{a} [63, 64] and opaque^{a} [63, 64] yeasts has revealed metabolic differences that may help to account for the functional differences among these cell types. The clearest case can be made for GUT cells, which, compared to white cells, exhibit general downregulation of pathways for utilization of glucose and iron uptake, with concomitant upregulation of pathways for utilization of N-acetylglucosamine and short chain fatty acids. Thus, GUT cell metabolism seems to be optimized for nutrients available in the distal mammalian digestive tract, the niche in which it thrives as a commensal.[16] By contrast, opaque$^{a/\alpha}$ and opaque^{a} cells upregulate pathways involved in oxidative respiration (for example, the Kreb's cycle), [15, 63, 64] whereas white $^{a/\alpha}$ and white^{a} cells upregulate fermentation pathways (for example, glucose uptake and, to varying degrees, glycolysis).[15, 63, 64] The transcriptome of gray cells shows differences in metabolic gene expression that are harder to categorize.[15] The functional importance of these cell type-specific metabolic signatures will hopefully be rationalized once the natural host niches of each cell type are identified.

Regulation of morphogenesis

Morphogenesis depends on environmental cues such as temperature and nutrient availability that signal through multiple pathways (Figure 2) and activate a variety of transcriptional regulatory circuits. Most of these pathways were initially characterized with respect to the yeast-to-hypha transition by white$^{a/\alpha}$ cells; however, several of these same signaling pathways also control discrete behaviors by additional cell types. The evolution of such elaborate systems to regulate morphogenesis speaks to the central importance of morphogenesis in *C. albicans* biology.

Environmental cues and their signaling pathways

On the basis of *in vitro* studies, various signals (mammalian body temperature, serum, N-acetylglucosamine (GlcNAc), low nitrogen, CO_2, peptidoglycan, and amino acids) have been shown to activate the fungal cAMP-PKA signaling pathway.
[84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100] In white$^{a/\alpha}$ cells, cAMP-mediated

signaling through the protein kinase A (PKA) complex activates transcription factors that promote the expression of hypha-specific genes and filamentation.[97] Alternatively, in white$^{\mathbf{a}}$ cells, PKA activation by GlcNAc or CO_2 promotes a switch to the opaque$^{\mathbf{a}}$ phenotype [65, 66, 86, 88] Similarly, the Cek1 MAP kinase pathway can promote either filamentation or mating in different cell types. In white$^{\mathbf{a/\alpha}}$ cells, nitrogen starvation or growth in an embedded matrix such as agar activates Cek1 to promote filamentation. [85, 101, 102, 103, 104, 105, 106] In *MTL* homozygous opaque$^{\mathbf{a}}$ cells, Cek1 activation by mating pheromones triggers the expression of genes required for mating.[107, 108, 109, 110, 111] In addition, various forms of cell stress (oxidative, osmotic, cell wall damage) affect filamentation indirectly via the Hog1 signaling pathway, which inhibits Cek1 and activates a transcriptional inhibitor of filamentation.[112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122] In white$^{\mathbf{a/\alpha}}$ cells exposed to alkaline pH, the RIM101 pH sensing pathway proteolytically activates the Rim101 transcription factor, leading to activation of hypha-specific genes and filamention.[123, 124, 125, 126] Additional, less well-described pathways negatively regulate filamentation in response to low oxygen levels and starvation, respectively. [127][128] Notably, the depicted pathways fail to account for certain observations in the host, such as the finding that white$^{\mathbf{a/\alpha}}$ yeasts appear to predominate in the mammalian GI tract[129], despite relatively high concentrations of GlcNAc and CO_2 in this niche, which would be expected to trigger filamentation or the white$^{\mathbf{a}}$-to-opaque$^{\mathbf{a}}$ switch based on *in vitro* evidence. Such discrepancies suggest that additional signaling pathways and/or crosstalk among existing pathways remain to be discovered. The multiplicity and complexity of the known signaling pathways suggest a model in which *C. albicans* continuously surveils the mammalian host, integrating a variety of signaling inputs to generate adaptive responses to the local environment.

Transcriptional regulation of morphogenesis

Remarkably, every morphological transition described in this article is regulated to some extent by the transcription factor, Efg1 (Figure 4A–E).[14, 15, 16, 130, 131, 132] The roles of Efg1 in *C. albicans* morphogenesis were deduced from phenotypes of *EFG1* mutants, with different cell shapes correlating with high or low levels of *EFG1* expression. In similar studies, Wor1 was shown to oppose Efg1 in the control of the white$^{\mathbf{a}}$-opaque$^{\mathbf{a}}$, white$^{\mathbf{a/\alpha}}$-gray-opaque$^{\mathbf{a/\alpha}}$, and white$^{\mathbf{a/\alpha}}$-GUT transitions (Figure 4C–E).[14, 15, 16, 73, 74, 75] Efg1 and Wor1 are fungal-specific transcription factors whose orthologs regulate diverse morphological transitions in different fungal species.[133, 134, 135, 136, 137, 138] In *C. albicans,* Efg1 and Wor1 have been demonstrated to bind to each other's promoters, where they are thought to mediate mutual transcriptional repression.[139, 140]

Regulation of *C. albicans* cell shape is more than a simple function of Efg1 and Wor1 levels, however, because each factor's activity is influenced by genotype at *MTL* and local environmental cues. For example, upon exposure to host serum, N-acetylglucosamine, high CO_2, nutrient depletion, and/or iron depletion, Efg1 promotes **a**/α white$^{\mathrm{a/\alpha}}$ cells to undergo the yeast-to-hypha transition;[130] however, under agar-embedded conditions, Efg1 promotes the *reverse* transition from hypha-to-yeast (Figure 4A).[132] In another case, under low oxygen, nutrient-depleted conditions, Efg1 promotes **a**/α hyphae and pseudohyphae to generate chlamydospores (Figure 4B).[33] In contrast, upon exposure to glucose and low CO_2, Efg1 promotes **a** and α opaque$^{\mathbf{a}}$ cells to switch to the white$^{\mathbf{a}}$ state (Figure 4C)[65] and certain

a/α clinical isolates to undergo opaque-to-white$^{a/\alpha}$ or gray-to-white$^{a/\alpha}$ switches (Figure 4D).[14, 15] Finally, Efg1 promotes **a**/α GUT-to-white$^{a/\alpha}$ cells to switch to the white$^{a/\alpha}$ state in all tested environments *other than* the mammalian intestinal tract (Figure 4E).[16]

A key unanswered question is how the genotype of the *MTL* locus and signals associated with different host niches contribute to discrete morphological outcomes. This challenge is compounded by the fact that cues such as N-acetylglucosamine and CO_2 promote distinct morphological switches in different contexts, as described above. Arguably the best characterized switch, in terms of its transcriptional regulation, is the white^{a}-opaque^{a} switch of *MTL* **a** and α cells (Figure 4C).[73, 74, 75, 139, 141, 142, 143, 144, 145] Importantly, this switch is controlled by numerous transcription factors in addition to the 'master regulators' Efg1 and Wor1,[73, 74, 75, 139, 141, 142, 143, 144, 145] which together form an interlocking circuit of positive and negative feedback loops.[139, 146] Under conditions in which pro-white^{a} and pro-opaque^{a} environmental cues are balanced (for example, glucose-containing medium maintained at 25°C), switching between white^{a} and opaque^{a} cell types occurs infrequently and stochastically in single cells, and both white^{a}-to-opaque^{a} and opaque^{a}-to-white^{a} switching occurs.[55] In this setting, cell morphology is thought to be programmed by the predominance of either Efg1 or Wor1 protein, such that switching might be triggered by events such as unequal distribution of Efg1 or Wor1 protein between a mother and daughter cell.[75, 139] In contrast, under environmental conditions that heavily favour the white^{a} (glucose-containing medium maintained in room air at 37°C) or opaque^{a} (N-acetylglucosamine-containing medium in ≥5% CO_2) state, switching occurs in a concerted fashion across the entire cell population.[55, 65, 66, 67] In these settings, it seems likely that potent environmental cues reinforce or inhibit transcriptional regulatory components in addition to Efg1 and Wor1 to produce a specific outcome. Broadly speaking, Efg1 and Wor1 can be envisioned as central hubs that link morphological switch-specific transcription factors and signaling molecules to a large variety of potential morphological outcomes.

Conclusions

Even within the morphologically diverse fungal kingdom, *C. albicans* stands out for its remarkable plasticity. Not only does it shift between single-celled yeasts and mycelial forms, similar to dimorphic fungi, but this continuously host-associated species also switches among at least six distinct yeast-like morphotypes. Recent observations of GUT, opaque$^{a/\alpha}$ and gray cells have provided fresh insights into the utility of morphological plasticity by showcasing *C. albicans* cell types that seem to be optimized for specific host niches, possibly via multiple mechanisms including metabolic polarization. Whereas hyphae and pseudohyphae predominate in most virulence models, with white$^{a/\alpha}$ yeasts also having an essential role in disseminated (bloodstream) infections, the newly described elongated yeasts may be more specialized for commensalism. For example, GUT cells were identified on the basis of their superior fitness in an intestinal commensalism model.[16] Meanwhile, opaque$^{a/\alpha}$ and opaque^{a} cells have been reported to outperform other cell types at skin colonization.[14, 82] More extensive, direct comparisons of all cell types in additional animal models will be required to validate and extend these initial observations. If true, niche-specific morphologic specialization would offer a potential rationale for the introduction of the white^{a}-to-opaque^{a} switch into the *C. albicans* mating program. That is, switching to the

opaque[a] cell type could have less to do with the mechanics of mating *per se* than in optimizing the fitness of mating-competent cells in the host niche in which mating occurs (a matter of continued speculation in the field).

Clearly, much remains to be learned of the signals, fungal signaling pathways, and transcriptional regulatory networks that control *C. albicans* morphogenesis. In addition, it is unclear whether the transitions between commensal and pathogenic cell types are regulated and potentially subject to pharmaceutical intervention. Given that *C. albicans* is the most common fungal commensal-pathogen of humans, the answers to these questions will have important implications for human health and disease.

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Glossary

Budding	Form of asexual reproduction by yeast cells, in which a new cell develops as a focal outgrowth of the mother cell, followed by detachment once growth is complete.
Cytokinesis	Division of the cytoplasm between mother and daughter cells after mitosis (or meiosis) is complete.
Dimorphic fungi	Set of human fungal pathogens that grow as mycelia in the environment but as yeasts (or spherules, in the case of *Coccidioides immitus*) in mammalian hosts. These pathogens include *Blastomyces dermatitidis, Coccidioides immitis, Histoplasma capsulatum, Paracoccidiodes brasiliensis, Penicillium marneffei, and Sporothrix schenckii.*
Meiosis	A type of cell division that produces four daughter cells, each with one-half of the DNA content of the mother. Used to generate sexually competent cells such as **a** and α cells in *S. cerevisiae*.
Mycelia	Multicellular, filamentous structures produced by repeated rounds of cell division by hyphal or pseudohyphal cells.
Suspensor cells	Terminal cells in mycelial networks that give rise to chlamydospores under nutrient poor and oxygen-depleted conditions.

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Key Points

- *Candida albicans* is a ubiquitous fungal component of the mammalian gut and skin microbiota
- *C. albicans* can infect most human tissues and causes superficial and disseminated disease syndromes in both healthy and immunocompromised hosts
- *C. albicans* shares with other fungi the ability to change shape in different environments. At least 9 different cell morphologies have been documented in this species
- Different *C. albicans* cell types vary in their ability to colonize the host or cause disease, as well as to inhabit different host niches. Metabolic differences appear to account for some of the differences in fitness.
- *C. albicans* has introduced an unusual cell type switch into its mating program
- Researchers have identified numerous environmental (host) signals that trigger *C. albicans* morphological transitions *in vitro*
- *C. albicans* signaling pathways transmit and integrate environmental information and induce morphological changes via fungal-specific transcription factors

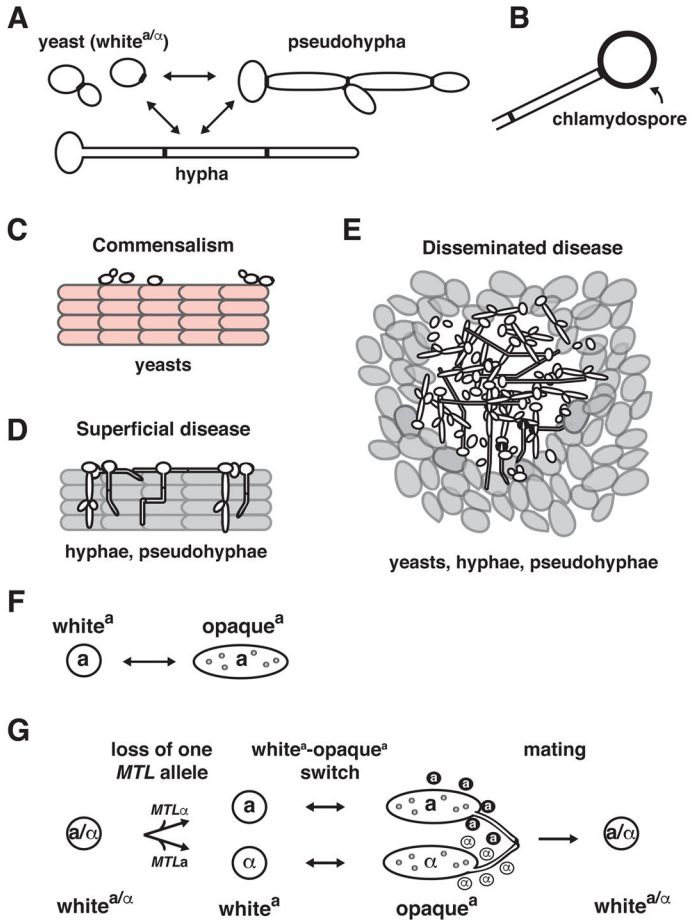

Figure 1. *C. albicans* Cell Type Transitions

A. *C. albicans* transitions reversibly among yeast (also known as white$^{a/\alpha}$), hypha, and pseudohypha cell types under different environmental conditions. B. Chlamydospores are generated by terminal (suspensor) cells of mycelia (multicellular hyphae or pseudohyphae) under adverse growth conditions. C and D. In mucocutaneous infection models, such as oropharyngeal candidiasis, yeasts are associated with commensalism (C), whereas the filamentous forms (hyphae and pseudohyphae) are associated with tissue invasion and damage (D). E. Yeasts, hyphae and pseudohyphae all seem to have roles in disseminated disease, for example in abscesses within host internal organs. F. *MTL***a** ("**a**") or *MTL*α ("α") cells can undergo an epigenetic switch between white^{a} and opaque^{a} phenotypes. White^{a} cells have the same appearance as typical white$^{a/\alpha}$ yeasts, while opaque^{a} cells are elongated and have "pimple" structures on their cell surface. G. Mating in *C. albicans* requires three events: Loss of one allele of *MTL* (*MTL*α or *MTL***a**) to generate a white^{a} phase **a** or α strain; an epigenetic switch from white^{a} to opaque^{a}; and pheromone sensing by opaque^{a} cells of opposite mating type, which triggers sexual filament production and mating.

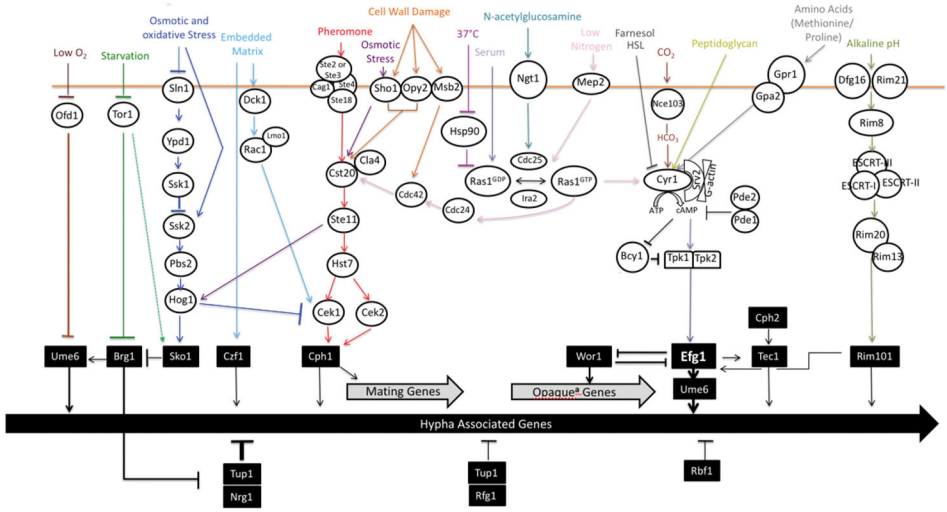

Figure 2. *C. albicans* Signaling and Morphogenesis

Numerous host signals and fungal signaling pathways have been implicated in the regulation of *C. albicans* cell shape. Based largely on *in vitro* analysis of wild-type *C. albicans* and specific gene deletion mutants, the signals and pathways depicted in this figure have been demonstrated to control the (white$^{a/\alpha}$) yeast-to-hypha transition and, in some cases, the white^{a}-to-opaque^{a} switch and mating. The **PKA pathway** (teal blue) incorporates signals via the GTPase Ras1 (gray) and Ras1-independent inputs resulting in the synthesis of cAMP from ATP by the adenylyl cyclase Cyr1 and cAMP-mediated activation of the two catalytic subunits (Tpk1 and Tpk2) of the PKA complex. Once activated, the PKA complex phosphorylates the downstream transcription factor Efg1, eliciting a potent effect on both filamentation and white-to-opaque^{a} switching. The **Cek1 Mitogen-Activated Protein Kinase pathway** (MAPK, navy blue) initiates a kinase signaling cascade in response to embedded growth (light blue), cell wall damage (navy blue), osmotic damage (beige), and low nitrogen (gray), ultimately phosphorylating the transcription factor Cph1 to induce filamentation. In opaque^{a} cells, mating pheromone (red) signals through the same upstream MAPK signaling cascade but leads to the additional phosphorylation of the MAPK Cek2 and activation of mating genes. The **Hog1 MAPK pathway** (gray blue) recognizes osmotic and oxidative stress through either the Sln1 two-component protein or the Sho1 adaptor protein and leads to phosphorylation of the MAPK Hog1. Activated Hog1 can inhibit both Cek1- and Brg1-mediated filamentation. The **RIM101 pathway** (green) senses alkaline pH via two putative receptors (Dfg16 and Rim21) that initiate a proteolytic signaling cascade that results in C-terminal cleavage of the transcription factor Rim101 by the protease Rim13 and activation of Efg1 and filamentation-specific genes. The **Ofd1 pathway** (pink) and **Tor1 pathway** (light green) respond to low oxygen and starvation, respectively, to regulate filamentation through the transcription factors, Brg1 and Ume6. **References** for signalling pathways are provided in the main text, and those for transcription factors (dark rectangles) are listed here:
46, 60, 73, 74, 75, 107, 111, 126, 127, 130, 139, 146, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167
. Note that additional transcription factors and some instances of regulation via Ume6 have been omitted for visual clarity.

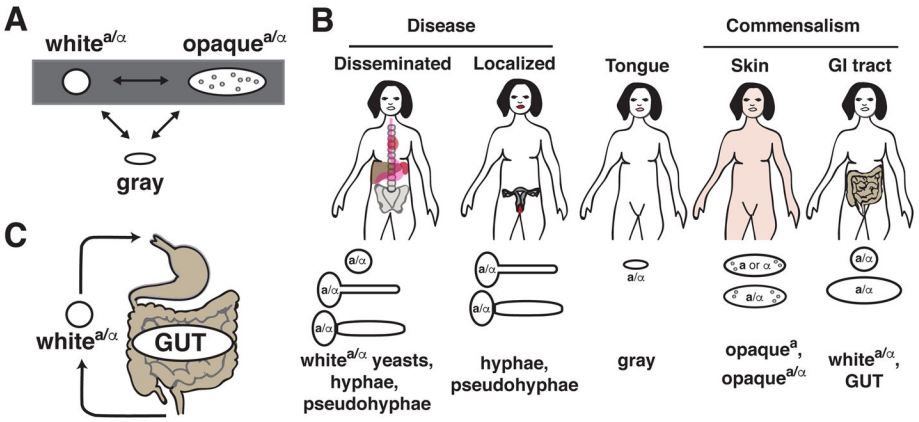

Figure 3. Opaque$^{a/\alpha}$, gray, and GUT cells

A) Certain *MTL***a**/α strains switch reversibly between standard white$^{a/\alpha}$ (round-to-oval) morphology and opaque$^{a/\alpha}$ morphology (elongated, with cell surface pimples). A subset of these strains can also switch to a third, gray (small, elongated, no pimples) morphology. B) Several *C. albicans* morphotypes exhibit enhanced fitness in specific host niches. *MTL***a**/α hyphae and pseudohyphae exhibit superior virulence in localized oral infection models, whereas white$^{a/\alpha}$ yeasts, hyphae and pseudohyphae are all required for virulence in disseminated infections. *MTL* heterozygous opaque$^{a/\alpha}$ and *MTL* homozygous opaque^{a} cells have both been reported to have superior fitness in colonizing skin, whereas *MTL***a**/α gray cells are the fastest proliferating cell type in an *ex vivo* tongue infection model. Finally, *MTL***a**/α GUT cells outcompete other cell types in the mammalian gastrointestinal tract, with a relative fitness of GUT≫white$^{a/\alpha}$ ≫opaque^{a}. C) GUT cells thrive within the digestive tract and rapidly revert to the white$^{a/\alpha}$ phenotype upon exit from the host, when signals required to maintain the GUT phenotype are removed. Thus, passage of *MTL***a**/α white cells through the mammalian gastrointestinal tract is required for the white$^{a/\alpha}$ -to-GUT switch.

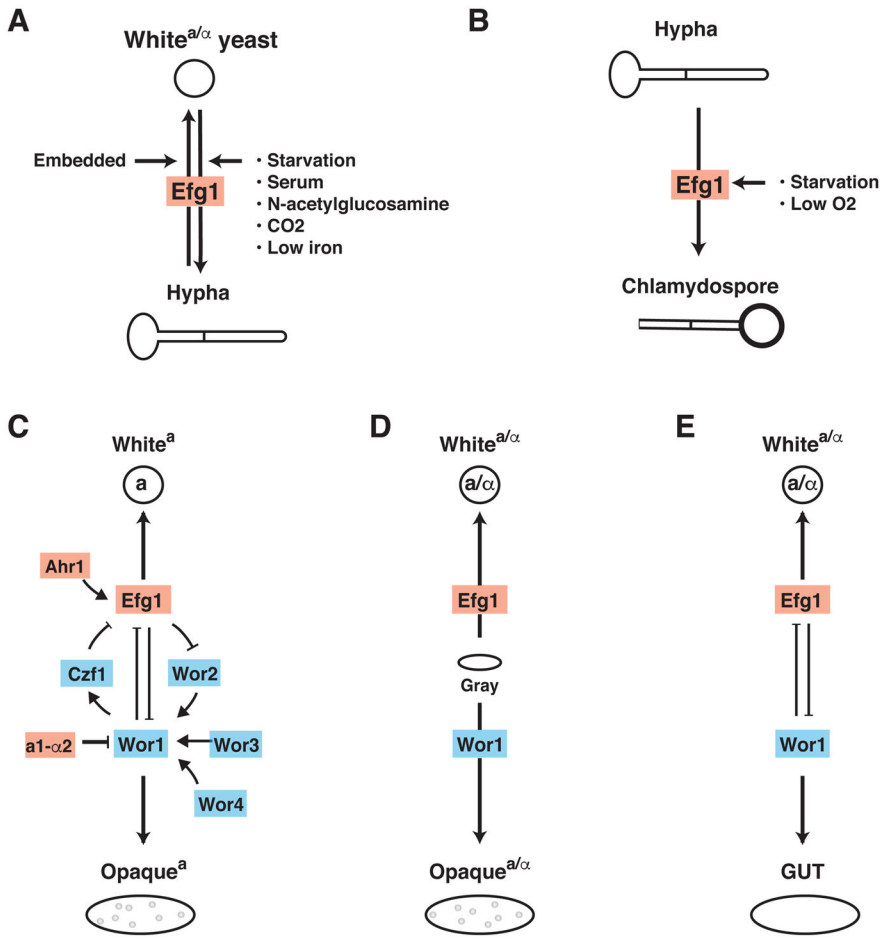

Figure 4. The Efg1 and Wor1 transcription factors have central roles in *C. albicans* morphological plasticity

Genetic studies have revealed roles for the transcription factors Efg1 and Wor1 in numerous morphological transitions. In each example below, arrows indicate activation, bars represent inhibition, and dashed lines indicate the direction of Efg1-promoted activity (with the exception of yeast-hypha morphogenesis (A), where Efg1 can support either transition, depending on environmental context). A) Efg1 promotes white$^{a/\alpha}$ cells to undergo the yeast-to-hypha transition upon exposure to host serum, N-acetylglucosamine, high CO_2, nutrient depletion, and/or iron depletion;[130] however, under agar-embedded conditions, Efg1 promotes the *reverse* transition from hypha-to-yeast.[132] B) Efg1 promotes chlamydospore production by white$^{a/\alpha}$ hyphal suspensor cells under nutrient poor, oxygen-depleted conditions.[51, 52] C) Efg1 promotes[131] and Wor1 opposes[73, 74, 75] the opaque^{a}-to-white^{a} switch, which is also controlled by additional transcription factors in a complex regulatory circuit. [139, 141, 142, 143, 144, 145] Under conditions in which neither cell type is favoured (glucose-containing medium maintained at 25°C), switching may occur in either direction on an infrequent, stochastic basis, depending on changes in the ratio of Efg1 to Wor1 protein in individual cells. [75, 139] Under conditions that strongly favour white^{a} (glucose-containing medium at 37°C) or opaque^{a} (N-acetylglucosamine-containing medium in ≥5% CO_2) cells, switching occurs in one direction across the entire cell population.[55, 65, 66, 67] D) Efg1 promotes the gray-to-white$^{a/\alpha}$ switch and Wor1 promotes the gray-to-opaque$^{a/\alpha}$ switch.[14,15]

Gray cells are favored under nutrient-rich conditions, whereas opaque$^{a/\alpha}$ cells are favored under nutrient-limited conditions in the presence of N-acetylglucosamine and elevated CO_2.[15] E) Efg1 promotes and Wor1 opposes the GUT-to-white$^{a/\alpha}$ switch.[16] White$^{a/\alpha}$ cells are favored under all tested conditions except for within the mammalian gastrointestinal tract.

Table 1

Features of *Candida albicans* cell types

	***MTL* Locus Genotype**	**Cell shape**	**Unicellular versus multicellular**	**Special morphological features**	***In vitro* inducing signals**	**Special functions**	**Host interactions**
Yeast (white (a/α))	**a**/α	Round-to-oval	Unicellular	N/A	Default cell shape under most *in vitro* conditions	Biofilm formation (conventional)	Virulence (bloodstream model); commensalism (mouth, skin, vagina and gastrointestinal tract)
Hypha *	**a**/α	Tube	Multicellular	N/A	37°C, N-acetylglucosamine, serum, immersion in agar, hypoxia, hypercarbia and alkaline pH	Thigmotropism; biofilm formation (conventional)	Induced endocytosis; active penetration of host epithelial cells; virulence (mouth, vagina and bloodstream models)
Pseudohypha *	**a**/α	Elongated ellipsoid	Multicellular	Indented cell-cell junctions	Hypha-inducing cues **	Biofilm formation (conventional)	Virulence (mouth, vagina and bloodstream)
Chlamydospore	**a**/α	Round-to-oval	Multicellular ***	Thick cell wall	Nutrient scarcity, hypoxia	Unknown	Unknown
White(a) and white(α)	**a**/Δ, **a/a** and α/Δ, α/α	Round-to-oval	Unicellular	N/A	37°C, glucose and alkaline pH	Biofilm formation (sexual)	Unknown
Opaque(a) and opaque(α)	**a**/Δ, **a/a** and α/Δ, α/α	Ellipsoid	Unicellular	Surface pimples	N-acetylglucosamine, hypercarbia and acidic pH	Mating	High fitness in a neonatal mouse skin colonization model
Opaque(a/α)	**a**/α	Ellipsoid	Unicellular	Surface pimples	Nutrient scarcity, N-acetylglucosamine and hypercarbia	Unknown	High fitness in a neonatal mouse skin colonization model
Gray(a/α)	**a**/α	Ellipsoid	Unicellular	Smallest cell type	Nutrient abundance	Unknown	High fitness in an *ex vivo* tongue infection model
GUT	**a**/α	Ellipsoid	Unicellular	N/A	Unknown	Unknown	High fitness in a mouse gastrointestinal commensalism model

GUT, gastrointestinally induced transition; *MTL,* mating-type-like.

* Please note that **a** and α cells form hyphae and pseudohyphae under certain environmental conditions, but these cell types have not been well characterized.

** Pseudohyphae arise as a subpopulation under most hypha-inducing conditions.

*** Chlamydospores are produced by the terminal cells of hyphae and pseudohyphae under nutrient-poor and oxygen-depleted conditions.