Candida albicans cell-type switching and functional plasticity in the mammalian host

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Abstract | *Candida albicans* is a ubiquitous commensal of the mammalian microbiome and the most prevalent fungal pathogen of humans. A cell-type transition between yeast and hyphal morphologies in *C. albicans* was thought to underlie much of the variation in virulence observed in different host tissues. However, novel yeast-like cell morphotypes, including opaque(**a**/α), grey and gastrointestinally induced transition (GUT) cell types, were recently reported that exhibit marked 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 and virulence potential, as well as the environmental cues and signalling pathways that are involved in the morphological transitions.

Budding
A 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.

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First described approximately 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 during infancy[8], and longitudinal molecular typing studies indicate that strains persist clonally for many years, with little evidence of 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 immunodeficiency, epithelial damage or microbial dysbiosis[10] (BOX 1). Ironically, the number of individuals who are vulnerable to infection with *C. albicans* 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 third or fourth most common cause of invasive bloodstream infections in hospitals in the United States[11–13]. 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 commensal colonization first established, and how does *C. albicans* persist for extended periods of time 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 that it encounters as a commensal and a pathogen? Some insights into these questions have been provided by a series of studies that linked newly described *C. albicans* cell types to niche-specific functional adaptations[14–16].

The fungal kingdom is characterized by vast morphological plasticity. Fungi range in size from the micrometre-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 largest living organism in the world[18]. Furthermore, many species of fungi 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*) in warm-blooded hosts[19–21]. Given that the entire life cycle of *C. albicans* occurs in mammalian hosts, less morphological plasticity would be expected 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 *C. albicans* cell types — yeasts, hyphae, pseudohyphae and chlamydospores — as well as yeast-like morphotypes, including opaque(**a**/α), grey and gastrointestinally induced transition (GUT) cells. We highlight emerging

Box 1 | *Candida albicans* occupies many niches in health and disease

The ability of *Candida 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 who have specific risk factors are vulnerable to *C. albicans* disease syndromes, which can involve almost 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 of *C. albicans* biofilm formation and infection of surrounding tissues. Patients who develop AIDS and others who have defective T cell immunity frequently suffer from oral thrush and invasive esophagitis. Gastrointestinal surgery can be complicated by the leakage of gut commensals, which puts patients at risk of post-operative infections, such as an intra-abdominal abscess. Prematurity is a major 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, due to haematological malignancy, organ transplant or cytotoxic chemotherapy, confer the highest risk for invasive disease. Immunocompromised patients as well as immunocompetent patients who have multiple risk factors (for example, hospitalization, treatment with antibiotics, catheters, surgery and other invasive procedures) are highly vulnerable to bloodstream candidiasis, which has a mortality rate of approximately 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.

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

Classic cell types

Yeasts, hyphae, pseudohyphae and chlamydospores were the first cell types of *C. albicans* to be described. These cell types 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 (reviewed in REFS 22–24) (FIG. 1a; TABLE 1), whereas pseudohyphae and chlamydospores are less well-understood (reviewed in REFS 22–25) (FIG. 1a,b; TABLE 1). Standard yeasts, also known as 'white' cells, have round-to-oval cell morphology, similar to that of *Saccharomyces cerevisiae*. Yeasts reproduce by budding, and nuclear division occurs at the junction between mother cell and daughter cell. As progeny cells detach completely from their mother cells after cytokinesis, yeasts are considered to be unicellular (reviewed in REF. 22; see also REF. 26). By contrast, hyphal cells are thin tube-shaped cells that resemble segments of a garden hose (FIG. 1a). Nuclear division occurs within hyphal daughter cells, followed by the migration of one progeny nucleus back into the mother cells. Hyphal cells remain firmly attached end-to-end following cytokinesis, such that subsequent rounds of cell division produce multicellular, sparsely branched, filamentous structures called mycelia. Ellipsoid-shaped pseudohyphal cells have features of both yeasts and hyphae (FIG. 1a), and some controversy remains regarding whether they represent a bona fide terminal cell type or an intermediate between the 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. Similar to 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 that are observed *in vitro* under certain harsh conditions, such as starvation and hypoxia[28] (reviewed in REF. 29) (FIG. 1b). Chlamydospores are produced by suspensor cells, which are cells at the distal ends of mycelial filaments. Nuclear division occurs within the suspensor cell parent, followed by the migration of a progeny nucleus to the nascent chlamydospore, which remains attached to its mother cell[30].

Virulence in yeasts, hyphae and pseudohyphae. Yeasts, hyphae and pseudohyphae can either propagate stably as the same cell type or generate other cell types through a process known as morphogenesis (FIG. 1a), depending on cues from the local environment (see below). Morphogenesis has long been a central focus of research in *C. albicans* because of links between each of these cell types and important host–fungal interactions. Traditionally, the filamentous forms of *C. albicans* (hyphae and pseudohyphae) were considered pathogenic, whereas yeasts were primarily viewed as commensal. 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 REF. 32). Moreover, hyphae express numerous cell type-specific virulence factors such as adhesins (for example, hyphal wall protein 1 (Hwp1), agglutinin-like protein 3 (Als3), Als10, factor activated 2 (Fav2) and Pga55), tissue-degrading enzymes (for example, secreted aspartyl protease 4 (Sap4), Sap5 and Sap6), antioxidant defence proteins (for example, superoxide dismutase 5 (Sod5)), and even a recently described cytolytic peptide toxin (extent of cell elongation protein 1 (Ece1))[27,33–36]. The increased virulence potential of hyphae compared with other cell types has been conclusively shown in superficial candidiasis models, such as models of oropharyngeal[37,38] and vulvovaginal[39] infection (FIG. 1c,d). For example, hyphae, but not yeasts, induce their endocytic uptake by cultured human oral epithelial cells through a specific interaction between the hyphal adhesin, Als3, and host epithelial cadherin (E-cadherin); internalized hyphae then proceed to damage the host cells[38]. Hyphae can also actively penetrate into oral epithelial cells, possibly through physical pressure and secreted enzymes[40,41]. Thus, in a reconstituted model of human oral epithelial tissue, invading hyphae trigger several pro-inflammatory signalling pathways in the host, whereas yeasts, which merely colonize the surface of the tissue without causing damage, trigger a more muted inflammatory response[37].

However, the simple dichotomy between virulent hyphae versus commensal yeasts does not account for observations of disseminated candidiasis, in which both cell types seem to contribute to disease. For example,

Cytokinesis
Division of the cytoplasm between a mother cell and daughter cell after mitosis (or meiosis) is complete.

Suspensor cells
Terminal cells in mycelial networks that produce chlamydospores under nutrient-poor and oxygen-depleted conditions.

Thigmotropism
The ability of hyphal tip cells to alter the direction of polarized growth in response to irregularities in an underlying surface.

yeasts, hyphae and pseudohyphae were all present in infected tissues that were recovered from human patients and animals with disseminated candidiasis[42–44] (FIG. 1e). Moreover, *C. albicans* mutants that are trapped as either yeasts or filaments are both defective in bloodstream infection models, which suggests that the ability to interconvert between different cell types is required for virulence (see, for example, REFS 45–47). 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 was surprising when a study that used 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, which supports 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 (reviewed in REFS 49, 50) (BOX 2), which is an attribute of *C. albicans* that is of substantial clinical importance. Together, these observations in localized versus 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].

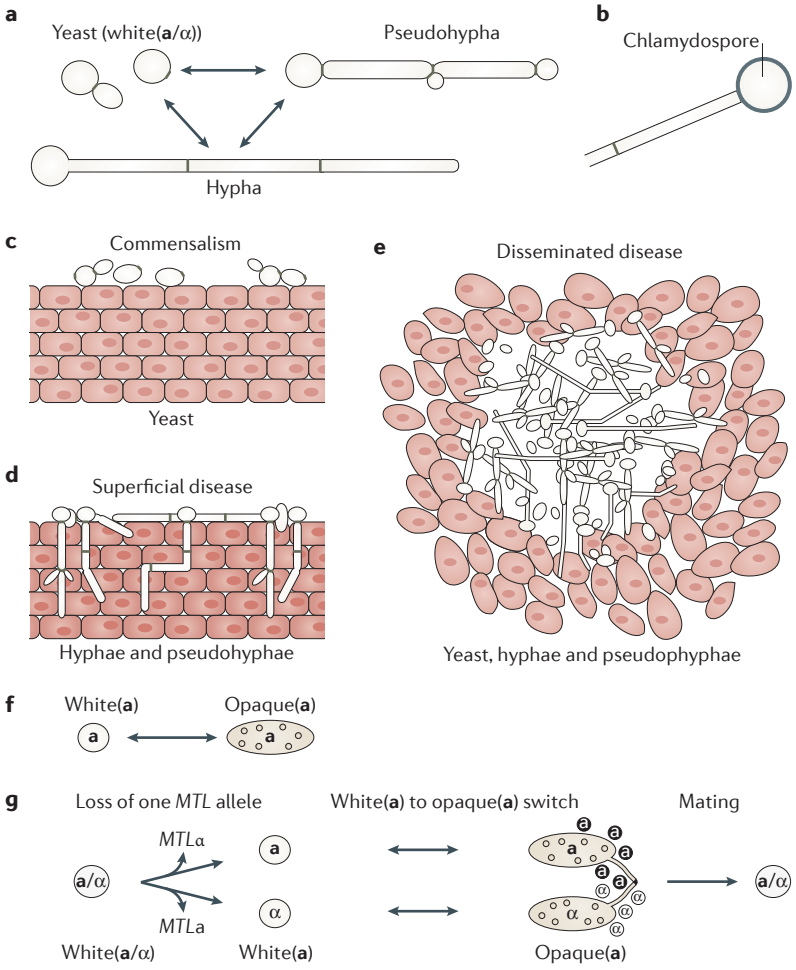


Figure 1 | ***C. albicans* cell type transitions. a** | *Candida albicans* transitions reversibly between yeast (also known as white(**a**/α)), hypha and pseudohypha cell types under different environmental conditions. **b** | Chlamydospores are produced by terminal (suspensor) cells of mycelia (multicellular hyphae or pseudohyphae) under adverse growth conditions. **c**,**d** | In mucocutaneous infection models, such as oropharyngeal candidiasis, yeasts are associated with commensalism (part **c**), whereas the filamentous forms (hyphae and pseudohyphae) are associated with tissue invasion and damage (part **d**). **e** | Yeasts, hyphae and pseudohyphae all seem to have roles in disseminated disease — for example, in abscesses within internal organs of the host. **f** | Mating-type-like (*MTL*) loci *MTL***a** ('**a**') or *MTL*α ('α') cells can undergo an epigenetic switch between white(**a** or α) and opaque(**a** or α) phenotypes. White(**a** or α) cells have the same appearance as typical white(**a**/α) yeasts, whereas opaque(**a** or α) cells are elongated and have 'pimple' structures on their cell surface. **g** | Mating in *C. albicans* requires three events: loss of one allele of the *MTL* locus (*MTL*α or *MTL***a**) to generate white(**a**) or white(α) strains; an epigenetic switch from white(**a** or α) to opaque(**a** or α); and pheromone sensing by opaque(**a** or α) cells of the opposite mating type, which triggers sexual filament production and mating.

Yeast-like morphotypes

In addition to the standard 'white' round-to-oval yeast morphology that is described above, *C. albicans* transitions into several more elongated yeast-like cell types (opaque, grey 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 (*MTL*) locus exhibit further alterations in their propensities for mating, filamentation, virulence, commensalism and/or biofilm formation, as described below and in BOX 2. The different types of white and opaque cells are not generally distinguished by genotype in the *C. albicans* literature. However, to clarify cell identity in this Review, we will introduce the convention of appending '(**a**/α)' to white or opaque cells that have the standard genotype of *MTL***a**/*MTL*α. An appended '(**a**)' will designate cells that contain only the *MTL***a** allele, '(α)' will designate cells that contain only the *MTL*α allele, and '(**a** or α)' will be used as a general term for cells that contain a single allele of the *MTL* locus (*MTL***a** or *MTL*α).

White(a or α) and opaque(a or α) cells. White(**a** or α) and opaque(**a** or α) cells were first described in a particular clinical isolate of *C. albicans*, WO-1, based on *in vitro* observations of rare but heritable changes in cell and colony morphology[55] (FIG. 1f; TABLE 1). White(**a** or α) WO-1 yeasts have an identical appearance to the standard white(**a**/α) yeasts that are described above and form similar, creamy white, shiny, domed colonies on solid media. However, on glucose-containing media that is maintained at room temperature, white(**a** or α) colonies occasionally (approximately 1 in 10,000 cell divisions) produce slower-growing sectors of

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	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
Grey(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 cells 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.

opaque(**a** or α) cells. Opaque(**a** or α) colony sectors seem slightly darker, matte and flattened compared with white(**a** or α) colonies. For undetermined reasons, opaque(**a** or α) cells also take up the dye phloxine B, which enables the rapid visualization of opaque(**a** or α) colonies and colony sectors that are stained bright pink on media that contain this dye. Microscopically, opaque(**a** or α) cells are elongated compared with white(**a** or α) cells, are approximately three-times larger (by volume) and have more pronounced vacuoles[56]. Additional opaque(**a** or α)-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[60,61], and changes in the expression of more than 1000 genes, including genes that are important for mating and respiration[62–64]. Similarly to yeast–hypha–pseudohypha morphogenesis (FIG. 2), switching between the white(**a** or α) and opaque(**a** or α) phenotypes is highly sensitive to environmental conditions: *N*-acetylglucosamine, ≥5% CO_2 and acidic pH all favour switching to the opaque(**a** or α) state[65–67], whereas glucose, low levels of CO_2, alkaline pH and mammalian body temperature promote the reverse switch back to the white(**a** or α) state[55].

Morphogenesis and mating type. The functional significance of the white(**a** or α)-to-opaque(**a** or α) switch was revealed with the discovery that opaque(**a** or α) *C. albicans* 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 REF. 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 that are secreted by mating partners of opposite mating type induce mutual cell cycle arrest, the production of polarized mating projections, and cell and nuclear fusion to produce diploid **a**/α cells. Wild-type *S. cerevisiae* exists in the diploid form except under

Meiosis
A type of cell division that produces four daughter cells, each containing half of the DNA content of the mother. This process is used to generate sexually competent cells such as **a** and α-cells in *Saccharomyces cerevisiae*.

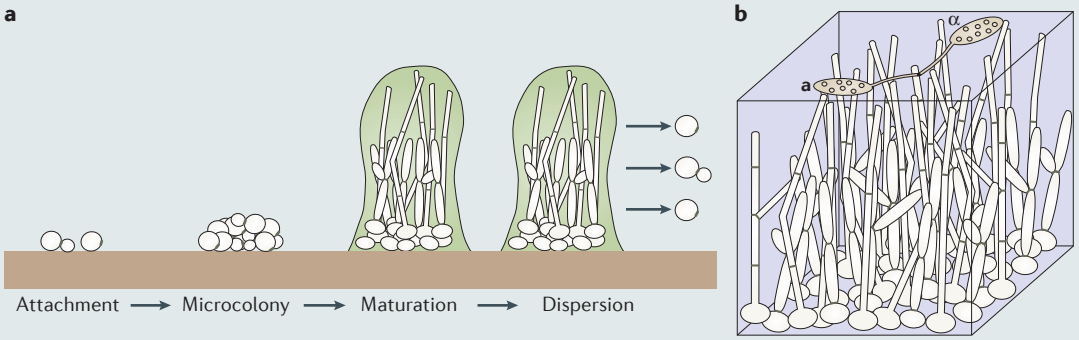
conditions of nutrient starvation, which triggers meiosis and the formation of hardy haploid spores. These spores germinate when nutrients become available and the mating cycle resumes.

In contrast to *S. cerevisiae, C. albicans* had 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 *MAT***a** and *MAT*α in *S. cerevisiae*[72]. Researchers generated '**a**' and 'α' cells by deleting *MTL***a** or *MTL*α from **a**/α strains through targeted gene disruption[70] or selection for the loss of one copy of chromosome 5 (REF. 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 that contained markers of both parental strains. One research group subsequently determined the relationship between allelism at *MTL*, opaque(**a** or α) 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(**a**) or opaque(α) states, respectively, and opaque(**a**) and opaque(α) cells are the mating-competent cell types in *C. albicans*[68] (FIG. 1g). The molecular mechanism that prevents the white(**a** or α)-to-opaque(**a** or α) switch in **a**/α cells is mediated by direct transcriptional repression of genes that are required for the switch by **a**1–α2, which is a heterodimeric transcription factor that is encoded by the combination of *MTL***a** with *MTL*α[63,73–75]. More recently, another study reported that white(**a**) and white(α) cells may also have a role in mating through the formation of specialized 'sexual' biofilms that constrain mating-competent opaque(**a**) and opaque(α) cells in space[76,77] (BOX 2).

Despite these advances in our understanding of the relationship between *MTL* genotype, white(**a** or α)-to-opaque(**a** or α) switching and mating competency, the larger contribution of sex to the biology of *C. albicans* 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 of *C. albicans* are heterozygous at the *MTL* locus and are therefore incapable of switching or mating[80,81]. Similarly, it remains unknown why *C. albicans*, together with its close relatives *Candida dubliniensis* and *Candida tropicalis*, introduced a baroque requirement for a white(**a** or α)-to-opaque(**a** or α) phenotypic switch into its mating programme, given that *S. cerevisiae* and the vast majority of other fungi mate efficiently without such a system. Some insights into the latter question are suggested by the recent discovery of three additional cell morphologies that have some features of opaque(**a** or α) cells in the *MTL* **a**/α genetic background (discussed below).

Box 2 | **Yeast, hyphae and pseudohyphae are required for biofilm formation**

Biofilms are communities of microorganisms that often form on solid surfaces in the environment and 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. *Candida albicans* white(**a**/α) cells form conventional biofilms in a stereotypical manner (reviewed in REFS 148,149; see the figure, part **a**). Biofilm formation is initiated when white(**a**/α)-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 that is composed of proteins, polysaccharides and nucleic acids. Biofilm dispersion is thought to occur when white(**a**/α) yeasts detach from a mature biofilm only to reattach at a second site. *C. albicans* white(**a** or α) cells have recently been shown to form sexual biofilms (see the figure, part **b**). These biofilms differ from conventional biofilms by several criteria, including increased permeability, decreased resistance to antibiotics and host immune cells, and promotion of chemotropism between opaque(**a**) and opaque(α) cells[76,77,149]. It has been proposed that a primary function of white(**a** or α) cell biofilms is to facilitate mating between sexually competent opaque(**a**) and opaque(α) cells[77].



Conventional biofilm
(White yeasts(**a**/α), hyphae(**a**/α) and pseudohyphae(**a**/α))

Sexual biofilm
(White yeasts(**a**), hyphae(**a**) and pseudohyphae(**a**))

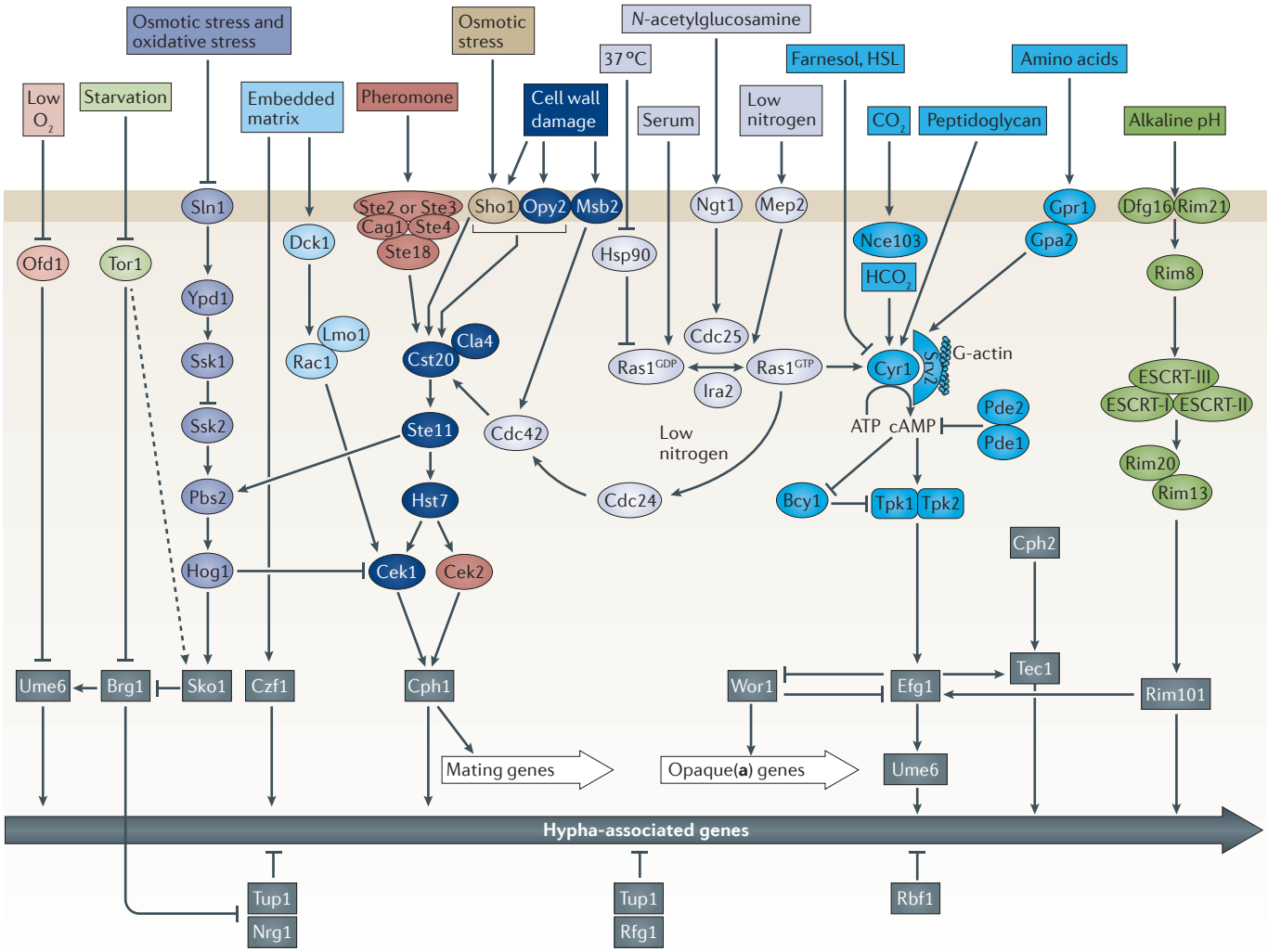


Figure 2 | ***C. albicans* signalling and morphogenesis.** Numerous host signals and fungal signalling pathways have been implicated in the regulation of cell shape in *Candida albicans*. Based largely on the *in vitro* analysis of wild-type *C. albicans* and specific gene deletion mutants, the signals and pathways that are depicted in this figure have been demonstrated to control the white(**a**/α) yeast-to-hypha transition and, in some cases, the white(**a** or α)-to-opaque(**a** or α) switch and mating. The protein kinase A (PKA) pathway (medium blue) incorporates signals through the GTPase Ras-like protein 1 (Ras1;grey) and Ras1-independent inputs that result in the synthesis of cyclic AMP (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 enhanced filamentous growth protein 1 (Efg1), eliciting a potent effect on both filamentation and white(**a** or α)-to-opaque(**a** or α) switching. Signalling through the PKA pathway is inhibited by the quorum sensing molecules farnesol and homoserine lactone (HSL). The Cek1 mitogen-activated protein kinase pathway (MAPK pathway, dark blue) initiates a kinase signalling cascade in response to embedded growth (light blue), cell wall damage (dark blue), osmotic stress (beige) and low nitrogen (grey), ultimately resulting in the phosphorylation of the transcription factor Cph1 to induce filamentation. In opaque(**a** or α) cells, mating pheromone (dark red) signals through the same upstream MAPK signalling cascade, but leads to the additional phosphorylation of the MAPK Cek2 and the activation of mating genes. The Hog1 MAPK pathway (purple) recognizes osmotic and oxidative stresses 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-mediated and biofilm regulator 1 (Brg1)-mediated filamentation. The Rim101 pathway (green) senses alkaline pH through two putative receptors (Dfg16 and Rim21) that initiate a proteolytic signalling cascade that results in carboxy-terminal cleavage of the transcription factor Rim101 by the protease Rim13 and the 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. See REFS 46,60,73–75,107,111,126,127,130,139,146,150–167 for further details about the transcription factors (dark grey rectangles) that are involved in the pathways in the figure. Note that additional transcription factors and some examples of regulation through Ume6 have been omitted for visual clarity. Cdc25, cell division control protein 25; ESCRT, endosomal-sorting complex required for transport; Gpa2, G protein α2 subunit; G-actin, globular actin; Gpr1, G protein- coupled receptor 1; Hsp90, heat shock protein 90; Nce103, carbonic anhydrase; Ngt1, *N*-acetylglucosamine transporter 1; Pde1, 3′,5′-cyclic nucleotide phosphodiesterase 1; $Ras1^{GDP}$, Ras-like protein 1 bound to GDP; Rfg1, repressor of filamentous growth 1; Wor1, white–opaque regulator 1.

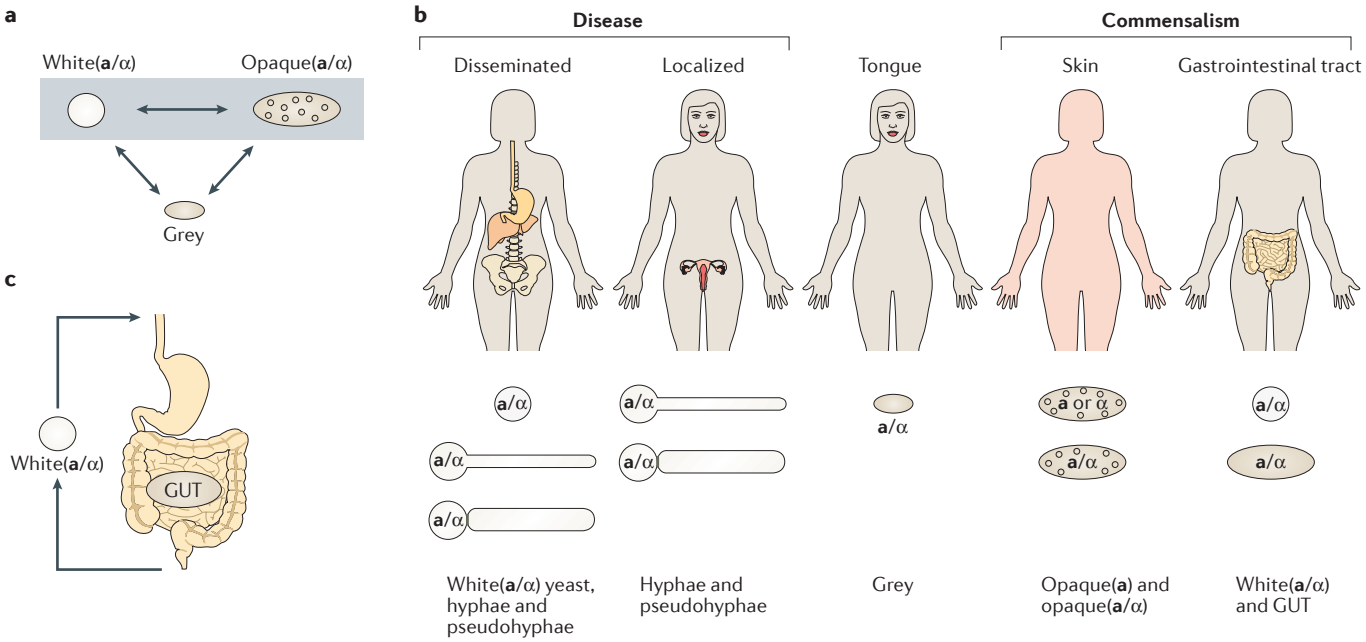


Figure 3 | **Opaque(a/α), grey and GUT cells. a** | Certain mating-type-like (*MTL*) locus **a**/α strains switch reversibly between standard white(**a**/α) morphology (round-to-oval) and opaque(**a**/α) morphology (elongated, with cell surface pimples). A subset of these strains can also switch to a third, grey morphology (small, elongated with no pimples). **b** | Several *Candida albicans* morphotypes exhibit enhanced fitness in specific host niches. **a**/α hyphae and pseudohyphae exhibit superior virulence in localized oral infection models, whereas white(**a**/α) yeasts, hyphae and pseudohyphae are all required for virulence in disseminated infections. *MTL* heterozygous opaque(**a**/α) and *MTL* homozygous opaque(**a** or α) cells have both been reported to have superior fitness during skin colonization, whereas grey(**a**/α) cells are the fastest proliferating cell type in an *ex vivo* tongue infection model. Finally, **a**/α gastrointestinally induced transition (GUT) cells outcompete other cell types in the mammalian gastrointestinal tract, with a relative fitness of GUT over white(**a**/α) over opaque(**a** or α). **c** | GUT cells thrive within the gastrointestinal tract and rapidly revert to the white(**a**/α) phenotype following exit from the host, when the signals that are required to maintain the GUT phenotype are removed. Thus, the passage of white(**a**/α) cells through the mammalian gastrointestinal tract is required for the white(**a**/α)-to-GUT switch.

Opaque(a/α) and grey cells. The opaque(**a**/α), grey and GUT (see below) cell types exhibit physical similarities to opaque(**a** or α) cells, but are functionally and genotypically distinct. A recent study discovered opaque(**a**/α) cells in a screen of 94 clinical isolates of *C. albicans* for morphological responses to opaque(**a** or α)-inducing signals[14]. This group had previously shown that the exposure of white(**a** or α) cells to 1% *N*-acetylglucosamine (as a sole carbon source) and 5% CO_2 induced 100% full-colony switching to the opaque(**a** or α) phenotype[66]. Using the same conditions, they found that approximately one-third of their **a**/α isolates developed opaque(**a** or α)-like (that is, bright pink staining with phloxine B) colony sectors. Moreover, opaque cells that were recovered from pink sectors were elongated, contained cell surface pimples and expressed several opaque(**a** or α)-specific genes (FIG. 3a; TABLE 1), similarly to traditional opaque(**a** or α) cells. However, unlike opaque(**a** or α) cells, opaque(**a**/α) cells were incapable of mating[14]. The same group subsequently discovered additional **a**/α isolates that switch between white(**a**/α), opaque(**a**/α) and a novel 'grey' phenotype[15] (FIG. 3a; TABLE 1). Grey 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(**a**/α)-to-opaque(**a**/α)-to-grey switching, the transition to grey cell morphology is induced by exposure to nutrient-rich growth medium (yeast extract peptone dextrose (YEPD)), whereas exposure to nutrient-poor medium (Lee's medium), *N*-acetylglucosamine and increased levels of CO_2 favour the opaque(**a**/α) phenotype[15].

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

GUT cells. *C. albicans* gastrointestinally induced transition (GUT) cells were discovered by means of a genetic screen for fungal mediators of commensalism in the

mammalian gastrointestinal 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 faeces to that in the infecting inoculum. Two mutants that affected a pair of mutually inhibitory transcription factors emerged because of their marked and opposite effects on commensal fitness: the enhanced filamentous growth 1 (*EFG1*)-knockout mutant was highly fit, outcompeting all other mutants and wild-type *C. albicans*, whereas the white–opaque regulator 1 (*WOR1*)-knockout mutant 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 gastrointestinal tract compared with standard laboratory conditions. Furthermore, forced expression of *WOR1* (*WOR1*^{OE}) through a strong heterologous promoter induced a hypercompetitive phenotype. Unexpectedly, after approximately 10 days of exposure to the mammalian model, a subset of the *WOR1*^{OE} yeasts that were 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(**a** or α) cells (and opaque(**a**/α) cells), GUT cells are elongated relative to isogenic white(**a**/α) cells and produce darker, flattened colonies that stain (weakly) with phloxine B (REF. 16 and B.A.G. and S.M.N., unpublished observations) (FIG. 3c; TABLE 1). Intriguingly, the initial appearance of the GUT phenotype coincided with a sharp increase in commensal fitness of the *WOR1*^{OE} strain, which suggests that the two phenotypes might be linked. Indeed, when GUT cells are introduced into naive animals, they are immediately hypercompetitive, unlike white(**a**/α) isolates of the same strain.

After demonstrating that GUT cells lack the classic features of white(**a**/α) and opaque(**a** or α) cells, it was hypothesized that this novel cell type might be specialized for commensalism in the mammalian gastrointestinal tract. In support of this hypothesis, it was shown that GUT cells are substantially more fit than both white(**a**/α) and opaque(**a** or α) cells in the gastrointestinal commensalism model, with a relative fitness of GUT over white(**a**/α) over opaque(**a** or α). This fitness advantage seems to be specific to gastrointestinal commensalism, as GUT cells proliferate more slowly than white(**a**/α) cells under standard laboratory conditions and are less virulent in a mouse bloodstream infection model. Furthermore, unlike opaque(**a** or α) 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**/α) to GUT (FIG. 3c). Whereas GUT cells thrive in the gastrointestinal tract, wild-type *C. albicans* strains rapidly revert to the white(**a**/α) phenotype following exit from animals, when signals that are 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**/α)-to-GUT switch.

Fitness and metabolism of yeast morphotypes. Comparative transcriptomics of white(**a**/α)[15,16], opaque(**a**/α)[15], grey[15], GUT[16], white(**a** or α)[63,64] and opaque(**a** or α)[63,64] yeasts has revealed metabolic differences that may help to account for the functional differences between these cell types. The clearest case can be made for GUT cells, which, compared with white(**a**/α) cells, exhibit general downregulation of pathways that are involved in iron uptake and the utilization of glucose, with concomitant upregulation of pathways involved in the utilization of *N*-acetylglucosamine and short-chain fatty acids. Thus, GUT cell metabolism seems to be optimized for nutrients that are available in the distal mammalian gastrointestinal tract, the niche in which it thrives as a commensal[16]. By contrast, opaque(**a**/α) and opaque(**a** or α) cells upregulate pathways that are involved in oxidative respiration (for example, Krebs cycle)[15,63,64], whereas white(**a**/α) and white(**a** or α) cells upregulate fermentation pathways (for example, glucose uptake and, to varying extents, glycolysis)[15,63,64]. The transcriptome of grey 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 several pathways (FIG. 2) and activate various transcriptional regulatory circuits. Most of these pathways were initially characterized with respect to the yeast-to-hypha transition by white(**a**/α) cells; however, several of these signalling pathways also control discrete behaviours by additional cell types. The evolution of such elaborate systems to regulate morphogenesis highlights the central importance of morphogenesis in the biology of *C. albicans*.

Environmental cues and their signalling pathways. Based on *in vitro* studies, various signals (mammalian body temperature, serum, *N*-acetylglucosamine, low nitrogen, CO_2, peptidoglycan and amino acids) have been shown to activate the fungal cyclic AMP (cAMP)–protein kinase A (PKA) signalling pathway[84–100]. In white(**a**/α) cells, cAMP-mediated signalling through the PKA complex activates transcription factors that promote the expression of hypha-specific genes and filamentation[97]. Alternatively, in white(**a** or α) cells, the activation of PKA by *N*-acetylglucosamine or CO_2 promotes a switch to the opaque(**a** or α) phenotype[65,66,86,88]. Similarly, the Cek1 (also known as Erk1) mitogen-activated protein kinase (MAPK) pathway can promote either filamentation or mating in different cell types. In white(**a**/α) cells, nitrogen starvation or growth in an embedded matrix, such as agar, activates Cek1 to promote filamentation[85,101–106].

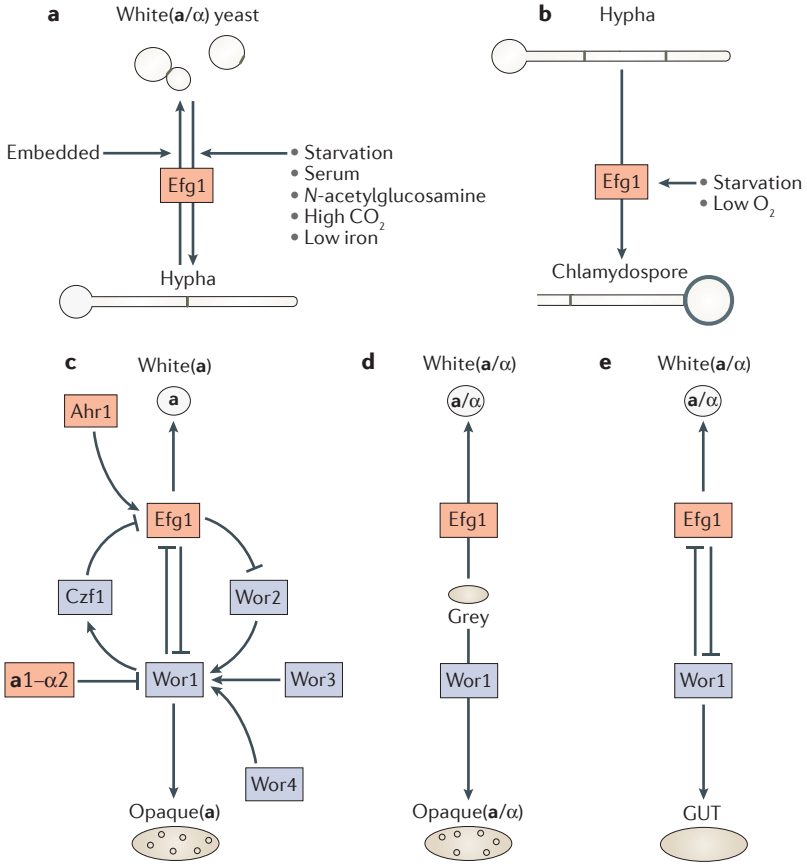


Figure 4 | **The Efg1 and Wor1 transcription factors have central roles in *Candida albicans* morphological plasticity.** Genetic studies have revealed roles for the transcription factors enhanced filamentous growth protein 1 (Efg1) and white–opaque regulator 1 (Wor1) in numerous morphological transitions. In each example in the figure, arrows indicate activation and bars represent inhibition. **a** | Efg1 promotes white(**a**/α) cells to undergo the yeast-to-hypha transition following exposure to host serum, *N*-acetylglucosamine, high levels of CO_2, nutrient depletion and/or iron depletion[130]; however, under agar-embedded conditions, Efg1 promotes the reverse hypha-to-yeast transition[132]. **b** | Efg1 promotes the production of chlamydospores by white(**a**/α) hyphal suspensor cells under nutrient-poor, oxygen-depleted conditions[51,52]. **c** | Efg1 promotes[131] and Wor1 opposes[73–75] the opaque(**a** or α)-to-white(**a** or α) switch, which is also controlled by additional transcription factors in a complex regulatory circuit. Additional regulators include **a**1–α2, Wor2, Wor3, Wor4, Czf1, adhesion and hyphal regulator 1 (Ahr1) and more than a dozen recently identified regulators that are not depicted in the figure[139,141–145]. Wor2, Wor3 and Wor4 induce the expression of *WOR1*, and Wor1 promotes the white(**a** or α)-to-opaque(**a** or α) switch; Wor1 also inhibits the expression of *EFG1* (both directly and indirectly; for example, through Czf1), thereby inhibiting the switch from opaque(**a** or α) to white(**a** or α). By contrast, Efg1 (induced by Ahr1) promotes the opaque(**a** or α)-to-white(**a** or α) switch and also (directly and indirectly; for example, through Wor2) inhibits the expression of *WOR1*, thus inhibiting the switch from white(**a** or α) to opaque(**a** or α). 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 in individual cells[75,139]. Under conditions that strongly favour white(**a** or α) cells (glucose-containing medium at 37 °C) or opaque(**a** or α) cells (*N*-acetylglucosamine-containing medium in ≥5% CO_2), switching occurs in one direction across the entire cell population[55,65–67]. **d** | Efg1 promotes the grey-to-white(**a**/α) switch and Wor1 promotes the grey-to-opaque(**a**/α) switch[14,15]. Grey cells are favoured under nutrient-rich conditions, whereas opaque(**a**/α) cells are favoured under nutrient-limited conditions in the presence of *N*-acetylglucosamine and increased CO_2 (REF. 15). **e** | Efg1 promotes and Wor1 opposes the gastrointestinally induced transition (GUT)-to-white(**a**/α) switch[16]. White(**a**/α) cells are favoured under all tested conditions except for within the mammalian gastrointestinal tract.

In *MTL* homozygous opaque(**a** or α) cells, the activation of Cek1 by mating pheromones triggers the expression of genes that are required for mating[107–111]. In addition, various forms of cell stress (oxidative, osmotic and cell wall damage) affect filamentation indirectly through the Hog1 signalling pathway, which inhibits Cek1 and activates a transcriptional inhibitor of filamentation[112–122]. In white(**a**/α) cells that are exposed to alkaline pH, the Rim101 pH sensing pathway proteolytically activates the Rim101 transcription factor, which leads to the activation of hypha-specific genes and filamention[123–126]. Additional, less well-described pathways negatively regulate filamentation in response to oxygen and nutrients[127,128]. Notably, the depicted pathways do not account for certain observations in the host, such as the finding that white(**a**/α) yeasts seem to predominate in the mammalian gastrointestinal tract[129], despite relatively high concentrations of *N*-acetylglucosamine and CO_2 in this niche, which would be expected to trigger filamentation or the white(**a** or α)-to-opaque(**a** or α) switch based on *in vitro* evidence. Such discrepancies suggest that additional signalling pathways and/or crosstalk between existing pathways remain to be discovered. The multiplicity and complexity of the known signalling pathways suggest a model in which *C. albicans* continuously surveys the mammalian host, integrating various signalling inputs to generate adaptive responses to the local environment.

Transcriptional regulation of morphogenesis. Remarkably, every morphological transition that has been described in this Review is regulated, to some extent, by the transcription factor Efg1 (REFS 14–16, 130–132) (FIG. 4). The roles of Efg1 in *C. albicans* morphogenesis were deduced from the 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 the activity of Efg1 in the control of the white(**a** or α)-to-opaque(**a** or α), white(**a**/α)-to-grey-to-opaque(**a**/α), and white(**a**/α)-to-GUT transitions[14–16,73–75] (FIG. 4c–e). Efg1 and Wor1 are fungal-specific transcription factors the orthologues of which regulate diverse morphological transitions in different fungal species[133–138]. 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].

The regulation of *C. albicans* cell shape is more than just a simple function of Efg1 and Wor1 levels, however, because the activity of each factor is influenced by genotype at the *MTL* locus and local environmental cues. For example, following exposure to host serum, *N*-acetylglucosamine, high levels of CO_2, nutrient depletion and/or iron depletion, Efg1 promotes white(**a**/α) cells to undergo the yeast-to-hypha transition[130]; however, under agar-embedded conditions, Efg1 promotes the reverse transition from hypha to yeast[132] (FIG. 4a). In another case, under low oxygen, nutrient-depleted conditions, Efg1 promotes **a**/α hyphae and pseudohyphae to produce chlamydospores[33] (FIG. 4b). By contrast, following exposure to glucose and low levels of CO_2, Efg1 promotes opaque(**a** or α) cells to switch to the white(**a** or α) state[65] (FIG. 4c) and certain clinical isolates to undergo

Dimorphic fungi
A set of human fungal pathogens that grow as mycelia in the environment but as yeast (or spherules, in the case of *Coccidioides immitis*) in mammalian hosts. These pathogens include *Blastomyces dermatitidis*, *C. immitis*, *Histoplasma capsulatum*, *Paracoccidioides brasiliensis*, *Talaromyces marneffei* (formerly known as *Penicillium marneffei*) and *Sporothrix schenckii*.

a grey-to-white(**a**/α) switch or a grey-to-opaque(**a**/α) switch[14,15] (FIG. 4d). Finally, Efg1 prevents the white(**a**/α)-to-GUT switch in all tested environments other than the mammalian gastrointestinal tract[16] (FIG. 4e).

A key unanswered question is how the genotype of the *MTL* locus and signals that are 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** or α)-to-opaque(**a** or α) switch of *MTL***a** and *MTL*α cells[73–75,139,141–145] (FIG. 4c). Importantly, this switch is controlled by numerous transcription factors in addition to the 'master regulators' Efg1 and Wor1[73–75,139,141–145], which together form an interlocking circuit of positive and negative feedback loops[139,146]. Under conditions in which pro-white(**a** or α) and pro-opaque(**a** or α) environmental cues are balanced (for example, glucose-containing medium that is maintained at 25 °C), switching between white(**a** or α) and opaque(**a** or α) cell types occurs infrequently and stochastically in single cells, and both white(**a** or α)-to-opaque(**a** or α) and opaque(**a** or α)-to-white(**a** or α) switching occurs[55]. In this setting, cell morphology is thought to be programmed by the predominance of either the Efg1 or Wor1 protein, such that switching might be triggered by events such as the unequal distribution of the Efg1 or Wor1 protein between a mother cell and daughter cell[75,139]. By contrast, under environmental conditions that heavily favour the white(**a** or α) state (glucose-containing medium maintained in room air at 37 °C) or opaque(**a** or α) state (*N*-acetylglucosamine-containing medium in ≥5% CO_2), switching occurs in a concerted fashion across the entire cell population[55,65–67]. 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 signalling molecules to a large variety of potential morphological outcomes.

Conclusions

Even within the morphologically diverse fungal kingdom, *C. albicans* stands out because of its remarkable plasticity. Not only does it shift between single-celled yeast and mycelial forms, similar to dimorphic fungi, but this continuously host-associated species also switches between at least six distinct yeast-like morphotypes. Recent observations of GUT, opaque(**a**/α) and grey 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 through several mechanisms, including metabolic polarization. Whereas hyphae and pseudohyphae predominate in most virulence models, with white(**a**/α) 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 based on their superior fitness in an intestinal commensalism model[16]. Meanwhile, opaque(**a**/α) and opaque(**a** or α) cells have been reported to outperform other cell types during 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 morphological specialization would provide a potential rationale for the introduction of the white(**a** or α)-to-opaque(**a** or α) switch into the *C. albicans* mating programme. That is, switching to the opaque(**a** or α) cell type could have less to do with the mechanics of mating per se than with optimizing the fitness of mating-competent cells in the host niche in which mating occurs (a matter of continued speculation in the field).

It is clear that much remains to be learned of the signals, fungal signalling pathways and transcriptional regulatory networks that control morphogenesis in *C. albicans*. 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 and pathogen of humans, the answers to these questions will have important implications for human health and disease.

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Competing interests statement
The authors declare no competing interests.