FUNGAL BIOLOGY REVIEWS 33 (2019) 180–189

British Mycological Society promoting fungal science

journal homepage: www.elsevier.com/locate/fbr

Review

Role of *Candida albicans* mating in genetic variability and adaptation to the host

Inês CORREIA[a], *Elvira ROMÁN*[b], *Daniel PRIETO*[b], *Susana HIDALGO-VICO*[b], *Rebeca ALONSO-MONGE*[b], *Jesús PLA*[b,*]

[a]*Biomolecular Structure and Function Group, Instituto de Biologia Molecular e Celular — IBMC, Instituto para a Investigação e Inovação em Saúde - i3S, Rua Alfredo Allen, 208, 4200-135, Porto, Portugal*
[b]*Departamento de Microbiología y Parasitología-IRYCIS, Facultad de Farmacia, Universidad Complutense de Madrid, Plaza de Ramón y Cajal s/n, E-28040, Madrid, Spain*

ARTICLE INFO

Article history:
Received 9 November 2018
Received in revised form
7 March 2019
Accepted 11 June 2019

Keywords:
Candida albicans
Epigenetic
Mating
Morphogenesis
Variability
White opaque

ABSTRACT

Since its discovery at the end of the XIX century, *Candida albicans* has emerged as one of the most important human pathogenic fungi. This yeast efficiently colonizes the gastrointestinal cavity of humans, which is an important source for gastrointestinal-mediated dissemination of the fungus to internal organs under immune suppression. Controlling colonization may therefore lead to the eradication of *C. albicans* which may, in turn, be a useful strategy in the prevention of candidiasis. Recent studies indicate that colonization is influenced by -and related to-the white opaque (**wo**) transition, an epigenetic transition that has been shown to mediate several aspects of the biology of this fungus. Efficient mating in *C. albicans* occurs by a two-step process which involves the conversion to a homozygous mating type cell followed by a transition to the opaque state. The discovery of the opaque cell as the mating competent phase of this fungus provided an interesting evolutionary example of the role of mating in the adaptation to a mammalian host in a pathogenic fungus. A full sexual cycle has not been observed; rather, after mating, return to a diploid state is achieved by concerted chromosome loss, being this an important source of genetic variability for this opportunistic pathogen.

1. Introduction

Candida species are the most prevalent fungal etiological agents of life-threatening invasive infections (called candidiasis), representing ~19 % of the infections in intensive care units of several developed and non-developed countries. This microorganism behaves as a harmless commensal of the gastrointestinal and vaginal tract of humans in a significant (albeit not precisely determined) percentage of individuals. Its proliferation is normally controlled by the host immune system, and defects in it can result in the development of disease (Gow *et al.*, 2012; Iliev and Leonardi, 2017; Leonardi *et al.*, 2018; Netea and Brown, 2012; Romani, 2011). Predisposing factors include underlying diseases such as

---

[*] *Corresponding author*. Fax: +34 91 3941745.
E-mail address: jpla@ucm.es (J. Pla).

https://doi.org/10.1016/j.fbr.2019.06.001

neutropenia, leukemia, physical injuries or disruption of the gut mucosal barriers. Under these conditions, the fungus is able to disseminate to target organs causing severe disease. *C. albicans* is therefore considered an opportunistic pathogen and it is today probably the best model to analyze human fungal pathogenesis. No unique pathogenic determinant has been made responsible for its virulence. Rather, studies from several laboratories have revealed several traits that contribute to it, such as the ability to grow with different morphologies, the presence of different adhesion molecules and lytic enzymes, its metabolic flexibility and its ability to respond to environmental stress via signaling pathways. A quite interesting feature of this fungus is its genetic variability and its ability to engage in morphogenetic differentiation programs, such as the white opaque transition, an epigenetic transition which influence several aspects of its adaptation to the host. We will review here key findings that relate to the discovery of its mating cycles with other aspects of its biology such as virulence, biofilm formation and adaptation to the commensal state, providing a historical perspective.

2. Morphogenesis

The first descriptions of the microorganism known today as *Candida albicans* were done in the first half of the nineteenth century. In 1841, F.T. Berg established that thrush was caused by a mould-like fungus. Robin named this fungus found in different human tissues as *Oidium albicans* and its presence was correlated with diverse superficial and mucosal diseases (i.e. *champignon du muguet* or thrush). From the very initial observations (Audrey, 1887) it was determined that thrush was associated with a fungus whose form varied according to the medium on which it grew. Therefore, its dimorphic nature -the presence of two different morphologies-was soon discovered (Bourguignon, 1906), although different works soon revealed a polymorphic (rather than dimorphic) nature (reviewed in Barnett, 2008). The naming of this fungus was controversial because it was named *Monilia albicans*, being *Monilia* a genus containing fungi that commonly grow in plants. However, *C. albicans* may only appear in vegetables as a consequence of occasional human contamination. It was reclassified in 1923 as *Candida* by considering the ability of this genus, *Candida,* but not *Monilia*, to infect humans (reviewed in Odds, 1988). The genus *Candida* was originally included in the former *Deuteromycetes* class, which included several fungi were no sexual cycle was discovered (imperfect fungi), but it is now incorporated in the class *Saccharomycetes* (Hibbett *et al.*, 2007) as new molecular biology methodologies have allowed a more precise phylogenetic classification.

As indicated, the term dimorphic transition was coined to refer to the environmentally-triggered conversion of yeast –to-hypha (and less frequently to the inverse) and received substantial attention given its potential role in virulence (Mitchell, 1998; Whiteway and Oberholzer, 2004). However, *C. albicans* can appear as a yeast (unicellular), a hypha, a pseudohypha or a chlamydospore (Fig. 1). Chlamydospores are thick-walled structures that develop under certain conditions such as the absence of light, specific nutrition conditions, low temperature and microaerophilia (Dujardin *et al.*, 1980; Montazeri and Hedrick, 1984). Their presence has been sometimes used to differentiate this organism from other *Candida* species, although other species like *Candida dubliniensis* also form chlamydospores (Staib and Morschhauser, 2007; Sullivan *et al.*, 2005). These structures appear rarely and they have been hypothesized to represent resistance forms although this has not been proved.

The conversion of yeast-to-hypha is favored under laboratory conditions at high (37 °C) temperature, neutral pH (≈7) and the presence of specific nutrients (N-acetylglucosamine, proline or serum), all these features mimicking the mammalian host environment. This process has special relevance in the biology of this fungus due to its implication in virulence and pathogenicity (Alonso-Monge *et al.*, 1999; Braun and Johnson, 1997; Lo *et al.*, 1997). It is currently accepted that both forms (yeast and hypha) play a separate role during infection (Jacobsen *et al.*, 2012) and that the ability to switch between both morphologies adds versatility to this microbe within the host. For example, while yeasts and hyphae are both commonly found in infected organs, one morphology may predominate in a particular organ (Lionakis *et al.*, 2011). Yeast cells, due to their smaller size, have been proposed to mediate fungal dissemination within the bloodstream. However, hyphae play a crucial role during invasion through the endothelial and epithelial layers (Saville *et al.*, 2003) (Moyes *et al.*, 2015; Naglik *et al.*, 2011) and they can inhibit the expression of human defensins in an oral experimental candidiasis model (Lu *et al.*, 2006). Hyphae also seem to be more resistant to phagocytosis, being capable to pierce and kill macrophages upon yeasts ingestion by different mechanisms (Ghosh *et al.*, 2009; Uwamahoro *et al.*, 2014). Different studies demonstrate that mice with specific immune defects (B-cell deficient, T-cell deficient or mice lacking both functional T and B cells) are able to survive to an infection caused by yeasts but not by hyphae, though yeast cells were able to display a lethal pathogenic effect in mice severely immunocompromised by cyclophosphamide-cortisone acetate treatment (Saville *et al.*, 2008). The different morphologies, therefore, represent different fungal adaptation mechanisms to environmental conditions found within the human host, having specific advantages during the stages of colonization, invasion and dissemination. Several authors have discussed the interest of using the dimorphic transition as targets to develop therapeutic drugs (Jacobsen *et al.*, 2012; Noble *et al.*, 2017; Shareck and Belhumeur, 2011).

3. Genetic variability

Although *C. albicans* is today a well-established model in fungal pathogenesis, early studies focused on the genetic identification of virulence traits revealed hard. *C. albicans* has no natural plasmids (such as the *S. cerevisiae* 2μ plasmid) to develop an efficient genetic manipulation system (De Backer *et al.*, 2000). The election of appropriate strains for genetic manipulation was also laborious. A first issue was the determination of its ploidy; the strikingly diverse frequency of isolation of auxotrophs (i.e. loss of heterozygosity) by mutagenesis from different clinical strains pointed towards the existence of natural heterozygosity among isolates (Whelan and

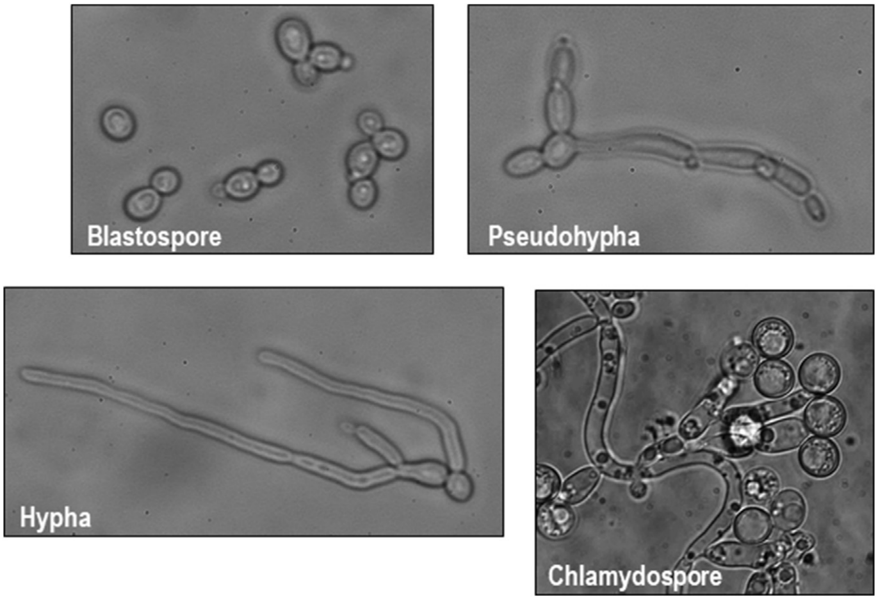

Fig. 1 – Distinct morphological states of *C. albicans*. The figure illustrates the different morphologies associated with *C. albicans*. Blastospores are the yeast unicellular form, with typical elliptical forms and asexual reproduction through budding. Bud elongation under defined environmental conditions triggers the formation of a germ tube that eventually produces a true hypha. Hyphae are different from pseudohyphae by the absence of cellular constrictions. Chlamydospores are round oval refractive cells with a thicker cell wall and are slightly larger than blastopores.

Magee, 1981) and diploidy. This was confirmed when combining precise measures of DNA content by flow cytometry and DNA renaturing studies (Riggsby *et al.*, 1982). The absence of a sexual cycle led to the development of an artificial parasexual cycle by generating protoplasts using cell wall lytic enzymes and subsequent fusion under osmotically protected conditions (Poulter *et al.*, 1981). Parasexual genetics found utility in the analysis of certain genetic traits such as auxotrophic markers or morphology (Gil *et al.*, 1988). Physical mapping of the genome by pulsed field electrophoresis revealed that *C. albicans* had an approximately 16 megabase genome organized in 8 pairs of homologous chromosomes (Chu *et al.*, 1993).

Several early studies using pulsed field electrophoresis reported a high variability of karyotypes among clinical isolates and laboratory strains (Chu *et al.*, 1993; Magee and Magee, 1987; Magee, 1993; Navarro-García *et al.*, 1995; Rustchenko-Bulgac, 1991; Rustchenko, 2007; Thrash-Bingham and Gorman, 1992). Sequencing of the clinical isolate SC5314 strain genome (a starting lineage for many molecular genetic studies-derived strains) (Braun *et al.*, 2005; Tzung *et al.*, 2001) revealed a 14.88 Mb size with the potential to encode 6354 genes (Braun *et al.*, 2005). The group of J. Berman, then, used comparative whole genome hybridization among *C. albicans* strains to demonstrate chromosomal translocations in several laboratory host strains that were routinely used in molecular biology studies (Selmecki *et al.*, 2005). For example, CAI4 (Fonzi and Irwin, 1993) frequently showed chromosome 2 trisomy (Selmecki *et al.*, 2005) in addition to chromosome 1 (Chen *et al.*, 2004). Changes in RM1000 (Negredo *et al.*, 1997) and its derived BWP17 strain (Wilson *et al.*, 1999) occurred during the disruption of the *HIS1* gene (Pla *et al.*, 1995) and resulted in the deletion of all genes telomeric to this gene in one chromosome.

Chromosomal reorganization in *C. albicans* was shown to occur dependent on growing conditions, such as carbon source or the presence of drugs such as 5-fluoroorotic acid, commonly used for the generation of mutant strains. For example, growing in sorbose frequently led to the loss of chromosome 5 (Janbon *et al.*, 1998), while growth in certain carbon sources such as D-arabinose led to chromosome 6 trisomy and chromosome 2 alterations (Rustchenko *et al.*, 1994). These changes were correlated with a distinct colony morphological appearance as occurred in the WO-1 strain (see later) but, most importantly, they were shown to have clinical significance. Fluconazole resistant strains frequently showed chromosome 5 modifications that ultimately lead to the altered expression of certain azole resistance genes (Selmecki et al., 2006, 2008). Interestingly, these changes occurred at higher rates during an experimental mouse infection (that is, *in vivo*) compared to those observed during standard laboratory *in vitro* growth conditions in liquid cultures (Forche et al., 2009, 2011), suggesting that they play a role in the generation of genetic variability during a mammalian infection, and probably, also during colonization. There are other mechanisms involved in the variability of the *C. albicans* genome and the loss of heterozygosity at certain *loci* may also be a consequence of mitotic recombination arising from gene conversion between homologues and subsequent crossovers.

4. Genetics of white-opaque switching

From the very initial observations of this fungus, a high diversity of colony morphologies on solid media was reported. The term phenotypic switching commonly refers to the variability of colonies on agar plates after prolonged periods of incubation. Slutsky referred to this in 1985 in *C. albicans* (Slutsky *et al.*, 1985) as a separate process to the already known yeast-to-hypha transition and which had similarities to the rough-smooth colony phenotype observed in some bacteria. A particular type of switching was observed for a strain called WO-1. This transition (called **wo**, from white-opaque) was spontaneous and occurred between two states that were easily distinguishable by their cellular morphology (Slutsky *et al.*, 1987). White cells grew as yeast like forms and developed standard cream colored colonies. Opaque cells were slightly elongated, had a prominent vacuole and cell surface irregularities (pimples) detectable by scanning electron microscopy and gave rise to easy distinguishable grey colonies on standard media plates (Fig. 2, upper panel); both morphotypes were easily distinguished by their preferential staining with the fluorochrome phloxine-B with white cells having a "normal" phloxine-B negative stain (dark pink) and opaque cells the less frequent phloxine-B positive pinky appearance (Fig. 2, lower panel). The term transition points towards a reversible -but heritable-trait, as white cells produced opaque cells but opaque cells could also lead to white cells and each cell type was stable under specific environmental conditions. After the discovery of the **wo** transition, a substantial effort was done to reveal the distinctive features of opaque cells. Differences in the composition of the cell wall, adhesion to different surfaces and measure recombination frequencies were reported. In addition, phase (white or opaque) specific genes were also identified (see (Soll, 2014b) for an excellent review).

While all these pioneering work highlighted important biological differences between white and opaque cells, it was only after the discovery of a mating type *locus* in *C. albicans* when the role of opaque cells was determined. Two independent simultaneous works demonstrated the ability of *C. albicans* to mate, already suggested by the inspection of its genome as the particular *C. albicans* strain sequenced (SC5314) had all orthologues of *S. cerevisiae* mating type genes and showed heterozygosity at the mating-type-like (MTL) genes MATa and MATα (Hull and Johnson, 1999), similar to what occurs in *S. cerevisiae* diploid cells (Heitman *et al.*, 2014). As mating genes lie on chromosome 5, the conditional loss of this chromosome by growing cells on sorbose allowed the construction of homozygous MTL **a/a** and MTL α/α diploids in the group of P. Magee; these diploids mated and generated recombinant, unicellular tetraploids (Magee and Magee, 2000). Similar results were obtained by genetic manipulation of mating type genes in A. Johnson's laboratory. In addition, engineered strains were able to mate (as determined by auxotrophy analysis and DNA content) following infections with strains of opposite mating type in an experimental model of systemic infection in mice (Hull *et al.*, 2000). Therefore, *C. albicans* was able to mate both in artificial and natural environments.

The relationship between mating type and switching was soon discovered. An analysis of different **wo** switcher strains revealed that they were all homozygous at the mating type *locus* (Lockhart *et al.*, 2002). This work demonstrated that a significant proportion of clinical isolates were mating type homozygous cells. It also showed that **wo** switchers are present in different *C. albicans* clades and therefore, mating competent cells exist in natural patients' populations of this microbe.

The process leading to homozygosity at the mating type occurs spontaneously and is environmentally induced by different mechanisms such as gene conversion, crossing over or even loss of one copy of chromosome 5 (where mating type *locus* resides) followed by duplication of the remaining copy (Wu et al., 2005, 2007). They would be responsible for the natural homozygosity found in approximately 3 % of the clinical isolates (Lockhart *et al.*, 2002; Odds *et al.*, 2007). MTL-homozygous cells then undergo a switch to the opaque phase and this change is epigenetic, each cell type being heritable for many generations without further alterations at the DNA sequence (Lockhart *et al.*, 2003; Soll *et al.*, 2003). The group of Johnson later discovered that MTLa1 and MTLα2 homeodomain proteins coordinately repress **wo** switching and that opaque cells are mating competent, being ~10^6 times more

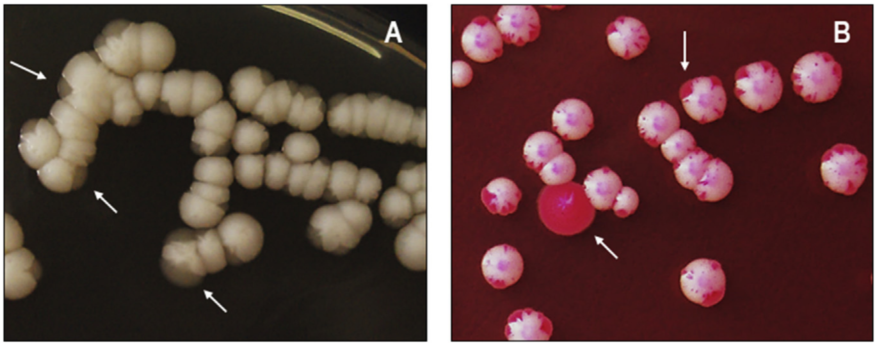

Fig. 2 — Macroscopic appearance of *C. albicans* opaque cells. A) A *C. albicans* wo switcher strain was plated on YPD agar plates and allowed to grow at room temperature (below 21 °C) for three days. Regions of opaque cells are shown (arrows). B) Phloxine B staining of a switcher strain. The incorporation of Phloxine B in YPD plates allows the clear identification of opaque cells as pink sectors of otherwise normal white colonies.

efficient in mating than the corresponding white cells (Miller and Johnson, 2002). MTL-homozygous opaque cells (a/a or α/α) sense opposite mating-type pheromones, resulting in the appearance of a *shmoo* (mating projection) and leading ultimately to nuclear fusion (Bennett and Johnson, 2006; Lockhart *et al.*, 2003).

As *C. albicans* is diploid, cell fusion produces a tetraploid (a/a α/α). Although meiosis-specific genes have been identified in the *C. albicans* genome based on their homology to those present in other fungi, a functional meiotic program has not been observed. Rather, these tetraploids undergo semi random chromosome loss to generate recombinant progeny with shuffled chromosomal combinations; these strains show different phenotypes on plates (Bennett and Johnson, 2003; Forche *et al.*, 2008) and, interestingly, specific meiotic genes (such as *SPO11*) may participate in mitotic recombination. Therefore, the parasexual cycle is an effective mechanism to generate genetic diversity. Interestingly, Alby et al. (2009) have shown evidence for an autocrine-like system; the production of pheromone α cells promotes same-sex mating, therefore being an alternative mechanism for genetic exchange within unisexual populations of *C. albicans*. Recent studies demonstrated that diploid strains can also give rise to rare mating-competent haploid strains (Hickman *et al.*, 2013). These strains have found usefulness in the functional analysis of specific genes in the laboratory, suggesting that chromosome loss allows cells to get rid of recessive lethal alleles.

Identification of the repressive role of the MTL *locus* on **wo** transition enabled the discovery of regulators of **wo** transition by different groups. The group of H. Liu identified Wor1 as a high copy suppressor of invasion defects of *S. cerevisiae flo8* mutants on solid media and showed how increased expression of *WOR1* bypassed the requirements for Flo8 in Flo11 expression (a Flo8 target) (Huang *et al.*, 2006). *wor1* mutants showed no apparent defects in growth and hyphal formation in **a**/α cells but, interestingly, they were blocked in the conversion to the opaque phase. No *WOR1* transcripts were detected in mating-type heterozygous diploid cells, consistent with *WOR1* being a mating-type regulated gene (Tsong *et al.*, 2003). Ectopic expression of *WOR1* from the constitutive *ACT1* promoter resulted in an en-masse conversion to the opaque phase (Huang *et al.*, 2006). Simultaneously, Wor1 was identified as regulator of the **wo** transition by screening of six potential putative **a**1-α2 targets (Zordan *et al.*, 2006). *WOR1* expression was shown to be repressed by the **a**1-α2 repressor complex, but expression of this gene from the *MET3* promoter was sufficient to relieve mating repression in normal diploid **a**/α cells, converting the whole population to the opaque phase. These authors demonstrated that Wor1 binds its own promoter and accumulated in the opaque phase, behaving as a self-sustained feedback mechanism to maintain the opaque phase once Wor1 levels exceed a certain threshold in the cell. Similar results were obtained by the group of D. Soll, who identified Wor1 (then called Tos9) as an interacting partner with the MTL **a**1-α2 corepressor complex via ChIP-chip immunoprecipitation analyses (Srikantha *et al.*, 2006).

Additional regulatory genes of the **wo** transition (*CZF1*, *WOR2, EFG1*, *AHR1* and *WOR3*) have been subsequently identified by different methodologies (Lohse *et al.*, 2013; Wang *et al.*, 2011; Zordan *et al.*, 2007). Efg1 is an essential regulator of morphogenesis, long time known to promote filamentation, chlamydospore formation (Sonneborn *et al.*, 1999a; Stoldt *et al.*, 1997) and to increase the frequency of conversion to the opaque phase (Sonneborn *et al.*, 1999b). Ahr1 is a repressor of this transition as overexpression of this gene promotes the opaque to white conversion, being this process dependent on Efg1. By an analysis of genome wide chromatin immunoprecipitation and transcriptional profiling, three of these regulators (Wor1, Wor2 and Efg1) were found to bind upstream of Ssn6 (Hernday *et al.*, 2016), a role for Ssn6 was hypothesized. Deletion of this gene results in an in-masse conversion of white to opaque cells indicating that it contributes to the stochastic nature of this conversion and represses the activation of Wor1 in white cells. More recently, a role for the general filamentation repressor *TUP1* has been evidenced in the Wor1 regulatory network. Tup1 is a Ssn6 corepressor whose deletion renders the cells locked in the filamentous state (Braun and Johnson, 1997). The group of H. Liu has demonstrated that this protein associates with Wor1 in immunoprecipitation assays (Alkafeef *et al.*, 2018). Although manipulation of *tup1* cells is difficult, they used conditional expression from the *MET3* promoter to show that its absence triggered the conversion to the opaque phase even in the absence of Wor1. They also demonstrated that Tup1 occupies Wor1 auto responsive elements at the *WOR1* promoter and that the absence of *TUP1* bypasses the requirement of *WOR1* for opaque cells formation, thus positioning this transcriptional corepressor downstream Wor1.

5. Role of mating and wo regulation in *C. albicans* biology

The white-opaque transition and its related mating program have relevance in several aspects of the biology of this fungus (Sasse *et al.*, 2013; Soll, 2014b). This is not surprising as approximately 400 genes are differently regulated in both phases. Although several mating-specific genes are up-regulated in the opaque phase, most of them do not seem to be involved in mating pointing towards a role in additional processes (Lan *et al.*, 2002; Tsong *et al.*, 2003; Tuch *et al.*, 2010). We will summarize here the involvement of **wo** switching in those processes that could mediate adaptation to different environments within a mammalian host such as biofilm formation, pathogenicity and commensalism.

C. albicans is able to form a multicellular biofilm both in the commensal and pathogenic state and many infections arise as a result of its ability to grow as a biofilm in medical devices such as venous or urinary catheters and artificial joints (Donlan and Costerton, 2002; Douglas, 2003). Switching plays an important role in the formation of biofilms. In addition to the biofilms produced by *C. albicans* heterozygous cells, homozygous (**a**/**a** or α/α) opaque cells produce pheromones that stimulate biofilm formation in white cells of the opposite mating type (Daniels *et al.*, 2006). The thickness of the biofilm is increased in the presence of a minor proportion ("seed") of opaque cells and the biofilm matrix is optimized to support mating, so that the chemotropism of opaque cells within this specialized environment is augmented. Although they have the same basic architecture as a conventional **a**/α biofilm, they are more permeable to low molecular weight compounds and are more susceptible to antifungal drugs such as fluconazole. These specialized biofilms are responsive to environmental conditions such as the presence of CO_2 and the type of the medium (Daniels *et al*., 2013; Park *et al*., 2013). Mating biofilms would have evolved by recruiting standard components from other pathways (pheromones, receptors and MAPK signaling cascade) to accommodate alternative transcription factors and develop a specific response. These "sexual" biofilms have been suggested as an adaptive mechanism to account for the putative selective pressure for *C. albicans* to mate within the host, which may be more difficult in standard biofilms (Soll, 2014a; Soll and Daniels, 2016).

Multiple studies have provided evidence for attenuated virulence of opaque cells compared to white cells. Opaque cells are less virulent in a mouse model of systemic infection and expression of the white specific gene *WH11* increases virulence (Kvaal *et al*., 1997). Interestingly, opaque cells colonize the skin more readily (Kvaal *et al*., 1999; Tsuboi *et al*., 1994) promoting the formation of cavities in the skin surface. Opaque cells are also capable of forming hyphae as do standard *C. albicans* cells, however they do not undergo the yeast-to-hypha transition under many conditions that induce it in white cells. This may be related to the decreased capacity of opaque cells to escape from phagocytes and to invade tissues (Anderson *et al*., 1989; Si *et al*., 2013). Opaque cells also seem to be more susceptible to reactive oxygen species (ROS) and therefore to phagocyte-mediated killing (Kolotila and Diamond, 1990), despite being able to avoid recognition and phagocytosis by macrophages and neutrophils. One possible mechanism could be loss of the capacity to release chemoattractants for PMNs (Geiger *et al*., 2004). *Drosophila* hemocyte-derived S2 and mouse RAW264.7 cell lines preferentially phagocytize white cells compared to opaque cells (Sasse *et al*., 2013); when mixed populations of white and opaque cells were mixed with neutrophils, white cells were more readily ingested in planktonic cultures but not under matrix embedded conditions which was thought to be caused by the lag in germ tube formation of opaque cells.

If the **wo** transition evolved to facilitate mating within the host, it may also play a role in commensalism, which is the natural state of *C. albicans* within an immunocompetent host. The development of commensal models (Bendel *et al*., 2002; Kinneberg *et al*., 1999; Koh, 2013; Neville *et al*., 2015; Prieto *et al*., 2016) has allowed identification of processes crucial for colonization of the mouse gut such as iron and glucose metabolism, signal transduction pathways and morphogenetic transitions (Noble, 2013; Perez *et al*., 2013; Pierce and Kumamoto, 2012; Prieto *et al*., 2014; Ramírez-Zavala *et al*., 2017; Vautier *et al*., 2015). Early work from the C. Kumamoto laboratory revealed a role for the Efh1 transcription factor in colonization, as this mutant showed increased colonization over wild type (wt) cells in the mouse gut and overexpression of Efh1 led to reduced colonization (White *et al*., 2007) suggesting that *C. albicans* was able to control its proliferation in this environment. In addition, *efg1* mutants showed enhanced fitness in this niche compared to wt cells (Pierce and Kumamoto, 2012). Interestingly, Efg1 was previously identified as a regulator of the white opaque (**wo**) transition (Sonneborn *et al*., 1999b).

The opaque phenotype cannot be maintained at physiological temperature (Slutsky *et al*., 1987; Rikkerink *et al*., 1988), at temperatures above 25 °C opaque cells revert to the white (Soll *et al*., 1993). This is consistent with white cells being mainly recovered from the kidneys of mice upon opaque cell injection (Kvaal *et al*., 1997) and it also supports the observation that opaque cells are able to colonize the skin, a niche with a lower temperature. Interestingly, the presence of high levels of CO_2, N-acetylglucosamine and anaerobic conditions promote the white-opaque transition even at 37 °C (Huang et al., 2009, 2010; Ramirez-Zavala *et al*., 2008), suggesting that this morphotype may play a role under defined host environments. In accordance with this, a novel morphological switch specific to the adaptation of the commensal lifestyle has been recently described. Upon passage through the mouse gastrointestinal tract, *C. albicans* cells with artificially augmented expression of *WOR1* (Pande *et al*., 2013) switched to a GUT (gastrointestinally induced transition) morphotype. GUT cells differ from opaque cells by surface ultrastructural details, by their *in vivo* fitness (low in opaque cells, high in GUT) and a differentiated transcriptomal program. In fact, cells overexpressing *WOR1* display an increased adhesion capacity specifically to gut mucosa (Prieto *et al*., 2017). In addition, a ''white-gray-opaque'' tristable phenotypic switching system has been discovered, which is dependent on the Wor1-Efg1 regulators (Tao *et al*., 2014). Gray cells can be cultured *in vitro*, they are smaller than GUT and opaque cells and exhibit a unique global gene expression profile. Gray cells express a MTL-dependent high aspartyl protease activity, mate less efficiently than opaque cells and have a distinct effect on virulence and organ lodging upon systemic and cutaneous infection indicating that they could play separate roles in *C. albicans* biology within the host.

Pathogenic fungi as *C. albicans*, may live either aerobically, with high levels of O_2 in some mucosal tissues, or inside the human body under different degrees of hypoxia (reviewed in (Ernst and Tielker, 2009)). Therefore, fungal adaptation to different niches is not only required due to varying availability of nutrients and metals but also of oxygen and carbon dioxide. Inside the human body, CO_2 levels are around 100-fold higher than in the atmosphere, ranging between 4.5 and 30 %. Microorganisms themselves can generate and secrete CO_2 into their microenvironment and there is evidence for a gaseous signaling pathway that regulates their morphology and, consequently, virulence (Hall *et al*., 2010). The availability of both elements can therefore modulate other virulence factors, such as the yeast-to-hypha transition in *C. albicans*: while CO_2 has been shown to induce filamentous growth (Klengel *et al*., 2005; Hall *et al*., 2010) and stabilize the opaque phenotype (Huang *et al*., 2009), it also stabilizes cells in the opaque state allowing for mating at 37 °C (Dumitru *et al*., 2007; Ramirez-Zavala *et al*., 2008), and enhances filamentation on agar as well (Setiadi *et al*., 2006; Sonneborn *et al*., 1999a).

6. Conclusions

The existence of a mating cycle in *C. albicans* has been found in other closely related yeasts within the CUG clade (Fitzpatrick *et al.*, 2006), although a high diversity in the mating type *locus* has been observed. As meiosis has not been detected in *C. albicans*, genetic diversity is generated by the formation of tetraploid cells and reduction to the natural diploid state by a parasexual cycle with chromosome reorganizations. Other species within the CUG clade show high diversity at the MTL *locus*, as well as the absence of specific genes. What is peculiar (although not specific) to *C. albicans* is the necessity to engage into a different program (white-opaque) to become mating competent. Such conversion is epigenetically regulated and promoted by various environmental conditions As *C. albicans* has no significant saprophytic life outside the host, the study of this transition during growth under laboratory conditions is somewhat artificial. The definition of the specific host conditions (niches, microenvironments and signals) that promote this process will be of primary importance and can be accomplished using appropriate genetic screenings. Furthermore, it is conceivable that with proper visualization tools, the mating process of *C. albicans* could be observed and analyzed *in vivo*, that is, within a mammalian host and in its native colonizing niches. This may ultimately lead to a more complete knowledge of the mechanisms that underlie genetic variability of this fungus and its adaptation to the commensal state. Both facts may enable the control of fungal populations in the human host in a future.

Acknowledgments

We thank Anja Forche for critical reading of the manuscript and very helpful comments. Work in our laboratory is supported by the Spanish Ministerio de Economía y Competitividad under Grant PGC2018-095047-B-100 and by Comunidad de Madrid under InGEMICS-CM S2017/BMD3691 (Group COMIPAT).

REFERENCES

Alby, K., Schaefer, D., Bennett, R.J., 2009. Homothallic and heterothallic mating in the opportunistic pathogen *Candida albicans*. Nature 460, 890–893.

Alkafeef, S.S., Yu, C., Huang, L., Liu, H., 2018. Wor1 establishes opaque cell fate through inhibition of the general co-repressor Tup1 in *Candida albicans*. PLoS Genet. 14, e1007176.

Alonso-Monge, R., Navarro-García, F., Molero, G., Díez-Orejas, R., Gustin, M., Pla, J., Sánchez, M., Nombela, C., 1999. Role of the mitogen-activated protein kinase Hog1p in morphogenesis and virulence of *Candida albicans*. J. Bacteriol. 181, 3058–3068.

Anderson, J., Cundiff, L., Schnars, B., Gao, M.X., Mackenzie, I., Soll, D.R., 1989. Hypha formation in the white-opaque transition of *Candida albicans*. Infect. Immun. 57, 458–467.

Audrey, C., 1887. Sur l'evolution du champignon du muguet. Rev. Med. 7, 586–595.

Barnett, J.A., 2008. A history of research on yeasts 12: medical yeasts part 1, *Candida albicans*. Yeast 25, 385–417.

Bendel, C.M., Wiesner, S.M., Garni, R.M., Cebelinski, E., Wells, C.L., 2002. Cecal colonization and systemic spread of *Candida albicans* in mice treated with antibiotics and dexamethasone. Pediatr. Res. 51, 290–295.

Bennett, R.J., Johnson, A.D., 2003. Completion of a parasexual cycle in *Candida albicans* by induced chromosome loss in tetraploid strains. EMBO J. 22, 2505–2515.

Bennett, R.J., Johnson, A.D., 2006. The role of nutrient regulation and the Gpa2 protein in the mating pheromone response of *Candida albicans*. Mol. Microbiol. 62, 100–119.

Bourguignon, G., 1906. Formes microbiennes du champignon du muguet. (morphologie et pathologie expérimentale). Cour d'Appel.

Braun, B.R., Johnson, A.D., 1997. Control of filament formation in *Candida albicans* by the transcriptional repressor *TUP1*. Science 277, 105–109.

Braun, B.R., Van Het, H.M., d'Enfert, C., Martchenko, M., Dungan, J., Kuo, A., Inglis, D.O., Uhl, M.A., Hogues, H., Berriman, M., Lorenz, M., Levitin, A., Oberholzer, U., Bachewich, C., Harcus, D., Marcil, A., Dignard, D., Iouk, T., Zito, R., Frangeul, L., Tekaia, F., Rutherford, K., Wang, E., Munro, C.A., Bates, S., Gow, N.A., Hoyer, L.L., Kohler, G., Morschhauser, J., Newport, G., Znaidi, S., Raymond, M., Turcotte, B., Sherlock, G., Costanzo, M., Ihmels, J., Berman, J., Sanglard, D., Agabian, N., Mitchell, A.P., Johnson, A.D., Whiteway, M., Nantel, A., 2005. A human-curated annotation of the *Candida albicans* Genome. PLoS Genet. 1, e1.

Chen, X., Magee, B.B., Dawson, D., Magee, P.T., Kumamoto, C.A., 2004. Chromosome 1 trisomy compromises the virulence of *Candida albicans*. Mol. Microbiol. 51, 551–565.

Chu, W.S., Magee, B.B., Magee, P.T., 1993. Construction of an *Sfi*I macrorestriction map of the *Candida albicans* genome. J. Bacteriol. 175, 6637–6651.

Daniels, K.J., Park, Y.N., Srikantha, T., Pujol, C., Soll, D.R., 2013. Impact of environmental conditions on the form and function of *Candida albicans* biofilms. Eukaryot. Cell 12, 1389–1402.

Daniels, K.J., Srikantha, T., Lockhart, S.R., Pujol, C., Soll, D.R., 2006. Opaque cells signal white cells to form biofilms in *Candida albicans*. EMBO J. 25, 2240–2252.

De Backer, M.D., Magee, P.T., Pla, J., 2000. Recent developments in molecular genetics of *Candida albicans*. Annu. Rev. Microbiol. 54, 463–498.

Donlan, R.M., Costerton, J.W., 2002. Biofilms: survival mechanisms of clinically relevant microorganisms. Clin. Microbiol. Rev. 15, 167–193.

Douglas, L.J., 2003. *Candida* biofilms and their role in infection. Trends Microbiol. 11, 30–36.

Dujardin, L., Walbaum, S., Biguet, J., 1980. Effect of glucose and nitrogen concentrations on the morphology of *Candida albicans* and the formation of chlamydospores in synthetic culture media. Mycopathologia 71, 113–118.

Dumitru, R., Navarathna, D.H., Semighini, C.P., Elowsky, C.G., Dumitru, R.V., Dignard, D., Whiteway, M., Atkin, A.L., Nickerson, K.W., 2007. *In vivo* and *in vitro* anaerobic mating in *Candida albicans*. Eukaryot. Cell 6, 465–472.

Ernst, J.F., Tielker, D., 2009. Responses to hypoxia in fungal pathogens. Cell. Microbiol. 11, 183–190.

Fitzpatrick, D.A., Logue, M.E., Stajich, J.E., Butler, G., 2006. A fungal phylogeny based on 42 complete genomes derived from supertree and combined gene analysis. BMC Evol. Biol. 6, 99.

Fonzi, W.A., Irwin, M.Y., 1993. Isogenic strain construction and gene mapping in *Candida albicans*. Genetics 134, 717–728.

Forche, A., Abbey, D., Pisithkul, T., Weinzierl, M.A., Ringstrom, T., Bruck, D., Petersen, K., Berman, J., 2011. Stress alters rates and types of loss of heterozygosity in *Candida albicans*. MBio 2.

Forche, A., Alby, K., Schaefer, D., Johnson, A.D., Berman, J., Bennett, R.J., 2008. The parasexual cycle in *Candida albicans* provides an alternative pathway to meiosis for the formation of recombinant strains. PLoS Biol. 6, e110.

Forche, A., Magee, P.T., Selmecki, A., Berman, J., May, G., 2009. Evolution in *Candida albicans* populations during a single passage through a mouse host. Genetics 182, 799–811.

Geiger, J., Wessels, D., Lockhart, S.R., Soll, D.R., 2004. Release of a potent polymorphonuclear leukocyte chemoattractant is regulated by white-opaque switching in *Candida albicans*. Infect. Immun. 72, 667–677.

Ghosh, S., Navarathna, D.H., Roberts, D.D., Cooper, J.T., Atkin, A.L., Petro, T.M., Nickerson, K.W., 2009. Arginine-induced germ tube formation in *Candida albicans* is essential for escape from murine macrophage line RAW 264.7. Infect. Immun. 77, 1596–1605.

Gil, C., Pomés, R., Nombela, C., 1988. A complementation analysis by parasexual recombination of *Candida albicans* morphological mutants. J. Gen. Microbiol. 134, 1587–1595.

Gow, N.A., van de Veerdonk, F.L., Brown, A.J., Netea, M.G., 2012. *Candida albicans* morphogenesis and host defence: discriminating invasion from colonization. Nat. Rev. Microbiol. 10, 112–122.

Hall, R.A., De Sordi, L., Maccallum, D.M., Topal, H., Eaton, R., Bloor, J.W., Robinson, G.K., Levin, L.R., Buck, J., Wang, Y., Gow, N.A., Steegborn, C., Muhlschlegel, F.A., 2010. CO(2) acts as a signalling molecule in populations of the fungal pathogen *Candida albicans*. PLoS Pathog. 6, e1001193.

Heitman, J., Carter, D.A., Dyer, P.S., Soll, D.R., 2014. Sexual reproduction of human fungal pathogens. Cold Spring Harb. Perspect. Med. 4.

Hernday, A.D., Lohse, M.B., Nobile, C.J., Noiman, L., Laksana, C.N., Johnson, A.D., 2016. Ssn6 defines a new level of regulation of white-opaque switching in *Candida albicans* and is required for the stochasticity of the switch. MBio 7, e01565-01515.

Hibbett, D.S., Binder, M., Bischoff, J.F., Blackwell, M., Cannon, P.F., Eriksson, O.E., Huhndorf, S., James, T., Kirk, P.M., Lucking, R., Thorsten Lumbsch, H., Lutzoni, F., Matheny, P.B., McLaughlin, D.J., Powell, M.J., Redhead, S., Schoch, C.L., Spatafora, J.W., Stalpers, J.A., Vilgalys, R., Aime, M.C., Aptroot, A., Bauer, R., Begerow, D., Benny, G.L., Castlebury, L.A., Crous, P.W., Dai, Y.C., Gams, W., Geiser, D.M., Griffith, G.W., Gueidan, C., Hawksworth, D.L., Hestmark, G., Hosaka, K., Humber, R.A., Hyde, K.D., Ironside, J.E., Koljalg, U., Kurtzman, C.P., Larsson, K.H., Lichtwardt, R., Longcore, J., Miadlikowska, J., Miller, A., Moncalvo, J.M., Mozley-Standridge, S., Oberwinkler, F., Parmasto, E., Reeb, V., Rogers, J.D., Roux, C., Ryvarden, L., Sampaio, J.P., Schussler, A., Sugiyama, J., Thorn, R.G., Tibell, L., Untereiner, W.A., Walker, C., Wang, Z., Weir, A., Weiss, M., White, M.M., Winka, K., Yao, Y.J., Zhang, N., 2007. A higher-level phylogenetic classification of the Fungi. Mycol. Res. 111, 509–547.

Hickman, M.A., Zeng, G., Forche, A., Hirakawa, M.P., Abbey, D., Harrison, B.D., Wang, Y.M., Su, C.H., Bennett, R.J., Wang, Y., Berman, J., 2013. The 'obligate diploid' *Candida albicans* forms mating-competent haploids. Nature 494, 55–59.

Huang, G., Srikantha, T., Sahni, N., Yi, S., Soll, D.R., 2009. CO_2 regulates white-to-opaque switching in *Candida albicans*. Curr. Biol. 19, 330–334.

Huang, G., Wang, H., Chou, S., Nie, X., Chen, J., Liu, H., 2006. Bistable expression of *WOR1*, a master regulator of white-opaque switching in *Candida albicans*. Proc. Natl. Acad. Sci. U S A 103, 12813–12818.

Huang, G., Yi, S., Sahni, N., Daniels, K.J., Srikantha, T., Soll, D.R., 2010. N-acetylglucosamine induces white to opaque switching, a mating prerequisite in *Candida albicans*. PLoS Path. 6, e1000806.

Hull, C.M., Johnson, A.D., 1999. Identification of a mating type-like locus in the asexual pathogenic yeast *Candida albicans*. Science 285, 1271–1275.

Hull, C.M., Raisner, R.M., Johnson, A.D., 2000. Evidence for mating of the "asexual" yeast *Candida albicans* in a mammalian host. Science 289, 307–310.

Iliev, I.D., Leonardi, I., 2017. Fungal dysbiosis: immunity and interactions at mucosal barriers. Nat. Rev. Immunol. 17, 635–646.

Jacobsen, I.D., Wilson, D., Wachtler, B., Brunke, S., Naglik, J.R., Hube, B., 2012. *Candida albicans* dimorphism as a therapeutic target. Expert. Rev. Anti. Infect. Ther. 10, 85–93.

Janbon, G., Sherman, F., Rustchenko, E., 1998. Monosomy of a specific chromosome determines L-sorbose utilization: a novel regulatory mechanism in *Candida albicans*. Proc. Natl. Acad. Sci. U S A 95, 5150–5155.

Kinneberg, K.M., Bendel, C.M., Jechorek, R.P., Cebelinski, E.A., Gale, C.A., Berman, J.G., Erlandsen, S.L., Hostetter, M.K., Wells, C.L., 1999. Effect of *INT1* gene on *Candida albicans* murine intestinal colonization. J. Surg. Res. 87, 245–251.

Klengel, T., Liang, W.J., Chaloupka, J., Ruoff, C., Schroppel, K., Naglik, J.R., Eckert, S.E., Mogensen, E.G., Haynes, K., Tuite, M.F., Levin, L.R., Buck, J., Muhlschlegel, F.A., 2005. Fungal adenylyl cyclase integrates CO_2 sensing with cAMP signaling and virulence. Curr. Biol. 15, 2021–2026.

Koh, A.Y., 2013. Murine models of *Candida* gastrointestinal colonization and dissemination. Eukaryot. Cell 12, 1416–1422.

Kolotila, M.P., Diamond, R.D., 1990. Effects of neutrophils and in vitro oxidants on survival and phenotypic switching of *Candida albicans* WO-1. Infect. Immun. 58, 1174–1179.

Kvaal, C., Lachke, S.A., Srikantha, T., Daniels, K., McCoy, J., Soll, D.R., 1999. Misexpression of the opaque-phase-specific gene *PEP1* (*SAP1*) in the white phase of *Candida albicans* confers increased virulence in a mouse model of cutaneous infection. Infect. Immun. 67, 6652–6662.

Kvaal, C.A., Srikantha, T., Soll, D.R., 1997. Misexpression of the white-phase-specific gene *WH11* in the opaque phase of *Candida albicans* affects switching and virulence. Infect. Immun. 65, 4468–4475.

Lan, C.Y., Newport, G., Murillo, L.A., Jones, T., Scherer, S., Davis, R.W., Agabian, N., 2002. Metabolic specialization associated with phenotypic switching in *Candida albicans*. Proc. Natl. Acad. Sci. U S A 99, 14907–14912.

Leonardi, I., Li, X., Semon, A., Li, D., Doron, I., Putzel, G., Bar, A., Prieto, D., Rescigno, M., McGovern, D.P.B., Pla, J., Iliev, I.D., 2018. CX3CR1(+) mononuclear phagocytes control immunity to intestinal fungi. Science 359, 232–236.

Lionakis, M.S., Lim, J.K., Lee, C.C., Murphy, P.M., 2011. Organ-specific innate immune responses in a mouse model of invasive candidiasis. J. Innate Immun. 3, 180–199.

Lo, H.J., Kohler, J.R., DiDomenico, B., Loebenberg, D., Cacciapuoti, A., Fink, G.R., 1997. Nonfilamentous *Candida albicans* mutants are avirulent. Cell 90, 939–949.

Lockhart, S.R., Pujol, C., Daniels, K.J., Miller, M.G., Johnson, A.D., Pfaller, M.A., Soll, D.R., 2002. In *Candida albicans*, white-opaque switchers are homozygous for mating type. Genetics 162, 737–745.

Lockhart, S.R., Zhao, R., Daniels, K.J., Soll, D.R., 2003. Alpha-pheromone-induced "shmooing" and gene regulation require white-opaque switching during *Candida albicans* mating. Eukaryot. Cell 2, 847–855.

Lohse, M.B., Hernday, A.D., Fordyce, P.M., Noiman, L., Sorrells, T.R., Hanson-Smith, V., Nobile, C.J., DeRisi, J.L., Johnson, A.D., 2013. Identification and characterization of a previously undescribed family of sequence-specific DNA-binding domains. Proc. Natl. Acad. Sci. U S A 110, 7660–7665.

Lu, Q., Jayatilake, J.A., Samaranayake, L.P., Jin, L., 2006. Hyphal invasion of*Candida albicans* inhibits the expression of human beta-defensins in experimental oral candidiasis. J Invest Dermatol 126, 2049–2056.

Magee, B.B., Magee, P.T., 1987. Electrophoretic karyotypes and chromosome numbers in *Candida* species. J. Gen. Microbiol. 133, 425–430.

Magee, B.B., Magee, P.T., 2000. Induction of mating in *Candida albicans* by construction of MTLa and MTLα strains. Science 289, 310–313.

Magee, P.T., 1993. Variations in chromosome size and organization in *Candida albicans* and *Candida* stellatoidea. Trends Microbiol. 1, 338–342.

Miller, M.G., Johnson, A.D., 2002. White-opaque switching in *Candida albicans* is controlled by mating-type locus homeodomain proteins and allows efficient mating. Cell 110, 293–302.

Mitchell, A.P., 1998. Dimorphism and virulence in *Candida albicans*. Curr. Opin. Microbiol. 1, 687–692.

Montazeri, M., Hedrick, H.G., 1984. Factors affecting spore formation in a *Candida albicans* strain. Appl. Environ. Microbiol. 47, 1341–1342.

Moyes, D.L., Richardson, J.P., Naglik, J.R., 2015. *Candida albicans*-epithelial interactions and pathogenicity mechanisms: scratching the surface. Virulence 6 (4), 338–346.

Naglik, J.R., Moyes, D.L., Wachtler, B., Hube, B., 2011. *Candida albicans* interactions with epithelial cells and mucosal immunity. Microb. Infect. 13, 963–976.

Navarro-García, F., Perez-Diaz, R.M., Magee, B.B., Pla, J., Nombela, C., Magee, P.T., 1995. Chromosome reorganization in *Candida albicans* 1001 strain. J. Med. Vet. Mycol. 33, 361–366.

Negredo, A., Monteoliva, L., Gil, C., Pla, J., Nombela, C., 1997. Cloning, analysis and one-step disruption of the *ARG5,6* gene of *Candida albicans*. Microbiology 143, 297–302.

Netea, M.G., Brown, G.D., 2012. Fungal infections: the next challenge. Curr. Opin. Microbiol. 15, 403–405.

Neville, B.A., d'Enfert, C., Bougnoux, M.E., 2015. *Candida albicans* commensalism in the gastrointestinal tract. FEMS Yeast Res. 15.

Noble, S.M., 2013. *Candida albicans* specializations for iron homeostasis: from commensalism to virulence. Curr. Opin. Microbiol. 16, 708–715.

Noble, S.M., Gianetti, B.A., Witchley, J.N., 2017. *Candida albicans* cell-type switching and functional plasticity in the mammalian host. Nat. Rev. Microbiol. 15, 96–108.

Odds, F.C., 1988. *Candida* and candidosis. Baillière Tindall, London.

Odds, F.C., Bougnoux, M.E., Shaw, D.J., Bain, J.M., Davidson, A.D., Diogo, D., Jacobsen, M.D., Lecomte, M., Li, S.Y., Tavanti, A., Maiden, M.C., Gow, N.A., d'Enfert, C., 2007. Molecular phylogenetics of *Candida albicans*. Eukaryot. Cell 6, 1041–1052.

Pande, K., Chen, C., Noble, S.M., 2013. Passage through the mammalian gut triggers a phenotypic switch that promotes *Candida albicans* commensalism. Nat. Genet. 45, 1088–1091.

Park, Y.N., Daniels, K.J., Pujol, C., Srikantha, T., Soll, D.R., 2013. *Candida albicans* forms a specialized "sexual" as well as "pathogenic" biofilm. Eukaryot. Cell 12, 1120–1131.

Perez, J.C., Kumamoto, C.A., Johnson, A.D., 2013. *Candida albicans* commensalism and pathogenicity are intertwined traits directed by a tightly knit transcriptional regulatory circuit. PLoS Biol. 11e1001510.

Pierce, J.V., Kumamoto, C.A., 2012. Variation in *Candida albicans EFG1* expression enables host-dependent changes in colonizing fungal populations. MBio 3, e00117-00112.

Pla, J., Pérez-Díaz, R.M., Navarro-García, F., Sánchez, M., Nombela, C., 1995. Cloning of the *Candida albicans HIS1* gene by direct complementation of a *C. albicans* histidine auxotroph using an improved double-ARS shuttle vector. Gene 165, 115–120.

Poulter, R., Jeffery, K., Hubbard, M.J., Shepherd, M.G., Sullivan, P.A., 1981. Parasexual genetic analysis of *Candida albicans* by spheroplast fusion. J. Bacteriol. 146, 833–840.

Prieto, A.D., Román, E., Correia, I., Pla, J., 2014. The HOG pathway is critical for the colonization of the mouse gastrointestinal tract by *Candida albicans*. PLoS One 9, e87128.

Prieto, D., Correia, I., Pla, J., Roman, E., 2016. Adaptation of *Candida albicans* to commensalism in the gut. Future Microbiol. 11, 567–583.

Prieto, D., Roman, E., Alonso-Monge, R., Pla, J., 2017. Overexpression of the transcriptional regulator *WOR1* increases susceptibility to bile salts and adhesion to the mouse gut mucosa in *Candida albicans*. Front. Cell. Infect. Microbiol. 7, 389.

Ramírez-Zavala, B., Mottola, A., Haubenreißer, J., Schneider, S., Allert, S., Brunke, S., Ohlsen, K., Hube, B., Morschhäuser, J., 2017. The Snf1-activating kinase Sak1 is a key regulator of metabolic adaptation and in vivo fitness of *Candida albicans*. Mol. Microbiol. 104 (6), 989–1007.

Ramirez-Zavala, B., Reuss, O., Park, Y.N., Ohlsen, K., Morschhauser, J., 2008. Environmental induction of white-opaque switching in *Candida albicans*. PLoS Pathol. 4, e1000089.

Riggsby, W.S., Torres-Bauza, L.J., Wills, J.W., Townes, T.M., 1982. DNA content, kinetic complexity, and the ploidy question in *Candida albicans*. Mol. Cell. Biol. 2, 853–862.

Rikkerink, E.H., Magee, B.B., Magee, P.T., 1988. Opaque-white phenotype transition: a programmed morphological transition in *Candida albicans*. J. Bacteriol. 170, 895–899.

Romani, L., 2011. Immunity to fungal infections. Nat. Rev. Immunol. 11, 275–288.

Rustchenko-Bulgac, E.P., 1991. Variations of *Candida albicans* electrophoretic karyotypes. J. Bacteriol. 173, 6586–6596.

Rustchenko, E., 2007. Chromosome instability in *Candida albicans*. FEMS Yeast Res. 7, 2–11.

Rustchenko, E.P., Howard, D.H., Sherman, F., 1994. Chromosomal alterations of *Candida albicans* are associated with the gain and loss of assimilating functions. J. Bacteriol. 176, 3231–3241.

Sasse, C., Hasenberg, M., Weyler, M., Gunzer, M., Morschhauser, J., 2013. White-opaque switching of *Candida albicans* allows immune evasion in an environment-dependent fashion. Eukaryot. Cell 12, 50–58.

Saville, S.P., Lazzell, A.L., Chaturvedi, A.K., Monteagudo, C., Lopez-Ribot, J.L., 2008. Use of a genetically engineered strain to evaluate the pathogenic potential of yeast cell and filamentous forms during *Candida albicans* systemic infection in immunodeficient mice. Infect. Immun. 76, 97–102.

Saville, S.P., Lazzell, A.L., Monteagudo, C., Lopez-Ribot, J.L., 2003. Engineered control of cell morphology in vivo reveals distinct roles for yeast and filamentous forms of *Candida albicans* during infection. Eukaryot. Cell 2, 1053–1060.

Selmecki, A., Bergmann, S., Berman, J., 2005. Comparative genome hybridization reveals widespread aneuploidy in *Candida albicans* laboratory strains. Mol. Microbiol. 55, 1553–1565.

Selmecki, A., Forche, A., Berman, J., 2006. Aneuploidy and isochromosome formation in drug-resistant *Candida albicans*. Science 313, 367–370.

Selmecki, A., Gerami-Nejad, M., Paulson, C., Forche, A., Berman, J., 2008. An isochromosome confers drug resistance in vivo by amplification of two genes, ERG11 and TAC1. Mol. Microbiol. 68, 624–641.

Setiadi, E.R., Doedt, T., Cottier, F., Noffz, C., Ernst, J.F., 2006. Transcriptional response of *Candida albicans* to hypoxia: linkage of oxygen sensing and Efg1p-regulatory networks. J. Mol. Biol. 361, 399–411.

Shareck, J., Belhumeur, P., 2011. Modulation of morphogenesis in *Candida albicans* by various small molecules. Eukaryot. Cell 10, 1004–1012.

Si, H., Hernday, A.D., Hirakawa, M.P., Johnson, A.D., Bennett, R.J., 2013. *Candida albicans* white and opaque cells undergo distinct programs of filamentous growth. PLoS Pathog. 9, e1003210.

Slutsky, B., Buffo, J., Soll, D.R., 1985. High-frequency switching of colony morphology in *Candida albicans*. Science 230, 666–669.

Slutsky, B., Staebell, M., Anderson, J., Risen, L., Pfaller, M., Soll, D.R., 1987. "White-opaque transition": a second high-frequency switching system in *Candida albicans*. J. Bacteriol. 169, 189–197.

Soll, D.R., 2014a. The evolution of alternative biofilms in an opportunistic fungal pathogen: an explanation for how new signal transduction pathways may evolve. Infect. Genet. Evol. 22, 235–243.

Soll, D.R., 2014b. The role of phenotypic switching in the basic biology and pathogenesis of *Candida albicans*. J. Oral Microbiol. 6.

Soll, D.R., Daniels, K.J., 2016. Plasticity of *Candida albicans* Biofilms. Microbiol. Mol. Biol. Rev. 80, 565–595.

Soll, D.R., Lockhart, S.R., Zhao, R., 2003. Relationship between switching and mating in *Candida albicans*. Eukaryot. Cell 2, 390–397.

Soll, D.R., Morrow, B., Srikantha, T., 1993. High-frequency phenotypic switching in *Candida albicans*. Trends Genet. 9, 61–65.

Sonneborn, A., Bockmuhl, D.P., Ernst, J.F., 1999a. Chlamydospore formation in *Candida albicans* requires the Efg1p morphogenetic regulator. Infect. Immun. 67, 5514–5517.

Sonneborn, A., Tebarth, B., Ernst, J.F., 1999b. Control of white-opaque phenotypic switching in *Candida albicans* by the Efg1p morphogenetic regulator. Infect. Immun. 67, 4655–4660.

Srikantha, T., Borneman, A.R., Daniels, K.J., Pujol, C., Wu, W., Seringhaus, M.R., Gerstein, M., Yi, S., Snyder, M., Soll, D.R., 2006. *TOS9* regulates white-opaque switching in *Candida albicans*. Eukaryot. Cell 5, 1674–1687.

Staib, P., Morschhauser, J., 2007. Chlamydospore formation in *Candida albicans* and *Candida dubliniensis* – an enigmatic developmental programme. Mycoses 50, 1–12.

Stoldt, V.R., Sonneborn, A., Leuker, C.E., Ernst, J.F., 1997. Efg1p, an essential regulator of morphogenesis of the human pathogen *Candida albicans*, is a member of a conserved class of bHLH proteins regulating morphogenetic processes in fungi. EMBO J 16, 1982–1991.

Sullivan, D.J., Moran, G.P., Coleman, D.C., 2005. Candida dubliniensis: ten years on. FEMS Microbiol. Lett. 253, 9–17.

Tao, L., Du, H., Guan, G., Dai, Y., Nobile, C.J., Liang, W., Cao, C., Zhang, Q., Zhong, J., Huang, G., 2014. Discovery of a "white-gray-opaque" tristable phenotypic switching system in candida albicans: roles of non-genetic diversity in host adaptation. PLoS Biol. 12, e1001830.

Thrash-Bingham, C., Gorman, J.A., 1992. DNA translocations contribute to chromosome length polymorphisms in *Candida albicans*. Curr. Genet. 22, 93–100.

Tsong, A.E., Miller, M.G., Raisner, R.M., Johnson, A.D., 2003. Evolution of a combinatorial transcriptional circuit: a case study in yeasts. Cell 115, 389–399.

Tsuboi, R., Ogawa, H., Bramono, K., Richardson, M.D., Shankland, G.S., Crozier, W.J., Sei, Y., Ninomiya, J., Nakabayashi, A., Takaiuchi, I., et al., 1994. Pathogenesis of superficial mycoses. J. Med. Vet. Mycol. 32 (Suppl 1), 91–104.

Tuch, B.B., Mitrovich, Q.M., Homann, O.R., Hernday, A.D., Monighetti, C.K., De La Vega, F.M., Johnson, A.D., 2010. The transcriptomes of two heritable cell types illuminate the circuit governing their differentiation. PLoS Genet. 6, e1001070.

Tzung, K.W., Williams, R.M., Scherer, S., Federspiel, N., Jones, T., Hansen, N., Bivolarevic, V., Huizar, L., Komp, C., Surzycki, R., Tamse, R., Davis, R.W., Agabian, N., 2001. Genomic evidence for a complete sexual cycle in *Candida albicans*. Proc. Natl. Acad. Sci. U S A 98, 3249–3253.

Uwamahoro, N., Verma-Gaur, J., Shen, H.H., Qu, Y., Lewis, R., Lu, J., Bambery, K., Masters, S.L., Vince, J.E., Naderer, T., Traven, A., 2014. The pathogen *Candida albicans* hijacks pyroptosis for escape from macrophages. MBio 5, e00003–e00014.

Vautier, S., Drummond, R.A., Chen, K., Murray, G.I., Kadosh, D., Brown, A.J., Gow, N.A., MacCallum, D.M., Kolls, J.K., Brown, G.D., 2015. *Candida albicans* colonization and dissemination from the murine gastrointestinal tract: the influence of morphology and Th17 immunity. Cell. Microbiol. 17, 445–450.

Wang, H., Song, W., Huang, G., Zhou, Z., Ding, Y., Chen, J., 2011. *Candida albicans* Zcf37, a zinc finger protein, is required for stabilization of the white state. FEBS Lett. 585, 797–802.

Whelan, W.L., Magee, P.T., 1981. Natural heterozygosity in *Candida albicans*. J. Bacteriol. 145, 896–903.

White, S.J., Rosenbach, A., Lephart, P., Nguyen, D., Benjamin, A., Tzipori, S., Whiteway, M., Mecsas, J., Kumamoto, C.A., 2007. Self-regulation of *Candida albicans* population size during GI colonization. PLoS Path. 3, e184.

Whiteway, M., Oberholzer, U., 2004. *Candida* morphogenesis and host-pathogen interactions. Curr. Opin. Microbiol. 7, 350–357.

Wilson, R.B., Davis, D., Mitchell, A.P., 1999. Rapid hypothesis testing with *Candida albicans* through gene disruption with short homology regions. J. Bacteriol. 181, 1868–1874.

Wu, W., Lockhart, S.R., Pujol, C., Srikantha, T., Soll, D.R., 2007. Heterozygosity of genes on the sex chromosome regulates *Candida albicans* virulence. Mol. Microbiol. 64, 1587–1604.

Wu, W., Pujol, C., Lockhart, S.R., Soll, D.R., 2005. Chromosome loss followed by duplication is the major mechanism of spontaneous mating-type locus homozygosis in *Candida albicans*. Genetics 169, 1311–1327.

Zordan, R.E., Galgoczy, D.J., Johnson, A.D., 2006. Epigenetic properties of white-opaque switching in *Candida albicans* are based on a self-sustaining transcriptional feedback loop. Proc. Natl. Acad. Sci. U S A 103, 12807–12812.

Zordan, R.E., Miller, M.G., Galgoczy, D.J., Tuch, B.B., Johnson, A.D., 2007. Interlocking transcriptional feedback loops control white-opaque switching in *Candida albicans*. PLoS Biol. 5, e256.