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Candida albicans pathogenicity mechanisms

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Abbreviations: AMP, antimicrobial peptide; Hsp, heat shock protein; sHsp, small Hsp; RHE, reconstituted human oral epithelium; RNS, reactive nitrogen species; ROS, reactive oxygen species

The polymorphic fungus *Candida albicans* is a member of the normal human microbiome. In most individuals, *C. albicans* resides as a lifelong, harmless commensal. Under certain circumstances, however, *C. albicans* can cause infections that range from superficial infections of the skin to life-threatening systemic infections. Several factors and activities have been identified which contribute to the pathogenic potential of this fungus. Among them are molecules which mediate adhesion to and invasion into host cells, the secretion of hydrolases, the yeast-to-hypha transition, contact sensing and thigmotropism, biofilm formation, phenotypic switching and a range of fitness attributes. Our understanding of when and how these mechanisms and factors contribute to infection has significantly increased during the last years. In addition, novel virulence mechanisms have recently been discovered. In this review we present an update on our current understanding of the pathogenicity mechanisms of this important human pathogen.

Introduction

The total number of eukaryotic species on Earth has recently been estimated at 8.7 million, with fungi making up approximately 7% (611,000 species) of this number.[1] Of all fungi, only around 600 species are human pathogens.[2] This relatively small group encompasses fungi that cause relatively mild infections of the skin (e.g., dermatophytes and *Malassezia* species), fungi that cause severe cutaneous infections (e.g., *Sporotrix schenkii*) and fungi that have the potential to cause life-threatening systemic infections (e.g., *Aspergillus fumigatus*, *Cryptococcus neoformans*, *Histoplasma capsulatum*, and *Candida albicans*). Indeed, *Candida* spp are the fourth most common cause of hospital-acquired systemic infections in the United States with crude mortality rates of up to 50%.[3,4] *C. albicans* can cause two major types of infections in humans: superficial infections, such as oral or vaginal candidiasis, and life-threatening systemic infections (for a comprehensive description of *C. albicans* infections see the second edition of *Candida and Candidiasis*[5]).

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C. albicans and to a lesser extent other *Candida* species are present in the oral cavity of up to 75% of the population.[6] In healthy individuals this colonization generally remains benign. However, mildly immunocompromised individuals can frequently suffer from recalcitrant infections of the oral cavity. These oral infections with *Candida* species are termed "oral candidiasis" (OC).[6] Such infections are predominantly caused by *C. albicans* and can affect the oropharynx and/or the esophagus of persons with dysfunctions of the adaptive immune system. Indeed, HIV is a major risk factor for developing OC. Further risk factors for developing OC include the wearing of dentures and extremes of age.[7]

It is estimated that approximately 75% of all women suffer at least once in their lifetime from vulvovaginal candidiasis (VVC), with 40–50% experiencing at least one additional episode of infection.[8,9] A small percentage of women (5–8%) suffer from at least four recurrent VVC per year.[10] Predisposing factors for VVC are less well defined than for OC and include diabetes mellitus, use of antibiotics, oral contraception, pregnancy and hormone therapy.[11] Despite their frequency and associated morbidity, superficial *C. albicans* infections are non-lethal. In stark contrast, systemic candidiasis is associated with a high crude mortality rate, even with first line antifungal therapy.[3,4,12] Both neutropenia and damage of the gastrointestinal mucosa are risk factors for the development of experimental systemic (disseminated) candidiasis.[13] Further risk factors include central venous catheters, which allow direct access of the fungus to the bloodstream, the application of broad-spectrum antibacterials, which enable fungal overgrowth, and trauma or gastrointestinal surgery, which disrupts mucosal barriers.[14]

During both superficial and systemic infection, *C. albicans* relies on a battery of virulence factors and fitness attributes. The major factors and fitness traits are discussed below.

Pathogenicity Mechanisms

The ability of *C. albicans* to infect such diverse host niches is supported by a wide range of virulence factors and fitness attributes. A number of attributes, including the morphological transition between yeast and hyphal forms, the expression of adhesins and invasins on the cell surface, thigmotropism, the formation of biofilms, phenotypic switching and the secretion of hydrolytic enzymes are considered virulence factors. Additionally, fitness attributes include rapid adaptation to fluctuations in

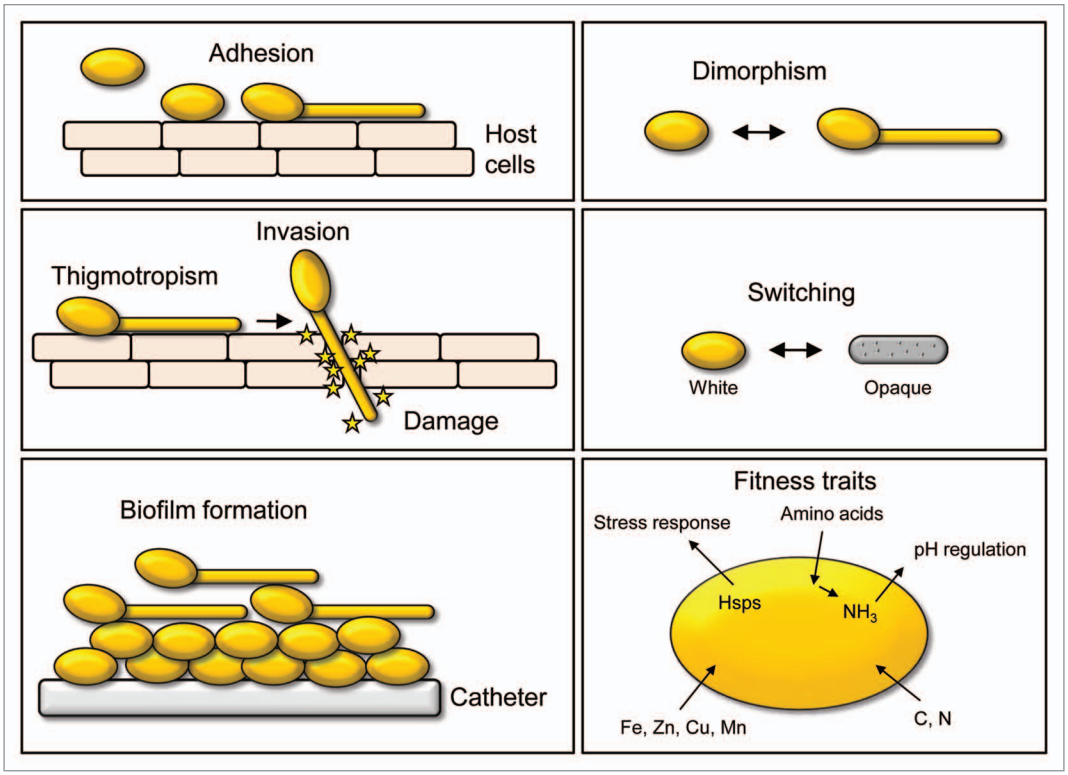

Figure 1. An overview of selected *C. albicans* pathogenicity mechanisms. Yeast cells adhere to host cell surfaces by the expression of adhesins. Contact to host cells triggers the yeast-to-hypha transition and directed growth via thigmotropism. The expression of invasins mediates uptake of the fungus by the host cell through induced endocytosis. Adhesion, physical forces and secretion of fungal hydrolases has been proposed to facilitate the second mechanism of invasion, i.e., fungal-driven active penetration into host cells by breaking down barriers. The attachment of yeast cells to abiotic (e.g., catheters) or biotic (host cells) surfaces can give rise to the formation of biofilms with yeast cells in the lower part and hyphal cells in the upper part of the biofilm. Phenotypic plasticity (switching) has been proposed to influence antigenicity and biofilm formation of *C. albicans*. In addition to these virulence factors, several fitness traits influence fungal pathogenicity. They include a robust stress response mediated by heat shock proteins (Hsps); auto-induction of hyphal formation through uptake of amino acids, excretion of ammonia (NH_3) and concomitant extracellular alkalinization; metabolic flexibility and uptake of different compounds as carbon (C) and nitrogen (N) sources; and uptake of essential trace metals, e.g., iron (Fe), zinc (Zn), copper (Cu) and manganese (Mn).

environmental pH, metabolic flexibility, powerful nutrient acquisition systems and robust stress response machineries (**Fig. 1**).[15]

Polymorphism. *C. albicans* is a polymorphic fungus that can grow either as ovoid-shaped budding yeast, as elongated ellipsoid cells with constrictions at the septa (pseudohyphae) or as parallel-walled true hyphae.[16] Further morphologies include white and opaque cells, formed during switching, and chlamydospores, which are thick-walled spore-like structures.[17] While yeast and true hyphae are regularly observed during infection and have distinct functions (as discussed below), the role of pseudohyphae and switching in vivo is rather unclear and chlamydospores have not been observed in patient samples.[18,19]

A range of environmental cues affect *C. albicans* morphology. For example, at low pH (< 6) *C. albicans* cells predominantly grow in the yeast form, while at a high pH (> 7) hyphal growth is induced.[20] Indeed, a number of conditions, including starvation, the presence of serum or N-acetylglucosamine, physiological temperature and CO_2 promote the formation of hyphae.[21] Morphogenesis has also been shown to be regulated by quorum sensing, a mechanism of microbial communication.[22] In *C. albicans*, the main quorum sensing molecules include farnesol, tyrosol and dodecanol.[23-25] Due to quorum sensing, high cell densities (> 10^7 cells ml^{-1}) promote yeast growth, while low cell densities (< 10^7 cells ml^{-1}) favor hyphal formation.

The transition between yeast and hyphal growth forms is termed dimorphism and it has been proposed that both growth forms are important for pathogenicity.[26] The hyphal form has been shown to be more invasive than the yeast form.[16] On the other hand the smaller yeast form is believed to represent the form primarily involved in dissemination.[27]

Mutants that are unable to form hyphae under in vitro conditions are generally attenuated in virulence.[28] However, hypha formation is linked to the expression of a subset of genes encoding virulence factors that are not involved in hyphal formation per se. Such hypha-associated proteins include the hyphal wall protein Hwp1, the agglutinin-like sequence protein Als3, the secreted aspartic proteases Sap4, Sap5 and Sap6 and the hypha-associated proteins Ece1 and Hyr1. Deletion of *HGC1*, which encodes a hypha-specific G1 cyclin-related protein, results in cells that grow normally in the yeast form but fail to produce hyphae. Nevertheless, the *hgc1*Δ/Δ mutant cells still express at least four

hypha-associated genes (*HWP1*, *ECE1, HYR1* and *ALS3*).[29,30] The finding that an *hgc1*Δ/Δ mutant was attenuated in a mouse model of systemic infection, supported the view that hyphal formation per se is an important virulence attribute.[29]

Adhesins and invasions. *C. albicans* has a specialized set of proteins (adhesins) which mediate adherence to other *C. albicans* cells to other microorganisms, to abiotic surfaces and to host cells.[31,32] Arguably the best studied *C. albicans* adhesins are the agglutinin-like sequence (ALS) proteins which form a family consisting of eight members (Als1–7 and Als9). The *ALS* genes encode glycosylphosphatidylinositol (GPI)-linked cell surface glycoproteins. Of the eight Als proteins, the hypha-associated adhesin Als3 is especially important for adhesion.[33-35] *ALS3* gene expression is upregulated during infection of oral epithelial cells in vitro and during in vivo vaginal infection.[36-39] Another important adhesin of *C. albicans* is Hwp1, which is a hypha-associated GPI-linked protein.[33,40,41] Hwp1 serves as a substrate for mammalian transglutaminases and this reaction may covalently link *C. albicans* hyphae to host cells. An *hwp1*Δ/Δ mutant displayed reduced adherence to buccal epithelial cells and displayed attenuated virulence in a mouse model of systemic candidiasis.[40,42,43]

Hwp1 and Als3 were also demonstrated to contribute to biofilm formation by acting as complementary adhesins.[44]

Morphology-independent proteins can also contribute to adhesion. These include GPI-linked proteins (Eap1, Iff4 and Ecm33), non-covalent wall-associated proteins (Mp65, a putative β-glucanase, and Phr1, a β-1,3 glucanosyl transferase), cell-surface associated proteases (Sap9 and Sap10) and the integrin-like surface protein Int1.[38,45]

C. albicans is a remarkable pathogen as it can utilize two different mechanisms to invade into host cells: induced endocytosis and active penetration.[37,38,45,46] For induced endocytosis, the fungus expresses specialized proteins on the cell surface (invasins) that mediate binding to host ligands (such as E-cadherin on epithelial cells[34] and N-cadherin on endothelial cells[47]), thereby triggering engulfment of the fungal cell into the host cell. Indeed, even killed hyphae are taken up, indicating that induced endocytosis is a passive process that does not require the activities of viable fungal cells.[46,48] Two invasins have been identified so far, namely Als3 (which also functions as an adhesin, see above) and Ssa1.[34,49] Ssa1 is a cell-surface expressed member of the heat shock protein 70 (Hsp70) family. Both *als3*Δ/Δ and *ssa1*Δ/Δ mutants exhibited reduced epithelial adherence and invasion, and reduced virulence in a murine model of oropharyngeal candidiasis.[38,49] Als3 and Ssa1 bind to host E-cadherin and likely induce endocytosis by a clathrin-dependent mechanism; however, macropinocytosis has also been implicated in *C. albicans* induced endocytosis.[34,46] In contrast, active penetration is a fungal-driven process and requires viable *C. albicans* hyphae.[36,46] It is still unclear exactly which factors mediate this second route of invasion into host cells. Fungal adhesion and physical forces are believed to be crucial.[36] Secreted aspartic proteases (Saps) have also been proposed to contribute to active penetration. Lipases and phospholipases, on the other hand, have not been shown to contribute to this process.[38,46]

In summary, invasion into host cells by *C. albicans* relies on two likely complementary mechanisms: induced endocytosis mediated by Als3 and Ssa1 and active penetration mediated by yet undefined molecular mechanisms.

Biofilm formation. A further important virulence factor of *C. albicans* is its capacity to form biofilms on abiotic or biotic surfaces. Catheters, dentures (abiotic) and mucosal cell surfaces (biotic) are the most common substrates.[50] Biofilms form in a sequential process including adherence of yeast cells to the substrate, proliferation of these yeast cells, formation of hyphal cells in the upper part of the biofilm, accumulation of extracellular matrix material and, finally, dispersion of yeast cells from the biofilm complex.[51] Mature biofilms are much more resistant to antimicrobial agents and host immune factors in comparison to planktonic cells.[50,51] The factors responsible for heightened resistance include the complex architecture of biofilms, the biofilm matrix, increased expression of drug efflux pumps and metabolic plasticity.[50] Dispersion of yeast cells from the mature biofilm has been shown to directly contribute to virulence, as dispersed cells were more virulent in a mouse model of disseminated infection.[52] The major heat shock protein Hsp90 was recently identified as a key regulator of dispersion in *C. albicans* biofilms.[53] In addition, Hsp90 was also required for biofilm antifungal drug resistance.[53]

Several transcription factors control biofilm formation. These include the transcription factors Bcr1, Tec1 and Efg1.[50] In a recent study, Nobile et al. investigated the transcriptional network regulating biofilm formation and identified further, previously unknown regulators of biofilm production.[54] These novel factors include Ndt80, Rob1 and Brg1. Deletion of any of these regulators (*BCR1*, *TEC1*, *EFG1*, *NDT80*, *ROB1* or *BRG1*) resulted in defective biofilm formation in in vivo rat infection models.[54]

Extracellular matrix production is controlled by additional factors. The zinc-responsive transcription factor Zap1 negatively regulates β-1,3 glucan, the major component of biofilm matrix.[55] Glucoamylases (Gca1 and Gca2), glucan transferases (Bgl2 and Phr1) and the exo-glucanase, Xog1, are positive regulators of β-1,3 glucan production.[55,56] While expression of *GCA1* and *GCA2* are controlled by Zap1, the enzymes Bgl2, Phr1 and Xog1 function independently of this key negative regulator.[56] Biofilms formed by mutants lacking *BGL2*, *PHR1* or *XOG1* were shown to be more susceptible to the antifungal agent, fluconazole, both in vitro and in vivo.[56] Furthermore, recent studies indicate that *C. albicans* biofilms are resistant to killing by neutrophils and do not trigger production of reactive oxygen species (ROS).[57] Evidence suggests that β-glucans in the extracellular matrix protect *C. albicans* from these attacks.[57]

Contact sensing and thigmotropism. An important environmental cue that triggers hypha and biofilm formation in *C. albicans* (see above) is contact sensing. Upon contact with a surface, yeast cells switch to hyphal growth.[58] On certain substrates, such as agar or mucosal surfaces, these hyphae can then invade into the substratum. Contact to solid surfaces also induces the formation of biofilms.[58] On surfaces with particular topologies (such as the presence of ridges) directional hyphal growth (thigmotropism) may occur.[59]

Brand et al. demonstrated that thigmotropism of *C. albicans* hyphae is regulated by extracellular calcium uptake through the calcium channels Cch1, Mid1 and Fig1.[59] Additional mechanisms include the polarisome Rsr1/Bud1-GTPase module.[60] Brand et al. also provided evidence that *C. albicans* thigmotropism is required for full damage of epithelial cells and normal virulence in mice.[61]

Therefore, the correct sensing and response to both abiotic (biofilm formation) and biotic (invasion) surfaces is important for pathogenicity.

Secreted hydrolases. Following adhesion to host cell surfaces and hyphal growth, *C. albicans* hyphae can secrete hydrolases, which have been proposed to facilitate active penetration into these cells.[62] In addition, secreted hydrolases are thought to enhance the efficiency of extracellular nutrient acquisition.[63] Three different classes of secreted hydrolases are expressed by *C. albicans*: proteases, phospholipases and lipases.

The family of secreted aspartic proteases (Saps) comprises ten members, Sap1–10. Sap1–8 are secreted and released to the surrounding medium, whereas Sap9 and Sap10 remain bound to the cell-surface.[63-65] Sap1–3 have been shown to be required for damage of reconstituted human epithelium (RHE) in vitro, and for virulence in a mouse model of systemic infection.[66,67] However, the relative contribution of Saps to *C. albicans* pathogenicity is controversial. Recent results indicate that Saps are not required for invasion into RHE and that Sap1–6 are dispensable for virulence in a mouse model of disseminated candidiasis.[68,69]

However, the observed expansion of Sap-encoding genes in *C. albicans* compared with its less pathogenic relatives suggests a role for these proteases in virulence.[70] Indeed, the large size of the Sap family itself makes it likely that a certain degree of functional redundancy may exist.

The family of phospholipases consists of four different classes (A, B, C and D).[71] Only the five members of class B (*PLB1–5*) are extracellular and may contribute to pathogenicity via disruption of host membranes.[72] Both *plb1*Δ/Δ and *plb5*Δ/Δ mutants have been shown to be attenuated in virulence in a mouse model of systemic infection.[73,74]

The third family of secreted hydrolases, the lipases, consists of 10 members (*LIP1–10*).[75,76] A *lip8*Δ/Δ mutant had reduced virulence in a mouse model of systemic infection, supporting a role for these extracellular hydrolases in *C. albicans* pathogenicity.[77]

pH-sensing and regulation. In the human host, *C. albicans* is exposed to a surrounding pH ranging from slightly alkaline to acidic.[78] Additionally, depending on the host niche, the environmental pH can be very dynamic. Therefore, *C. albicans* must be able to adapt to changes in pH.[78] The pH of human blood and tissues is slightly alkaline (pH 7.4), while the pH of the digestive tract ranges from very acidic (pH 2) to more alkaline (pH 8), and the pH of the vagina is around pH 4.[78] Neutral to alkaline pH can cause severe stress to *C. albicans*, including malfunctioning of pH-sensitive proteins, and impaired nutrient acquisition (as a consequence of a disrupted proton gradient).[78] Among the first proteins identified as being important for adaptation to changing pH were the two cell wall β-glycosidases Phr1 and Phr2.[79] *PHR1* is expressed at neutral-alkaline pH. In contrast, *PHR2* is mainly expressed at acidic pH.[80] Correspondingly, Phr1 is required for systemic infections, and Phr2 is essential for infections of the vagina.[81]

C. albicans senses pH via the Rim101 signal transduction pathway. In this pathway, environmental pH is gauged by the plasma membrane receptors Dfg16 and Rim21.[78] Activation of these receptors leads to induction of a signaling cascade, finally leading to activation of the major pH-responsive transcription factor Rim101 via its proteolytic cleavage. Rim101 then enters the nucleus and mediates pH-dependent responses.[78] A *dfg16*Δ/Δ mutant had reduced virulence in a mouse infection model of systemic candidiasis,[82] and a *rim20*Δ/Δ mutant had attenuated virulence in a mouse corneal infection model.[83,84] Finally, a *rim101*Δ/Δ mutant had reduced virulence in both a systemic mouse model of hematogenously disseminated candidiasis[85] and a murine model of oropharyngeal candidiasis.[86] Together these data demonstrate that the Rim101 pathway, and pH sensing in general, are critical for *C. albicans* virulence.

C. albicans is not only able to sense and adapt to environmental pH, but can also modulate extracellular pH, actively alkalinizing its surrounding environment under nutrient starvation and, thereby, autoinducing hypha formation.[87,88] The molecular mechanisms underlying this are beginning to be uncovered and appear to involve the uptake of amino acids and probably other amine-containing molecules, such as polyamines, in the absence of glucose. *C. albicans* then cleaves these substrates intracellularly with the urea amidolyase Dur1,2, and exports the resulting ammonia through the Ato (**a**mmonia **t**ransport **o**utward) export proteins. The extrusion of ammonia leads to an alkalinization of the extracellular milieu, which in turn promotes hyphal morphogenesis.[87] Hyphal formation itself is considered a key virulence factor of *C. albicans* as non-filamentous mutants are attenuated in virulence (see above).[28] Therefore, *C. albicans* senses, adapts to and, strikingly, also actively modulates extracellular pH. All these features contribute to its remarkable capacity to co-exist as a commensal, and to prevail as a fungal pathogen in humans.

Metabolic adaptation. Nutrition is a central and fundamental prerequisite for survival and growth of all living organisms. Metabolic adaptability mediates the effective assimilation of alternative nutrients in dynamic environments.[89] This metabolic flexibility is particularly important for pathogenic fungi during infection of different host niches.[90,91] Glycolysis, gluconeogenesis, and starvation responses are all thought to contribute to host colonization and pathogenesis, but their specific contribution may be highly niche-specific and is still only partially understood. In healthy individuals *C. albicans* is predominantly found as part of the gastrointestinal microbiome. Although the concentration of nutrients in this environment can be naturally high, growth of the fungus is believed to be controlled through competition with other members of the intestinal microbial flora.[90] During disseminated candidiasis in susceptible individuals, *C. albicans* gains access to the bloodstream. Blood is relatively rich in glucose (6–8 mM),[90] the preferred nutrient source of most fungi. However, phagocytic cells (macrophages and neutrophils) can efficiently phagocytose *C. albicans*. Once

inside a macrophage or neutrophil, however, the nutritional environment completely changes for the fungus. Not only does the phagocyte produce highly reactive intermediates like ROS, reactive nitrogen species (RNS) and antimicrobial peptides (AMPs), it also restricts the availability of nutrients, thereby creating an environment of nutrient starvation.[92] Prompt and efficient metabolic plasticity is therefore required for adaptation of *C. albicans* to such a hostile host milieu. Inside macrophages, the fungus initially switches from glycolysis to gluconeogenesis and a starvation response (activation of the glyoxylate cycle). Lipids and amino acids are proposed to serve as nutrient sources within macrophages.[93]

In addition to metabolic flexibility, the fungus has also evolved ways to escape from macrophages by inhibiting the production of antimicrobial effectors and inducing hyphal formation. Hyphae formed inside phagocytic cells can pierce through the host immune cell by mechanical forces and can permit escape.[93,94] During systemic candidiasis, fungal cells can disseminate to virtually every organ within the human host, each with potentially different availability of nutrients. In the liver for example, *C. albicans* has access to large quantities of glycogen, the main storage molecule of glucose. The brain has high concentrations of glucose and vitamins as potential nutrient sources.[91] In other tissues, *C. albicans* faces relatively poor glucose concentrations and uses alternative metabolic pathways to utilize host proteins, amino acids, lipids and phospholipids. The fungus can use secreted proteases (see above) to hydrolyse host proteins. It was recently shown that adaptation to different nutrient sources by *C. albicans* not only promotes survival and growth, but also affects virulence.[95] Growth on alternative carbon sources, such as lactate or amino acids, rendered the fungus more resistant to environmental stresses and increased its virulence potential in both a mouse model of systemic candidiasis, and a murine vaginal infection model.[95] Furthermore, the glyoxylate cycle has been shown to be required for full virulence in *C. albicans*.[96] Uptake of amino acids, and likely also polyamines, affects the virulence of *C. albicans* by allowing the fungus to autoinduce hypha formation through extracellular alkalinization (**Fig. 1**).[87,88]

In summary, during infection the main nutrient sources for *C. albicans* are likely to be host-derived glucose, lipids, proteins and amino acids, depending on the anatomical niche. Besides being able to use these different nutrients individually, the ability of *C. albicans* to rapidly and dynamically respond to host and pathogen-induced changes in micro-environmental nutrient availability contributes to its success as a pathogen.

Environmental stress response. A robust stress response contributes to the survival and virulence of *C. albicans* by facilitating the adaptation of the fungus to changing conditions and protecting it against host-derived stresses. Phagocytic cells of the immune system produce oxidative and nitrosative stresses. pH-stress occurs, for example, in the gastrointestinal and urogenital tract.[89] Stress-responsive regulatory pathways, as well as downstream targets, were shown to be essential not only for efficient stress adaptation, but also for full virulence of the fungus.[89] In fact, several mutants lacking genes encoding regulators of stress response or detoxifying enzymes are attenuated in virulence. Cellular responses to stresses include heat shock-, osmotic-, oxidative- and nitrosative-stress responses.[89]

The heat shock response is mediated by heat shock proteins (see below) which act as molecular chaperones to prevent deleterious protein unfolding and aggregation. Additionally, thermal stress leads to trehalose accumulation in *C. albicans*, which is thought to act as a "chemical chaperone" by stabilizing proteins prone to unfolding.[89] However, the exact function of trehalose accumulation following thermal insults remains unknown.

The osmotic stress response results in intracellular accumulation of the compatible solute glycerol to counteract loss of water due to the outward-directed chemical gradient. Glycerol biosynthesis is mediated by the glycerol 3-phosphatase Gpp1 and the glycerol 3-phosphate dehydrogenase Gpd2.[36] Both *gpp1*Δ/Δ and *gpd2*Δ/Δ mutants were shown to have reduced capacity to damage oral epithelial cells in vitro. However, this was probably due to an inability to generate hyphal turgor pressure and mechanical forces (see above) rather than heightened sensitivity to osmotic stress in this infection model.[36]

Reactive oxygen species (ROS), such as peroxide, superoxide anions, and hydroxyl radicals, induce an oxidative stress response.[89] Catalase Cta1 and superoxide dismutases, Sod1 and Sod5, are crucial for efficient detoxification of ROS in *C. albicans*, and are required for full virulence in mouse models of systemic candidiasis.[97-99]

Neutrophils also produce reactive nitrogen species (RNS), which induce a nitrosative stress response in phagocytosed *C. albicans* cells. The major protein implicated in detoxification of RNS is the flavohemoglobin-related protein Yhb1. Deletion of Yhb1 renders *C. albicans* cells sensitive to RNS and attenuates virulence in a mouse model of systemic candidiasis.[100]

In fungi, environmental signals, including stress signals, are sensed and transmitted by mitogen-activated protein (MAP) kinase pathways through sequential phosphorylation events.[101] The three main MAP kinase signaling pathways in *C. albicans* are the Mkc1-, Hog1- and Cek1-MAP kinase pathway.[101]

The Mkc1 (**M**AP **k**inase from *C. albicans*) pathway is primarily involved in maintaining cellular integrity, cell wall biogenesis, invasive growth under embedded conditions and biofilm formation.[101] Mkc1 is activated upon oxidative and osmotic stress conditions.

The Hog1 (**H**igh **o**smolarity **g**lycerol response) pathway mediates the response to osmotic, oxidative and thermal stress, morphogenesis and cell wall formation.[101] Under osmotic stress, activated Hog1 leads to glycerol accumulation.[101]

The Cek1 (**C**andida **E**RK-like **k**inase) pathway mediates filamentation, mating and likely also adaptation to thermal stress.[101,102] Mutants in all three pathways (*mkc1*Δ, *hog1*Δ or *cek1*Δ) were all attenuated in virulence in mouse infection models, highlighting the importance of the stress response during infection.[103-105]

In addition to the sequential cascade of activation in the three MAP kinase pathways, environmental signals also trigger cross-talk between these pathways. For example, activated Hog1 both represses the Cek1-pathway and activates the Mkc1 pathway.[101] Moreover, certain signals, like oxidative or osmotic stress are

sensed by more than one pathway. This interweaved MAP kinase sensing network probably engenders fine-tuning of a robust adaptive response.

Heat shock proteins. The heat shock response is a conserved reaction of living organisms to stressful conditions such as high temperature, starvation and oxidative stress.[106,107] Such stresses can induce protein unfolding and nonspecific protein aggregation, ultimately leading to cell death. In order to prevent this detrimental fate, cells produce heat shock proteins (Hsps).[106] These specialized proteins act as chaperones and prevent protein unfolding and aggregation by binding to their clients and stabilizing them.[108] Six major Hsps have been identified in *C. albicans*: Hsp104, Hsp90, Hsp78, two Hsp70 proteins (Ssa1 and Ssa2) and Hsp60. *HSP104* encodes a Hsp required for proper biofilm formation, and virulence in a *Caenorhabditis elegans* infection model.[109] Hsp90 is a major Hsp in *C. albicans* and regulates drug resistance, morphogenesis, biofilm formation and virulence.[53,110-113] *HSP78* encodes an uncharacterized Hsp that is transcriptionally upregulated in response to phagocytosis by macrophages.[93] The two *C. albicans* Hsp70 family members, Ssa1 and Ssa2 (stress-70 subfamily A), are expressed on the cell surface and function as receptors for antimicrobial peptides, e.g., Ssa2 binds histatin 5.[114-116] An *ssa2*Δ/Δ mutant had increased resistance to histatin 5, but was dispensable for virulence in mouse models of disseminated and oropharyngeal candidiasis.[49,114,116] Ssa1 also acts as an invasin.[49] An *ssa1*Δ/Δ mutant had attenuated virulence in mouse models of both disseminated and oropharyngeal candidiasis.[49] Finally, *HSP60* encodes a putative mitochondrial Hsp of unknown function. An *hsp60*Δ/*HSP60* heterozygous mutant has increased sensitivity to elevated temperatures, indicating that Hsp60 might be required for thermal stress tolerance.[117]

Expression of Hsps is mainly controlled by the transcription factor heat shock factor 1 (Hsf1).[118,119] Hsf1 is phosphorylated in response to heat stress and induces transcription of Hsp-encoding genes via binding to heat shock elements (HSEs) in their promoters.[119] *C. albicans* Hsf1 is essential for viability and a mutant that is unable to activate Hsf1 displays attenuated virulence in a mouse model of systemic candidiasis.[15]

Small heat shock proteins. In addition to the above mentioned heat shock proteins, six small Hsps (sHsps) have also been identified in *C. albicans*.[120] sHsps are low-molecular-mass chaperones that prevent protein aggregation.[121,122] Upon heat, or other forms of stress, cells express sHSPs which transition from an oligomeric to a multimeric state and bind aggregated proteins.[123] In these chaperone-aggregate complexes, client proteins are held ready for disaggregation and refolding by other major Hsps, such as Hsp104.[124]

C. albicans is predicted to encode six sHsps: Hsp31, Hsp30, Hsp21, two Hsp12 proteins and Hsp10.[120] As yet only Hsp12 and Hsp21 have been investigated. Hsp12 is expressed in response to different stresses, including heat shock and oxidative stress. Deletion of both *HSP12* genes did not influence virulence of *C. albicans* in a Drosophila infection model.[125] It should be noted, however, that the fly infection experiment was performed at 30°C, and it remains to be investigated how the mutant behaves at physiological temperature in a mammalian host.

We recently investigated the function of Hsp21 and showed that this sHsp is crucial for regulation of intracellular levels of trehalose. Deletion of *HSP21* resulted in impaired thermotolerance, enhanced sensitivity toward oxidative stress, and strongly attenuated virulence in a mouse model of systemic candidiasis.[102] Importantly, Hsp21 is not found in humans. These results indicate that sHsps can act as virulence factors and might represent attractive drug targets.

Metal acquisition. Trace metals are essential for the growth and survival of all living organisms including humans, animals, plants, bacteria and fungi. Among the most important metals are iron, zinc, manganese and copper, all of which are essential for the proper function of a large number of proteins and enzymes. Pathogenic microorganisms, as well as their respective hosts, have evolved elaborate mechanisms to acquire or restrict access to these metals.[126]

To date, the most widely investigated transition metal with regard to pathogenesis is iron. *C. albicans* acquires this metal by different strategies, including a reductive system, a siderophore uptake system and a heme-iron uptake system.[127] The reductive system mediates iron acquisition from host ferritin, transferrin or the environment. The adhesin and invasin Als3 (see above) was shown to be the receptor for ferritin.[30] Despite the fact that an *als3*Δ/Δ mutant had normal virulence in a mouse infection model of disseminated candidasis,[128] deletion of *ALS3* resulted in reduced capacity to damage oral epithelial host cells in vitro, suggesting that Als3-mediated iron acquisition from host ferritin contributes to iron acquisition depending on the stage of the infection.[30,35] Although *C. albicans* does not synthesize its own siderophores, the fungus uses an uptake system to steal iron from siderophores produced by other microorganisms, also known as xeno-siderophores. The only described siderophore transporter in *C. albicans* is Sit1. A *sit1*Δ/Δ mutant exhibited normal virulence in a mouse model of disseminated candidiasis. However, the mutant was strongly impaired in its capacity to damage ex vivo human keratinocyte tissue.[129] Finally, the heme-iron uptake system promotes iron acquisition from hemoglobin and heme-proteins and is mediated by the heme-receptor gene family members *RBT5*, *RBT51*, *CSA1*, *CSA2* and *PGA7* (*RBT6*).[127,130] An *rbt5*Δ/Δ mutant has normal virulence in mice, however, this may be due to functional redundancy.[127,131] The role of the other four heme-binding proteins (Rbt51, Csa1, Csa2 and Pga7) in virulence has not yet been investigated.

Zinc is the second most abundant metal in most living organisms.[126] Our group has recently uncovered a previously undescribed mechanism of zinc acquisition by *C. albicans*.[132] The fungus secretes the zinc-binding protein Pra1 (**p**H-**r**egulated **a**ntigen 1), which, analogous to siderophore-mediated iron acquisition, acts as a zincophore by binding extracellular zinc and re-associating with the fungal cell. Re-association of Pra1 is mediated by the zinc transporter Zrt1.[132] Despite enhanced virulence of a *pra1*Δ/Δ mutant in mice,[133] deletion of *PRA1* strongly reduces the capacity of *C. albicans* to damage endothelial cells in vitro in the absence of exogenous zinc, suggesting that zinc acquisition plays an important role during certain steps of infection.[132] Copper and manganese are also essential for fungal growth; however,

the mechanisms by which *C. albicans* acquires these metals is currently poorly understood. A putative manganese transporter, Ccc1,[120] and a copper transporter, Ctr1,[134] have been identified, although their roles in virulence have not yet been determined. Therefore, future studies are required to elucidate the role of these other essential metals in *C. albicans* pathogenicity.

Conclusions

Understanding the pathogenicity mechanisms that *C. albicans* uses during infection is crucial for the development of new antifungal therapies and diagnostics. Classically, antifungal drugs were designed to exert fungicidal activities, i.e., to kill the pathogenic microorganism. Recently however, specifically targeting virulence factors has been proposed as a new and promising antifungal strategy.[135] Several virulence factors, such as dimorphism, the secretion of proteases and the expression of adhesins and invasins, have been suggested as attractive targets,[26,135] and recent investigations have further broadened our understanding of the *C. albicans* factors and activities which contribute to virulence. The heat shock response, including major and small heat shock proteins, has emerged as a promising drug target. Specifically, those Hsps which are unique to fungi and do not occur in humans (for example Hsp21) represent good candidates for specific drug targets. Another avenue of research is the interplay between host nutritional immunity and fungal nutrient acquisition systems. In particular, interfering with the iron, zinc, manganese or copper homeostasis mechanisms of pathogenic microorganisms may represent promising therapeutic strategies. As our detailed understanding of fungal pathogenicity mechanisms improves, the potential for developing novel therapeutic and diagnostic strategies expands.

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