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"The Fungal Cell Wall: An Armour and a Weapon for Human Fungal Pathogens"
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Impact of the environment upon the *Candida albicans* cell wall and immune evasion

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Abstract

The fungal cell wall is an essential organelle that maintains cellular morphology and protects the fungus from environmental insults. For fungal pathogens such as *Candida albicans,* it provides a degree of protection against attack by host immune defences. However, the cell wall also presents key epitopes that trigger host immunity, and attractive targets for antifungal drugs. Rather than being a rigid shield, it has become clear that the fungal cell wall is an elastic organelle that permits rapid changes in cell volume and the transit of large liposomal particles such as extracellular vesicles. The fungal cell wall is also flexible in that it adapts to local environmental inputs, thereby enhancing the fitness of the fungus in these microenvironments. Recent evidence indicates that this cell wall adaptation affects host-fungus interactions by altering the exposure of major cell wall epitopes that are recognised by innate immune cells. Therefore, we discuss the impact of environmental adaptation upon fungal cell wall structure and immune evasion, focussing on *C. albicans* and drawing parallels with other fungal pathogens.

Introduction

The ascomycete fungus, *Candida albicans*, is carried as a relatively harmless commensal by most healthy individuals in their oral cavity, or urogenital and gastrointestinal tracts. In general, the local epithelial barriers, innate immune defences and microbiota limit the colonisation and outgrowth by *C. albicans* cells. However, the perturbation of any of these local defences often leads to local mucosal infection (*thrush*) (1,2). Most women suffer at least one episode of vaginitis in their lifetime, and oral thrush is common in babies, the elderly, diabetics, and HIV patients. *C. albicans* is the most common cause of fungal mucosal infections (3). In neutropenic patients, whose immune defences are severely compromised, *C. albicans* can cause systemic infections of the blood and internal organs (4,5). Despite the availability of several classes of antifungal drug, including azoles, polyenes, echinocandins, and flucytosine (6), these systemic infections display about 40% mortality (7,8). This, combined with the emergence of resistance to the current antifungal drugs in clinical use, means that there is a clear need for the development of new, more effective antifungals (7).

From a clinical perspective, the fungal cell wall represents an attractive target for the development of new antifungal drugs (6,9). This is because human cells lack a cell wall, whereas the cell wall is essential for the viability of fungal pathogens such as *C. albicans* (10-12). Therefore, drugs that target cell wall biosynthesis or function are less likely to perturb human cells. The *C. albicans* cell wall also represents the first point of direct contact with the host, and cell wall molecules are exploited as key recognition targets by our immune defences. For this reason, the cell wall is also an attractive target for the development of vaccines and immunotherapeutics that might prevent or combat *Candida* infections. In addition, structural distinctions between the cell walls of pathogenic fungal species (13) represent a point of leverage for the development of the novel diagnostics that are required to accelerate the diagnosis, and thereby improve the prognoses of life-threatening systemic infections (7). Therefore, a comprehensive understanding of the structure and function of the fungal cell wall is vital for the elaboration of the new antifungal drugs, immunotherapies, diagnostics, and vaccines that ultimately will improve patient outcomes.

From the perspective of the fungus, the cell wall is a vital organelle that requires significant metabolic and energetic investment to construct. (The wall comprises about 30% of the dry weight of a yeast cell (14). The cell wall provides protection against environmental insults (9). It maintains cell shape and osmotic integrity, asserting the cellular morphology

driven by the regulatory apparatus that establishes the balance between isotropic and polarised growth, generating morphogenetic transitions between yeast, pseudohyphal and hyphal growth forms in response to the environmental conditions (15). Yet the cell wall is an elastic, not a rigid structure, which permits the transit of large liposomes and extracellular vesicles (16,17), as well as rapid changes in cell volume in response to osmotic challenges (18). Furthermore, rather than being a relatively inert shield, the cell wall responds to local inputs as the fungus adapts to environmental change (19-21). Therefore the fungal cell wall is a remarkable organelle that is simultaneously robust but elastic, and stable but flexible.

In this article we focus on the cell wall of the major pathogen, *C. albicans*. We review the structure of the *C. albicans* cell wall, its stability and elasticity; how the cell wall responds to environmental challenges, whether natural or therapeutic; and how changes in the *C. albicans* cell wall affect host-fungus interactions. We then discuss parallels with other fungal pathogens before suggesting key questions for the future.

Structure and synthesis of the *C. albicans* cell wall

Significant differences exist between the cell walls of the major fungal pathogens of humans (9,13). Nevertheless, some of the macromolecular building blocks that comprise the cell wall are conserved across most of these fungal species. These consist of β-1,3- and β-1,6-glucan, chitin and mannoproteins. Additionally, some fungal cell walls contain melanin, chitosan and β-1,4-glucan (9,22-24). A combination of microscopy, biochemistry and molecular genetics has shown clearly that the *C. albicans* cell wall comprises two main layers: an inner layer of chitin and glucan cross-linked together, and an outer layer of mannan fibrils that are covalently attached to this inner layer via their anchoring mannoproteins (Figure 1).

Chitin is a linear homopolymer of β-1,4-linked *N*-acetylglucosamine, which forms anti-parallel chains linked by intra-chain hydrogen bonds. Chitin accounts for only about 2-3% of the dry weight of the *C. albicans* yeast cell wall. Yet it is a strong fibrous structural component of the inner layer that contributes significantly to the overall integrity of the cell wall. *C. albicans* mutants with impaired chitin synthesis present with a disordered cell wall architecture and display osmotic instability (25,26). In *C. albicans*, a small fraction of chitin (less than 5%) is deacetylated to chitosan by one or more chitin deacetylases, making chitin fibrils more elastic and protecting them from the action of hostile chitinases (26).

In *C. albicans* chitin is synthesized by a family of four chitin synthases, representing three different classes of chitin synthase that generate chitin microfibrils of different lengths (25). Together, these enzymes engineer the chitin skeleton in the fungal cell wall and septum. Chs1 is an essential Class II enzyme that is required for the synthesis of the primary septum. Chs3 is a Class IV enzyme, which is usually located in the tip of buds and hyphal cells and synthesizes the majority of chitin found in the fungal cell wall and septum. Chs2 and Chs8 also contribute to cell wall integrity during normal growth and stress conditions. These Class I enzymes account for most of the chitin synthase activity that is measurable *in vitro*, and indeed the deletion of *CHS2* alone reduces *in vitro* chitin synthase activity by 80–91% (11,26-28).

β-Glucan is the major structural polysaccharide of the *C. albicans* cell wall, accounting for 50–60% of the dry weight of the yeast cell wall (22,23). β-Glucan is composed of chains of glucose residues linked via β-1,3- or β-1,6 linkages. β-1,3-Glucan fibrils represent the main structural component of the *C. albicans* cell wall, and chitin, β-1,6-glucan and mannoproteins are covalently attached to this β-1,3-glucan network in the inner layer of the wall. β-1,3-Glucan is synthesized at the plasma membrane and extruded into extracellular space by the beta-1,3-glucan synthase complex, which consists of catalytic subunits encoded by *GSC1/FKS1* and

GSC2/FKS2, and a small regulatory GTPase encoded by *RHO1* (29,30). β-1,3-Glucan synthase is essential for fungal viability and is the target of echinocandin drugs, such as caspofungin (10).

β-1,6-Glucan is less abundant than β-1,3-glucan. Branched β-1,6-glucan structures are crosslinked to β-1,3-glucan in the inner layer of the *C. albicans* cell wall, providing an additional platform for the covalent anchoring of some cell wall mannoproteins (31). A number of genes are involved in β-1,6-glucan biosynthesis, including *KRE5*, *KRE6*, *KRE9*, *BIG1* and *SKN1.* Although it is not clear where β-1,6-glucan synthesis occurs, it does involve enzymes localised in the endoplasmic reticulum and the Golgi apparatus (32).

The mannoproteins in the *C. albicans* cell wall are frequently heavily decorated with *N*- and/or *O*-linked oligosaccharides (23,33). Together with phospholipomannans, these represent up to 30-40% of the dry weight of the cell wall. The *O*-mannans are relatively short linear carbohydrate polymers comprised of two to six α-1,2-linked mannose units. Their synthesis requires the activities of *PMR1*, the *PMT* gene family, *MNT1*, and *MNT2* (34-37). The addition of the first mannose residue to the polypeptide chain is catalysed by *O*-mannosyltransferases (encoded by *PMT* genes), whilst Mnt1 and Mnt2 are responsible for the addition of the first and second α-1,2-mannose units into the α-mannose backbone. The resultant *O*-linked oligosaccharides are thought to promote a rod-like conformation to the serine-threonine-rich repeats to which they are generally attached (38).

The outer layer of the *C. albicans* cell wall is composed of highly branched *N*-linked oligosaccharide structures that are covalently linked to asparagine residues in the mannoproteins. These *N*-mannans contain a *N*-glycan core, to which are attached long branched chains with an α-1,6-mannose backbone and side chains of oligomannosides linked via α-1,2 or α-1,3 bonds (23). The synthesis of the *N*-linked oligosaccharide core structure occurs in the endoplasmic reticulum and involves the sequential addition of sugar residues by glycosyltransferases, encoded by asparagine-linked glycosylation (*ALG*) genes. The mannosyltransferase Och1 catalyses the addition of the first alpha-1,6-mannose (39) and the branched oligosaccharide structure is then added to the nascent protein by the oligosaccharyltransferase complex. After the initial glycosylation, the mannoprotein is further modified in the ER and Golgi apparatus. Golgi resident enzymes, encoded by members of the *KTR/KRE/MNT* and *MNN* gene families, process and elongate the *N*-linked as well as *O*-linked oligosaccharides (40-42). Phosphomannan is a β-1,2-mannose moiety linked to the branched *N*-glycan via a phosphodiester bond. A similar moiety can be linked to lipid domains creating the phospholipomannans. Different enzymes from the *MNN* and *BMT* families participate in the synthesis of phosphomannan and phospholipomannans (42-44).

There are two main classes of cell wall mannoprotein in *C. albicans* based on the nature of their linkage to cell wall polymers. GPI-anchored proteins, which are the most abundant class of cell wall mannoprotein, are covalently attached via their carboxy-terminal glycosylphosphatidylinositol (GPI) anchor to β-1,6-glucan which, in turn, is linked to β-1,3-glucan (31). Pir proteins (proteins with internal repeats) are less abundant, and these are covalently linked directly to β-1,3-glucan (31,45).

Cell wall proteins provide anchors for the mannan outer layer of the cell wall. They contribute to the structural integrity of the cell wall, and some are cell wall remodelling enzymes responsible for generating essential covalent linkages between cell wall components (18,46,47). Transglycosylases from the GH72 family catalyse glucan remodelling, and their inactivation affects growth, morphology and virulence. For example, *PHR1* and *PHR2* (pH-responsive genes 1 and 2) encode members of this family, and they catalyse the pH-regulated cross-links between β-1,6- and β-1,3-glucans. *PHR1* plays a crucial role in the formation of

the hyphal cell wall and in pathogenesis (47,48). The GPI-anchored yapsin-like aspartic proteases Sap9 and Sap10 have functions in cell surface integrity and cell separation during budding, while the *CRH* family of chitin-glucanosyltransferases (Crh11, Crh12, Utr2) are involved in formation of linkages between β-1,3-glucan and chitin (46). The degree of cross-linking between components of the cell wall is important for its organization and integrity, as this determines its elasticity, resistance and porosity. This is evidenced by the deletion or over-expression of genes encoding cell wall remodelling enzymes, which results in altered sensitivity to cell wall disrupting agents, such as Congo Red, Calcofluor White, SDS, and high Ca^{2+} concentrations (18,46,47).

Besides its structural role, the cell wall promotes *C. albicans* pathogenicity, for example through adhesion, invasion and damage. Adhesins are important not only for fungal colonisation, but also for biofilm formation and interactions with other microbes. Most of the known adhesins are GPI anchored proteins, and many are members of multigene families such as the *ALS* and *HWP* gene families (49,50). Some adhesion genes, such as *ALS3* and *HWP1*, are expressed during hypha formation, which is why this morphotype is particularly adherent. The HWP adhesin family is required for adhesion to host cell proteins, biofilm formation, cell-cell aggregation and mating (49,51). *HWP1*, *HWP2*, and *RBT1* expression is induced not only during hypha formation but also during mating of opaque cells. Another member of this family, *EAP1,* is expressed in both yeast and hyphal cells and is differentially regulated during yeast phenotypic switching (51,52). Als3 acts both as an adhesin and an invasin as it binds to a host receptor on epithelial or endothelial cells to induce endocytosis (53). Als3 also enables iron acquisition by binding transferrin and has effects on host cell damage and cytokine induction (54).

The general structure of the *C. albicans* cell wall has been reasonably well understood for some time (9,22,23). However, recent technological advances are extending our knowledge of this field. For example, atomic force microscopy is providing direct information about cell wall structure and elasticity (55). Also, super-resolution microscopy has shown that phosphomannans, which are negatively charged, are critical for glucan masking at the cell surface (56). Furthermore, high resolution electron tomography has permitted the development of the first scale model of *C. albicans* cell wall architecture (Megan Lenardon and Neil Gow, personal communication). This deeper understanding of *C. albicans* cell wall biosynthesis, structure and organisation is helping the development of new therapies and diagnostics.

In the past, the fungal cell wall was often portrayed as a rigid shield-like structure in which, for example, chitin was compared to the steel in reinforced concrete. However, it has become clear that the cell wall is actually a surprisingly elastic structure. *C. albicans* releases extracellular vesicles which carry diverse cargo (including enzymes, toxins and nucleic acids, for example) that are believed to function in cell-to-cell communication, metabolism, and pathogenesis (16,57,58). These extracellular vesicles vary in size from 50 to 850 nm, and yet these membrane-bound compartments are able to transit through the cell wall into the surrounding milieu (16). Another study has demonstrated that large liposomal particles can gain entry to the cell by traversing the *C. albicans* cell wall. Transmission electron microscopy has revealed that Ambisome particles of 60 to 80 nm, which are much larger than the predicted pore size of the cell wall (approximately 6 nm), are able to transit through the cell wall whilst both particle and cell wall retain their integrity (17). The remarkable elasticity of the *C. albicans* cell wall is further reflected in the ability of *C. albicans* cells to undergo rapid and dramatic changes in volume in response to acute hyper-osmotic stress (18). Therefore, the cross-linked polymers of the *C. albicans* cell wall have evolved to provide an elastic and flexible structure, not a rigid shield.

Cell wall remodelling in response to damage

The *C. albicans* cell wall is a dynamic structure that changes in response to morphogenetic triggers, other environmental inputs, genetic perturbation, and antifungal treatment. Transcriptomic, proteomic and biochemical studies from a number of research groups have revealed condition-specific programmes of cell wall protein expression (33) and carbohydrate content or synthesis (21). A complex network of signalling pathways regulates this cell wall adaptation. These pathways include the cell wall integrity pathway, high osmolarity glycerol (Hog1) mitogen activated protein kinase (MAPK) signalling, the calcineurin-calmodulin pathway, the protein kinase A (PKA) pathway, the Cek1 MAPK pathway, mitochondrial reactive oxygen species (ROS) signalling, casein kinase I (Yck2, Yck3), and the heat shock transcription factor (Hsf1)-Hsp90 auto-regulatory circuit (59-64) (Figure 2). This complexity probably reflects the absolute requirement to retain cell wall homeostasis in the face of a diverse range of environmental inputs and challenges.

This complex regulatory network presents an issue for antifungal therapy because, following exposure to an agent that compromises cell wall integrity, the network provides mechanisms for compensatory changes to the fungal cell wall. The major classes of antifungal drug impose major insults on the cell wall by targeting the synthesis or structural integrity of the cell wall and plasma membrane. Echinocandins, such as Caspofungin, target β-glucan biosynthesis via the catalytic subunit of glucan synthase, Fks1. The inhibition of Fks1, and hence β-glucan synthesis, by Caspofungin induces compensatory activities in the form of increased chitin synthesis and deposition in the cell wall (65). This elevation in cell wall chitin then protects cells against further Caspofungin treatment both *in vitro* and *in vivo,* thereby compromising the efficacy of the antifungal drug (66).

Azole drugs, such as fluconazole, target lanosterol 14-α-demethylase (Erg11) on the ergosterol biosynthesis pathway. This induces significant changes in plasma membrane rigidity and integrity. Although azoles do not appear to affect the cell wall directly, proteomics and cell wall sensitivity assays have shown that fluconazole treatment indirectly perturbs the integrity of the cell wall (67).

Clearly, genetic perturbation of cell wall components can significantly affect cell wall architecture. The loss of β-glucan synthase (Fks1) is lethal to *C. albicans* (10,29). However, only one of the four chitin synthase genes (*CHS1*) is essential for viability (12,68), due to compensatory changes in chitin synthesis rescuing the loss of other *CHS* genes (25). In general, cell wall mannoproteins themselves are rarely essential for viability, but the inactivation of some specific GPI-anchored proteins can perturb integrity of the *C. albicans* cell wall (69,70). Mutations with more general effects upon the localisation or mannosylation of GPI anchored cell wall proteins also affect the sensitivity of *C. albicans* to cell wall stresses (34-36,39,71,72). This type of approach, involving the analysis of cellular responses to the disruption of cell wall genes or to cell wall perturbing agents, has helped to elucidate the roles of specific proteins or protein families in virulence-related phenotypes, such as adhesion and biofilm formation. In the context of this article, it has also highlighted key mechanisms underlying cell wall maintenance and homeostasis.

The cell wall integrity signalling pathway drives the main compensatory changes in the cell wall that are initiated in response to antifungal drugs, other cell wall stresses, and genetic insults. This pathway has been evolutionarily conserved across those fungi investigated, and has been extensively studied in the model yeast, *Saccharomyces cerevisiae*. The cell wall integrity pathway responds to the activation of cell wall stress sensors by up-regulating the

expression of cell wall synthesis genes via a highly conserved MAPK signalling cascade (Figure 2). In *S. cerevisiae*, signalling via the cell wall integrity pathway is initiated by the membrane proteins Wsc1, Wsc2, Wsc3, Mid2, and Mtl1, which act as cell integrity sensors (73). Upon loss of cell wall integrity, these sensors interact with Rom2 to activate Rho1, which then activates protein kinase C (Pkc1). Pkc1 signals to a MAPK module comprising Bck1, which activates MKK1/2, which phosphorylate and activate the MAPK, Slt2. Slt2 then activates the transcription factors Rlm1 and Swi4/6, which induce the expression of genes that include the cell wall synthesis machinery (73).

C. albicans has homologs for many components of the cell wall integrity pathway (74-76). Mutations in many affect the virulence of *C. albicans*, as well as its cell wall integrity, which suggests a key role for the cell wall integrity pathway in host niches. Furthermore, some components of this PKC-MAPK module in *C. albicans* have broader roles than their homologs in *S. cerevisiae*. For example, Mkc1, the *C. albicans* homolog of the *S. cerevisiae* MAPK Slt2, has an expanded role in regulating cellular morphogenesis under certain conditions (75). In addition, in *C. albicans,* Cas5 (rather than Rlm1) appears to be the transcription factor that plays the major role in controlling key gene outputs of the cell wall integrity pathway (77). The cell wall integrity pathway also engages in cross talk with other important signalling pathways that include the cAMP-PKA, target of rapamycin (TOR), and Hog1 pathways, which helps to coordinate the response to specific stressors (78,79) (Figure 2). It is worth noting that the cell wall integrity pathway also regulates important virulence traits in other fungi, for example capsule synthesis in *Cryptococcus neoformans* (80) and drug resistance and virulence in *Aspergillus fumigatus* (81).

Cell wall remodelling in response to environmental change

The ability of the *C. albicans* cell wall to remodel itself in response to sub-lethal concentrations of cell wall damaging agents (above) reflects the fact that cell wall remodelling is simply an important component of the normal adaptive responses of this fungus to environmental change. Yeast-hypha morphogenesis is one of the most studied adaptive responses of *C. albicans* because of the importance of this reversible morphological transition for host-fungus interactions and virulence (82-84). A range of environmental stimuli trigger hyphal development, including temperatures above 36°C, neutral pH, serum, bacterial peptidoglycan, high CO_2 levels, release from quorum sensing and nutrient starvation. The resultant yeast-to-hypha transition is accompanied by shifts in the carbohydrate and proteomic content of the cell wall (27,85-87). The cell walls of *C. albicans* hyphae can have up to two-fold less mannan, three-fold more glucan, and five times more chitin than the walls of yeast cells (27). Furthermore, changes in glucan structure are associated with hypha formation (88). These changes in cell wall structure attenuate Dectin-1 mediated recognition of hyphae by innate immune cells, which compounds the physical challenges associated with the phagocytosis of mycelia (88-91).

Carbon source availability differs significantly between host niches. For example, glucose concentrations are about 0.06–0.1% in the bloodstream, but are essentially zero in the colon (92), whilst significant amounts of short chain fatty acids, such as lactate, are present in the vagina and colon (93,94). Changes in carbon source have been found to exert major effects on the architecture and content of the *C. albicans* cell wall. Although the relative amounts of chitin, glucan and mannan remain similar, *C. albicans* cells grown on lactate, rather than glucose, have a thinner and less elastic cell wall (18,20). These changes in cell wall architecture correlate with changes in the cell wall proteome and secretome. In particular,

the levels of certain chitinases increase (Cht1, Cht3), as do the cell wall remodelling enzymes Pga4, Phr1, Phr2, Pir1 and Xog1 (95).

The availability of essential micronutrients such as iron and zinc also varies between host niches, and this is exacerbated by the host's attempts to deprive invading pathogens of these micronutrients via nutritional immunity (96-98). Therefore, the ability to scavenge iron and zinc is critical for fungal pathogenicity and tissue invasion (99,100). In *C. albicans*, adaptation to iron starvation triggers changes in the expression of genes encoding cell wall proteins, biosynthetic enzymes and cross-linking enzymes (e.g. Als2, Bgl2, Cht2, Mnt4, Phr2, Pir1, Scw11) (101) and the elevation of Hwp1 and Rbt5 in the cell wall proteome (19). Recent data from our laboratory has shown that iron limitation is also accompanied by significant changes in cell wall architecture (unpublished). Changes in zinc availability also affect the *C. albicans* cell wall. Adaptation to zinc deprivation yields more adherent *C. albicans* cells that expose less mannan, but more chitin at their cell surface (102). Also, zinc mobilisation is linked to PKA signalling (103), which influences cell wall remodelling (59).

Host niches also vary significantly in their ambient pH. For example, the bloodstream is maintained at around pH 7.4, whereas the vaginal mucosa varies from pH 4 to pH 5 (1,104), and the major compartments of the gastrointestinal tract range from pH 2 to pH 7.5 (105-107). This type of change in ambient pH has a significant effect upon the *C. albicans* cell wall. When cells are exposed to low pH, the chitin content of the cell wall increases and the mannan fibrils in the outer layer of the cell wall become shorter and more disorganised (108). The expression of cell wall protein genes is also affected by changes in ambient pH. For example, exposure to alkaline pHs leads to the up-regulation of genes encoding cell wall biosynthetic enzymes (Kre6, Ecm38), modifying enzymes (Cht2, Crh1, Phr1), adhesins (Als3, Hwp1) and other cell wall mannoproteins (Hyr1, Rbt1, Rbt4) (109-111). Growth at an alkaline pH also induces the expression of cell wall and secreted proteins that play important roles in host-fungus interactions, such as the zincophore Pra1 and the candidalysin precursor, Ece1 (100,111,112).

Oxygen levels vary dramatically between host niches, approaching zero in the human colon and in some fungal lesions (113-115). Adaptation to hypoxia drives changes to the architecture of the *C albicans* cell wall (Figure 3), yielding a thinner inner glucan-chitin layer and thinner mannan outer layer (64). Hypoxia up-regulates *ECM33,* which is important for cell wall biogenesis and integrity (116), and *ALG2,* which encodes a putative mannosyltransferase (117). There is also an increase in the abundance of specific GPI-anchored proteins in the cell wall proteome (Hwp1, Pir1, Rbt5) (19). Hypoxic regulation of cell wall changes depend largely upon a combination of mitochondrial, PKA and Efg1 signalling (64,117).

Changes in ambient temperature also affect the *C. albicans* cell wall. When *C. albicans* yeast cells grow at 42°C their chitin content increases relative to cells grown at 30°C (118). Furthermore, the levels of the cell wall β-glucan glycosidases, Phr1 and Phr2, and chitin transglycosidases, Crh11 and Utr2, increase during growth at 42°C (118). In addition, temperature has an effect on the branched *N*-mannan composition of the cell wall: there is a decrease in β-1,2-linked mannose and an increase in α-1,3-linked mannose during growth at higher temperatures (119). Unsurprisingly, the perturbation of thermal regulatory processes in *C. albicans* also affects the cell wall. For example, depletion of the molecular chaperone Hsp90, which regulates the transcription factor Hsf1, affects the chitin content of the cell wall and leads to an increase in the thickness of both the inner and outer layers of the wall (120).

As discussed above, exposure to antifungal drugs or to cell wall stresses triggers cell wall remodelling. Other types of environmental stress also affect the cell wall. *C. albicans* is exposed to oxidative stress during phagocytic attack, and the cell wall provides a first line of

defence against the oxidative damage caused by reactive oxygen species (ROS). ROS detoxifying enzymes such as superoxide dismutases (Sod4, Sod5) and catalase (Cat1) are found at the cell surface (121,122). Furthermore, the peroxidase Tsa1 is localised to the hyphal cell wall (123). All of these are up-regulated upon encountering oxidative stress (122-124). Oxidative stress also influences cell wall architecture by inducing elongation of β-1,2-linked mannose side chains (125).

Changes in osmolarity drive changes in cell volume. Under these circumstances, the elasticity of the cell wall underlies the ability of *C. albicans* cells to adjust their volume without incurring fatal rupturing of the wall or plasma membrane (18). This cell wall elasticity is dependent on the expression of the CHR family of transglycosylases (Chr11, Chr12, Utr2) (18). Adaptation to osmotic shock is dependent on signalling through the MAP kinases Hog1 and Mkc1, both of which regulate cell wall synthesis and remodelling (75,126-128) (Figure 2).

Quorum sensing also influences cell wall biogenesis by modulating yeast hypha morphogenesis and PKA signalling at high cell densities (Figure 2). *C. albicans* generates farnesol, which accumulates at high cell densities. Farnesol attenuates the activity of adenyl cyclase, thereby down-regulating PKA activity (129). Farnesol also inhibits hyphal development by blocking Ubr1-mediated protein degradation of Nrg1, which represses hyphal development (130,131).

Clearly the cell wall is a flexible organelle that responds to local environmental inputs. These adaptive changes in cell wall structure and organisation directly affect the fitness of the fungus in these microenvironments. However, they also affect the fitness of the fungus indirectly in these microenvironments by influencing host-fungus interactions (below).

The cell wall in immune surveillance

As mentioned above, the cell wall is the first point of direct contact between *C. albicans* cells and innate immune cells. The cell wall polymers chitin, β-glucan and mannan are present on diverse fungal pathogens (13). The immune system has evolved to recognise these cell wall polymers as key epitopes, or pathogen-associated molecular patterns (PAMPs) (132). Professional phagocytes (including neutrophils, macrophages and dendritic cells) and non-professional phagocytes (such as epithelial and endothelial cells) express an array of fungal-sensing receptors, or pattern recognition receptors (PRRs) (133,134). These host receptors detect *C. albicans* PAMPs, many of which are located at the cell surface, and this recognition elicits innate immune responses (135-137).

Chitin is located in the inner layer of the *C. albicans* cell wall, in relatively low abundance compared to the other main cell wall components. Consequently most of the chitin in the lateral cell wall is largely shielded by the outer layer of mannan fibrils. Nevertheless, chitin is exposed at the cell surface in bud/birth scars and at sites of cell wall damage and does act as a PAMP (132). Chitin is thought to undergo degradation into small particles (<1 um) during the inactivation of fungal cells by neutrophils and macrophages and by chitinase digestion (138). These chitin particles are recognised by the mannose receptor (MR) and, thereafter, intracellularly by NOD2 and TLR9, eliciting an anti-inflammatory programme that includes elevated IL-10 expression and the dampening of TNF-α levels (138). Furthermore, *C. albicans* chitin suppresses the generation of nitric oxide by macrophages and shifts macrophage polarization from a pro-inflammatory M1 state towards anti-inflammatory M2 activation (139).

β-Glucan is highly immunogenic and the recognition of this PAMP is critical for antifungal immunity. While most β-glucan is buried in the inner layer of the *C. albicans* cell wall and masked by mannan fibrils (56,140), some β-glucan is exposed at bud scars and at small

puncta over the lateral cell surface (89; Bain *et al.* unpublished). β-Glucan recognition occurs predominantly through the C-type lectin receptor (CLR), Dectin-1 (133). Dectin-1 mediated recognition of β-glucan promotes formation of a phagocytic synapse, which activates pro-inflammatory signalling through Syk/CARD9, driving the respiratory burst and the release of cytokines such as TNF-α, IL-6 and IL-12 (141). In addition, the recognition of β-glucan by Dectin-1 triggers phagocytosis, phagosome maturation and ultimately clearance of the offending fungal cell (142).

The critical importance of Dectin-1 in anti-*Candida* immunity is highlighted by the susceptibility of Dectin1 knockout mice to lethal infection (143), as well as by the association of a genetic polymorphism in human Dectin-1, which disrupts β-glucan recognition by phagocytes and abrogates cytokine expression, with familial recurrent vulvovaginal candidiasis (144). Furthermore, elevated β-glucan exposure on *C. albicans* cells correlates with their reduced fitness in the gastrointestinal tract (145), probably because this exposure enhances Dectin-1-mediated clearance of the fungal cells from the gut. In addition to activating phagocytosis and pro-inflammatory functions, Dectin-1-mediated sensing of *C. albicans* β-glucan enables "trained immunity" via epigenetic reprogramming of monocyte metabolism to drive enhanced protection against secondary infections (146). Also, a link to adaptive immunity was demonstrated in a study showing that Dectin-1 expressed on dendritic cells controls CD4+ T cell-mediated gut immunity in mice (147).

These observations illustrate the central importance of Dectin-1 in anti-*Candida* innate and adaptive immune defences. However, additional receptors contribute to the recognition of β-glucan. CR3 (Mac1, CD11b/CD18) is an integrin expressed on several myeloid and lymphoid cell types with affinity for a variety of ligands including iC3b-opsonized target cells (148). The I-domain of the CD11b subunit can bind β-glucan (149) and contributes, along with Dectin-1, to the recognition of *C. albicans* hyphae by macrophages (150). Other β-glucan receptors include the glycosphingolipid Lactosylceramide, the scavenger receptors SCARF and CD36, and CD23 (151-153).

As stated above, the frond-like mannan fibrils that decorate the outer cell wall limit the exposure of β-glucan to immune recognition (56). Nevertheless, the mannan fibrils themselves contain molecular signatures that potentiate host immune responses (132). *N*-mannan is detected by the mannose receptor, which promotes the oxidative burst and Th1/Th17 responses to control *C. albicans* infection (154). DC-SIGN (SIGN-R in mice), which is expressed by dendritic cells, also binds fungal *N*-mannan. This leads to interactions with plasma membrane "pickets", such as CD44, that connect the *N*-mannan-DC-SIGN synapse to the cytoskeleton, thereby stabilising phagocytic binding to the target *C. albicans* cells (155).

The PRRs Dectin-2 and Dectin-3 (MCL, ClecSF8) recognise hyphal α-mannan (156-158). Indeed, hetero-dimerization of Dectin-2 with Dectin-3 drives a more potent NFkB response than either of these receptors alone (158). The Mincle (macrophage inducible Ca^{++}-dependent lectin) receptor also recognises α-mannan in the *C. albicans* cell wall to drive TNF-α production, thereby promoting protection against systemic infection in mice (137,159). In humans, Mincle expression on monocytes is non-phagocytic, but drives pro-inflammatory responses, whereas Mincle expression on neutrophils mediates phagocytosis and killing of *C. albicans* (160). *C. albicans* α-mannans are also recognised by CD23, resulting in NFkB activation (153).

Mannose-binding lectin (MBL) is a secreted circulatory PRR that supports opsono-phagocytosis, and mice that lack MBL succumb to lethal *C. albicans* infections (161). Gut epithelial cells secrete MBL upon sensing *C. albicans* to regulate gut homeostasis and control infection (162). Galectin-3, which is expressed in the cytoplasm of host cells and in body fluids

(163), has direct fungicidal activity against *C. albicans* cells (164). Galectin-3 binds fungal β-1,2 mannoside residues, which are found in phospholipomannan and occasional side branch caps of *N*-mannan chains in the outer wall of *C. albicans* (165) . Meanwhile the PRR Langerin recognises mannan and β-glucan and is the dominant receptor on Langerhans cells, which are specialised dendritic cells that are positioned within the epidermis to sample *Candida* species during gut colonisation (166,167).

The role of Toll-Like Receptors (TLRs) in mammalian antifungal defences was initially suggested by a *Drosophila melanogaster* study that revealed the regulation of drosomycin by the Toll pathway (168). Subsequently, TLR2 and TLR4 were shown to modulate cytokine production during candidiasis (169). These TLR receptors recognise phospholipomannan and *O*-linked mannan in the *C. albicans* cell wall, respectively (170-172).

Host receptors do not act efficiently in isolation. Instead, sensing of fungal targets is best achieved by collaboration between PRRs and the multi-valent engagement of multiple PAMPs on the cell surface. The inflammatory programme is maximised by co-stimulation of TLR and CLR and activation of MyD88 and Syk/CARD9 pathways, respectively. For example, Dectin-1 and TLR2 cooperate to drive TNF-α production following recognition of *C. albicans* β-glucan (173,174). Dectin-1 also mediates cooperative signalling with CR3 and SIGN-R1 (175,176) and, as mentioned above, the paired engagement of Dectin-2 and Dectin-3 synergistically boosts inflammatory responses (158). Our understanding of fungal recognition, combinatorial signalling and effector function is limited, and this is further complicated by the context of immune cells involved, their activation status and the nature of the fungal target encountered.

The cell wall in immune evasion

Most studies of fungal immunology have focussed on the immune cell – the receptors and their ligands, mechanisms of intracellular and cytokine signalling, and phagocytosis, for example. Less attention has been paid to the fungus and in particular to the impact of fungal adaptation upon PAMP exposure. Indeed most fungal immunology studies have examined fungal cells that were grown under standardised, but non-physiological conditions *in vitro.* Yet, as described above, *C. albicans* remodels its cell wall in response to environmental change. It is therefore unsurprising that the conditions under which *C. albicans* is grown significantly affect PAMP exposure, and thereby, the outcome of host-fungus interactions (91). It is becoming clear that, in reality, *C. albicans* is a moving target for the immune system.

Early indications that *C. albicans* is a moving immunological target arose from Wheeler's work showing that dynamic morphogenetic changes during infection affect the degree of β-glucan exposure on the fungal cells (177). In part this effect appears to be mediated by the damage that neutrophil extracellular traps cause to the fungal cell surface *in situ*, and the subsequent fungal cell wall remodelling and repair, which is largely mediated by Hog1-dependent processes (178).

The paradigm of the moving immunological target was clearly demonstrated by the observation that exposing *C. albicans* cells to physiological levels of lactate (a metabolite generated in the vagina and gut by host cells and the microbiota) triggers β-glucan masking at the fungal cell surface (179). *C. albicans* cells detect extracellular lactate via the receptor Gpr1, which signals through Gpa2, PKA, Crz1 and Ace2, leading to reduced β-glucan exposure at the cell surface (64,179) (Figure 3). This results in decreased macrophage phagocytosis, lower rates of neutrophil recruitment to sites of infection, and decreased production of the pro-inflammatory cytokines TNF-α and MIP-1 (179). This work, together with the correlative studies of Sem and co-workers (145), suggests that *C. albicans* exploits local

environmental signals to evade immune recognition and thereby enhance its fitness in certain host niches.

More recently, *C. albicans* has been shown to trigger β-glucan masking and immune evasion in response to hypoxia (64,115). During the development of a lesion, oxygen concentrations are lower through the combined activities of the infecting *C. albicans* cells and the neutrophils in immune infiltrates that form in an attempt to clear these fungal cells. The resultant hypoxic microenvironment activates β-glucan masking by the *C. albicans* cells, thereby protecting them from clearance by the surrounding neutrophils (115). The hypoxic signal is transduced via the mitochondrion, which leads to PKA-mediated β-glucan masking (64) (Figure 3). More recently, we have shown that iron depletion also promotes β-glucan masking in *C. albicans* (Pradhan *et al.* unpublished). Iron depletion is highly relevant to systemic infection as the fungus becomes exposed to iron-limiting conditions in tissues as a consequence of the nutritional immunity imposed by immune infiltrates around fungal lesions (98). Clearly, *C. albicans* is able to exploit the local signals in certain host niches to evade immune recognition.

Other host niches appear to trigger PAMP exposure and inflammation, rather than PAMP masking and immune evasion. *C. albicans* cells that are exposed to the relatively low ambient pH of the human vagina tend to expose higher levels of β-glucan and chitin at their surface than cells grown at neutral pH of the bloodstream, for example (108). The elevated chitin exposure appears to be mediated by a reduction in chitinase (Cht2) expression via Bcr1 and Rim101 signalling (Figure 3). The exposed fungal cells are phagocytosed more efficiently by macrophages and neutrophils, they stimulate increased production of pro-inflammatory cytokines, and they recruit immune cells more efficiently to infection sites (108). These observations appear to resonate with the inflammatory behaviour of *C. albicans* during vulvovaginal candidiasis (180).

Artificial environmental inputs, such as antifungal drugs, also convert *C. albicans* into a moving immunological target. Exposure to sub-inhibitory concentrations of caspofungin increases β-glucan exposure in *C. albicans* to sufficient levels to elicit a potent TNF-α response from macrophages (140). This caspofungin-mediated β-glucan exposure is relevant *in vivo* during infection (177). A heightened immune response to *C. albicans* can also be caused by mannan grazing by *Bacteroidetes* (a Gram-negative member of the gut microbiota), possibly via trimming of the outer fibrillar layer of the cell wall to reveal the underlying β-glucan (181).

Parallels with other fungal pathogens

C. albicans is not the only fungal pathogen to evade host immune responses by masking a major PAMP in their cell wall. *A. fumigatus*, *C. neoformans*, *Histoplasma capsulatum*, and other dimorphic fungal pathogens have evolved effective mechanisms to avoid Dectin-1-mediated immune responses. These fungal pathogens mask PAMPs via two major mechanisms: firstly, by physically masking the PAMP with non-stimulatory cell wall molecules; or secondly, by hydrolase-mediated remodelling of the exposed PAMP.

A. fumigatus is the most common cause of invasive mould infections in immunocompromised patients (7). The initial host-pathogen interaction, and an important stage for immune evasion, occurs between conidia, lung epithelial cells, and resident alveolar macrophages. The *A. fumigatus* cell wall contains pro-inflammatory PAMPs, such as galactomannans and β-glucan, which stimulate robust antifungal immune responses and clearance mechanisms (182-184). *A. fumigatus* PAMP exposure peaks with conidial swelling and early hyphal germination, but is masked in mature hyphae and ungerminated conidia

(185). These ungerminated conidia mask their cell wall PAMPs under a rodlet layer composed of DHN-melanin and the hydrophobic RodA protein (186).

RodA masks Dectin-1- and Dectin-2-mediated detection of *A. fumigatus* conidial PAMPs, and this promotes early immune evasion and fungal survival in the host (187). DHN-melanin plays an important role in preventing phagosomal acidification, thereby enhancing virulence (188,189). However, DHN-melanin is also a PAMP and the ligand for the newly characterised host PRR, MelLec (184). Sensing of DHN-melanin by MelLec is important for the control of systemic *A. fumigatus* infection and MelLec polymorphisms are associated with increased risk of aspergillosis in certain cohorts of transplant patients (184).

The protective rodlet layer is lost during *A. fumigatus* germination to reveal the underlying PAMPs. However, *A. fumigatus* hyphae synthesize a cell wall polysaccharide, galactosaminogalactan, which masks β-glucan whilst mediating adherence to host cells (190). *A. fumigatus* mutants with defects in galactosaminogalactan biosynthesis display increased β-glucan exposure, they are attenuated in their virulence, and they induce hyper-inflammation in mice (190).

C. neoformans is another environmentally prevalent human fungal pathogen that causes disease in immunocompromised patients (7,191). Despite its clinical significance, relatively little is known about how the immune system recognises *C. neoformans* (192). In addition to the conserved carbohydrate polymers that typically form fungal cell walls (e.g. chitin, β-glucans, and mannans (13), *C. neoformans* possesses a unique polysaccharide capsule, primarily composed of glucuronoxylomannan, which masks its cell wall PAMPs. Glucuronoxylomannan is recognised by the receptor TLR4, but TLR4 engagement is not sufficient to induce TNF-α or influence mouse susceptibility to cryptococcosis (193,194). Interestingly, the collectin SP-D binds to glucuronoxylomannan *in vitro*, and its interaction with *C. neoformans* cells facilitates fungal protection from macrophage killing (195). This suggests a possible proactive immune evasion role for *C. neoformans* capsule beyond simply passively shielding cell wall PAMPs (195).

Acapsular *C. neoformans* mutants are avirulent and are phagocytosed more efficiently than encapsulated cells (195), which is likely due to the unmasking of the underlying immune-stimulatory PAMPs. These appear to include mannoprotein moieties recognised by the Mannose Receptor (196), as mice lacking the Mannose Receptor are more susceptible to infection than wild-type mice (197). Other major receptors, such as Dectin-1, Dectin-2, and Dectin-3, are not essential for *in vivo* defences against cryptococcosis (198-200).

The virulence of dimorphic fungal pathogens, such as *H. capsulatum, Blastomyces dermatitidis* and *Paracoccidioides brasiliensis*, has been linked to α-1,3-glucan in their cell walls. α-1,3-Glucan blocks the recognition of cell wall β-1,3-glucan via Dectin-1 by physically masking β-1,3-glucan (201). Consequently, *P. brasiliensis* and *B. dermatitidis* mutants with low α-1,3-glucan production display decreased virulence in mouse models of infection (202,203). However, this association between *H. capsulatum* virulence and α-1,3-glucan is dependent on strain chemotype. *H. capsulatum* strains of chemotype II require α-1,3-glucan for virulence (204) and the inactivation of α-1,3-glucan synthesis attenuates *H. capsulatum* virulence in mice (205).

In addition to physically masking β-1,3-glucan with α-1,3-glucan, *H. capsulatum* also enzymatically reduces β-glucan exposure in its cell wall (206). This is achieved by expressing Eng1, an endoglucanase that hydrolyses β-(1,3)-glycosyl linkages. Eng1 decreases β-glucan exposure at the cell surface, thereby reducing Dectin-1-mediated recognition of *H. capsulatum* cells, and enhancing the virulence of *H. capsulatum* (206). Therefore the combined effects of physical masking (via α-1,3-glucan) and enzymatic trimming (via Eng1) provide *H. capsulatum*

with effective PAMP masking mechanisms (Figure 3). No doubt these contribute to the inability of innate immune cells to control *H. capsulatum* infection, with macrophages ultimately serving as a reservoir for disseminated infection (206). Interestingly, Eng1 homologs exist in other important fungal pathogens, including *C. albicans*, suggesting that PAMP trimming mechanisms might contribute to immune evasion in these fungi.

Conclusions and outlook

To summarise, the fungal cell wall is a remarkable organelle that retains a high degree of elasticity and permeability, whilst retaining sufficient tensile strength and spatial integrity to preserve the morphology of the cell. In this way the cell wall is able to protect the fungal cell against certain acute environmental stresses, whilst permitting communication with the host or local microbiota through the release of large extracellular vesicles. Furthermore, through a complex signalling network that regulates cellular adaptation and cell wall synthesis, the cell wall is responsive to a wide variety of environmental challenges. This cell wall remodelling allows a fungal pathogen to evade the potentially lethal effects of certain antifungal drugs or debilitating mutations, and of local cell wall stresses imposed by host niches.

However, the cell wall is also a point of fragility for a fungal pathogen, as it carries immuno-stimulatory epitopes that can trigger antifungal host defences. Therefore the cell wall has a major influence upon host-fungus interactions. Nevertheless, the ability to remodel the cell wall has provided fungal pathogens with the capacity to evolve effective immune evasion strategies that either mask or remove cell surface PAMPs. *C. albicans,* in particular, has "learned" to exploit a variety of host-derived signals to activate β-glucan masking and immune evasion, including lactate, iron deprivation and hypoxia.

A number of fascinating questions remain to be answered. For example, what is the exact nature and frequency of the covalent cross-links between the major cell wall polymers in the *C. albicans* cell wall? And how, together with the properties of these polymers, do these cross-links promote the remarkable elasticity and morphological stability of the cell wall? The development of monoclonal or recombinant antibodies that are specific for particular cross-links would permit the frequency and spatial distribution of these linkages to be analysed *in situ* on the *C. albicans* cell wall. This would be particularly interesting in the context of environmental or genetic changes that affect cell wall elasticity and/or morphology (e.g. (18,20,46,48).

It would be fascinating to screen for host inputs that influence β-glucan exposure in *C. albicans* and thereby affect immune evasion. A number of specific host inputs have been identified already, but an unbiased screen of host signals has yet to be reported. Then, given the complexity and diversity of host niches, it would be important to test combinations of inputs to establish which signals are most influential in particular niches, and to test PAMP exposure on cells isolated directly from these niches. For example, recent data (e.g. (207) suggests that lactate-mediated β-glucan masking might dominate over pH-mediated β-glucan exposure during vulvovaginal infection. But what signals dominate in the gastrointestinal tract, and how does this affect *C. albicans* colonisation of the colon, for example?

It is also important to understand exactly how do *C. albicans* cells mask β-glucan at their cell surface – by covering it with mannan or by trimming via an Eng1-like activity (56,208)? Does β-glucan masking attenuate *C. albicans*-phagocyte interactions by simply delaying phagocytic recognition, or does masking (also) reduce the dynamics of phagocytic uptake and/or phagolysosomal maturation?

These questions are not simply of academic interest. A better understanding of the intricacies of cell wall structure and biogenesis is likely to reveal new therapeutic targets that will compromise this essential organelle. Furthermore, a better understanding of the immune evasion strategies exploited by fungal pathogens might reveal ways in which PAMP masking might be blocked. This type of drug might provide a potential means of augmenting antifungal immunotherapies. Time will tell.

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FIGURE LEGENDS

Figure 1: Architecture of the *C. albicans* cell wall. The model of the cell wall structure illustrates the organisation of chitin and glucan in the inner cell wall, and the *N*-mannan fibrils of the outer cell wall, which are linked to the inner cell wall via the GPI-anchored proteins from which these fibrils radiate. GPI-proteins are attached to β-1,6-glucan which, in turn, is linked to β-1,3-glucan, whereas Pir proteins are linked directly to β-1,3-glucan. The cartoon, which is taken with permission from (13), is compared with a transmission electron micrograph of the *C. albicans* cell wall (upper panel), which is an expanded region (blue box) from a micrograph of a complete cell (lower panel). The diameters of inner and outer layers of the cell wall are each about 0.14 μm across (64).

Figure 2: A complex network of signalling pathways regulates cell wall synthesis and remodelling in *C. albicans*. Cell wall remodelling depends on the cell integrity pathway (red) (74,75). Cell wall damage is thought to be detected by Wsc1/2/4, which activates protein kinase C (Pkc1) via Rho1. This leads to activation of the Mkc1 MAPK module which triggers cell wall remodelling via the transcription factor Rlm1 but primarily via Cas5 (76,77,209). The Hog1 pathway (blue) also contributes to the control of cell wall synthesis and remodelling (59). Cell wall or osmotic stress down-regulates Sln1, which leads to the activation of the Hog1 MAPK module via the Ypd1 and Ssk1 phosphorelay (126,127,210,211). Hog1 then modulates cell wall largely via the transcription factor Sko1, which also represses Brg1 (212). Hog1 is down-regulated by the phosphatases Ptp2/3, which are activated by TOR signalling (grey) (212). Hog1 also activates Mkc1 signalling, and represses the Cek1 pathway (dark green) (213). Msb2 acts in concert with Sho1 to activate the Cek1 pathway in response to osmotic stress or cell wall damage, and Opy2 also contributes to Cek1 activation via Cst20 (214-217). Cek1 activates Cph1 which is thought to contribute to cell wall remodelling during hyphal development. Morphogenesis is also activated by cAMP-PKA signalling (purple), which leads to cell wall remodelling. In response to a variety of environmental inputs, the Gpr1-Gpa2 and Ras modules activate adenyl cyclase (Cyr1), which leads to cAMP accumulation and inactivation of the PKA regulatory subunit Bcy1 (218). This leads to activation of the PKA catalytic subunits (Tpk1, Tpk2) which stimulates a network of transcription factors (Efg1,

Ume6, Brg1) and releases Nrg1-Tup1-mediated repression to activate hypha-specific genes, hyphal development and cell wall remodelling (27,85-87,219). This pathway is repressed by quorum sensing (brown), which inhibits adenyl cyclase (Cyr1), and also stabilises the repressor Nrg1 via Ubr1, Cup9 and Sok1 (129,130,220). Calcium (Ca^{++}) – camodulin (Cmd1) – calcineurin (Cna/b) signalling (lime green) also plays an important role in cell wall remodelling (59,221,222). Activation of this pathway, possibly via the stretch-activated channel Mid1, leads to the up-regulation of the transcription factor Crz1, which promotes cell wall remodelling (223,224).

Figure 3: Environmental factors modulate the exposure of cell surface epitopes to promote immune evasion or inflammation. A number of factors have been shown to influence the exposure of key epitopes (PAMPs) on the *C. albicans* cell surface. Caspofungin treatment leads to β-glucan exposure via the cell integrity (Mkc1) pathway (red) (140,177). In contrast, host-derived lactate triggers β-glucan masking via Gpr1-Gpa2 and PKA signalling (purple) (64,179). Hypoxia also initiates β-glucan masking, but this is mediated by mitochondrial signalling (grey), which then activates the PKA pathway (purple) (64). Micronutrient depletion leads to morphological changes that coincide with elevated chitin exposure at the cell surface (102). This might be transduced via PKA signalling (103). Growth in acidic pHs leads to increased β-glucan and chitin exposure (108). The increase in chitin exposure is mediated by Bcr1 and Rim101 signalling (108).

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