**Introduction**

In *Candida albicans*, like in *Saccharomyces cerevisiae*, the basal layer of the mature cell wall consists of a network of β-1,3- and β-1,6-glucans and chitin and functions as a skeletal layer. This basal layer is covered by an external layer of highly glycosylated, covalently anchored wall proteins radiating from the cell surface, which are directly involved in the first contacts between the fungal pathogen and host cells. The majority of the covalently bound wall proteins are modular glycosylphosphatidylinositol (GPI)-proteins. In their final form, wall-bound GPI-proteins usually consist of a C-terminal, truncated GPI-anchor that attaches them to the β-glucan layer, followed by a heavily glycosylated serine/threonine-rich spacer domain that often includes repeats, and an N-terminally located functional domain protruding from the cell surface [1]. At a given time-point >20 different covalently bound wall proteins can be identified [2,3] that are involved in processes such as adhesion, biofilm formation, wall remodeling, iron acquisition, and coping with immune responses. Importantly, the wall proteome is highly dynamic and continuously adapts to the specific conditions that *C. albicans* encounters in the host environment. In this review we examine the role of wall proteins in infection-related processes and assess their potential as targets for antifungal and vaccine development.

**Why Do Most Wall Proteins Form Families?**

*C. albicans* is able to thrive in many host niches, including the skin, mucosal surfaces, the bloodstream, and internal organs. Wall proteins are subject to the surrounding conditions and come into contact with highly diverse, niche-associated, extracellular matrix proteins from the host as well as with bacterial surface proteins. This probably explains the evolution of many wall protein families with individual members showing optimal functionality dependent on environmental conditions and infection sites [1]. For example, the environmental pH strongly affects the wall proteome, revealing the preferred usage of specific family members at acidic and neutral pH [4]. Interestingly, invasive growth is generally associated with hyphal growth, and comparison of the wall proteomes of yeast and hyphal cells revealed a core set of hypha-associated wall proteins under various hyphal growth-inducing conditions (Als3, Hwp1, Hwp2, Hyr1, Plb5, and Sod5) [2,5]. The two largest wall protein families are the Als family [6] and the Hyr/If family [7]. The family of agglutinin-like sequence (ALS) proteins consists of eight GPI-modified, elongated, broad-specificity adhesins with an immunoglobulin-like N-terminal domain that can interact with a wide variety of host proteins [8]. Some Als proteins possess amyloid-forming sequences, which could play a role in forming biofilms [9]. Fascinatingly, Als3 has multiple functions, including ferritin binding [10] as well as binding to E-cadherin, thereby facilitating iron uptake and active internalization of *C. albicans* by host cells, respectively [11]. This supports that proteins of a family share a particular function, but might also have additional functions that are not conserved throughout the family. Intriguingly, Hyr1, one of the 12 GPI-proteins belonging to the If/Hyr family, is strongly hypha-associated and confers resistance to neutrophil killing [12] through its N-terminal domain. Although the domain structure within the family is variable, the N-terminal domain is strongly conserved in all family members (Figure 1) [7]. This hints at a more general, niche-specific role of the family in evading immune cells under different growth conditions.

**What Is the Role of Wall Proteins in Iron Acquisition?**

One of the most restricted nutrients in the human body is iron. Because of its reactive nature, but also in order to restrict growth of invading microorganisms, free iron is highly limited in the host and mainly found in association with proteins, either as a prosthetic group like in hemoglobin and myoglobin, stored inside ferritin, transported by transferrin, or liganded by lactoferrin. *C. albicans* has evolved a number of strategies to scavenge iron from these complexes. Of the five Rbt5 family proteins, which belong to the CFEM superfamily and are characterized by an internal domain containing eight invariantly spaced cysteines [13], Csa1, Pga7, Pga10, and Rbt5 are found attached both to the plasma membrane and the wall, while Csa2 is secreted [3,14–16]. It has been shown that Csa1, Pga10, and Rbt5 are involved in heme binding [17]. As the expression of *CSA1*, *CX5*, *PGA7*, *PGA10*, and *RBT5* is co-regulated under various conditions, including iron restriction, the question arises whether the Rbt5 family proteins might act as a relay system, similar to bacterial iron uptake systems [18]. As mentioned above, Als3 is also important for iron acquisition as a receptor for ferritin, an iron-storage host molecule that contains about 30% of the total human iron pool. Without Als3, *C. albicans* is unable to grow with ferritin as its sole iron source [10].

**Which Wall Proteins Allow *C. albicans* to Cope with the Host Immune Response?**

*C. albicans* has evolved various mechanisms to avoid or counteract the immune response. The cell wall is the first line of...
defense, but also a target for the immune system due to its immunogenic epitopes. For example, the receptor dectin-1, which is mainly expressed on dendritic cells and macrophages, recognizes the β-glucan of the cell wall and leads to the activation of pro-inflammatory cytokines [19]. However, the mannoprotein coat largely prevents the detection of the underlying β-glucan layer. Additionally, the wall protein Hyr1 effectively reduces immune cell killing of C. albicans [12]. In support of its protective role, heterologous expression of Hyr1 in Candida glabrata also mitigates immune cell killing, suggesting a direct function of the protein. C. albicans also has two wall-bound, morphotype-associated superoxide dismutases (Sod4, Sod5) [14]. These cell wall–resident superoxide dismutases (Sods) detoxify reactive oxygen species (ROS) to H₂O₂, which is subsequently converted into H₂O and O₂ by catalase activity [20]. Proteins of the Hyr/Iff family confer resistance to neutrophil and phagocyte killing through an unknown mechanism [12]. Possibly, like in S. cerevisiae, proteases situated on the cell wall process the trans-membrane signaling protein Msb2 and liberate the extracellular domain Msb2*. Msb2* is able to bind to antimicrobial peptides (AMPs) in a dose-dependent manner and confers resistance [21].

**How Do the Wall and its Proteins Cope with Surface Stress?**

Cell shape is mainly determined by the skeletal polysaccharides of the wall, which are important for resisting the internal turgor pressure and shielding the cell from external mechanical forces. Nonetheless, remodeling of the wall is required, for example, during isotropic growth and cell separation, and for coping with surface stress. Remodeling of the wall is mediated by wall- and plasma membrane-resident, carbohydrate-active enzymes that detach, re-arrange, and re-attach carbohydrates. The main wall-bound proteins involved are a chitinase (Cht2), transglucosylases (Phr1, Phr2, Pga4), and chitin transglycosylases (Crh11, Utr2). The secretory aspartyl proteases Sap9 and 10, and Pir1, a predicted β-glucan cross-linking protein, also seem to be involved [3,23]. In contrast to Sap1 to 8, Sap9 and 10 are GPI-modified, yapsin-like proteases that are retained at the cell surface [23]. Interestingly, Sap9 has been implicated in the processing and shedding of other wall proteins, most notably, the chitinase Cht2 and Pir1 [24]. The levels of wall-bound Sap9 seem largely morphotype-independent, but its levels increase in conjunction with surface stress conditions as observed in response to fluconazole [3].

Strikingly, when C. albicans is grown on a poor carbon source such as lactate (found in the vaginal fluid and together with acetate...
which Wall Proteins Are Promising Targets for Other fungi as well.

antimicrobial peptides found in body fluids, epithelial layers, and survive other surface stresses, including membrane-perturbing surface-stress conditions ([3,25] and (Heilmann et al., unpublished remodeling proteins that is conserved in the response to several

Which Wall Proteins Are Promising Targets for Vaccine Development?

A vaccine that could be administered to high-risk groups, e.g., pre-surgery, or to women suffering from recurrent vaginitis, would be an important asset. As stated earlier, the functional domain of wall proteins is almost exclusively situated in the N-terminal region, while the C-terminal part is mainly of structural importance. This is reflected in the various vaccines that are currently being developed (reviewed in [1,26]). For example, mice immunized with the recombinantly expressed N-terminal domain of Als3 become resistant to infections by C. albicans as well as Staphylococcus aureus [27]. The N-terminal domains of Als1 and Hyl1, and a short immunogenic peptide from the N-terminal domain of Hwp1 conjugated to a β-1,2-linked mannotrioside, have been used similarly as C. albicans vaccines [1,12]. Notably, these four vaccine targets are strongly associated with hyphal growth, suggesting that hyphal epitopes might be more easily recognized by the immune system as a threat, since they are associated with the breach of host tissue. Invasive growth in vivo is not only associated with hyphal growth, but probably also with iron restriction and thus with increased levels of the iron acquisition proteins in the wall [15]. Relevantly, all five members of the Rbt5 family contain an identical sequence (with Csa1 containing four copies) that could represent a prime target. Developing this approach further, it is conceivable to combine immunogenic epitopes from the N-terminal functional region of a selection of wall proteins in a single recombinant protein for use as a multi-component vaccine. In summary, the evolution of wall protein families in the human fungal pathogen C. albicans allows survival in diverse host niches and has resulted in an impressive plasticity of the wall proteome. The exposure of wall proteins on the surface together with their critical functions, and the use of single- or multi-component vaccines, makes them promising targets for combating fungal infections.

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References

1. Klis FM, de Koster CG, Bruil S (2011) A mass spectrometric view of the fungal wall proteome. Future Microbiol 6: 941–951.
2. Heilmann CJ, Sorgo AG, Silikuas AR, Dekker HL, Bruil S, et al. (2011) Hyphal induction in the human fungal pathogen Candida albicans reveals a characteristic wall protein profile. Microbiology 157: 2997–3007.
3. Sorgo AG, Heilmann CJ, Dekker HL, Bekker M, Bruil S, et al. (2011) Effects of fluconazole on the secretome, the wall proteome, and wall integrity of the clinical fungus Candida albicans. Eukaryot Cell 10: 1071–1081.
4. Sosinska GJ, de Koning LJ, de Groot PW, Maunders EM, Dekker HL, et al. (2011) Mass spectrometric quantification of the adaptations in the wall proteome of Candida albicans in response to ambient pH. Microbiology 157: 136–146.
5. Staab JF, Bradley SD, Fidel PL, Sundstrom P (1999) Adhesive and mammalian transglutaminase substrate properties of Candida albicans Hwp1. Science 283: 1533–1538.
6. Hoyer LL, Green CB, Oh SH, Zhao X (2008) Discovering the secrets of the Candida albicans agglutinin-like sequence (ALS) gene family–a sticky pursuit. Med Mycol 46: 1–15.
7. Reisrane A, Coruza A, Da Costa G, Richard ML (2011) Unexpected role for a serine/threonine-rich domain in the Candida albicans Hwp1 protein family. Eukaryot Cell 10: 1317–1330.
8. Salgado PS, Yan R, Taylor JD, Burchell L, Jones R, et al. (2011) Structural basis for the broad specificity to host-cell ligands by the pathogenic fungus Candida albicans. Proc Natl Acad Sci U S A 108: 15775–15779.
9. Lipke PN, Garcia MC, Altemeis D, Ramsook CB, Klotz SA, et al. (2012) Strengthening relationships: amyloids create adhesion nanodomains in yeasts. Trends Microbiol 20: 59–65.
10. Almeida RS, Brunke S, Albrecht A, Thewes S, Laue M, et al. (2008) the hyphal-associated adhesin and invasion Als3 of Candida albicans mediates iron acquisition from host ferritin. PLoS Pathog 4: e1000217.
11. Liu Y, Filler SG (2011) Candida albicans Als3, a multifunctional adhesin and invasion. Eukaryot Cell 10: 168–173.
12. Luo G, Ibrahim AS, Spellberg B, Noble CJ, Mitchell AP, et al. (2010) Candida albicans Hyl1p confers resistance to neutrophil killing and is a potential vaccine target. J Infect Dis 201: 1719–1728.
13. Kulkarni RD, Kelkar HS, Dean RA (2003) An eight-cysteine-containing CFEM domain unique to a group of fungal membrane proteins. Trends Biochem Sci 28: 118–121.
14. Sorgo AG, Heilmann CJ, Dekker HL, Bruil S, de Koster CG, et al. (2010) Mass spectrometric analysis of the secretome of Candida albicans. Yeast 27: 661–672.
15. Sosinska GJ, de Groot PW, Triceira de Mattos MJ, Dekker HL, de Koster CG, et al. (2008) Hypoxic conditions and iron restriction affect the cell-wall proteome of Candida albicans grown under vagina-simulative conditions. Microbiology 154: 510–520.
16. Weissman Z, Shemer R, Conibear E, Kornitzer D (2008) An endo-lytic mechanism for haemoglobin-iron acquisition in Candida albicans. Mol Microbiol 69: 201–217.
17. Weissman Z, Kornitzer D (2004) A family of Candida cell surface haem-binding proteins involved in haemin and haemoglobin-iron utilization. Mol Microbiol 53: 1209–1220.
18. Braun Y, Hamke K (2011) Recent insights into iron import by bacteria. Curr Opin Chem Biol 15: 328–334.
19. Brown GD, Netea MG (2012) Exciting developments in the immunology of fungal infections. Cell Host Microbe 11: 422–424.
20. Frohner IE, Bourgeois G, Yatey K, Majer O, Kuchler K (2009) Candida albicans cell surface superoxide dismutase degrades host-derived reactive oxygen species to escape innate immune surveillance. Mol Microbiol 71: 240–252.
21. Szafrański-Schneider E, Swidergall M, Cottier F, Tielker D, Roman E, et al. (2012) Msb2 shedding protects Candida albicans against antimicrobial peptides. PLoS Pathog 8: e1002501.
22. Puri S, Kumar R, Chadha S, Tati S, Conti HR, et al. (2012) Secreted aspartic protease cleavage of Candida albicans Msb2 activates Cek1 MAPK signaling affecting biofilm formation and oropharyngeal candidiasis. PLoS One 7:e46020.
23. Albrecht A, Folk A, Pichova I, Naglik JR, Schaller M, et al. (2006) Glycosylphosphatidylinositol-anchored proteases of Candida albicans target proteins necessary for both cellular processes and host-pathogen interactions. J Biol Chem 281: 688–694.
24. Schaad U, Heyken A, de Groot PW, Hiller E, Mock M, et al. (2010) Protoytic cleavage of covalently linked cell wall proteins by Candida albicans Sap9 and Sap10. Eukaryot Cell 10: 98–109.
25. Ene IV, Heilmann CJ, Sorgo AG, Walker LA, de Koster CG, et al. (2012) Carbon source-induced reprogramming of the cell wall proteome and secretome modulates the adherence and drug resistance of the fungal pathogen Candida albicans. Proteomics doi: 10.1002/pmic.201200228.
26. Vecchiarelli A, Pericolini E, Gabrielli E, Pietrella D (2012) New approaches in the development of a vaccine for mucosal candidiasis: progress and challenges. Front Microbiol 3: 294.
27. Spellberg B, Ibrahim AS, Yeaman MR, Liu L, Fu Y, et al. (2008) The antifungal vaccine derived from the recombinant N terminus of Als3 protects mice against the bacterium Staphylococcus aureus. Infect Immun 76: 4574–4580.