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Klis, F.M.; Brul, S.

Published in:
Eukaryotic Cell

DOI:
[10.1128/EC.00142-15](https://doi.org/10.1128/EC.00142-15)

[Link to publication](#)

Citation for published version (APA):

Klis, F. M., & Brul, S. (2015). Adaptations of the secretome of *Candida albicans* in response to host-related environmental conditions. *Eukaryotic Cell*, 14(12), 1165-1172. <https://doi.org/10.1128/EC.00142-15>

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Adaptations of the Secretome of *Candida albicans* in Response to Host-Related Environmental Conditions

Frans M. Klis, Stanley Brul

Department of Molecular Biology and Microbial Food Safety, Swammerdam Institute for Life Sciences, University of Amsterdam, Amsterdam, The Netherlands

The wall proteome and the secretome of the fungal pathogen *Candida albicans* help it to thrive in multiple niches of the human body. Mass spectrometry has allowed researchers to study the dynamics of both subproteomes. Here, we discuss some major responses of the secretome to host-related environmental conditions. Three β -1,3-glucan-modifying enzymes, Mp65, Sun41, and Tos1, are consistently found in large amounts in culture supernatants, suggesting that they are needed for construction and expansion of the cell wall β -1,3-glucan layer and thus correlate with growth and might serve as diagnostic biomarkers. The genes *ENG1*, *CHT3*, and *SCW11*, which encode an endoglucanase, the major chitinase, and a β -1,3-glucan-modifying enzyme, respectively, are periodically expressed and peak in M/G_1 . The corresponding protein abundances in the medium correlate with the degree of cell separation during single-yeast-cell, pseudohyphal, and hyphal growth. We also discuss the observation that cells treated with fluconazole, or other agents causing cell surface stress, form pseudohyphal aggregates. Fluconazole-treated cells secrete abundant amounts of the transglucosylase Phr1, which is involved in the accumulation of β -1,3-glucan in biofilms, raising the question whether this is a general response to cell surface stress. Other abundant secretome proteins also contribute to biofilm formation, emphasizing the important role of secretome proteins in this mode of growth. Finally, we discuss the relevance of these observations to therapeutic intervention. Together, these data illustrate that *C. albicans* actively adapts its secretome to environmental conditions, thus promoting its survival in widely divergent niches of the human body.

The fungal pathogen *Candida albicans* is a highly specialized inhabitant of warm-blooded animals (mammals and birds). It preferentially colonizes mucosal surfaces and the skin but can also invade deeper-lying tissues and cause systemic infections that are difficult to treat and frequently lethal (1). To survive under the challenging and divergent conditions associated with the various mucosal surfaces in the human body, *C. albicans* disposes of a wide arsenal of virulence traits that help it to cope with antimicrobial peptides, the complement system, engulfment by macrophages, antibodies, hypoxic conditions, iron restriction, etc. A fascinating trait is its ability to switch reversibly between various growth forms, including among others the single-cell yeast form, which is especially suitable for dispersion of the fungus; the hyphal form, which facilitates adhesion to host tissues and promotes invasive growth and escape from engulfment by immune cells; and an intermediate, pseudohyphal growth form. *C. albicans* also forms biofilms (surface-associated microbial communities), which clinically speaking represent a highly relevant mode of growth and in which yeast, pseudohyphal, and hyphal cells cooccur and become encapsulated by substantial amounts of extracellular, macromolecular material. Biofilm formation on abiotic surfaces of medical devices and prostheses and on teeth has therefore been extensively studied (2, 3). The first contacts between *C. albicans* and host cells occur predominantly at the cell surface, and this presumably explains why the external protein coat of *C. albicans* cell walls consists of a wide variety of glycoproteins with specialized functions, many of which are under tight control, thus promoting survival under diverse stress conditions (4, 5). Equally important, *C. albicans* secretes a variety of glycoproteins that help to forage for nutrients by degrading host proteins, lipids, and glycogen, while others acquire iron and zinc ions and provide protection against antimicrobial peptides. Other glycoproteins help to form and strengthen biofilms and to accumulate extracellular matrix material. Together, we designate these secreted proteins as the secre-

tome *sensu stricto* (see below). The introduction of mass spectrometry in protein research has made it possible to study the protein assortment of entire cells or tissues and also well-defined subsets of proteins (subproteomes such as the cell wall proteome and the secretome), not only qualitatively but also quantitatively. This review discusses recent mass spectrometric explorations of the dynamics of the secretome of *C. albicans* depending, for example, on growth form and pH or in response to cell surface stress. For complementary reviews, the reader is referred to reference 4, which includes an extensive section about secretome proteins, and to a more recent review (6). In addition, the *Candida albicans* PeptideAtlas and the *Candida* Genome Database are recommended for detailed information about mass spectrometrically identified peptides (7, 8).

CLASSIFICATION OF MAJOR SECRETOME PROTEINS (*SENSU STRICTO*) OF *C. ALBICANS*

In this review, we will restrict ourselves to the secretome in the narrow sense of the word (secretome *sensu stricto*), that is, we will discuss only those proteins that possess an N-terminal signal peptide for entering the classical secretory pathway and lack internal transmembrane sequences. The major advantages of analyzing

Accepted manuscript posted online 9 October 2015

Citation Klis FM, Brul S. 2015. Adaptations of the secretome of *Candida albicans* in response to host-related environmental conditions. *Eukaryot Cell* 14:1165–1172. doi:10.1128/EC.00142-15.

Address correspondence to Frans M. Klis, F.M.Klis@uva.nl.

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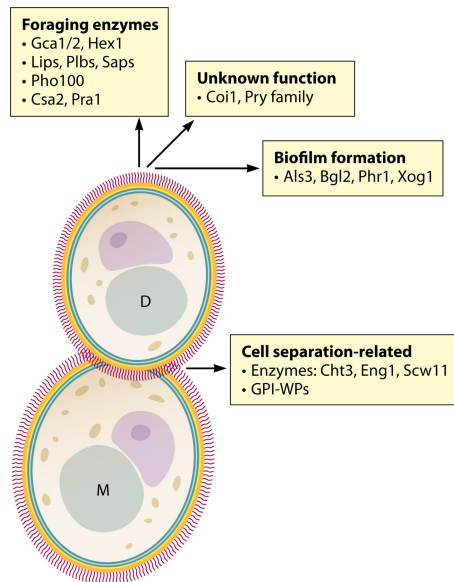


FIG 1 Major features of the secretome (*sensu stricto*) of *C. albicans*. The wall proteins are represented as short line segments perpendicular to the cell surface. M, mother cell; D, daughter cell; GPI-WP, GPI-modified wall protein. The role of Csa2 in heme binding is speculative (6, 40, 41). Note that Als3 and Phr1 are possibly directly released from the cell wall by Sap9/10 activity (47).

this protein category separately are that it is physiologically well defined, is limited in size, and is not affected by accidentally released proteins. This facilitates statistical analysis of the data (9), simplifies discussion of the results, and leads to physiologically relevant conclusions and testable hypotheses. For more information about the secretome in the wide sense of the word (*secretome sensu lato*), which includes a number of known cytosolic proteins, we refer to references 6 and 10.

The experimentally identified secretome proteins (*sensu stricto*) (currently, about 70 [9–15]) can be classified into two major groups: nonglycosylphosphatidylinositol (non-GPI) proteins, which lack a C-terminal signal sequence for the addition of a GPI anchor in the endoplasmic reticulum, and GPI proteins (Fig. 1). To avoid an excessively long list, this review focuses on the more abundant secretome proteins that are observed under more than one growth condition and/or have a known or predicted function.

NON-GPI PROTEINS

Modification of cell wall polysaccharides. Eleven enzymes (distributed over six families of glycosylhydrolases [GHs]) are involved in glycan chain elongation and branching and in glycan degradation (for example, during cell separation and emergence of a new bud or hyphal branch). Throughout this paper, we will follow the GH family classification according to the CAZy (for carbohydrate-active enzymes) database (16).

(i) **β -1,3-Glucan-modifying enzymes.** β -1,3-Glucan-modifying enzymes include Xog1 (GH5); Tos1 (GH16); Bgl2, Mp65, and Scw11 (GH17); Eng1 (GH81); Sim1 and Sun41 (GH132); and Dag7 (Barwin-like endoglucanase domain, PF03330) and GH (unspecified) (8).

(ii) **Chitin-degrading enzymes.** Chitin-degrading enzymes include Cht1 and Cht3 (GH18); they carry out degradation of the primary septum between mother and daughter cells, thus initiat-

ing cell separation. Note that the GH18 family member Cht2 is a GPI protein.

Three of the 11 corresponding genes (*CHT3*, which encodes the major chitinase; *ENG1*, which encodes an endo- β -1,3-glucanase; and *SCW11*, which encodes a β -1,3-glucan-modifying enzyme) are periodic genes with maximal expression in the M/G₁ period of the cell cycle and are target genes of the transcription factor Ace2 (17–20), consistent with a role in cell separation. Agar-grown colonies, mucosal biofilms, and biofilms formed on abiotic surfaces produce abundant amounts of extracellular matrix material (3, 21–23). The transglucosylase Bgl2 and the exoglucanase Xog1 (together with the GPI-modified, putative β -1,3-glucan-elongating enzyme Phr1) seem to be directly involved in formation and modification of extracellular matrix material in biofilms (24).

Nutrient acquisition. (i) **Glucosylases.** Glucosylases include Gca1 and Gca2 (GH31); the predicted substrates are glycogen and starch (4, 16, 25, 26). Interestingly, maltose, which is a degradation product of both polysaccharides, is a carbon source known to promote hyphal growth (27). It has been suggested previously that Gca1 and Gca2 are directly involved in promoting matrix production in biofilms by enzymatic release of soluble β -1,3-glucan fragments from insoluble β -1,3-glucan chains (28). However, Gca1 and Gca2 possess the hallmarks of an α -glucosidase/glucosylase, cleaving α -1,4-glucosidic linkages (8), and hence, it seems unlikely that they might cleave β -1,3-glucosidic linkages, which have a spatial structure highly different from that of α -1,4-glucosidic linkages.

(ii) **N-Acetylhexosaminidase.** *HEX1* is a glucose-repressed gene (29). Hex1 (GH20) is found in the periplasm and in the culture supernatant (29). It is possibly involved in the release of GlcNAc residues from host tissues for use as a carbon or nitrogen source (29). Note that GlcNAc also acts as a signaling molecule and induces and maintains hyphal growth in glucose-derepressed cells (30–33).

(iii) **Lipid degradation by Lips and Plbs.** Lipids are degraded by lipases (Lips) including Lip1 to Lip6 and Lip8 to Lip10 and by phospholipases (Plbs) including Plb1 and Plb2, reviewed in references 4 and 34.

(iv) **Protein degradation by aspartyl proteases.** Protein degradation by aspartyl proteases includes, for example, degradation of mucins and host immune proteins (4, 34–37), by aspartyl proteases such as Sap1 to Sap8. Note that Sap9 and Sap10 are cell surface-associated GPI proteins (38).

(v) **Metal ion acquisition.** Metal ion acquisition includes acquisition of zinc by Pra1 (39) and, probably, acquisition of heme (iron) by Csa2 (6, 12, 40, 41). Intriguingly, Pra1 is also involved in immune evasion (42, 43).

Pry family. The Pry family (11) consists of five members: Pry1, Rbe1, Rbt4, and two uncharacterized open reading frames (ORFs) (19.6200 and 19.2336). Rbe1 and Rbt4 have been often identified in culture supernatants (10–14). Interestingly, whereas Rbe1 is much more abundant in yeast culture supernatants, Rbt4 is much more abundant in hyphal culture supernatants (11–13) (see also Table 2). The virulence of an $\Delta rbe1 \Delta rbt4$ double deletion strain in the mouse model for systemic infection is strongly diminished (11), but the precise function of the Pry family proteins is still unknown.

Coi1. Coi1 is a small protein of 191 amino acids that is relatively abundant and is consistently found in the medium under all

growth conditions tested (10–14). Homologous proteins are found in only a limited number of *Candida* spp.—*Candida dubliniensis*, *Candida orthopsilosis*, *Candida parapsilosis*, and *Candida tropicalis*—and in *Lodderomyces elongisporus* (8). Its function is unknown.

Signaling protein Msb2. The signaling protein Msb2 is located in the plasma membrane and involved in signaling through activation of the Cek1 mitogen-activated protein (MAP) kinase (44). Although Msb2 has an internal transmembrane sequence, it is included in this classification and in the secretome *sensu stricto*, because it has a large extracellular, highly *O*-glycosylated domain that is shed into the medium and consistently identified in culture supernatants (10–14, 45). Interestingly, it also serves as a broad-range protectant against antimicrobial peptides (45, 46).

GPI PROTEINS

GPI proteins are targeted to the plasma membrane or become covalently linked to the β -glucan layer of the cell wall through their GPI anchor or are found at both locations. However, most GPI proteins are also identified in culture supernatants (10–14). There are several (possible) explanations for their presence.

Target proteins of Sap9 and Sap10. Some wall-bound GPI proteins are released by the surface-bound aspartyl proteases Sap9 and Sap10, such as Cht2 (47). Other candidates for active and controlled release, such as the adhesion protein Als3 and the transglucosylase Phr1, will be discussed below.

GPI proteins from the neck region. In single-cell yeast cultures, wall-bound GPI proteins are released from the neck region during cell separation. The wall between mother cell and growing bud is continuous, and complete cell separation therefore requires not only degradation of the primary septum by chitinase activity but also degradation of the lateral wall in the neck region.

Accidental release. Especially in shaken cultures, wall-bound GPI proteins might be released during emergence of a new bud or hyphal branch, which requires localized cell wall softening, or during periods of isotropic growth, which requires insertion of new cell wall polysaccharides and wall proteins into the existing wall.

Wall protein precursors may be washed out into the medium before they become covalently linked to the glucan-chitin network, especially in shaken cultures.

DYNAMICS OF THE SECRETOME OF *C. ALBICANS*

In this section, the term “apparent abundance” is introduced. It is defined as the number of spectral counts per protein divided by the total number of spectral counts of all secretome proteins (*sensu stricto*) and expressed as a percentage (48). This is a semi-quantitative measure that allows comparison of the individual contributions of the secretome proteins and, importantly, allows estimating and comparing the fold changes of individual secretome proteins upon changes in environmental conditions, including conditions that induce growth as single yeast, pseudohyphal, or hyphal cells. We prefer the use of apparent abundances to that of normalized spectral abundance factors (NSAFs [9, 10, 49]). In the latter approach, the number of spectral counts per protein is normalized for protein length, which for nonglycosylated proteins results in more accurate estimates of protein abundance. However, secretome proteins *sensu stricto* frequently contain long, heavily *O*-glycosylated sequences, which rarely result in detectable tryptic peptides and thus lead to serious underestimations of pro-

TABLE 1 Three prominent secretome (*sensu stricto*) proteins of *C. albicans* grown under various conditions

Protein	Apparent abundance (%) ^a		
	Yeast	Hyphal	FCZ ^b
Mp65	12.4	14.3	8.5
Sun41	8.0	8.6	7.4
Tos1	9.9	12.4	8.7

^a Based on data from references 12 to 14. Yeast cultures were grown at 30°C and pH 7.4; hyphal cultures were grown at 37°C and pH 7.4.

^b FCZ, fluconazole-treated cells grown at 37°C and pH 7.4. They form pseudohyphal aggregates (14).

tein abundances (for example, about 10-fold in the case of Msb2 [7, 8]) and decreased accuracy. A rough estimate of the number of secretome proteins per cell present in yeast culture supernatants, based on the data in references 12 and 50 and assuming an average protein mass of 40 kDa, is about 4×10^5 to 5×10^5 .

Three prominent secretome proteins. The three β -glucan-modifying enzymes Mp65 (GH17), Sun41 (GH132), and Tos1 (GH16) belong to the most prominent (detectable) secretome proteins, both in single-cell yeast culture supernatants and hyphal culture supernatants and during pseudohypha-like growth induced by fluconazole (Table 1). Similar values for their apparent abundances have been obtained under diverse growth conditions (6, 9, 10, 12–14). Conceivably, they are involved in various ways in the construction and remodeling of the β -1,3-glucan layer in the cell wall during growth (8, 16, 51–55). Their combined apparent abundance accounts for one-fourth to one-third of all secretome proteins. Consistent with this, the gene sequences of Mp65, Sun41, and Tos1 have a relatively high codon bias index (*MP65*, 0.71; *SUN41*, 0.64; *TOS1*, 0.59 [8]), suggesting that these genes are strongly expressed. Surprisingly, although Mp65 lacks a C-terminal GPI anchor to connect it covalently to the β -glucan network, it is usually also found in (hot-SDS-extracted) cell walls (10, 13, 14, 30, 37, 40). Scw11 and Sim1 belong to the same families as Mp65 and Sun41, respectively, and both show high apparent abundances in single-cell yeast cultures (Tables 2 to 4). However, in hyphal cultures and to a lesser extent also in pseudohyphal-growth cultures, their apparent abundances are considerably lower, suggesting that Scw11 and Sim1 might play a direct role in cell separation.

Yeast state-enriched secretome proteins: yeast versus hyphal cultures. In single-cell yeast cultures, the daughter cells become separated from the mother cell, a process that requires chitinase activity to degrade the primary septum formed during cytokinesis and β -1,3-endoglucanase activity to degrade the β -1,3-glucan layer in the lateral wall of the neck region. Conceivably, also transglucosylase activity is needed for repair activity. This results in much higher apparent abundances of the cell separation enzymes Cht3 and Eng1 and the potential repair enzyme Scw11 in the culture solution of unicellular budding yeast than in hyphal cells (Table 2). Although the apparent abundances of Bgl2, Cht1, Dag1, Rbe1, Sim1, and Xog1 in the culture solution of hyphal cultures are also strongly reduced, the corresponding genes do not seem to be periodically expressed (8, 17).

Hyphal state-enriched secretome proteins: hyphal versus yeast cultures. Many host-related chemical and physical conditions, such as a neutral pH, a temperature of 37°C, low oxygen levels and high CO₂ concentrations, the presence of GlcNAc (de-

TABLE 2 Yeast state- and hyphal state-enriched secretome proteins

Change and protein	Apparent abundance (%) ^a		Fold change
	Yeast	Hyphal	
Yeast to hyphal change			
Cht3 ^b	8.6	1.9	4.5
Scw11 ^b	8.6	1.9	4.5
Xog1	8.4	1.2	7.1
Sim1	7.8	2.6	3.0
Eng1 ^b	7.3	ND ^d	>28
Bgl2	3.6	0.5	7.5
Rbe1	3.0	ND	>12
Cht1	2.5	ND	>9
Dag7	2.3	ND	>9
Hyphal to yeast change			
Sap6	ND	13.1	>62
Rbt4	1.8	9.7	5.7
Als3^c	ND	9.3	>44
Sap4	ND	8.8	>42

^a Based on data from reference 12. Yeast cultures were grown at 30°C and pH 7.4; GlcNAc-induced hyphal cultures were grown at 37°C and pH 7.4 (12).

^b Periodically expressed genes with maximal expression in the M/G₁ phase of the cell cycle.

^c The GPI protein is in bold.

^d ND, not detected.

rivatives) or serum, iron restriction, and low glucose concentrations, promote hyphal growth (30, 56–58). The natural inducer *N*-acetylglucosamine (GlcNAc) offers several advantages. Under glucose-derepressing conditions, which occur in many niches in the human body, GlcNAc and also GlcNAc derivatives strongly initiate and sustain hyphal growth (31, 32, 59). GlcNAc is, for example, found in the polysaccharide hyaluronan, which consists of alternating residues of glucuronic acid and GlcNAc and is an abundant component of the extracellular matrix of epithelial and connective tissues (60) and is a known inducer of hyphal growth (59). An additional advantage of using GlcNAc to induce hyphal growth is that it, in contrast to bovine serum, allows (mass spectrometric) analysis of the hyphal secretome (12). GlcNAc-induced hyphal cultures contain four additional proteins in the culture medium that become about as equally prominent as Mp65, Sun41, and Tos1 (Tables 1 and 2), namely, the aspartyl proteases Sap4 and Sap6; the Pry family protein Rbt4, whose function is unknown; and the GPI-modified adhesion protein Als3 (Tables 1 and 2). This is consistent with the strongly increased expression levels of the corresponding genes in GlcNAc-induced hyphal cultures (31; see also reference 61) for serum-induced hyphal cultures. Of interest, Als3 is a hypha-specific adhesive GPI protein that is targeted to the cell wall and required for biofilm formation (62); it is also known to promote the formation of mixed fungal-bacterial biofilms through its N-terminal adhesion domain (63, 64). This raises the question of whether biofilm matrix material might become enriched with released Als3 and whether it might promote the cohesion of the extracellular matrix material. It is unknown whether Als3 is passively released from the cell wall or is released through a regulated, enzymatic process, possibly involving the GPI proteins Sap9 and/or Sap10 (38, 47). The secreted aspartyl proteases Sap4 and Sap6 are strongly associated with the yeast-to-hypha transition (65). Note that the presence of

TABLE 3 Main features of the secretomes of fluconazole-supplemented cultures

Change and protein	Apparent abundance (%) ^a		Fold change
	FCZ	Yeast	
FCZ to yeast			
Phr1^b	10.4	ND	>49
Yeast to FCZ			
Cht3 ^c	4.3	8.6	2.0
Scw11 ^c	5.6	8.6	1.5
Sim1	4.2	7.8	1.9
Eng1 ^c	0.3	6.7	22
Cht1	0.3	2.5	7.5

^a Based on data from references 12 and 14. Yeast cultures were grown at 30°C and pH 7.4 (12); fluconazole (FCZ)-treated cultures were grown at 37°C and pH 7.4. ND, not detected.

^b The GPI protein is in bold.

^c Periodically expressed genes with maximal expression in the M/G₁ phase of the cell cycle.

an ammonium salt as a rich nitrogen source in the culture medium at neutral pH does not seem to interfere with the accumulation of Sap4 and Sap6 at high levels in the medium (Table 2).

Major changes in apparent abundance in the secretome of fluconazole-treated cells. Pseudohyphal (or pseudohypha-like) growth can be induced in various ways, for example, by treating single-cell yeast cultures with sublethal concentrations of the antifungal compound fluconazole (14). Fluconazole blocks an Erg11-mediated demethylation step of the planar ring structure during ergosterol synthesis, and this results in the formation of structurally suboptimal sterols and increased fluidity of the plasma membrane (66). The cells respond by suppression of cell separation and by increased phosphorylation of the MAP kinase Mkc1 (14), which mediates the cell wall integrity pathway. Pseudohypha-like cell aggregates are formed, which separate into single cells when treated with exogenous chitinase (14). These effects are not specific for fluconazole but also occur when the cells are treated with the detergent SDS, grown at the harmful temperature of 42°C, or treated with the cell wall construction-perturbing compound calcofluor white or Congo red (14, 51) (Fig. 2). This suggests that many forms of plasma membrane stress or cell wall stress, together here referred to as cell surface stress, will cause pseudohypha-like growth and constitutively activate the cell wall integrity pathway. Indeed, cell separation suppression is a common phenotype among all genetic mutants that have to cope with a weakened cell wall resulting from defective *N*- or *O*-glycosyla-

TABLE 4 Main features of secretomes of low-pH-grown cultures

Change and protein	Apparent abundance (%) at pH ^a :		Fold change
	4	7.4	
pH 4 to pH 7.4 change			
Utr2^b	8.0	3.2	2.5
Bgl2	7.4	2.8	2.6
P1b4.5^b	6.0	ND	>32
Pir1	3.6	1.1	3.2

^a Data based on reference 14. ND, not detected.

^b GPI proteins are in bold.

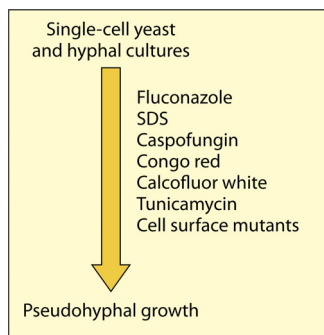


FIG 2 Cell surface stress induces pseudohyphal growth. For further discussion, see the text and references 14 and 75.

tion of secretory glycoproteins (67–70) or lack proteins involved in cell wall formation, such as Bgl2 (71); Ecm33, which is a GPI protein required for cell wall integrity (72); the secretome enzyme Sun41 (51, 52); and the GPI-modified and cell surface-located aspartyl proteases Sap9 and Sap10 (38), as well as Pir1, a putative cross-linker of β -glucan chains (73), and this list could be easily extended. Interestingly, *CHT3* transcription is markedly down-regulated in micafungin-treated cells (74), suggesting that this inhibitor of β -1,3-glucan synthesis might also induce cell separation suppression. Similar observations have been described by O’Meara and coworkers, who treated wild-type cultures with serum in combination with various antifungal drugs at sublethal concentrations and found that under these conditions hyphal growth was suppressed and, instead, pseudohyphal cell clusters were formed (see Fig. 2c in reference 75). A relevant implication of these observations is that “cell separation suppression” as a phenotype does not necessarily prove that the mutated gene is directly involved in cell separation.

Consistent with the partial suppression of cell separation in fluconazole-treated cells, resulting in pseudohypha-like growth, the major chitinase *Cht3* (GH18), the endo- β -1,3-glucanase *Eng1* (GH81), and the β -1,3-glucan-modifying enzyme *Scw11* are all decreased in the supernatants of fluconazole-treated cultures, although less so than in hyphal cultures (Tables 2 and 3). Other chitin- and β -1,3-glucan-modifying enzymes, namely, *Cht1* (GH18) and *Sim1* (GH132), are also decreased in fluconazole-treated and hyphal cells. How exactly suppression of cell separation is regulated in response to cell surface stress is currently unknown. As *CHT3*, *ENG1*, and *SCW11* are known target genes of the transcription factor *Ace2* (18–20), a regulator of cell separation that controls the expression of *M/G₁*-specific genes, it seems likely that fluconazole treatment and many other forms of cell surface stress lead to suppression of *Ace2* activity in cell separation. Possibly, cell separation is suppressed through phosphorylation of the transcription factor *Efg1*, which in turn represses *Ace2* target genes and promotes pseudohypha-like growth (76).

Another striking observation is the high apparent abundance of the GPI protein *Phr1* in the medium of fluconazole-treated cells, whereas it is not detectable in the medium of single-cell yeast cultures and hyphal cultures. This observation is consistent with the finding that the antifungal compounds ketoconazole and caspofungin stimulate the expression of *PHR1* (77). *Phr1* (GH72) is a pH-responsive transglucosylase and probably involved in expansion of the β -1,3-glucan layer (78, 79). It also plays an impor-

tant role in the accumulation of β -1,3-glucan in the extracellular matrix of biofilms, possibly by extending the β -1,3-glucan chains there and thus increasing the cohesiveness of the biofilm (24). This raises the interesting question of whether biofilm-associated *Phr1* in fluconazole-treated cells might contribute to protection against fluconazole. It is currently unknown how *Phr1* is released from the cell surface. However, *Phr1* contains two consecutive lysine residues (K_{451} and K_{452}), suggesting that it might be a substrate of Sap9/10 (47).

Finally, the culture medium of fluconazole-treated yeast cultures contains several low-abundance secretory proteins that so far have not been observed under other growth conditions (13). This does not necessarily prove that this secretome response is specific for fluconazole-treated cells, because, as we have seen above, many other conditions lead to cell surface stress, and this might be in itself enough to trigger the release of members of this subset of proteins.

Secretome proteins increased at low pH. Acidic conditions are found on the skin (80); in the vagina, due to formation of lactic acid by vaginal epithelial cells and by lactobacilli (81); and in dental plaque, where they are caused by the formation of lactic acid by *Streptococcus mutans* (82). Two GPI-modified wall proteins (*Plb4.5* and *Utr2*) and the β -1,3-glucan-linked wall protein *Pir1* become much more abundant in the culture medium upon lowering the environmental pH, either because at acidic pH their incorporation into the cell wall becomes less efficient or because they are actively released from the wall (Table 4). The apparent abundance of the non-GPI, β -1,3-glucan-modifying protein *Bgl2* (GH17) also shows a considerable increase. As this protein contributes to β -1,3-glucan accumulation in biofilms (24), this might mean that at acidic pHs biofilms produce more extracellular matrix.

Lactate-induced secretome proteins. Although at many infection sites glucose levels are low and, consequently, *C. albicans* cells are in a glucose-derepressed state, depending instead on alternative carbon sources for growth, glucose-grown cells are often used for research. The results obtained in this way can therefore not always be directly extrapolated to glucose-derepressed cells (83). For example, the levels of three secretome proteins, glucoamylase(s) *Gca1/2* (GH31), the aspartyl protease *Sap7*, and the exo- β -1,3-glucanase *Xog1* (GH15), strongly increase when lactate, a nonfermentable carbon source and a much poorer carbon source than glucose, is used to support growth (9, 84). On the other end of the spectrum, there are also a number of secretome proteins whose apparent abundances strongly decrease in lactate-grown cultures (9). The induction of hyphal growth by GlcNAc as discussed above is already blocked at a glucose concentration of 20 mM (33), and this represents another striking example of the importance of selecting the appropriate carbon source and/or glucose concentration in *Candida* research.

CONCLUDING REMARKS

Similarly to the wall proteome, the secretome of *C. albicans* operates at the fungus-host interface, and pronounced changes in abundance of individual proteins occur in distinct host niches. For example, the switch from single-cell yeast growth to hyphal growth results in the abundant secretion of the aspartyl proteases *Sap4* and *Sap6*, which significantly contribute to virulence (85). As expected, the pH of the host niche also strongly affects the composition of the secretome. The ability to form biofilms, in which

yeast, pseudohyphal, and hyphal cells are found, with the concurrent accumulation of extracellular matrix material and the often strongly lowered susceptibility to antifungal agents, is another important virulence trait. Several secretome proteins, such as Als3, Bgl2, Mp65, Phr1, Xog1, and Sun41, are believed to be involved in biofilm formation (8). In view of their high apparent abundances under widely diverging growth conditions, the secretome proteins Mp65, Sun41, and Tos1 seem attractive candidates for diagnostic purposes. In addition, as *Candida* infections are usually associated with invasive, hyphal growth, and as Als3, Rbt4, Sap4, and Sap6 become abundant in the supernatants of hyphal cultures, these proteins also are potential diagnostic candidates. Two secretome proteins, Als3 (actually, a recombinant protein that covers the N-terminal immunoglobulin-like domain of Als3) and Sap2, are currently being actively pursued for vaccine development (86–88). Als3 is an abundant, multifunctional GPI-modified cell wall protein (86, 89, 90) and as such is a highly attractive vaccine candidate. The finding that Als3 also becomes an abundant secretome protein in hyphal cultures (12) with a proposed role in biofilm formation further increases its attractiveness. It has been argued that a multivalent vaccine might be more effective to combat the various types of *Candida* infections (91). Conceivably, a recombinant protein consisting of two or three of the most immunogenic epitopes from various cell wall proteins (92) and from secretome proteins such as the aspartyl proteases Sap4 and Sap6 (12, 85); the three secretome proteins involved in the formation of biofilm matrix beta-glucan (Bgl2, Phr1, and Xog1) (24); Sun41 and Sim1, which form a two-member family and are synthetically lethal (52); and the Pry proteins Rbe1 and Rbt4 (11), each separated from the other by a nonimmunogenic linker sequence, might be an option. Similar approaches might be considered for other pathogenic fungi. Finally, many regulatory pathways have been shown to be involved in controlling the abundance of individual secretome proteins, but more-systematic studies of how the secretome as a whole is regulated are still scarce.

ACKNOWLEDGMENTS

We thank Alice Sorgo and Clemens Heilmann for their stimulating involvement in the research presented here.

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