Differential surface localization and temperature-dependent expression of the *Candida albicans* CSH1 protein

David R. Singleton¹ and Kevin C. Hazen¹,²

Departments of Pathology¹ and Microbiology², University of Virginia Health System, PO Box 800904, Charlottesville, VA 22908-0904, USA

Cell-surface hydrophobicity (CSH) in *Candida albicans* contributes to virulence and can be conveniently regulated in planktonic cultures by altering growth temperature. The CSH1 gene is the first candidate gene that has been demonstrated to play a role in affecting the CSH phenotype. However, the primary amino acid sequence of the CSH1 gene product suggests that the protein should be restricted to the cytoplasm. A majority of the protein appears to demonstrate that localization. Cell-surface biotinylation and limited glucanase digestion were used to determine and estimate the relative amount of Csh1p in the extracellular compartment in comparison to the cytoplasmic pool. Additionally, Western and Northern blotting were used to assess expression of the CSH1 gene under different growth conditions. Compared with cells grown at 23 °C, the total cellular levels of Csh1p are significantly greater at elevated growth temperatures. Detection of Csh1p on the cell surface correlates with the level of overall protein expression. The temperature-dependent regulation and surface presentation of Csh1p suggests a mechanism for regulating the CSH phenotype.

Abbreviation: CSH, cell-surface hydrophobicity.

INTRODUCTION

The cell wall of pathogenic fungi represents the initial point of interaction between the host and pathogen. The cell wall provides the pathogen with a structure that can enable the organism to evade active and passive immune surveillance of the host, and also enable it to adhere to and penetrate into tissue. The dynamic nature of the fungal cell wall is a significant virulence trait that allows the yeast to overcome host defences by altering these interactions.

The wall of the commensal yeast *Candida albicans* is composed of a tight matrix of carbohydrate and protein (Chauhan et al., 2002; Kapteyn et al., 2000; Klis et al., 2001). Carbohydrate classes in the cell wall can be broadly separated as glucans, mannans and chitin, which are composed of high-molecular-mass polymers of glucose, mannose and N-acetylglucosamine, respectively. Proteins within the cell wall matrix are typically highly glycosylated mannoproteins, which are cross-linked to other carbohydrate moieties within the wall (Klis et al., 2002). Biosynthesis of wall components occurs by the addition and maturation of core carbohydrate residues to glycoproteins during transit through the secretory pathway, and by de novo synthesis of glycan polymers within the wall matrix.

Cell-surface hydrophobicity (CSH) has been implicated as a virulence contributor for *C. albicans* (Hazen & Hazen, 1989; Hazen, 1989). Cells in candidal lesions are a mixture of yeast and hyphae, and are highly hydrophobic. Additionally, hydrophobic cells attach to cultured endothelial and epithelial cells more readily in static and flow adhesion assays, and are more resistant to killing by macrophages than hydrophilic cells. The hydrophobic state of the cell wall is conveniently regulated in the laboratory by growth temperature and analysis of cell wall extracts from hydrophobic cells has resulted in the identification of a number of protein candidates with hydrophobic character. Further analysis has indicated that hydrophobic and hydrophilic cells are biochemically similar, but the cell walls are ultrastructurally unique (Hazen & Hazen, 1993).

The first gene product to be cloned that affected the hydrophobic status of the cell was recognized on the basis of reactivity with the 6C5-H4CA (6C5) mAb (Glee et al., 1995, 1996). The antibody was raised against hydrophobic cell wall extracts and recognizes a single 38 kDa protein which we have referred to as Csh1p (Singleton et al., 2001). Csh1p (*C. albicans* unannotated genome build 19, ORF 10206; http://www-sequence.stanford.edu/group/candida; this gene product is identical in sequence to the IFD4 predicted gene product at http://genolist.pasteur.fr/CandidaDB) is one of a family of closely related proteins in *C. albicans* of unknown function, which are highly homologous to a family of *Saccharomyces cerevisiae* aryl-alcohol dehydrogenases. Ablation of the family of seven gene

---

INFORMATION:

**Correspondence**
Kevin C. Hazen
khazen@virginia.edu

**Received** 21 July 2003
**Revised** 31 October 2003
**Accepted** 5 November 2003

**Abbreviation:** CSH, cell-surface hydrophobicity.
products in *S. cerevisiae* had no discernible phenotype (Delneri et al., 1999), although knockout of the single gene in *C. albicans* resulted in a statistically significant reduction in CSH and a concurrent reduction in adhesion in a static assay (Singleton et al., 2001). Comparison of the csh1 knockout derivative with a wild-type strain has indicated that the knockout is still virulent, but shows a lag in the time-course of disease progression in a mouse infection model (unpublished data).

Inspection of the Csh1p sequence suggested that the protein should demonstrate cytoplasmic localization as opposed to being in the cell wall, as no N-terminal signal sequence directs targeting to the secretory pathway is present. Accordingly total cell extracts released significantly more antigen than limited glucanase digestion of the cell wall (Singleton et al., 2001). However, reproducible amounts of Csh1p are released under conditions maintaining cell integrity (Glee et al., 1996; Masuoka et al., 1999; Singleton et al., 2001) and the mAb is effective in blocking fungal adhesion in static and flow adhesion assays (Glee et al., 2001), suggesting that the protein is present to some degree in the cell wall. Supporting the cell wall localization of Csh1p, a number of presumptively cytoplasmic proteins have been described in the *C. albicans* literature to be present within the cell wall and playing a role in *Candida* pathogenesis (for review, see Chaffin et al., 1998). The presence of these proteins within the cell wall has been explained as the result of pre-existing or possibly novel transport mechanisms to the cell surface, or by heterologous cell lysis and reassociation of cytoplasmic proteins with neighbouring cells. The latter explanation does not adequately account for the amount of protein recoverable from intact cells.

To address the intriguing presence of Csh1p within the cell wall, we have characterized the nature of attachment of Csh1p to the wall and have labelled surface-accessible protein specifically. Several techniques to categorize peripherally and covalently attached cell wall materials were used to release Csh1p relative to the total cellular pool. A membrane-impermeable surface-labelling reagent was also used to tag surface-accessible Csh1p to allow the comparison of labelled protein with total Csh1p. The results show that the presence of Csh1p on the cell wall is correlated with the level of expression of the protein.

**METHODS**

**Strains and growth.** *C. albicans* strain LGH1095 (Hazen & Hazen, 1987) (ATCC MYA-2719) was grown in either in minimal media [YNB2G (0-67% Difco yeast nitrogen base plus 2% glucose) or Sabouraud’s glucose broth (SD; 1% casein hydrolysate plus 2% glucose, powder pre-mix from Difco)] or rich medium (YPD; 1% yeast extract, 2% bacto-peptone, 2% glucose) in liquid culture at 23, 30 or 37°C with shaking at 100 r.p.m. A csh1/csh1::URA3 knockout derivative of *C. albicans* strain SC5314 (Singleton et al., 2001) and an isogenic reintegrant csh1/csh1::CSH1::URA3 (this study) were grown under the same conditions. The reintegrant was generated by targeting a cassette containing the *CSH1* ORF ligated to the *C. albicans* URA3 gene to one *csh1* allele of a 5-fluoroorotic-acid-resistant derivative of the *csh1/csh1::URA3* knockout using standard *C. albicans* genetic techniques (Wilson et al., 2000).

**Protein extraction.** Proteins from yeast were extracted with a variety of methods to isolate total proteins and cell wall proteins preferentially. For total cell lysates, cell pellets from 5 ml cultures were suspended in a 1-5 ml microfuge tube in 500 µl ddH₂O plus a protease inhibitor cocktail of 1 mM AEBSF (Roche), 1 µM pepstatin A (Roche) and 1 mM EDTA (Baker). A volume of glass beads equal in size to the cell pellet was added and the suspension was vortexed 4°C for 5 minutes. After vortexing, 250 µl concentrated reducing Laemmli SDS-PAGE sample buffer was added and the sample was boiled for 3 min, followed by centrifugation at 10 000 g for 3 min to pellet insoluble material (Singleton et al., 2001).

For preferential extraction of cell wall materials, two methods were used. In the first method, cell pellets from 5 ml cultures (approx. 5×10⁶ cells) were suspended in a 1-5 ml microfuge tube in 500 µl 0-1 M Tris/HCl, pH 7-6/80-1 M EDTA/0-9 M sorbitol plus AEBSF (Roche) and pepstatin A (Roche) as above. The spheroplasting enzyme Zymolyase 100T (ICN) was added as a 50 µl aliquot of a 0-3 mg ml⁻¹ stock (30 µg ml⁻¹ final concn) and a 50 µl aliquot of diluted 2-mercaptoethanol (30 mM final concn). Cells were treated for 60 min at 37°C with gentle shaking (modified from Glee et al., 1995). Cells were gently pelleted from the extract by centrifugation at 1000 g for 5 min and the supernatant was boiled with reducing sample buffer as above. In the second method, cell pellets were suspended in 0-5 ml 0-1 M Tris/HCl, pH 9-4, plus the protease inhibitor cocktail, and 1 M DTT was added to a final concentration of 10 mM. Cells were incubated for 15 min at 37°C with gentle shaking, then pelleted as above (Cleve et al., 1996). Supernatants were again boiled with reducing sample buffer.

**Cell surface labelling.** Surface proteins of yeast were biotinylated with sulfo-LC-NHS-biotin (Pierce, after Masuoka et al., 2002) and isolated by precipitation with immobilized avidin (Pierce). Stationary-phase cultures of *C. albicans* were grown as above in YNB2G, pelleted and washed with water followed by 100 mM sodium phosphate buffer. After counting, 5×10⁸ cells were suspended in 2-5 ml cold NaPO₄, pH 8, buffer and 250 µl sulfo-LC-NHS-biotin (10 mg ml⁻¹ stock in ddH₂O) was added. Biotinylation was carried out for 1 h, then the cells were washed sequentially four times with ice-cold 50 mM NaPO₄, pH 6, then once with 10 mM NaPO₄, pH 7-4. After the final wash, cells were suspended in 3 ml of the pH 7-4 buffer and split into three tubes for protein extractions as above, with the exception that sample buffer was not added to the samples when finished. Following extraction, supernatants were brought up to 1 ml final volume by the addition of 100 µl 10× Tris-buffered saline (TBS, pH 7-4), 100 µl of a 50% slurry of TBS-washed streptavidinagarose and ddH₂O to volume. Biotinylated proteins were allowed to bind to the matrix while rotating at room temperature. Beads were pelleted at 1000 g for 30 s and washed four times with TBS. Washed beads were suspended in 100 µl reducing sample buffer and boiled prior to electrophoresis and blotting.

**Two-dimensional electrophoretic separation.** Samples were prepared from a csh1/csh1 derivative of *C. albicans* strain CA14 and an isogenic *CSH1* reintegrant by glass bead breakage as described above. Protein concentration was determined by a modified BCA assay (Pierce) of supernatants from broken cells and 100 µg protein was precipitated using a universal protein precipitation reagent (UPPA; Genotech). Precipitated protein was dissolved in equilibration buffer [8 M urea, 2% CHAPS, 0-5% pH 3–10 IPG buffer (Pharmacia), 0-002% bromophenol blue, 0-28% DTT] for application to 18 cm pH 3–10 immobilized gradient strips (Pharmacia) and separated to isoelectric equilibrium using a four-step voltage protocol. For this procedure, strips were rehydrated under load with.
the sample for 12 h at 30 °C, then the sample was electrophoresed for 500 V h (500 V maximum), 1000 V h (1000 V max.), 32 000 V h (8000 V max.) for a total run time of 18 h, and 33 862 V h. Following the run, strips were equilibrated in 50 mM Tris/HCl, pH 8-8.6 M urea/30 % glycerol/2 % SDS/0-002 % bromophenol blue prior to application onto 12 % denaturing PAGE slab gels for electrophoretic separation by mass. Gels were transferred to nitrocellulose for Western blot analysis with mAbs 6C5-H4CA or YOL1/34 (Accurate Chemical). Blots were blocked in 4 % non-fat dry milk in phosphate-buffered saline plus 0.1 % Tween-20 (PBS-Tween), incubated in affinity-purified primary antibody solution overnight at 4 °C and an HRP-conjugated isotype appropriate secondary antibody for 1 h at room temperature. Blots were developed using HRP-dependent chemiluminescence and visualized by exposure to film.

RNA isolation. Total RNA was prepared from cells grown to stationary phase as above in 1 ml YNB2G culture using a method from Ausubel et al. (1999). Cells were collected by centrifugation at 2000 g for 5 min and suspended in 400 μl AE buffer (50 mM NaOAc, pH 5-3, 10 mM EDTA). Cells were broken by vortexing in 40 μl 10 % SDS and 500 μl phenol (pre-equilibrated with AE buffer). The mixture was incubated at 65 °C for 10 min, vortexed and placed on dry ice for 4 h. Phases were separated by centrifugation at 10 000 g for 10 min and the aqueous phase was extracted with an equal volume of chloroform/isoamyl alcohol (24:1). RNA was precipitated overnight at −20 °C from the aqueous phase by the addition of 0-1 vols 3 M NaOAc and 2-5 vols 100 % EtOH. RNA was pelleted by centrifugation at 10 000 g for 15 min at 4 °C, rinsed with cold 70 % EtOH and dissolved in 10 mM Tris/HCl, pH 8, 1 mM EDTA. RNA was quantified by spectroscopy and 10 μg total RNA from each sample was separated by denaturing formaldehyde-agarose electrophoresis (Ausubel et al., 1999), followed by transfer to charged nylon membranes for Northern blotting.

RESULTS

Temperature-dependent expression of CSH1

Temperature dependent expression of Csh1p was demonstrated by growing yeast cultures in identical medium at 23, 30 or 37 °C to early stationary phase. Following protein extraction by bead breakage, total cellular protein levels of Csh1p were assessed by Western blot analysis, using tubulin as a loading control (Fig. 1). In the csh1/csh1 knockout, virtually no immunoreactivity to mAb 6C5-H4CA was observed at 23 °C in comparison to the readily detectable signal from a CSH1 reintegrant derivative. Steady-state expression of the CSH1 gene was followed by Northern blotting of total RNA isolated from aliquots drawn from stationary-phase cultures of C. albicans strain LGH1095 or the CSH1 reintegrant grown at 23, 30 or 37 °C (Fig. 2a), or from cultures of LGH1095 at intervals during growth to stationary phase at either 23 or 37 °C (Fig. 2b). Lanes were normalized to total RNA loaded (ethidium bromide staining of rRNA subunits). Fig. 2(a) demonstrates that in both strain backgrounds, CSH1 mRNA levels are higher in cultures grown at lower temperatures, in contrast to the increased expression of protein at temperatures greater than 23 °C. Fig. 2(b) demonstrates that RNA levels peak during exponential growth phase and decrease in stationary phase at 37 °C. CSH1 RNA levels in 23 °C-grown cells are maximal at 26 h post-inoculation. The CSH1 probe used for Northern blotting hybridizes strongly with the

---

**Fig. 2.** Steady-state levels of CSH1 RNA at different growth temperatures. (a) Total RNA was isolated from the CSH1 reintegrant (left) and strain LGH1095 (right) at the indicated growth temperatures. Equal amounts by mass of total RNA were loaded for each sample and separated on 1-2 % formaldehyde agarose gels for subsequent transfer to a nylon membrane for Northern blotting. Blots were hybridized under high stringency conditions with a probe fragment corresponding to the whole CSH1 ORF (final wash at 65 °C with 0-1 x SSC/1 % SDS). Samples were controlled for loading and to assess sample integrity by ethidium bromide staining to visualize 25S and 18S ribosomal subunits. (b) Total RNA was isolated from strain LGH1095 at either 23 or 37 °C at time points during liquid culture growth after inoculation into pre-equilibrated medium. Samples were removed for RNA isolation at 4-5 h post-inoculation (lane 1, early exponential phase), 11 h post-inoculation (lane 2, mid-exponential phase) and 26 h post-inoculation (lane 3, early stationary phase.)
predicted gene in Southern analysis as assessed under high stringency conditions. A second, weakly hybridizing band on Southern blots indicates that a related gene is also recognized to a much lesser extent by this probe (data not shown).

**Paralogue specificity of the anti-Csh1p mAb**

To address whether the residual Western blot signal in the csh1/csh1 knockout is the result of cross-reactivity of the mAb 6C5 with one of the gene paralogues, cell lysates were separated by two-dimensional electrophoresis and analysed by Western blotting with mAb 6C5 (Fig. 3). In the reintegrant derivative, an intensely immunoreactive spot is visible, along with a set of less intense immunoreactive spots of variable isoelectric points and approximately the same molecular mass. In the csh1/csh1 knockout, the intense spot and the spots focusing at the anodic end of the gel are absent, and the two spots at the cathodic end appear to be undiminished in intensity from the knockin derivative. This suggests that a minor portion of the 6C5 signal in wild-type *C. albicans* is the result of cross-reactivity of the antibody with proteins of similar mass and isoelectric points.

**Extraction of Csh1p from intact cells**

To determine whether Csh1p is extractable with agents which solubilize cell-wall-associated materials, cells from 26 h liquid culture were treated with Zymolyase under conditions maintaining cell integrity to solubilize cell wall and periplasmic materials without contamination of cytoplasmic contents. Extraction of cell walls with Zymolyase resulted in the release of Csh1p from cells grown at 30 and 37 °C as assessed by Western blotting with non-detectable release of cytoplasmic tubulin under the same conditions (Fig. 4). Cells were also treated with alkaline-DTT according to Cleves *et al.* (1996), used by the authors to remove peripherally associated cell wall materials. Total cell lysates prepared by bead breakage in parallel resulted in significant release of Csh1p along with tubulin. DTT extraction using these experimental conditions is inefficient at extracting Csh1p and comparably loaded gels do not consistently demonstrate the presence of Csh1p (Fig. 4). DTT extraction of Csh1p can be visualized in Western blots of overloaded gels (data not shown); however, the intermittent presence of the tubulin signal complicates the interpretation of these results by suggesting that some degree of cytoplasmic leakage occurs with this treatment in *C. albicans*.

**Accessibility of Csh1p to surface labelling**

To further address the surface accessibility of Csh1p, surface proteins of intact cells were labelled on ice with a membrane-impermeable biotinylation reagent. Following extensive washing of labelled cells, cell wall proteins were released by glass bead breakage and biotinylated proteins were adsorbed to immobilized avidin. Csh1p was readily detectable by immunoblotting of the adsorbed material from biotin-labelled 37 °C-grown cells and absent in material from unlabelled cells (Fig. 5). The amount of surface-accessible Csh1p that is pulled down by immobilized avidin is a small fraction of the total cellular pool of Csh1p. A tubulin signal by immunodetection was absent from the pulled down material and is therefore not labelled with the surface biotinylation reagent.

---

**Fig. 3.** Recognition of CSH1 paralogues by mAb 6C5-H4CA. Cell extracts were obtained from the csh1/csh1 knockout and CSH1 reintegrant as in Fig. 1 from cultures grown at 30 °C. Equal amounts of protein from each sample were initially separated by first dimension isoelectric focusing across a pH 3–10 gradient. Following first dimension separation, the samples were further resolved by second dimension PAGE separation on 12% gels and then transferred to nitrocellulose for Western blotting with mAb 6C5-H4CA. The relevant portions of the two blots are shown, with the acidic and basic directions of the isoelectric focusing separation indicated.

**Fig. 4.** Partial extractability of Csh1p under conditions maintaining cell integrity. Cell extracts were obtained from *C. albicans* strain LGH1095 grown at 23, 30 or 37 °C. Samples were prepared for separation by PAGE on 12% gels and Western blotting by glass bead breakage (lanes 1), by Zymolyase extraction in 1 M sorbitol (lanes 2) or by extraction with 10 mM DTT in 100 mM Tris/HCl, pH 9.4 (lanes 3). Blots were probed either with anti-Csh1p antibody 6C5-H4CA (C) or with the anti-tubulin antibody YOL1/34 (T).
Expression of Csh1p on hydrophobic cells

To demonstrate the surface presence of Csh1p on hydrophobic cells at 37 °C, one half of a 20 ml 26 h culture of LGH1095 cells grown at 37 °C to stationary phase in SD broth was used as an inoculum into fresh prewarmed SD broth. Reinoculation of a cell population with the hydrophilic phenotype to low cell density in fresh medium results in the rapid shift of the cell population to the hydrophobic phenotype (Hazen & Hazen, 1988). After 1 h of additional growth at 37 °C, the culture was pelleted and CSH of the reinoculated culture was compared to the remaining stationary-phase culture. The stationary-phase culture of 37 °C-grown cells was composed exclusively (100%) of hydrophobic cells, with a generally unubded morphology, whereas the reincubated culture contained greater than 50% hydrophobic cells. The stationary-phase culture of 23 °C-grown cells contained exclusively (100%) hydrophobic cells. Additionally, cells with the hydrophobic phenotype typically generated elongated buds and germination tubes measuring on average less than one cell diameter. A significant number of cells with the hydrophobic phenotype retained the yeast cell morphology. Cell-surface proteins were extracted from intact cells with Zymolyase as before for subsequent analysis by Western blotting. Protein release and gross protein profiles as assessed by Ponceau S staining of blots from each of the cell cultures were essentially identical (not shown), despite the significant differences in CSH, and relative amounts of tubulin and Csh1p by Western blotting of lysates were comparable (Fig. 6).

DISCUSSION

In previous studies, we identified the CSH1 gene product as a significantly expressed hydrophobic protein, the genetic ablation of which led to a measurable decrease in CSH. Analysis of the Csh1p protein sequence indicated that the protein shows cytoplasmic localization and the steady-state pool of the protein does seem to be primarily cytoplasmic. Given the paradox between the predicted cytoplasmic localization of Csh1p and its presumed function at the cell wall, the present work was initiated to characterize the association of Csh1p with the cell wall. The present study demonstrates temperature-dependent expression of the C. albicans CSH1 gene product and that a portion the total cellular pool of Csh1p is cell-wall-associated.

Expression of Csh1p is significantly higher in early-stationary-phase cultures that have been grown at 37 °C as opposed to 23 °C, and the surface detection of the protein is strongly correlated with high levels of intracellular Csh1p expression. Cells grown at 23 °C do have readily detectable levels of the protein, however, so the lack of detection of Csh1p on the surface of these cells may therefore be due to the limits of detection of the protein using the antibody. The steady-state RNA levels of the CSH1 gene inversely reflect that of the protein levels in early-stationary-phase cultures. This discrepancy may be the result of differences in relative population maturity between cultures grown at different temperatures, as cultures grown at 37 °C enter stationary phase somewhat more rapidly (approx. 2 h earlier) than those grown at 23 °C. Transcription levels and steady-state RNA levels would then be lower in the 37 °C cultures than in the 23 °C cultures. This is supported by the observation that mid-exponential-phase cultures grown at 37 °C show higher
levels of CSH1 RNA than 23 °C cultures. For the purposes of examining protein expression in the context of CSH, these are the culture conditions generating populations with virtually 100 % hydrophobic or hydrophilic cells. C. albicans is unique among common pathogenic yeasts in that hydrophobicity can be significantly varied in standard in vitro culture conditions. However, hydrophobic cell populations rapidly develop a hydrophobic phenotype upon reinoculation into fresh medium at low cell number (Hazen & Hazen, 1988), even at 37 °C. Expression levels of Csh1p in early-stationary-phase hydrophobic cells and in reinoculated cells is comparable both in total cell lysates and in surface-accessible pools. This result suggests that hydrophobic cells in early stationary phase might be in a state that allows the population to have a pre-existing pool of proteins that directly affect hydrophobicity readily available for surface presentation upon a change in the environment and a concurrent change in the growth rate of the organism.

A number of proteins have been described as cell wall components in C. albicans that lack a defined N-terminal signal sequence for insertion into the secretory pathway. Typically these proteins were reported to be potent elicitors of the host immune response (Ponton et al., 2001) and cell wall localization was later demonstrated by microscopy or other methods. These antigens are frequently glycolytic enzymes and include phosphoglycerate kinase, pyruvate kinase, glyceraldehyde-3-phosphate dehydrogenase and enolase, all of which have been shown by biochemical methods to be major expressed proteins secreted by regenerating spheroplasts. A number of heat-shock proteins and chaperonins have also been postulated to be significant cell wall components (Pitarch et al., 1999, 2002). No enzymic function in the cell wall has been described for these antigens and presumably any other function of them in the wall is fortuitous and unrelated to the well described biochemical function. A report describing the cross-reactivity of the C. albicans alcohol dehydrogase protein with antibodies directed to human fibronectin receptor suggested the possibility that this protein may function as an adhesin to host extracellular matrix proteins (Klotz et al., 2001). No interaction of ADH1p with fibronectin was demonstrated and recombinant ADH1p was unable to inhibit fungal cell adhesion in vitro.

The presence of glycolytic enzymes in the fungal cell wall is controversial, primarily because of the lack of a proven mechanism of export (Klis et al., 2002). Speculation on the exact mechanism has focused on heterologous cell lysis and reassociation of pre-synthesized antigen on adjacent cells. Such a mechanism is not extant for Csh1p. Analysis of cell viability in 26 h cultures indicates that greater than 99 % of the cells in the population are metabolically active (data not shown), suggesting the absence of a paracrine-like mechanism for Csh1p association with the cell wall. Similarly, a mechanism used by some moulds to deposit hydrophobins on the wall (McCabe & Van Alfen, 1999) is not likely as culture filtrates lack detectable Csh1p (unpublished results). Alternatively, proteins might be exported via a non-traditional secretory mechanism. Secretion of the S. cerevisiae a mating factor is facilitated by the STE6 transporter that translocates the mature polypeptide across the plasma membrane. However, the STE6p transporter system is responsible for translocating only a single, small peptide. Recently, Delgado et al. (2003) demonstrated that a chimera between GAPDH and a non-secreted invertase was able to direct secretion of the construct to the cell wall in S. cerevisiae. Truncation of the GAPDH fusion partner led the authors to map the region necessary for chimera secretion to the N-terminal region of GAPDH. In mammalian systems, a non-traditional secretory pathway is proposed to explain the secretion of the cytokines IL-1β, bFGF and TNF-α, which also lack an N-terminal signal sequence (Rubartelli & Sitia, 1997). The mechanism of export of IL-1β is unclear, although the majority of the protein is cytoplasmic and it appears to bypass the endoplasmic reticulum to Golgi pathway. No class of proteins demonstrating a secretion pathway analogous to that of IL-1β has been described in lower eukaryotes.

A notable alternative to the two export pathways discussed above has been described in S. cerevisiae and may provide an explanation for the appearance of Csh1p in C. albicans. Cleves and co-workers proposed an alternative export pathway for the transport of a heterologously expressed galectin (Cleves et al., 1996; Cleves, 1997). Expression of mammalian galectin-1 in S. cerevisiae resulted in the extracellular appearance of the mature protein in a sec18-independent manner, indicating that it is secreted by a mechanism that bypasses traditional vesicular transport. Release of extracellular galectin was accomplished by treatment of intact cells with DTT in alkaline conditions, presumably by releasing a secreted fraction peripherally associated with the cell wall. The authors went on to describe an endogenous S. cerevisiae protein (NCE3p) that uses the same export mechanism, and two candidate gene products (NCE1p/2p) of unknown function that were responsible for the export of this class of proteins (Cleves et al., 1996). Ablation of either NCE1 or NCE2 prevented secretion of heterologously expressed galectin, confirming their roles in effecting secretion of galectin in a sec14-independent manner. NCE1 and NCE2 showed no homology with any other described transporters and to date the mechanism by which they effect secretion of galectin is still unclear. Analysis of the unannotated C. albicans genome (http:// www-sequence.stanford.edu/group/candida) has identified the presence of homologues of the NCE1, 2 and 3 proteins, indicating the potential conservation of a non-classical export pathway between the two fungal species and suggesting a possible mechanism for Csh1p export in C. albicans. Studies are under way to address this possibility.

Use of Zymolyase extraction and cell-surface biotinylation allowed us to demonstrate the presence of Csh1p in the C. albicans cell wall. Previously, we have demonstrated that ablation of the CSH1 gene product results in a moderate,
although significant decrease in CSH (Singleton et al., 2001), and antibodies recognizing Csh1p are effective in blocking fungal cell adhesion (Glee et al., 2001; Masuoka et al., 1999); however, the antibody is not reproducibly effective in indirect immunofluorescence. These results taken together present circumstantial evidence that Csh1p has a surface presence. Our present results confirm by several methods that Csh1p can be biochemically detected at the cell surface. Other workers reporting the surface presence of glycolytic enzymes in C. albicans have not described the relative surface presence to total cellular pool, although it must be assumed that any glycolytic enzymes must be functioning within the cytoplasm. The fraction of Csh1p associated with the extracellular compartment is small in comparison with the total cell-associated Csh1p associated with the extracellular compartment is must be functioning within the cytoplasm. The fraction of Csh1p associated with the extracellular compartment is small in comparison with the total cell-associated Csh1p and is reminiscent of the amount of cell-associated IL1β. Release of Csh1p from the cell wall and the ability to surface-label the protein is correlated with the total level of Csh1p, suggesting that surface presentation of this protein is not a default pathway for it.

CSH status in C. albicans is mediated by a combination of proteins and post-translational modifications within the cell wall. Previous work has demonstrated that the proteinaceous makeup of cell walls from hydrophobic and hydrophilic yeasts are very similar (Hazan et al., 1990; Hazen & Hazen, 1993), and mannans extracted from each are also similar, with the exception that the length of the acid-labile fraction (composed of β-1,2-oligomannose) of N-glycans from hydrophobic yeasts are generally longer than in hydrophilic cells (Masuoka & Hazen, 1997, 1999). Characterization of proteins extracted from hydrophobic yeasts has enabled us to identify potential gene products that might contribute to the CSH phenotype on the basis of the behaviour of the proteins during hydrophobic interaction chromatography (Masuoka et al., 1999; Singleton et al., 2001). CSH contributes to virulence in mice and can be regulated experimentally by changes in growth conditions of cultures. The regulation of the cellular protein components that affect CSH may allow us to identify factors that regulate the CSH phenotype itself.

ACKNOWLEDGEMENTS

We thank James Masuoka for helpful discussions during the course of the work. Sequence data for C. albicans was obtained from the Stanford DNA Sequencing and Technology Center website (http://www-sequence.stanford.edu/group/candida). Sequencing of C. albicans was accomplished with the support of the NIDCR and the Burroughs Welcome Fund. This work was supported by PHS grants RO1AI043997 from the National Institute of Allergy and Infectious Diseases.

REFERENCES


