Transcriptional Responses of *Candida albicans* to Epithelial and Endothelial Cells[∇][†]

Hyunsook Park,[1] Yaoping Liu,[1] Norma Solis,[1] Joshua Spotkov,[1] Jessica Hamaker,[2]
Jill R. Blankenship,[2]‡ Michael R. Yeaman,[1,3] Aaron P. Mitchell,[4]‡
Haoping Liu,[4] and Scott G. Filler[1,3]*

*Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center, Torrance, California[1]; Columbia University, New York,
New York[2]; David Geffen School of Medicine at UCLA, Los Angeles, California[3]; and Department of Biological Chemistry,
College of Medicine, University of California—Irvine, Irvine, California[4]*

***Candida albicans* interacts with oral epithelial cells during oropharyngeal candidiasis and with vascular
endothelial cells when it disseminates hematogenously. We set out to identify *C. albicans* genes that govern
interactions with these host cells in vitro. The transcriptional response of *C. albicans* to the FaDu oral epithelial
cell line and primary endothelial cells was determined by microarray analysis. Contact with epithelial cells
caused a decrease in transcript levels of genes related to protein synthesis and adhesion, whereas contact with
endothelial cells did not significantly influence any specific functional category of genes. Many genes whose
transcripts were increased in response to either host cell had not been previously characterized. We con-
structed mutants with homozygous insertions in 22 of these uncharacterized genes to investigate their function
during host-pathogen interaction. By this approach, we found that *YCK2*, *VPS51*, and *UEC1* are required for
C. albicans to cause normal damage to epithelial cells and resist antimicrobial peptides. *YCK2* is also necessary
for maintenance of cell polarity. *VPS51* is necessary for normal vacuole formation, resistance to multiple
stressors, and induction of maximal endothelial cell damage. *UEC1* encodes a unique protein that is required
for resistance to cell membrane stress. Therefore, some *C. albicans* genes whose transcripts are increased upon
contact with epithelial or endothelial cells are required for the organism to damage these cells and withstand
the stresses that it likely encounters during growth in the oropharynx and bloodstream.***

Candida albicans is the most common cause of oropharyn-
geal candidiasis in patients with human immunodeficiency vi-
rus infection or AIDS, Sjogren's syndrome, diabetes mellitus,
and head and neck cancers (52, 54, 55, 61, 74). This organism
is also the most common cause of hematogenously dissemi-
nated candidiasis in neonates and in adult patients with central
venous catheters, cancer, or recent surgery (3, 23, 51). The
predominance of *C. albicans* in these infections suggests that
this organism expresses unique virulence factors that enable it
to cause disease in susceptible hosts.

Some aspects of the pathogenesis of oropharyngeal candidi-
asis and disseminated candidiasis are comparable. For exam-
ple, during the development of oropharyngeal candidiasis, the
organism adheres to and invades the epithelial cells of the oral
mucosa (8, 14, 30, 31, 40, 69, 78). Similarly, during the initia-
tion of hematogenously disseminated candidiasis, *C. albicans*
adheres to and invades the endothelial cell lining of the blood
vessels (19, 21, 49, 57). In addition, this organism invades both
oral epithelial cells and endothelial cells in vitro by inducing its
own endocytosis (14, 15, 19, 49, 57, 78). This endocytosis re-
quires functional host cell microfilaments and is induced in
part by Als3 on the fungal surface binding to E-cadherin on
epithelial cells and N-cadherin on endothelial cells (19, 47, 49,
50, 57). Invasion of both cell types by live *C. albicans* results in
damage and eventual death of these host cells (47, 48, 50, 59,
78). Moreover, the capacity of *C. albicans* to form hyphae is
required for maximal virulence during both oropharyngeal and
disseminated candidiasis (34, 47, 62).

There are also distinct differences between the pathogenesis
of oropharyngeal and disseminated candidiasis. The microen-
vironment of the oropharynx, which contains epithelial cells,
antimicrobial salivary proteins, and numerous species of bac-
teria, is distinct from that of the blood vessels, which contain
endothelial cells, blood cells, and plasma proteins, and is nor-
mally sterile. Furthermore, some *C. albicans* genes, such as
TPK2 and *CKA2*, are required for normal virulence in a murine
model of oropharyngeal candidiasis, but not disseminated can-
didiasis (9, 47). In contrast, *C. albicans IRS4* and *NRG1* are
necessary for maximal virulence during disseminated candidi-
asis, but not oropharyngeal candidiasis (4, 41, 43). These find-
ings have led us to hypothesize that different virulence deter-
minants and regulatory pathways enable *C. albicans* to initiate
and maintain an infection in different host microenvironments.

Here we set out to test this hypothesis through transcrip-
tional profiling and subsequent functional analysis. We have
used the interactions of *C. albicans* with epithelial and endo-
thelial cells as surrogates for animal models of disease in order
to compare these distinct pathogen-cell interactions under
controlled in vitro conditions. Our results distinguish gene

* Corresponding author. Mailing address: Division of Infectious
Diseases, Los Angeles Biomedical Research Institute at Harbor-
UCLA Medical Center, 1124 W. Carson St., Torrance, CA 90502.
Phone: (310) 222-3813. Fax: (310) 782-2016. E-mail: sfiller@ucla.edu.
‡ Present address: Department of Microbiology, Carnegie Mellon
University, Pittsburgh, PA 15213.

TABLE 1. Strains used in this study

Strain	Genotype	Source or reference
CAI4-URA	*ura3Δ::imm*[434]*/ura3Δ::imm*[434]*::URA3*	Y. Fu (59)
36082	Wild type (human blood isolate)	American Type Culture Collection
7392	Wild type (human oral isolate)	T. Patterson (50)
BWP17	*ura3Δ::λimm*[434]*/ura3Δ::λimm*[434]*his1::hisG/his1::hisG arg4::hisG/arg4::hisG*	A. Mitchell (75)
DAY286	*ura3Δ::λimm*[434]*/ura3Δ::λimm*[434]*pARG4::URA3::arg4::hisG/arg4::hisG*	A. Mitchell (13)
DAY185	pARG4::*URA3::arg4::hisG/arg4::hisG* pHIS1::*his1::his1/his1::hisG*	A. Mitchell (13)
EpKO1	*atm1*-Tn7::*UAU1/atm1*-Tn7::*URA3*	This study
EpKO2	orf19.4656-Tn7::*UAU1/*orf19.4656-Tn7::*URA3*	This study
EpKO3	orf19.4793-Tn7::*UAU1/*orf19.4793-Tn7::*URA3*	This study
EpKO4	orf19.2398-Tn7::*UAU1/*orf19.2398-Tn7::*URA3*	This study
EpKO5	*clp1*-Tn7::*UAU1/clp1*-Tn7::*URA3*	This study
EpKO6	*opt26*-Tn7::*UAU1/opt26*-Tn7::*URA3*	This study
EpKO7	*cdc47*-Tn7::*UAU1/cdc47*-Tn7::*URA3*	This study
EpKO8	orf19.1504-Tn7::*UAU1/*orf19.1504-Tn7::*URA3*	This study
JJH34	*yck2*-Tn7::*UAU1/yck2*-Tn7::*URA3*	This study
JJH34H	*yck2*-Tn7::*UAU1/yck2*-Tn7::*URA3*::pHIS1	This study
JJH34C	*yck2*-Tn7::*UAU1/yck2*-Tn7::*URA3*::pHIS1-YCK2	This study
BL35-1	*vps51*-Tn7::*UAU1/vps51*-Tn7::*URA3*	This study
BL35-2	*vps51*-Tn7::*UAU1/vps51*-Tn7::*URA3*	This study
BL35-1H	*vps51*-Tn7::*UAU1/vps51*-Tn7::*URA3*::pHIS1	This study
BL35-1C	*vps51*-Tn7::*UAU1/vps51*-Tn7::*URA3*::pHIS1-VPS51	This study
BL36	orf19.6168-Tn7::*UAU1/*orf19.6168-Tn7::*URA3*	This study
BL37	orf19.1766-Tn7::*UAU1/*orf19.1766-Tn7::*URA3*	This study
BL38	orf19.3740-Tn7::*UAU1/*orf19.3740-Tn7::*URA3*	This study
BL39	orf19.4142-Tn7::*UAU1/*orf19.4142-Tn7::*URA3*	This study
BL40	orf19.4791-Tn7::*UAU1/*orf19.4791-Tn7::*URA3*	This study
BL41-1	*uec1*-Tn7::*UAU1/uec1*-Tn7::*URA3*	This study
BL41-2	*uec1*-Tn7::*UAU1/uec1*-Tn7::*URA3*	This study
BL41-1H	*uec1*-Tn7::*UAU1/uec1*-Tn7::*URA3*::pHIS1	This study
BL41-1C	*uec1*-Tn7::*UAU1/uec1*-Tn7::*URA3*::pHIS1-UEC1	This study
BL42	orf19.3664-Tn7::*UAU1/*orf19.3664-Tn7::*URA3*	This study
BL43	orf19.4894-Tn7::*UAU1/*orf19.4894-Tn7::*URA3*	This study
BL44	orf19.1980-Tn7::*UAU1/*orf19.1980-Tn7::*URA3*	This study
BL45	orf19.1939-Tn7::*UAU1/*orf19.1939-Tn7::*URA3*	This study
BL46	orf19.6392-Tn7::*UAU1/*orf19.6392-Tn7::*URA3*	This study
BL48	orf19.403-Tn7::*UAU1/*orf19.403-Tn7::*URA3*	This study
BL88	orf19.1148-Tn7::*UAU1/*orf19.1148-Tn7::*URA3*	This study

expression patterns and functional requirements for each of these pathogenic interactions and lend overall support to the evolving view that niche-specific responses are critical for infection (6).

MATERIALS AND METHODS

***C. albicans* strains and growth conditions.** The *C. albicans* strains used in this study are listed in Table 1. All strains were maintained on YPD agar (1% yeast extract [Difco], 2% peptone [Difco], and 2% glucose plus 2% Bacto agar). *C. albicans* were selected on synthetic complete medium (2% dextrose, 0.67% yeast nitrogen base [YNB] with ammonium sulfate, and auxotrophic supplements). For use in the experiments, the strains were grown in YPD broth at 30°C in a shaking incubator overnight. The resulting blastospores were harvested by centrifugation and enumerated with a hemacytometer as previously described (19). The strains were adjusted to the desired concentration in RPMI 1640 medium and warmed to 37°C prior to adding them to the host cells.

Epithelial and endothelial cells. The FaDu oral epithelial cell line, originally isolated from a pharyngeal carcinoma, was purchased from the American Type Culture Collection. This cell line was maintained in Eagle's minimum essential medium with Earle's balanced salt solution (Irvine Scientific) containing 10% fetal bovine serum, 1 mM pyruvic acid, 2 mM L-glutamine, 0.1 mM nonessential amino acids, 100 IU/ml penicillin, and 100 IU/ml streptomycin. Endothelial cells were harvested from human umbilical veins by the method of Jaffe et al. (28). The cells were grown in M-199 medium (Gibco) supplemented with 10% fetal bovine serum, 10% defined bovine calf serum, and 2 mM L-glutamine with penicillin and streptomycin (16). All cell cultures were grown at 37°C in a humidified environment containing 5% CO_2.

Microscopic observation of *C. albicans*. *C. albicans* blastospores were added to tissue culture-treated plastic, or 95% confluent FaDu oral epithelial cells and human umbilical vein endothelial cells at 10^5 blastospores per cm^2. After incubation for various times at 37°C, the cells were rinsed once with Hanks balanced salt solution (Irvine Scientific) to remove the unbound organisms and then fixed with 3% paraformaldehyde. The cells were washed three times with phosphate-buffered saline and imaged using differential interference contrast with a Leica Microsystems confocal microscope.

The vacuolar morphology of the *C. albicans* strains was visualized by pulse-chase staining with FM4-64 by a minor modification of the method of Subramanian et al. (68). Briefly, each strain was grown to log phase in YPD broth at 30°C. The cells were harvested by centrifugation and resuspended in YPD broth, after which FM4-64 (Invitrogen) was added to achieve a final concentration of 25 µM. The cells were incubated at 30°C for 30 min and then harvested by centrifugation. They were resuspended in fresh YPD broth and incubated for an additional 90 min. During the last 60 min of this incubation, a polyclonal anti-*C. albicans* antibody (Biodesign International) conjugated with Alexa Fluor 488 (Molecular Probes) was added to the medium to label the cell surface of the organisms. Next the cells were rinsed once in phosphate-buffered saline, resuspended in YNB broth (0.17% YNB, 2% glucose), and imaged by confocal microscopy.

Adherence and endocytosis assay. The time course of *C. albicans* adherence to and endocytosis by oral epithelial and endothelial cells was determined by our standard differential fluorescence assay, as described previously (47, 48). Briefly, the host cells were grown on 12-mm-diameter glass coverslips and inoculated with 10^5 blastospores of *C. albicans* CAI4-URA in RPMI 1640 medium. After, 45, 90, and 180 min, the nonadherent organisms were removed by rinsing with Hanks balanced salt solution, after which the cells were fixed with 3% paraformaldehyde. The adherent, extracellular organisms were stained with the anti-*C. albicans* rabbit antiserum conjugated with Alexa Fluor 568 (red fluorescence;

Molecular Probes). Next, the host cells were permeabilized in 0.5% Triton X-100, after which the cell-associated organisms (the adherent plus endocytosed organisms) were stained with anti-*C. albicans* antiserum conjugated with Alexa Fluor 488 (green fluorescence). The coverslips were mounted inverted on a microscope slide, and the number of endocytosed and cell-associated organisms was determined by viewing the cells with an epifluorescent microscope. At least 100 organisms were counted on each coverslip, and organisms that were partially internalized were scored as being endocytosed. Each experiment was performed in triplicate on at least three separate occasions.

Damage assay. The time course of oral epithelial and endothelial cell damage caused by *C. albicans* was determined using a ^{51}Cr release assay with a minor modification of our previously described method (19, 47, 70). Briefly, the oral epithelial or endothelial cells were grown to 95% confluence in a 24-well tissue culture plate and loaded with ^{51}Cr. They were infected with 10^6 blastospores of *C. albicans* CAI4-URA to yield the same ratio of organisms to host cells as was used in the transcriptional profiling experiments (see below). After 45, 90, and 180 min, the medium was aspirated from each well and the ^{51}Cr content was determined by gamma counting. Next, the cells were lysed by the sequential application of 6 N NaOH and RadiacWash (Atomic Products), after which the ^{51}Cr content of the lysate was measured. The amount of ^{51}Cr released by epithelial or endothelial cells infected with the various *C. albicans* strains was compared with the amount of ^{51}Cr released by uninfected host cells to calculate the specific release of ^{51}Cr using the following formula: (experimental release − spontaneous release)/(total incorporation − spontaneous release). Experimental release was the amount of ^{51}Cr released into the medium by cells infected with *C. albicans*. Spontaneous release was the amount of ^{51}Cr released into the medium by uninfected host cells. Total incorporation was the sum of the amount of ^{51}Cr released into the medium and remaining in the host cells. Each experiment was performed in triplicate on at least three separate occasions.

Isolation of *C. albicans* RNA. *C. albicans* cells suspended in prewarmed RPMI 1640 medium were added to 15-cm-diameter polystyrene tissue culture plates containing either oral epithelial or endothelial cells. As a control, organisms were added to empty tissue culture plates that did not contain host cells (hereafter called "polystyrene"). In all experiments, the final concentration of organisms was 5×10^5 cells/cm^2 and the same RNA extraction procedure was used for both the experimental and control conditions. The organisms were incubated with the host cells or plastic for 45, 90, and 180 min. At the end of each incubation period, the nonadherent organisms were removed by rinsing with ice-cold distilled water. To reduce the amount of host cell RNA, 10 ml of mammalian cell RNA extraction buffer (4 M guanidine thiocyanate, 25 mM sodium citrate, 0.5% Sarkosyl [*N*-lauroyl-sarcosine], and 0.1 M β-mercaptoethanol) (10) was added to each dish. This buffer lyses the host cells but leaves the fungal cells intact (18). The fungal cells were collected by centrifugation at 4°C, washed once with ice-cold diethyl pyrocarbonate-treated water, and snap-frozen in liquid nitrogen. Next, the cells were thawed on ice and the fungal RNA was extracted by the hot phenol method (32). The quality of RNA was determined using a Bioanalyzer (Agilent, Inc.).

To verify that the RNA extraction procedure did not significantly alter the gene expression profile of *C. albicans*, an alternative approach was also used. *C. albicans* cells suspended in prewarmed RPMI 1640 medium were added to either empty polystyrene tissue culture plates or tissue culture plates containing endothelial cells. After 90 min, the nonadherent organisms were removed by rinsing once with ice-cold distilled water. Next, 8 ml of ice-cold diethyl pyrocarbonate-treated water was added to each dish, and the cells were removed with a cell scraper. They were vortexed vigorously for 30 s to lyse the host cells, after which the fungal cells were collected by a 2-min centrifugation at 4°C. They were quickly resuspended in 400 μl TES buffer (10 mM Tris, 10 mM EDTA, 0.5% sodium dodecyl sulfate [SDS]) and then snap-frozen in liquid nitrogen. The total time from rinsing the cells to freezing them in liquid nitrogen was less than 5 min. The total RNA was extracted from these cells as described above. This RNA was used for the confirmatory real-time PCR experiments described below.

***C. albicans* microarray.** The microarrays were constructed using the *C. albicans* Genome Oligo set (Qiagen), which consists of 70-mer oligonucleotide probes designed from 6,266 predicted open reading frames (ORFs) in assembly 6 of the *C. albicans* Genome Sequencing Project. Because multiple *ALS* genes were misassembled in this assembly, we added custom-designed oligonucleotides to detect *ALS1*, *ALS2*, *ALS3*, *ALS4*, *ALS5/6*, *ALS7*, and *ALS9*. In addition, we added probes to detect the following genes that were not represented in the original set of oligonucleotides: *CDC24*, *CLN2*, *EFG1*, *HDA1*, *HOS2*, *HSP12*, *MAD2*, *PCL2*, *PLC2.3*, *PHO11*, *RBT2*, *RBT4*, *RBT7*, *RHO3*, *SNF1*, *SRA1*, *STT4*, *TEM1*, *TPK1*, and *TPK2*. The oligonucleotides were spotted onto glass slides.

cDNA labeling and microarray hybridization. Preparation and labeling of the cDNA and hybridization with the microarrays were performed following stan-

dard protocols (http://microarrays.org/). Briefly, 10 μg of total RNA was reverse transcribed with Superscript II reverse transcriptase (Invitrogen) in the presence of 5-(3-aminoallyl)-2′-dUTP (aa-dUTP), using both oligo(dT) and random primers (Stratagene). The cDNAs from the control and experimental conditions were coupled with Cy3 or Cy5 monoreactive dyes (Amersham Biosciences), mixed at 1:1 ratio, and concentrated in a Microcon-30 spin column (Millipore). The concentrated probes were hybridized with the microarray slides in hybridization buffer 3 (Ambion) at 50°C for 16 h. The arrays were visualized with 428 array scanner (Affymetrix). At least three hybridizations were performed for each time point, host cell type, and strain of *C. albicans*.

Data analysis. The scanned images of both channels were quantified using ImaGene 5.0 (Biodiscovery). Spots were quantified as the median value of all pixel intensities in the spot region. The local background values were calculated from the area surrounding each feature and subtracted from the respective spot signal values. The data from each array were normalized by locally weighted linear regression (lowess) analysis using the MIDAS program (http://www.tigr.org/software/).

Strain CAI4-URA, which was originally derived from the clinical isolate SC5314 (59), was used as the reference strain, and its response to both oral epithelial cells and endothelial cells was determined. To increase the power of our analysis, we also determined the transcriptional response to oral epithelial cells of another oral isolate, strain 7392 (50). We combined the data from both CAI4-URA and 7392 and considered a gene to have a significant change in transcript levels in response to oral epithelial cells only when the following two criteria were met: (i) there had to be at least a twofold difference in transcript levels between the two conditions, and (ii) this difference had to be statistically significant by the Cyber-*t* test ($P \le 0.05$) (71). We also determined the transcriptional response to endothelial cells of both CAI4-URA and the blood isolate, strain 36082 (17), and analyzed the data in a similar manner. Strain 7392 adheres to, invades, and damages oral epithelial cells in vitro and is virulent in a mouse model of oropharyngeal candidiasis (50; H. Park and S. G. Filler, unpublished data). Similarly, strain 36082 adheres to, invades, and damages endothelial cells in vitro and is virulent in a mouse model of disseminated candidiasis (17, 19, 25, 27, 38).

Real-time PCR. *C. albicans* CAI4-URA was incubated with oral epithelial cells, endothelial cells, or polystyrene as in the microarray experiments. Next *C. albicans* RNA was extracted, treated with DNase I (Ambion), and then used to synthesize cDNA with Moloney murine leukemia virus reverse transcriptase (Ambion). Quantitative real-time PCR was carried out using the SYBR green PCR kit (Applied Biosystems) and an ABI 7000 real-time PCR system (Applied Biosystems) following the manufacturer's protocol. The primers used in these experiments are listed in Table S1 in the supplemental material. The results were analyzed by the threshold cycle ($2^{-\Delta\Delta C_T}$) method (33) using the transcript level of the *C. albicans ACT1* gene (Ca*ACT1*) as the endogenous control. The mRNA levels for each gene were determined in at least two biological replicates, and the results were combined.

Construction of insertion mutants. *C. albicans* mutants with homozygous insertions in genes that were verified to be upregulated in response to oral epithelial cells or endothelial cells were constructed using the Tn7-*UAU1* transposon (13, 44). Insertion cassettes were obtained from a library of Tn7-*UAU1* insertions in *C. albicans* CAI4 DNA that were sequenced from one end. The insertion sites for each clone are listed at http://www.tigr.org/tigr-scripts/e2k1/qzhao/page.cgi? num=1, and the specific sequence from the end of each insertion may be found through the Seq_ID link. Because insertion cassettes for orf19.403 and orf19.1148 were not present in this library, they were constructed using the Tn7-*UAU1* transposon using the GPS-M mutagenesis system (NEB), following the manufacturer's protocol. Each cloned DNA insert, including the Tn7-*UAU1* insertion, was excised from the plasmid backbone by digestion with NotI, and then transformed into strain BWP17 (75). Arg$^+$ Ura$^+$ clones with homozygous insertions were selected as previously described (13, 44). The presence of homozygous insertions was verified by whole-cell PCR using the primer sets listed in Table S1 in the supplemental material. The homozygous insertion mutant strains were transformed with pGEM-HIS1 (75), linearized with NruI to generate His$^+$ strains.

Complementation of insertion mutants. The same approach was used to complement the *yck2/yck2*, *vps51/vps51*, and *uec1/uec1* insertion mutants. DNA fragments encompassing the protein-coding regions of *YCK2* (orf19.7001), *VPS51* (orf19.5568), or *UEC1* (orf19.4646) plus ~1,000 bp of upstream sequence and 500 bp of downstream sequence were amplified from genomic DNA of strain CAI4-URA with high-fidelity polymerase. The primers were YCK2 comp-5 and YCK2 comp-3 for *YCK2*, VPS51 comp-f and VPS51 comp-r for *VPS51*, and UEC1 comp-f and UEC1 comp-r for *UEC1* (see Table S1 in the supplemental material). Each fragment was cloned into pGEM-T Easy. The fragment contain-
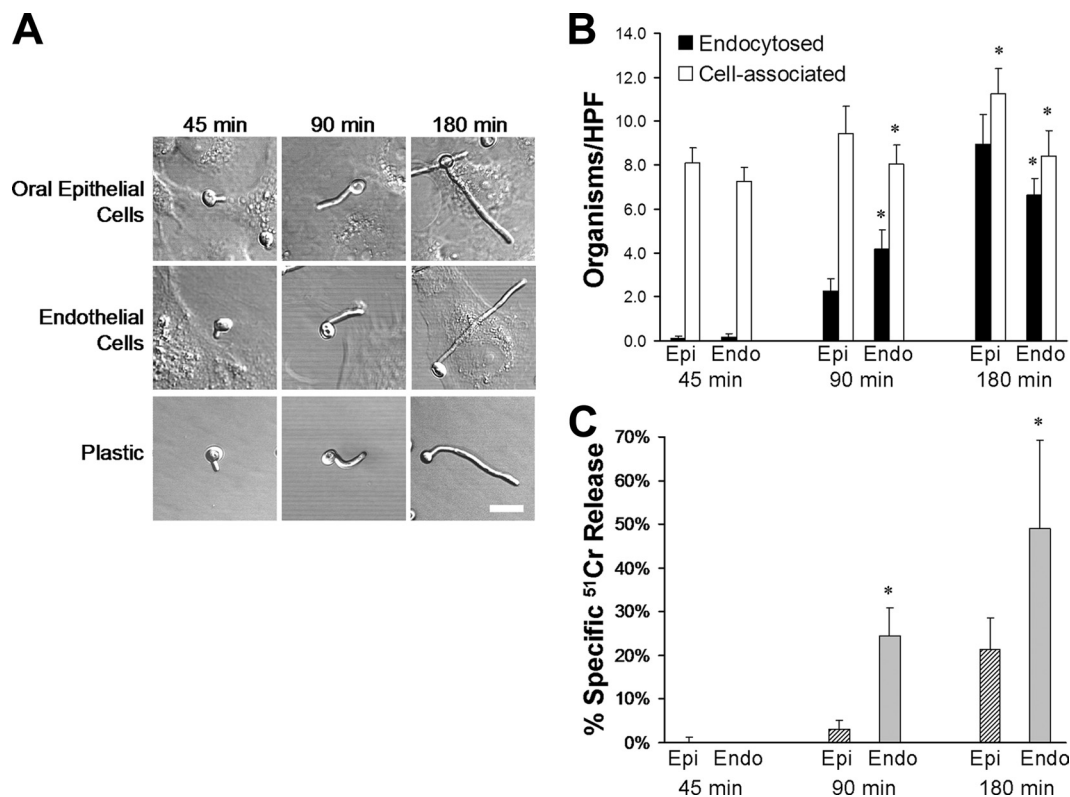
FIG. 1. Time course of *C. albicans* interaction with host cells and polystyrene. Blastospores of *C. albicans* strain CAI4-URA were incubated with FaDu oral epithelial cells, human umbilical vein endothelial cells, or polystyrene. (A) At the indicated times, the cells were fixed and the organisms were imaged by differential interference contrast. The scale bar indicates 5 μm. All images were taken at the same magnification. (B) The number of organisms that were endocytosed by and cell associated with the host cells at the indicated times was determined by a differential fluorescence assay. Results are means ± standard deviations from three independent experiments. ∗, $P = < 0.015$ compared to oral epithelial cells at the same time point. (C) The extent of *C. albicans*-induced host cell damage was measured using a ^{51}Cr release assay. Results are the means ± standard deviations from three independent experiments. ∗, $P < 0.001$ compared to oral epithelial cells at the same time point. Epi, oral epithelial cells; Endo, endothelial cells; HPF, high-powered field.

ing *YCK2* was then subcloned into the SphI site of pGEM-HIS1 to yield pHIS1-YCK2. The fragments containing *VPS51* and *UEC1* were subcloned into the NruI site of pGEM-HIS1 to produce pHIS1-VPS51 and pHIS1-UEC1, respectively. pHIS1-YCK2 was linearized with PacI and transformed into strain JJH34. pHIS1-VPS51 and pHIS1-UEC1 were linearized with SalI and transformed into strains BL35 and BL41, respectively. Correct integration of the complementation plasmids was verified by PCR.

Susceptibility to antimicrobial peptides and environmental stressors. Agar dilution assays were used to test the susceptibility of the various *C. albicans* strains to protamine and environmental stressors. Serial 10-fold dilutions of the strains (range 10^4 to 10^1 CFU) in 10 μl were plated onto YPD agar containing 2.8 mg/ml protamine sulfate (Sigma-Aldrich), 200 μg/ml Congo red, 1 M NaCl, 0.05% SDS, or 5 mM H_2O_2 and incubated at 30°C. The plates were photographed after 5 days in the protamine experiments and after 2 days when the other stressors were tested.

The susceptibility of the *C. albicans* strains to human β-defensin 2 was determined by a radial diffusion assay as previously described (77). Briefly, late-logarithmic-phase organisms were suspended in 1% agarose (pH 7.5) at a final concentration of 10^6 organisms/ml. The agar was solidified in petri dishes, and 4-mm-diameter sample wells were bored. Ten micrograms of human β-defensin 2 was added to each well, after which the dishes were overlaid with YNB agar. After incubation at 30°C for 2 days, the diameters of the zone of growth inhibition around each of the wells were measured.

Statistical analysis. Differences in *C. albicans* adherence to, endocytosis by, and damage to the two different types of host cells were compared by analysis of variance. *P* values of ≤0.05 were considered to be significant.

Microarray data accession numbers. The transcriptional profiling data have been deposited in Gene Expression Omnibus (GEO; http://www.ncbi.nlm.nih .gov/geo/) under accession no. GSE5340 (transcriptional response of *C. albicans*

to oral epithelial cells) and GSE5344 (transcriptional response of *C. albicans* to endothelial cells).

RESULTS

Interactions of *C. albicans* with oral epithelial cells and endothelial cells. The interaction of *C. albicans* with host cells includes three features: germination of *C. albicans* to produce hyphae, binding to and endocytosis by host cells, and damage to the host cells. We first compared germination of *C. albicans* after contact with FaDu oral epithelial cells, endothelial cells, or polystyrene. We added blastospores of *C. albicans* to the different host cells or polystyrene and observed the morphology of the organisms. After 45 min, the blastospores had just begun to germinate (Fig. 1A). At subsequent time points, the resulting hyphae progressively elongated. We tested three different clinical isolates of *C. albicans*, and all had similar morphologies at these time points (data not shown). At each time point, the hyphae that formed in the presence of either host cell type were of similar length to those grown on polystyrene. Thus, the host cells did not visibly influence candidal germination or hyphal elongation at the time points studied. These findings also indicate that differences discussed below in the transcriptional profile of *C. albicans* exposed to these condi-

tions were the result of factors other than differences in morphology.

Next, we compared the time course of *C. albicans* adherence to, invasion of, and damage to oral epithelial cells and endothelial cells. At the 45-min time point, slightly more organisms adhered to the epithelial cells than to the endothelial cells, and very few organisms had been endocytosed (Fig. 1B). There was no detectable damage to either type of host cell at this time (Fig. 1C). After 90 min, 24% of the cell-associated organisms had been endocytosed by oral epithelial cells, whereas 50% of the cell-associated organisms had been endocytosed by endothelial cells. The epithelial cells experienced significantly less *C. albicans*-induced damage than did endothelial cells at this time. By 180 min, endocytosis was largely complete as the majority of cell-associated organisms had been endocytosed by both types of host cell. It is noteworthy that the oral epithelial cells endocytosed significantly more organisms than the endothelial cells, yet *C. albicans* caused ~50% less damage to the epithelial cells than to the endothelial cells. The delayed endocytosis of *C. albicans* by oral epithelial cells and the relative resistance of these cells to *C. albicans*-induced damage are consistent with the model that *C. albicans* interacts differently with epithelial cells than endothelial cells.

C. albicans responded differently to oral epithelial cells compared to endothelial cells. We used microarray analysis to determine the transcriptional response of *C. albicans* to contact with oral epithelial cells and endothelial cells. Organisms grown on polystyrene were used as the reference condition. The response of strain CAI4-URA, which was derived from the sequence strain SC5314, to both cell types was determined. To increase the power of our analysis, we also determined the oral epithelial cell response of strain 7392, which was isolated from a patient with oropharyngeal candidiasis. As our goal was to determine the core response of *C. albicans* to oral epithelial cells, we focused our analysis on genes whose mRNA levels changed in the same direction in both 7392 and CAI4-URA (73). Similarly, we determined the endothelial cell response of strain 36082, which was isolated from a patient with candidemia, and focused on genes whose transcript levels changed in both this strain and in CAI4-URA.

The transcript levels of only 29 *C. albicans* genes changed significantly in response to both epithelial and endothelial cells at 45 min, and the transcript levels of all of these genes were decreased (see Table 3). A complete list of *C. albicans* genes whose transcript levels changed significantly in response to either host cell type is provided in Table S2 in the supplemental material. The mRNA levels of 116 genes changed in response to epithelial cells at 90 and/or 180 min (Table 3). Interestingly, contact with endothelial cells did not alter the transcript levels of any of these genes. These results indicate that the response of *C. albicans* to oral epithelial cells is substantially different from its response to endothelial cells.

Different strains of *C. albicans* were used in the epithelial cell experiments compared to the endothelial cell experiments. Therefore, it was possible that some of the differences in the response of *C. albicans* to the two types of host cells were due to the difference in *C. albicans* strains. To evaluate this possibility, we analyzed the microarray data of strain CAI4-URA alone to oral epithelial cells and endothelial cells. As expected, when the response of a single strain was used, many more

TABLE 2. Number of *C. albicans* genes with significant changes in transcript levels in response to FaDu oral epithelial cells and endothelial cells by microarray analysis

Time	Direction of change	No. of genes[a]			% of uncharacterized genes	
		Epithelial cells	Endothelial cells	Both cell types	Epithelial cells	Endothelial cells
45 min	Increase	31	9	29[b]	53	44
	Decrease	211	54		36	35
90 min	Increase	8	35	0	38	43
	Decrease	54	19		32	33
180 min	Increase	12	33	0	33	27
	Decrease	32	23		38	36

[a] Results represent the combined data from *C. albicans* strains CAI4-URA and 7392 for the epithelial cell data and strains CAI4-URA and 36082 for the endothelial cell data.
[b] All genes had a decrease in transcript levels.

genes had changes in transcript levels. A total of 1,424 genes had significant changes in transcript levels after 45, 90, and 180 min of contact with epithelial or endothelial cells compared to polystyrene. However, only 160 (11%) of these genes had transcript levels that changed in the same direction in response to both cell types. These results support our conclusion that the response of *C. albicans* to oral epithelial cells is substantially different from its response to endothelial cells.

Next, we used Gene Ontology (GO) term analysis to determine the functional categories of the *C. albicans* genes whose transcript level changed in response to epithelial and endothelial cells. A large percentage of these genes did not have GO terms associated with them (Table 2). As a result, the genes whose transcripts were increased in response to either type of host cell were not significantly enriched for any GO term. Also, no GO term was significantly overrepresented among the genes whose transcripts were decreased in response to endothelial cells. Interestingly, the genes with reduced transcript levels in response to epithelial cells were significantly enriched in three functional categories. Genes related to adhesion were overrepresented at all three time points ($P < 0.04$ at each time point) (Table 3). Also, genes involved in ribonucleoprotein complex biogenesis were significantly enriched at 45 min ($P = 0.004$). Finally, genes related to the protein catabolic process were overrepresented at 90 min. Collectively, these data suggest that *C. albicans* responds to contact with epithelial cells by reducing expression of adhesins. There is also decreased protein synthesis that may be counteracted by a reduction in protein catabolism.

Functional analysis of differentially induced genes. We used real-time PCR using RNA from strain CAI4-URA to verify the increased transcript levels of selected genes identified in the microarray experiments. The verification experiments focused on genes for which Tn7-UAU1 transposon insertion cassettes (7, 13, 42) were available and whose function had not been studied previously. Real-time PCR assays verified that the transcripts of 13 *C. albicans* genes were significantly increased in response to oral epithelial cells compared to polystyrene (Table 2). Similarly, we confirmed that the transcripts of 15 *C. albicans* genes were significantly increased in response to en-

TABLE 3. Overrepresented functional categories of *C. albicans* genes whose transcript levels were decreased in response to
FaDu oral epithelial cells[a]

Time	GO term	*P* value	Genes annotated to the term
45 min	Biological adhesion	0.04	*ALS1, ALS3, ALS9, MNT1, MP65, PDE1, RFX2, SAP6, SUN41*
	Ribonucleoprotein complex biogenesis	0.004	*DIP2, HCR1, MPP10, NIP7, RIA1, RLP24, RPB4, RPL3, RPP0, RPS15, RPS21, RPS5, RPS6A, RPS7A, RPS8A, TIF5, UTP20, YST1,* orf19.1466, orf19.1833, orf19.2384, orf19.3481, orf19.4479, orf19.501, orf19.6197, orf19.7422
90 min	Protein catabolic process	0.006	*CDC48, DOG1, RAD16, RAD7, RPN4, SAP1, SAP6,* orf19.6672
	Biological adhesion	0.02	*ALS3, HWP1, MNT1, SAP1, SAP6*
180 min	Adhesion to host	0.008	*ALS3, ALS5, HWP1, TDH3*

[a] The combined data from *C. albicans* strains CAI4-URA and 7392 were used this analysis.

dothelial cells. These measurements confirm our microarray results, thus strengthening the argument that *C. albicans* interacts with and responds differently to these distinct host cell types.

To verify that the observed changes in gene transcript levels were induced by contact with the host cells and not by the RNA isolation procedure, we infected endothelial cells and polystyrene with *C. albicans* CAI4-URA for 90 min. Next, the host cells were lysed in ice-cold distilled water, after which the *C. albicans* cells were collected and then snap-frozen. The total time between adding the distilled water and snap-freezing the fungal cells was less than 5 min. We then used real-time PCR to measure the transcript levels of *YCK2*, orf19.5568, and orf19.4646. When Ca*ACT1* was used as the endogenous control gene, the transcripts of *YCK2*, orf19.5568, and orf19.4646 increased by 1.8-, 2.2-, and 1.8-fold, respectively, in response to endothelial cells compared to polystyrene. A similar increase was also observed when either Ca*TDH3* or Ca*TEF1* was used as the endogenous control gene (data not shown), indicating that Ca*ACT1* was an appropriate endogenous control gene for the real-time PCR experiments.

For genes whose transcripts were found by real-time PCR to be increased by at least 1.5-fold in response to epithelial cells or endothelial cells, we constructed homozygous insertion mutants using the Tn*7-UAU1* transposon insertion cassettes. We generated mutant strains with homozygous insertions in 8 of the 13 genes whose transcripts were increased in response to epithelial cells and 14 of the 15 genes whose transcripts were increased in response to endothelial cells (Tables 1 and 4). Among the six genes for which we were unable to construct homozygous insertion mutants, orf19.202 and orf19.3260 have essential orthologs in *Saccharomyces cerevisiae* (*MCM*7 and *CAB3*, respectively). The others are predicted to participate in vital cellular processes, including cell wall biogenesis, transcription, or mitochondrial function, so that homozygous mutations may cause lethality or very slow growth.

The 22 insertion mutants were screened for defects in virulence-related phenotypes, including hyphal formation and capacity to damage epithelial and endothelial cells (34, 47, 59, 62). In addition, because *C. albicans* is likely exposed to host antimicrobial peptides during oropharyngeal and disseminated candidiasis, the mutants were tested for their susceptibility to protamine. Protamine is a helical cationic polypeptide that is often used to screen for antimicrobial peptide susceptibility (22, 76).

We found that mutants with insertions in *YCK2* (orf19.7001), orf19.5568, orf19.4646, and orf19.1939 had defects in at least one of the following characteristics: hyphal formation, host cell damage, and protamine resistance (Table 4). These phenotypes were shared with at least one additional independent insertion mutant for each gene. To verify that each insertion mutation caused the phenotypic defect, the mutants were complemented with the respective wild-type allele. This approach linked the mutant phenotypes to insertions in *YCK2*, orf19.5568, and orf19.4646. *YCK2* encodes the highly conserved serine/threonine kinase, casein kinase 1 (CK1). orf19.5568 has no *S. cerevisiae* ortholog, but the predicted protein product has limited homology to *S. cerevisiae* Vps51, a component of the Vps53 complex. This complex is required for fusion of endosome-derived vesicles with the late Golgi compartment (11, 53, 64). Based on our phenotypic analysis below, we suggest the provisional name "*VPS51*" for orf19.5568. orf19.4646 specifies a unique protein that does not share significant homology with any other protein, including the predicted proteins of the closely related species *Candida dubliniensis*. Because expression of orf19.4646 was increased in response to endothelial cells, we named it "*UEC1*" (for *u*pregulated by *e*ndothelial *c*ells).

YCK2 governs cell polarity and is necessary for maximal epithelial cell damage. *YCK2* mRNA was increased in response to contact with either oral epithelial or endothelial cells, as indicated by real-time PCR measurements (Table 4). The *yck2/yck2* insertion mutant produced multiple short hyphae on epithelial cells, endothelial cells, and plastic, suggesting that it had a defect in polarized growth (Fig. 2) (data not shown). It had significantly reduced capacity to damage oral epithelial cells, but not endothelial cells, and this defect was reversed by complementation (Fig. 3). Prior studies indicate that mutant germination defects are invariably associated with endothelial cell damage defects (48, 59), so the *yck2/yck2* mutant is novel in separating these two phenotypes.

The *yck2/yck2* mutant also had impaired response to stress. It had markedly increased susceptibility to protamine, Congo red, and SDS (Fig. 4). As predicted by the protamine results, this mutant also had increased susceptibility to human β-defensin 2 (Fig. 5), which is made by oral epithelial cells (1, 29, 39), All defects of the *yck2/yck2* mutant were due to the insertions in *YCK2* because complementing this mutant with a wild-type copy of *YCK2* restored the wild-type phenotype in all assays. Collectively, these results suggest that in *C. albicans*,

TABLE 4. List of genes for generation of insertion mutant strains

ORF19 no.	Gene name	Possible function	Fold change by[a]: Microarray Epithelial	Microarray Endothelial	Real-time PCR Epithelial	Real-time PCR Endothelial	TIGR insertion ID	Insertion mutation	Hyphae	Host cell damage (% of wild type ± SD)[b] Epithelial	Endothelial	Protamine susceptibility
orf19.348		Uncharacterized	2.2	1.0	2.5	ND[c]	CAGOO23	No				
orf19.1077	ATM1	Member of MDR subfamily of ABC family	2.2	1.1	3.5	ND	CAGQD15	Yes	Normal	91 ± 5	ND	Normal
orf19.4656		Uncharacterized	2.2	−1.1	3.8	ND	CAGPD46	Yes	Normal	112 ± 4	ND	Normal
orf19.4793		Uncharacterized	2.0	−1.1	1.6	ND	CAGAQ76	Yes	Normal	111 ± 15	ND	Normal
orf19.6266		Uncharacterized, CCR4-NOT core complex	2.0	−1.1	2.5	ND	CAGB385	No				
orf19.2398		Uncharacterized	2.1	1.2	2.5	ND	CAGN836	Yes	Normal	114 ± 8	ND	Normal
orf19.6931	CLP1	Uncharacterized, probable cleavage/polyadenylation factor	2.0[d]	−1.5	1.9	ND	CAGI909	Yes	Normal	109 ± 24	ND	Normal
orf19.200	THO1	Uncharacterized	2.1[e]	−1.1[e]	2.5[e]	ND	CAGDN85	No				
orf19.4655	OPT6	Putative oligopeptide transporter	2.4[e]	−1.7[e]	3.1[e]	ND	CAGFI33	Yes	Normal	94 ± 9	ND	Normal
orf19.7001	YCK2	Casein kinase I (by homology)	2.2[e]	−1.4[e]	3.5[e]	2.0[d]	CAGDI41	Yes	Short/multiple	48 ± 8	79 ± 8	Increased
orf19.202	CDC47	Cell division control protein	2.5[e]	−1.5[e]	1.6[e]	ND	CAGCJ23	No				
orf19.1504		Uncharacterized, putative patatin-like phospholipase	2.6	1.1	4.3	ND	CAGBR73	Yes	Normal	107 ± 13	ND	Normal
orf19.3455	LPE10	Uncharacterized, putative magnesium transporter activity	2.7	2.5	2.6	ND	CAGDF12	No				
orf19.4922		Uncharacterized	2.3	1.0	1.6	ND	CAGF844	Yes	Normal	122 ± 10	ND	Normal
orf19.3260	SIS2	Uncharacterized	−1.2	2.1[d]	ND	3.0[d]	CAGB279	No				
orf19.5568	VPS51	Protein targeting to vacuole (by homology)	1.2[d]	2.1[d]	2.2[d]	2.9[d]	CAGDP76	Yes	Short	2 ± 13	3 ± 4	Increased
orf19.6168		Uncharacterized, possible structural constituent of cytoskeleton	1.2	2.3	ND	2.4	CAGEP09	Yes	Normal	ND	97 ± 9	Normal
orf19.1766	IFO3	Uncharacterized, similar to Streptomyces coelicolor putative hydrolase	1.3	2.1	ND	1.8	CAGL436	Yes	Normal	ND	80 ± 3	Normal
orf19.3740	PGA23	Putative glycosylphosphatidylinositol-anchored protein with unknown function	1.0[d]	2.7[d]	ND	2.8[d]	CAGN829	Yes	Normal	ND	99 ± 2	Normal
orf19.4142		Uncharacterized	1.0[d]	2.4[d]	ND	1.6[d]	CAGC641	Yes	Normal	ND	71 ± 16	Normal
orf19.4791		Uncharacterized	−1.8[d]	2.0[d]	ND	2.2[d]	CAGE249	Yes	Normal	ND	107 ± 9	Increased
orf19.4646	UEC1	Uncharacterized	1.2	2.0	1.1	3.4	CAGLQ62	Yes	Thickened	36 ± 18	91 ± 5	Normal
orf19.3664	HSP31	Heat shock protein (by homology)	−1.8	2.2	ND	4.9	CAGJM81	Yes	Normal	ND	84 ± 13	Normal
orf19.4894		Uncharacterized	−1.5[d]	3.4[d]	ND	2.3[d]	CAGKC82	Yes	Normal	ND	73 ± 11	Normal
orf19.1980	GIT4	Putative glycerophosphoinositol permease	1.1[d]	2.5[d]	ND	1.7[d]	CAGP819	Yes	Normal	ND	77 ± 12	Normal
orf19.1939		Uncharacterized	−1.1[d]	2.1[d]	ND	1.5[d]	CAGM083	Yes	Normal	ND	67 ± 6	Normal
orf19.6392		Uncharacterized	−1.1[d]	2.3[d]	ND	1.5[d]	CAGJP50	Yes	Normal	ND	96 ± 16	Normal
orf19.403	PCL2	Cyclin-dependent protein kinase	−1.3[d]	2.3[d]	ND	2.0[d]	—[f]	Yes	Normal	ND	94 ± 6	Normal
orf19.1148	CIS312	Uncharacterized	1.0	2.4	ND	2.5	—[f]	Yes	Normal	ND	96 ± 6	Normal

[a] After 45 min of incubation unless otherwise specified.
[b] The wild-type control strain was DAY185.
[c] ND, not determined.
[d] After 90 min of incubation.
[e] After 180 min of incubation.
[f] —, insertion cassettes for orf19.403 and orf19.1148 were made in the present study.
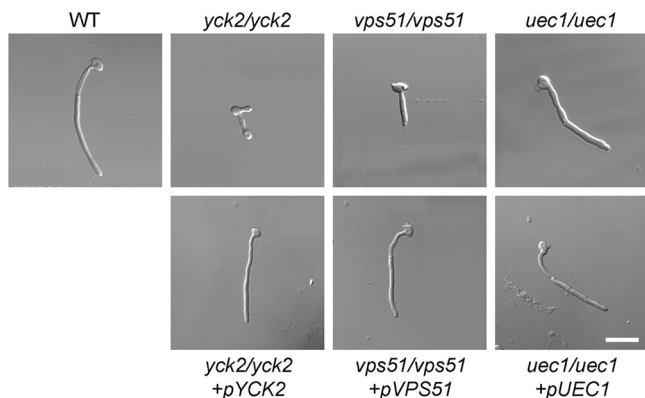
FIG. 2. Morphology of the *yck2/yck2*, *vps51/vps51*, and *uec1/uec1* insertion mutants. Blastospores of the indicated strains were incubated on plastic for 180 min and then were fixed and imaged by differential interference contrast. The scale bar indicates 5 μm. All images were taken at the same magnification. WT, wild-type strain DAY185.

YCK2 governs polarized growth, capacity to damage epithelial cells, and resistance to environmental stress.

VPS51 is required for hyphal elongation, maximal damage to host cells, and stress resistance. *VPS51* mRNA levels were also increased in response to contact with either oral epithelial or endothelial cells, as indicated by real-time PCR measurements (Table 4). The *vps51/vps51* insertion mutant produced unbranched hyphae that were shorter than those of the wild-type strain when it was grown on epithelial cells, endothelial cells, or plastic (Fig. 2) (data not shown). This mutant had a severe defect in its capacity to damage both epithelial and endothelial cells (Fig. 3). Furthermore, the *vps51/vps51* insertion mutant had significantly increased susceptibility to all stressors tested (Fig. 4 and 5). To verify that *VPS51* did indeed specify a protein involved in vacuolar function, we stained the *vps51/vps51* insertion mutant with FM4-64, which stains the vacuolar membrane (72). The vacuoles of this mutant were highly fragmented (Fig. 6), similar to the vacuolar morphology of the *S. cerevisiae vps51Δ* mutant (53). In contrast, the vacuoles of the wild-type cells were much larger and either single or multilobed. Complementing the *vps51/vps51* insertion mutant with a wild-type copy of *VPS51* rescued all of these defects. Therefore, *VPS51* is necessary in *C. albicans* for normal vacuolar morphology as well as many virulence-related phenotypes.

UEC1 is required for maximal epithelial cell damage and protamine resistance. *UEC1* mRNA levels were increased by contact with endothelial cells, but not epithelial cells (Table 4). Under all conditions tested, the *uec1/uec1* insertion mutant produced hyphae that were as long as those of the wild-type strain but slightly thicker (Fig. 2) (data not shown). Although *UEC1* transcript levels were not increased by contact with epithelial cells, the *uec1/uec1* insertion mutant had significantly reduced capacity to damage these cells (Fig. 3). However, it did not have an endothelial cell damage defect. The *uec1/uec1* insertion mutant also had significantly increased susceptibility to protamine, SDS, and human β-defensin 2, as well as a slight increase in susceptibility to NaCl (Fig. 4 and 5). These defects were rescued by complementing the insertion mutant with a wild-type copy of *UEC1*. Taken together, these results indicate that *UEC1* is necessary for *C. albicans* to cause normal damage
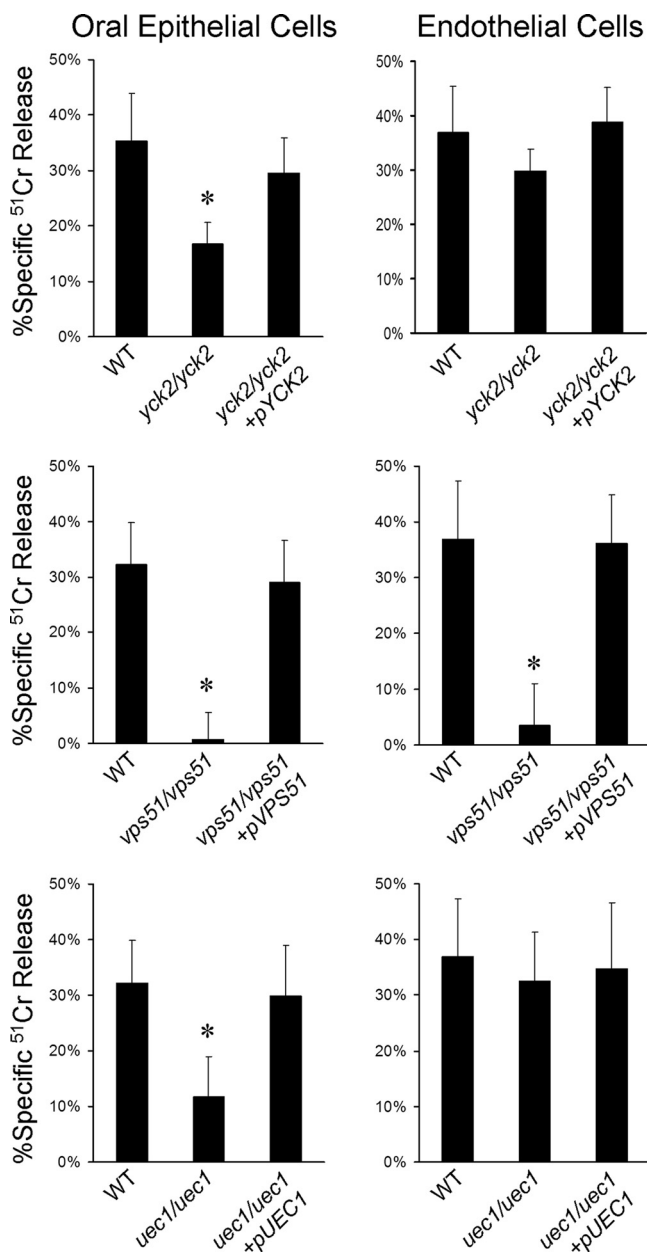

FIG. 3. Damage to oral epithelial cells and endothelial cells caused by the *yck2/yck2*, *vps51/vps51*, and *uec1/uec1* insertion mutants. The indicated strains were incubated with FaDu oral epithelial cells or endothelial cells for 180 min, after which the extent of host cell damage was assessed using a ^{51}Cr release assay. ∗, $P < 0.01$ compared to both the wild-type (WT) strain (DAY185) and complemented strains.

epithelial cells in vitro, perhaps by governing cell membrane integrity.

DISCUSSION

The results presented here demonstrate that *C. albicans* interacts differently with oral epithelial cells compared to endothelial cells. *C. albicans* hyphae were endocytosed more slowly by epithelial cells and caused less damage to these cells than endothelial cells. Also, the transcriptional response of *C.*
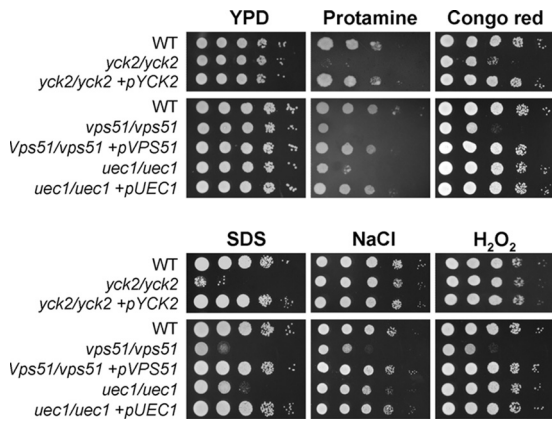
FIG. 4. Susceptibility of the *yck2/yck2*, *vps51/vps51*, and *uec1/uec1* insertion mutants to stressors. Serial 10-fold dilutions of the indicated strains were plated onto YPD agar containing 2.8 mg/ml protamine sulfate, 200 μg/ml Congo red, 1 M NaCl, 0.05% SDS, or 5 mM H₂O₂ and incubated at 30°C. The plates containing protamine were photographed after 5 days, and the other plates were photographed after 2 days. WT, wild-type strain (DAY185).

FIG. 5. Susceptibility of the *yck2/yck2*, *vps51/vps51*, and *uec1/uec1* insertion mutants to human β-defensin 2. The susceptibility of the indicated strains of *C. albicans* to human β-defensin 2 was measured using a radial diffusion assay. The zones of growth inhibition were imaged after incubation at 30°C for 2 days. WT, wild-type strain (DAY185).

albicans to epithelial cells was different from its response to endothelial cells. Interestingly, the *C. albicans* genes whose transcripts were increased in response to either of these cell types were also different from those that have been reported to be induced by exposure to monocytes or neutrophils (35, 58). These divergent responses to different types of host cells likely enable *C. albicans* to survive and proliferate in diverse anatomic sites within the host.

To our knowledge, the transcriptional response of *C. albicans* to endothelial cells has not been determined previously. However, two other groups of investigators have studied the response of this organism to contact with vaginal, cervical, and intestinal epithelial cell lines as compared to polystyrene (60, 65). Both groups found that contact with epithelial cells caused only a two- to fourfold change in *C. albicans* gene transcript levels. This magnitude of differential transcript levels is comparable to the results reported here. Sohn et al. (65) found that, when *C. albicans* was added to either vaginal or intestinal cell lines, the transcript levels of most genes changed within 30 to 60 min of contact; the mRNA of very few genes changed after 120 min of contact with these epithelial cells. Our results reported here lead to a similar conclusion.

The prior epithelial cell interaction studies (60, 65) reported that contact with epithelial cells increased mRNA levels of *C. albicans* cell surface protein genes, including *ALS2*, *ALS5*, *HWP1*, *PRA1*, and *PGA7*. In contrast, we found that the transcript levels of some adhesin genes actually fell in response to epithelial cells. Sohn et al. (65) noted that *HWP1* transcripts were induced simply by contact with a solid support, so it is possible that that *HWP1* transcript levels were stimulated more strongly by contact with polystyrene than by contact with epithelial cells. Also, *PRA1* orthologs in *S. cerevisiae* and *Aspergillus fumigatus* are members of a zinc-responsive regulon (36, 63), and recent results verify the induction of *C. albicans PRA1* by zinc limitation (Nobile et al., unpublished data). Thus, it seems likely that competition for zinc by host cells and *C. albicans* cells may have caused the *PRA1* induction reported by
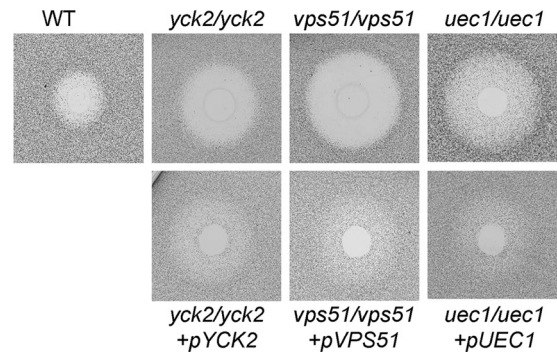
Sandovsky-Losica et al. (60), and our failure to detect its up-regulation may simply reflect a higher zinc level in our medium. Other key differences between these other microarray studies and ours include differences in the epithelial cell type, growth media, and strains of *C. albicans* used.

The microarray studies also suggested that contact with oral epithelial cells resulted in an early reduction of protein synthesis followed by a decrease in protein catabolism. Inhibition of genes involved in protein synthesis also occurs when the organism is ingested by macrophages and neutrophils, and in response to nitric oxide (20, 24, 35). The downregulation of genes involved in protein synthesis may be a response to stress (24, 35). It is known that oral epithelial cells can inhibit the growth of *C. albicans* (66, 67). Even though we did not observe any reduction in growth or hyphal elongation at the time points studied, it is possible that inhibition of protein synthesis may have preceded growth inhibition, which might have been detectable after longer incubation times.

Zakikhany et al. (78) used microarrays to analyze the transcript levels of *C. albicans* during infection of reconstituted human epithelium in vitro. They also analyzed *C. albicans* gene expression in samples from patients with pseudomembranous oropharyngeal candidiasis. In these analyses, the reference condition was *C. albicans* blastospores grown to the mid-log phase in YPD broth. Because contact with epithelial cells induces hyphal formation, the microarray data of Zakikhany et al. were enriched in hyphal-associated genes, such as *ALS3*, *HYR1*, *RBT1*, and *ECE1* (78). In the present experiments, the reference condition (contact with polystyrene) caused the organisms to form hyphae, and thus increased transcript levels that were induced by hyphal formation alone were not detected. However, Zakikhany et al. found that a large proportion of genes with increased transcript levels during the early phase of infection had not been previously characterized (78), similar to the results presented here.

The main value of our expression profiling results comes from the implication of new genes in the process of *C. albicans*-host cell interaction. Our analysis of mutants defective in up-regulated genes indicates that a relatively modest change in gene expression can nonetheless be biologically significant. Of
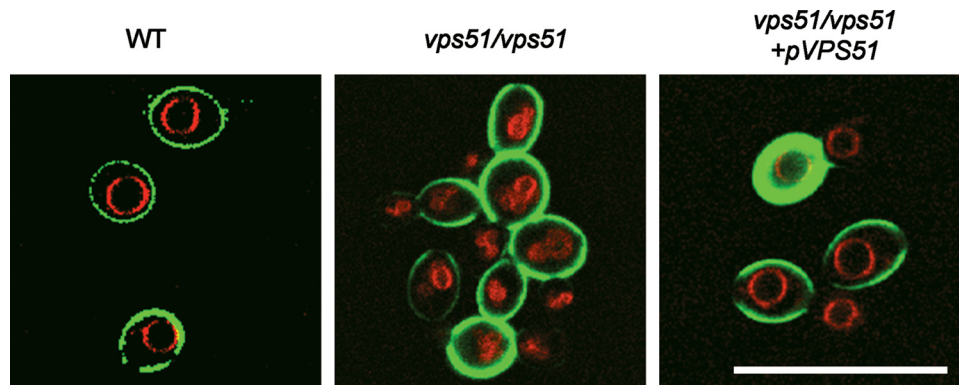
| WT | vps51/vps51 | vps51/vps51 +pVPS51 |

FIG. 6. Abnormal vacuolar morphology of the *vps51/vps51* insertion mutant. Blastospores of the indicated strains were grown to the mid-log phase and then pulse-chased with FM4-64 to label their vacuolar membranes. The cell walls were stained with an Alexa Fluor 488-labeled anti-*C. albicans* antibody, after which the cells were imaged by confocal microscopy. The cell walls are shown in green, and the vacuolar membranes are show in red. The scale bar indicates 5 μm. WT, wild-type strain (DAY185).

the 22 unique insertion mutants reported here, 3 (14%) had defective capacity to damage epithelial or endothelial cells. In contrast, our previous screen of unselected *C. albicans* insertion mutants yielded only 3 (1.6%) of 183 mutants with consistent defects (9). Therefore, the use of microarray data to guide candidate gene selection significantly improves the efficiency of virulence-associated gene identification.

Mutants with insertion in *YCK2*, *VPS51*, and *UEC1* all had defects in damaging oral epithelial cells, while only the *vps51/vps51* insertion mutant had a defect in damaging endothelial cells. We also found that the wild-type strain of *C. albicans* caused less damage to epithelial cells than to endothelial cells. We hypothesize that oral epithelial cells may be relatively resistant to *C. albicans*-induced damage because oral epithelial cells frequently encounter *C. albicans* even in normal hosts, whereas endothelial cells do not. Our microarray data also suggest the possibility that oral epithelial cells may actually induce downregulation of some *C. albicans* genes (such as those involved in protein synthesis) that are likely required for the organism to cause maximal epithelial cell damage.

Damage to epithelial and endothelial cells requires that *C. albicans* adheres to and invades these cells and that it secretes lytic enzymes (9, 26, 43, 47, 50). Therefore, it is probable that the *yck2/yck2*, *vps51/vps51*, and *uec1/uec1* insertion mutants have defects in one or more of these processes. Furthermore, mutants with reduced capacity to damage epithelial cells or endothelial cells in vitro have a high probability of having attenuated virulence in mouse models of oropharyngeal or hematogenously disseminated candidiasis (9, 47, 59). Collectively, our results suggest that *YCK2*, *VPS51*, and *UEC1* may influence the virulence of *C. albicans*.

It was notable that all three insertion mutants also had increased susceptibility to the antimicrobial peptides, protamine and human β-defensin 2. *C. albicans* is exposed to antimicrobial peptides, such as histatins and defensins, when it is in the oral cavity. The organism also encounters defensins and antimicrobial chemokines as it disseminates hematogenously and interacts with leukocytes and endothelial cells. Our finding that three of the genes that were upregulated in response to either epithelial or endothelial cells were required for *C. albicans* to resist antimicrobial peptides suggests that contact with

these host cells induces a protective reaction that enables *C. albicans* to withstand these peptides. The capacity to tolerate antimicrobial peptides is important for *C. albicans* virulence because the extent of antimicrobial peptide resistance is directly related to virulence in animal models of disseminated candidiasis (22, 76).

C. albicans Yck2 shares extensive homology with *S. cerevisiae* Yck2 and slightly less homology with *S. cerevisiae* Yck1, which are the two plasma membrane-associated isoforms of CK1 in this yeast. BLAST searches of the *C. albicans* genome did not identify a *YCK1* ortholog, suggesting that *C. albicans* has only a single plasma membrane-associated isoform of CK1. In *S. cerevisiae*, Yck2 and Yck1 are required for normal bud morphogenesis, cytokinesis, and endocytosis (2, 56). Strains of *S. cerevisiae* with reduced plasma membrane CK1 activity have defects in cell polarity (56). The early branching phenotype of the *C. albicans yck2/yck2* insertion mutant suggests that Yck2 is also required for maintenance of cell polarity in *C. albicans*.

Vps51 was first identified in *S. cerevisiae*, where it functions in a complex with Vps52, Vps53, and Vps54 (53, 64). This complex is required for retrograde protein traffic from the early endosome to the late Golgi compartment. Mutants that lack any of the subunits of this complex have missorting of vacuolar proteins, abnormal Golgi membrane proteins, and fragmented vacuoles (12, 53). While vacuoles are not required for growth under nutrient-rich conditions, they are important for *S. cerevisiae* to resist stress due to starvation, changes in environmental pH, and hyperosmolarity (5). The Vps51-54 complex has not been studied previously in *C. albicans*. However, a *vps11Δ/vps11Δ* mutant of *C. albicans*, which does not contain a vacuole, has delayed germination and increased susceptibility to osmotic stress due to glycerol or high NaCl (45). It also has markedly reduced secretion of secreted aspartyl proteinases and lipases, enzymes that have been implicated in damaging host cells (26, 46). The abnormal vacuole, shortened hyphae, and increased susceptibility to environmental stress of the *C. albicans vps51/vps51* insertion mutant are consistent with the probable role of Vps51 in vacuolar function. The impaired capacity of the *vps51/vps51* insertion mutant to damage epithelial and endothelial cells is probably due to multiple

factors, including abnormal hyphal formation and possibly reduced secretion of hydrolytic enzymes.

Our recommendation that orf19.5568 be named "*VPS51*" is based upon three considerations. First, as discussed above, the *C. albicans* mutant has several phenotypes that would be expected to result from a Vps51 defect. Second, although the homology between CaVps51 and ScVps51 is limited (64% similarity over 56 residues; $P = 4.6e^{-06}$), it spans the most highly conserved region of ScVps51 among fungi (residues 90 to 145; see http://www.yeastgenome.org/cache/fungi/YKR020W.html). Third, we note that ScVps51 does not have a closer homolog than CaVps51 among *C. albicans* predicted proteins. Thus, this gene name recommendation is based upon independent lines of evidence.

UEC1 encodes a unique 145-amino-acid protein. The function of Uec1 cannot be inferred from its primary amino acid sequence as it has no close orthologs and Pfam analysis does not reveal any conserved domains. Although *UEC1* is listed as a dubious ORF in the Candida Genome Database, our results suggest that it is a functional gene for the following reasons. First, the microarray and real-time PCR data indicate that *UEC1* is transcribed. Second, homozygous insertions in the *UEC1* locus induced a mutant phenotype. In principle, these properties might be expected for a 5′ regulatory transcript, as has been described for the *S. cerevisiae SER3* gene (37). However, we were able to complement the *uec1/uec1* insertion mutant with a wild-type copy of *UEC1*, integrated ectopically at the *HIS1* locus, to restore the wild-type phenotype. This observation argues that the *uec1*::Tn*7*-*UAU1* insertion phenotype does not result from a *cis*-acting effect on a neighboring gene. While the exact function of Uec1 remains to be determined, our finding that the *uec1/uec1* insertion mutant had increased susceptibility to the cell membrane's stressors, antimicrobial peptides and SDS, suggests that Uec1 may be required for maintenance of cell membrane integrity.

In summary, our microarray analysis demonstrates that the transcriptional response of *C. albicans* to oral epithelial cells is significantly different from its response to vascular endothelial cells. Furthermore, a significant fraction of the *C. albicans* genes whose transcript levels are increased upon contact with either of these host cells are uncharacterized. Some of these genes, such as *YCK2*, *VPS51*, and *UEC1*, are required for the organism to damage host cells and resist the types of environmental stress that it likely encounters during growth in the oropharynx and bloodstream.

REFERENCES

1. **Abiko, Y., Y. Jinbu, T. Noguchi, M. Nishimura, K. Kusano, P. Amaratunga, T. Shibata, and T. Kaku.** 2002. Upregulation of human beta-defensin 2 peptide expression in oral lichen planus, leukoplakia and candidiasis. an immunohistochemical study. Pathol. Res. Pract. **198:**537–542.
2. **Babu, P., J. D. Bryan, H. R. Panek, S. L. Jordan, B. M. Forbrich, S. C. Kelley, R. T. Colvin, and L. C. Robinson.** 2002. Plasma membrane localization of the Yck2p yeast casein kinase 1 isoform requires the C-terminal extension and secretory pathway function. J. Cell Sci. **115:**4957–4968.
3. **Bader, M. S., S. M. Lai, V. Kumar, and D. Hinthorn.** 2004. Candidemia in patients with diabetes mellitus: epidemiology and predictors of mortality. Scand. J. Infect. Dis. **36:**860–864.
4. **Badrane, H., S. Cheng, M. H. Nguyen, H. Y. Jia, Z. Zhang, N. Weisner, and C. J. Clancy.** 2005. *Candida albicans IRS4* contributes to hyphal formation and virulence after the initial stages of disseminated candidiasis. Microbiology **151:**2923–2931.
5. **Banta, L. M., J. S. Robinson, D. J. Klionsky, and S. D. Emr.** 1988. Organelle assembly in yeast: characterization of yeast mutants defective in vacuolar biogenesis and protein sorting. J. Cell Biol. **107:**1369–1383.
6. **Brown, A. J., F. C. Odds, and N. A. Gow.** 2007. Infection-related gene expression in *Candida albicans*. Curr. Opin. Microbiol. **10:**307–313.
7. **Bruno, V. M., and A. P. Mitchell.** 2005. Regulation of azole drug susceptibility by Candida albicans protein kinase CK2. Mol. Microbiol. **56:**559–573.
8. **Cawson, R. A., and K. C. Rajasingham.** 1972. Ultrastructural features of the invasive phase of *Candida albicans*. Br. J. Dermatol. **87:**435–443.
9. **Chiang, L. Y., D. C. Sheppard, V. M. Bruno, A. P. Mitchell, J. E. Edwards, Jr., and S. G. Filler.** 2007. *Candida albicans* protein kinase CK2 governs virulence during oropharyngeal candidiasis. Cell. Microbiol. **9:**233–245.
10. **Chomczynski, P., and N. Sacchi.** 1987. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal. Biochem. **162:**156–159.
11. **Conibear, E., J. N. Cleck, and T. H. Stevens.** 2003. Vps51p mediates the association of the GARP (Vps52/53/54) complex with the late Golgi t-SNARE Tlg1p. Mol. Biol. Cell **14:**1610–1623.
12. **Conibear, E., and T. H. Stevens.** 2000. Vps52p, Vps53p, and Vps54p form a novel multisubunit complex required for protein sorting at the yeast late Golgi. Mol. Biol. Cell **11:**305–323.
13. **Davis, D. A., V. Bruno, L. Loza, S. G. Filler, and A. P. Mitchell.** 2002. *C. albicans* Mds3p, a conserved regulator of pH responses and virulence identified through insertional mutagenesis. Genetics **162:**1573–1581.
14. **Eversole, L. R., P. A. Reichart, G. Ficarra, A. Schmidt-Westhausen, P. Romagnoli, and N. Pimpinelli.** 1997. Oral keratinocyte immune responses in HIV-associated candidiasis. Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endod. **84:**372–380.
15. **Farah, C. S., R. B. Ashman, and S. J. Challacombe.** 2000. Oral candidosis. Clin. Dermatol. **18:**553–562.
16. **Filler, S. G., B. O. Ibe, A. S. Ibrahim, M. A. Ghannoum, J. U. Raj, and J. E. Edwards, Jr.** 1994. Mechanisms by which *Candida albicans* induces endothelial cell prostaglandin synthesis. Infect. Immun. **62:**1064–1069.
17. **Filler, S. G., B. O. Ibe, P. M. Luckett, J. U. Raj, and J. E. Edwards, Jr.** 1991. *Candida albicans* stimulates endothelial cell eicosanoid production. J. Infect. Dis. **164:**928–935.
18. **Filler, S. G., A. S. Pfunder, B. J. Spellberg, J. P. Spellberg, and J. E. Edwards, Jr.** 1996. *Candida albicans* stimulates cytokine production and leukocyte adhesion molecule expression by endothelial cells. Infect. Immun. **64:**2609–2617.
19. **Filler, S. G., J. N. Swerdloff, C. Hobbs, and P. M. Luckett.** 1995. Penetration and damage of endothelial cells by *Candida albicans*. Infect. Immun. **63:**976–983.
20. **Fradin, C., P. De Groot, D. MacCallum, M. Schaller, F. Klis, F. C. Odds, and B. Hube.** 2005. Granulocytes govern the transcriptional response, morphology and proliferation of *Candida albicans* in human blood. Mol. Microbiol. **56:**397–415.
21. **Fu, Y., A. S. Ibrahim, D. C. Sheppard, Y. C. Chen, S. W. French, J. E. Cutler, S. G. Filler, and J. E. Edwards.** 2002. *Candida albicans* Als1p: an adhesin that is a downstream effector of the *EFG1* filamentation pathway. Mol. Microbiol. **44:**61–72.
22. **Gank, K. D., M. R. Yeaman, S. Kojima, N. Y. Yount, H. Park, J. E. Edwards, Jr., S. G. Filler, and Y. Fu.** 2008. *SSD1* is integral to host defense peptide resistance in *Candida albicans*. Eukaryot. Cell **7:**1318–1327.
23. **Hajjeh, R. A., A. N. Sofair, L. H. Harrison, G. M. Lyon, B. A. Arthington-Skaggs, S. A. Mirza, M. Phelan, J. Morgan, W. Lee-Yang, M. A. Ciblak, L. E. Benjamin, L. T. Sanza, S. Huie, S. F. Yeo, M. E. Brandt, and D. W. Warnock.** 2004. Incidence of bloodstream infections due to *Candida* species and in vitro susceptibilities of isolates collected from 1998 to 2000 in a population-based active surveillance program. J. Clin. Microbiol. **42:**1519–1527.
24. **Hromatka, B. S., S. M. Noble, and A. D. Johnson.** 2005. Transcriptional response of Candida albicans to nitric oxide and the role of the YHB1 gene in nitrosative stress and virulence. Mol. Biol. Cell **16:**4814–4826.

25. Ibrahim, A. S., S. G. Filler, M. A. Ghannoum, and J. E. Edwards, Jr. 1993. Interferon-gamma protects endothelial cells from damage by *Candida albicans*. J. Infect. Dis. **167:**1467–1470.

26. Ibrahim, A. S., S. G. Filler, D. Sanglard, J. E. Edwards, Jr., and B. Hube. 1998. Secreted aspartyl proteinases and interactions of *Candida albicans* with human endothelial cells. Infect. Immun. **66:**3003–3005.

27. Ibrahim, A. S., F. Mirbod, S. G. Filler, Y. Banno, G. T. Cole, Y. Kitajima, J. E. Edwards, Jr., Y. Nozawa, and M. A. Ghannoum. 1995. Evidence implicating phospholipase as a virulence factor of *Candida albicans*. Infect. Immun. **63:**1993–1998.

28. Jaffe, E. A., R. L. Nachman, C. G. Becker, and C. R. Minick. 1973. Culture of human endothelial cells derived from umbilical veins. Identification by morphologic and immunologic criteria. J. Clin. Investig. **52:**2745–2756.

29. Joly, S., C. Maze, P. B. McCray, Jr., and J. M. Guthmiller. 2004. Human β-defensins 2 and 3 demonstrate strain-selective activity against oral microorganisms. J. Clin. Microbiol. **42:**1024–1029.

30. Kamai, Y., M. Kubota, Y. Kamai, T. Hosokawa, T. Fukuoka, and S. G. Filler. 2002. Contribution of *Candida albicans ALS1* to the pathogenesis of experimental oropharyngeal candidiasis. Infect. Immun. **70:**5256–5258.

31. Kamai, Y., M. Kubota, T. Hosokawa, T. Fukuoka, and S. G. Filler. 2001. New model of oropharyngeal candidiasis in mice. Antimicrob. Agents Chemother. **45:**3195–3197.

32. Kohrer, K., and H. Domdey. 1991. Preparation of high molecular weight RNA. Methods Enzymol. **194:**398–405.

33. Livak, K. J., and T. D. Schmittgen. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. Methods **25:**402–408.

34. Lo, H. J., J. R. Kohler, B. DiDomenico, D. Loebenberg, A. Cacciapuoti, and G. R. Fink. 1997. Nonfilamentous *C. albicans* mutants are avirulent. Cell **90:**939–949.

35. Lorenz, M. C., J. A. Bender, and G. R. Fink. 2004. Transcriptional response of *Candida albicans* upon internalization by macrophages. Eukaryot. Cell **3:**1076–1087.

36. Lyons, T. J., A. P. Gasch, L. A. Gaither, D. Botstein, P. O. Brown, and D. J. Eide. 2000. Genome-wide characterization of the Zap1p zinc-responsive regulon in yeast. Proc. Natl. Acad. Sci. USA **97:**7957–7962.

37. Martens, J. A., P. Y. Wu, and F. Winston. 2005. Regulation of an intergenic transcript controls adjacent gene transcription in *Saccharomyces cerevisiae*. Genes Dev. **19:**2695–2704.

38. Mayer, C. L., S. G. Filler, and J. E. Edwards, Jr. 1992. *Candida albicans* adherence to endothelial cells. Microvasc. Res. **43:**218–226.

39. Meyer, J. E., J. Harder, T. Gorogh, J. B. Weise, S. Schubert, D. Janssen, and S. Maune. 2004. Human beta-defensin-2 in oral cancer with opportunistic *Candida* infection. Anticancer Res. **24:**1025–1030.

40. Montes, L. F., and W. H. Wilborn. 1968. Ultrastructural features of host-parasite relationship in oral candidiasis. J. Bacteriol. **96:**1349–1356.

41. Murad, A. M., P. Leng, M. Straffon, J. Wishart, S. Macaskill, D. MacCallum, N. Schnell, D. Talibi, D. Marechal, F. Tekaia, C. d'Enfert, C. Gaillardin, F. C. Odds, and A. J. Brown. 2001. *NRG1* represses yeast-hypha morphogenesis and hypha-specific gene expression in *Candida albicans*. EMBO J. **20:**4742–4752.

42. Nobile, C. J., V. M. Bruno, M. L. Richard, D. A. Davis, and A. P. Mitchell. 2003. Genetic control of chlamydospore formation in *Candida albicans*. Microbiology **149:**3629–3637.

43. Nobile, C. J., N. Solis, C. L. Myers, A. J. Fay, J. S. Deneault, A. Nantel, A. P. Mitchell, and S. G. Filler. 2008. *Candida albicans* transcription factor Rim101 mediates pathogenic interactions through cell wall functions. Cell. Microbiol. **10:**2180–2196.

44. Norice, C. T., F. J. Smith, Jr., N. Solis, S. G. Filler, and A. P. Mitchell. 2007. Requirement for *Candida albicans* Sun41 in biofilm formation and virulence. Eukaryot. Cell **6:**2046–2055.

45. Palmer, G. E., A. Cashmore, and J. Sturtevant. 2003. *Candida albicans VPS11* is required for vacuole biogenesis and germ tube formation. Eukaryot. Cell **2:**411–421.

46. Paraje, M. G., S. G. Correa, M. S. Renna, M. Theumer, and C. E. Sotomayor. 2008. *Candida albicans*-secreted lipase induces injury and steatosis in immune and parenchymal cells. Can. J. Microbiol. **54:**647–659.

47. Park, H., C. L. Myers, D. C. Sheppard, Q. T. Phan, A. A. Sanchez, J. E. Edwards, Jr., and S. G. Filler. 2005. Role of the fungal Ras-protein kinase A pathway in governing epithelial cell interactions during oropharyngeal candidiasis. Cell. Microbiol. **7:**499–510.

48. Phan, Q. T., P. H. Belanger, and S. G. Filler. 2000. Role of hyphal formation in interactions of *Candida albicans* with endothelial cells. Infect. Immun. **68:**3485–3490.

49. Phan, Q. T., R. A. Fratti, N. V. Prasadarao, J. E. Edwards, Jr., and S. G. Filler. 2005. N-cadherin mediates endocytosis of *Candida albicans* by endothelial cells. J. Biol. Chem. **280:**10455–10461.

50. Phan, Q. T., C. L. Myers, Y. Fu, D. C. Sheppard, M. R. Yeaman, W. H. Welch, A. S. Ibrahim, J. E. Edwards, and S. G. Filler. 2007. Als3 is a *Candida albicans* invasin that binds to cadherins and induces endocytosis by host cells. PLoS Biol. **5:**e64.

51. Rangel-Frausto, M. S., T. Wiblin, H. M. Blumberg, L. Saiman, J. Patterson, M. Rinaldi, M. Pfaller, J. E. Edwards, Jr., W. Jarvis, J. Dawson, and R. P. Wenzel. 1999. National epidemiology of mycoses survey (NEMIS): variations in rates of bloodstream infections due to *Candida* species in seven surgical intensive care units and six neonatal intensive care units. Clin. Infect. Dis. **29:**253–258.

52. Redding, S. W., R. C. Zellars, W. R. Kirkpatrick, R. K. McAtee, M. A. Caceres, A. W. Fothergill, J. L. Lopez-Ribot, C. W. Bailey, M. G. Rinaldi, and T. F. Patterson. 1999. Epidemiology of oropharyngeal *Candida* colonization and infection in patients receiving radiation for head and neck cancer. J. Clin. Microbiol. **37:**3896–3900.

53. Reggiori, F., C. W. Wang, P. E. Stromhaug, T. Shintani, and D. J. Klionsky. 2003. Vps51 is part of the yeast Vps fifty-three tethering complex essential for retrograde traffic from the early endosome and Cvt vesicle completion. J. Biol. Chem. **278:**5009–5020.

54. Revankar, S. G., O. P. Dib, W. R. Kirkpatrick, R. K. McAtee, A. W. Fothergill, M. G. Rinaldi, S. W. Redding, and T. F. Patterson. 1998. Clinical evaluation and microbiology of oropharyngeal infection due to fluconazole-resistant *Candida* in human immunodeficiency virus-infected patients. Clin. Infect. Dis. **26:**960–963.

55. Rhodus, N. L., C. Bloomquist, W. Liljemark, and J. Bereuter. 1997. Prevalence, density, and manifestations of oral *Candida albicans* in patients with Sjogren's syndrome. J. Otolaryngol. **26:**300–305.

56. Robinson, L. C., C. Bradley, J. D. Bryan, A. Jerome, Y. Kweon, and H. R. Panek. 1999. The Yck2 yeast casein kinase 1 isoform shows cell cycle-specific localization to sites of polarized growth and is required for proper septin organization. Mol. Biol. Cell **10:**1077–1092.

57. Rotrosen, D., J. E. Edwards, Jr., T. R. Gibson, J. C. Moore, A. H. Cohen, and I. Green. 1985. Adherence of *Candida* to cultured vascular endothelial cells: mechanisms of attachment and endothelial cell penetration. J. Infect. Dis. **152:**1264–1274.

58. Rubin-Bejerano, I., I. Fraser, P. Grisafi, and G. R. Fink. 2003. Phagocytosis by neutrophils induces an amino acid deprivation response in *Saccharomyces cerevisiae* and *Candida albicans*. Proc. Natl. Acad. Sci. USA **100:**11007–11012.

59. Sanchez, A. A., D. A. Johnston, C. Myers, J. E. Edwards, Jr., A. P. Mitchell, and S. G. Filler. 2004. Relationship between *Candida albicans* virulence during experimental hematogenously disseminated infection and endothelial cell damage in vitro. Infect. Immun. **72:**598–601.

60. Sandovsky-Losica, H., N. Chauhan, R. Calderone, and E. Segal. 2006. Gene transcription studies of *Candida albicans* following infection of HEp2 epithelial cells. Med. Mycol. **44:**329–334.

61. Sangeorzan, J. A., S. F. Bradley, X. He, L. T. Zarins, G. L. Ridenour, R. N. Tiballi, and C. A. Kauffman. 1994. Epidemiology of oral candidiasis in HIV-infected patients: colonization, infection, treatment, and emergence of fluconazole resistance. Am. J. Med. **97:**339–346.

62. Saville, S. P., A. L. Lazzell, C. Monteagudo, and J. L. Lopez-Ribot. 2003. Engineered control of cell morphology in vivo reveals distinct roles for yeast and filamentous forms of *Candida albicans* during infection. Eukaryot. Cell **2:**1053–1060.

63. Segurado, M., R. López-Aragón, J. A. Calera, J. M. Fernández-Abalos, and F. Leal. 1999. Zinc-regulated biosynthesis of immunodominant antigens from *Aspergillus* spp. Infect. Immun. **67:**2377–2382.

64. Siniossoglou, S., and H. R. Pelham. 2002. Vps51p links the VFT complex to the SNARE Tlg1p. J. Biol. Chem. **277:**48318–48324.

65. Sohn, K., I. Senyurek, J. Fertey, A. Konigsdorfer, C. Joffroy, N. Hauser, G. Zelt, H. Brunner, and S. Rupp. 2006. An in vitro assay to study the transcriptional response during adherence of *Candida albicans* to different human epithelia. FEMS Yeast Res. **6:**1085–1093.

66. Steele, C., J. Leigh, R. Swoboda, and P. L. Fidel, Jr. 2000. Growth inhibition of *Candida* by human oral epithelial cells. J. Infect. Dis. **182:**1479–1485.

67. Steele, C., J. Leigh, R. Swoboda, H. Ozenci, and P. L. Fidel, Jr. 2001. Potential role for a carbohydrate moiety in anti-*Candida* activity of human oral epithelial cells. Infect. Immun. **69:**7091–7099.

68. Subramanian, S., C. A. Woolford, and E. W. Jones. 2004. The Sec1/Munc18 protein, Vps33p, functions at the endosome and the vacuole of *Saccharomyces cerevisiae*. Mol. Biol. Cell **15:**2593–2605.

69. Sundstrom, P., E. Balish, and C. M. Allen. 2002. Essential role of the *Candida albicans* transglutaminase substrate, hyphal wall protein 1, in lethal oroesophageal candidiasis in immunodeficient mice. J. Infect. Dis. **185:**521–530.

70. Tsuchimori, N., L. L. Sharkey, W. A. Fonzi, S. W. French, J. E. Edwards, Jr., and S. G. Filler. 2000. Reduced virulence of *HWP1*-deficient mutants of *Candida albicans* and their interactions with host cells. Infect. Immun. **68:**1997–2002.

71. Tusher, V. G., R. Tibshirani, and G. Chu. 2001. Significance analysis of microarrays applied to the ionizing radiation response. Proc. Natl. Acad. Sci. USA **98:**5116–5121.

72. Vida, T. A., and S. D. Emr. 1995. A new vital stain for visualizing vacuolar membrane dynamics and endocytosis in yeast. J. Cell Biol. **128:**779–792.

73. Williams, R. M., M. Primig, B. K. Washburn, E. A. Winzeler, M. Bellis, C. Sarrauste de Menthiere, R. W. Davis, and R. E. Esposito. 2002. The Ume6

regulon coordinates metabolic and meiotic gene expression in yeast. Proc. Natl. Acad. Sci. USA **99:**13431–13436.

74. **Willis, A. M., W. A. Coulter, C. R. Fulton, J. R. Hayes, P. M. Bell, and P. J. Lamey.** 1999. Oral candidal carriage and infection in insulin-treated diabetic patients. Diabet. Med. **16:**675–679.

75. **Wilson, R. B., D. Davis, and A. P. Mitchell.** 1999. Rapid hypothesis testing with *Candida albicans* through gene disruption with short homology regions. J. Bacteriol. **181:**1868–1874.

76. **Yeaman, M. R., S. S. Soldan, M. A. Ghannoum, J. E. Edwards, Jr., S. G.**

Filler, and A. S. Bayer. 1996. Resistance to platelet microbicidal protein results in increased severity of experimental *Candida albicans* endocarditis. Infect. Immun. **64:**1379–1384.

77. **Yount, N. Y., and M. R. Yeaman.** 2004. Multidimensional signatures in antimicrobial peptides. Proc. Natl. Acad. Sci. USA **101:**7363–7368.

78. **Zakikhany, K., J. R. Naglik, A. Schmidt-Westhausen, G. Holland, M. Schaller, and B. Hube.** 2007. In vivo transcript profiling of *Candida albicans* identifies a gene essential for interepithelial dissemination. Cell. Microbiol. **9:**2938–2954.