Normal Adaptation of Candida albicans to the Murine Gastrointestinal Tract Requires Efg1p-Dependent Regulation of Metabolic and Host Defense Genes

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Although gastrointestinal colonization by the opportunistic fungal pathogen Candida albicans is generally benign, severe systemic infections are thought to arise due to escape of commensal C. albicans from the gastrointestinal (GI) tract. The C. albicans transcription factor Efg1p is a major regulator of GI colonization, hyphal morphogenesis, and virulence. The goals of this study were to identify the Efg1p regulon during GI tract colonization and to compare C. albicans gene expression during colonization of different organs of the GI tract. Our results identified significant differences in gene expression between cells colonizing the cecum and ileum. During colonization, effg1− null mutant cells expressed higher levels of genes involved in lipid catabolism, car- nitine biosynthesis, and carnitine utilization than did colonizing wild-type (WT) cells. In addition, during laboratory growth, effg1− null mutant cells grew to a higher density than WT cells. The effg1− null mutant grew in depleted medium, while WT cells could grow only if the depleted medium was supplemented with carnitine, a compound that promotes the metabolism of fatty acids. Altered gene expression and altered growth capability support the ability of effg1− cells to hypercolonize naive mice. Also, Efg1p was shown to be important for transcriptional responses to the stresses present in the cecum environment. For example, during colonization, SOD5, encoding a superoxide dismutase, was highly upregulated in an Efg1p-dependent manner. Ectopic expression of SOD5 in an effg1− null mutant increased the fitness of the effg1− null mutant cells during colonization. These data show that EFG1 is an important regulator of GI colonization.

Candida albicans is a medically important opportunistic pathogen that commonly colonizes the human gastrointestinal (GI) tract, skin, and genitourinary tracts (1–4). Normally, C. albicans benignly interacts with its host, but it can cause serious disease when a host becomes immunocompromised (5–9). C. albicans is capable of causing several different types of diseases, including gastrointestinal candidiasis, yeast infections, thrush, skin infections, and systemic infections (4, 10). Systemic infections are thought to be caused by endogenous C. albicans escaping the GI tract and circulating via the bloodstream to infect deep tissues such as the lungs, kidney, and liver (11–14).

As a colonizing organism of the GI tract, C. albicans grows mainly in the yeast form (15), but as a pathogen, the virulence of C. albicans is often associated with its ability to form hyphal cells (16–18). Hyphal morphology is induced by an array of environmental signals which converge through a network of transcription factors, allowing C. albicans to fine-tune its responses to changes in the environment (19–24).

Several transcription factors that regulate morphogenesis also regulate the behavior of C. albicans during GI tract colonization. Ehf1p is relatively highly expressed during GI tract colonization and regulates persistence in the GI tract (15, 25). Cph2p (26) is required for normal levels of GI tract colonization (27). Cph2p and Tec1p (28), another regulator of morphogenesis, both regulate gene expression during colonization (27).

The transcription factor Efg1p was identified as a major regulator of hyphal formation under most laboratory conditions (25, 29). An effg1− null mutant was attenuated for virulence in a systemic model of candidiasis and was more susceptible to phagocytosis (17, 30). Efg1p regulates gene expression in response to se-
shows that during gastrointestinal colonization, the \( \text{Efg1p} \) regulon differs from that seen during laboratory growth.

To understand how \( \text{Efg1p} \) regulates GI tract colonization, we used microarray analysis to globally identify genes that are regulated by \( \text{Efg1p} \) during GI tract colonization in the cecum and ileum. These data show that \( \text{Efg1p} \) regulates the expression of genes involved in metabolism and host interactions in colonizing cells. Genes that contribute to the hypercolonization of the \( \text{efg1}^- \) null mutant and its increased sensitivity to the host immune response during GI tract colonization were identified.

**MATERIALS AND METHODS**

**Animal models.** Five- to 7-week-old BALB/c mice (NCI) were treated with antibiotics (tetracycline, 1 mg/ml; streptomycin, 2 mg/ml; gentamicin, 0.1 mg/ml) as described previously (15). Mice were tested for fungal contamination prior to each experiment. Mice were inoculated with 0.1 ml \( \text{C. albicans} \) cells at \( 5 \times 10^6 \) cells/ml by oral gavage. Colonization was tested over time by collecting fresh fecal pellets and plating homogenates on yeast extract-peptone-dextrose (YPD) agar plus streptomycin (100 \( \mu \)g/ml) and ampicillin (50 \( \mu \)g/ml) (YPD-SA). Mice were sacrificed at 3 days postinoculation to obtain cecum and ileum contents for RNA isolation. Combined results from at least 2 experiments are shown for all data. Homogenates of kidney, liver, and tongue were plated on YPD-SA. No colonization of these organs was detected in any experiment. All experiments were done in compliance with regulatory guidelines defined by the Tufts University IACUC committee.

For competition experiments, colonies on YPD-SA plates were replica plated on yeast extract-peptone-sucrose (YPD)-nourseothricin (Nou) (200 \( \mu \)g/ml) and the ratio of drug-sensitive to drug-resistant cells was measured. The competitive index (CI) is defined as the number of \( \text{efg1}^- \) colonies/number of wild-type (WT) colonies, the number of \( \text{efg1}^- \) \( \text{ACT1pr}\text{-SOD5} \) colonies/number of WT colonies, or the number of \( \text{efg1}^- \) \( \text{ACT1pr}\text{-SOD5} \) colonies/number of \( \text{efg1}^- \) colonies at various time points, divided by the input ratio. For \( \text{efg1}^- \) /WT competitions, the WT strain was Nou'. For \( \text{efg1}^- \) \( \text{ACT1pr}\text{-SOD5} \) competitions, the \( \text{efg1}^- \) and WT strains were Nou'. At some time points, total colonization levels were below the level of detection and a ratio could not be determined; these samples were not included in the analysis. For samples in which one strain fell below the limit of detection, the CI is shown as 0.01.

**RNA isolation.** For the reference sample, WT cells were grown in laboratory culture at 37°C in YPD medium to log phase. Due to the high temperature of the culture, some degree of filamentation occurred in the reference sample, and some hyphal genes were expressed to some extent. Laboratory-grown cells and contents of cecum or ileum taken at 3 days postinoculation were mixed with RNA-Later (Ambion) and frozen at \(-80°C\). RNA was extracted from laboratory-grown samples using mechanical disruption and an RNA Easy minikit (Qiagen) with on-column DNase I digestion. Samples of mouse GI tract contents were filtered through 250-\( \mu \)m polypropylene mesh (Small Parts, Inc.) and then pelleted and extracted using the Purelink kit TRiZol extraction procedure (Invitrogen) with DNase I digestion. Two ceca or 4 ilea were combined per sample. Samples were stored at \(-80°C\) and were sent to the Whiteway lab at the CNRC, Montreal, Canada, for microarray analysis. RNA concentration was determined with a NanoDrop ND-1000 spectrophotometer, and RNA quality was tested using the Agilent 2100 BioAnalyzer (Agilent Technologies) with the RNA 6000 Nano LabChip. Reverse transcription of up to 3 \( \mu \)g of RNA using a modified oligo(dT)/T7-oligo(dT) primer, amplification, and coupling to Cy3/Cy5 dyes was done using the Amino Allyl MessageAmp II RNA amplification kit (Ambion [now Life Technologies]) kit catalog no. AM1753).

For reverse transcription-PCR (RT-PCR) confirmation, cDNA was generated from 1 to 10 \( \mu \)g RNA with Superscript II or III reverse transcription (Invitrogen) and an oligo(dT) primer. Samples were heated to 70°C for 10 min, and the enzyme was added. The sample was then incubated at 42°C or 50°C, respectively, for 1.5 h. Following addition of 2.5 M NaOH, the reaction mixture was incubated at 37°C for 15 min, and 2 M HEPES free acid was then added to neutralize the pH and cDNA was purified using QiAquick columns (Qiagen). Concentrations were determined using a NanoDrop spectrophotometer, and samples were stored at \(-20°C\).

**Microarray hybridization.** \( \text{C. albicans} \) microarrays (double-spotted 6,394 invariant 70-mer oligonucleotides) were obtained from the Bio-technology Research Institute microarray facility center (http://www.nrc-cnrc.gc.ca/eng/services/bri/microarray.html), Montreal, Canada. Slides were prehybridized for 2 h at 42°C, and probes were denatured at 96°C. Hybridization was done overnight at 42°C in a “Slide Booster” chamber (Advalytix) (36). Microarray samples were used with at least 3 biological replicates.

**Microarray analysis.** Microarray slides were scanned using the “ScanArray Gx” scanner (Perkin-Elmer) at a 10-\( \mu \)m resolution, and quantification was done with QuantArray software. Data analysis was done with “GeneSpring GX 7.3 Expression Analysis” software (Agilent Technologies) and Microsoft Excel. Gene expression in colonizing cells was compared directly to gene expression in a reference sample of WT cells grown in exponential phase in YPD medium at 37°C by hybridization of differentially labeled samples of each to the same array. Differences in expression between the reference and experimental samples were considered significant if the value was \( >1.5 \) or \( <0.67 \) and the \( P \) value \((t\ test)\) was \( <0.05 \). Additional analysis was done with gene ontology (GO) term analysis using the Candida Genome Database (http://www.candidagenome.org).

For comparison of gene expression in colonizing WT cells and colonizing mutant cells, values obtained from the microarrays were divided. Genes with ratios of \( >1.3 \) or \( <0.7 \) with at least one \( P \) value \((<0.05\) were designated differentially expressed.

For additional comparisons of gene expression in colonizing cells and cells grown under laboratory conditions, we calculated the number of genes expected to be up- or downregulated both in colonizing cells and under another condition based on the frequencies of up- or downregulated genes under various conditions. This number was compared to the number of genes actually observed to be up- or downregulated both in colonizing cells and under another condition, and statistical significance was evaluated using the chi-square test, with a \( P \) value \((<0.05\) considered significant. For analysis of \( \text{Efg1p} \)-dependent gene expression, this analysis was performed with 5,977 genes that were not differentially expressed in response to hyphal induction conditions.

**Strains.** All \( \text{C. albicans} \) strains used in this study are listed in Table S1 in the supplemental material. A CAI-4 strain carrying two copies of chromosome 1 was used. Strains CAI-4 and HLC67 were kindly provided by W. Fonzi and G. Fink, respectively. Escherichia coli strain XL1-Blue or DH5\( \alpha \) was used for propagating plasmids.

**Plasmids.** To construct a strain in which \( \text{SOD5} \) was overexpressed, the Cipl10 \( \text{ACT1pr}\text{-glUC59} \) plasmid (37) was digested with HindIII and NheI and the gLUC-PGAS9 fusion was replaced with the \( \text{SOD5} \) open reading frame (ORF) downstream of the actin promoter \((\text{ACT1pr})\). The plasmid was linearized with Stul and transformed into strain CAI-4 and the \( \text{efg1}^- \) strain using URA3 as the selectable marker. Proper integration at the \( \text{RPS1} \) locus was confirmed by PCR.

Drug-resistant wild-type or \( \text{efg1}^- \) null strains were constructed by digesting the plasmid iSAT (15) with BsgI and transforming into CAI-4 or HLC67 using \( \text{URA3} \) as the selectable marker. The resulting strains (JPY105 and JPY106) are resistant to nourseothricin on plates containing sucrose as the carbon source.

**Media and growth conditions.** YPD (1% yeast extract, 2% peptone, 2% glucose) and YPS (1% yeast extract, 2% peptone, 2% sucrose) were used as rich growth media. Minimal CM medium lacking uracil was made as previously described (38). Log-phase cells were grown to an optical density (OD) of 1 in YPD at 30°C. For mouse inoculations, strains were grown at 37°C in YPD for 21 to 24 h.
Growth curves. Strains were grown overnight in YPD at 30°C and diluted to 5 × 10^6 cells/ml the next morning. Cell density was measured immediately after dilution and at various time points by counting the number of cells per ml using a hemacytometer. Cultures were diluted into YPD, YP alone, or YP plus 2% ethanol, acetate, glycerol, or oleic acid (3:5 ratio of oleate to Tween 80), and cells were then grown at 30°C. For experiments with depleted media, WT C. albicans was grown in YPD for 5 days at 30°C and centrifuged, and the culture supernatant was filter sterilized. The culture supernatant (depleted medium) was then reincubated with either WT or efg1^- null mutant cells. Growth is shown as fold change above the inoculum level. Growth curves on various carbon sources were repeated with at least three independent replicates. Growth in glucose and in the depleted medium was done in triplicate with at least two replicates.

RT-PCR. Primers used to detect expression of genes are listed in Table S2 in the supplemental material. Quantitative RT-PCRs (qRT-PCRs) were performed with SYBR green reaction mix (Qiagen) with reference dye ROX using an MX3000p instrument (Stratagene). Each sample was tested in triplicate, and melting curve analysis in addition to agarose gel electrophoresis was used to confirm primer specificity. Samples tested with no cDNA showed no detectable signal. Standard curves were generated and all results normalized to the level of actin (ACT1) expression in each respective sample. Results for all samples are presented normalized to the level of expression for each respective gene in wild-type cells grown in YPD at 30°C to an OD of 1.

Microarray data accession number. The microarray data have been deposited in the GEO database under accession number GSE41771.

RESULTS

A general program of C. albicans gene expression within the host. Previous studies examined the effects of the cecum environment on gene expression by wild-type (WT) C. albicans (27). Here, we extended these studies to examine gene expression in WT C. albicans cells colonizing either the cecum or the ileum. There are considerable differences between the ileum and cecum environments. The ileum has a more alkaline pH and increased levels of oxygen relative to those in the cecum (39, 40). After passage of gut contents through the ileum, where many nutrients are absorbed, the remaining indigestible polysaccharides are fermented by bacteria to produce short-chain fatty acids (SCFA), and there are greater numbers of commensal bacteria in the cecum than in the ileum (41–43).

To understand similarities and differences between fungal cells colonizing the cecum and the ileum, BALB/c mice were inoculated by oral gavage with C. albicans (WT or mutant strains), as described in Materials and Methods. Fecal pellets were collected on days 1 and 3 postinoculation, and cecum contents were collected on day 3 postinoculation. These samples were homogenized and plated to measure colonization levels (Fig. 1, squares). In addition, cecum and ileum contents were harvested at day 3 for gene expression analysis. RNA was extracted from the GI tract contents, amplified, and labeled for hybridization to microarrays (see Materials and Methods). For each microarray, gene expression was measured relative to a reference sample of WT C. albicans cells grown in the laboratory to log phase in rich medium at 37°C. Hybridization, scanning, and analysis of microarray results using GeneSpring were performed (see Materials and Methods). Genes which were 1.5-fold up- or downregulated with a P value of <0.05 were considered to be significantly differentially expressed. Gene expression ratios between microarrays were then used to define genes differentially expressed between cells colonizing the cecum or the ileum and between WT C. albicans and efg1^- null mutants.

Differential expression of selected genes was confirmed by real-time qRT-PCR (Fig. 2 and 3). Relative to the reference sample of WT cells grown under laboratory conditions, WT C. albicans cells in the cecum or ileum differentially expressed 604 genes and 772 genes, respectively (Table 1; see Table S3 in the supplemental material) (GEO accession number GSE41771). Roughly half of these genes were differentially regulated by C. albicans cells in both organs. For upregulated genes, in the cecum, 111/189 (59%) were common between both organs, and in the ileum, 111/307 (36%) were common. For downregulated genes, 212/415 (51%) were common in the cecum, and in the ileum, 212/415 (46%) were common. These 323 genes constituted a common group of differentially regulated genes affected during colonization of the cecum and ileum (see Table S4 in the supplemental material).

Analysis of GO terms enriched in the common genes showed that the genes upregulated during colonization were enriched for genes involved in pathogenesis (Table 2). Consistent with this observation, 49% of the common genes were also upregulated in cells recovered from other body sites following infection of mammalian hosts, based on previous microarray experiments (33, 34, 44) (examples are indicated in Table 2 and in Table S4 in the supplemental material).

Common upregulated genes involved in host interactions included HYRI (encoding a hypha-induced cell wall protein [46, 47]), SAP4, -5, and -6 (encoding secreted aspartyl proteases [48–50]), ALS3 (encoding an adhesin [51, 52]), and HWPI (encoding a hyphal wall protein [53, 54]) (Table 2 and Fig. 2). These genes have all been described as being upregulated during hyphal growth (23, 31, 51, 55) as well as during experimental infection (33, 45, 56). Therefore, consistent with previous data (15), cells colonizing both the cecum and the ileum have gene expression patterns that are similar to those in hyphal cells even though most colonizing cells are yeast.

Another GO category that was enriched in the common genes

FIG 1 Altered colonization of the GI tract by some C. albicans mutants. Cells of strain CKY101 (WT; squares), CKY136 (efg1^- null mutant; diamonds), JPY109 (efg1^- efh1^- double null mutant; circles), or CKY138 (efg1^- cph1^- double null mutant; triangles) were inoculated into BALB/c mice by oral gavage as described in Materials and Methods. Colonization was measured in fresh fecal pellets collected on days indicated or in cecum contents harvested on day 3 postinoculation. C. albicans CFU per gram of material was determined. Each symbol represents a sample from an individual mouse; bars indicate geometric means. Asterisks indicate statistically significant differences between WT and mutant colonization: *, P < 0.05; **, P < 0.01; ***, P < 0.001 (Mann-Whitney test).
was the carbohydrate metabolic process category (Table 2; see Table S4 in the supplemental material). Several of the genes in this category were also upregulated in cells recovered from other sites of infection (Table 2). This category included genes involved in galactose metabolism (e.g., GAL1 and GAL10) and genes required for gluconeogenesis (e.g., FBP1 [57] and PCK1 [58]). Expression of the latter genes increases in the laboratory in the absence of glucose, and upregulation of these pathways in colonizing cells is consistent with low availability of glucose in the cecum. Genes involved in alcohol metabolism, such as ALD5 (encoding aldehyde dehydrogenase), were upregulated in cells colonizing both organs. In addition, CTN1 (encoding carnitine acetyltransferase) was upregulated in the ileum and cecum as shown by microarray results or qRT-PCR (Fig. 3). Upregulation of both fermentation and gluconeogenesis as observed previously (27, 59) may indicate that some colonizing fungal cells have access to nutrients for fermentation, while others are in a nutrient-poor local environment.

Gene expression consistent with increased uptake and utilization of several essential nutrients was observed in cells colonizing the cecum and ileum. Genes involved in phosphate transport (PHO84 and PHO89) were upregulated (see Table S4 in the supplemental material; qRT-PCR results are shown in Fig. 2). Expression of PHO84 by invasive C. albicans cells in both the liver and kidney has been previously observed (33, 34), suggesting a general need for phosphate transport activity during growth within a host.

TABLE 1 Summary of C. albicans gene expression changes during colonization

<table>
<thead>
<tr>
<th>Strain and site</th>
<th>No. of genes</th>
<th>Upregulated</th>
<th>Downregulated</th>
<th>Total differential</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strain</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WT</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cecum</td>
<td>189</td>
<td>415</td>
<td>604</td>
<td></td>
</tr>
<tr>
<td>Common</td>
<td>111</td>
<td>212</td>
<td></td>
<td></td>
</tr>
<tr>
<td>efh1− null mutant</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cecum</td>
<td>71</td>
<td>184</td>
<td>255</td>
<td></td>
</tr>
<tr>
<td>Ileum</td>
<td>226</td>
<td>499</td>
<td>725</td>
<td></td>
</tr>
<tr>
<td>Common</td>
<td>27</td>
<td>109</td>
<td></td>
<td></td>
</tr>
<tr>
<td>efh1− efh1− null mutant</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cecum</td>
<td>85</td>
<td>154</td>
<td>239</td>
<td></td>
</tr>
<tr>
<td>Ileum</td>
<td>222</td>
<td>395</td>
<td>617</td>
<td></td>
</tr>
<tr>
<td>Common</td>
<td>34</td>
<td>99</td>
<td></td>
<td></td>
</tr>
<tr>
<td>efh1− cph1− null mutant</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cecum</td>
<td>336</td>
<td>472</td>
<td>808</td>
<td></td>
</tr>
<tr>
<td>Ileum</td>
<td>441</td>
<td>345</td>
<td>986</td>
<td></td>
</tr>
<tr>
<td>Common</td>
<td>149</td>
<td>236</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

A * compared to the reference sample of WT laboratory-grown cells.
B Number of genes upregulated (or downregulated) relative to the reference sample in C. albicans cells from the ileum and from the cecum.

Increased expression of the hemoglobin utilization gene RBT5 (60, 61) compared to that in the reference sample was observed by microarray analysis and confirmed by qRT-PCR (Fig. 2). Upregulation of two other hemoglobin utilization genes, PGA10 (60, 61) and PLA10 (60, 61) was also significant compared to the reference sample of WT laboratory-grown cells (Table S4).
and \textit{HMX1} (60, 62), was observed in cells from the cecum. Up-regulation of hemoglobin uptake and utilization genes in cells taken from the gut suggests a role for this activity during colonization.

Consistent with other reports (33, 45, 56), a significant stress response was detected in colonizing WT cells relative to laboratory-grown cells in both the cecum and the ileum. The increased expression of several stress response genes, such as \textit{DDR48}, was detected (see Table S4 in the supplemental material). The expression of heat shock genes, including \textit{HSP60}, \textit{HSP78}, and \textit{HSP104}, also increased in colonizing cells, despite the fact that the reference sample was grown at physiological temperature (see Table S4 in the supplemental material). Genes involved in the oxidative stress response were also highly induced. \textit{SOD5}, encoding a superoxide dismutase (63, 64), was the most highly upregulated gene in our study (Fig. 2; see Table S4 in the supplemental material). \textit{CAT1}, encoding the major catalase in the cell (65), and \textit{HSP104}, encoding a superoxide dismutase (63, 64), were also upregulated during colonization relative to in laboratory-grown cells (Fig. 3; see Table S4 in the supplemental material). Based on these results, \textit{C. albicans} undergoes various types of stress during colonization, including oxidative stress, and expresses a number of genes to counteract these effects.

As a second way to analyze the patterns of gene expression associated with GI colonization, we compared gene expression in cells recovered from the GI tract with expression in cells exposed to various conditions in the laboratory. Using the frequencies of up- or downregulated genes under a laboratory condition, we calculated the number of genes expected to be up- or downregulated both in the cecum and ileum and under that condition and compared that number to the observed number of similarly regulated genes. Comparing the common genes differentially expressed during GI colonization with other conditions, statistically significant enrichment was detected for genes upregulated in response to hyphal induction (67, 68), temperature shock (69), osmotic shock (69), carbon and nitrogen starvation (30), growth in galactose (70), and growth at alkaline pH (71), consistent with previous analysis of gene expression in the cecum (27). Thus, the common genes expressed in cells colonizing both organs included well-characterized genes that are regulated under laboratory conditions.

In contrast, genes upregulated and downregulated only in the cecum were not enriched for any GO process categories (Table 2). These genes likely have functions that are uncharacterized during laboratory growth and are colonization specific.

In summary, there was significant overlap in gene expression between cells colonizing the cecum and ileum. Genes involved in host interactions, carbohydrate metabolism, and hyphal growth were expressed at higher levels in both organs relative to in laboratory-grown cells. In addition, cells in the ileum exhibited stress responses similar to those of cells colonizing the cecum, suggesting that these responses are part of a general response to the host GI tract environment.

**Niche-specific gene expression by WT \textit{C. albicans} in the ileum.** While many features of gene expression were common in cells colonizing the two organs, there were also some significant differences in gene expression patterns between cells from the cecum and those from the ileum. Over half of the genes that were differentially expressed between colonizing cells in the ileum and the reference sample of laboratory-grown cells were not similarly regulated in the cecum (Table 1). Genes annotated to the GO term acetyl coenzyme A (acyetyl-CoA) metabolism (\textit{CIT1} [encoding citrate synthase], \textit{ACS1} [encoding a putative acetyl-CoA synthase], and \textit{MDH1-1} [encoding malate dehydrogenase]) were enriched among genes upregulated by WT \textit{C. albicans} in the ileum but not the cecum relative to the reference sample (see Table S5 in the supplemental material). Cells growing under hypoxic conditions have decreased levels of respiration, consistent with the decreased expression of tricarboxylic acid (TCA) cycle genes observed in the cecum relative to the ileum. These data show that acetyl-CoA metabolism and the TCA cycle are active during colonization of the ileum.

Relative to the reference sample, expression of \textit{PHO89} and \textit{PHR1}, alkaline pH-induced genes (71, 72), was higher in the ileum than in the cecum (see Table S5 in the supplemental material). Consistent with this observation, the pH of the digestive tract is

**Table 2 GO categories enriched among \textit{C. albicans} genes differentially expressed during growth in both the ileum and cecum**

<table>
<thead>
<tr>
<th>Regulated in WT cells</th>
<th>GO term</th>
<th>Corrected P value</th>
<th>Genes*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regulated in both cecum and ileum</td>
<td>Pathogenesis</td>
<td>0.0008</td>
<td>RBT1, UME6, HXX1, DAF1, ALS3, TPS2, orf19.3070.1, PHR1, SAP6, SAP5, SAP4, VPS21, RBT4, CAT1, HSP104, HXX1, DAF1, TPS2, orf19.3070.1, Gal1, Gal10, Gal7, PHR1, orf19.3982, orf19.5365, GLK4, FBPI, HSP104, orf19.6423, SKN1, PCK1, PUT2, PUT1, PRO3</td>
</tr>
<tr>
<td>Regulated in cecum only</td>
<td>Pathogenesis</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>Regulated in ileum only</td>
<td>Acetyl-CoA metabolic process</td>
<td>0.002</td>
<td>ACS1, CNT3, CIT1, CNT1, MVD, KGD1, FUM12, MNT3, orf19.1092, ALG2, orf19.1761, orf19.1995, DFG10, MNN2, ALG11, CWHi8, orf19.3085, OST1, orf19.7426, PMT5</td>
</tr>
</tbody>
</table>

*Underlined genes are upregulated under other in vivo conditions such as in catheters, kidneys, liver, or oral lesions (33, 34, 44, 45).
alkaline in the ileum and closer to neutral in the cecum. These genes are both activated by the RIM101 pathway under alkaline conditions (73, 74). We observed that RIM101 was upregulated in the cecum by qRT-PCR (Fig. 3) and in the ileum by microarray analysis (see Table S3 in the supplemental material). However, when a rim101 null mutant was inoculated into BALB/c mice by oral gavage, the mutant was able to colonize at WT levels as measured in fecal pellets and GI tract organs (data not shown). Therefore, although Rim101p is required for systemic virulence (73), it is not required for GI colonization.

One major group of genes downregulated by WT C. albicans in the ileum was genes involved in glycosylation. This category included genes responsible for N-linked glycosylation, hexosylation, and mannosylation (e.g., MNT2, MNT3, and PMT5), all of which were downregulated in cells colonizing the ileum (see Table S5 in the supplemental material). Because a decreased inflammatory response in response to C. albicans mutants defective in glycosylation was observed (75), decreased or altered patterns of glycosylation may allow colonizing cells to avoid the immune response. In addition, cells with deficiencies in mannosylation are more resistant to phagocytosis by neutrophils (76).

In summary, there were differences in gene expression when C. albicans was colonizing the ileum or the cecum. In addition, there were similar patterns of gene expression in the two organs. There was also considerable similarity in gene expression between colonizing cells and cells causing infection of various body sites. Thus, many aspects of C. albicans gene expression during colonization represent a general adaptation to growth within a host.

Efg1p-dependent gene expression during colonization. Efg1p is a major regulator of GI colonization dynamics (35). We observed that in the GI tract of a naïve host, efg1 null mutant cells were able to grow to higher levels than WT cells. However, when an immune response was mounted by the host, efg1 null mutant cells were hypersusceptible to that response in comparison to WT cells. As Efg1p is a major regulator of gene expression during laboratory growth of C. albicans (23, 25, 31, 55), it is likely that altered gene expression during colonization underlies the altered colonization dynamics exhibited by the efg1 null mutant.

To understand the effect of Efg1p on gene expression during colonization, microarray analysis of gene expression in colonizing efg1 null mutant cells was conducted. BALB/c mice were colonized with the efg1 null strain of C. albicans, and fecal pellets were plated to determine the level of colonization. As previously observed (35), the efg1 null mutant strain showed increased colonization relative to WT C. albicans at day 3 in both the fecal pellets and the cecum contents (P < 0.0001 and P = 0.0024, respectively [Mann-Whitney test]) (Fig. 1). Cecum and ileum contents were collected at day 3 postinoculation and used to determine gene expression in colonizing cells.

Compared to a WT laboratory reference sample, 255 genes were differentially expressed in the cecum in the absence of Efg1p and 725 genes were differentially expressed in the ileum (Table 1). The number of common genes that were differentially expressed in both organs was relatively small. For upregulated genes, in the cecum, 27/71 (38%) were common, and in the ileum, 27/226 (12%) were common. For downregulated genes, in the cecum, 109/184 (59%) were common, and in the ileum, 109/499 (22%) were common. Thus, in the efg1 null mutant compared to the WT, there was a greater effect of the niche on gene expression patterns.

Gene expression in colonizing WT and efg1 null mutant cells was compared by calculating ratios between the microarray measurements for each strain; expression of a gene was considered different in the two strains if the ratio was >1.3 or <0.7 and at least one microarray measurement had a P value of <0.05. Using these criteria, the efg1 null mutant differentially expressed 504 genes in the cecum and only 241 genes in the ileum compared to the WT strain colonizing each respective organ (see Table S6 in the supplemental material). Therefore, Efg1p had a greater effect on gene expression in the cecum than in the ileum. Other transcription factors that are redundant with Efg1p may be active in cells colonizing the ileum.

Previous studies showed that a few hypha-regulated genes are expressed independently of Efg1p during colonization, despite being regulated by Efg1p during laboratory growth (15). In these studies, we observed that a subset of genes previously identified as Efg1p dependent also require Efg1p for their expression in colonizing cells. The genes encoding the adhesin Als3p, the hypha-regulated cell surface protein Hya1p, the secreted aspartyl proteases Sa4p to -6p, and the superoxide dismutase Sod5p all required Efg1p for their expression during colonization (Fig. 2; see Table S6 in the supplemental material). However, other genes, including the hyphal wall protein gene HWPI, the pH-responsive glycosidase gene PHR1, and the hemoglobin utilization gene RBT5 (Fig. 2; see Table S3 in the supplemental material), were not affected by the absence of Efg1p in colonizing cells. Therefore, genes regulated by hyphal growth in the laboratory are expressed during colonization, but only a subset of these genes require Efg1p for their expression.

We analyzed the genes that were differentially expressed during colonization by efg1 null mutant cells in comparison to WT colonizing cells to detect enriched categories of genes. Genes expressed at lower levels in efg1 null mutant cells than in WT cells were involved mainly in host interactions. GO term analysis of these genes revealed enrichment for genes involved in pathogenesis (RBT1, MNT2, UE06, SOD5, RCK2, ALS3, TPS2, WHI1, CDC42, HSX11, SAP6, SAP5, SAP4, orf19.60, RBT4, CAT1, and HSP21; P = 8.8 × 10^-7) and genes involved in the response to oxidative stress (CIP1, orf19.1340, SOD5, RCK2, AHP1, TPS2, DDR48, CAT1, HSP21, GPX2, and orf19.86; P = 0.0015).

Analysis of genes expressed at higher levels in efg1 null mutant cells recovered from the ileum than in WT cells from the ileum showed that genes involved in cellular lipid catabolism (PLB4.5, POX1 to -3, PXP2, orf19.3070.1, and POT1; P = 0.024) were enriched (Fig. 4; see Tables S5 and S6 in the supplemental material). Further analysis of related metabolic pathways revealed several additional genes that showed the same pattern, i.e., higher expression in colonizing efg1 null mutant cells than in colonizing WT cells (Fig. 4A). In order to catabolize lipids, carnitine is used as a shuttle to transfer acetyl-CoA generated through catabolism of lipids into mitochondria for generation of ATP via the TCA cycle. Several genes involved in carnitine biosynthesis or encoding carnitine acetyltransferase were more highly expressed in the efg1 null mutant during colonization of the ileum (e.g., AGP2, CAT2, and orf19.6306) (Fig. 4; see Table S5 in the supplemental material). Also, the expression of CTN1, another carnitine acetyltransferase, was higher in the absence of Efg1p in the cecum than in WT C. albicans as measured by qRT-PCR (Fig. 3). Enhanced carnitine biosynthesis and utilization would be expected to increase the ability of the efg1 null mutant
strain to utilize nonfermentable carbon sources present in the GI tract. Thus, although carnitine metabolism is not required for virulence in systemic infections (77), it may contribute to growth in the GI tract, where short-chain fatty acids (SCFA) produced by bacteria would be available as carbon sources. Thus, Efg1p negatively regulates expression levels of genes involved in lipid catabolism during colonization.

To characterize other types of Efg1p-responsive genes, we compared gene expression in colonizing efg1Δ null mutant cells with expression in WT cells grown under various laboratory conditions. Because Efg1p has a large effect on expression of hyphal genes, we filtered the data to remove from consideration 343 genes whose transcription is influenced by Efg1p during colonization of the ileum. Reduced expression of stress-related genes during colonization of the ileum (upregulated genes compared to the reference sample), was less significant than in the efg1Δ null mutant strain (Fig. 5). Therefore, in colonizing cells, Efg1p is critically important for mounting gene expression responses to stresses due to the cecum environment. In the ileum, the effect of the efg1Δ null mutation on gene expression in response to these stresses was not as great (data not shown). This finding is consistent with the relatively low number of genes whose transcription is influenced by Efg1p during colonization of the ileum. Reduced expression of stress-related genes during colonization by the efg1Δ null mutant may underlie the hypersusceptibility of this mutant strain to the host immune response. Thus, Efg1p is a critical regulatory factor for adaptation of C. albicans to the cecum environment.

**Effect of Ef1p and Cph1p on Efg1p-dependent gene expression patterns.** To understand the role of Ef1p in the regulation of gene expression during colonization further, we expanded our study to include the efg1Δ efh1Δ and efg1Δ cph1Δ double null mutant strains of C. albicans. Ef1p (25) is a homolog of Efg1p that regulates the persistence of C. albicans in the GI tract (15). Cph1p, another transcription factor, regulates the yeast-to-hyphal transition through a mitogen-activated protein (MAP) kinase pathway (19, 78). Cph1p appears to be partially redundant with Efg1p, and previous studies showed that deletion of both CPH1 and EFG1 caused additive defects in morphogenesis and virulence of C. albicans in the bloodstream (17, 19).

![FIG 4 Differences in expression of genes involved in lipid catabolism and carnitine metabolism between colonizing WT and efg1Δ null mutant cells. (A) Illustration of metabolic pathways. Genes more highly expressed in colonizing efg1Δ null mutant cells than in colonizing WT cells are shown in red. (B) Microarray data for RNA samples of C. albicans strain CKY101 (WT; blue), CKY136 (efg1Δ null mutant; red), JPY109 (efg1Δ efh1Δ double null mutant; yellow), or CKY138 (efg1Δ cph1Δ double null mutant; green) recovered from the ileum of colonized mice. Gene names are indicated below the graph.](http://ec.asm.org/)

![FIG 5 Overlap in upregulated genes between colonizing C. albicans cells and C. albicans cells grown under laboratory conditions. Microarray data generated in this study or from published studies were compared. For this analysis, all genes that are differentially expressed during hyphal growth were removed from consideration, as described in text. From the frequency of upregulated (nonhyphal) genes during colonization and the frequency of upregulated (nonhyphal) genes during growth under the indicated laboratory condition, the number of genes predicted to be upregulated under both conditions by chance alone was calculated (open bars). Closed bars indicate the number of nonhyphal genes that were observed to be upregulated under both conditions. Strains were CKY101 (WT; red), CKY136 (efg1Δ null mutant; dark blue), and JPY109 (efg1Δ efh1Δ double null mutant; light blue). For WT cells, differences between expected and observed were statistically significant for all conditions shown (P < 0.05 by chi-square test).](http://ec.asm.org/)
These double null mutant strains were inoculated into BALB/c mice, and at days 1 and 3 postinoculation, fecal pellets were plated to determine colonization levels. The efg1- efh1- double null strain hypercolonized at day 3 in the fecal pellets and cecum contents (P = 0.0305 and P = 0.0304, respectively), similar to the case for the efg1- null mutant alone (Fig. 1). In contrast, addition of a cph1- deletion to an efg1- null mutant resulted in a return to WT initial levels of colonization (Fig. 1). At later time points postinoculation, the double null mutant, like the efg1- single null mutant, was outcompeted by WT cells in a competition experiment (data not shown).

Microarray analysis was used to determine the global gene expression patterns from cells colonizing the cecum and ileum. The results showed that patterns of gene expression in the efg1- single null and efg1- efh1- double null strains were similar, consistent with their similar colonization phenotypes. These results provide further confirmation of the patterns of Efg1p-dependent gene expression described above.

Comparisons were made with WT C. albicans colonizing each organ, more genes were differentially regulated in the cecum (498 genes) than in the ileum (205 genes) by the efg1- efh1- double null strain (see Table S7 in the supplemental material). As shown in Fig. 5, up-regulation in the cecum of C. albicans genes that respond to starvation, with 3-amino-nitroazole (3-AT), absence of non-glucose carbon sources, or stress was markedly reduced in the double mutant compared to WT C. albicans. In some cases, the double null mutant was more defective than the efg1- single null mutant, arguing for partial redundancy between Efg1p and Efhl1p in the regulation of gene expression. Overall, the efg1- efh1- double null mutant had patterns of gene expression similar to those of the efg1- null mutant, supporting previous data that these two mutants show partial redundancy and express similar genes during laboratory growth (25).

In contrast, more genes were differentially expressed by the efg1- cph1- double null mutant (cecum, 649; ileum, 340) than by either the efg1- single null or the efg1- efh1- double null mutant, relative to WT gene expression in the gut (see Tables S6, S7, and S8 in the supplemental material). This finding mirrors the observation that during laboratory growth, more genes are altered in expression in the efg1- cph1- double null mutant than in the efg1- single null mutant relative to WT cells (25). For example, expression of several genes encoding cell surface adhesion molecules was reduced in the efg1- cph1- strain relative to WT colonizing cells, particularly in the ileum, and expression of genes involved in sugar transport was increased in efg1- cph1- double null relative to WT colonizing cells in the ileum. These differences in gene expression between WT and efg1- cph1- double null colonizing cells do not appear to influence colonization levels initially, since cells of both strains colonized at similar levels (Fig. 1). However, at later times postinoculation, reduced expression of adhesion molecules may contribute to the competitive disadvantage of the double null mutant relative to WT C. albicans.

Interestingly, some genes that were more highly expressed in the efg1- null mutant in the ileum were expressed at close to WT levels in the efg1- cph1- double null mutant. A systematic search for such genes showed that genes in the GO category fatty acid catabolic process were enriched in this set of genes. As shown in Fig. 4, several genes involved in lipid catabolism and carnitine biosynthesis or utilization (XP2, ORF19.3936, ORF19.3070.1, POT1, CAT2, and ORF19.6306) showed this pattern of expression. The lack of higher expression of these genes may contribute to the lack of hypercolonization by the efg1- cph1- double null strain (Fig. 1). Therefore, during colonization, the addition of the cph1- deletion reversed the effects of the efg1- null mutation on expression of some genes, in contrast to the partial redundancy between Efg1p and Cph1p observed under other conditions (17, 25).

Finally, we identified a number of genes that were regulated by Efg1p in all three mutants, specifically, 104 genes in the cecum and 49 in the ileum (see Table S9 in the supplemental material). Interestingly, 54/104 (52%) and 18/49 (37%) of these genes are uncharacterized ORFs (see Table S9 in the supplemental material). This observation suggests that a large number of Efg1p functions may be specific to host interaction during colonization. Other genes coregulated between the three efg1- mutants included known host interaction genes such as ALS1 and ALS3 (encoding adhesins), SAP4 to -6 (encoding secreted aspartyl proteases), and SOD5 (encoding superoxide dismutase), as well as the heat shock genes HSP30, -21, and -78. The expression of several transcription factors, including Tec1p, Rim101p, and Um69, also appeared to be affected by Efg1p. Therefore, in colonizing cells, many genes involved in host interactions showed regulation by Efg1p in all 3 mutants.

Increased growth of efg1- null mutant cells compared to WT cells under laboratory conditions. When introduced into the GI tract of a naive mouse, the efg1- null mutant reached a higher population density (Fig. 1). This mutant also showed alterations in metabolic gene expression during colonization compared to WT colonizing cells (Fig. 4). To determine whether the mutant strain could grow to a higher level under other conditions, WT and efg1- null mutant strains were grown in the laboratory in rich medium under yeast growth conditions at 30°C. The concentration of cells in the culture was measured by counting the number of cells/ml with a hemacytometer, as differences in cellular morphology and size alter optical density. During exponential phase growth in glucose-containing medium, both strains grew well, and the experimentally determined doubling times during this phase (WT, 58 min; efg1-, 70 min) were similar to previously published values (WT, 57.6 min; efg1-, 69 min) (17). However, at the end of exponential-phase growth, wild-type cells paused briefly before resuming growth at a lower rate, while efg1- null mutant cells continued to grow (P = 0.0036; 6.25 h [Student’s t test]) (Fig. 6A). The efg1- null mutant strain then reached a higher density during post-exponential phase than wild-type C. albicans (P = 0.0013; 48 h.) (Fig. 6B). Therefore, increased growth of the efg1- null mutant was observed under laboratory conditions as well as during colonization.

To determine if the efg1- null mutant grew better on alternative carbon sources than WT C. albicans, growth was measured in the presence of glucose, ethanol, acetate, and oleate. As a control, both WT and efg1- null cells were grown in YP (yeast extract and peptone) in the absence of any carbon source. WT and efg1- null strains were inoculated at 5 × 106 cells/ml, and cells were counted at 5, 24, and 48 h postinoculation. During log-phase growth, the WT and efg1- null mutant grew at an equal rate on all carbon sources except oleate. Increased growth of the efg1- null mutant was observed on oleate as early as 5 h postinoculation (data not shown). After 24 h of growth, while no difference in growth between the WT and efg1- null mutant strains in YP was noted, the efg1- null mutant grew to 2- to 3-fold-higher levels than WT cells in several carbon sources (glucose, P = 0.0005;
fermentable carbon sources, such as acetate and ethanol (77, 79, 80), the increased expression of genes involved in carnitine metabolism by the efg1" null mutant may contribute to its enhanced growth.

To determine whether addition of carnitine would allow WT cells to grow in depleted medium, carnitine was added to this medium. In the presence of added carnitine, WT cells were able to grow \((P = 0.0181\) after 4 h) (Fig. 6). Similar trends in growth were observed over a period of 24 h (data not shown). In contrast, efg1" null mutant cells were able to grow in the absence of additional carnitine \((P = 0.0007)\). When carnitine was added, the efg1" null mutant was able to undergo further growth \((P = 0.0042)\). The ability of the efg1" null mutant to grow in the absence of additional carnitine may reflect increased carnitine biosynthesis as well as increased levels of transferase activity.

Thus, we showed that during colonization, some genes required for carnitine metabolism were expressed at higher levels in efg1" null mutant cells than in WT cells. Additionally, the efg1" null mutant strain grew to a higher density than the WT on non-fermentable carbon sources and in the gut. The mutant also grew on depleted medium, while the WT strain required the addition of carnitine in order to grow. Therefore, higher expression of carnitine metabolism genes likely contributes to the enhanced growth capabilities of the efg1" null mutant.

**Overexpression of SOD5 increases the fitness of the efg1" null mutant.** The results of our microarray analysis demonstrated that multiple genes involved in protection against host defenses (e.g., SOD5, CAT1, and DDR48) were regulated by Efg1p during colonization of the GI tract. One of these, SOD5, was among the most highly upregulated genes when expression by WT *C. albicans* in the GI tract was compared to expression in laboratory culture. Sod5p is involved in detoxification of reactive oxygen species (ROS) produced by host cells, such as macrophages and neutrophils (63, 64). Neutropenia is a major risk factor for disseminated candidiasis (9, 81), arguing that production of ROS by neutrophils is an important component of the host immune response to *C. albicans*. During colonization of the GI tract, *C. albicans* upregulated several ROS defense genes, including SOD5, in an Efg1p-dependent manner. Therefore, reduced expression of these genes may contribute to the observed hypersusceptibility of the efg1" null mutant to the host immune response during colonization (35).

To test this hypothesis, SOD5 was ectopically expressed from the ACT1 promoter and the construct was integrated into the genome of an efg1" null mutant strain (efg1" ACT1pr-SOD5 strain). Expression of SOD5 from the ACT1 promoter was not observed to alter morphology or culture density during post-exponential phase in laboratory medium.

To determine the effect of ectopic expression of SOD5 in the efg1" null mutant, cells of the efg1" ACT1pr-SOD5 strain were mixed with cells of the efg1" null mutant strain (without ectopic expression of SOD5) or with cells of a WT strain and inoculated by oral gavage into BALB/c mice. The efg1" null and WT strains carried nourseothricin resistance. Fecal pellets were collected and plated; the numbers of nourseothricin-resistant and -sensitive colonies were measured. The competitive index (CI) (defined as the ratio of sensitive CFU and resistant CFU divided by their ratio in the inoculum) was measured in fecal pellets at several time points. When the efg1" ACT1pr-SOD5 strain was competed against the efg1" null mutant, both strains competed equally at
Ectopic expression of SOD5 increases the fitness of the efg1 null mutant during colonization. Competition experiments were done in BALB/c mice to determine the effect of overexpression of SOD5 in the absence of EFG1 during colonization. Strains were inoculated at a 1:1 ratio, and nourseothricin (Nou) resistance was used to differentiate between strains. Competitions included the following: efg1– Nou'/WT Nou', efg1– ACT1pr-SOD5 Nou'/WT Nou', and efg1– ACT1pr-SOD5 Nou'/efg1– 'Nou'. The CI was determined as the ratio of the number of drug-sensitive colonies to the number of drug-resistant colonies, normalized to the input ratio. Each symbol represents an individual mouse; bars are the geometric means. (A) CI in fecal pellet samples at days 1, 6, and 18 postinoculation. SC, stomach contents; CW, cecum wall. *, P < 0.05; **, P < 0.01; ***, P < 0.0001 (Mann-Whitney test comparing efg1– ACT1pr-SOD5/WT and efg1– ACT1pr-SOD5/efg1– competitions).

DISCUSSION

Using microarray analysis, we have shown that EFG1 is a major regulator of gene expression in C. albicans cells that are colonizing the gastrointestinal tract. Efg1p is also required for normal GI colonization dynamics (35). Therefore, in addition to its known role in pathogenesis, EFG1 plays a key role in commensal colonization. By regulating genes involved in both commensal and pathogenic growth of C. albicans, Efg1p is well positioned to play a role in the transition between the two lifestyles and is thus a critical regulator of host-C. albicans interactions.

Relative to laboratory-grown cells, cells colonizing the GI tract showed altered expression of metabolic genes. Gene expression in the ileum consistent with increased acetyl-CoA metabolism was noted in colonizing cells. Increased acetyl-CoA metabolism would allow cells to utilize nonfermentable carbon sources, thus enhancing GI tract colonization. Cells lacking Efg1p showed increased transcription of genes involved in carnitine biosynthesis and utilization. These observations suggest that during colonization, efg1– null mutant cells are better able to utilize carnitine to metabolize nonfermentable carbon sources than WT C. albicans. In support of this hypothesis, we observed that the efg1– null mutant was able to grow to a higher density than WT C. albicans during growth on glucose, and it grew to an even higher relative density on nonfermentable carbon sources, such as ethanol and acetate. Under nutrient-limiting conditions (depleted medium), the efg1– null mutant was able to grow, while WT cells could grow only if carnitine was added to the depleted medium. Growth of the efg1– null mutant without additional carnitine suggests that the mutant is better able to utilize carbon sources available in the depleted medium than WT C. albicans, consistent with the gene expression patterns in colonizing cells.

Since altered carnitine metabolism appears to enhance the growth of the efg1– null mutant strain, it may influence colonization of the GI tract. Commensal bacteria, such as Bacteroides, are known to produce a majority of the metabolic by-products present in the GI tract, specifically short-chain fatty acids such as acetate and propionate (41, 82, 83). Therefore, short-chain fatty acids would be abundant carbon sources present in the cecum for C. albicans to utilize. Increased utilization of these nonfermentable carbon sources would thus promote growth of the efg1– null mu-
tant to higher levels than WT *C. albicans* in this environment. Indeed, the hypercolonization of the *efg1*− null mutant is first observed in organs of the distal GI tract such as the cecum, potentially due to high levels of short-chain fatty acids in this environment. Therefore, altered metabolism of the *efg1*− null mutant, particularly carnitine utilization, likely contributes to the hypercolonization of this mutant in the GI tract.

*SOD5* was one of the genes most highly upregulated by WT *C. albicans* during colonization, and its expression decreased in the absence of Efg1p. *SOD5*, encoding a superoxide dismutase required for the detoxification of reactive oxygen species (ROS) produced by host immune cells, is required for virulence in systemic infections (63, 64). In a competition experiment, an *efg1*− null mutant overexpressing *SOD5* colonized at higher levels than an *efg1*− null mutant strain without *SOD5* expression, at later times postinoculation (Fig. 7). When mice deficient in the production of ROS were orally inoculated with *C. albicans*, both the *efg1*− null mutant and WT cells killed mice with equal efficiency (84). This observation suggests that a major defect of the *efg1*− null mutant strain is its sensitivity to ROS production by the host.

We observed that in a healthy host, the *efg1*− null mutant was more sensitive than WT *C. albicans* to the host immune response that develops over time during colonization (35). In contrast, in an immunocompromised host, the *efg1*− null mutant strain was able to maintain high levels of colonization (35). During colonization, neutrophils recruited to the gut produce ROS as an antimicrobial defense in addition to phagocytosis. Thus, ROS production is a major part of the host immune response to *C. albicans* during both colonization and systemic infections.

Coregulation of metabolism and host interactions by the EFG1 transcription factor allows *C. albicans* to choose between two survival strategies: increased growth or protection from the host immune response. In a healthy host, *C. albicans* limits its growth (15) and expresses genes that will protect it against the host immune system. Overgrowth of *C. albicans* may indicate a change toward increased pathogenesis in a colonizing population. Changes in Efg1p activity under different conditions cause global changes in gene expression and change host-fungus interactions. High Efg1p activity promotes the expression of genes involved in host defense, while low Efg1p activity derepresses metabolic gene expression, leading to increased colonization. Consistent with this, Efg1p expression increases over time during GI tract colonization of a healthy host but remains low in an immunocompromised host (35). Phenotypic variation of Efg1p activity during colonization may produce subpopulations of cells with different characteristics, enabling host-dependent shaping of the colonizing population and altered expression of genes involved in metabolism and host defense.

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