Transcriptional Regulators Cph1p and Efg1p Mediate Activation of the Candida albicans Virulence Gene SAP5 during Infection

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The opportunistic fungal pathogen Candida albicans can cause superficial as well as systemic infections. Successful adaptation to the different host niches encountered during infection requires coordinated expression of various virulence traits, including the switch between yeast and hyphal growth forms and secretion of aspartic proteinases. Using an in vivo expression technology that is based on genetic recombination as a reporter of gene activation during experimental candidiasis in mice, we investigated whether two signal transduction pathways controlling hyphal growth, a mitogen-activated protein kinase cascade ending in the transcriptional activator Cph1p and a cyclic AMP-dependent regulatory pathway that involves the transcription factor Efg1p, also control expression of the SAP5 gene, which encodes one of the secreted aspartic proteinases and is induced by host signals soon after infection. Our results show that both transcriptional regulators are important for SAP5 activation in vivo. SAP5 expression was reduced in a cph1 mutant, although filamentous growth in infected tissue was not detectably impaired. SAP5 expression was also reduced, but not eliminated, in an efg1 null mutant, although this strain grew exclusively in the yeast form in infected tissue, demonstrating that in contrast to in vitro conditions, SAP5 activation during infection does not depend on growth of C. albicans in the hyphal form. In a cph1 efg1 double mutant, however, SAP5 expression in infected mice was almost completely eliminated, suggesting that the two signal transduction pathways are important for SAP5 expression in vivo. The avirulence of the cph1 efg1 mutant seemed to be caused not only by the inability to form hyphae but also by a loss of expression of additional virulence genes in the host.

The yeast Candida albicans is a member of the microflora on mucosal surfaces of healthy people, but it can also cause superficial and life-threatening systemic infections, especially in immunocompromised patients. The success of C. albicans both as a colonizer and as an infectious microorganism probably depends on many different characteristics of the fungus, including its ability to adhere to a variety of host tissues, its ability to switch between yeast and hyphal growth forms, and its secretion of hydrolytic enzymes, such as proteinases and phospholipases (3, 5, 24). Since C. albicans encounters different body niches during an infection, a flexible reaction to different environmental conditions is a prerequisite for optimal adaptation to the host at different infection stages. For example, the switch from yeast growth to hyphal growth may facilitate tissue invasion and evasion of macrophages, and the generation of yeasts budding off from the hyphae may allow more efficient spread through the bloodstream during disseminated infection and multiplication in infected tissues. Accordingly, mutants restricted to either the yeast form or the hyphal form are attenuated in terms of virulence (2, 18). The secreted aspartic proteinases (Saps) are also necessary for full virulence of C. albicans (6, 10, 14, 28). Different roles have been suggested for the Saps based on in vitro experiments; these roles include nitrogen supply (30), adherence (33), degradation of host barriers (4, 23), and evasion of host defense mechanisms (11, 25). C. albicans has a large gene family encoding Saps (21), and it is likely that the individual Sap isoenzymes evolved for optimal adaptation to specific functions or host niches. In fact, by using an in vivo expression technology (IVET) that is based on genetic recombination as a reporter of gene expression and allows detection of gene activation in single cells, we could demonstrate that individual SAP genes were differentially activated during infection depending on the host niche and the infection stage (31).

It is likely that expression of different virulence traits has to be coordinated, and common signal transduction pathways may be used to ensure induction of an appropriate set of virulence genes in response to environmental signals. A mitogen-activated protein kinase cascade ending in the transcription factor Cph1p and a cyclic AMP-dependent signaling pathway ending in the transcriptional regulator Efg1p are both necessary for induction of filamentous growth in C. albicans, depending on the conditions used (17, 18). Expression of some of the SAP genes, SAP4 to SAP6, in vitro has been linked to the hyphal form of C. albicans (9, 34). This linkage was confirmed by the finding that in contrast to expression in the wild-type parent strain, expression of SAP4 to SAP6 was not detectable in an efg1 mutant in vitro under conditions in which the strain failed to form hyphae (29). However, recent studies have dem-
onstrated that the pattern of expression of virulence genes in pathogenic microorganisms in an infected host can be totally different from the expression pattern observed in vitro, including the dependence on regulatory factors (15, 16). Indeed, when IVET was used to study expression of the \textit{SAP} genes during infection, we observed that the \textit{SAP5} gene, one of the putative hypha-specific genes, was induced very soon after contact with the host, at a time when no hyphae were detected in the infected animals (31). We suggested that expression of \textit{SAP5} during infection might be activated by signals that also induce hyphal growth, possibly involving the same signal transduction pathways, but might be independent of the hyphal morphology itself. Therefore, in the present study we investigated whether the transcriptional regulators \textit{CPH1} and \textit{EFG1}, representing the two best-studied signaling pathways regulating hyphal formation in \textit{C. albicans} (7, 35), were also necessary for \textit{SAP5} activation under experimental infection conditions. The \textit{SAP5} gene was chosen for this analysis because activation of this gene was observed in a large proportion of infecting cells and a negative influence of a regulatory mutation on \textit{SAP5} expression in vivo would be detected most easily (31). In addition, an \textit{efg1} mutation strongly attenuates virulence and prevents tissue invasion, so that certain host niches that induce activation of specific virulence genes are not accessible to an \textit{efg1} mutant (18). Since the \textit{SAP5} gene is activated very soon after infection, it was possible to study a possible effect of regulatory mutations on gene expression in a body location that is reached by both wild-type and mutant strains (i.e., under comparable in vivo conditions).

**MATERIALS AND METHODS**

**Strains and growth media.** The \textit{C. albicans} strains used in this study are listed in Table 1. The strains were maintained on minimal agar (6.7 g of yeast nitrogen base without amino acids [Bio 101, Vista, Calif.] per liter, 20 g of glucose per liter, 0.77 g of complete supplement medium [Bio 101] per liter, 15 g of agar per liter). For routine growth of the strains, YPD liquid medium (10 g of yeast extract per liter, 20 g of peptone per liter, 20 g of glucose per liter) was used. For \textit{SAP5} promoter activity studies, cells were grown overnight in YCB-BSA (23.4 g of yeast carbon base per liter, 4 g of bovine serum albumin per liter; pH 4.0).

**TABLE 1. \textit{C. albicans} strains used in this study**

| Strain(s) | Parent | Genotype* | Reference |
|-----------|--------|-----------|-----------|
| CAI4      | CAI4   | \(\Delta u r a 3: i m m 4 3 4:: u r a 3: i m m 4 3 4\) | 8 |
| CFI1      | CAI4   | \(A C T 1 / a c t 1:: F R T - M P A ^ { - } - F R T \) | 32 |
| S2F15B    | CFI1   | \(s a p 2 - 1:: P _ { S A P 2 } - e c a F L P - U R A 3 / S A P 2 - 2\) | 31 |
| SSF12A    | CFI1   | \(s a p 5 - 1:: P _ { S A P 5 } - e c a F L P - U R A 3 / S A P 5 - 2\) | 31 |
| SSF12B    | CFI1   | \(S A P 5 - 1 / s a p 5 - 2:: P _ { S A P 5 } - e c a F L P - U R A 3\) | 31 |
| JKC18     | CAI4   | \(c p h 1:: hi s G / c p h 1:: hi s G\) | 17 |
| CFI2      | JKC18  | \(A C T 1 / a c t 1:: F R T - M P A ^ { - } - F R T \) | This study |
| C2SF1A and C2SF1B | CFI2 | \(s a p 2 - 1:: P _ { S A P 2 } - e c a F L P - U R A 3 / S A P 2 - 2\) | This study |
| C2SF1A and C2SF1B | CFI2 | \(s a p 5 - 1:: P _ { S A P 5 } - e c a F L P - U R A 3 / S A P 5 - 2\) | This study |
| HLC67     | CAI4   | \(e f g 1:: h i s G / e f g 1:: h i s G\) | 18 |
| CFI3      | HLC67  | \(A C T 1 / a c t 1:: F R T - M P A ^ { - } - F R T \) | This study |
| C3SF1D and C3SF1E | CFI3 | \(s a p 2 - 1:: P _ { S A P 2 } - e c a F L P - U R A 3 / S A P 2 - 1\) | This study |
| C3SF1A    | CFI3   | \(S A P 5 - 1 / s a p 5 - 2:: P _ { S A P 5 } - e c a F L P - U R A 3\) | This study |
| C3SF1B    | CFI3   | \(s a p 5 - 1:: P _ { S A P 5 } - e c a F L P - U R A 3 / S A P 5 - 2\) | This study |
| HLC69     | CAI4   | \(c p h 1:: h i s G / c p h 1:: h i s G / e f g 1:: h i s G / e f g 1:: h i s G\) | 18 |
| CFI4      | HLC69  | \(A C T 1 / a c t 1:: F R T - M P A ^ { - } - F R T \) | This study |
| C4SF1B and C4SF1C | CFI4 | \(s a p 2 - 1:: P _ { S A P 2 } - e c a F L P - U R A 3 / S A P 2 - 2\) | This study |
| C4SF1A    | CFI4   | \(S A P 5 - 1 / s a p 5 - 2:: P _ { S A P 5 } - e c a F L P - U R A 3\) | This study |
| C4SF1B    | CFI4   | \(s a p 5 - 1:: P _ { S A P 5 } - e c a F L P - U R A 3 / S A P 5 - 2\) | This study |

* Apart from the features indicated all strains are identical to their parents.

Screening for mycophenolic acid (MPA)-sensitive colonies was performed after 2 days of growth at 30°C on minimal agar containing 1 µg of MPA ml⁻¹, which resulted in generation of large MPA⁻ and small MPA⁺ colonies (32). Uridine (100 µg ml⁻¹) was added to the media to support growth of \(\Delta u r a 3\) mutant strains.

**\textit{C. albicans} transformation.** \textit{C. albicans} strains were transformed by electroporation (13) with the following gel-purified linear DNA fragments: a \textit{SacI}-\textit{SacI} fragment from pAFI3 (32) containing the \textit{FRT-MPA⁻-FRT} cassette between flanking \textit{ACT1} sequences (Fig. 1A), an \textit{XbaI}-\textit{SacI} fragment from pSFL53 (31) containing a \(P_{S A P 5}-e c a F L P\) reporter gene fusion (Fig. 2A), and an \textit{XbaI}-\textit{SacI} fragment from pSFL213 (31) containing a \(P_{S A P 5}-e c a F L P\) reporter gene fusion

**FIG. 1.** Integration of the deletable \textit{FRT-MPA⁻-FRT} cassette into one of the \textit{ACT1} alleles of strains JKC18 (\(c p h 1 / c p h 1\)), HLC67 (\(e f g 1 / e f g 1\)), and HLC69 (\(c p h 1 / c p h 1\) \(e f g 1 / e f g 1\)). (A) Integration scheme. The \textit{ACT1} coding region is represented by the open arrow, and the \textit{MPA⁻} marker is represented by the grey arrow. The 34-bp \textit{FRT} site is not drawn to scale. The probe used for verification of correct integration by Southern hybridization is represented by the black bar, and the diagnostic \textit{BglII} sites are shown. Bg, \textit{BglII}; ScI, \textit{SacI}. (B) Southern hybridization of \textit{BglII}-digested genomic DNA of parent strains and their transformants carrying the \textit{FRT-MPA⁻-FRT} cassette with an \textit{ACT1}-specific probe. The positions of the fragments are indicated on the right, and molecular sizes (in kilobases) are indicated on the left. Lane 1, CAI4; lane 2, CFI1; lane 3, JKC18; lane 4, CFI2; lane 5, HLC67; lane 6, CFI3; lane 7, HLC69; lane 8, CFI4.
For the in vivo experiments, 8- to 12-week-old female BALB/c mice (Harlan, Borchen, Germany) were used. To prepare the inoculum, C. albicans yeast cells grown overnight in YPD broth at 30 °C were washed twice in phosphate-buffered saline (PBS) (Gibco, Karlsruhe, Germany) and resuspended in the same buffer. The phenotype of injected cells was controlled by spreading the inocula on MPA indicator plates. The percentage of MPA-sensitive cells was always less than 1%.

Mice were each infected intraperitoneally with 1 × 10⁷ blastoconidia in 1.0 ml of PBS. At 30 min postinfection C. albicans cells were recovered by peritoneal lavage with 10 ml of PBS. Cells that adhered to the liver surface at 4 h but had not yet invaded (as determined by microscopic examination) were recovered after the peritoneal cavity was first washed to remove nonadherent cells. The organ was then cut out and homogenized with Tenbroeck tissue grinders (Wheaton Scientific) in 10 ml of sterile distilled water. After 24 and 48 h cells that adhered to or had invaded the liver were recovered in the same way. Aliquots of the lavages and the homogenates were spread on indicator plates to determine the percentage of MPA-sensitive cells.

**Histology and alanine aminotransferase (ALT) activity determination.** For histological examination, organs were dissected, and blocks of tissue were fixed in a 10% formaldehyde solution in PBS. The tissue samples were further processed by using standard methods for paraffin embedding and cutting. Five-micrometer sections of the organs were cut. To stain the C. albicans cells, the periodic acid-Schiff reaction was used. The tissue sections were incubated in a 1% solution of periodic acid in distilled water for 5 min, and this was followed by washing with distilled water and incubation with Schiff reagent (Sigma), which consisted of 1% (wt/vol) pararosaniline HCl and 4% (wt/vol) sodium bisulfite in hydrochloric acid (0.25 mol/liter). The periodic acid-Schiff reagent reaction mixture was developed in tap water for 10 min. The tissue sections were counterstained with hematoxylin and eosin (H&E) or with the periodic acid-Schiff reaction for 1 h.
stained with Mayer's hemalum solution (Merck) for 10 s, dehydrated, and embedded in DePeX (Serva, Heidelberg, Germany).

ALT activity, an indicator of liver damage in infected mice, was determined as described previously (14).

RESULTS

Construction of *C. albicans* reporter strains in *cph1* and *efg1* mutant backgrounds. So that we could use IVET to analyze the influence of *CPH1* and *EFG1* on virulence gene expression during infection, we integrated the deletable MPA resistance marker, flanked by direct repeats of the minimal FLP recombination target (FRT), into the genome of the *cph1* mutant JKCI8, the *efg1* mutant HLC67, and the *cph1 efg1* double mutant HLC69 (Fig. 1A). Southern hybridization analysis demonstrated that the resulting MPA-resistant transformants, CFI2 (Fig. 1B, lane 4), CFI3 (lane 6), and CFI4 (lane 8), had the deletable marker integrated into one of the *ACT1* alleles in the same way as in previously described strain CFI1 (lane 2), as shown by the appearance of an expected 3.5-kb fragment in addition to the 3.9-kb wild-type fragment (lanes 1 to 8) hybridizing with an *ACT1*-specific probe.

A fusion of the *ecaFLP* reporter gene to the *SAP5* promoter (P~SAP5~) was then integrated into the various signal transduction mutants carrying the deletable MPA* marker (Fig. 2A). The two *SAP5* alleles in strain CAI4 and its derivatives can be distinguished by a *BglII* restriction site polymorphism. As previously shown for strains S5F12A and S5F12B (Fig. 2B, lanes 3 and 4) carrying the P~SAP5~-*ecaFLP* fusion in a wild-type background, derivatives of the *efg1* mutant CFI3 and the *cph1 efg1* double mutant CFI4 were isolated in which the reporter gene fusion was integrated into one of the two possible *SAP5* alleles (strains C3S5F1A and C3S5F1B [lanes 11 and 12] and strains C4S5F1A and C4S5F1B [lanes 15 and 16]). All of the transformants of strain CFI2 tested had the reporter gene fusion integrated into the *SAP5*-1 allele; however, the original wild-type fragment did not disappear in these transformants, as was expected after allelic replacement (lanes 7 and 8). Close examination of the Southern blot revealed that the relative signal intensity of the band corresponding to the *SAP5*-1 allele was stronger in strain JKCI8 (lane 5) and its derivative, CFI2 (lane 6), than in the other parent strains, CAI4 (lane 1), HLC67 (lane 9), and HLC69 (lane 13), and their derivatives, CFI1 (lane 2), CFI3 (lane 10), and CFI4 (lane 14). This suggests that duplication of the *SAP5*-1 allele had occurred in our copy of strain JKCI8 and was maintained in all of its derivatives. Two independent transformants carrying the P~SAP5~-*ecaFLP* fusion integrated into one of the duplicated *SAP5*-1 alleles in the *cph1* mutant background, strains C2S5F1A and C2S5F1B (lanes 7 and 8), were used for further analysis.

To control for a possible effect of the *cph1* and *efg1* mutations on FLP activity, a P~SAP2~-*ecaFLP* fusion was also integrated into strains CFI2, CFI3, and CFI4 (Fig. 3A), since the *SAP2* promoter could be induced by growth of the cells in YCB-BSA. The two *SAP2* alleles in strain CAI4 and its derivatives could be distinguished by *ClaI* restriction site polymorphism. Wild-type control strain S2F15B contained the P~SAP2~-*ecaFLP* fusion integrated into the *SAP2*-1 allele (Fig. 3B, lane 3). To ensure optimal comparability, the reporter fusion was integrated into the same *SAP2* allele in two independent trans-
The complex environments encountered by pathogens within their host during an infection can influence gene expression patterns in a way that is very different from the organism’s response to the simpler parameters that regulate gene activity in vitro (19). In vivo, the induction of virulence genes can depend on regulatory factors which are not required for expression in vitro, and conversely, the dependence of virulence gene expression on specific regulators in vitro may be bypassed in the host by the use of alternative signaling pathways, illustrating the importance of studying regulation of virulence genes in the context of bona fide host-pathogen interactions (15, 16).

The recombination-based IVET detects expression of a target gene whenever the promoter is sufficiently activated in a cell to result in recombinase-mediated marker excision. As in a previous study (31), expression of the SAP5 gene was observed only in a subpopulation of cells recovered from infected tissue. The heterogeneity in expression status can be explained in several ways. Both yeasts and hyphae were present in the infected host, and the different morphological forms probably differed with respect to gene expression. It is also likely that fungal cells reisolated from whole organs in fact inhabited many different microniches, only some of which may have induced SAP5 expression. Apart from these factors, it can be assumed that individual cells in a given population differ in the activation status of genes even in the same environment, so that in some cells the promoter activity is below the threshold that is necessary to detect gene expression by IVET. As long as no differences are observed in the infection progress of two strains that are compared, a reduction in the number of cells in which a target gene is detectably activated can be assumed to be caused by reduced promoter activity in the population. This should have been the case for the cph1 mutants at all infection stages which we investigated, since we did not observe major differences in the capacities of these strains to infect internal organs in our experimental model system compared with the wild-type controls, in agreement with observations made by other workers (18). In contrast, the efg1 and cph1 efg1 mutants were unable to form hyphae and invade the internal organs. Especially at the late infection stages, when wild-type cells had invaded deep tissue, the different host niches in which wild-type and mutant cells were located, and not the defective signal transduction pathways alone, contributed to differences in gene expression. Nevertheless, at the early infection stages the mutants had access to the same host niches as the wild-type strains (i.e., the peritoneal cavity and the surfaces of the parenchymatous organs). Since in the wild-type strains SAP5 activation was clearly detectable in these host niches, the failure of the mutants to induce SAP5 at a significant level indicates the importance of EFG1 for SAP5 expression at this infection stage. At later times, even in the absence of hyphal formation and tissue invasion, reduced but significant SAP5 activation was observed also with the efg1 single mutants, which may have been induced by changes in the host environment caused by the infecting C. albicans cells. It seems that this activation was mediated by the Cph1p transcription factor, because almost no SAP5 induction was detected in the cph1 efg1 double mutants, indicating the

In contrast, the negative effect of cph1 inactivation on SAP5 expression was less pronounced than the effect of the efg1 mutation but was nevertheless clearly detectable at all infection stages (17.3% ± 3.3%, 17.5% ± 2.6%, and 18.9% ± 6.9% MPA lines cells after 4, 24, and 48 h, respectively), although the invasiveness of the cph1 mutant was comparable to that of the wild type. In the absence of both CPH1 and EFG1, SAP5 expression was almost completely eliminated, since very few MPA-sensitive cells were recovered at all times investigated (1.1% ± 0.6%, 4.5% ± 2.1%, and 1.9% ± 0.8% MPA lines cells after 4, 24, and 48 h, respectively). In each genetic background, the two independently constructed reporter strains gave comparable results. Together, these results demonstrated that SAP5 activation can occur in the absence of CPH1 or EFG1 and independent of hyphal growth, but both transcriptional regulators are necessary for full SAP5 activation in the host.
importance of the two signal transduction pathways for SAP5 activation in an infected host. Interestingly, Efg1p also seems to negatively regulate expression of some genes directly or indirectly, since expression of the SAP2 gene was derepressed in all strains lacking a functional EFG1 gene.

Transcripts hybridizing with a probe specific for the SAP4 to SAP6 genes have also been detected during hyphal growth of C. albicans in vitro (9, 29). Because of the high levels of homology of the three genes, Northern hybridization did not reveal whether all three genes or only one or two of them were expressed under these conditions. In contrast, using antisera that recognize this subgroup of Saps, Borg-von Zepelin et al. observed expression of SAP4 to SAP6 only after phagocytosis by murine peritoneal macrophages and not in hyphae alone (1). We tried to detect SAP5 expression in vitro by incubating the strains containing the P_{SAP5}::caFLP reporter fusion in a wild-type background in RPMI medium containing serum at 37°C, conditions that favor hyphal growth of C. albicans. Although germ tube formation and formation of hyphae were induced very efficiently, SAP5 expression was hardly detected, since very few cells became MPA sensitive (data not shown). This result was confirmed with strains carrying a different reporter gene, GFP, under the control of the SAP5 promoter. The fluorescence of the cells was hardly above the background level (unpublished observations), although like FLP, GFP has been demonstrated to be a useful reporter of SAP2 expression under SAP2-inducing conditions (22). Therefore, the transcripts detected by other workers during hyphal growth either may correspond to SAP4 and/or SAP6 but not SAP5 or SAP5 expression under hyphal formation conditions in vitro is below the detection limit of our reporter systems. These observations, which are in agreement with those of Borg-von Zepelin et al. (1), suggest that SAP5 activation during infection is much stronger than the possible low-level expression under hypha-inducing conditions in vitro.

An issue to be considered when our IVET is used is that some of the colonies obtained after cells recovered from infected tissue are plated may be derived from more than one cell. If only one of the cells contains the MPA marker, this gives rise to an MPA colony, so the actual percentage of MPA cells is underestimated. Microscopic analysis demonstrated that there was no clumping of cells that might be responsible for the reduced percentage of MPA colonies of the efg1 and cph1 efg1 mutants. However, since the wild-type strains and the cph1 single mutants form hyphae in infected tissue, many colonies of these strains are derived from mycelial fragments, which can consist of several unseparated cells. Therefore, the percentages of MPA cells of these strains may in fact have been somewhat higher than the observed percentages of MPA colonies, and the difference between the wild type and the cph1 and cph1 efg1 mutants may have been even more pronounced. However, this would only emphasize our conclusion that full activation of the SAP5 gene during infection depends on CPH1 and EFG1.

We observed that our copy of cph1 mutant JKC18 contained a duplication of one of the SAP5 alleles. In addition, a change from heterozygosity to homozygosity for the SAP2 gene occurred during the construction of strain CF13, which was derived from efg1 mutant HLC67. Such changes may be induced by genetic manipulation of strains (transformation, fluoroorotic acid selection) but may also occur due to natural genomic alterations (26, 27). These changes were detected only later when the reporter fusions were introduced into the loci and only because we made efforts to distinguish between the two alleles of the target locus. Nonspecific genomic alterations in derivatives of a parent strain may therefore be more common than suggested by the usually limited analyses of genetically engineered strains. However, we do not think that the additional copy of SAP5 or the homozygosity for SAP2 influenced our results for SAP5 regulation by CPH1 and EFG1, since no such alterations were observed in the reporter strains derived from the cph1 efg1 double mutant. The contribution of both
Although the ability to grow in the hyphal form is certainly but also by the loss of expression of additional virulence genes. It seems to be caused not only by the non-completely eliminated in vivo, at least in the infection model used in this study. Therefore, the avirulence of the strains JKC18, HLC67, and HLC69.

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REFERENCES

1. Borg-von Zepelin, M., S. Beggh, K. Boggian, D. Sanglard, and M. Monod. 1998. The expression of the secreted aspartyl proteinases Sap1 to Sap6 from Candida albicans in murine macrophages. Mol. Microbiol. 28:543–554.

2. Braun, B. R., W. S. Head, M. X. Wang, and A. D. Johnson. 1999. Evidence for degradation of gastrointestinal mucin by Candida albicans –efg1 mutants demonstrate that SAP5 can be expressed in the absence of hyphal formation in vivo, although SAP5 activation occurred at a reduced level in these strains due to the involvement of EFG1 in SAP5 induction. In the absence of both transcription factors, SAP5 expression was almost completely eliminated in vivo, at least in the infection model used in this study. Therefore, the avirulence of the cph1 efg1 mutant seems to be caused not only by the nonfilamentous phenotype but also by the loss of expression of additional virulence genes. Although the ability to grow in the hyphal form is certainly important for the virulence of C. albicans, it will be difficult to assess the contribution of morphology per se since many other cellular characteristics change upon the yeast-hypha transition (12). These observations illustrate the complex regulatory networks that control and coordinate virulence gene expression in C. albicans within its host.

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The acquisition of a chromosomally integrated, single-copy GFP gene in Candida albicans, and its use as a reporter of gene regulation. Mol. Gen. Genet. 257:412–420.

Morschhäuser, J., R. Virlka, T. K. Kohrhoen, and J. Hacker. 1997. Degradation of human subendothelial extracellular matrix by proteinase-secreting Candida albicans. FEMS Microbiol. Lett. 153:349–355.

Navarro-Garcia, F., M. Sanchez, C. Nombela, and J. Pla. 2001. Virulence genes in the pathogenic yeast Candida albicans. FEMS Microbiol. Rev. 25:245–268.

Rüchel, R. 1986. Cleavage of immunoglobulins by pathogenic yeasts of the genus Candida. Microbiol. Sci. 3:316–319.

Rustchenko, E. P., D. H. Howard, and F. Sherman. 1994. Chromosomal alterations of Candida albicans are associated with the gain and loss of assimilating functions. J. Bacteriol. 176:3231–3241.

Rustchenko, E. P., D. H. Howard, and F. Sherman. 1997. Variation in assimilating functions occurs in spontaneous Candida albicans mutants having chromosomal alterations. Microbiology 143:1765–1778.

Sanglard, D., B. Hube, M. Monod, F. C. Odds, and N. A. Gow. 1997. A triple deletion of the secreted aspartyl proteinase genes SAP4, SAP5, and SAP6 of Candida albicans causes attenuated virulence. Infect. Immun. 65:3539–3546.

Schürholz, K., K. Sprünger, M. Köhler, M. Whiteley, D. V. Thomas, and C. Csink. 2000. Repression of hyphal proteinase expression by the mitogen-activated protein (MAP) kinase phosphatase Cpp1 of Candida albicans is independent of the MAP kinase Cek1p. Infect. Immun. 68:7159–7161.

Staib, F. 1965. Serum-proteins as nitrogen source for yeastlike fungi. Sabouraudia 4:187–193.

Staib, P., M. Kretschmar, T. Nöth, H. Hof, and J. Morschhäuser. 2000. Differential activation of a Candida albicans virulence gene family during infection. Proc. Natl. Acad. Sci. USA 97:6102–6107.

Staib, P., M. Kretschmar, T. Nöth, G. Köhler, M. Hoch, J. Hacker, and J. Morschhäuser. 1999. Host-induced, stage-specific virulence gene activation in Candida albicans during infection. Mol. Microbiol. 32:333–346.

Watts, H. J., F. S. Cheah, B. Hube, D. Sanglard, and N. A. Gow. 1998. Altered adherence in strains of Candida albicans harbouring null mutations in secreted aspartic proteinase genes. FEMS Microbiol. Lett. 159:129–135.

White, T. C., and N. Agabian. 1995. Candida albicans secreted aspartyl proteinases: isoenzyme pattern is determined by cell type, and levels are determined by environmental factors. J. Bacteriol. 177:5215–5221.

Whiteway, M. 2000. Transcriptional control of cell type and morphogenesis in Candida albicans. Curr. Opin. Microbiol. 3:582–588.