Fig1 facilitates calcium influx and localizes to membranes destined to undergo fusion during mating in Candida albicans

Meng Yang  
*Washington University School of Medicine in St. Louis*

Alexandra Brand  
*University of Aberdeen*

Thyagarajan Srikantha  
*University of Iowa*

Karla J. Daniels  
*University of Iowa*

David R. Soll  
*University of Iowa*

*See next page for additional authors*

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Fig1 Facilitates Calcium Influx and Localizes to Membranes Destined To Undergo Fusion during Mating in Candida albicans

Meng Yang, Alexandra Brand, Thyagarajan Srikantha, Karla J. Daniels, David R. Soll, and Neil A. R. Gow
Aberdeen Fungal Group, School of Life Sciences & Medicine, Institute of Medical Science, University of Aberdeen, Foresterhill, Aberdeen AB25 2ZD, United Kingdom, and Department of Biology, University of Iowa, Iowa City, Iowa 52242

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Few mating-regulated genes have been characterized in Candida albicans. C. albicans FIG1 (CaFIG1) is a fungus-specific and mating-induced gene encoding a putative 4-transmembrane domain protein that shares sequence similarities with members of the claudin superfamily. In Saccharomyces cerevisiae, Fig1 is required for shmoo fusion and is upregulated in response to mating pheromones. Expression of CaFIG1 was also strongly activated in the presence of cells of the opposite mating type. CaFig1-green fluorescent protein (GFP) was visible only during the mating response, when it localized predominantly to the plasma membrane and perinuclear zone in mating projections and daughter cells. At the plasma membrane, CaFig1-GFP was visualized as discontinuous zones, but the distribution of perinuclear CaFig1-GFP was homogeneous. Exposure to pheromone induced a 5-fold increase in Ca\(^{2+}\) uptake in mating-competent opaque cells. Uptake was reduced substantially in the fig1Δ null mutant. CaFig1 is therefore involved in Ca\(^{2+}\) influx and localizes to membranes that are destined to undergo fusion during mating.

Candida albicans is part of the normal microflora in the gastrointestinal tract and is also a clinically important opportunistic pathogen in humans. A parasexual mating pathway in this obligately diploid fungus has been investigated intensively over the last 10 years, and the studies have shown similarities to and distinct differences from the orthologous mating process in Saccharomyces cerevisiae. C. albicans mating may occur on or within the human body and has been shown to occur either through heterothallic or homothallic interactions (2, 16, 23, 28, 35, 36). Transcriptome studies showed that the mating response involved the upregulation of a specific set of genes via the transcription factor Cph1, which also regulates virulence factor expression and promotes filamentous growth. This suggests that the mating response in C. albicans may directly influence host-pathogen interactions as well as creating novel recombinant genotypes via genetic recombination (7, 55). Although the activity of pheromone-regulated transcription factors has been described, the function of their effectors has received less attention.

Unlike the case for mating in the haploid yeast S. cerevisiae, diploid C. albicans cells must first become homoygous at the mating-type locus (MTL), which promotes the switch from white yeast-shaped cells to mating-competent, bean-shaped opaque cells (22, 33, 36). The mechanism that leads to homozygosity in vivo is not well understood, but the genotype can be generated in vitro either by deletion of the MTL\(\alpha\) or MTL\(\alpha\) genes or by plating cells on \(L\)-sorbose, which causes loss of one copy of chromosome 5 bearing the MTL (24, 35). In vitro, MTL-homozygous cells arise predominantly by the loss of one chromosome 5 homolog, followed by duplication of the retained homolog (51). MTL-homozygous cells switch to the opaque form at high frequency to produce mating-competent, pheromone-secreting mating partners that form shmoo mating projections (34). In the process of shmoo formation, pheromone-dependent chemotropism can occur in a biofilm which stabilizes chemotropism gradients, hence, facilitating the directed growth of mating projections toward each other (16). Chemotropism is followed by fusion and the formation of a tetraploid daughter cell (6).

In S. cerevisiae, calcium influx is essential for cell survival and efficient fusion of gametes during the mating process (17). In C. albicans, directional growth responses depend on the influx of calcium ions through calcium channels in the plasma membrane (10, 12). Two calcium uptake systems in S. cerevisiae have been identified (12, 37, 38). The high-affinity calcium uptake system (HACS), comprising the Mdr1-Chcl complex, is activated in low-Ca\(^{2+}\) medium and in response to alkaline, cold, iron, and endoplasmic reticulum (ER) stress (8, 40, 48). A third protein, Fig1 (mating factor-induced gene 1), was identified as a component of the low-affinity calcium uptake system (LACS) that was activated during growth in rich medium (37). In C. albicans, deletion of calcium channel gene CCH1 or MID1 reduced Ca\(^{2+}\) uptake during vegetative growth, but deletion of FIG1 had no measurable effect on calcium ion transport in yeast or hyphal cells (12). However, Cafig1Δ null mutants had altered hyphal tropic responses, suggesting that CaFIG1 was expressed at low levels during vegetative growth (12). FIG1 expression was upregulated in both S. cerevisiae and C. albicans in response to mating pheromone (34, 38). In S. cerevisiae, FIG1 expression increased 5- to 7-fold in response to...
The role of Fig1 in mating pheromone and *S. cerevisiae* Fig1-green fluorescent protein (ScFig1-GFP) was distributed homogeneously in the plasma membrane of shmoo and to occasional reticular or punctate intracellular foci (38). Deletion of *FIG1* resulted in incomplete fusion between the tips of mating shmoo, which was thought to be due to the loss of a calcium-dependent membrane repair mechanism (1, 38). Transcription profiling in *C. albicans* suggested that Fig1 function could be likewise predominately associated with the mating process because white/opaque switching and exposure to mating pheromone caused a significant increase in *FIG1* gene expression (34).

There is no clear Fig1 homologue outside the fungal kingdom (17, 54), but the protein sequence contains characteristics found in the large mammalian MMP22/EMP/MP20/claudin superfamily (NCBI accession number pfam00822), the members of which selectively limit or promote paracellular ion flux across epithelial tight junctions and regulate the expression of proteins at the cell surface (44, 49). Sequence characteristics include four putative transmembrane domains and a conserved claudin motif [G(F)GxGx(C)n(C), where *Φ* is a hydrophobic amino acid and *n* is any number] in the large first extracellular loop (29, 45). Claudin-like proteins in fungi include Sur7 in *C. albicans* and Dnl1 in *Schizosaccharomyces pombe*. Sur7 is an eisosome protein involved in plasma membrane organization and Dnl1 in *S. cerevisiae* protein involved in plasma membrane organization and Dni1 in *Schizosaccharomyces pombe*.

**TABLE 1. Strains used in this study**

| Strain | Genotype | MTL | Reference(s) |
|--------|----------|-----|--------------|
| CAI4   | *ura3Δ* *iro1Δ*::imm434/*ura3Δ* *iro1Δ*::imm434 | a/α | 19, 20 |
| NGY152 | CAI4/Clp10, as CAI4, *RPS1*::pCIp10 | a/α | 11 |
| NGY372 | As CAI4, *fig1Δ*::dp200/*fig1Δ*::dp200/RPS1::pCIp10 | a/α | 12 |
| NGY493 | As CAI4, *FIG1*/*FIG1*-GFP | a/α | This study |
| NGY494 | As CAI4, *FIG1*/*FIG1*-GFP | a/α | This study |
| NGY495 | As CAI4, *FIG1*/*FIG1*-GFP | a/α | This study |
| NGY496 | As CAI4, *RPS1*::pCIp10::placpoly6-FIG1 | a/α | This study |
| NGY497 | As CAI4, *RPS1*::pCIp10::placpoly6-FIG1 | a/α | This study |
| NGY498 | As CAI4, *RPS1*::pCIp10::placpoly6-FIG1 | a/α | This study |
| NGY499 | CAI4 | a/– | This study |
| NGY500 | CAI4 | a/– | This study |
| NGY501 | As CAI4, *fig1Δ*::dp200/*fig1Δ*::dp200/RPS1::pCIp10 | a/α | This study |
| NGY502 | As CAI4, *fig1Δ*::dp200/*fig1Δ*::dp200/RPS1::pCIp10 | a/α | This study |
| NGY576 | As CAI4, *fig1Δ*::dp200/*FIG1*/*FIG1*-GFP (A) | a/α | This study |
| NGY577 | As CAI4, *fig1Δ*::dp200/*FIG1*/*FIG1*-GFP (B) | a/α | This study |

*Independent transformant.*
**TABLE 2. Oligonucleotide primers used in this study**

| Primer (reference) | Sequence (5'→3') |
|--------------------|------------------|
| FIG1-GFP-F         | TTTTTTGGCTTGTGTTTATCATGTTGGATGGAAAATTTGAAGTAAATTGAATGGAAACATCCTGGCAACACCAAACAAACACCCGCTGGTGATACAATATTTGGTGGTGGTTCTAAAGGTGAAGAATTTGATGAATTTGTTTATGTCAGTAGAAGACTATAAAC |
| FIG1-GFP-R         | ATTTTCTTCTTCACTGTGTCG |
| FIG1-plac-F        | GGCTACATTCCTACTCCTGTTCG |
| FIG1-plac-R        | TGTAAACATCCTCAATTGTACCC |
| mtlα3 (35)         | TTTCGAGTACATTCTGGTCGCG |
| mtlα5 (35)         | GTTTGGGTTCCTTCTTCTCATT |
| ClicP10-GS         | GTACATTTCTACTTCTGTTCTG |

* Underlining indicates region of homology with genomic DNA flanking the 3' end of the FIG1 gene.

**RESULTS**

Ornithogli Fig1 structure and conserved motifs. Researchers have identified Fig1 orthologues in the sequenced genomes from the *Ascomycota* group but not in those from the basidiomycetes, *Ustilago* *maydis*, or zygomycete *Rhizopus oryzae* (42). Sequence alignment using ClustalW showed that all orthologues contained 4 potential transmembrane (TM) domains, with two conserved motifs in the predicted extracellular loop region between TM1 and TM2 (Fig. 1A and B). The gilcine-cysteine motif was conserved in all species, with the exception of *Debaromyces hansenii*, and located at the C terminus of TM1. The second domain, G6FGXGx(1-3)C, is a feature of the claudin superfamily. With the exception of the *Pezizomycotina* subgroup that consisted of 3 *Aspergillus* species, all species contained 1 to 4 potential N-glycosylation sites in the TM1-TM2 intervening region. Within the claudin super-
family, the positioning of N-glycosylation sites between TM1 and TM2 is a characteristic of the EMP (epithelial membrane protein) subgroup (44).

**FIG1 deletion reduces calcium uptake under mating conditions.** To determine whether Fig1 is involved in Ca\(^{2+}\) uptake during mating in *C. albicans*, mating-competent a or α (MTL-homozygous) fig1 deletion mutants were generated from a parent MTL-a/homozygous strain, NGY372 (12). Homozygous fig1 a (NGY502) and fig1α (NGY501) strains underwent normal white/opaque switching (data not shown). The accumulation of \(^{45}\)Ca\(^{2+}\) in a 1:1 mixed population of opaque mating-competent control strains, CAI4 a (NGY500) and CAI4α (NGY499), increased 5-fold over a 12-h period, indicating that Ca\(^{2+}\) uptake occurs during mating in *C. albicans* (Fig. 2A). A similar increase was observed when the CAI4 a strain was exposed to apheromone (Fig. 2B). Deletion of *FIG1* did not affect Ca\(^{2+}\) accumulation in unstimulated cells, but reduced levels of uptake occurred in fig1 null mutants in mating populations and in fig1 MTLα cells that were treated with apheromone.

*FIG1 expression is activated by α-pheromone and mating.* Activation of the *FIG1* promoter was assayed using strains bearing the *FIG1* promoter inserted upstream of the *LACZ* reporter gene. Opaque-phase MTLα and MTLa control strains carrying the *FIG1*-LacZ reporter construct (*FIG1*LacZ a and *FIG1*LacZ α strains) were cross-streaked on solid YPD agar and incubated at room temperature for 48 h. Whole-plate images were photographed following cell lysis and overlay of X-Gal. Blue pigment was visible only where streaks of cells of opposite mating types carrying the *FIG1*-LacZ reporter intersected, indicating that *FIG1* expression was activated when mixed populations of MTLa and MTLα occurred together but not in populations with homogeneous mating types (Fig. 3A). As predicted, fainter blue pigment was observed at the intersection of Fig1p-LacZ-carrying strains of either mating type with cells carrying no reporter. Shmoo formation and cell fusion were observed in all cell samples picked from the cross-streaked intersections and viewed by light microscopy (Fig. 3B), indicating that the *FIG1* promoter was activated in cells of both mating types.
Opaque-phase \( \text{MTL}^a \) and \( \text{MTL}^\alpha \) control strains carrying the \( \text{FIG1p-LacZ} \) reporter construct (\( \text{FIG1p-LacZ}^a \) and \( \text{FIG1p-LacZ}^\alpha \) strains) were mixed in a 1:1 ratio in liquid MLM and incubated at 25°C for 12 h. Colorimetric analysis showed that exposure to cells of opposite mating types induced a 16-fold increase of \( \text{FIG1} \) expression compared to that of populations of a single mating type (Fig. 3C). Opaque, mating-competent \( \text{FIG1p-LacZ}^a \) cells grown in liquid YPD medium were exposed to increasing concentrations of synthetic 13-mer \( \alpha \)-pheromone for a period of 12 h. \( \alpha \)-Pheromone at a concentration of 0.1 \( \mu \)g/ml failed to induce a detectable increase of \( \text{FIG1} \) promoter activity over that observed in unstimulated cells, but activity increased in a dose-dependent manner on exposure to 10-fold increases in the \( \alpha \)-pheromone concentration (Fig. 3C). From this, the concentration of pheromone produced by the mixture of mating cells in 12-h cultures was estimated to be around 5 \( \mu \)g/ml.

**Dynamics of Fig1-GFP expression.** To visualize Fig1-GFP expression and localization in single cells during shmoo formation and mating, an enhanced GFP cassette containing the \( \text{URA3} \) auxotrophic marker was fused to the C terminus of the Fig1 protein in the \( \text{FIG1/FIG1} \) and \( \text{fig1}/\text{FIG1} \) genetic backgrounds. \( \text{FIG1-GFP} \) was therefore under the transcriptional control of the native \( \text{FIG1} \) promoter (21). To test whether the Fig1-GFP fusion was functional, strains harboring a single GFP-tagged allele of \( \text{FIG1} \) were assayed for their ability to reorient their growing tips on contact with ridges in the substrate. In previous studies, the \( \text{fig1}/\text{fig1} \) null mutant was significantly defective in this thigmotropic response (12). The two \( \text{fig1}/\text{FIG1-GFP} \) independent transformants (NGY576 and NGY577) exhibited thigmotropic responses that were not significantly different from those of the control strain, unlike the null mutant, which was defective in thigmotropism (Fig. 4A). This suggests that \( \text{FIG1-GFP} \) was functional. The same result was observed for the \( \text{FIG1/FIG1-GFP} \) (NGY493) strain. This strain was used to generate mating-competent \( \text{FIG1-GFP}^a \) (NGY494) and \( \text{FIG1-GFP}^\alpha \) (NGY495) strains by selection on \( \gamma \)-sorbitose, followed by PCR screening for the two mating type alleles. Opaque cells were picked and restreaked from sectored white cell colonies grown in the presence of phloxine B. \( \text{FIG1-GFP}^a \) cells were exposed to 50 \( \mu \)g/ml \( \alpha \)-pheromone for a period of 12 h, and images were captured using time-lapse fluorescence microscopy. Fig1-GFP was detectable at the site of shmoo formation 40 to 60 min after the addition of \( \alpha \)-pheromone, as the emerging shmoo tip itself became visible (Fig. 4B). Fig1-GFP localized primarily to the shmoo apices. Fig1-GFP associated with the entire shmoo structure during the early stages but became tip biased in more elongated cells after 4 h of pheromone exposure (Fig. 4C). The same Fig1-GFP distribution was observed in populations of mating cells on YPD agar (Fig. 5A).

**Contrasting distribution patterns of Fig1-GFP in the shmoo plasma membrane and the nuclear periphery.** Fig1-GFP was visualized by fluorescence microscopy in shmoo produced in populations of \( \text{MTL}^a \) and \( \text{MTL}^\alpha \) cells mixed at a ratio of 1:1 (Fig. 5A). By using deconvolution microscopy, we observed Fig1-GFP as discontinuous zones at the periphery of the shmoo and in continuous intracellular structures within the shmoo, the body of the mother cell, and the newly formed daughter cell (Fig. 5A). In order to identify the intracellular structures associated with Fig1-GFP, mating cells were stained with DAPI (for nuclei), FM4-64 (for vacuoles), and CFW (for walls). The discontinuous microdomains of Fig1-GFP were immediately adjacent to the CFW-stained cell wall, suggesting
that Fig1-GFP localized to the plasma membrane (Fig. 5B). In contrast, the continuous zones of Fig1-GFP surrounded the nuclear regions, suggesting that it localized to the proximal ER or the nuclear periphery (Fig. 5C and 6). Perinuclear Fig1-GFP persisted during nuclear migration and fusion and was still present at the nuclear periphery in the newly formed daughter cell (Fig. 5A, panels 4 and 5). Elongated DAPI-stained structures that were not associated with Fig1-GFP were observed in the shmoo, possibly representing the localization of mitochondrial DNA (Fig. 6C and F). Fig1-GFP was not detectable in the FM4-64-stained endosomal and vacuolar membranes (Fig. 6). This suggests that Fig1-GFP was not targeted to the vacuole for degradation at a significant rate in shmooing cells.

DISCUSSION

Fig1 as a member of the claudin superfamily. Fig1 is a member of a fungus-specific family of proteins that contains sequence characteristics found in the large mammalian PMP22/EMP/MP20/claudin (pfam00822). Claudins combine as dynamic multimeric complexes at the cell membrane. It is thought that their basic function lies in cell-cell adhesion. Claudins are also found in the epithelia of invertebrates, which lack tight junctions, where they are thought to perform a signaling function (50). The family includes regulators of solute movement through epithelial tight junctions, scaffolding proteins for the assembly of adhesion and receptor complexes, and subunits for the membrane delivery of the large α subunit of voltage-dependent calcium channels (reviewed by Van Itallie and Anderson [45]). In mammalian epithelia, claudins form ion-selective intercellular pores, but there is no evidence that they directly admit ions across the plasma membrane into the cell. Although Fig1 is involved in Ca\(^{2+}\) uptake, its lack of homology to any known ion influx channel suggests that its role may be as an indirect facilitator of Ca\(^{2+}\) influx.

We generated a series of mutant strains that were of the MTL\(α\) or MTL\(a\) mating type in order to study Fig1 activity under conditions in which the gene was predicted to be highly expressed. Specifically, the aim was to determine whether Fig1 is involved in Ca\(^{2+}\) uptake in C. albicans and to understand more about its role during polarized growth and mating.

Fig1-GFP localizes to shmoo apices during mating. In a time course study, expression of Fig1-GFP, controlled by the native CaFig1 promoter, was easily detectable by fluorescence mi-
croscopy on exposure of a cells to α-pheromone. Fig1-GFP was observed at the site of mating projection formation, approximately 50 min after exposure to mating pheromone. Fig1-GFP was visible at the cell periphery and in reticular structures throughout the cell but localized predominantly within the mating projection. The reticular distribution in C. albicans contrasted with the pattern observed using a ScFig1::H9252 gal fusion protein during shmoo formation in S. cerevisiae, where Fig1 appeared as punctate spots at the cell periphery (17). By use of deconvolution microscopy of C. albicans cells, we found the distribution of Fig1-GFP at the cell membrane to be discontinuous, which suggested that it may be present in microdomains. CFW staining showed that Fig1-GFP appeared in flattened zones in apposition with the cell wall. The intracellular reticular structures carrying Fig1-GFP localized within bright, perinuclear regions that persisted throughout shmoo formation and cell-cell fusion and in nuclei of the newly formed daughter cell. It is possible that the perinuclear localization of Fig1-GFP resulted from its biosynthesis in the ER. However, many other mating-induced genes, such as FUS1 and PRM1, whose expression is induced in other fungi during mating, do not accumulate in the ER or the nuclear periphery (18, 41). Furthermore, Fig1-GFP was not visualized in the FM4-64-stained vacuolar membrane during shmoo elongation. Low levels of expression of ScFig1::βgal were visible in the perinuclear zone in S. cerevisiae cells prior to treatment with mating pheromone, supporting the possibility that Fig1 may have a second nuclear function (17). It has been reported that in mammalian cells, perinuclear Ca\textsuperscript{2+} release affects nuclear Ca\textsuperscript{2+} much more strongly than distal Ca\textsuperscript{2+} influx and a sustained rise in nuclear [Ca\textsuperscript{2+}] is required for calcineurin-NFAT (the Ca\textsuperscript{CRZ1} product homologue) and cyclic AMP response binding element (CREB) signaling (13, 27, 31, 43). Fig1 may also have a general role in limiting membrane damage or promoting membrane fusion of the nuclear envelope and at the plasma membrane, both of which undergo fusion during mating.

Mating-dependent Fig1 expression and Ca\textsuperscript{2+} uptake in C. albicans. Ca\textsuperscript{2+} uptake increased approximately 5-fold in mixtures of opaque MTLα or MTLα cells, and in MTLα cells treated with α-pheromone (10 μg/ml), an 8- to 10-fold increase in Ca\textsuperscript{2+} uptake compared to that of untreated controls was observed. Together, these results demonstrate that exposure to mating pheromone in C. albicans mating-competent cells causes a significant increase in the intracellular [Ca\textsuperscript{2+}], as seen in S. cerevisiae (38). Deletion of FIG1 in C. albicans reduced Ca\textsuperscript{2+} uptake to 50% of the control level, confirming that Fig1 is involved in Ca\textsuperscript{2+} influx but implying that mating also in-

**FIG. 4.** Temporal expression and localization of Fig1-GFP during shmoo formation and extension. (A) Cells expressing a single copy of FIG1-GFP show normal contact-sensing responses to a ridged substrate (error bars represent standard errors of the means [n = 3]). Asterisk indicates a significant difference compared to the control strain (P < 0.01; Dunnett’s t test). (B) Bean-shaped mating-competent FIG1-GFP cells in modified Lee’s medium were treated with 50 μg/ml α-pheromone, and image capture commenced immediately for a period of 100 min. (C) Cells were treated at 0 h (time zero) with 50 μg/ml α-pheromone. Image capture started at 4 h and continued until 13 h. Scale bars = 5 μm.
Involves Fig1-independent Ca\(^{2+}\) uptake. The Fig1-LacZ reporter construct was activated in both MTL mating types, demonstrating that this pathway forms part of a general mating response and is not MTL specific. In addition, the level of activity of the Fig1-LacZ reporter construct increased in a pheromone dose-dependent manner. Taken together, these results suggest a positive correlation between pheromone concentration, Fig1 expression, and Ca\(^{2+}\) uptake. Mid1-Cch1, the HACS complex, may therefore also contribute to Ca\(^{2+}\) uptake during mating in *C. albicans*. HACS expression is influenced by the calcineurin-CRZ1 Ca\(^{2+}\)-dependent signaling pathway in *C. albicans*, but its expression is not thought to be highly regulated (26). We have not been able to visualize Cch1-GFP or Mid1-GFP in *C. albicans*, but in *S. cerevisiae*, Mid1-GFP was visualized at the plasma membrane and in an ER-like zone around the nucleus in nonmating conditions and did not relocalize to the shmoo on treatment with mating pheromone (25, 32, 53). The highly enriched and spatially distinct domains of Fig1-GFP at the shmoo plasma membrane suggest that the functions of the two Ca\(^{2+}\) uptake systems differ during mating and that Fig1 might be involved in the generation of Ca\(^{2+}\) signals at specific sites.

It has been reported that both chelation of extracellular Ca\(^{2+}\) and deletion of FIG1 dramatically reduced mating efficiency of *C. albicans* (3). Our finding that Fig1 is involved in 50% of the Ca\(^{2+}\) uptake during mating confirms its importance in the mating process.

**Mating-independent Fig1 expression.** We previously identified Fig1 as a protein required for the normal thigmotropic response by *C. albicans*, where FIG1 deletion significantly reduced the ability of hyphal tips to reorient on contact with ridges in the underlying substrate; this role of Fig1 is in contrast to its mating-dependent functions (12). All hyphal tropisms we have studied to date have been shown to depend on Ca\(^{2+}\) influx, but deletion of FIG1 did not affect Ca\(^{2+}\) uptake during vegetative growth. Although FIG1 mRNA was detected under all growth conditions tested, including in kidney tissue isolated from infected mice, a Fig1-GFP construct in yeasts or hyphae could not be visualized. This suggests that Fig1 is present at very low levels during vegetative growth but

![Fig. 5](http://ec.asm.org/)

**FIG. 5.** Fig1-GFP localization in shmoos and fused cells. (A) FIG1-GFP a (NGY494) and FIG1-GFP a (NGY495) cells were mixed at a ratio of 1:1, spotted on 2% YPD agar, and incubated for 48 h at 25°C. Cells were taken from the plate and viewed by fluorescence microscopy. Fig1-GFP was observed in shmoos (panels 1 and 2), a zygote (panel 3), and daughter cells (panels 4 and 5). Cell wall chitin was stained with 100 μg/ml CFW. Fig1-GFP (green) localized in microdomains adjacent to the CFW-chitin wall layer (blue; arrows) (B) and more homogeneously adjacent to DAPI-stained perinuclear regions (enlarged image) (C). DIC, differential interference contrast. Scale bars = 5 μm.

![Fig. 6](http://ec.asm.org/)

**FIG. 6.** Fig1 in shmooing cells from mating mixtures of FIG1-GFP a and FIG1-GFP a cells stained with DAPI (200 μg/ml) and FM4-64 (1 μg/ml). Scale bars = 5 μm.
that even basal level expression of Fig1 influences hyphal growth and orientation.

It is possible that the function and activity of Fig1 depend on its colocalization with other effectors. Two lines of evidence in S. cerevisiae suggest that ScFig1 has Ca\(^{2+}\)-related and Ca\(^{2+}\)-unrelated functions. First, output from the aequorin/Ca\(^{2+}\) reporter peaked during mating, while Fig1-Myc levels remained high, which suggested that Fig1 was still required in the plasma membrane after mating-induced Ca\(^{2+}\) uptake was complete (38). Second, mating-induced rapid cell death was found to be dependent on Fig1 but independent of its Ca\(^{2+}\) uptake activity (54). The involvement of other effectors in Fig1 function is suggested by the observation that membrane localization of Fig1 was necessary but not sufficient for LACS activity (38). In C. albicans, Fig1-GFP localization appeared to be perinuclear and at the plasma membrane. Both membranes are sites of membrane perturbation and fusion during mating. Fig1 was proposed to be necessary in S. cerevisiae for limiting the zone of membrane fusion during mating (38). FIG1 deletion in S. cerevisiae resulted in defective cell fusion during mating, with undissolved wall material at the fusion site, which was rescued by the addition of extracellular Ca\(^{2+}\). Such fusion defects were not observed in fig1 null mutants of C. albicans, but Fig1 could nevertheless function to stabilize membranes in response to perturbation during polarized growth, possibly by limiting membrane expansion during critical stages in morphological change. Two observations provide evidence for this. First, FIG1 deletion resulted in hypha formation on solid SD medium, while the control strain grew as yeast (12). This suggests that Fig1 may be a negative regulator of polarized growth in certain conditions. Second, FIG1 deletion decreases the likelihood that hyphae will reorient the growth axis on contact with a small obstacle (12). Thus, although Fig1 is undoubtedly up-regulated as part of the mating signaling pathway, its role may well be more generally related to membrane stability during morphological transitions, whether internally or externally induced. Fig1 therefore participates in the polarized growth of both mating projections and vegetative fungal hyphae, presumably by regulating calcium ion uptake at sites of polarized tip growth. FIG1 expression represents a convenient marker for the mating process, but low levels of Fig1 in nonmating cells also influence the physiology of growth and development of C. albicans.

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REFERENCES

1. Aguilar, P. S., A. Engel, and P. Walter. 2007. The plasma membrane proteins Prm1 and Fig1 ascertain fidelity of membrane fusion during yeast mating. Mol. Biol. Cell 18:547–556.
2. Alby, K., D. Schaefer, and R. J. Bennett. 2009. Homothallic and heterothallic mating in the opportunistic pathogen Candida albicans. Nature 460:890–893.
3. Alby, K., D. Schaefer, R. K. Sherwood, S. K. Jones, Jr., and R. J. Bennett. 2010. Identification of a cell death pathway in Candida albicans during the response to pheromone. Eukaryot. Cell 9:1690–1701.
4. Alvarez, F. J., L. M. Douglas, A. Rosebrock, and J. B. Konopka. 2008. The Sur7 protein regulates plasma membrane organization and prevents intracellular cell wall growth in Candida albicans. Mol. Biol. Cell 19:5214–5225.
5. Anderson, J. M., and D. R. Soll. 1987. Unique phenotype of opaque cells in the white-opaque transition of Candida albicans. J. Bacteriol. 169:559–558.
6. Bedell, G. W., and D. R. Soll. 1979. Effects of low concentrations of zinc on the growth and dimorphism of Candida albicans: evidence for zinc-resistant and -sensitive pathways for meiosis formation. Infect. Immun. 26:348–354.
7. Bennett, R. J., M. G. Miller, P. R. Chua, M. E. Maxon, and A. D. Johnson. 2005. Nuclear fusion occurs during mating in Candida albicans and is dependent on the KAR3 gene. Mol. Microbiol. 55:1046–1059.
8. Bonilla, M., and K. W. Cunningham. 2003. Mitogen-activated protein kinase stimulation of Ca\(^{2+}\) signaling is required for survival of endoplasmic reticulum stress in yeast. Mol. Biol. Cell 14:4296–4305.
9. Brand, A., and N. A. Gow. 2009. Mechanisms of hypha orientation of fungi. Curr. Opin. Microbiol. 12:350–357.
10. Brand, A., K. Lee, B. Vesey, and N. A. Gow. 2009. Calcium homeostasis is required for contact-dependent helical and sinuous tip growth in Candida albicans hyphae. Mol. Microbiol. 71:1155–1164.
11. Brand, A., D. M. MacCallum, A. J. P. Brown, N. A. R. Gow, and F. C. Odds. 2004. Ectopic expression of URA3 can influence the virulence phenotypes and survival of Candida albicans but can be overcome by targeted reinteg-ration of URA3 at the RPS10 locus. Eukaryot. Cell 3:900–909.
12. Chen, J., J. Chen, S. Lane, and H. Liu. 2002. A conserved mitogen-activated protein kinase pathway is required for mating in Candida albicans. Mol. Microbiol. 46:1335–1344.
13. Clemente-Ramos, J. A., et al. 2009. The tetraspan protein Dnl1p is required for correct membrane organization and cell wall remodelling during mating in Cryptococcus neoformans. Mol. Microbiol. 73:695–709.
14. Daniels, K. J., T. Srikantha, S. R. Lockhart, C. Pujol, and D. R. Soll. 2006. Opaque cells signal white cells to form biofilms in Candida albicans. EMBO J. 25:2240–2252.
15. Erdman, S. E., L. Lin, M. Maleczynski, and M. Snyder. 1998. Pheromone-regulated genes required for yeast mating differentiation. J. Cell Biol. 140:461–483.
16. Fleissner, A., S. Diamond, and N. L. Glass. 2009. The Saccharomyces cerevi- siae PRM1 homolog in Neurospora crassa is involved in vegetative and sexual cell fusion events but also has postfermentation functions. Genetics 181:497–510.
17. Fonzi, W. A., and M. Y. Irwin. 1993. Isogenic strain construction and gene mapping in Candida albicans. Genetics 134:717–728.
18. Gollob, G. M., J.-E. O’Connor, L. I. Garcia, S. I. Martinez, E. Herrero, and L. del Castillo Agudo. 2001. Isolation of a Candida albicans gene, tightly linked to URA3, coding for a putative transcription factor that suppresses a Saccharomyces cerevisiae a1 mutation. Yeast 18:301–311.
19. Gomis-Nejad, M., J. Berman, and C. Gale. 2001. Casettes for the PCR-mediated construction of green, yellow, and cyan fluorescent protein fusions in Candida albicans. Yeast 18:859–864.
20. Hull, C. M., and A. D. Johnson. 1999. Identification of a mating type-like locus in the assexual pathogenic yeast Candida albicans. Science 285:1271–1275.
21. Hull, C. M., R. M. Raisner, and A. D. Johnson. 2000. Evidence for mating of the “asexual” yeast Candida albicans in a mammalian host. Science 289:307–310.
22. Janbon, G., F. Sherman, and E. Rustchenko. 1999. Appearance and properties of L-sorbos-2-chitobiose-utilizing mutants of Candida albicans obtained on a selective plate. Genetics 153:653–664.
23. Kanazaki, M., et al. 1999. Molecular identification of a eukaryotic, stretch-activated nonscavenger cation channel. Science 285:882–886.
24. Karababa, M., et al. 2006. CRZ1, a target of the calcineurin pathway in Candida albicans. Mol. Microbiol. 59:1429–1451.
25. Kornhauser, J. M., et al. 2002. CREB transcriptional activity in neurons is regulated by multiple, calcium-specific phosphorylation events. Neuron 34:221–233.
26. Lacke, S. A., S. R. Lockhart, K. J. Daniels, and D. R. Soll. 2003. Skin facilitates Candida albicans mating formation. Infect. Immun. 71:4970–4978.
27. Lal-Nag, M., and P. Morin. 2009. The claudins. Genome Biol. 10:235.
28. Lenardon, M. D., R. K. Whitton, C. A. Munro, D. Marshall, and N. A. R. Gow. 2007. Individual chitin synthase enzymes synthesize microfibrils of differing structure at specific locations in the Candida albicans cell wall. Mol. Microbiol. 66:116–1173.
29. Lipp, P., D. Thomas, M. J. Berridge, and M. D. Bootman. 1997. Nuclear calcium signalling by individual cytoplasmic calcium puffs. EMBO J. 16:7166–7173.
30. Locke, G. D., M. Bonilla, L. Liang, Y. Takita, and K. W. Cunningham. 2000. A homolog of voltage-gated Ca\(^{2+}\) channels stimulated by depletion of secretory Ca\(^{2+}\) in yeast. Mol. Cell. Biol. 20:6686–6694.
33. Miller, M. G., and A. D. Johnson. 2002. In Candida albicans, white-opaque switchers are homoyzogous for mating type. Genetics 162:737–745.
34. Lockhart, S. R., R. Zhao, K. J. Daniels, and D. R. Soll. 2003. Alpha-pheroemone-induced “shmooing” and gene regulation require white-opaque switching during Candida albicans mating. Eukaryot. Cell 2:847–855.
35. Magee, B. B., and P. T. Magee. 2000. Induction of mating in Candida albicans by construction of MTLa and MTLalpha strains. Science 289:310–313.
36. Miller, M. G., and A. D. Johnson. 2002. White-opaque switching in Candida albicans is controlled by mating-type locus homeodomain proteins and allows efficient mating. Cell 110:293–302.
37. Muller, E. M., E. G. Locke, and K. W. Cunningham. 2001. Differential regulation of two Ca2+ influx systems by pheromone signaling in Saccharomyces cerevisiae. Genetics 159:1527–1538.
38. Muller, E. M., N. A. Mackin, S. E. Erdman, and K. W. Cunningham. 2003. Fig1p facilitates Ca2+ influx and cell fusion during mating of Saccharomyces cerevisiae. J. Biol. Chem. 278:38461–38469.
39. Munro, C. A., et al. 2007. The PKC, HOG and Ca2+ signalling pathways co-ordinately regulate chitin synthesis in Candida albicans. Mol. Microbiol. 63:1399–1413.
40. Peiter, E., M. Fischer, K. Sidaway, S. K. Roberts, and D. Sanders. 2005. The Saccharomyces cerevisiae Ca2+ channel Cch1pMid1p is essential for tolerance to cold stress and iron toxicity. FEBS Lett. 579:5697–5703.
41. Proszynski, T. J., R. Klemm, M. Bagnat, K. Gaus, and K. Simons. 2006. Plasma membrane polarization during mating in yeast cells. J. Cell Biol. 173:861–866.
42. Rispaal, N., et al. 2009. Comparative genomics of MAP kinase and calcium-calceurin signalling components in plant and human pathogenic fungi. Fungal Genet. Biol. 46:287–298.
43. Shibasaki, F., E. R. Price, D. Milan, and F. McKeon. 1996. Role of kinases and the phosphatase calceurin in the nuclear shuttling of transcription factor NF-AT4. Nature 382:370–373.
44. Taylor, V., A. A. Welcher, EST Program Amgen, and U. Suter. 1995. Epithelial membrane protein-1, peripheral myelin protein 22, and lens membrane protein 20 define a novel gene family. J. Biol. Chem. 270:22824–22833.
45. Van Itallie, C. M., and J. M. Anderson. 2006. Claudins and epithelial paracellular transport. Annu. Rev. Physiol. 68:403–429.
46. Veses, V., and N. A. Gow. 2008. Vascular dynamics during the morphogenetic transition in Candida albicans. FEMS Yeast Res. 8:1339–1348.
47. Vesses, V., et al. 2005. ABG1, a novel and essential Candida albicans gene encoding a vacular protein involved in cytokinesis and hyphal branching. Eukaryot. Cell 4:1088–1101.
48. Viladevall, L., et al. 2004. Characterization of the calcium-mediated response to alkaline stress in Saccharomyces cerevisiae. J. Biol. Chem. 279:43614–43624.
49. Wadhera, M., H. Su, L. K. Gordon, L. Goodglick, and J. Braun. 2003. The tetraspan protein EMP2 increases surface expression of class I major histo-compatibility complex proteins and susceptibility to CTL-mediated cell death. Clin. Immunol. 107:129–136.
50. Wu, V. M., J. Schulte, A. Hirschi, U. Tepass, and G. J. Beitel. 2004. Sinuous is a Drosophila claudin required for septate junction organization and epithelial tube size control. J. Cell Biol. 164:313–323.
51. Wu, W., C. Pujol, S. R. Lockhart, and D. R. Soll. 2005. Chromosome loss followed by duplication is the major mechanism of spontaneous mating-type locus homoyzogosis in Candida albicans. Genetics 169:1311–1327.
52. Wu, W., S. R. Lockhart, C. Pujol, T. Srikantha, and D. R. Soll. 2007. Heterozygosity of genes on the sex chromosome regulates Candida albicans virulence. Mol. Microbiol. 64:1567–1604.
53. Yoshimura, H., T. Tada, and H. Iida. 2004. Subcellular localization and oligomeric structure of the yeast putative stretch-activated Ca2+ channel component Mid1. Exp. Cell Res. 293:185–195.
54. Zhang, N. N., et al. 2006. Multiple signaling pathways regulate yeast cell death during the response to mating pheromones. Mol. Biol. Cell 17:3429–3442.
55. Zhao, R., et al. 2005. Unique aspects of gene expression during Candida albicans mating and possible G1 dependency. Eukaryot. Cell 4:1175–1190.