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Supplemental materials and methods

Disruption of the *C. albicans GIN4, HSL1,* and *SWE1* homologues

Disruption cassettes, consisting of either *URA3, ARG4,* or *HIS1* selection markers, flanked by a 60-bp sequence of the target gene, were generated by PCR (Wilson et al., 2000) using oligonucleotide primers listed in Table S1 . Disruptions were confirmed by PCR amplification using primers that anneal to regions flanking the disrupted gene. Two successive rounds of gene deletion using a different nutritional marker were used to create a homozygous null strain. This PCR analysis of the putative *hsl1*Δ/*hsl1*Δ strain demonstrated that it had been created by a gene conversion event during the second round of transformation. To confirm that no part of the *HSL1* gene remained, two further pairs of primers were designed to amplify sequences within the *HSL1* coding region (Table S1, *HSL1* confirmation sets). Both failed to amplify a product from the putative deletant, even though both amplified fragments of the predicted size from the *hsl1*Δ/*HSL1* heterozygote (unpublished data).

Construction of *MET3-GIN4* and *MET3-HSL1* fusions

A 330- and 350-bp fragment from the 5′ end of ORF19.663 (*GIN4*) and ORF19.4308 (*HSL1*), respectively, were generated by PCR (see Table S1 for primers) and cloned into the BamHI–PstI site of pCaDIS (Care et al., 1999) creating plasmids pDIS-*GIN4* and pDIS-*HSL1*. The resulting plasmids were linearized with AgeI (pDIS-*GIN4*) and XcmI (pDIS-*HSL1*) and transformed into strains that were heterozygous for the respective deletion. The correct insertion of *MET3-GIN4* or *MET3-HSL1* was confirmed by PCR using an internal pCaDIS primer (RC8con) and a flanking primer that annealed within the 3′ portion of the open reading frame (Table S1, *gin4*Δ checking reverse, *hsl1*Δ checking reverse).

Construction of the *swe1D/swe1D gin4D/MET3-GIN4* strain

A homozygous *swe1*Δ/*swe1*Δ strain was generated using *HIS1* and *ARG4* as selection markers as described in Disruption of the *C. albicans GIN4, HSL1,* and *SWE1* homologues. One copy of *GIN4* was then deleted using a *URA3*-based disruption cassette (Wilson et al., 2000). The *ura3* marker was regenerated, as in the *URA*-blaster procedure (Fonzi and Irwin, 1993), and a copy of the pDIS*MET3-GIN4* integrated using *URA3* as a selection marker.

Cloning of *C. albicans* SWE1

The *C. albicans SWE1* gene was amplified from genomic DNA using primers SWTAfwd and SWTArev (Table S1) followed by ligation into the TA cloning vector pYES2.1/V5-His-TOPO (Invitrogen) according to the manufacturer's protocol.

Creation of COOH-terminal GFP fusions

The in-frame insertion of a COOH-terminal GFP tag containing the *HIS1* marker was performed as described previously (Gerami-Nejad et al., 2001) with the following modification: due to low integration efficiencies of the *GIN4-GFP* fusion cassette, two rounds of PCR were performed to increase the flanking homologous DNA to >90 bp (Table S1). This resulted in a marked increase in the yield of positive integrants, which were confirmed by PCR.

Protein extraction and Western blotting

Total protein extracts were prepared using the following modified protocol that allows accurate determination of protein concentration by the Bradford assay. Cells were washed once in STOP solution (1× PBS + 10 mM NaN_3 + 50 mM NaF) and once in 20% TCA, and resuspended in 200 μl of 20% (wt/vol) TCA plus glass beads. Cells were disrupted by five 12-s cycles of agitation in a Rybolyser (Hybaid) set at a speed of 6.5. Extracts were separated from glass beads by piercing a hole at the bottom of the eppendorf tube and centrifuging at 7,000 rpm for 5 min. TCA precipitated proteins were recovered by centrifugation for 5 min at 28,000 *g*. The pellet was washed in a 1:1 mixture of cold ethanol/diethyl ether followed by resuspension in 1% (wt/vol) SDS, 0.1 M Tris, pH 8.9, until the TCA had been neutralized. The suspension was then incubated at 37°C for 3 h and vortexed frequently. The suspension was centrifuged for 5 min at 14,000 rpm and the supernatant concentrated using a Microcon YM –30 column (Millipore). Equal quantities of protein samples were resolved by SDS-PAGE and electroblotted onto Hybond-C membrane (Amersham Biosciences). Blocking and antibody incubations were performed in 5% wt/vol dried milk in 1× PBS, 0.1% (vol/vol) Tween 80. The primary antibody was a dual mouse monoclonal anti-GFP (Roche Biosciences) used at a working concentration of 1/4,000. The secondary anti-

Table S1. **Oligonucleotides used**

*gin4*Δ delete forward primer	TAATCATCCACAGAAGATCGGACCTTGGAAATTAGGTAAAACTTTAGGAAGAGGTGCCACG TTTTCCCAGTCACGACGTT
*gin4*Δ delete reverse primer	ACAATTTCTGATTTTTGATTAAAATCTCTTTGATTAATCTTAATTCTAAATTTACAACTCTGTGG AATTGTGAGCGGATA
*gin4*Δ checking forward	ATGCCACATTCAAGACAACC
*gin4*Δ checking reverse	TTATCTAAAACACCTTCTTT
Construction of *MET3-GIN4*	GAAGGATCCATGCCACATTCAAGACAACC
Construction of *MET3-GIN4*	GAACTGCAGCAAGATAAAGAGCCTTAGATG
*swe1*Δ delete forward primer	ATACCAGTAGCACACCAATGGCCAACAATAATCCCACTAATGACAGCACAATCTCCTCTCG TTTTCCCAGTCACGACGTT
*swe1*Δ delete reverse primer	AATTGCCCAGCAAACTATTATAACTAAAATTAGTCGAATTCGGGGATCCATCACCAGCACTG TGGAATTGTGAGCGGATA
*swe1*Δ checking forward primer	ATGGATTCAAACCCGTGTCAAG
*swe1*Δ checking reverse primer	TATTCATCAGGTGGACTACC
*hsl1*Δ delete forward primer	ATCATTCATCTTCGATGAATGTGGATAAGGTGGTTCAATCAGTTACAAATGCCACTAAGCG TTTTCCCAGTCACGACGTT
*hsl1*Δ delete reverse primer	GATGATGAATTTATTAAATCAATAACTTCAATTTTAAATTTCAATTTTCTACCATTTGCATGTGG AATTGTGAGCGGATA
*hsl1*Δ checking forward primer	CAGTTGTTAATAGACGGTCATCAC
*hsl1*Δ checking reverse primer	GATTATAAACGTGACAATATTGAC
HSL1 confirmation primer set 1-forward	TTTGGGTAGAGGTTCTACTG
Primer set 1-reverse	TTCTGGTGAAGCATAATGAG
Primer set 2-forward	CGCAAGCACAGCTAACACTG
Primer set 2-reverse	TTGACAACATATGGAGATCG
Construction of *MET3-HSL1*	GAAGGATCCATGTCAACAGTTGTTAATAG
Construction of *MET3-HSL1*	GAACTGCAGGTAGCATCTTCTTTAGAGCG
RC8con (*MET3* promoter)	TATGCGATTGTGGCTCATAGTAACG
GIN4-GFP tagging forward primer	ACAACTGATACTTTATTTTCTGAAATTGAAAAGGTCTTACTCAAAGAAGGTGTTTTAGATAAA GGTGGTGGTTCTAAAGGTGAAGAATTATT
GIN4-GFP extension forward primer	AAGATTAATCAAAGAGATTTTAATCAAAAATCAGAAATTGTTTGTGTTAGAGTGAAAGGATC TAAAGTTACAACTGATACTTTATTTTCTGAAATTGAA
GIN4-GFP tagging reverse primer	TAAGAAATAAAACAAGCCAAATTAGTATACTGAAAAAAAAAATTCATAAATAATGCATCATTG TTGAATTCCGGAATATTTATGAGAAAC
GIN4-GFP extension reverse primer	TATTAATGAAGAAGATTTGCAAAAATGAATTGTAGTTTAAAATTATCATAGTTATATAATATAC ATAAGAAATAAAACAAGCCAAATTAGTATACT
GIN4-GFP checking forward primer	CCCAAACATGAAATGTCTAC
GIN4-GFP checking reverse primer	TTAATGAGACAATTCAAATTG
CDC10-GFP tagging forward primer	TGAAGAACGCCTCTGGTGTGCCAAATGCTCCTATGTTCCAATCAACTACAGGTACTGCTGC TGCTAGAGGTGGTGGTTCTAAAGGTGAAGAATTAT
CDC10-GFP extension forward primer	CAAATAGACAATCCCAACTTCAAAAAGATCAAGGGCAAACCTCACAACAATCAAACCAAGA TTTGAAGAACGCCTCTGGTGTGCCAAATGCTC
CDC10-GFP tagging reverse primer	ACGCGTTTTGCTTTTCAACAAACACACAAAAGAAGAGGAATACAAAAAAGTAAAATCACATT ATATCAATAACAAACATGAATTCCGGAATATTTATGAGAAAC
CDC10-GFP extension reverse primer	AAATATAAAATCTCGAGTGGGAAAAAAAAAATACACACACACATAAATATTTTTGTTCATACG CGTTTTGCTTTTCAACAAACACACAAA
CDC10-GFP checking forward primer	ATTGAAGATGTTAGTCAATGTG
CDC10-GFP checking reverse primer	CTCGTTAGTTAATTGTTGTC
SWE1 cloning (SWTAfwd)	GCAATGGATTCAAACCCGTGTCAAG
SWE1 cloning (SWTArev)	CACTTATTCATCAGGTGGACTACC

body was goat anti–mouse conjugated to HRP (DakoCytomation) used at a working concentration of 1/12,000. Detection was performed using the Supersignal West Femto kit (Pierce Biotechnology Inc.). Images were acquired with a GeneGnome chemiluminescence detector and quantified using GeneTools 3.00.22 (both purchased from Syngene). No protein of comparable size to Gin4-GFP (200 kD) was readily available to use as loading control and we found that smaller proteins such as actin or tubulin gave inconsistent results. Preliminary experiments established that the most reliable way of demonstrating equal loading was to measure total proteins transferred to the membrane as visualized by Ponceau S staining. To do this, membranes were incubated in Ponceau S for 30 s before blocking. Desaturation of the membranes was achieved by washing three times in water. Images were recorded using a UVI gel documentation system (UVI Tec)

Table S2. **Ectopically expressed Gin4-GFP overcomes the inability of pseudohyphae to form hyphae**

Strain	*GIN4* on/off or native promoter pseudohyphae (PH) or yeast (Y)	Hyphae after preincubation for: 2 h (*n*)	6 h (*n*)
		%	%
MET3-GIN4-GFP	*GIN4* on PH	90.5 (169)	67.1 (167)
MET3-GIN4-GFP	*GIN4* off PH	85.6 (194)	9.1 (121)
GIN4-GFP	Native promoter (PH)	17.3 (231)	5.3 (152)
GIN4-GFP	Native promoter (Y)	79.8 (208)	47.2 (165)

and quantified using Quantity One software (Bio-Rad Laboratories). The total amount of protein measured in each lane was then used to normalize the signal from the anti-GFP antibody.

Transformation procedures

For *C. albicans,* an overnight yeast culture was inoculated into fresh medium to a cell density of 2.5×10^6 cells/ml and incubated with vigorous shaking to 2×10^7 cells/ml. The cells were washed in water and 100 mM lithium acetate (Sigma-Aldrich) and resuspended in 100 mM lithium acetate at a cell density of 2×10^9 cells/ml. For each transformation, 50 μl of cells were collected by centrifugation and resuspended in of transformation mix (240 μl 50% [wt/vol] PEG 3350, 36 μl 1 M lithium acetate, 10 μl 10 mg/ml denatured herring sperm DNA (CLONTECH Laboratories, Inc.) and 5–10 μg transforming DNA in 74 μl solution). The mix was incubated at 30°C for 90 min followed by 42°C for 30 min. Transformations were washed once in water and plated onto the appropriate selective medium. *S. cerevisiae* cells were transformed by the lithium acetate procedure (Gietz et al., 1995).

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