Supplemental methods

Yeast strains and cell culture

The *dbr1Δ* strain (QMY1) used in our microarray study was derived from strain SN87, which is defective for both *HIS1* and *LEU2*, by replacing the two alleles of the *DBR1* coding sequence with *HIS1* and *LEU2* markers, as described (Noble and Johnson 2005). A matched wild-type control strain (QMY23) was generated by integration of the *HIS1* and *LEU2* markers at the endogenous *LEU2* locus of SN87. Strain QMY23 was also used in our *RPL30* assays. For our *SPR28* assays, we selected for mating-type **a**/**a** cells by growth of strain SN87 on sorbose medium (Magee and Magee 2000), and then isolated spontaneous opaque-phase cells (Miller and Johnson 2002).

Except where noted, all cell collections were carried out by centrifugation at 2,800*g* for 5 min at 4ºC, followed by rapid freezing in liquid nitrogen. For *RPL30* analysis, cells were grown in YEPD (1% yeast extract, 2% peptone, 2% glucose) at 23ºC to an OD_{600} of 0.8, rapidly equilibrated to 37ºC or 16ºC in water baths, then grown for 30 min (37ºC) or 2 h (16ºC). Cells were collected by centrifugation (15,800*g*, 1 min) at room temp (37ºC sample) or 4ºC (16ºC sample). For *SPR28* analysis, mating-competent opaque cells were grown overnight at 23ºC to an OD_{600} of ~1 in SC media (Sherman 2002) supplemented with 100 mg/l uridine. Cells were collected, rinsed in water, and suspended in SpiderM media (1% nutrient broth, 1% mannitol, 4 g/l K_2HPO_4, pH 7.2) to an OD_{600} of 1.0. Either the 13-mer form of *C. albicans* alpha factor mating pheromone (Genemed Synthesis) at 10 mg/ml in 10% DMSO, or a 10% DMSO control was added at a 1,000-fold dilution to cell cultures, which were then grown for 4 h at 23ºC (Bennett and Johnson 2006). Cells exposed to alpha factor were confirmed by light microscopy to have formed mating projections.

For our first microarray experiment, cells were grown in YEPD at 30ºC to an OD_{600} of ~1.4. We examined numerous alternative growth conditions for our subsequent microarray. **a**/**a** and alpha/alpha opaque cells were isolated as described above for strain SN87. *Opaque **a**/**a** cells*: cells were grown in SC + uridine at 23ºC to an OD_{600} of ~1. *Mating cells*: **a**/**a** and alpha/alpha opaque cells were grown overnight at 23ºC in SC + uridine and resuspended to an OD_{600} of 1.0 in SpiderM. Cells of opposite mating-type were mixed and grown 4 h at 23ºC (Bennett and Johnson 2006). *Nitric oxide stress*: cells were grown at 30ºC to an OD_{600} of 1.0 in YEPD supplemented with 80 mM HEPES, pH 7.5, treated with 1 mM dipropylenetriamine NONOate (Cayman Chemicals), grown for 30 min, and collected by centrifugation at room temp (Hromatka et al. 2005). *Starvation*: cells were grown at 30ºC in YEPD to an OD_{600} of ~1.3, washed in water, resuspended in minimal media (0.7% yeast nitrogen base) and grown for 50 min at 30ºC. *Stationary phase*: cells were grown at 30ºC in YEPD to an OD_{600} of ~13.8. *Filamentous cells*: cells were grown at 30ºC in YEPD to an OD_{600} of ~12, diluted 1:10 in YEPD supplemented with 10% fetal bovine serum (Invitrogen), and grown for 1 h (Kadosh and Johnson 2005). Production of hyphal filaments was confirmed by light microscopy. *Galactose*: Cells were grown at 30ºC in YEPGal (1% yeast extract, 2% peptone, 2% galactose) to an OD_{600} of ~1.4.

RNA extraction and cDNA synthesis

RNA was extracted from frozen cells pellets derived from 10 ml cultures. 2 ml of an acid phenol/$CHCl_3$ mixture (5:1) was added to each pellet, followed by 2 ml of AES buffer (50mM Na acetate (pH 5.3), 10mM EDTA, 1% SDS). Pellet was incubated at 65ºC for 10 min, with

vortexing every min, then transferred to ice for 5 min. 2 ml $CHCl_3$ was added, followed by vigorous hand mixing (15 sec), and centrifugation (2,800*g*, 5 min, 4ºC). Aqueous phase was mixed with 2 ml phenol/$CHCl_3$/IAA (25:24:1), and extracted using a 15 ml heavy phase lock tube (Eppendorf) according to manufacturer's protocol. Extraction was repeated using 2 ml $CHCl_3$, and RNA was precipitated using standard protocols (Sambrook and Russell 2001).

Prior to cDNA synthesis, RNA for RT-PCR analysis was treated with RQ1 DNase (Promega) using manufacturer's protocol. To verify DNase treatment, we ensured that products were not PCR-amplified in the absence of reverse transcription (samples for *SPR28* analysis) and that short intergenic sequences amplified robustly from DNA were not amplified from cDNA (all samples). To prepare cDNA, total RNA was suspended to 500 ng/µl with 500 ng/µl random 9-mer oligos in 50 mM HEPES-KOH (pH 8.3), 75 mM KCl. Samples were heated to 65ºC for 5 min, chilled on ice for 5 min, then brought to room temp. To this was added an equal volume of 50 mM HEPES-KOH (pH 8.3), 75 mM KCl, 6 mM $MgCl_2$, 20 mM DTT, dNTPs (1 mM each) and RNaseH$^-$ MMLV reverse transcriptase according to manufacturer's recommendation. Reactions were incubated at room temp for 15 min, then 42ºC for 2 h. RNA was degraded by treatment with an equal volume of 300 mM NaOH, 30 mM EDTA at 65ºC for 10 min, chilled on ice, and neutralized with the same volume of 300 mM HCl. cDNA was purified on DNA clean & concentrator spin columns (Zymo Research) using manufacturer's protocol. For synthesis of cDNAs for microarrays, RNAs were not DNase treated, and 1 mM dTTP was substituted with 0.6 mM dTTP and 0.4 mM aminoallyl-dUTP.

RT-PCR analysis

cDNA was amplified by PCR using primers in the first and second exons (*RPL30*) or in the first and third exons (*SPR28*). To verify representative intron predictions, cDNAs were amplified using primers in the exons flanking the predicted introns. Reactions contained each primer at 1 µM, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, dNTPs at 200 µM each, Taq DNA polymerase according to manufacturer's recommendation, and for every µl reaction volume, the amount of cDNA derived from 1 ng RNA. Reactions were incubated at 94ºC for 4 min, cycled 32 times through 94ºC (15 sec), 57ºC (30 sec) and 72ºC (1 min), and then held at 72ºC for 7 min. Products were resolved on 1.5% agarose/TBE gels (Sambrook and Russell 2001) containing 100 ng/ml ethidium bromide. Gels were documented using an AlphaImager 2200 (Alpha Innotech). Lanes were plotted and peak volumes quantitated using ImageJ v1.37 (rsb.info.nih.gov).

To identify *RPL30* RT-PCR products, bands were gel-purified and cloned, and representative clones were sequenced. *SPR28* RT-PCR products were cloned directly, without gel-purification, and representative clones sequenced. A subset of the cDNAs used to verify intron predictions were sequenced either directly (when the spliced form predominated) or after cloning.

GO term analysis

The *S. cerevisiae* GO term annotation file (v1.1294) was downloaded from the Gene Ontology Consortium (www.geneontology.org). The annotation of GO terms for *C. albicans* genes was too limited for our analysis. We generated an alternative GO term annotation by mapping terms from *S. cerevisiae* genes onto their *C. albicans* orthologs. We used a complex ortholog map that takes into account gene duplications (Tsong et al. 2006), and assigned all associated *S. cerevisiae* GO terms to the corresponding *C. albicans* ORFs. For intron-containing genes, which often differ substantially from previous annotations, we also used orthologs determined individually in

the course of our annotation (Supplementary Table S1). In total, our GO terms cover 64% of predicted *C. albicans* genes. We performed an over-representation analysis on the GO terms of both *S. cerevisiae* and *C. albicans* intron-containing genes, compared to the corresponding total gene list from each organism, using ErmineJ v2.1.12 (microarray.cpmc.columbia.edu/ermineJ).

References

Bennett, R.J. and Johnson, A.D. 2006. The role of nutrient regulation and the Gpa2 protein in the mating pheromone response of *C. albicans*. *Mol Microbiol* **62:** 100-119.

Hromatka, B.S., Noble, S.M., and Johnson, A.D. 2005. Transcriptional response of *Candida albicans* to nitric oxide and the role of the *YHB1* gene in nitrosative stress and virulence. *Mol Biol Cell* **16:** 4814-4826.

Kadosh, D. and Johnson, A.D. 2005. Induction of the *Candida albicans* filamentous growth program by relief of transcriptional repression: a genome-wide analysis. *Mol Biol Cell* **16:** 2903-2912.

Magee, B.B. and Magee, P.T. 2000. Induction of mating in *Candida albicans* by construction of MTLa and MTLalpha strains. *Science* **289:** 310-313.

Miller, M.G. and Johnson, A.D. 2002. White-opaque switching in *Candida albicans* is controlled by mating-type locus homeodomain proteins and allows efficient mating. *Cell* **110:** 293-302.

Noble, S.M. and Johnson, A.D. 2005. Strains and strategies for large-scale gene deletion studies of the diploid human fungal pathogen *Candida albicans*. *Eukaryot Cell* **4:** 298-309.

Sambrook, J. and Russell, D.W. 2001. *Molecular cloning: A laboratory manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Sherman, F. 2002. Getting started with yeast. *Methods Enzymol* **350:** 3-41.

Tsong, A.E., Tuch, B.B., Li, H., and Johnson, A.D. 2006. Evolution of alternative transcriptional circuits with identical logic. *Nature* **443:** 415-420.