

GENETICS

Supporting Information

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**Comparative Transcriptome Analysis of the CO₂ Sensing Pathway
Via Differential Expression of Carbonic Anhydrase
in *Cryptococcus neoformans***

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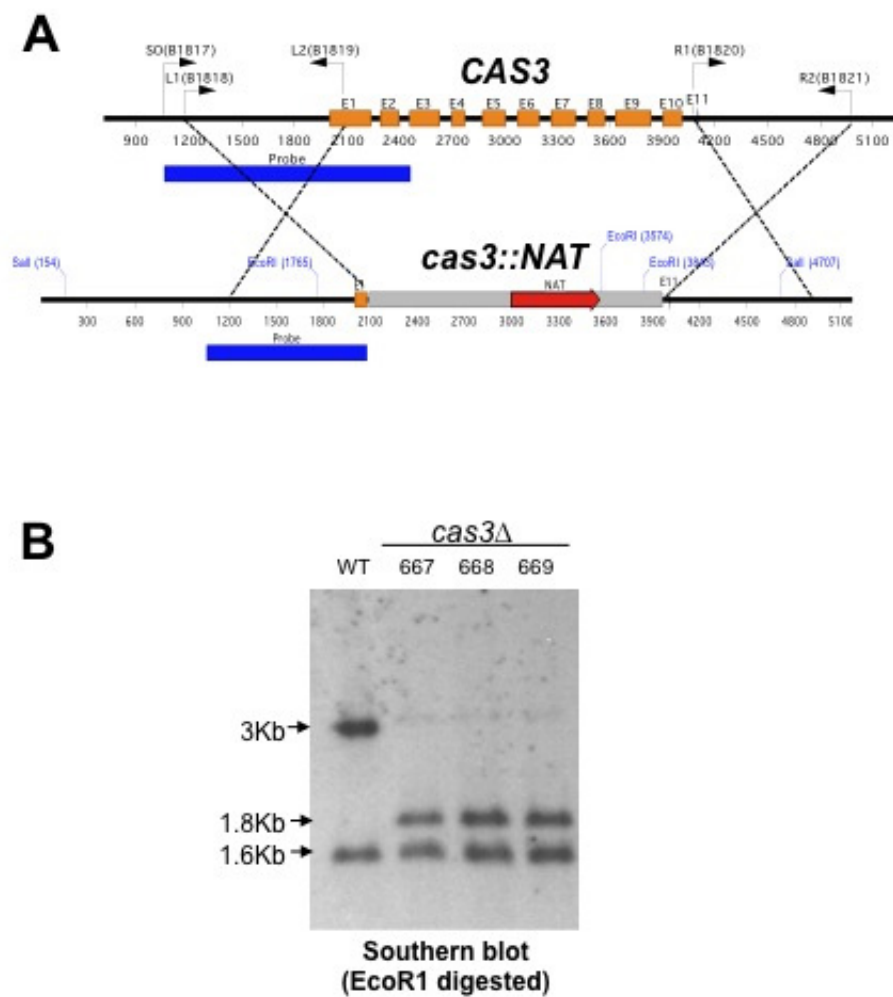


FIGURE S1.—Deletion of the *CAS3* gene. (A) The overlap PCR transformation strategy for disruption of the *CAS3* gene. Primers for overlap PCR and diagnostic PCR are indicated as bent arrows. (D) Southern hybridization was performed to verify positive *cas3Δ* mutants.

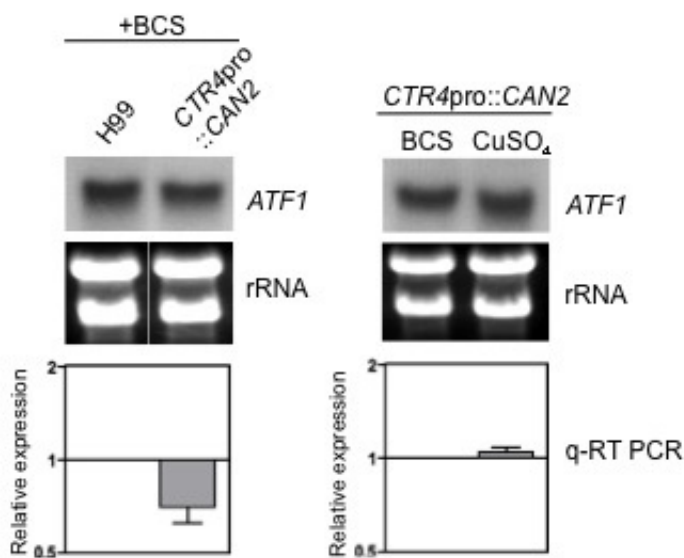


FIGURE S2.—Transcript levels of *ATF1* by overexpression or suppression of *CAN2* expression. Northern blot (top panel) and quantitative RT-PCR (qRT-PCR, bottom panel) show transcript levels of *ATF1* in the wild-type and the $P_{CTR4}::CAN2$ strains grown in YNB+BCS medium (left panel) or the $P_{CTR4}::CAN2$ grown in YNB+BCS and YNB+CuSO₄ medium. For qRT-PCR, data obtained from three independent biological replicates with three technical replicates were normalized by using *ACT1* as a control. Relative gene expression indicates normalized *ATF1* expression levels to those of the wild-type strain (left panel) or the $P_{CTR4}::CAN2$ strain in YNB+BCS medium (right panel).

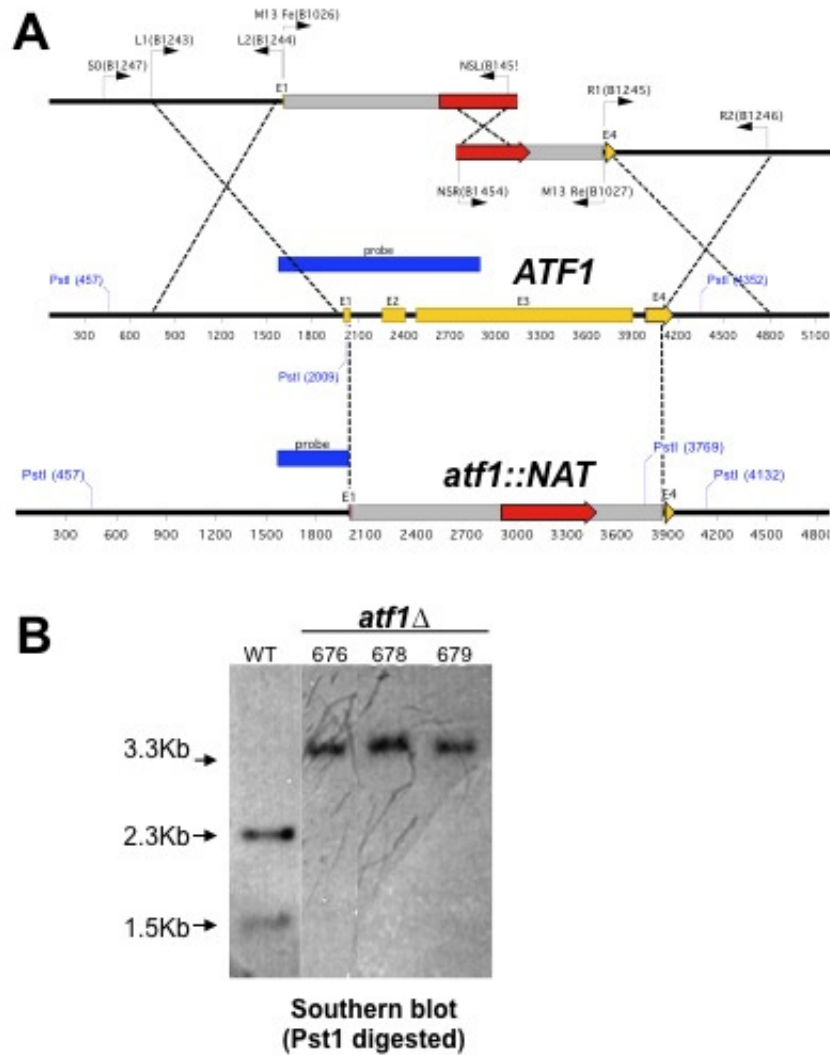


FIGURE S3.—Deletion of the *ATF1* gene. (A) The *NAT* split-marker transformation strategy for disruption of the *ATF1* gene. Primers for the double-joint PCR and diagnostic PCR are indicated as bent arrows. (D) Southern hybridization was performed to verify positive *atf1* Δ mutants.

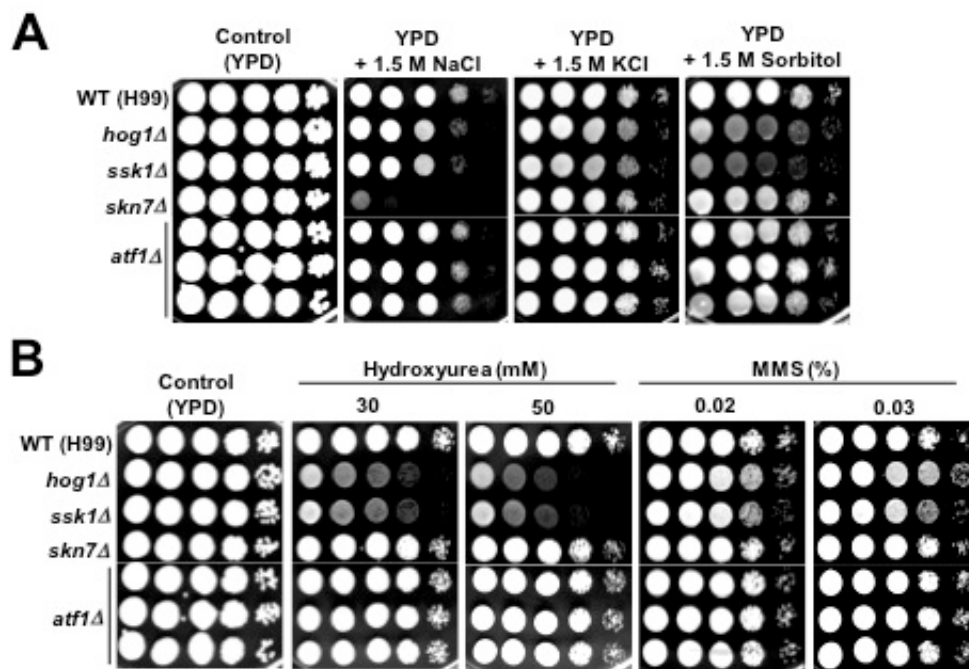


FIGURE S4.—The role of Atf1 in osmotic and genotoxic stress response. Each *C. neoformans* strain (the wild-type H99 strain and *hog1Δ* [YSB64], *ssk1Δ* [YSB261], *skn7Δ* [YSB349], *atf1Δ* [YSB676, YSB678, and YSB679] mutant strains) was grown overnight at 30°C in liquid YPD medium, 10-fold serially diluted (1–10⁴ dilutions), and spotted (3 μl of dilution) on YPD agar containing the indicated concentrations of NaCl, KCl, sorbitol, methylmethane sulfonate (MMS), and hydroxyurea (HU). Cells were incubated at 30°C for 72 h and photographed.

TABLE S1

***C. neoformans* strains and primers used in this study**

Strain	Genotype	Parent	Reference
H99	<i>MATα</i>		(Perfect <i>et al.</i> , 1993)
H99-Eunich	<i>MATα</i>	H99	
BE7-151	<i>MATα atf1Δ::HYG</i>	H99-Eunich	
YSB64	<i>MATα hog1Δ::NAT-STM#177</i>	H99	(Bahn <i>et al.</i> , 2005)
YSB261	<i>MATα ssk1Δ::NAT-STM#205</i>	H99	(Bahn <i>et al.</i> , 2006)
YSB349	<i>MATα skn7Δ::NAT-STM#201</i>	H99	(Bahn <i>et al.</i> , 2006)
YSB733	<i>MATα P_{CTR}::CAN2</i>	H99	This study
YSB734	<i>MATα P_{CTR}::CAN2</i>	H99	This study
YSB735	<i>MATα P_{CTR}::CAN2</i>	H99	This study
YSB667	<i>MATα cas3Δ::NAT-STM#122</i>	H99	This study
YSB668	<i>MATα cas3Δ::NAT-STM#122</i>	H99	This study
YSB669	<i>MATα cas3Δ::NAT-STM#122</i>	H99	This study
YSB676	<i>MATα atf1Δ::NAT-STM#220</i>	H99	This study
YSB678	<i>MATα atf1Δ::NAT-STM#220</i>	H99	This study
YSB679	<i>MATα atf1Δ::NAT-STM#220</i>	H99	This study

Primer Name	Sequence	Comment
B79	TGTGGATGCTGGCGGAGGATA	<i>ACT1</i> promoter screening oligo
B93	CTATGCTGTAATGACTGAGCC	CAN2 CR2
B354	GCATGCAGGATTCGAGTG	NAT/CTR Left
B355	GATTGGTGAAGTCGTTGTCC	NAT/CTR Right
B356	CATTTTCGCTTGCCATAAGTG	CAN2 CSO1
B357	CACCTTCTTGATTGTAGGGG	CAN2 CL1
B358	<u>CACTCGAATCCTGCATGCAAACCCCAACCAACCCCGATTG</u>	CAN2 CL2
B359	<u>CGACAACGACTTCACCAATCATGCCTTTCCACGCTGAACC</u>	CAN2 CR1
B1026	GTAAAACGACGGCCAGTGAGC	M13F extended
B1027	CAGGAAACAGCTATGACCATG	M13R extended
B1243	GAGTAGAGGAGTGGATTGGG	ATF1-L1 for the left flanking region
B1244	<u>GCTCACTGGCCGTCGTTTTACCCATTGTTCAAGCAGAGC</u>	ATF1 L2 for the left flanking region
B1245	<u>CATGGTCATAGCTGTTTCCTGGGCTTACTGCTATCTTGATGC</u>	ATF1 R1 for the right flanking region
B1246	GAAAGAGGAACTACATACTGGGTC	ATF1 R2 for the right flanking region
B1247	GTCTTTTGCTCCTTGAAACC	ATF1 screening oligo paired with B79
B1377	ATTGATTCCAGCAGACCCC	ATF1 southern probe paired with B1780

B1454	AAGGTGTTCCCCGACGACGAATCG	NAT Split marker Right (NSR)
B1455	AACTCCGTCGCGAGCCCCATCAAC	NAT Split Marker Left (NSL)
B1780	ACCAGGTGTAAACCCAGTCC	ATF1 southern probe paired with B1377
B1817	AAAACCTGGGCGGAACACAG	CAS3 screening oligo paired with B79
B1818	CGTCGGACATTCATCAATCTAC	CAS3 L1 for the left flanking region
B1819	<u>GCTCACTGGCCGTCGTTTTACTTGGCAGGAAGAGAGTTATG</u>	CAS3 L2 for the left flanking region
B1820	<u>CATGGTCATAGCTGTTTCCTGGCATAGTGTGGCTGACTTTAG</u>	CAS3 R1 for the right flanking region
B1821	ATGGTCCCCTGCTCACTATCC	CAS3 R2 for the right flanking region
B1829	CCGAAGCCTTTTGTGACTAC	CAS3 southern probe oligo

Each *NAT-STM#* indicates the *Nat^r* marker with a unique signature tag.

References for Table S1

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TABLE S2

Microarray data generated by this study

Table S2 is available for download as an Excel file at <http://www.genetics.org/cgi/content/full/genetics.110.118315/DC1>.

TABLE S3

Genes regulated by differential expression of *CAN2* in the *CAN2* promoter replacement strain growing in either BCS or Copper-containing medium

Table S3 is available for download as a Word (.doc) file at <http://www.genetics.org/cgi/content/full/genetics.110.118315/DC1>.

TABLE S4**Genes regulated by differential expression of *CAN2* between the wild-type strain and the *CAN2* promoter replacement strain growing in BCS-containing medium**

Table S4 is available for download as a Word (.doc) file at <http://www.genetics.org/cgi/content/full/genetics.110.118315/DC1>.

TABLE S5**List of Can2-dependent genes in *C. neoformans***

Table S5 is available for download as a Word (.doc) file at <http://www.genetics.org/cgi/content/full/genetics.110.118315/DC1>.