Transcriptional profiling of the *Candida albicans* Ssk1p receiver domain point mutants and their virulence

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Abstract

The Ssk1p response regulator of *Candida albicans* is required for oxidant adaptation, survival in human neutrophils, and virulence in a disseminated murine model of candidiasis. The activities of the Ssk1p are signaled through the HOG1 MAP kinase pathway (HOG=hyperosmotic glycerol). Further, we have previously shown that the amino acid residues D556 and D513 of the Ssk1p receiver domain are critical to the activities of the Ssk1p in oxidant stress adaptation and morphogenesis. Herein, we use transcriptional profiling to explain the oxidant sensitivity and defect in morphogenesis of two point mutants (D556N and D513K, respectively) compared to a WT strain. In the D556N mutant compared to WT cells during oxidative stress (5mM H_2O_2), a down regulation of genes associated with redox homeostasis and oxidative stress occurred that accounted for about 5% of all gene changes, including among others, *SOD1* (superoxide dismutase), *CAP1* (transcription factor required for some forms of oxidant adaptation), and three genes encoding glutathione biosynthesis proteins (*GLR1, GSH1, and GSH2*). Mutant SSK26 (D513K) was not sensitive to peroxide but was impaired in its yeast to hyphal transition. We noted down regulation of genes associated with morphogenesis and cell elongation. Virulence of each mutant was also evaluated in a rat vaginitis model of candidiasis. Clearance of the *SSK1* null mutant (strain SSK21) and SSK25 from the vaginal canal was significantly greater than wild type (CAF2-1) or the D513K mutant (D513K), indicating that a specific change in a single amino acid of the Ssk1p may alter the ability of this strain to colonize the rat vaginal mucosa.

Keywords

response regulator; two-component proteins; point mutants

Introduction

Signal transduction pathways contribute to many cell functions including adaptation to external and internal stress conditions. Environmental stress such as high oxidants, either self-generated, from microbial competitors, or during phagocytosis by neutrophils or macrophages, requires perception of that signal by cells and relay via phosphotransfer to MAPK pathways. Subsequently, gene transcription occurs such that cells can adapt to oxidants. This paradigm has been suggested based upon mutant data in, for example, *C. albicans*, where strains lacking key phosphotransfer proteins are unable to adapt and

consequently are more sensitive to specific oxidants or unable to undergo morphogenesis (yeast → hyphal transition). The role of anti-oxidant encoding genes in virulence has been established to further substantiate their importance. In *Candida albicans*, the HOG1 MAPK pathway and upstream two component signaling proteins such as Ssk1p are critical to oxidant adaptation and virulence (Alonso-Monge *et al.*, 2003, 1999; Arana *et al.*, 2005; Chauhan *et al.*, 2006, 2003; Enjalbert *et al.*, 2006; 1999; San Jose *et al.*, 1996; Smith *et al.*, 2004). The HOG1 pathway of *Saccharomyces cerevisiae* is critical to osmoadaptation (reviewed in Hohmann, 2002; Maeda *et al.*, 1994; Posas *et al.*, 1998, 1996).

The Ssk1p, a key phosphotransfer upstream protein in the HOG MAPK pathway of *C. albicans*, has previously been shown to provide an oxidant adaptation function (Chauhan, *et al.*, 2003). A null mutant lacking Ssk1p was oxidant sensitive, more easily killed by human neutrophils than a parental, WT strain of *C. albicans*, and avirulent in a mouse model of hematogenously disseminated candidiasis (Chen *et al.*, 2005; Calera *et al.*, 2000; Calera *et al.*, 1999). During peroxide stress in the *ssk1* deletion mutant (strain SSK21), the downstream Hog1p MAPK was unphosphorylated *in vitro* (Chauhan *et al.*, 2003). However, the same mutant exhibited a minor sensitivity to osmotic shock with NaCl and in this stress condition, Hog1p was phosphorylated, indicating that Ssk1p is required for down stream signaling to Hog1 in oxidant but is not critical to adaptation during osmotic stress. In addition to *C. albicans*, the Hog1 homologue (saKa) of *Aspergillus fumigatus* has also been shown to provide oxidant and nutrient adaptation functions (Chen *et al.*, 2006; Xue *et al.*, 2004). In *Cryptococcus neoformans*, processes such as stress adaptation and sexual reproduction require Hog1p, some of which is regulated by Ssk1p, although Skn7p can also provide stress adaptation in *C. neoformans* and *C. albicans* (Bahn *et al.*, 2006; Singh *et al.*, 2004; Wormley *et al*, 2005). Oxidant adaptation in fungi including human pathogenic fungi is described in Chauhan *et al.*, 2006 and Moyer-Rowley, 2003.

Structure-function studies of the *C. albicans* Ssk1p response regulator protein have identified key residues within the receiver domain of this protein that are required for phosphotransfer from Ypd1p, the up stream histidine phosphotransfer (Hpt) intermediate protein (Menon *et al.*, 2006; Calera *et al.*, 1999). Ypd1p-Ssk1p phosphotransfer has been studied extensively in *S. cerevisiae* (Janiak-Spens *et al.*, 1999; Porter *et al.*, 2005, 2003; Stock and West, 2002; Thompson and Kay, 2000; West and Stock, 2001). In *C. albicans*, the D556 of the response regulator (receiver) domain, which is thought to be the site of phosphotransfer from the histidine phosphotransfer protein Ypd1p, corresponds to the D554 of *Saccharomyces cerevisiae* (Hohmann, 2002). In *C. albicans*, mutation of D556 (D556N) resulted in peroxide sensitivity and non-translocation of Hog1p to the nucleus even though the Hog1p is phosphorylated (Menon *et al.*, 2006). In comparison, Hog1p phosphorylation and its nuclear translocation is unaffected in the D556N mutant (SSK25) when cells are stressed in 1.5 M NaCl.

The D513 residue of the response regulator domain is believed to be required for stabilization of the protein during phosphotransfer from Ypd1p. However, in a D513K point mutant (SSK26), peroxide resistance was similar to WT cells but this strain was unable to convert to hyphae in normally inducing conditions (Menon *et al.*, 2006). To examine these mutants for transcript changes, we performed microarray analysis in YPD medium with or without hydrogen peroxide (WT and D556N) or in M199 medium (WT and D513K) at 37°C and compared their transcriptional profiles. Further, we evaluated the consequence of these point mutations in virulence by comparing strains in a rat model of vaginitis.

Materials and methods

Strains and culture conditions

Candida albicans strains used in this study are described in Table 1. Inocula for all experiments were prepared in YPD (1% yeast extract, 2% peptone and 2% glucose) at 30°C with shaking at 200 rpm, cells were washed, and adjusted to a specific cell number in YPD broth (see below) with/without 5mM H_2O_2 or M199 for microarray experiments. For the oxidative stress experiments, we used strains CAF2-1 (WT) and SSK25 (D556N) since the latter but not SSK26 was hypersensitive to peroxide stress. Wt and SSK25 were grown overnight in YPD broth at 30°C. Overnight cultures were than washed, and diluted to an OD_{600} of 0.1 in 500 ml of fresh YPD broth medium and grown at 30°C until cells reached mid-log phase (OD_{600} ~0.5). The mid-log phase cells of both CAF2-1(WT) & SSK25 (D556N) were then stressed with 5mM H_2O_2 for 10 min. At 0 and 10 min post-treatment, cultures were harvested by centrifugation at $3000 \times$ g for 10 min, cells were washed, and the cell pellet was rapidly frozen prior to RNA extraction for microarray experiments. For the microarrays with strains CAF2-1 (WT) and the SSK26 (D513K), cells were grown overnight in YPD, washed, diluted to an appropriate inoculum, added to 500-ml of M199 (pH 7.0), and harvested immediately (0 time) or grown at 37°C for 3 hr in liquid Medium 199 (pH 7.0), a time in which germination approached 90%. After 3h, cultures were harvested by centrifugation at $3000 \times$ g for 10 min, washed, and the cell pellet thus obtained was quick frozen and stored at −80°C for RNA extraction.

Microarray analysis

Total RNA for microarray analysis was extracted with the hot phenol method (Chauhan *et al.*, 2003). Samples were stored at −80°C. DNA microarrays were fabricated at the University of Wisconsin Biotechnology Center by spotting *C. albicans* specific ~75 mer oligonucleotides on slides obtained from Qiagen (Valencia, CA) which correspond to roughly 6000 open reading frames (ORFs). Twenty micrograms of total RNA from each sample was used for microarray analysis. cDNA synthesis, and labeling of samples with Cy3 and Cy5 dyes was performed according to the SuperScript Indirect cDNA Labeling kit (Invitrogen). The Cy3 and the Cy5 labeled samples were hybridized to the array in 25% formamide, 5X SSC, and 0.1% SDS at 42°C overnight (~16 h). Following hybridization, the microarrays were washed at room temperature with the following (2 min each): 2X SSC + 0.1 % SDS, 1X SSC, and 0.2X SSC. The microarrays were then immediately dried and scanned with a GenePix 4000 microarray scanner (Molecular Devices). Two arrays per strain/RNA/condition were run to assess the consistency of data (i.e., technical replicates). For microarray data analysis, the TIGR microarray software suite (http://www.tm4.org/) was used. Experimental data from each hybridization was normalized using a LOWESS analysis and standard-deviation regulation, so that each gene in an array received a normalized expression value. Finally, the normalized data was imported into TIGR Mev (version 3.03), and it was further normalized using total intensity normalization and processed using low intensity cutoff filter, low percentage cutoff and variance data filters. This data was then analyzed by using Self Organizing Tree Algorithm (SOTA) to identify genes that are differentially expressed in mutant over WT strain. We used a >2-fold increase and a 0.5-fold decrease in both mutants compared to WT CAF2-1 strains of *C. albicans* samples to identify up- or down regulated genes.

RT-PCR

For verification of microarray data, RT-PCR was performed with strains CAF2-1, SSK25 (D556N), and SSK26 (D513K) (Table 1) using conditions described above, i.e., SSK25 with or without peroxide, and SSK26 (cultures were compared in YPD and M199). In brief, cultures were grown in 5-ml of YPD at 30°C overnight. These cultures were then used to

inoculate 50-ml of YPD in order to obtain an initial optical density (OD_{600}) between 0.1 and 0.15. All strains were then grown at 30°C until they reached mid log phase (OD_{600} ~0.5), cells were harvested by centrifugation, and total RNA was extracted using the hot phenol method (Chauhan *et al.*, 2003). RT-PCR was performed using 1 µg of total RNA with primer sets for genes whose transcription was altered in each mutant (Table 2). Samples were processed by the one-step RT-PCR kit according to the manufacturer's instructions (Qiagen, Valencia, CA.). 10 µl of each PCR amplification reaction was separated on a 1% agarose gel and stained with ethidium bromide. *ACT1* served as an internal loading control.

Rat vaginitis model

The virulence of strains CAF2-1 (*SSK1/SSK1*), SSK21 (*ssk1/ssk1*), SSK25 (*ssk1/SSK1* D556N), and SSK26 (*ssk1/SSK1* D513K) in a rat model of experimental vaginal candidiasis was evaluated according to the protocol described by De Bernardis *et al.*, 2002. Each *C. albicans* strain was grown in YPD medium at 28°C overnight. Cells were harvested by centrifugation, washed twice in calcium-and magnesium-free phosphate-buffered saline (PBS; Gibco-BRL) and suspended to a required density on the basis of hemocytometer counts prior to use. Ovariectomized, female, Wistar rats (80 to 100 g; Charles River, Wilmington, MA.) were injected subcutaneously with 0.5mg of estradiol benzoate (Benzatrone; Samil, Rome, Italy). Six days after the first estradiol treatment, groups of five rats were inoculated intravaginally with 10^7 yeast cells per 0.1 ml of each strain tested. The inoculum was dispensed into the vaginal cavity through a syringe equipped with a multipurpose calibrated tip (Combitip; PBI, Milan, Italy). Enumeration of *C. albicans* in the vaginal cavity was achieved by culturing 0.1-ml samples (using a calibrated plastic loop, Disponoi; PBI), obtained from each animal at designated times from 0 – 28 days, on Sabouraud agar containing chloramphenicol (50µg/ml). Cultures were incubated at 28°C for 48 to 72 h. For each time point per strain, a total of four Saboraud agar plates were inoculated and the average cfu/ml of vaginal fluid was obtained. Experiments were repeated at least one additional time.

Results

Microarray analysis of genes down regulated during oxidative stress in SSK25

For these experiments, strain SSK25 was compared to WT since we have previously reported its sensitivity to oxidants such as hydrogen peroxide (Menon *et al.*, 2006). Cells of WT and SSK25 (D556N) were grown under identical conditions, *i.e.*, YPD at 30°C with or without 5mM H_2O_2 for 10 min and their transcriptional profiles compared. The effect of oxidative stress on the transcription of WT and strain SSK25 resulted in a change of approximately 256 transcripts (both up and down regulated genes). The three largest groups of transcriptionally altered genes in peroxide-treated SSK25 (D556N) either encode proteins related to cell growth and maintenance (35%), energy metabolism/pathways (20%), or unknown functions (20%) (Supplementary data, S1).

Of all transcriptional changes, we chose to focus upon the family of redox homeostasis & oxidative stress response genes, since the SSK25 mutant was peroxide sensitive (Menon *et al*, 2006). Of those, we observed 11 genes assigned an anti-oxidant function that were down-regulated to the greatest extent (see methods) in SSK25 compared to the WT cells (about 4.3% of the total changes) In Table 3, microarray data are expressed as a differential expression ratio (SSK25/WT) in the presence of peroxide. Adaptation to oxidative stress requires the induction of genes that play a role in detoxification of the reactive oxygen intermediates (ROI), like superoxide, peroxides, and hydroxide radicals. To verify the change in transcription of oxidative stress genes listed in Table 3, we performed RT-PCR of those genes. Each of the down regulated genes listed in Table 3 as determined by micorarray

also was down regulated in strain SSK25 as determined by RT-PCR compared to wt cells (Figure 1). In this regard, the down regulation was either greater for peroxide stress (*GSH1, ARH1, GSH2, TSA1* and *CAP1*) or occurred about equally in strain SSK25 with or without peroxide stress, for example *FKH2, GLR1, TRR1,* and *SOD1*. Two significantly up regulated genes were also identified by microarray analysis (Table 3) (*HSP30, TPS3*), but their regulation was verified by RT-PCR reactions only for *HSP30* (Figure 1). The decreased transcription of the genes that are involved in oxidative stress adaptation in strain SSK25 may explain in part the oxidant sensitive phenotype of the mutant compared to the strain CAF2-1.

Other oxidative stress profiling studies in *C. albicans* have revealed induction of genes that function in the adaptation to reactive oxygen species (Smith *et al.*, 2004; Enjalbert *et al.*, 2006). In these studies, most of the core functions required for tolerance to peroxide stress are involved in vacuolar protein sorting, cell wall biosynthesis and maintenance, transcription and protein synthesis. Transcription of genes encoding proteins involved in mitochondrial function, ubiquitin processing, and the pentose phosphate pathway were also up regulated in response to oxidative stress.

Microarray analysis of genes down regulated in SSK26 (D513K) grown in the hyphal inducing medium M199

We also compared the transcriptional profiles of strains SSK26 and CAF2-1. In these experiments, strains were grown in Medium 199 (37°C) because of the constitutive yeast morphology of strain SSK26 compared to the filamentous growth of CAF2-1. Profiling of wild type and SSK26 (D513K) under non-inducing and hyphal-inducing conditions revealed that a total of 283 genes were affected, a majority of which were up regulated in the wild type strain under these hyphal inducing conditions (data not shown). In this case, those genes that encode functions related to cellular metabolism and cell maintenance displayed the largest number of transcriptional changes (40%) (Supplementary data, S2). Since strain SSK26 is defective in its conversion of yeast to hyphae, we focused on the gene group associated with among other things, cell elongation, and cytoskeleton functions (5% of total change). The following genes were down regulated in SSK26 compared to WT cells: *HYR1, HWP1, ECE1, MIG1, GCN4, RFG1 (ROX1), MSB1* (cell polarity), *RBF1, RIM101, HAC1, HAP5, TUP1, NRG1, EFG1,* and *CPH1* (Table 4). Most of these genes either are required for or up regulated during hyphal growth of the organism (Kumamoto & Vinces, 2005). Again, RT-PCR was done to verify microarray data (Figure 2). As with strain SSK25, transcriptional changes in SSK26 either were specific for conditions (in this experiment) inducing hypha formation (M199 pH 7.0) or occurred regardless of whether cells were induced (M-199 medium) or not induced to form hyphae (YPD medium). Thus genes *NRG1, EGF1, CPH1, TUP1, RBF1, HYR1, MIG1, GCN4, HAC1, RIM101,* and *HWP1* were all down regulated in both growth conditions (Figure 2). Genes down regulated in M-199 medium more than YPD included, *ECE1, SPT3, HAP5,* and *MSB1* (Figure 2). Surprisingly, genes encoding yeast transcription factors or suppressors of hyphal growth (*TUP1, MIG1, RBF1, and NRG1*) were also down regulated in strain SSK26 even though SSK26 constitutively produces yeast growth (Table 4, and Figure 2).

Virulence in a rat model of candida vaginitis—The virulence of each point mutant as well as strain SSK21 (*ssk1/ssk1*) and the wild type strain CAF2-1 was evaluated in a rat model of candida vaginitis (Table 5). This model measures persistence on the vaginal mucosa as a disease index. Our data indicate that Ssk1p is required for optimal colonization of the rat vaginal mucosa and that maximal virulence also requires the D556 residue in the receiver domain. It would appear that mutation to D513 (D513K), even though a non-conservative change, has little effect on virulence. The reduced vaginal colonization by

strain SSK21 may be related to the down regulation of the host recognition protein Als1p (<u>a</u>gglutinin-<u>l</u>ike <u>s</u>equences) in this strain (Chauhan *et al.*, 2003;Hoyer, 2001). Strain SSK21 (*ssk1/ssk1*) is also less adherent to human esophageal tissue *in vitro* and that phenotype may be due to the same transcriptional change just described (Li *et al.*, 2002;Bernhardt, *et al.*, 2001).

Discussion

The Hpt (<u>h</u>istidine <u>p</u>hospho<u>t</u>ransfer protein) Ypd1p of fungi such as *C. albicans* (Calera *et al.*, 2000) and *S. cerevisiae* (Hohmann, 2000) is phosphorylated by the Sln1p membrane-associated histidine kinase. The Ssk1p response regulator protein in turn catalyzes and is the substrate for phosphate transfer from Ypd1p during unstressed conditions such that phosphorylated Ssk1p is unable to activate the Ssk2/22 MAPKKK of the HOG1 MAPK pathway. In oxidatively- or osmotically-stressed cells, phosphotransfer does not occur among these proteins and unphosphorylated Ssk1p in turn now activates the HOG1 MAPK via the Ssk2/22 (MAPKKK) and Pbs2p (MAPKK) proteins resulting in cell adaptation to stress (Arana *et al.*, 2005; Walia and Calderone, 2008).

Phosphotransfer from Ypd1p requires the D556 amino acid residue of the Ssk1p receiver domain. Paradoxically, since the Ssk1p of the D556N mutant is not phosphorylated, then that mutant should behave similarly to peroxide resistant wild type cells at comparable concentrations of the inhibitor. However, the D556N mutant is oxidant sensitive (Menon *et al*, 2006). Our explanation of this observation relates to the fact that for adaptation to oxidant stress to occur, phosphorylated Hog1p (via Ssk1p activation of the HOG1 MAPK pathway) must translocate to the nucleus for transcriptional regulation of stress genes. We have previously shown that during peroxide stress, in fact while Hog1p is phosphorylated, it is not translocated to the nucleus; hence, the D556N mutant is oxidant sensitive. Also, it would appear that the D556 residue, in addition to its phosphorylation role, also is either directly or indirectly required for Hog1p translocation. Interestingly, the D556N mutant is not osmosensitive, but Hog1p is both phosphorylated and translocated to the nucleus (Menon *et al*, 2006). In this case, we have previously shown in *C. albicans* that the Ssk1p-driven Hog1 MAPK pathway is not critical to osmoadaptation (Chauhan, *et al*, 2003). Based upon this observation, we speculate that the Sho1p–Ypd1p-Pbs1p-Hog1p driven pathway (or another) appears to be more important to provide osmoadaptation.

In *E. coli*, the D13 residue of the CheY RR protein is critical to the phosphorylation of D57 (D556 in *C. albicans*) and forms part of a divalent metal binding site that is required for flagella rotation and chemotaxis (Stock *et al*, 1985). Because of the critical role this residue plays in phosphotransfer among RR proteins, we also examined its function in the Ssk1p of *C. albicans* (Menon *et al*, 2006). We found that the D513K mutant like wt cells was oxidant- and osmoresistant, and that Hog1p was phosphorylated and able to translocate to the nucleus under both stress conditions. However, a major difference in the D513K mutant versus the D556N mutant was that in the D513K mutant, Hog1p was constitutively phosphorylated in the absence of stress and in hyphal-inducing media (Menon *et al.*, 2006). We hypothesized that constitutive activation of Hog1p is at least partially responsible for the morphogenesis defect of D513K (failure to undergo hyphal conversion), analogous to the sustained phosphorylation of Hog1p in *S. cerevisiae* which results in at least a partial activation of the pheromone response pathway in cells that are osmotically stressed (Yale and Bohnert, 2001).

In order to investigate the transcriptional profiling of the D556N and D513K mutants, we used microarray analysis. In these experiments, entire arrays were done of both mutants, but data presented above focused upon gene families that might reflect the oxidant sensitive

phenotype in the D556N, while in the D513K mutant, gene families associated with morphogenesis functions were highlighted. Since the morphogenesis phenotype of D556N and the oxidant phenotype of D513K each was like wt cells, our mutants were only compared to wt cells. However, the entire microarray analysis of each mutant is available as supplementary data, S1 and S2.

Transcriptional profiling of the D556N point mutant correlates with its peroxide sensitivity, as 9 genes associated with oxidative stress adaptation were down regulated as determined by microarray and that result was verified by RT-PCR during peroxide stress. As for the D513K mutant, 15 genes (by microarray) with functions associated with morphogenesis in *C. albicans* were altered transcriptionally compared to wt cells. By RT-PCR, of these 15 genes, transcriptional changes fell into 2 categories; four genes were down regulated in M199 only (M199 medium induces hyphal formation), while nine genes were down regulated in both YPD and M199. Thus, the phenotype of each mutant correlated with transcriptional changes as verified by RT-PCR from microarray data.

To investigate the requirement of the D556 and D513 amino acids in virulence, we used the rat model of vaginitis which is distinguished from the human disease in at least two important ways. First, cellular infiltrates including neutrophils are not observed and second, the rat vaginal canal does not harbor a commensal population of *C. albicans* (De Bernardis *et al.*, 2002). Nevertheless, the model has been useful in evaluating a number of *C. albicans* mutants for virulence. We show that the D556N mutation but not D513K affects colonization. The reasons for this difference in virulence are not understood. In summary, we have identified D556 as an important residue in at least the oxidative stress response and virulence, probably through its activation of the HOG1 MAPK pathway. As candidiasis continues to be an important disease of patients (Wisplinghoff, 2004), Ssk1p may be useful target in drug discovery. In fact, recent data indicate that ssk1 deletion mutants are hypersensitive to fluconazole and voriconazole both of which are cidal to the mutant (Chauhan *et al*, 2007).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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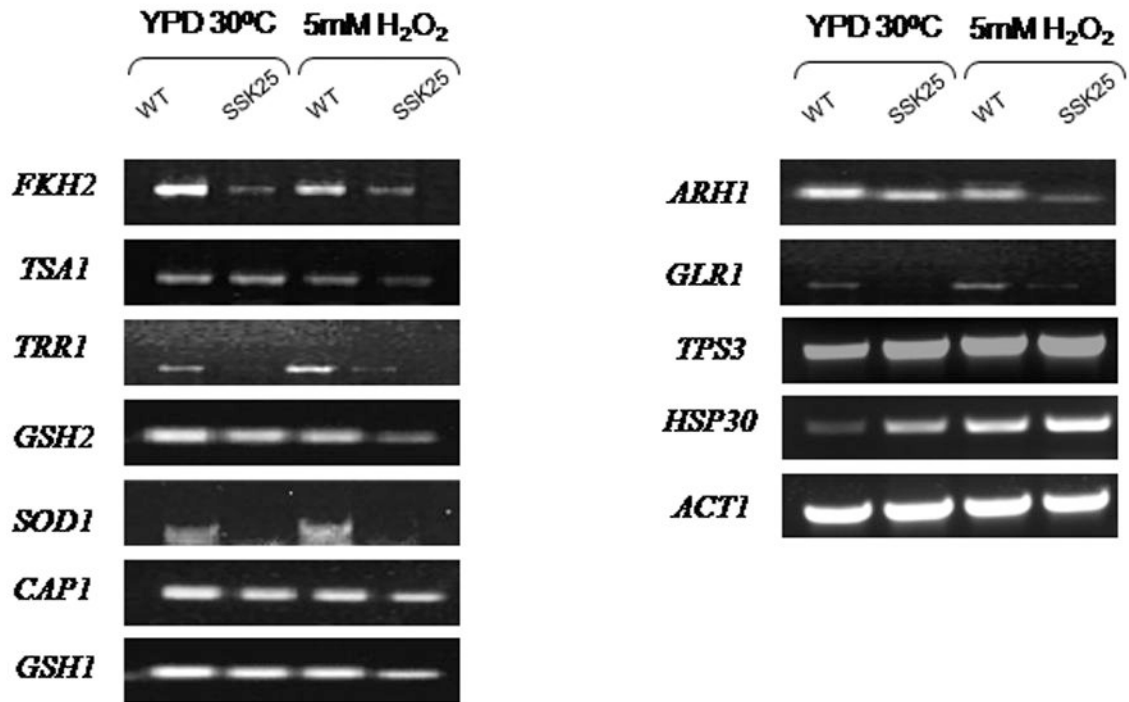
Figure 1.
RT-PCR of SSK25 genes that are down regulated during peroxide stress (YPD + 5mM H_2O_2) compared to WT cells. The genes represented were chosen for analysis based upon microarray data of only those associated with redox homeostasis and oxidative stress response. CAF2-1 and D556N were grown in YPD + peroxide as described in the text. RT-PCR reactions are shown for both strains with and without H_2O_2. *ACT1* was used as a loading control
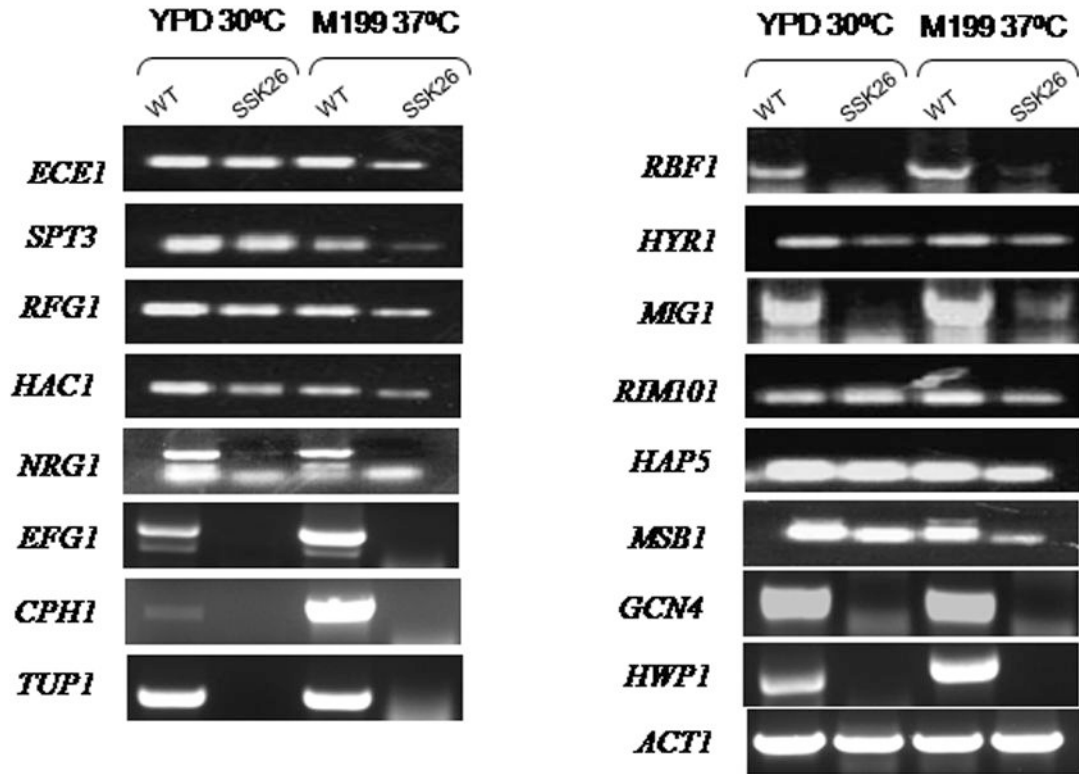
Figure 2.
RT-PCR of genes from SSK26 (D513K) that are down regulated compared to WT during growth in YPD or M-199 medium. The genes represented were chosen for analysis based upon microarray data of only those associated with cell cycle, DNA replication and cell elongation and include reactions for both strains grown in YPD or M199 medium. *ACT1* was used as a loading control

Table 1

Strains of *C .albicans* used in this study

Strains	Genotype	Reference
CAF2-1	*URA3/ura3Δ∷imm434*	Fonzi & Irwin, 1998
SSK21	*ssk1:hisG-URA3-hisG/ssk1∷hisG ura3Δ∷imm434/ura3Δ∷imm434*	Calera *et al.*, 2000
SSK25	*ssk1∷hisG/SSK1* (D556N)-*URA3 ura3Δ∷imm434/ura3Δ∷imm434*	Menon *et al.*, 2006
SSK26	*ssk1∷hisG/SSK1* (D513K)-*URA3 ura3Δ∷imm434/ura3Δ∷imm434*	Menon *et al.*, 2006

Table 2

Primer sets for RT-PCR reactions.

Gene	Sequence (5' – 3')
ECE1-F	GCTTTATCTTCTCAAGCTGCC
ECE1-R	GAAAGACTTTGTTAAACTCGTCG
SPT3-F	GTTGGTGTAGGAGCCG
SPT3-R	GCCATATCATATAGAG
RFG-F	CTTCCTAATAATAAATC
RFG-R	GATTATAGTCTCAATG
HAC1-F	CATGTTGAAAAACAAGAA
HAC1-R	GAATCATTTCAGTTGG
NRG1-F	AATTGACGGTGGTTGCACGTTGTC
NRG1-R	CATGTTGGCCATGGACATTGGTGT
EFG1-F	ACAACCAGGTCAACAGACTGGACA
EFG1-R	ACCAGACACATTACTGCCACCACT
CPH1-F	ACACAGAAGAGGATCAACGACGCA
CPH1-R	AGCAGTAGTAGCAACTGCACCAGA
TUP1-F	AGTGTTCAATGTCACCACCGGAGA
TUP1-R	CTTGCCTTCCAAGTGCCACAACTT
RBF1-F	GCGCAAGCTCAAAGAGAACACCAA
RBF1-R	TGCTGTTGCAGTTGTTGACTCTGG
HYR1-F	GGTGGAATTCAAGGTTTCCATG
HYR1-R	GTCGTAAAAACTCTGGTGCGTC
MIG-F	CAGCAACAATATTACCAAC
MIG-R	CAACAATCAACAACAACAG
RIM101-F	GGATACTACTCAAACAAC
RIM101-R	GCACAAGCTCCACAGC
HAP5-F	GACGACCAGCTTCCGCAG
HAP5-R	CATGCAGGAGGACATTAACTGC
MSB1-F	GCTGGTATTATGGACCCAGC
MSB1-R	CACCAACTATGTGTTTGTGTCC
GCN4-F	TATTGGAGTCGGGTTTCAGCACCA
GCN4-R	GCAACAACAACTTCACCACCAGCA
HWP1-F	ACCCACAACAACAACCACAAGAGC
HWP1-R	TGTTTCAGTGGCTGGAGCAGTAGA

Gene	Sequence (5' – 3')
FKH2-F	GCTCCAGAGGAGAAAACCAATG
FKH2-R	CATACGCATTACTAGTCGTTAGG
TSA1-F	GGCTCCAGTCGTTCAACAAC
TSA1-R	GATCATATTTAGTCCGAGCTTAG
TRR1-F	GCTGCTGGTGGTCAATTGAC
TRR1-R	CATCTTCGACAAGGACCTCAC
GSH2-F	GTTATGTACCCACCCAACTTTG
GSH2-R	GGAGATTATCGTTGGATCGTCG
SOD1-F	GGTTAAAGCTGTCGCTGTTG
SOD1R	CTACCGTTAGAACCGAGTAG
CAP1-F	GTTTCTACCGGTACACCTC
CAP1-R	GACAACTTTGATCTTGC
GSH1-F	GAGTTTATCAGCGGCAGCACC
GSH1-R	GAGAGGTTGGACCAAATCATC
ARH1-F	GATACGGGGTAGCACCAGATC
ARH1-R	GTTGAAACACCCCTCGATTTCC
GLR1-F	CCTCTGCAAGAAGAGCTGCCAAAT
GLR1-R	AATGTAACCAGCACCGACAATGGC
TPS3-F	ACCCGCAGCTGCTATTCCAGATAA
TPS3-R	TGTCGCGAACCATCTTGGGAACTA
HSP30-F	GCTTGGTTCTTGGGTTGGCCATTA
HSP30-R	AACACCAGTGTCTGGAACACCTGA

Table 3

Genes that encode redox potential or anti-oxidant proteins that are differentially expressed in strain D556N Ssk1p response regulator by microarray analysis are listed.

Gene name	Description	Differential expression ratio[1]
GSH1	Gamma-glutamylcysteine	0.18
GLR1	Glutathione reductase, Cap1-induced	0.16
GSH2	Glutathione synthase	0.30
TSA1	Alkyl hydroperoxide peroxidase	0.24
TRR1	Thioredoxin reductase	0.23
SOD1	Cu,ZN-superoxide dismutase	0.28
ARH1	Adrenodoxin reductase and ferredoxin-NADP+ reductase	0.18
FKH2	Forhkead transcription factor	0.21
CAP1	Transcription factor, oxidative stress	0.50
HSP30	Heat shock protein, response to stress	5.10
TPS3	trehalose-phosphate synthase, regulatory subunit	2.90

[1]Differential expression is calculated as the following ratio: D556N/WT under conditions of peroxide stress (YPD + 5mM H_2O_2).

Table 4

Microarray analysis of genes that encode morphogenesis functions that are differentially expressed in the Ssk1p response regulator D513K point mutant, strain SSK26.

Gene name	Description	Differential expression ratio
HYR1	Hyphal regulated protein	0.33[1]
HWP1	Hyphal cell wall protein	0.37
ECE1	Cell elongation protein	0.31
MSB1	Cell polarity-related protein	0.28
MIG1	Transcriptional hyphal suppressor	0.15
HAC1	Transcription factor	0.5
TUP1	Repressor of hyphal formation	0.14
RFG1	Hyphal regulator	0.4
GCN4	Transcriptional activator, filamentous growth	0.22
RIM101	Alkaline induction of filamentation	0.27
HAP5	Transcription, morphogenesis	0.29
RBF1	Transcription factor	0.18
NRG1	Transcriptional repressor, hyphal growth	0.17
EFG1	Transcription factor, hyphal growth	0.19
CPH1	Transcription factor, hyphal growth	0.2

[1] As in Table 3, data are expressed as D513K/WTt in Medium199, 37°C.

Table 5

Vaginal colonization by strains of *C. albicans* (CAF2-1, wt; SSK21,*ssk1/ssk1;* SSK25, D556N; and SSK26, D513K). Rats were infected intravaginally with 10^7 yeast cells. Vaginal samples were taken at the times indicated, aliquots plated on Sabouraud agar medium containing chloramphenicol, and cfu per ml determined.

CFU/ml VAGINAL FLUID × 10^3				
DAYS	**CAF2-1**	**SSK21**	**SSK25**	**SSK26**
0	>100	>100	>100	>100
1	>100	60±2	50.5 ± 1.8	75.8 ± 1.8
2	95 ± 3.1	58±2.4	43.2 ± 2.6	67.6 ±1.4
5	77 ± 2.5	46±2	41.4 ± 2.5	60.2 ± 1.3
7	60.7 ± 1.1	40±1.5	35.6 ± 1.8	53.6 ± 1.2
14	38.5 ± 1.1	19±2.2	11.2 ± 0.8	28.8 ± 2.8
21	19.6 ± 1.2	12±1.7	4.8 ± 1.4	12.8 ±1.2
28	0	0	0	1.2 ± 0.6