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The *Candida albicans* histidine kinase Chk1p: Signaling and cell wall mannan

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ABSTRACT

Several published functions associated with the *CHK1* histidine kinase of *Candida albicans* resemble those of the MAPK Cek1p and its cognate receptor Sho1p (*SSU81*). To explore this further, we have compared mutants lacking the proteins mentioned above and have constructed a double *sho1*/*chk1*Δ null mutant to determine relationships among these proteins. We observed that the sensitivity to Congo red (CR), calcofluor white (CW), as well as clumping of cells, was slightly increased in the double mutant compared to the single *chk1*Δ or *sho1*Δ mutants. However, Cek1p phosphorylation via Sho1p, which occurs during log phase growth in the presence or absence of CR in Wt cells, does not require Chk1p. These data suggest that Chk1p and Sho1p are components of parallel but independent signal pathways. In addition, bulk mannan of strains was analyzed by GLC/MS and GPC MALLS and NMR. Compared to Wt and a *CHK1* gene-reconstituted strain (CHK23) that contained high, intermediate and low Mw mannan species, we found that the mannan of strains CHK21 (*chk1*Δ null), the *cek1*Δ null, and the double mutant consisted only of low Mw mannan. The *sho1*Δ null mutant only demonstrated a reduced intermediate type of mannan. Alcian blue binding was lower in *cek1*Δ, *chk1*Δ, and the double *sho1*/*chk1*Δ null mutant lacking high and intermediate Mw mannan than in the *sho1*Δ null which had a partial loss of intermediate Mw mannan only. We conclude that the Chk1p HK is part of a functionally similar but parallel pathway to the Sho1p-Cek1p pathway that confers resistance to the cell wall inhibitors CR and CW. However, a functional relationship in mannan biosynthesis of Chk1p and Cek1p exists that only partially requires Sho1p.

1. Introduction

Signal transduction via MAPK pathways is critical to the adaptation of fungi and other microorganisms to their environment. For human pathogenic fungi, expression of virulence factors, morphogenesis, stress adaptation, and drug resistance are associated with signaling via MAPK pathways. In *Candida albicans*, at least four MAPK pathways have been identified and their functions partially described, including Cek1 (growth and cell wall construction), Cek2 (mating), Mkc1 (cell wall integrity and stress adaptation), and Hog1 (cell wall and stress adaptation) (Alonso-Monge et al., 2006). In yeast, the Hog1 MAPK (high osmolarity glycerol) consists of three upstream, phosphotransfer proteins, including a membrane-bound, histidine kinase (HK, Sln1p), a histidine intermediate protein (Hpt, Ypd1p) that in turn is critical to the phosphotransfer from Sln1p to the third protein, the Ssk1p response regulator protein. Ssk1p then activates the Hog1 MAPK pathway during stress (Hohmann, 2002). Compared to bacteria which accomplish phosphotransfer on two proteins (hence the name 2-component), the eukaryotic system is referred to as 3-component signal transduction (Beier and Gross, 2006). The role of the *C. albicans* Hog1 MAPK pathway in adaptation to high osmolarity, oxidative stress, morphogenesis, cell wall biosynthesis, and virulence has been documented (Alonso-Monge et al., 2006, 2003; Arana et al., 2007, 2005; Calera et al., 2000a,b; Chauhan and Calderone, 2008; Chauhan et al., 2006; Eisman et al., 2006; San Jose et al., 1996).

C. albicans has two additional HK proteins that are found in other fungi but not in *Saccharomyces cerevisiae*, including Chk1p and Nik1p, both of which have been extensively studied (Alex et al., 1998; Calera et al., 1998; Calera and Calderone, 1999; Calera et al., 1999; Kruppa and Calderone, 2006; Kruppa et al., 2004a,b, 2003; Li et al., 2004, 2002; Nagahashi et al., 1998; Selitrennikoff et al., 2001; Srikantha et al., 1998; Torosantucci et al., 2002; Yamada-Okabe et al., 1999). Thus, in *C. albicans*, there are 3 HK

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proteins, although both the Chk1p and Nik1p have not been assigned to a MAPK pathway.

Three-component proteins of fungi may be important drug targets, an hypothesis based upon their conservation among human pathogens and important functions in virulence, such as the Sln1p homologue of *Blastomyces dermatitidis* that is required for dimorphism and transcription of virulence factors as well as in *Cryptococcus neoformans* of which functions have been assigned to stress response adaptation, drug sensitivity, sexual development, and virulence (Bahn et al., 2006; Nemecek et al., 2006). Interestingly, for *C. albicans*, a double histidine kinase mutant (*sln1/nik1*Δ) could not be isolated suggesting that these proteins play a major role in growth of the organism (Yamada-Okabe et al., 1999).

Functions have been assigned to the Chk1p of *C. albicans* based upon mutants lacking the gene (Calera and Calderone, 1999; Calera et al., 1999; Kruppa et al., 2004a,b, 2003; Li et al., 2002; Nagahashi et al., 1998; Yamada-Okabe et al., 1999). For example, a deletion mutant lacking *CHK1* (strain CHK21) is avirulent in a murine model of hematogenously disseminated candidiasis and has decreased levels of adherence to human esophageal cells (Bernhardt et al., 2001; Calera et al., 1999; Li et al., 2002). Further, the mutant heavily agglutinates *in vitro* (Calera and Calderone, 1999), and this phenotype, along with its inability to bind to host cells, suggests that the mutant has alterations in its cell surface. To that end, we have demonstrated that changes occur in the proportions of β-glucans and by Western blot that the acid-stable mannan side chain is truncated (Kruppa et al., 2003).

Common phenotypes have been reported for *CHK1* (herein), *SHO1*, and *CEK1* null mutants, including their sensitivity to Congo red that is not observed in other MAPK signal pathway mutants (Alonso-Monge et al., 2006; Eisman et al., 2006; Roman et al., 2005). Also, both the *chk1*Δ and *sho1*Δ mutants clump extensively *in vitro*, but those data were reported using different media (Calera and Calderone, 1999; Roman et al., 2005). As stated above, Sho1p is an upstream protein of the Cek1p MAPK pathway. To determine functional relationships among these proteins, a double *chk1/sho1*Δ mutant was constructed and phenotypically compared to single mutants of *CEK1*, *CHK1*, and *SHO1*. We have demonstrated that Chk1p and the Cek1p MAPK (and partially Sho1p) have common functions in mannan biosynthesis, but Chk1p is part of a parallel but independent pathway of Sho1p or Cek1p in regard to CR and CW resistance.

2. Materials and methods

2.1. Strains and growth conditions

All *Candida albicans* strains previously described or constructed in this study are listed in Table 1. Cells were grown at 30 °C in YNB medium consisting of 2% glucose, 0.67% yeast nitrogen base without amino acids, supplemented with uridine (25 μg ml^{-1}) or in YPD broth. A *sho1/chk1*Δ double mutant (strain REP36-1) was constructed as described below. Null mutants in the MAPK genes *hog1*Δ, *mkc1*Δ, *cek1*Δ, and *cek2*Δ were used in agglutination experiments (Chen et al., 2002; Eisman et al., 2006; San Jose et al., 1996). The Sho1R (REP5) and Cek1R (CK43B-R1) are reintegrant strains of the *sho1*Δ and *cek1*Δ null mutants, respectively (Table 1).

2.2. Construction of the sho1/chk1Δ double mutant

Disruption of *SHO1* was completed in strain CHK22, a Uri$^-$ mutant lacking the *CHK1* gene, described in Table 1. Standard molecular biology procedures were used for construction of a double *sho1/chk1*Δ null mutant (REP36-1) using the same disruption cassette described by Roman et al. (2005).

2.3. Drop plate assays with inhibitors

We followed standard procedures for these assays (Chauhan et al., 2003). To determine the sensitivity of strains to Congo red (CR) and calcofluor white (CW), we used drop plates containing 50 μg ml Congo Red (CR) (ICN Biomedicals Inc.) or 24 μg/ml of CW in YPD agar. Cells for assays were prepared as overnight

Table 1
Candida albicans strains used in this study.

Strains	Relevant genotype	Reference
CAF2	*ura3*Δ*::imm434/URA3*	Fonzi and Irwin (1993)
CAI4	*ura3*Δ*::imm434/*Δ*ura3*Δ*::imm434*	Fonzi and Irwin (1993)
CHK21	*ura3*Δ*::imm434/ura3*Δ*::imm43* *chk1*Δ*::hisG/chk1*Δ*::hisG-URA3-hisG*	Calera and Calderone (1999)
CHK22	*ura3*Δ*::imm434/ura3*Δ*::imm43* *chk1*Δ*::hisG/chk1*Δ*::hisG*	Calera and Calderone (1999)
CHK23	*ura3*Δ*::imm434/ura3*Δ*::imm434* *chk1*Δ*::hisG/CHK1::URA3-hisG*	Calera and Calderone (1999)
Rm100	ura3Δ::imm434/ura3Δ::imm434 his1Δ::hisG/his1Δ::hisG-URA3-hisG	Roman et al. (2005)
REP3	*ura3*Δ*::imm434/ura3*Δ*::imm434 his1*Δ*::hisG/his1*Δ*::hisG* *sho1*Δ*::SHO1-GFP*	Roman et al. (2005)
REP5	*ura3*Δ*::imm434/ura3*Δ*::imm434 his1*Δ*::hisG/his1*Δ*::hisG* *sho1*Δ*::hisG/sho1*Δ*::hisG-URA3-hisG::SHO1-GFP*	Roman et al. (2005)
REP36-1	*ura3::imm434/ura3::imm434 chk1*Δ*::hisG/chk1*Δ*::hisG sho1*Δ*::hisG/sho1::hisG-URA3-hisG*	This study
REP1	*ura3*Δ*::imm434/ura3*Δ*::im434 hisG/his*Δ*::hisG SHO1/sho1::hisG-URA3-hisG*	
CK43B-16	*cek1*Δ*::hisG/cek1*Δ*::hisG-URA3-hisG*	Csank et al. (1998)
CK43B-RI	*ura3/ura3 cek1*Δ*:: hisg/cek1*Δ*::CEK1-URA3*	Csank et al. (1998)
CM1613	*mkc1*Δ*::hisg/mkc1*Δ*::hisG-URA3-hisG* *ura3*Δ*::imm434/ura3*Δ*::imm434*	Novarro-Garcia et al. (2005)
CNC13	*ura3*Δ*::imm434/ura3*Δ*::imm434his1*Δ*::hisG/his1*Δ*::hisGhog1::hisG-URA3-his/hog1::hisG* P_{CHK1}*-lacZ-URA3-CHK1/CHK1*	Eisman et al. (2006)
CHK-lacZ	*ura3*Δ*::imm434/ura3*Δ*::imm434* P_{CHK1}*-lacZ-URA3-CHK1/CHK1*	Li et al. (2004)
REP3-lacZ	*sho1*Δ*::hisG/sho1*Δ*::hisGura3*Δ*::imm434/ura3*Δ*::imm434* P_{CHK1}*-lacZ-URA3-CHK1/CHK1*	This work
CK43B-lacZ	*cek1*Δ*::hisG/cek1*Δ*::hisG ura3*Δ*::imm434/ura3*Δ*::imm434*	This work