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The Swi/Snf chromatin remodeling complex is essential for hyphal development in *Candida albicans*

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Abstract The ability of dimorphic transition between yeast and hyphal forms in *Candida albicans* is one of the vital determinants for its pathogenicity and virulence. We isolated *C. albicans SWI1* as a suppressor of the invasive growth defect in a *Saccharomyces cerevisiae* mutant. Expression of *C. albicans SWI1* in *S. cerevisiae* partially complemented the growth defect of a *swi1* mutant in the utilization of glycerol. Swi1 is in a complex with Snf2 in *C. albicans*, and both proteins are localized in the nucleus independent of the growth form. Deleting *SWI1* or *SNF2* in *C. albicans* prevented true hyphal formation and resulted in constitutive pseudohypha-like growth in all media examined. Furthermore, *swi1/swi1* mutant was defective in hypha-specific gene expression and avirulent in a mouse model of systemic infection. These data strongly suggest the conserved Swi/Snf complex in *C. albicans* is required for hyphal development and pathogenicity.

Keywords: *SWI1*; *SNF2*; Swi/Snf complex; Hyphal development; Virulence; *Candida albicans*

1. Introduction

Candida albicans is emerging as one of the predominant human opportunistic fungal pathogens and can cause mucosal as well as systemic candidiasis, especially in immunocompromised patients, such as HIV-infected or organ-transplanted individuals, etc. [1]. *C. albicans* can adopt one of three growth forms: yeast, pseudohyphal, or hyphal form, depending on external stimuli. The pathogenicity of *C. albicans* correlates with its capacity to convert between yeast and hyphal forms, and accordingly, loss of switching capacity results in decreased virulence or avirulence [2–6].

Several transcription factors have been shown to regulate hyphal development in *C. albicans*. These include Cph1 [7], Efg1 [4,8], Tec1 [9], Cph2 [10], Czf1 [11], Rim101 [12], Mcm1 [13], Fkh2 [2], Tup1 [14], and Nrg1 [3,15]. Deletion of *TUP1* or *NRG1* causes constitutive filamentation and expression of hypha-specific genes, while *EFG1* or *TEC1* is required for hyphal development and the induction of hypha-specific genes. Recently, we have shown that Flo8 is essential for hyphal development and also the expression of hypha-specific genes [16]. Regulators and their signal transduction pathways for filamentous growth are well conserved between *C. albicans* and *Saccharomyces cerevisiae*. Homologs including Ste12 (Cph1), Tec1, Phd1 (Efg1), Flo8, and Nrg1, play similar roles in the pseudohyphal growth of *S. cerevisiae*. Ste12/Tec1 are targets of conserved mitogen activated protein kinases (MAPK), and Flo8 is downstream of the cAMP/protein kinase A pathway [17,18]. Like hypha-specific genes, the transcription of *FLO11* is under the positive regulation of Ste12 (Cph1), Tec1, Flo8, and Phd1 (Efg1), as well as the negative regulation of Nrg1 and Tup1/Ssn6 complex [19,20].

In addition to sequence specific regulators, modulation of the chromatin state also plays a major role in the regulation of gene expression in eukaryotes [21]. Chromatin structure can be affected by chromatin remodeling complexes. The yeast Swi/Snf complex is an ATP-dependent chromatin remodeling complex that can activate or repress transcription and is conserved throughout eukaryotes [22]. The Swi/Snf complex activates transcription by remodeling nucleosomes, thereby permitting the increased access of transcription factors to their binding sites. The complex contains 11 different subunits, including Swi1 to bind nucleosome and activators [23,24], and Snf2, a highly conserved DNA-dependent ATPase required for the chromatin remodeling activity. Swi/Snf is able to bind to both DNA and nucleosomes with high affinity, but without DNA sequence specificity [25]. Interestingly, gene expression microarray analysis has shown that the mRNA levels of only a small subset (about 5%) of *S. cerevisiae* genes are significantly affected by the loss of Swi/Snf activity [26]. Many studies have shown that the Swi/Snf complex can interact with sequence-specific transcriptional factors, which may recruit the complex to specific genes for chromatin remodeling [27].

Here, we report the cloning of a *C. albicans* homolog of *S. cerevisiae SWI1* as a suppressor of a *flo8* mutant in invasive growth. Deletion of *SWI1* or *SNF2* in *C. albicans* blocks hyphal development and the expression of hyphal specific genes under all hyphal induction conditions examined. And *swi1/swi1* mutant is avirulent in a mouse model of systemic infection. We suggest that a conserved Swi/Snf complex in *C. albicans* is required for its hyphal development.

2. Materials and methods

2.1. Plasmid construction

All plasmids and primers used in this study were listed on Tables 1 and 2, respectively. Plasmid pCF37 containing 3.8 kb insertion of *C. albicans SWI1* was screened out in *C. albicans* genomic library by

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Table 1
Plasmids

Plasmid	Description	Reference
YEp24	2μ origin and *S. cerevisiae URA3* in pBR322	[46]
BD1	*S. cerevisiae SWI1* in YEp24	[46]
pCF37	3.8 kb *C. albicans SWI1* in pRS202	This study
pCUB6	Substitution of *S. cerevisiae URA3* by *C. albicans URA3* in pNKY50	[36]
pSWI1-KO	Replacement of 1.8 kb in pCF37 with 4.0 kb *HisG-URA3-HisG* from pCUB6	This study
pSNF2-KO	0.9 and 1.0 kb of *C. albicans SNF2* in pCUB6	This study
pBES116	*URA3* vector, integration at *ADE2*	[47]
pBES116-SWI1	3.8 kb *SWI1* in pBES116	This study
p584	*ACT1p-GFP-URA3-GFP* in p471	[28]
pSWI1-GFP	0.9 and 1.2 kb of *C. albicans SWI1* in p584	This study
pSNF2-GFP	1.3 and 1.2kb of *C. albicans SNF2* in p584	This study
p4FLAG-SNF2	0.9 kb of *SNF2* and 3 × FLAG in pFLAG-Act1	This study

suppression of invasive growth defect of *S. cerevisiae flo8* mutant [16]. A *Bcl*I fragment of pCF37 was replaced with *HisG–URA3–HisG* fragment from pCUB6 to generate plasmid pSWI1-KO. pBES116-SWI1 was generated by fusion of 3.8 kb *Kpn*I–*Not*I fragment containing *C. albicans SWI1* from pCF37 to pBES116. 0.9 kb PCR fragment (primers 1 and 2) and 1.2 kb PCR fragment (primers 3 and 4) amplified from pCF37 were digested with *Kpn*I–*Xho*I and *Sac*II, respectively, and sequentially inserted into plasmid p584 [28] to produce plasmid pSWI1-GFP. pSNF2-GFP was also generated with the same strategy by sequential insertion of 1.3 and 1.2 kb PCR products with primers 5, 6 and primers 7, 8 into p584. Two PCR fragments, 0.9 kb (primers 9 and 10) and 1.0 kb (primers 11 and 12), were digested with *Bam*HI–*Bgl*II and *Bam*HI–*Sph*I, respectively, and sequentially ligated to pCUB6 to generate pSNF2-KO. A 1.0 kb PCR product (primers 13 and 14) containing the C-terminal *SNF2* coding region and a 3× FLAG tag was inserted into *Bam*HI–*Sph*I site of pFLAG-Act1 to generate p4FLAG-SNF2.

2.2. Strains and culture conditions

The *S. cerevisiae* and *C. albicans* strains used in this study are listed in Table 3. Yeast strains were grown in YPD or SC medium at 30 °C for prototrophic selection, except that SC + 2% glycerol was used for a growth rate test of the *S. cerevisiae swi1* mutant. *C. albicans* strains were grown in YPD for yeast form, and induced to hyphal form in serum (GIBCO), Lee's medium or embedded condition described previously [16,29].

Table 2
Primers

Primer	Sequence
1	5′-GGGGATCCGGACACCTACACCAAAACA
2	5′-CCGCTCGAGCCATTCACACCCTGCCATA
3	5′-TCCCCGCGGTACCTTGAGATTTGGCTGTAACA
4	5′-TCCCCGCGGCCATGTCTGATTGGTTGAATG
5	5′-ATGGTACCCTGATAACTTGGCGGAAATG
6	5′-AAGCTCGAGAATGAAGTAACTTCTTGTAAAC
7	5′-TACCCGCGGCCATGAATCGTCAACCTACAAG
8	5′-TCCCCGCGGTACCACTAGATGAAGTTGATGCTG
9	5′-CGGGATCCATGAATCGTCAACCTACAAGAGAG
10	5′-GAAGATCTGTTGTTGAAGGGCATATTGTTG
11	5′-CAGGATCCGAACAGAAGAGTCTACACCAG
12	5′-ACATGCATGCGTTCCACAAGTGTTCTATACC
13	5′-GAGGATCCTCTGACGACGATGATGACAATG
14	5′-ACATGCATGCCTTGTCATCGTCATCCTTGTAA TCGATGTCATGATCTTTATAATCACCGTCATGGT CTTTGT AGTCATCAAAATTTGCTGGTGTAGACTC
15	5′-GCCATCATCCACCATGCTCC
16	5′-GTGCTACTGAGCCGGCATCTC
17	5′-TGCTCCAGGTACTGAATCCGC
18	5′-GGCAGATGGTTGCATGAGTGG
19	5′-CCTCAGTGCTGCATTAGAAGTTG
20	5′-GATGAAGCAGACATAGATTCGG
21	5′-CTTGAGTGTTCTTGCTTTCGC
22	5′-GCTGATCTCATGAAGTTGTCAC

2.3. C. albicans strain construction

To delete *SWI1*, pSWI1-KO was digested with *Hin*dIII and transformed into CAI4 to generate heterozygotes CAM1a and CAM1b. CAM1b was streaked onto 5-fluoro-orotic acid (5-FOA) plate to select for the Ura− strain CAM2. *swi1/swi1* homozygotes CAM3 (Ura+) and CAM4 (Ura−) were screened out by subsequent transformation with the *Hin*dIII-digested pSWI1-KO fragment. By the same strategy, *snf2/snf2* null mutants CAM41 (Ura+) and CAM42 (Ura−) were generated through two rounds of transformation with a *Pst*I-digested pSNF2-KO DNA fragment.

To express GFP fusion proteins, the wild-type strain CAI4 was transformed with *Kpn*I-digested pSWI1-GFP to generate CAM20, which was selected on 5-FOA to generate CAM21. Similarly, *Kpn*I-digested pSNF2-GFP DNA fragment was introduced into CAI4 for GFP-Snf2 expression, and then the strain was transformed with *Stu*I-digested pFLAG-Act1 to produce Ura+ strain CAM68.

Table 3
Yeast strains

S. cerevisiae strains

Strain	Genotype	Reference
DY878	*MATa ade2 gal3 his3 leu2 lys2 trp1Δ1 ura3*	[46]
DY2024	*MATa swi1::HisG ade2 gal3 his3 leu2 lys2 trp1Δ1 ura3*	[46]

C. albicans strains

Strain	Genotype	Reference
SC5314	Wild type	[36]
CAI4	*ura3::imm434/ura3::imm434*	[36]
CAM1a	*ura3::imm434/ura3::imm434 SWI1/swi1::HisG-URA3-HisG*	This study
CAM1b	*ura3::imm434/ura3::imm434 swi1::HisG-URA3-HisG/SWI1*	This study
CAM2	*ura3::imm434/ura3::imm434 swi1::HisG/SWI1*	This study
CAM3	*ura3::imm434/ura3::imm434 swi1::HisG/swi1::HisG-URA3-HisG*	This study
CAM4	*ura3::imm434/ura3::imm434 swi1::HisG/swi1::HisG*	This study
CAM20	*ura3::imm434/ura3::imm434 SWI1/SWI1::GFP-URA3-GFP*	This study
CAM21	*ura3::imm434/ura3::imm434 SWI1/SWI1::GFP*	This study
CAM38	*ura3::imm434/ura3::imm434 SWI1/SWI1::GFP RP10/RP10:: FLAG-URA3*	This study
CAM42	*ura3::imm434/ura3::imm434 snf2::HisG/snf2::HisG*	This study
CAM43	*ura3::imm434/ura3::imm434 SWI1/SWI1::GFP SNF2/SNF2:: 4FLAG-URA3*	This study
CAM68	*ura3::imm434/ura3::imm434 SNF2/SNF2::GFP RP10/RP10:: FLAG-URA3*	This study