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Functional analysis of ScSwi1 and CaSwi1 in invasive and pseudohyphal growth of *Saccharomyces cerevisiae*

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Here we reported that, in *Saccharomyces cerevisiae*, deleting Swi1 (ScSwi1), a core component in Swi/Snf complex, caused defects of invasive growth, pseudohyphal growth, *FLO11* expression, and proper cell separation. Re-introduction of *SWI1* into the *swi1* mutants could suppress all defects observed. We also showed that overproducing Swi1 could suppress the defect of *flo8* cells in pseudohyphal growth in diploids, but not invasive growth in haploids. Overexpression of *SWI1* could not bypass the requirement of Ste12 or Tec1 in invasive growth or pseudohyphal growth. We concluded that the Swi/Snf complex was required for *FLO11* expression and proper cell separation, and both the *FLO8* and *STE12* genes should be present for the complex to function for the invasive growth but only the *STE12* gene was required for the pseudohyphal growth. Ectopic expression of *Candida albicans* SWI1 (CaSWI1) could partially complement the defects examined of haploid *Scswi1* mutants, but failed to complement the defects examined of diploid *Scswi1*/*Scswi1* mutants. Overexpressing CaSwi1 mitigated invasive and pseudohyphal growth defects resulting from deletions in the MAP kinase and cAMP pathways. The integrity of *S. cerevisiae* Swi/Snf complex is required for invasive and filamentous growth promoted by overexpressing CaSwi1.

Keywords ScSwi1; CaSwi1; invasive growth; pseudohyphal growth; *FLO11*; *Saccharomyces cerevisiae*

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Introduction

Saccharomyces cerevisiae is one of the dimorphic fungi, which shows invasive growth, pseudohyphal growth (collectively referred to as filamentous growth) and yeast growth. MAPK and cAMP/PKA pathways have been well characterized required for invasive growth of haploid cells and pseudohyphal growth diploid cells. Ste12/Tec1, transcription factors of conserved MAPK pathway, binds cooperatively to consensus filamentous responsive elements of target genes, and Flo8 is a transcription factor downstream of cAMP/PKA pathway, whereas Phd1 modulates filaments independently of MAPK or cAMP/PKA pathway, and all these signaling converge on a unique target gene, *FLO11* [1].

Swi/Snf complex is a global conserved regulator by repositioning nucleosomes to remodel chromatin in an ATP-dependent manner [2,3]. In *S. cerevisiae*, Swi/Snf complex has been shown to play vital roles in transcription initiation (activation and repression), transcription elongation, damage-induced DNA repair, DNA replication, and heat shock responses, etc [2,4–8].

In mammalian cells, altered activity of Swi/Snf complex correlated to cell morphology in tumor development. Expression of BRG1, a core component of human Swi/Snf complex with ATPase activity, promoted filamentation of actin in BRG1-deficient cells [9], and expression of ATPase-deficient BRG1 enhanced attachment of cells to extracellular matrix [10]. However, re-introduction of SNF5, also a core member of Swi/Snf complex, into SNF5-deficient malignant rhabdoid tumor cell line, causes destruction of actin stress fiber network and disappearance of extracellular focal adhesion [11]. *Candida albicans* is a polymorphic human opportunistic

pathogen and the ability of transition among different forms is considered to correlate with its pathogenicity [12,13]. We have also characterized that a conserved Swi/Snf complex in this fungus was required for hyphal development and virulence by antagonizing the repression from Tup1 on hypha-specific gene expression and actin organization [14,15], and binding of Swi/Snf complex to promoters of hypha-specific genes is subsequent to nucleosome acetyltransferase of histone H4 (NuA4 complex), which is recruited by Efg1 the transcription factor of cAMP/PKA pathway in *C. albicans* [16]. In *S. cerevisiae*, evidences showed that Swi/Snf complex could remodel the chromosomal structure of *FLO1* promoter by antagonizing Tup1/Ssn6 repressive complex [17]. Recently, components of Swi/Snf complex were isolated as the transcription activators of *FLO11* in *S. cerevisiae* 133d strain, and disruption of these components caused defects in biofilm formation, invasive growth, and decreased *FLO11* expression [18]. Moreover, Ste12/Tec1 could recruit Swi/Snf complex to *STA1* promoter [19], which is almost identical to *FLO11* promoter [20], suggesting similar regulatory mechanism of Swi/Snf complex in filamentous growth via *FLO11*.

Here we reported that the deletion of *SWI1* in *S. cerevisiae* caused defect of filamentous growth, *FLO11* expression, and proper cell separation. Moreover, overproducing *S. cerevisiae* Swi1 (ScSwi1) and *C. albicans* Swi1 (CaSwi1) in *S. cerevisiae* mediated different actions in invasive and pseudohyphal growth.

Materials and Methods

Strains and culture conditions

The *S. cerevisiae* strains used in this study are listed in **Table 1**. All strains are in ∑1278b genetic background. Yeast strains were routinely grown in 1% yeast extract, 2% peptone, 2% glucose (YPD) or synthetic complete (SC) medium at 30°C. For filamentous growth, synthetic low-ammonia medium (SLAD) allowed for pseudohyphal growth and YPD medium used for invasive growth of *S. cerevisiae*, respectively, were prepared as described previously [21,22]. Appropriate yeast strains were streaked on SLAD plates, incubated for 1 week at 30°C and then photographed. For invasive growth observation, strains were carefully patched on YPD plates and allowed to grow at 30°C for 5−7 days. Cells that penetrated into agar were photographed after gentle wash with de-ionized water on agar surface [21,22].

Plasmid construction

All plasmids used in this work are listed in **Table 2**. Plasmid pNKY50-SWI1 containing *S. cerevisiae URA3*

Table 1 *Saccharomyces cerevisiae* strains in this study

Strain	Genotype	Reference
L5528	*MAT***a** *ura3-52 his3::hisG*	[33]
HLY850	*MAT***a** *flo8::hisG ura3-52*	[34]
HLY367	*MAT***a** *ste7::LEU2 leu2::hisG ura3-52*	[33]
HLY362	*MAT***a** *ste12::LEU2 leu2::hisG ura3-52*	[33]
HLY2000	*MAT***a** *tec1::HIS3 ura3-52*	[34]
L5783	*MAT***a**/α *ura3-52/ura3-52*	[21]
HLY852	*MAT***a**/α *flo8::hisG/flo8::hisG ura3-52/ura3-52*	[35]
HLY351	*MAT***a**/α *ste7::LEU2/ste7::LEU2 ura3-52/ura3-52 leu2::hisG/leu2::hisG*	[33]
HLY352	*MAT***a**/α *ste12::LEU2/ste12::LEU2 ura3-52/ura3-52 leu2::hisG/leu2::hisG*	[33]
HLY2002	*MAT***a**/α *tec1::HIS3/tec1::HIS3 ura3-52/ura3-52*	[34]
L6235	*MAT***a**/α *phd1::hisG/phd1::hisG ste12::LEU2/ste12::LEU2 ura3-52/ura3-52*	[36]
SM1	*MAT***a** *ura3-52 his3::hisG swi1::HisG-URA3-HisG*	This study
SM2	*MAT***a** *ura3-52 his3::hisG swi1::HisG*	This study
SM3	*MAT***a**/α *ura3-52/ura3-52 swi1::HisG-URA3-HisG/SWI1*	This study
SM4	*MAT***a** *ura3-52 swi1::HisG-URA3-HisG*	This study
SM5	*MATα ura3-52 swi1::HisG-URA3-HisG*	This study
SM6	*MATα ura3-52 swi1::HisG*	This study
SM7	*MAT***a**/α *ura3-52/ura3-52 his3::hisG/HIS3 swi1::HisG-URA3-HisG/swi1::HisG*	This study
SM8	*MAT***a**/α *ura3-52/ura3-52 his3::hisG/HIS3 swi1::HisG/swi1::HisG*	This study

Table 2 Plasmids in this study

Plasmid	Description	Reference
YEp24	2 μ origin and *S. cerevisiae URA3* in pBR322	[37]
BD1	ScSwi1 in YEp24	[37]
pCF37	CaSwi1 in pRS202	[14]
pNKY50	Vector containing *HisG-URA3-HisG*	[23]
pNKY50-SWI1	0.9 kb of 5′ fragment and 0.7 kb of 3′ fragment of *ScSWI1* in pNKY50	This study

flanked by 5′ fragment and 3′ fragment of *SWI1* was constructed as following. The 0.9 kb of 5′ fragment amplified with primers CTGAATTCATACTTCTCTTC TCCTCTC and GAAGATCTGCCTGTGCTATTGTTG TTA, and the 0.7 kb of 3′ fragment amplified with primers GAGGATCCGATCGCAACAGTAACAAC and CATGCATGCTGGATTAGTGAATAACTGG from genomic DNA of wild-type strain L5528 were sequentially inserted into *Eco*RI/*Bgl*II and *Bam*HI/*Sph*I sites of plasmid pNKY50 [23], respectively.

Strain construction

Transformations of *S. cerevisiae* strains were performed as described previously [24]. The procedure for construction of *S cerevisiae* haploid *swi1* and diploid *swi1*/*swi1* mutants and genotypes of respective strains were described in **Fig. 1**. Briefly, URA^{+} his^{-} *swi1* strain SM1 was constructed from wild-type of **a** mating type strain L5528 transformed with *Eco*RI-linearized pNKY50-SWI1 and screened on SC-ura plate. The ura^{-} his^{-} *swi1* strain SM2 was the derivate of SM1 after loop-out of one copy of *URA3-HisG* on 5-FOA plates. For construction of diploid mutants, *Eco*RI-linearized pNKY50-SWI1 was introduced into diploid wild strain L5783 to generate *SWI1*/*swi1* heterozygote SM3, which was then induced for sporulation [25]. The spored *swi1* mutant SM4 and SM5 were URA^{+} and screened out on SC-ura plate, and **a** or α mating types were identified by mating to the **a** type strain L5528 [25]. The his^{+} ura^{-} α strain SM6 was from SM5 on 5-FOA plates, and mated with his^{-} URA^{+} *swi1* strain SM2 of **a** mating type to give rise to *SWI1*-distrupted homozygote SM7, which was the parental diploid strain of ura^{-} *swi1* null mutant SM8. Genotypes of all strains in construction were verified by Southern blot.

Southern blot and Northern blot

Genomic DNA isolation, Southern blot hybridization, total RNA extraction by hot phenol, Northern blot hybridization were demonstrated as previously described [26]. All probes were randomly labeled with Random Primers DNA Labeling System (Invitrogen) with [α-^{32}P]-dATP. The probe for Southern blot was 0.7 kb of *SWI1* 3′ fragment, and in Northern blot *FLO11* probe was amplified with primers ATGCCTAACTTCCAAA TTCAATTC and CTGGATGGAGTTGGTACTGGAGC, and *ACT1* probe was amplified with primers GGTGAC GACGCTCCTCGTGC and TGAGGTAGAGAGAAA CCAG from genomic DNA of strain L5528.

Results

Knockout of *ScSwi1*

We constructed *swi1* mutants in ∑1278b genetic background by homologous recombination. Most of *SWI1* coding region including the fragment for ARID domain was substituted by a *HisG-URA3-HisG* insertion, whose loop-out with only one copy of *HisG* left could be screened out on a 5-FOA plate [**Fig. 1(A)**]. We successfully disrupted *SWI1* in **a** mating-type haploid strain L5528 and knocked out one copy of *SWI1* in diploid strain L5783. After sporulation, α mating-type haploid *swi1* mutant strain could be readily obtained. The α ura^{-} *swi1* derivative was mated with **a** *swi1* mutant strain generating diploid *swi1*/*swi1* mutant strain [**Fig. 1(B)**]. The genotypes of all mutant strains were verified by Southern blot [**Fig. 1(C)**].

Haploid *swi1* mutant is defective in invasive growth and *FLO11* expression

In *S. cerevisiae*, haploid cells can invade into agar in search for nutrients upon glucose starvation, which correlates with some changes on cell-wall structure, such as abundant disposition of cell-wall protein Flo11, which is required for flocculation, cell adhesion, and filamentous growth in yeast [22]. After continuous incubation on YPD plates for 1 week, wild-type cells exhibited perfect invasive growth, whereas *swi1* mutant cells lost its

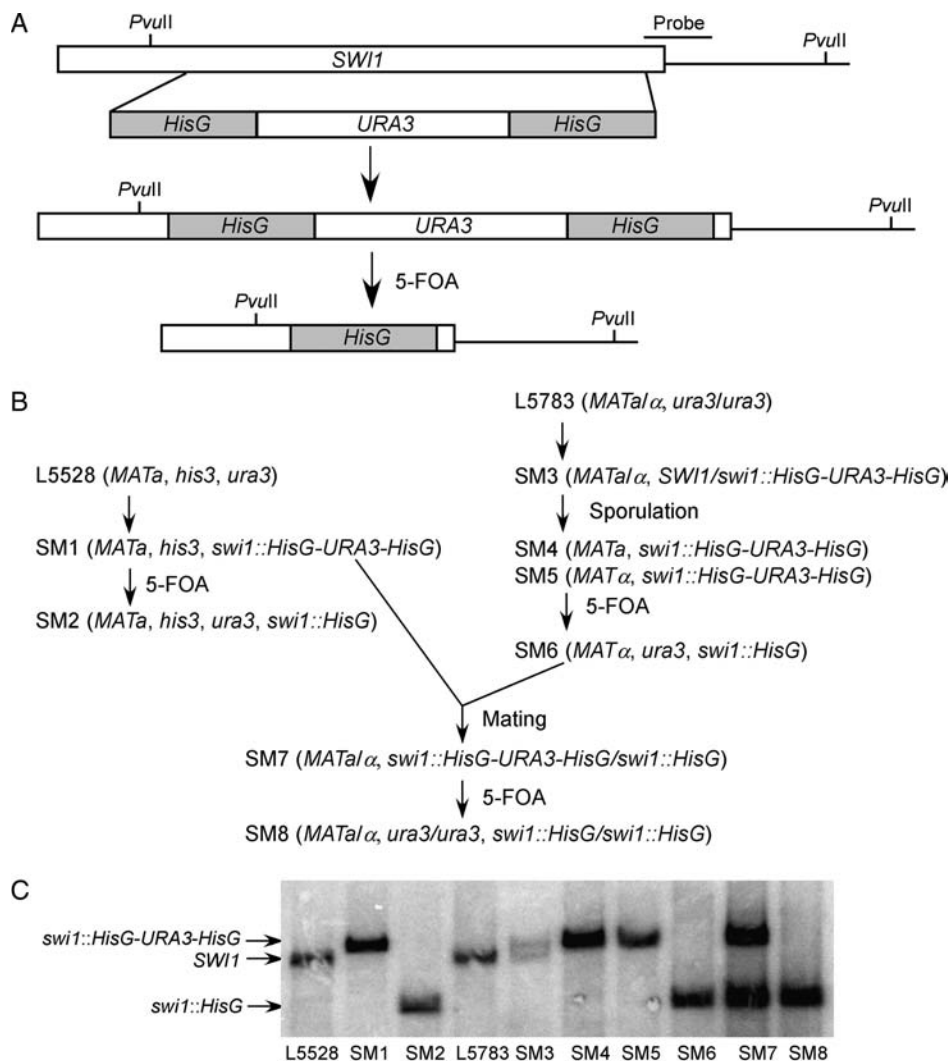

Figure 1 Deletion of ScSwi1 (A) Strategy for disruption of *SWI1*. (B) Construction of haploid or diploid *SWI1*-deletion mutants in *S. cerevisiae*. (C) Southern blot analysis of various strains during construction. Genomic DNAs were digested with *Pvu*II and hybridized with 0.7 kb probe of 3′ fragment of ScSwi1 as indicated.

invading ability, which was consistent with high-expression level of *FLO11* in wild-type cells but null in *swi1* mutant. Re-introduction of *SWI1* in high copy number vector in *swi1* mutant could fully restore the invasive growth and gave higher *FLO11* expression than wild-type (**Fig. 2**), suggesting that the Swi1 is essential for invasive growth and *FLO11* expression in haploid cells.

Diploid *swi1*/*swi1* mutant is defective in pseudohyphal growth and *FLO11* expression

In diploid cells, nitrogen depletion can induce pseudohyphal growth on solid medium, where cells are elongated, attached, and budded in unipolar pattern [1]. On SLAD medium, diploid wild-type cells grew in typical pseudohyphae and formed branched filamentous colonies. In contrast, *swi1*/*swi1* mutant cells were defective in pseudohyphal growth and displayed irregular smooth-edged colonies [**Fig. 3(A)**]. Consistent to these phenotypes, *FLO11* expression was prominent in wild-type cells but undetectable in *swi1*/*swi1* mutant cells [**Fig. 3(B)**]. Overexpression of *SWI1* in *swi1*/*swi1* mutant could exhibited more flouring pseudohyphal growth and even higher *FLO11* expression than wild-type (**Fig. 3**), indicating that the Swi1 is essential for pseudohyphal growth and *FLO11* expression in diploid cells.

Deletion of *SWI1* affects cell separation

In *S. cerevisiae*, haploid cells proliferate in axial pattern, and separate well after budding [27]. Although haploid

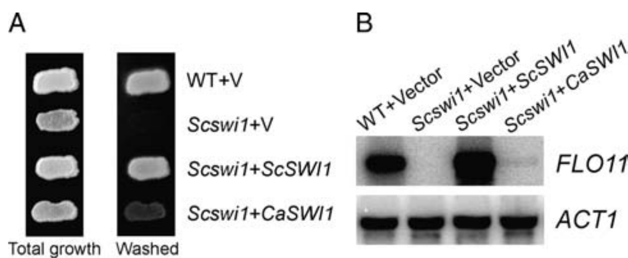

Figure 2 Invasive growth assay (A) Haploid **a** mating type strains including WT+Vector (L5528+YEp24), *swi1*+Vector (SM2+YEp24), *swi1+SWI1* (SM2+BD1), and *swi1+CaSWI1* (SM2+pCF37) were patched on YPD plates, incubated at 30°C for 7 days and photographed after gentle wash with water. (B) Strains in (A) were cultured overnight and re-inoculated in SC-ura medium for further 6 h at 30°C. Total RNA was extracted and hybridized with *FLO11* and *ACT1* probes.

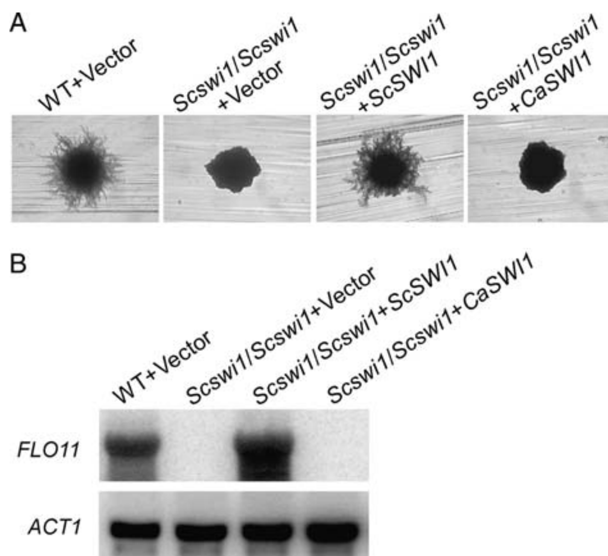

Figure 3 ScSwi1 is essential for pseudohyphal growth (A) Diploid strains including WT+Vector (L5783+YEp24), *swi1/swi1*+Vector (SM8+YEp24), *swi1/swi1+SWI1* (SM8+BD1), and *swi1/swi1+CaSWI1* (SM8+pCF37) were patched on SLAD plates, incubated at 30°C for 7 days and photographed. (B) Strains in (A) were cultured overnight and re-inoculated in SC-ura medium for further 6 h at 30°C. Total RNA was extracted by hot phenol and hybridized with *FLO11* and *ACT1* probes.

swi1 mutant cells budded in axial pattern, but most cells attached together to form aggregates (averagely about 30 cells per aggregate) [**Fig. 4(A)**]. Diploid yeast cells adopt bipolar budding pattern [27], daughter cells bud at the opposite sites of previous budding scars, which could be obviously observed in diploid wild-type cells [**Fig. 4(B)**]. The bipolar budding pattern was not altered in diploid *swi1/swi1* mutant cells, but the budded cells were attached and aggregated (averagely about 20 cells

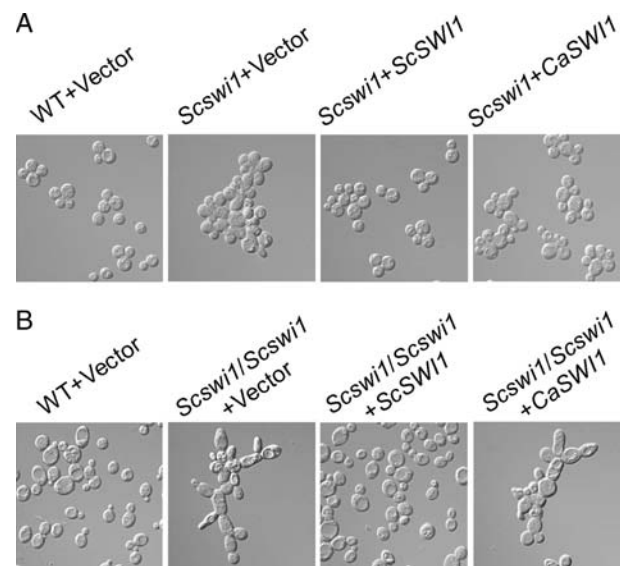

Figure 4 Cell morphology assay (A) Haploid **a** mating type strains including WT+Vector (L5528+YEp24), *swi1*+Vector (SM2+YEp24), *swi1+SWI1* (SM2+BD1), and *swi1+CaSWI1* (SM2+pCF37). (B) Diploid strains including WT+Vector (L5783+YEp24), *swi1/swi1*+Vector (SM8+YEp24), *swi1/swi1+SWI1* (SM8+BD1), and *swi1/swi1+CaSWI1* (SM8+pCF37) were cultured overnight, re-inoculated in SC-ura medium for further 6 h at 30°C and photographed.

per aggregate) in pseudohyphae-like form [**Fig. 4(B)**]. Re-introduction of *SWI1* restored the phenotypes of haploid *swi1* and diploid *swi1/swi1* mutants to wild-type (**Fig. 4**). These results suggest that Swi1 is required for proper cell separation in *S. cerevisiae*.

Caswi1 partially complemented haploid *Scswi1* mutant, but not diploid mutant, in cell separation, invasive growth, and *FLO11* expression

CaSwi1, which is required for the hyphal development in *C. albicans*, was recognized as the homologue of ScSwi1, since it could partially suppress the defect of glycerol utilization in haploid *Scswi1* mutant in S288C background [14]. Consistent with the previous observations, in our $\sum$1278b background strains, overexpression of *CaSWI1* could also partially complement haploid *Scswi1* mutant in the defects of invasive growth, *FLO11* expression, and cell separation (averagely about 10 cells per aggregate) [**Figs. 2** and **4(A)**]. However, unlike in haploid cells, cells carrying high copies of *CaSWI1* still showed phenotypes identical to *Scswi1/Scswi1* mutant including pseudohyphal growth, *FLO11* expression, and cell separation (**Figs. 3** and **4**), suggesting CaSwi1 could not complement ScSwi1 in diploid cells and CaSwi1 could not simply replace it.

Swi1 promoting invasive growth requires Ste12 and Flo8

MAPK pathway downstream transcription factors Ste12 and cAMP/PKA pathway downstream transcription factor Flo8 are required for invasive growth and *FLO11* expression in *S. cerevisiae* haploid cells [28]. We introduced a high copy ScSwi1 expression vector into haploid strains defective in components of MAPK or cAMP/PKA pathway, respectively. Overexpression of *SWI1* could not promote invasive growth [**Fig. 5(A)**] and *FLO11* expression [**Fig. 5(C)**] in *ste7*, *ste12*, *tec1*, or *flo8* mutant. In wild-type cells, overproducing Swi1 could elevate *FLO11* expression level. These results suggest that overexpressing Swi1 can not bypass the requirement of Ste7, Ste12, Tec1, and Flo8 in invasive growth and *FLO11* expression in haploids. The Swi1 promotion depends on both MAPK and cAMP/PKA pathways.

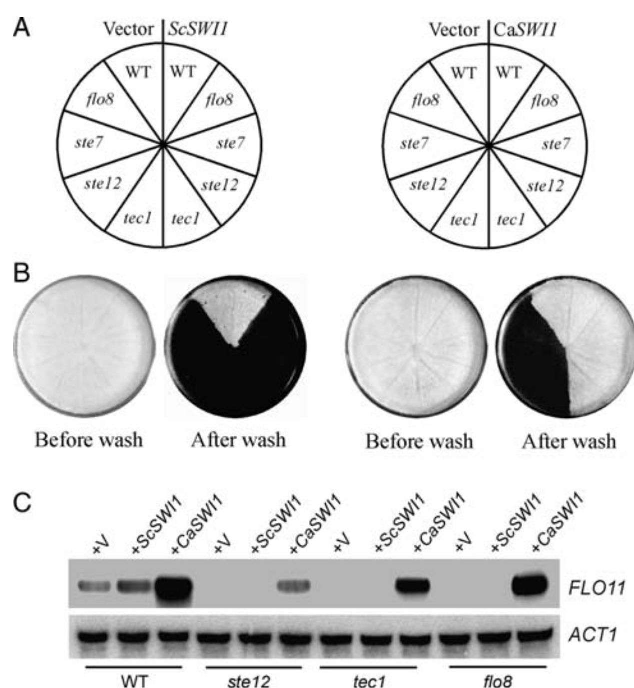

Figure 5 Activation of invasive growth by *ScSWI1* or *CaSWI1* in *S. cerevisiae* *S. cerevisiae* haploid **a** mating type wild-type strain (L5528), *flo8* (HLY850), *ste7* (HLY367), *ste12* (HLY362), and *tec1* (HLY2000) mutants carrying vector (YEp24) in (A and B), *ScSWI1* (BD1) in (A) or *CaSWI1* (pCF37) in (B), respectively, were grown on YPD medium at 30°C for 5–7 days, washed gently and photographed. (C) Haploid wild-type strain (L5528), *ste12* (HLY362), *tec1* (HLY2000), and *flo8* (HLY850) mutants carrying vector YEp24 (A and B), *ScSWI1* (BD1) or *CaSWI1* (pCF37) were cultured overnight and re-inoculated in SC-ura medium for further 6 h at 30°C. Total RNA was extracted by hot phenol and hybridized with *FLO11* and *ACT1* probes.

Swi1 activating pseudohyphal growth requires Ste12 but not Flo8

To examine the effect of overexpressing *SWI1* in pseudohyphal growth of diploid cells, we introduced the high copy *SWI1* expression vector into diploid mutant strains, respectively. Overexpression of *SWI1* could not activate pseudohyphal growth [**Fig. 6(A)**] and *FLO11* expression [**Fig. 6(B)**] in mutants of MAPK pathway including *ste12*/*ste12*, *tec1*/*tec1*, *ste7*/*ste7* and a double mutant *ste12*/*12 phd1*/*phd1*. In contrast, overproducing Swi1

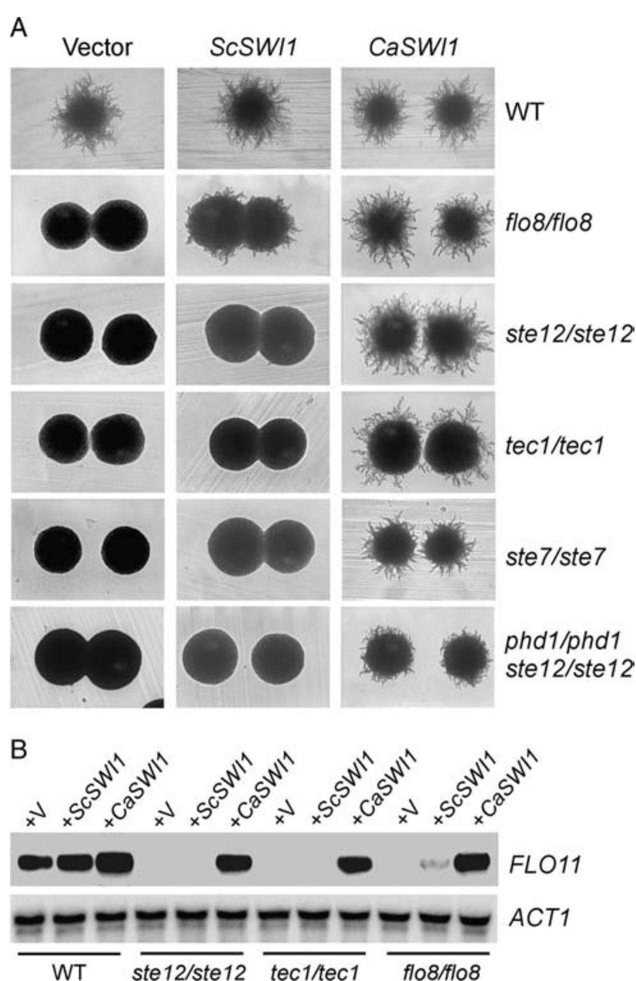

Figure 6 Different modules of *ScSWI1* and *CaSWI1* in activation of pseudohyphal growth in *S. cerevisiae* (A) *S. cerevisiae* diploid strain wild-type (L5783), *flo8/flo8* (HLY852), *ste7/ste7* (HLY351), *ste12/ste12* (HLY352), *tec1/tec1* (HLY2002), and *phd1/phd1 ste12/ste12* (L6235) mutants carrying vector (YEp24), *ScSWI1* (BD1), *CaSWI1* (pCF37), respectively, were grown on SLAD medium at 30°C for 7 days. (B) Diploid strain wild-type (L5783), *ste12/ste12* (HLY352), *tec1/tec1* (HLY2002), and *flo8/flo8* (HLY852) mutants carrying vector (YEp24), *ScSWI1* (BD1), *CaSWI1* (pCF37), respectively, were cultured overnight and re-inoculated in SC-ura medium for further 6 h at 30°C. Total RNA was extracted by hot phenol and hybridized with *FLO11* and *ACT1* probes.

partially suppressed the defect of *flo8*/*flo8* cells in pseudohyphal growth and *FLO11* expression (**Fig. 6**). Overexpression of *SWI1* increased the *FLO11* expression level and pseudohyphal growth in wild-type cells. These results suggest that the Swi1 activation in pseudohyphal growth and *FLO11* expression depends on MAPK pathway but not cAMP/PKA pathway in diploid cells.

Ectopic expression of *CaSwi1* promotes invasive and pseudohyphal growth in *S. cerevisiae*

To examine whether the CaSwi1 has an activation effect on invasive or pseudohyphal growth in *S. cerevisiae*, we introduced a high copy *CaSWI1* expression vector into haploids and diploids. Overexpression of CaSwi1 promoted *FLO11* expression in *S. cerevisiae* wild-type cells and exhibited a stronger *FLO11* expression than strain overexpressing ScSwi1 [**Figs. 5(C)** and **6(B)**]. Contrast to ScSwi1, overproducing CaSwi1 suppressed the invasive and pseudohyphal growth defects in all mutants examined [**Figs. 5(B)** and **6(A)**]. Consistent with the phenotype, the *FLO11* expression level was restored in *CaSWI1*-overexpression strain. In haploids, overproducing CaSwi1 resulted in the *FLO11* expression increased by 7 folds in wild-type, 5 folds in *flo8*, 3 folds in *tec1*, 1 fold in *ste12*, respectively, compared with that in wild-type cells without carrying *CaSWI1* [**Fig. 5(C)**]. In diploids, overproducing CaSwi1 also increased the *FLO11* expression level by 1–3 folds in wild-type and mutant cells [**Fig. 6(B)**]. These results suggest that the CaSwi1 acts as an activator in promoting invasive or pseudohyphal growth via enhancement of the *FLO11* expression and bypasses the requirement of MAPK or cAMP/PKA pathways.

Discussion

In this study, we have successfully deleted the *ScSWI1* gene in ∑1278b background, including fragment coding for DNA-binding ARID domain. Previous study showed lethality after full deletion of *SWI1* but viability via insertion mutation in 133d background [18]. However, the C-terminus of Swi1 (780 amino acids of total 1314 amino acids) has been demonstrated to fully complement the *SWI1* deletion though in S288C background, indicating Swi1-C was sufficient for Swi1 functions [29]. Thus, our deletion of *SWI1* (more than 1000 amino acids) was sufficient for functional analysis of Swi1. Loss of ScSwi1 decreased the stability of ScSwi3 in *S. cerevisiae* [30], and ScSwi3 served as the scaffold subunit of Swi/Snf complex and was required for maintenance of the full structural integrity of this complex [31,32], suggesting that deleting ScSwi1 caused disassembly of the Swi/Snf complex. Our data showed that loss of ScSwi1 resulted in defect of invasive growth, pseudohyphal growth, proper cell separation, and *FLO11* expression. We have previously demonstrated a *C. albicans* Swi/Snf complex is required for full cell separation, hyphal development and expression of hypha-specific genes [14], and invasion into agar [15]. The *CaSWI1* can partially complement the growth defect of a *Scswi1* mutant (non ∑1278b background strain) in the utilization of glycerol [14]. Although the *CaSWI1* can also partially complement the defects of SM2 (*Scswi1* mutant, ∑1278b background strain) in invasive growth and cell separation, but failed to complement pseudohyphal growth or cell separation in SM8 (*Scswi1*/*Scswi1* mutant, ∑1278b background strain), suggesting that the CaSwi1 could not simply replace the ScSwi1 in Swi/Snf complex, and the integrity of native *S. cerevisiae* Swi/Snf complex is essential for filamentous growth and for the activation by CaSwi1.

With loss of transcription factors of cAMP/PKA or MAPK pathway, such as Flo8, Ste12, or Tec1, mutants are prevented from expressing *FLO11* and filamentous growth. Overexpression of *ScSWI1* promoted filamentous growth and *FLO11* expression in wild-type cells but not in some mutant cells. Overproducing ScSwi1 can suppress the defect of *flo8* cells in pseudohyphal growth in diploids, but not invasive growth in haploids. Overexpression of *SWI1* cannot bypass the requirement of Ste12 or Tec1 in invasive growth or pseudohyphal growth. Our data suggest that in haploid cells, both cAMP/PKA and MAPK pathways are required for invasive growth and *FLO11* expression mediated by ScSwi1, presence of either pathway is insufficient for recruitment of the Swi/Snf complex to *FLO11* promoter for further transcriptional activation. On the other hand, in diploid cells, only MAPK pathway but not cAMP/PKA pathway is required for Swi/Snf activation in pseudohyphal growth and *FLO11* expression. The Swi/Snf complex may be recruited to *FLO11* promoter by other transcription factors instead of the Flo8 during transcriptional activation.

In existence of endogenous ScSwi1, overexpressing CaSwi1 in *S. cerevisiae* not only promotes filamentous growth and *FLO11* expression in wild-type but also in mutants with the deletion of MAPK or cAMP/PKA pathway components. Higher *FLO11* expression level and stronger filaments formation are observed when the strain overexpressed with *CaSWI1*, suggesting that either

MAPK or cAMP/PKA pathway is sufficient for activation of the CaSwi1, or alternatively, the CaSwi1 may be recruited by some other transcriptional factors to filamentous response elements of the *FLO11* promoter mediate the activation of *FLO11* expression and filamentous growth.

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