EUKARYOTIC CELL, Nov. 2009, p. 1780–1791 Vol. 8, No. 11
1535-9778/09/$12.00 doi:10.1128/EC.00190-09
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Mss11, a Transcriptional Activator, Is Required for Hyphal Development in *Candida albicans*[▽]

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Received 30 June 2009/Accepted 28 August 2009

Candida albicans* undergoes a morphological transition from yeast to hyphae in response to a variety of stimuli and growth conditions. We previously isolated a LisH domain containing transcription factor Flo8, which is essential for hyphal development in *C. albicans*. To search the putative binding partner of Flo8 in *C. albicans*, we identified *C. albicans* Mss11, a functional homolog of *Saccharomyces cerevisiae* Mss11, which also contains a LisH motif at its N terminus. *C. albicans* Mss11 can interact with Flo8 via the LisH motif by in vivo coimmunoprecipitation. The results of a chromatin immunoprecipitation (ChIP) assay showed that more Mss11 and Flo8 proteins bound to the upstream activating sequence region of *HWP1* promoter in hyphal cells than in yeast cells, and the increased binding of each of these two proteins responding to hyphal induction was dependent on the other. Overexpression of *MSS11* enhanced filamentous growth. Deletion of *MSS11* caused a profound defect in hyphal development and the induction of hypha-specific genes. Our data suggest that Mss11 functions as an activator in hyphal development of *C. albicans*. Furthermore, overexpression of *FLO8* can bypass the requirement of Mss11 in filamentous formation, whereas overexpression of *MSS11* failed to promote hyphae growth in *flo8* mutants. In summary, we show that the expression level of *MSS11* increases during hyphal induction, and the enhanced expression of *MSS11* may contribute to cooperative binding of Mss11 and Flo8 to the *HWP1* promoter.

The human fungal pathogen *Candida albicans* can cause superficial mucosal infections, severe surface infections and, in particular, life-threatening systemic diseases in immunocompromised patients (48). In recent years, the deaths due to *C. albicans* infections increased dramatically, despite the use of antifungal therapies (4). *C. albicans* can undergo reversible morphological transitions between yeast, pseudohyphal, and hyphal growth forms (6). Its ability to switch from yeast to hyphal growth in response to various signals is essential for its pathogenicity (20, 29, 35). Multiple signaling pathways and transcription factors are involved in regulating morphogenesis of *C. albicans* (13). Among them, the Efg1/Flo8-mediated cyclic AMP (cAMP)-dependent protein kinase A (PKA) pathway plays a predominant role in hyphal development and virulence (30). Many mutants in the cAMP/PKA pathway (*cdc35*, *tpk1*, *tpk2*, *efg1*, and *flo8*) are defective in hyphal formation and show decreased pathogenicity (8, 32).

In *C. albicans*, many transcriptional regulators, including Cph1 (33), Tec1 (45), and Flo8 (8), are identified based on sequence similarity and functional complementation to their homologues in *S. cerevisiae*. ScFlo8, a transcription factor critical for invasive growth and flocculation in haploids and pseudohyphal growth in diploids of *S. cerevisiae* (34), functions downstream of the cAMP/PKA pathway (44). The *C. albicans FLO8* homolog was identified by functional complementation of a *S. cerevisiae flo8* mutant. The *C. albicans* Flo8 is essential for hyphal development and hypha-specific gene expression. *flo8/flo8* mutants are unable to form hyphae in all liquid-inducing media but generate increased filaments under embedded conditions at low temperature. Flo8 can interact with Efg1, which is a potential target of the cAMP/PKA pathway, and they share a common set of target genes (8). Like ScFlo8, CaFlo8 also contains a LUFS domain (LUG/LUH, Flo8, single-stranded DNA-binding protein) (12). LUFS domain containing proteins play important roles in regulating critical developmental processes. LUG (Leunig) is a key regulator of flower-specific gene expression during flower development in *Arabidopsis* (12). The single-stranded DNA-binding protein (Ssdp) regulates the activity of LIM-homeodomain protein complexes in *Drosophila* (52). Within the LUFS domain, there is a lissencephaly type 1-like homology motif (LisH) (15). The LisH motif is widespread among many proteins, some of which are implicated in human diseases (1, 17, 46). The crystal structure of the N-terminal domain of mouse LIS1 shows that the LisH motif is a thermodynamically very stable dimerization domain and forms a tightly associated homodimer with a four-helix antiparallel bundle core (25), suggesting that Flo8 might interact with another LisH domain containing protein to regulate gene expression. In fact, a previous study in *Saccharomyces diastaticus* showed that Flo8 and Mss11, another LisH-containing transcription factor, function as a heterodimer to activate *STA1* expression (27).

MSS11 was originally isolated as a multicopy suppressor of *STA10* phenotype and an activator of *STA2* gene in *S. cerevisiae* (54). It plays a central role in the regulatory network involved in the activation of *FLO11*, which is essential for invasive/filamentous growth and flocculation (36). The Mss11 activity is independent of the presence of activators, including Tec1, Flo8, and Phd1, or the repressors Nrg1, Nrg2, Sok2, and

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▽ Published ahead of print on 4 September 2009.

TABLE 1. *C. albicans* and *S. cerevisiae* strains used in this study

Strain	Genotype	Source or reference
C. albicans		
SC5314	Wild type	18
CAF2-1	*URA3/ura3::λimm434*	18
CAI4	*ura3::λimm434/ura3::λimm434*	18
CSL1	*ura3::λimm434/ura3::λimm434 MSS11/mss11::hisG-URA3-hisG*	This study
CSL2	*ura3::λimm434/ura3::λimm434 MSS11/mss11::hisG*	This study
CSL3	*ura3::λimm434/ura3::λimm434 mss11::hisG/mss11::hisG-URA3-hisG*	This study
CSL4	*ura3::λimm434/ura3::λimm434 mss11::hisG/mss11::hisG*	This study
CCF3	*ura3::λimm434/ura3::λimm434 flo8::hisG/flo8::hisG-URA3-hisG*	8
CCF4	*ura3::λimm434/ura3::λimm434 flo8::hisG/flo8::hisG*	8
CSL5	*ura3::λimm434/ura3::λimm434 mss11::hisG/mss11::hisG flo8::hisG/flo8::hisG-URA3-hisG*	This study
RM1000	*ura3::λimm434/ura3::λimm434 his1::hisG/his1::hisG*	42
CSL6	*ura3::λimm434/ura3::λimm434 his1::hisG/his1::hisG RP10::ACT1p-MSS11-4FLAG-HIS1 FLO8/ FLO8-13MYCFLAG-URA3*	This study
CSL7	*ura3::λimm434/ura3::λimm434 his1::hisG/his1::hisG RP10::ACT1p-MSS11-4FLAG-HIS1*	This study
CSL8	*ura3::λimm434/ura3::λimm434 his1::hisG/his1::hisG RP10::ACT1p-FLO8-13MYCFLAG-HIS1 ADE2::ADH1p-MSS11-3HA-URA3*	This study
CSL9	*ura3::λimm434/ura3::λimm434 his1::hisG/his1::hisG RP10::ACT1p-FLO8-13MYCFLAG-HIS1 ADE2::ADH1p-MSS11ΔLisH-3HA-URA3*	This study
CSL10	*ura3::λimm434/ura3::λimm434 his1::hisG/his1::hisG RP10::ACT1p-MSS11-13MYCFLAG-HIS1 ADE2::ADH1p-FLO8-3HA-URA3*	This study
CSL11	*ura3::λimm434/ura3::λimm434 his1::hisG/his1::hisG RP10::ACT1p-MSS11-13MYCFLAG-HIS1 ADE2::ADH1p-FLO8ΔLisH-3HA-URA3*	This study
CSL12	*ura3::λimm434/ura3::λimm434 MSS11/MSS11-13MYCFLAG-URA3*	This study
CSL13	*ura3::λimm434/ura3::λimm434 flo8::hisG/flo8::hisG MSS11/MSS11-13MYCFLAG-URA3*	This study
CSL14	*ura3::λimm434/ura3::λimm434 FLO8/FLO8-13MYCFLAG-URA3*	This study
CSL15	*ura3::λimm434/ura3::λimm434 mss11::hisG/mss11::hisG FLO8/FLO8-13MYCFLAG-URA3*	This study
S. cerevisiae		
MLY61	*ura3-52/ura3-52 MAT***a**/α	37
MLY181a/α	*Δmss11::G418/Δmss11::G418 ura3-52/ura3-52 MAT***a**/α	38
BY4742	*MATα flo8-1 his3 leu2 lys2 ura3*	EUROSCARF
BY4742 *mss11Δ*	*MATα flo8-1 his3 leu2 lys2 ura3 mss11Δ::KanMX4*	EUROSCARF
CZS1	*MATα his3 lys2 ura3 flo8-1Δ::FLO8-LEU2*	This study
CZS2	*MATα his3 lys2 ura3 flo8-1Δ::FLO8-LEU2 mss11Δ::KanMX4*	This study
EGY48 (p8op-lacZ)	*MATα his3 trp1* $LexA_{op(\times 6)}$*-leu2* $LexA_{op(\times 8)}$-lacZ $_{flo8\text{-}1}$	23

Sfl1 and the signaling proteins Ras2, Kss1, and Tpk2 (19, 51). Genetic analysis shows that Mss11 is positioned downstream of Flo8 in regulating *FLO11* expression. In *S. diastaticus*, Mss11 plays an important role in activation of the *STA1* gene, which encodes an extracellular glucoamylase. The *STA1* gene is activated by sequentially binding of transcriptional activators Ste12, Tec1, Flo8, and Mss11 and the Swi/Snf chromatin-remodeling complex. Furthermore, it has been shown that Mss11 and Flo8 bind cooperatively to the *STA1* promoter to activate expression of the gene (27). However, the function of *C. albicans* Mss11 remains to be characterized.

In searching for the binding partner of Flo8 in *C. albicans*, we identified *C. albicans* Mss11 by sequence comparison and functional complementation. Like Flo8, *C. albicans* Mss11 acts as an activator in hyphal development and hypha-specific gene expression. We further investigated the interaction between Mss11 and Flo8 and their relationship in hyphal development.

MATERIALS AND METHODS

Strains and culture conditions. The *C. albicans* and *S. cerevisiae* strains used in the present study are listed in Table 1. Yeast strains were routinely grown on YPD (1% yeast extract, 2% peptone, 2% glucose) medium or on synthetic complete medium with 2% glucose (SCD) for selection of prototropic strains. The pseudohyphal colony formation and flocculent phenotype of *S. cerevisiae* were examined as described previously (34, 38). *C. albicans* strains were grown in YPD at 25°C for yeast form and in Lee medium or YPD containing 10% serum (Gibco) at 37°C for hyphal induction. YPS (1% yeast extract, 2% peptone, 2% sucrose) with 1% agar was used for colony morphology assay under embedded conditions (7).

Plasmid and strain construction. SC5314 genomic DNA was used as a template for all PCR amplifications of *C. albicans* genes. All constructs were verified by DNA sequencing. The plasmids used in the present study are listed in Table 2. The primers used for PCR amplification are listed in Table 3.

The pVT102U-CaMSS11 plasmid was constructed for complementation assays in *S. cerevisiae mss11* mutants. A PCR fragment (primers 1 and 2) containing the Ca*MSS11* coding sequence was subcloned into the BamHI-SstI site of pVT102U to express CaMss11 under the control of *ADH1p* in *S. cerevisiae*. To examine the flocculation phenotype in a background with a functional Sc*FLO8* gene, the wild-type Sc*FLO8* coding sequence was amplified from a Σ strain CGx68 genomic DNA (22) with primers 3 and 4, and inserted into the BamHI-SstI site of YIplac128 to create YIplac128-ScFLO8. BglII-digested YIplac128-ScFLO8 was introduced into strains BY4742 and BY4742 *mss11Δ* to replace the *flo8-1* mutant allele with wild-type Sc*FLO8*, generating strains CZS1 and CZS2, respectively. pGilda-CaMSS11 for expression of LexA-CaMss11 fusion protein under the *GAL1* promoter in *S. cerevisiae* was constructed by inserting a 2.07-kb PCR fragment (primers 5 and 6) containing the Ca*MSS11* coding sequence into the BamHI-NcoI site of pGilda.

The YEplac195-MSS11Δ plasmid for *C. albicans MSS11* disruption was constructed as follows. Primers 7 and 8 were used to amplify the upstream region of the *MSS11* open reading frame (ORF) from *C. albicans* genomic DNA; the PCR product was inserted into the PstI-XbaI site of YEplac195 (21), generating YEplac195-MSS11ΔN. Then, the downstream region of *MSS11* ORF (primers 9 and 10) was inserted into the KpnI-SstI site of YEplac195-MSS11ΔN to generate YEplac195-MSS11ΔNΔC. A 4-kb fragment containing the *HISG-URA3-HISG* cassette digested from pCUB6 with BglII and BamHI was subcloned into the BamHI site of YEplac195-MSS11ΔNΔC to create YEplac195-MSS11Δ. To de-

TABLE 2. Plasmids used in this study

Plasmid	Description	Source or reference
pVT102U	*S. cerevisiae URA3*/2μ vector with *ADH1* promoter and terminator	53
pVT102U-CaMSS11	1.93-kb full-length *C. albicans MSS11* in pVT102U	This study
YIplac128	2μ, *LEU2*-based vector	21
YIplac128-ScFLO8	3.21-kb full-length *S. cerevisiae FLO8* in YIplac128	This study
pGilda	2μ, *HIS3*-based vector carrying the LexA DNA-binding domain	Clontech
pGilda-CaMSS11	2.07-kb full-length *C. albicans MSS11* in pGilda	This study
pJG4-5	2μ, *TRP1*-based vector carrying the B42 activation domain	23
YEplac195	2μ, *URA3*-based vector	21
pCUB6	Substitution of *S. cerevisiae URA3* by *C. albicans URA3* in pNKY50	18
YEplac195-MSS11Δ	0.72-kb upstream region and 0.80-kb downstream region of *MSS11* ORF and *hisG-URA3-hisG* in YEplac195	This study
pBA1	*C. albicans ADH1* promoter in pBES116	8
pBA1-MSS11	2.17-kb full-length *C. albicans MSS11* in pBA1	This study
pBA1-FLO8	2.45-kb full-length *C. albicans FLO8* in pBA1	8
pBES116	*URA3* vector, digest with AscI for integration at *ADE2*	16
pBES116-MSS11	3.09-kb *C. albicans MSS11* fragment including 1.04-kb *MSS11* promoter region in pBES116	This study
pPR671	*C. albicans ACT1* promoter, 13×myc-FLAG, *HIS1*	8
pPR672	2.45-kb full-length *C. albicans FLO8* in pPR671	8
pFLAG-Act1	*C. albicans ACT1* promoter, FLAG, *URA3*	50
pPR677	2.45-kb full-length *C. albicans FLO8* in *URA3* vector with 13×myc-FLAG	This study
pPR678	1.92-kb full-length *C. albicans MSS11* in pPR671	This study
pPR679	1.92-kb full-length *C. albicans MSS11* in *URA3* vector with 13×myc-FLAG	This study
pFLAG-Act1-HIS1	*C. albicans ACT1* promoter, FLAG, *HIS1*	This study
p4FLAG-MSS11	1.92-kb full-length *C. albicans MSS11* and 3×FLAG in pFLAG-Act1-HIS1	This study
pYPB1-ADHpt	*C. albicans ADH1* promoter and *ADH1* terminator on a *C. albicans URA3*/*ARS*/2μ vector	28
BES-ADH	*C. albicans URA3* vector with *ADH1* promoter and terminator, digest with AscI for integration at *ADE2*	This study
ADH-3HA	*C. albicans ADH1* promoter, 3×HA, *URA3*	This study
ADH-3HA-MSS11	1.92-kb full-length *C. albicans MSS11* in ADH-3HA	This study
ADH-3HA-MSS11ΔN	1.69-kb *C. albicans MSS11ΔN* in ADH-3HA	This study
ADH-3HA-FLO8	2.45-kb full-length *C. albicans FLO8* in ADH-3HA	This study
ADH-3HA-FLO8ΔN	2.10-kb *C. albicans FLO8ΔN* in ADH-3HA	This study

lete *MSS11 in C. albicans*, YEplac195-MSS11Δ was digested with PstI and SstI, and then transformed into CAI4 to produce *MSS11*/*mss11* strains (CSL1). CSL1 was streaked onto a 5-fluoroorotic acid plate to select for the Ura^- strain CSL2. *mss11*/*mss11* homozygotes CSL3 (Ura^+) and CSL4 (Ura^-) were screened out by subsequent transformation. The disruption was confirmed by Southern blotting.

pBA1-MSS11 was constructed for expression of Ca*MSS11* under the control of *ADH1p* in *C. albicans*. The full-length coding sequence of *MSS11* was amplified with primers 11 and 12 and inserted into the BglII-ClaI site of pBA1 to generate pBA1-MSS11. pBES116-MSS11 was for expression of Ca*MSS11* under its own promoter for complementation assays in *C. albicans*. A 3.09-kb fragment containing the entire *MSS11* ORF and 1.04 kb of upstream sequence was inserted into pBES116 at the PstI-KpnI site. Primers 13 and 14 were used for the PCR amplification.

Plasmids for expression of 13×Myc fusion proteins were constructed as follows. The *HIS1* fragment of pPR672 (8) (digested with HindIII) was replaced with the *URA3* fragment from pFLAG-Act1 (50) to generate plasmid pPR676. pPR676 was digested with BamHI and StuI to delete the *ACT1* promoter, generating pFLO8-13MYC-FLAG-URA3 (pPR677) and then digested with SpeI for targeting the integration into the *FLO8* locus of CAI4 and *mss11*/*mss11* strains under *URA3* selection. The *FLO8* fragments in pPR672 and pPR677 (digested with BamHI and MluI) were replaced with the *MSS11* coding sequence (primers 15 and 16) to generate plasmids pPR678 and pPR679, respectively. Strains CAI4 and *flo8*/*flo8* were transformed with SpeI-digested pPR679 to express the Mss11-13Myc fusion protein under the endogenous promoter. pPR672 and pPR678 were digested with StuI to integrate into the genomic *RP10* locus for expression of 13×Myc fusion proteins under the *ACT1* promoter.

pFLAG-Act1-HIS1 is a derivative of pFLAG-Act1 (50), in which the *URA3* fragment in the HindIII site was substituted by *HIS1* fragment from pPR671. A 2.0-kb PCR product (primers 1 and 17) containing the entire *MSS11* coding region and a 3×FLAG tag was inserted into the BamHI-SphI site of pFLAG-Act1-HIS1 to create p4FLAG-MSS11. The StuI-digested p4FLAG-MSS11 DNA fragment was introduced into the RM1000 strain for 4FLAG-Mss11 expression, and then the strain was transformed with SpeI-digested pPR677 to express Flo8 under its own promoter. Expression of Mss11 and Flo8 in YPD medium at 30°C was verified by Western blot analysis using anti-FLAG-conjugated peroxidase antibody (Sigma) and anti-c-myc antibody (Santa Cruz), respectively.

To express hemagglutinin (HA) fusion proteins under the control of *ADH1* promoter, a 4.5-kb PCR product (primers 18 and 19) amplified from pBES116 was digested with NotI and XbaI and then ligated with a fragment (with the same restriction sites) containing the *ADH1* promoter and *C. albicans URA3* gene from pYPB1-ADHpt (28) to generate BES-ADH. The synthetic 3×HA fragment (GGATCCATGTACCCATACGATGTTCCAGATTACGCTGGTTACCCATACGATGTTCCAGATTACGCTGGTTACCCATACGATGTTCCAGATTACGCTATCGATATCCCATGGAGATCTACGCGT [the introduced multiple cloning sites are underlined]) was placed into the BamHI-MluI site of the BES-ADH plasmid, generating the vector ADH-3HA. DNA fragments encoding full-length Mss11 (primers 20 and 21), C-terminal Mss11 (primers 22 and 21), full-length Flo8 (primers 23 and 24), and C-terminal Flo8 (primers 25 and 24) were inserted into the ClaI-MluI site of ADH-3HA to create expression vectors for coimmunoprecipitation assays.

Northern blotting. RNA extraction and Northern blotting were performed as described previously (10). The *HWP1* and *ECE1* PCR products (41) were used for probing Northern blots. The *ACT1* probe was used as previously described (31). The ImageQuant 5.2 Software is used to evaluate the relative density of different bands on the scanned image of a gel.

Immunoprecipitation. Protein extraction was performed as described previously (8). To determine whether Mss11 interacts with Flo8 in vivo, crude extract of *C. albicans* cells was precleared with protein G-agarose (Roche) and then incubated with 2.0 μg of anti-c-myc antibody (Santa Cruz). After incubation for 2 h at 4°C, ~40 μl of protein G-agarose suspension was added to precipitate the immunocomplex. The proteins were subjected to Western blotting with anti-FLAG peroxidase conjugated antibody (Sigma). For the immunoprecipitation shown in Fig. 6, 2.0 μg of anti-HA antibody (Santa Cruz) was added to the lysates, and the immunocomplex was detected by using anti-c-myc antibody.

TABLE 3. Primers used in this study

Primer	Sequence (5′–3′)[a]	Purpose and features
1	GCAGGATCCATACCACATAGATGTCTAAAC	pVT102U-CaMSS11
2	GCAGAGCTCTTATTATTCATAACCTGGACC	
3	CTAGGATCCAATGCTGGCTCTAGTAGTAAC	YIplac128-ScFLO8
4	CTAGAGCTCCAATGAGTGTACATCAACCAG	
5	CTAGGATCCGAATACCACATGAGATG	pGilda-CaMSS11
6	CTGCCATGGTAGTACATAAGAAGAGC	
7	GCACTGCAGGTTGCAATATGGAGATAAGAA	YEplac195-MSS11ΔN
8	GCCTCTAGAAATAAGTTCCCTTACAACTTG	
9	GCAGGTACCGTATGCCACCTAATCAGAATC	YEplac195-MSS11ΔNΔC
10	GCAGAGCTCTTATATTAAGCGGTTGTGCGA	
11	CTAAGATCTGAGATGTCTAAACCACCACCT	pBA1-MSS11
12	CTGATCGATATCAAGAATACCCTCTACCTC	
13	GTCCTGCAGGTATTATCGATTACAG	pBES116-MSS11
14	CTAGGTACCAGTACATAAGAAGAGC	
15	CGGGATCCCTGAATACCACATGAGATGTCT	pPR678 and pPR679
16	GTAGACGCGTCGTTCATAACCTGGACCTGATC	
17	ACATGCATGC**CTTGTCATCGTCATCCTTGTAATCGATGTCATGATCTTTAT AATCACCGTCATGGTCTTTGTAGTC**TTCATAACCTGGACCTGA	p4FLAG-MSS11
18	CTGCGCGGCCGCGATCTGTTTAGCTGGTGATTT	BES-ADH
19	TGTCTAGAGGTGTTGAAATGTTCTTGTTG	
20	CTGATCGATATGTCTAAACCACCACCTC	ADH-3HA-MSS11
21	CTAACGCGTTTATTCATAACCTGGACCTG	
22	CTGATCGATGATAATAGTGGTCCTGATC	ADH-3HA-MSS11ΔN
23	CTGATCGATATGAATCATAAACAAGTACTACCAG	ADH-3HA-FLO8
24	CATACGCGTTCTAATCGCCATTTTCAATTGGATC	
25	CTGATCGATAGTCAACTTCCTCTTATACAG	ADH-3HA-FLO8ΔN

[a] Restriction sites are underlined. The boldface sequence in primer 17 is 3×FLAG.

ChIP analyses. Overnight cultures were grown in YPD for 6 h at 25°C to an optical density at 600 nm of 0.8 for yeast growth or in YPD plus 10% serum for 3 h at 37°C for hyphal induction. Chromatin immunoprecipitations (ChIPs) were performed as described previously (39). Cells were formaldehyde cross linked and lysed using lysis buffer (50 mM HEPES-KOH [pH 7.5], 140 mM NaCl, 1 mM EDTA, 1% Triton X-100, and 0.1% sodium deoxycholate). Then, the DNA was sheared by sonication three times for 10 s at high power on a Bioruptor, with incubation on ice for 20 s between sonication pulses. To precipitate the Myc-tagged proteins, 8 μl of monoclonal Myc antibody (Abcam) was used in an immunoprecipitation volume of 400 μl (~2 mg of chromatin proteins). DNA derived from the whole-cell extract, and the immunoprecipitation eluate was analyzed by PCR or quantitative PCR.

RESULTS

Identification of *C. albicans MSS11*. We have reported that the transcription factor Flo8 is essential for hyphal development in *C. albicans* (8). In *S. diastaticus*, Flo8 and Mss11 bind cooperatively to the *STA1* promoter and function as a heterodimer in activating *STA1* expression (27). Furthermore, Mss11 is a transcription factor playing important roles in flocculation, filamentous growth, starch metabolism, and activation of gene expression in *S. cerevisiae* (2, 19, 38, 51, 54). To determine whether a similar molecular mechanism exists in *C. albicans*, we searched the NCBI database (http://www.ncbi.nlm.nih.gov/BLAST/) for the *S. cerevisiae* Mss11 homolog and found a *C. albicans* protein designated Mss11 (orf19.6309) that shares similar structural features with *S. cerevisiae* Mss11 (ScMss11). Like ScMss11, CaMss11 contains a LisH motif at its N terminus, a glutamine-rich region in the central part and asparagine-rich regions at the C terminus. As shown in Fig. 1A, both CaFlo8 and ScFlo8 contain a LisH motif and a glutamine-rich region but lack asparagine-rich regions. In contrast to other LisH motif containing proteins in which the glutamine-rich regions are located at the right part of the motif, the glutamine-rich region of ScFlo8 is located at the left part of the LisH motif. The Ca*MSS11* gene encodes a putative protein of 637 amino acids, with a small region (amino acids 29 to 101) that is highly similar to a region (amino acids 51 to 150) in the ScMss11 (Fig. 1B). The conserved region, named the LUFS domain (12), exists in many regulators, including CaFlo8, ScFlo8, LEUNIG, and Ssdp. The LUFS domain consists of a conserved LisH motif in its N-terminal part and a coiled-coil region in the C terminus, and the two regions are separated by a linker varying in length. In the *C. albicans* genome, there are five LisH motif-containing proteins: Flo8, putative Mss11, potential SET3 histone deacetylase complex component Sif2, potential SAGA complex component Taf5, and a putative uncharacterized protein. In addition, sequence analysis shows that Mss11 and Flo8 in *C. albicans* contain the LUFS domain (Fig. 1B).

Although CaMss11 and ScMss11 share similar structural features, the sequence similarity between them is very low. To determine whether CaMss11 is a functional homolog (ortholog) of ScMss11, we examined the ability of CaMss11 to complement the flocculation and filamentous growth defect in Sc*mss11* mutants. An expression plasmid, pVT102U-CaMSS11, containing Ca*MSS11* ORF under the control of *ADH1* promoter, was introduced into the Sc*mss11* mutant strains. To eliminate the effect of Sc*FLO8* mutation on the nonflocculent phenotype of Sc*mss11* mutants, a wild-type Sc*FLO8* gene was integrated into strains BY4742 and BY4742 *mss11*Δ to replace the *flo8-1* mutant allele. Ectopically expressed Ca*MSS11* suppressed the nonflocculent phenotype of a haploid Sc*mss11* mutant (Fig. 1C) and the filamentous

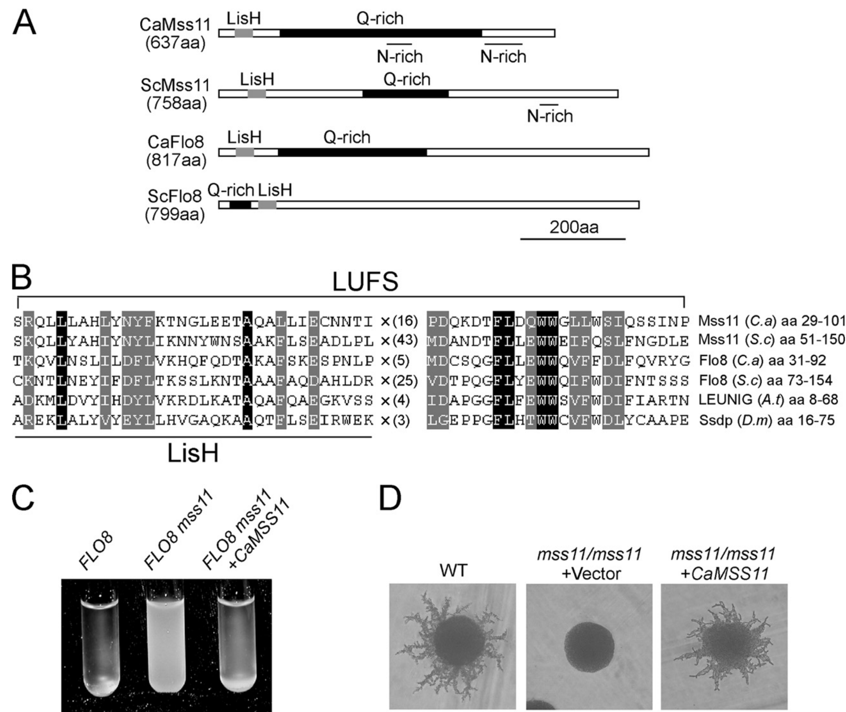

FIG. 1. *C. albicans* Mss11 is a functional homolog of *S. cerevisiae* Mss11. (A) Schematic depiction of the LisH domain (gray box), glutamine-rich (Q-rich) regions (black box), and asparagine-rich (N-rich) regions (underlined areas) in the CaMss11, ScMss11, CaFlo8, and ScFlo8. aa, amino acids. (B) Sequence alignment of the LUFS domain between CaMss11 and other regulatory proteins. Identical residues are shaded in black and conserved residues are shaded in gray. The multiplication sign (×) and subscript numbers indicate spacing between the motifs in the LUFS domain. (C) Ectopically expressed Ca*MSS11* suppresses the nonflocculent phenotype of a haploid *mss11* mutant. *S. cerevisiae* strains CZS1 (*FLO8*) carrying pVT102U and CZS2 (*FLO8 mss11*) carrying pVT102U or pVT102U-CaMSS11 were grown in SCD to saturation, allowed to settle for 5 min, and then photographed. (D) Ectopically expressed Ca*MSS11* suppresses the pseudohyphal growth defect of a diploid *mss11* mutant. *S. cerevisiae* strains MLY61 (WT) carrying pVT102U and MLY181a/α (*mss11*/*mss11*) carrying pVT102U or pVT102U-CaMSS11 were grown on SLAD plates at 30°C for 5 days.

growth defect of a diploid Sc*mss11* mutant (Fig. 1D). The results show that Ca*MSS11* can functionally complement Sc*mss11* mutant in both flocculation and filamentous growth.

Overexpression of *MSS11* promotes filaments formation in *C. albicans*. ScMss11 functions as a transcriptional activator in *S. cerevisiae*. To examine whether CaMss11 also acts as an activator in filamentous growth of *C. albicans*, a single copy of Ca*MSS11* under the control of *ADH1* promoter was introduced into the wild-type strain at the *ADE2* locus. As shown in Fig. 2A, wild-type strains formed wrinkled colonies in Lee's medium at 25°C, while the Ca*MSS11*-overexpressing strains formed wrinkled colonies surrounded by long filaments. To analyze the activating effect of CaMss11 on gene expression, we fused CaMss11 with a LexA DNA-binding domain and used *lacZ* as a reporter for measuring the transcriptional activity of CaMss11 in the yeast system. The β-galactosidase activity assays with X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) as a substrate showed that the LexA-CaMss11 fusion protein could promote expression of the *lexAop-lacZ* reporter with or without the existence of the B42 activation domain (Fig. 2B), a finding consistent with the result of a Gal4-ScMss11 fusion in activating *lacZ* expression (19). Our data suggested that *C. albicans* Mss11 functions as a transcriptional activator in filamentous growth and gene expression.

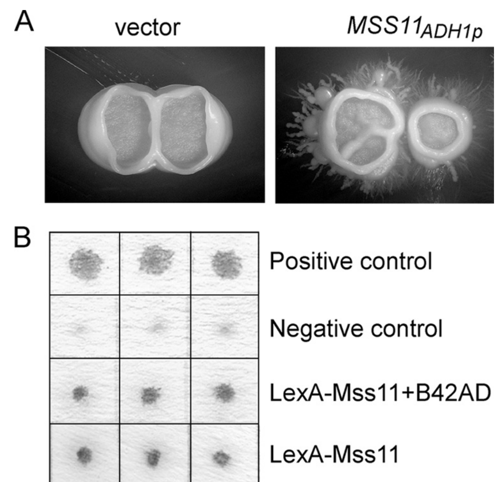

FIG. 2. CaMss11 functions as an activator in hyphal development. (A) Overexpression of *MSS11* promotes filamentous growth in *C. albicans*. Strain CAI4 (wild type) carrying pBA1 or pBA1-MSS11 was grown on Lee's plates at 25°C for 10 days. (B) CaMss11 exhibits strong transcriptional activity in *S. cerevisiae*. For the β-galactosidase assay, strain EGY48 carrying pGilda-CaMSS11 was transformed with or without vector pJG4-5. Three corresponding transformants were tested for the level of reporter gene expression using X-Gal. pSH17-4 was a positive control plasmid for LexA-AD fusion protein. pRFHM1 was a negative control plasmid for expression of a LexA-human lamin C fusion protein (43). The results are representative of three independent experiments.

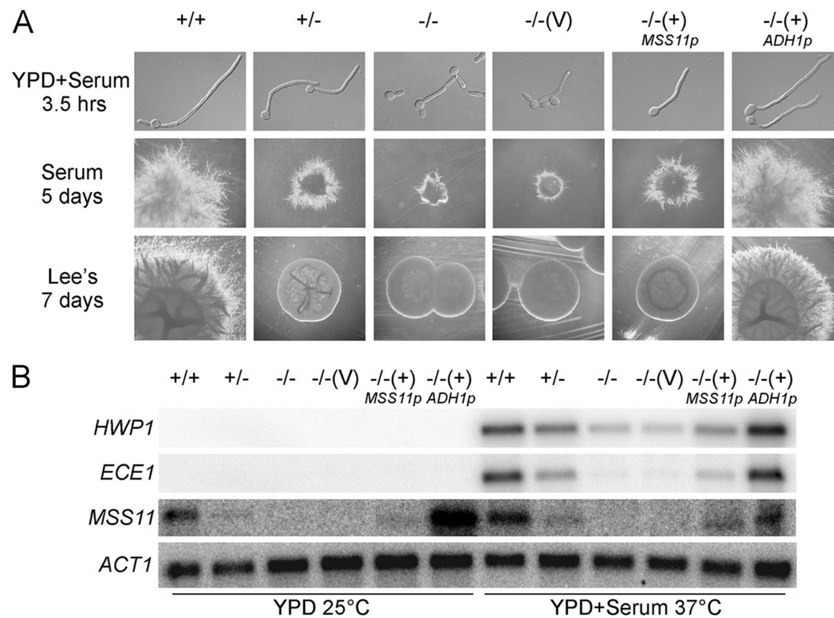

FIG. 3. Mss11 is required for hyphal development and the expression of hypha-specific genes in *C. albicans*. (A) Effects of *MSS11* disruption on hyphal development. Cells were induced in YPD plus 10% serum for 3.5 h at 37°C (top). Colony morphology of *mss11*/*mss11* mutants (middle and bottom rows). Strains were plated on solid serum-containing medium and solid Lee's medium, and incubated at 37°C for 5 and 7 days, respectively. (B) *mss11*/*mss11* mutants were defective in the induction of hypha-specific genes. Cells were grown in YPD plus 10% serum at 37°C for 3.5 h or grown in YPD at 25°C for 6 h and collected for RNA extraction and Northern analysis. The image for the *MSS11* hybridization was obtained after two weeks of exposure. Image for the *HWP1*-, *ECE1*-, and *ACT1*-probed filters were obtained after 3 h of exposure. Strains shown in panels A and B: wild type (CAF2-1), *MSS11*/*mss11* (CSL1, +/−), *mss11*/*mss11* (CSL3, −/−), *mss11*/*mss11* + vector [CSL4 + pBA1, −/−(V)], *mss11*/*mss11* + *MSS11* [CSL4 + pBES116-MSS11, $-/-(+)_{\mathrm{MSS11p}}$], *mss11*/*mss11* + *ADH1p-MSS11* [CSL4 + pBA1-MSS11, $-/-(+)_{\mathrm{ADH1p}}$].

Deletion of *MSS11* prevents hyphal formation. To elucidate the role of *C. albicans* Mss11 in hyphal development, we constructed an *mss11*/*mss11* null mutant by sequential gene disruption using a *hisG-URA3-hisG* cassette in *C. albicans*. Successful deletion of the *MSS11* was confirmed by Southern (data not shown) and Northern blot analysis (Fig. 3B). Deletion of the *MSS11* caused a significant reduction of filaments formation in response to hyphal induction (Fig. 3A). In liquid serum-containing medium, wild-type cells formed true hyphae, whereas the *mss11*/*mss11* mutant cells displayed pseudohyphalike form with a low percentage (ca. 20%) of stunted hyphae. On solid serum containing medium, the wild-type strain produced florid filamentous colonies, while the *mss11*/*mss11* mutants formed small downy colonies without long filaments (Fig. 3A). The *mss11*/*mss11* mutant strain displayed a more severe defective phenotype of hyphal development in Lee's medium. In contrast to the wild-type strain which developed extensive filaments, the *mss11*/*mss11* mutant formed smooth colonies even after incubation for 7 days at 37°C on solid Lee medium. The defects of *mss11*/*mss11* mutant strains in hyphal development could be rescued by integrating a wild-type *MSS11* under the *ADH1* promoter (Fig. 3A). Interestingly, *MSS11* exerted its effects on hyphal development in a dosage-dependent manner. Reintroducing a single copy of *MSS11* under its own promoter could not fully restore the ability of *mss11*/*mss11* mutants to form filaments. Similarly, *MSS11*/*mss11* heterozygotes also showed reduced filaments in solid serum-containing medium. More obviously, strains containing a single copy of *MSS11* could not promote filaments formation and formed smooth-edged colonies on solid Lee's medium (Fig. 3A). Therefore, our data indicate that Mss11 is required for hyphal development in *C. albicans*.

To explain the dosage effect of Mss11 on filamentous growth, we examined the transcription of *MSS11* under the control of the *ADH1* promoter and its own promoter. The transcription levels of *MSS11* in *MSS11*/*mss11* heterozygotes and a single-copy *MSS11* revertant under its endogenous promoter were lower than that in *MSS11*/*MSS11* wild-type cells. The transcription level of *MSS11 in* single copy *MSS11* revertant under the *ADH1* promoter was 10-fold higher than that under its own promoter in yeast growth condition, but only 2-fold higher in hyphal growth condition (Fig. 3B). This is consistent with the results reported by Swoboda et al. that the level of *ADH1* mRNA fluctuates during the yeast-to-hyphal transition (49). Considering the dosage and positioning effect of *URA3* in filamentation, we examined the phenotypes of *MSS11*/*mss11* and *mss11*/*mss11* mutants in the media containing additional uridine and observed similar phenotypes to that shown in Fig. 3A. In addition, we also compared the phenotypes between the *MSS11*/*mss11* and *mss11*/*mss11* mutant strains containing a single copy of *URA3* at the *ADE2* locus or at the *MSS11* locus. The two sets of strains shared similar phenotypes in the media with or without additional uridine. Therefore, the gene dosage effect of *MSS11* in affecting filamentation was not due to different expression level of *URA3* or its different positions in the genome.

The expression of hypha-specific genes correlates with hyphal morphogenesis in *C. albicans*. *HWP1*, which serves as a mammalian transglutaminase substrate responsible for *C. albicans*-host interaction (47), was highly induced in wild-type

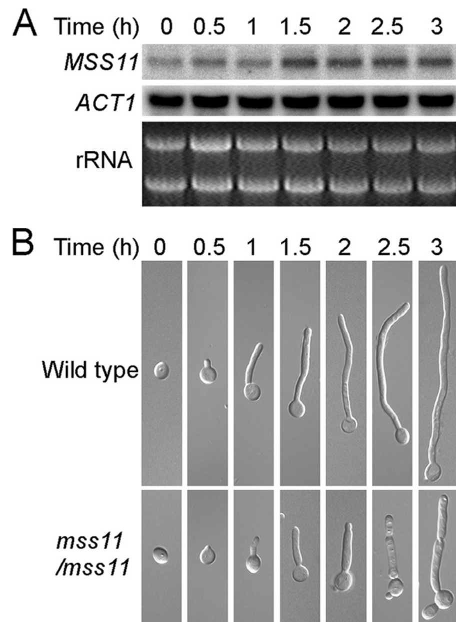

FIG. 4. The enhanced expression of *MSS11* during hyphal induction is associated with the process of cell elongation in *C. albicans*. (A) *MSS11* transcript level increases in response to serum at 37°C. For Northern analysis, RNA was extracted from the wild-type strain (SC5314) after the indicated times of growth in liquid YPD plus 10% serum medium at 37°C. (B) Time-lapse microscopy of CAF2-1 and *mss11*/*mss11* cells in liquid YPD containing 10% serum at 37°C.

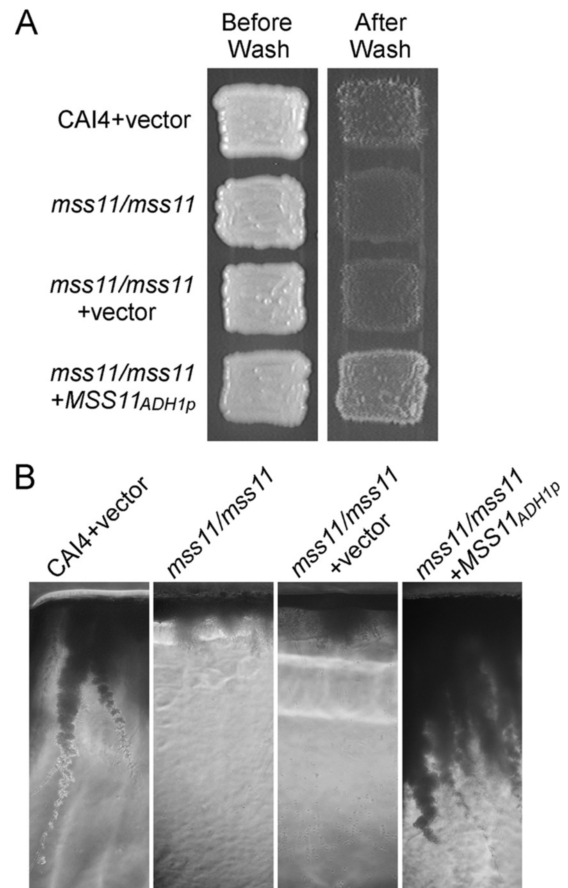

FIG. 5. Invasive growth assay of *C. albicans*. Strains of the wild type (CAI4 + pBA1), *mss11*/*mss11* (CSL3), *mss11*/*mss11* + vector (CSL4 + pBA1), and *mss11*/*mss11* + *ADH1p-MSS11* (CSL4 + pBA1-MSS11) were patched on solid YPD and incubated at 30°C for 2 days. (A) The plates were photographed before and after washing. (B) After washing with water, the agar containing cells was cut into slices and photographed.

cells under hypha-inducing conditions but was reduced 10-fold in *mss11*/*mss11* mutant cells (Fig. 3B). As expected, the expression of *ECE1*, another hypha-specific gene (3), was reduced 60-fold in *mss11*/*mss11* cells compared to that in wild-type cells. Consistent with the phenotypes, the *MSS11*/*mss11* heterozygote as well as the single-copy *MSS11* revertant under the control of the endogenous *MSS11* promoter showed decreased expression of *HWP1* and *ECE1*, whereas the *MSS11* revertant under the control of the *ADH1* promoter could fully restore the transcription of *HWP1* and *ECE1* (Fig. 3B).

To examine the expression level of *MSS11* during hyphal development, wild-type (SC5314) cells were cultured overnight in YPD at 25°C and then released to liquid serum-containing medium for hyphal induction at 37°C and collected at various times up to 3 h for Northern blot analysis. The expression of *MSS11*, which was at a low level in yeast cells induced in response to serum, then reached the highest level in hyphal cells, which was ~3-fold higher than that in yeast cells (Fig. 4A). To distinguish the effect of temperature switch on the expression of *MSS11*, we performed Northern analysis to examine the expression level of *MSS11* in YPD at 37°C. Although the transcription of *MSS11* was enhanced slightly (~1.2-fold) in YPD at 37°C compared to that in YPD at 25°C but unable to reach the level that in YPD plus serum at 37°C (data not shown). Therefore, the increased expression of *MSS11* responding to serum at 37°C was not due to the effect of temperature shift. Considering the existence of elongated cells in YPD at 37°C, the slightly enhanced *MSS11* expression seems to correlate with the process of hyphal elongation. Consistently, deletion of Mss11 inhibited the cells from forming long filaments in serum-containing medium (Fig. 4B). The upregulated expression of *MSS11* in hyphal cells reflected the regulatory activity of *Mss11* in response to hyphal induction.

A previous study of *S. cerevisiae* showed that the *mss11* mutant was severely defective in haploid invasive growth (51). To investigate the function of *C. albicans* Mss11 in the invasive growth, the strains were patched on YPD plates, incubated at 30°C for 2 days, and then washed with water for observation. Most of the wild-type cells on the surface were washed off, while some of them remained and penetrated into agar which formed invasive filaments under the agar (Fig. 5). The *mss11*/*mss11* mutant cells were easily washed off and showed a very weak invasive growth. The invasive growth defect of *mss11*/*mss11* mutant cells could be rescued by integrating a wild-type *MSS11* under the *ADH1* promoter. In fact, overexpressed Mss11 caused more cells to stick to the agar surface and promoted invasive filaments formation (Fig. 5). These observations suggest that the function of Mss11 in the invasive growth is conserved between *S. cerevisiae* and *C. albicans*.

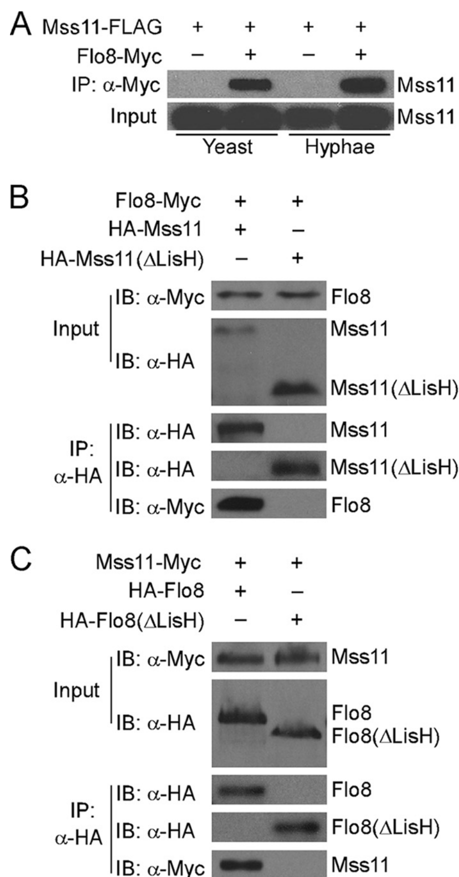

FIG. 6. Mss11 interacts with Flo8 in vivo. (A) Mss11 interacts with Flo8 in yeast and hyphal cells. *C. albicans* CSL6 (Mss11-FLAG Flo8-Myc) and CSL7 (Mss11-FLAG) were grown at 25°C in YPD for yeast growth, and at 37°C in YPD + 10% serum for hyphal growth. Protein lysates were subjected to immunoprecipitation with anti-Myc antibody, and the precipitated proteins were separated by sodium dodecyl sulfate–8% polyacrylamide gel electrophoresis and probed with peroxidase-conjugated anti-FLAG. As an input control, cell lysates were analyzed by Western blotting with the peroxidase-conjugated anti-FLAG antibody. (B and C) Mss11 interacts with Flo8 via the LisH motif. Total proteins were extracted from *C. albicans* CSL8 (HA-Mss11 Flo8-Myc), CSL9 (HA-Mss11ΔN Flo8-Myc), CSL10 (HA-Flo8 Mss11-Myc), and CSL11 (HA-Flo8ΔN Mss11-Myc) after growth in YPD at 30°C to an optical density at 600 nm of ~1.0. The tagged proteins were pulled down with anti-HA antibody. Western blots were probed with anti-HA antibody or anti-Myc antibody. The relative amounts of protein loaded in the input control in panels A to C were 1% of the cell extracts used for immunoprecipitation.

Mss11 and Flo8 interact in vivo. Mss11 and Flo8 are required for hyphal development and expression of hypha-specific genes in *C. albicans*. In addition, both Mss11 and Flo8 contain the LisH motif (amino acids 29 to 61 in Mss11 and amino acids 31 to 63 in Flo8), which is a thermodynamically stable dimerization domain. To define the relationship between Mss11 and Flo8, we examined the interaction between the two transcription factors by immunoprecipitation analysis. C-terminal 4FLAG-tagged Mss11 from *ACT1* promoter at the *RP10* locus was coexpressed with C-terminal 13Myc-tagged Flo8, which is under the control of its own endogenous promoter. As shown in Fig. 6A, the Mss11-4FLAG could be coimmunoprecipitated with anti-Myc antibody under both yeast and hyphal growth conditions, suggesting that Mss11 and Flo8 can interact in vivo and that the interaction is not affected by growth forms. The interaction between Mss11 and Flo8 is specific since the interaction was undetectable in the control strain that carried Mss11-4FLAG and Act1-13Myc fusion protein (data not shown).

Considering the protein level of Mss11 and Flo8 may affect their interactions, we detected the Mss11 expressing by Western blot analysis, and revealed that the expression level of *MSS11* from the *ACT1* promoter at the *RP10* locus was almost same as that from its own promoter (data not shown). Therefore, the coimmunoprecipitation assay in Fig. 6A should represent the Mss11-Flo8 interaction in native situation. Tagging with 13Myc, 4FLAG, and 3HA may change the conformation of Mss11 and Flo8 and further affect their cellular functions. We introduced all of the N- or C-terminally tagged Flo8 and Mss11 shown in Fig. 6A, B, and C into *flo8/flo8* or *mss11/mss11* mutants, respectively. All of the tagged Flo8 or Mss11 can complement the defect of the mutants in filamentous growth (data not shown), indicating that the tagged Mss11 and Flo8 in Fig. 6 were all functional.

To determine whether the interaction between Mss11 and Flo8 is mediated by the LisH motif, the N-terminal 73 amino acids of Mss11 was removed. The truncated Mss11 lacking the LisH motif was fused with a 3HA tag at its N terminus (HA-Mss11ΔLisH) and subjected to the coimmunoprecipitation assay with Flo8-13Myc. The interaction between Mss11 and Flo8 was abolished by deletion of the LisH motif in Mss11 (Fig. 6B). To verify that the LisH motif in Flo8 is also required for the Mss11-Flo8 interaction, Flo8 with an N-terminal truncation of 119 amino acids was fused with a 3HA tag at its N terminus (HA-Flo8ΔLisH) and coexpressed with the Mss11-13Myc under the control of *ACT1* promoter. The coimmunoprecipitation analysis with the truncated Flo8 protein indicated that the LisH motif of Flo8 is necessary for interaction with Mss11 (Fig. 6C). The results suggest that Mss11 interacts with Flo8 through the LisH motif.

Deletion of *MSS11* weakens the binding of Flo8 to the *HWP1* promoter in hyphal cells. Considering the *mss11/mss11* mutants are defective in the expression of *HWP1*, we reasoned that Mss11 might play an important role in transcriptional activation of *HWP1*. To test this possibility, a ChIP assay was performed to determine the presence of Myc-tagged Mss11 on the *HWP1* promoter. The *ADE2* promoter was served as a negative control as described in a previously published study utilizing the ChIP assay (39). As shown in Fig. 7A, Mss11-Myc expressed from its own endogenous promoter can bind to a specific region (roughly from −1381 to −1101) on the *HWP1* promoter, a UAS region essential for the induction of *HWP1* (26), but not to the surrounding regions of the *HWP1* promoter. The promoter binding of Mss11 was detected under both yeast and hyphal growth conditions (Fig. 7A), and a significant enhancement in Mss11-Myc binding during hyphal induction was observed ($P < 0.01$) (Fig. 7B), a finding consistent with the phenomena of increased expression of *MSS11* in response to serum at 37°C (Fig. 4A). ChIP analysis with Flo8-Myc showed that Flo8 bound to the same UAS region as Mss11 in the *HWP1* promoter (Fig. 7A). Interestingly, the Flo8 binding was increased in hyphal cells compared to that in yeast cells

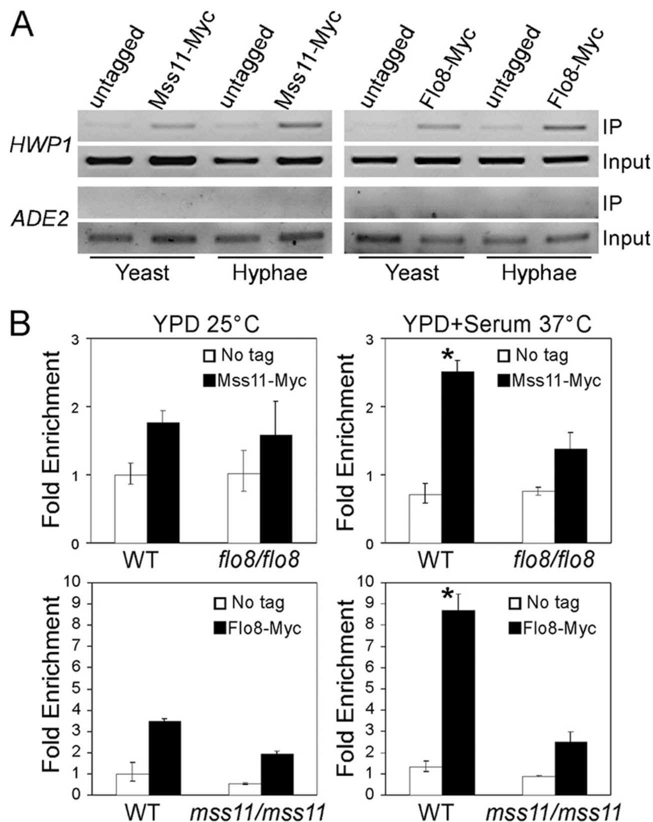

FIG. 7. Mss11 and Flo8 are present at the promoter of *HWP1*. (A) ChIP of Mss11 and Flo8 at a specific region of the *HWP1* promoter (approximately positions −1381 to −1101) in yeast and hyphal cells. Strains CAF2-1 (no tag control), CSL12 (Mss11-Myc), and CSL14 (Flo8-Myc) were grown in either YPD at 25°C or YPD plus 10% serum at 37°C. (B) The enhanced binding of Mss11 and Flo8 proteins in the hyphal state depends on each other. For ChIP of Mss11 in the wild-type (CSL12) or *flo8/flo8* (CSL13) strains (top row), wild-type (CAF2-1) and *flo8/flo8* (CCF3) were used as no-tag controls, respectively. For ChIP of Flo8 in the wild-type (CSL14) or *mss11/mss11* (CSL15) strains (bottom row), wild-type (CAF2-1) and *mss11/mss11* (CSL3) were used as no-tag controls, respectively. DNA in the ChIP reactions was determined by quantitative PCR with primers at approximately positions −1381 to −1101 of the *HWP1* promoter. The enrichment value was then presented as a ratio of immunoprecipitation versus input. Bars indicate standard deviation. *, $P < 0.05$ compared to the wild-type strain carrying Myc-tagged proteins under yeast growth condition. Cells were grown either in YPD at 25°C for yeast growth or in YPD plus 10% serum at 37°C for hyphal growth.

(Fig. 7B), although the transcriptional level of *FLO8* was unchanged during hyphal induction (data not shown).

Since Mss11 can interact with Flo8 via the LisH motif, we speculated that the two proteins might cooperate to achieve the hyphal induced transcriptional activation of *HWP1*. Therefore, we performed a ChIP assay to examine whether Flo8 is required for Mss11-binding to the *HWP1* promoter or vice versa. In the yeast growth condition, deletion of *MSS11* or *FLO8* has minor effect on the promoter binding of Flo8 or Mss11, respectively (Fig. 7B). However, lack of Mss11 or Flo8 has significant effect on each other's binding in hyphal growth condition, the ChIP signal of Mss11 in *flo8/flo8* mutant or Flo8 in *mss11/mss11* mutant remained almost unchanged during hyphal induction (Fig. 7B). The data suggested that Mss11 and Flo8 influence each other's enhanced binding to the *HWP1* promoter in hyphal cells.

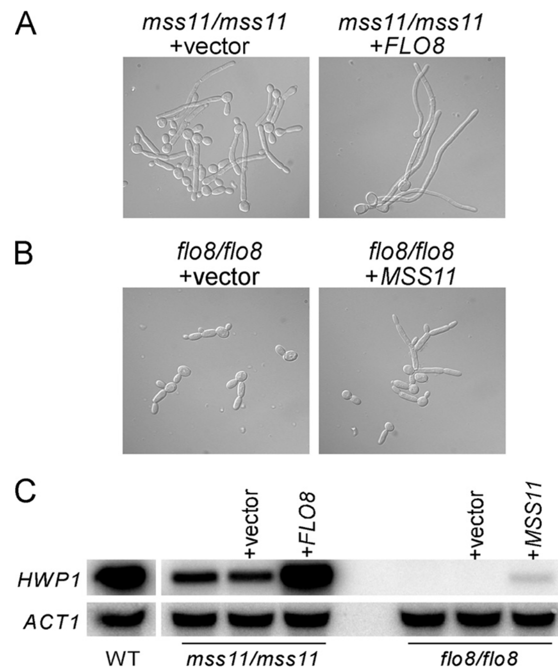

FIG. 8. Mss11-stimulated hyphal development in *C. albicans* requires Flo8. (A) *FLO8* overexpression suppresses the defect of *mss11/mss11* mutant in filamentous growth. (B) Effect of *MSS11* overexpression in the *flo8/flo8* mutant. In panels A and B, the cells were induced in YPD plus 10% serum for 3.5 h at 37°C. (C) Northern analysis of *HWP1* in the wild-type (CAF2-1), *mss11/mss11* (CSL3), *mss11/mss11* + vector (CSL4 + pBA1), *mss11/mss11* + *ADH1p-FLO8* (CSL4 + pBA1-FLO8), *flo8/flo8* (CCF3), *flo8/flo8* + vector (CCF4 + pBA1), and *flo8/flo8* + *ADH1p-MSS11* (CCF4 + pBA1-MSS11) strains. The experiment in panel C was carried out under the same conditions as in panels A and B.

Overexpression of *MSS11* cannot promote hyphal growth in *flo8/flo8* mutant under aerobic conditions. To define the functional relationship between Mss11 and Flo8, we performed reciprocal epistatic analysis. The filamentation defect in the *mss11/mss11* mutant could be suppressed by overexpression of *FLO8* (Fig. 8A), whereas the hyphal formation defect of the *flo8/flo8* mutant was hardly suppressed by overexpression of *MSS11* (Fig. 8B). The *flo8/flo8* mutant carrying *MSS11* under the *ADH1* promoter only generated pseudohyphalike cells and exhibited a very low level of *HWP1* expression when induced by serum (Fig. 8C), indicating that the presence of Flo8 is required for Mss11-mediated transcriptional activation of filamentous growth. In agreement with the phenotype, we observed that the *HWP1* expression defect was restored by overexpressing *FLO8* in the *mss11/mss11* mutant (Fig. 8C). This result suggested that overproduction of Flo8 can activate *HWP1* expression and hyphal growth in the absence of Mss11. We further constructed an *mss11/mss11 flo8/flo8* double mutant for morphological observation. As expected, the double mutant strain failed to form filaments in the serum-containing medium, resulting in a phenotype similar to that of the *flo8/flo8* mutant (Fig. 9A).

Mss11 and Flo8 have different effects on hyphal growth under embedded condition at low temperature. It has been

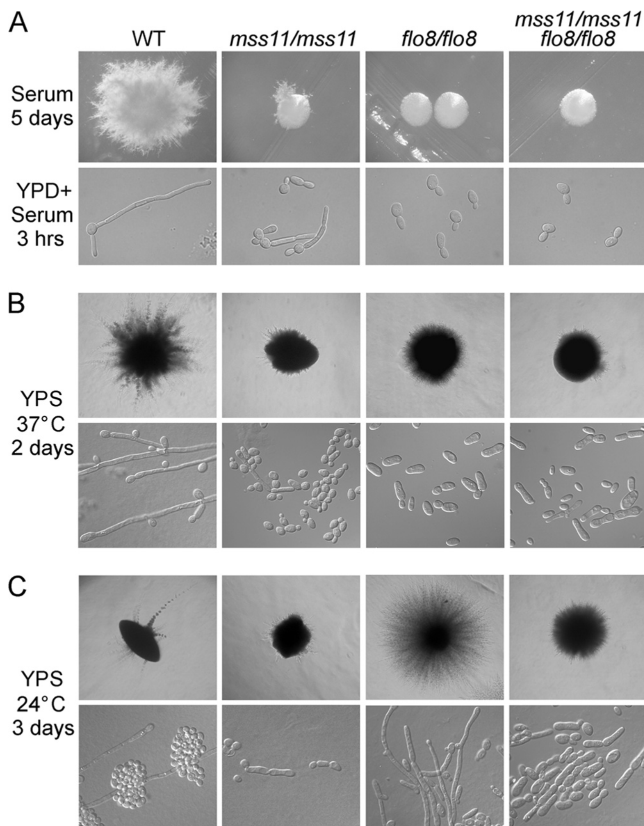

FIG. 9. Effects of Mss11 and Flo8 on hyphal growth under embedded condition. (A) *C. albicans mss11/mss11 flo8/flo8* cells are unable to form hyphae in response to serum. Strains were streaked on the solid serum-containing medium and incubated at 37°C for 5 days or grown in liquid medium (YPD plus 10% serum) at 37°C for 3 h. (B and C) Deletion of *MSS11* inhibits filaments formation under microaerophilic condition. Cells were plated with molten YPS agar and grown at 37°C for 2 days (B) or at 24°C for 3 days (C). Colony and cell morphologies of the wild-type strain (CAF2-1), the *mss11/mss11* mutant (CSL3), the *flo8/flo8* mutant (CCF3), and the *mss11/mss11 flo8/flo8* double mutant (CSL5) are shown.

reported that Flo8 functions as an activator at 37°C and as a repressor at 24°C under the embedded condition (8). To clarify the role of Mss11 in filamentous growth under the embedded condition, we examined the phenotype of the *mss11/mss11* mutant at high and low temperatures under microaerophilic conditions. After growth in YPS agar at 37°C for 2 days, wild-type colonies generated long heterogeneous filaments, and *mss11/mss11* or *flo8/flo8* single mutant, as well as *mss11/mss11 flo8/flo8* double mutant produced colonies surrounded with very short filaments (Fig. 9B). At 24°C, the wild type formed limited amounts of filaments after 3 days of incubation, while the *mss11/mss11* single mutant formed smooth colonies surrounded with very short filaments, and the *flo8/flo8* mutant produced fluffy colonies with long homogeneous filaments consisting of true hyphal cells. Although the *mss11/mss11 flo8/flo8* double mutant strains formed colonies surrounded with homogeneous short filaments at 24°C, deletion of Mss11 exhibited a significant inhibitory effect on cell elongation in the *flo8/flo8* mutant, the *mss11/mss11 flo8/flo8* mutant cells could not develop into true hyphae (Fig. 9C). In contrast to Flo8, Mss11 functions as an activator in hyphal development under microaerophilic conditions at both temperatures.

DISCUSSION

***C. albicans* Mss11 is a transcriptional activator interacting with Flo8 via the LisH motif.** We identified *C. albicans* Mss11 as a LisH motif containing protein through a search of the NCBI database. Sequence analysis shows that a LisH motif, which is found in 114 eukaryotic proteins (15), is located at the N terminus of CaMss11, similar to that of *S. cerevisiae* Mss11. Previous studies suggest a central role for ScMss11 in the regulatory network that controls invasive growth in *S. cerevisiae* (51). By homology, CaMss11 is also important for *C. albicans* to invade the agar. Flo8, a transcription factor essential for hyphal development and virulence in *C. albicans*, also possesses a conserved LisH motif. The crystal structure of the N-terminal domain of mouse LIS1 shows that the LisH motif is a thermodynamically very stable dimerization domain (25), suggesting a common function of the LisH motif. Sif2, an integral component of the Set3 complex (SET3C), has been reported to function as a tetramer via the N-terminal LisH-containing domain in *S. cerevisiae* (9). Therefore, it is possible that the LisH motif in Flo8 is involved in dimerization. In fact, Flo8 and Mss11 have been found to function as a heterodimer in activating *STA1* expression in *S. diastaticus* (27), although it remains to be defined whether the interaction is through the LisH motif. Here, we focus on the role of Mss11 in the morphological transition which is considered to be necessary for virulence of *C. albicans*.

Mss11 is involved in the transcriptional activation of hypha-specific genes necessary for hyphal development. Deleting *MSS11* dramatically impairs hyphal formation in response to serum and the expression of hypha-specific genes, such as *HWP1* and *ECE1*. ChIP analysis with the Mss11-Myc shows that Mss11 is located at the UAS region of the *HWP1* promoter, and the Mss11 binding increases in hyphal cells. Furthermore, Mss11 functions as a transcriptional activator because the $LexA_{DB}$-Mss11 fusion protein has high transcriptional activity in *S. cerevisiae*. Taken together, our results suggest that Mss11 plays a role as a transcriptional activator in regulating the transcriptional program during hyphal induction.

The *MSS11* transcript is regulated in response to serum at 37°C. The Northern blot analysis indicates that the expression level of *MSS11* is enhanced during hyphal induction. In addition, constitutively overexpressed *MSS11* promoted filamentation in the wild-type strain. Based on the expression pattern of *MSS11*, which was enhanced after 1.5 h in serum-containing medium, we infer that perhaps Mss11 is required for the elongation of hyphal cells. The regulation of *MSS11* expression could explain the morphological characteristics of *mss11/mss11* mutant cells which form stunted hyphae.

Changing the expression level is common for *C. albicans* to regulate the activity of transcription factors during hyphal development. It was previously reported that Nrg1, a repressor of filamentous growth in *C. albicans*, is downregulated during hyphal induction (5). Tec1, a transcription factor that regulates hyphal development in *C. albicans*, has been shown to be upregulated by serum-induced filamentous formation at 37°C (45). The expression level of *SSN6* which encodes an important

factor of morphological conversion declines significantly in response to serum (24). Our observation provides an inducible mechanism for induction of hyphal development by Mss11.

Cooperative regulation of Mss11 and Flo8 in hyphal development of *C. albicans*. In this report, several lines of evidence suggest that Mss11 cooperates with Flo8 to regulate the expression of hypha-specific genes. An in vivo interaction between Mss11 and Flo8 was detected by immunoprecipitation in *C. albicans*, which is consistent with the notion that LisH motifs presented in both proteins contribute to dimerization. Furthermore, Mss11 and Flo8 bind to the same UAS region of the *HWP1* promoter. Interestingly, there are more Mss11 and Flo8 proteins bound at this region in hyphal cells than in yeast cells, and the enhanced binding of both proteins depends on each other. Therefore, Mss11 might cooperate with Flo8 to regulate the hyphal transcriptional program in *C. albicans*, such as found between the Mss11 and Flo8 proteins in activation of *STA1* expression in *S. diastaticus*. Another well-demonstrated cooperating pair in *S. cerevisiae* includes Ste12 and Tec1 (11). The transcription factors Ste12 and Tec1 bind cooperatively to enhancer elements called FREs (for filamentation and invasion response elements) and program transcription that is specifically responsive to the mitogen-activated protein kinase signaling components required for filamentous growth in *S. cerevisiae* (40). The *C. albicans* APSES proteins Efg1 and Efh1 cooperate to repress an alternative pathway of true hyphal formation in embedded or microaerophilic conditions (14). With coordinated activation and repression, *C. albicans* is able to integrate the signals from different pathways and specifically regulate the expression of target genes.

In contrast to the role of *S. cerevisiae* Mss11 which is genetically positioned downstream of Flo8 in regulating invasive growth and *FLO11* expression (51), *C. albicans* Mss11 seems to act upstream of Flo8 since the CaMss11 is unable to promote hyphal development in the absence of the Flo8 in aerobic conditions. Loss of *FLO8* nearly blocked the activation effect of Mss11 on hyphal formation, whereas overproducing Flo8 can suppress the hyphal growth defect of the *mss11/mss11* mutant. It is possible that the increased expression of *MSS11* during hyphal induction enhances the cooperative binding of Mss11 and Flo8 to the *HWP1* promoter and further activates the expression of *HWP1*. These results, combined with the fact that *flo8/flo8* mutant strains have a more severe defect in hyphal development than *mss11/mss11* mutant strains, suggest that *C. albicans* Flo8 may play a central role in controlling the hyphal formation and activating the expression of hypha-specific genes by interacting with additional regulators. Indeed, Flo8 can interact with Efg1, a potential target of the cAMP/PKA pathway, and regulate the expression of hypha-specific genes (8). Interestingly, *C. albicans* Mss11 is essential for hyphal growth in microaerophilic conditions. Mss11 always functions as an activator under embedded conditions, whereas Flo8 is a dual functional regulator which acts as an activator at high temperature and as a repressor at low temperature. Loss of *MSS11* in the *flo8/flo8* mutant weakens the derepression effect of *FLO8* disruption on hyphal formation at low temperature, indicating that the *C. albicans* Mss11 is required for hyphal growth of *flo8/flo8* mutants in matrix.

In summary, we have identified a *C. albicans* transcription factor Mss11 that plays an important role in promoting hyphal development and is upregulated in response to serum. This enhanced expression of *MSS11* increases the cooperative binding of Mss11 and Flo8 to the *HWP1* promoter during hyphal induction, leading to transcriptional activation.

ACKNOWLEDGMENTS

We thank Joseph Heitman and Florian F. Bauer for kindly providing *S. cerevisiae mss11* strains. We thank Haoping Liu for helpful suggestions.

This study was supported by the Chinese National Natural Science Foundation (grants 30600008 and 30830003), the Chinese Academy of Sciences Foundation (KSCX2-YW-R-107), and Chinese 863 grant 2006AA02Z178.

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