Wangella dermatitidis is a dematiaceous (black) fungal pathogen of humans. First identified as an agent of subcutaneous chromoblastomycosis, it is today considered a paradigm for the emerging mycosis known as phaeohyphomycosis, because this polymorphic fungus also causes systemic and sometimes fatal disease, as well as chronic superficial, cutaneous, and subcutaneous infections (13, 24). Systemic phaeohyphomycosis caused by W. dermatitidis includes respiratory, intestinal, cardiac, and cerebral diseases (6, 27, 47). In the manner of most dematiaceous pathogens of humans, W. dermatitidis is a saprophyte, although isolations from nature are infrequent and most strains are from patients (22, 23). It is also asexual and thus is not classifiable by traditional morphological methods. Nonetheless, numerous molecular phylogenetic sequence analyses indicate that W. dermatitidis belongs to the phylum Ascomycota, the subphylum Pezizomycotina, and the class Chaetothyriomycetes (12). Thus, W. dermatitidis and yeast species such as Saccharomyces cerevisiae and Candida albicans are classified molecularly in the same phylum, but in different subphyla, whereas W. dermatitidis and numerous other conidigenous mold species, such as Aspergillus spp., Penicillium marneffei, and Fusarium oxysporum, are classified in the same subphylum but different classes (http://www.ncbi.nlm.nih.gov/Taxonomy/).

The predominant vegetative morphotype of W. dermatitidis is a budding yeast cell, but because of its polymorphism, it also produces pseudohyphae, moniliform hyphae, true hyphae, and sclerotic forms (see the comprehensive review by Szaniszlo [44]). At the light microscope level, the pseudohyphal and moniliform hyphal morphotypes appear similar, because both have constrictions at septal regions. However, they can be distinguished by noting that the cells of a moniliform hypha do not separate at septal regions, get progressively longer as the filament elongates, and may eventually terminate in the production of true hyphae having parallel sidewalls in optical section. In contrast, pseudohyphae are simply polarized chains of relatively normal size yeast that separate poorly. The differences between the two are even more evident at the transmission electron microscope level (31). Most importantly, and in the manner also of the true hyphae, the moniliform hyphae are distinguished from pseudohyphae by the former having septa in a true hyphal fashion, whereas the latter are devoid of such structures. In stark contrast, sclerotic cells and sclerotic bodies are isotropically enlarged, nonpolarized morphotypes that may become divided by one internal transverse septum or multiple intersecting septa (14, 45). Yeast, pseudohyphae, moniliform hyphae, true hyphae, and sclerotic forms are all found in the infected tissues. Finally, conidia of W. dermatitidis are produced by relatively undifferentiated conidiophores that develop from aerial moniliform and true hyphae (32).

Yeast-to-hyphal transition in W. dermatitidis has been critically monitored by transmission electron microscopy (31). As yeast cells age on agar media, they first become isotropically enlarged, thick-walled cells that contain one or more large lipid bodies. These cells are then competent to produce moniliform and true hyphae if subcultured in fresh media. Moniliform and true hyphal growth in W. dermatitidis can also be induced...
under nitrogen limitation conditions and in temperature-sensitive (ts) hyphal-form mutants (44, 50, 53). On the other hand, sclerotic morphotypes can be induced in nutrient-rich media at pH 2.5, by calcium limitation at pH 6.5, and by culture of certain ts multicellular-form mutants (Mc2/cdc1 and Mc3/cdc2) at the restrictive temperature (5, 14, 44, 45). Also in W. dermatitidis, WdCDC42 encodes a Rho/Rac member of small GTP-binding proteins, and the dominant active mutation CDC42G14V activates sclerotic growth in the wild-type strain and represses filamentous growth in both the wild type and the ts hyphal-form mutant Hf1 at 37°C (53).

APSES (Neurospora crassa Asm1p, S. cerevisiae Phd1p, Aspergillus nidulans StuAp, C. albicans Efg1p, and S. cerevisiae Sok2p) transcription factors have diverse effects on cellular development and differentiation in fungi. Among conidigenous Ascomycota, StuAp of the obligate filamentous fungus A. nidulans is best known for its involvement in conidiation (26). Strains with mutations in STUA lack phialides and metulae. In a similar manner, deletion of PmSTUA in the dimorphic conidigenous fungus P. marneffei also affects conidiation but does not influence its yeast-phase cells or their transition to hyphae (2). By comparison, deletion of FoSTUA in F. oxysporum represses the development of macroconidia and activates the growth of chlamydospores (30). Deletion of AfSTUA in Aspergillus fumigatus also results in abnormal conidiophores and conidia, and microarray analysis of this species shows that AfStuAp activates numerous other genes involved in development and virulence, including genes encoding secondary metabolites in the period preceding conidiogenesis (37).

Among ascomycetous yeast species, overexpression of the APSES transcription factor gene PHD1 in S. cerevisiae activates pseudo-hyphal growth and deletion of SOK2 accelerates pseudo-hyphal growth to the level caused by PHD1 overexpression (10, 51). In mutants lacking SOK2, PHD1 is up-regulated to activate the expression of FLO11, which encodes a cell wall glycosylphosphatidylinositol protein required for pseudo-hyphal growth and biofilm formation (33, 34). However, in the dimorphic human pathogenic yeast C. albicans, mutants lacking the APSES transcription factor gene EFG1 are defective in hyphal growth under aerobic conditions, as well as in virulence (20, 43), white-phase colony production (41), and chlamydospore formation (40). Genes regulated by Efg1p include some involved in cell wall synthesis, metabolism, and cellular differentiation (7, 16, 17, 28, 38). In addition, Efg1p is known to function downstream of protein kinase A in yeast-hyphal transitions (1), and overexpression of EFG1 has been shown to lead to production of pseudohyphae instead of true hyphae. Interestingly, the transcript level of EFG1 is decreased to its lowest level during the induction of hyphal growth, and Efg1p must be repressed to allow continued apical growth (43, 46). Furthermore, under hypoxic conditions at incubation temperatures lower than 37°C, deletion of EFG1 activates filamentous growth. In this case, another set of genes is regulated, including particularly those for fatty acid biosynthesis that require Efg1p for activation (36).

Although a number of genes of W. dermatitidis encode proteins that when defective affect its morphology, nothing is known about how transcription factors control the cellular morphogenesis of this fungal pathogen of humans. Therefore, the purpose of this study was to investigate the importance of the gene WdSTUA, and particularly how its encoded APSES transcription factor affects yeast-hyphal transitions. The results reported provide numerous new insights into the importance of the APSES family of transcription factors in fungi in general, and particularly in the pathogenic fungus W. dermatitidis, a polymorphic, conidigenous black mold with yeast-phase predominance.

**MATERIALS AND METHODS**

**Strains, culture conditions, and microscopy.** The W. dermatitidis and S. cerevisiae strains used in this research are listed in Table 1. Routine culture of W. dermatitidis was on the nutrient-rich yeast maintenance medium yeast extract-peptone-dextrose (YPD) agar (YPD) and in YPD broth (YPDB) as described previously (19). For the detection of hyphal growth and conidium production, W. dermatitidis was grown on less-rich potato dextrose agar (PDA; Difco Scientific, Detroit, MI), corn meal agar (CMA; Difco Scientific), corn meal dextrose agar (CMDA; Difco Scientific), Sabouraud dextrose agar (Difco Scientific), and synthetic low-ammonium dextrose agar (SLADA) (30). Yeast extract-peptone-maltose agar (YPMA) (52, 53) was used for the induction of the glaC promoter. For the induction of sclerotic cells and sclerotic bodies, W. dermatitidis was grown in pH 2.5 modified Caspex Dox broth (50). For slide cultures, a thin square block of PDA on a slide was inoculated on each side and then overlaid with a coverslip.
prior to incubation over water in a closed petri dish. Colony morphology pictures were taken with a Canon Powershot G3 camera attached to an Olympus model SZ-III dissecting microscope fitted with a ScopeTronix Maxview Plus adapter. Light microscopy pictures were obtained with a Leica DFC camera and a Leica DMLB upright microscope.

Degenerate PCR and library screening. Degenerate primers with designs based on the conserved APSES domains of the StuAp was used for A. midlandensis and P. marneffei and Asn1p of N. crassa had the sequences that follow: WFR1, AACRCCMIGGTAIACGXCAXCXYTXTG; WSR1, IGGDATCACCACCCYTTXARTTCTGATXG (where R is A or G; M is A or C; X is T, C, or G; Y is C or T; and D is A, T, or G). PCR amplifications were carried out with 2 μM of the primers, 0.5 μM deoxynucleoside triphosphate, 0.5 μM genomic DNA, and 0.5 U/μl Tag polymerase mixture in 1.5 mM MgCl₂, 50 mM KCl, and 10 μM Tris-HCl (pH 9.0) buffer. The PCR conditions were as follows: 5 min at 94°C for premelting; 50 cycles of 1 min at 94°C for denaturation, 1 min at 55°C for annealing, and 1 min at 72°C for extension; and 7 min at 72°C for completion of the extension. The resulting PCR products were then cloned into the pGEM-T Easy vector (Promega, Madison, WI) and sequenced. After sequencing showed that plasmid pT-WdStuA386 contained an APSES sequence of 386 bp, the full gene, which we named WdStuA, was cloned from a previously constructed W. dermatitidis genomic DNA library (9) by hybridization using the 386-bp WdStuA PCR product as a probe. The DECA prime II DNA labeling kit (Ambion, Inc., Austin, TX) was used to label the probe with [α-32P]dATP (Dupont NEN, Boston, MA). From 104 colonies screened, one positive cosmid was isolated. To subclone the fragments containing WdStuA, Southern analysis of the cosmid digested by different restriction enzymes was also carried out with the 386-bp WdStuA probe. After the fragments with WdStuA were identified, they were isolated by precipitation with Xhol, HindIII, and SstI and subcloned into the corresponding sites of the pBSKS vector (Stratagene, La Jolla, CA), yielding plasmids pBSKS-W1, pBSKS-W2, and pBSKS-W5, respectively. DNA sequencing of the plasmids was performed by the Core Facility of Institute of Cellular and Molecular Biology, University of Texas at Austin, using BigDye technology (Applied Biosystems, Foster City, CA). The locations of introns, first predicted in silico by alignments and by consensus splice sequences, were then confirmed by sequencing of the cDNA, which was amplified by degenerate RT-PCR (RT-PCR) using the One-Step RT-PCR kit (Qiagen, Valencia, CA) with the genomic DNA sequence.

Deletion of WdStuA in W. dermatitidis. To create the WdStuA deletion construct, which contained the hph selection marker gene (19) flanked by WdStuA 5' and 3' sequences, a 0.8-kb PstI-Sall fragment corresponding to the 5' region of WdStuA was first released from pBSKS-W5 and then ligated into vector pBSKS to generate pBSKS-W85. Next a 1.4-kb Sall-PstI hph gene sequence from pCB1636 (Fungal Genetics Stock Center, University of Kansas Medical Center) was inserted into the Sall site of pBSKS-W4 to obtain pBSKS-W85. After a 0.6-kb 3' region of WdStuA was amplified from pBSKS-W3 by PCR with primers WSF5 (GTCTCCTAGTCTCAGGAAATCGAAG; the introduced Xhol restriction digestion enzyme recognition site is underlined) and WSR5 (GAAGATCTAATGCCACACCTCCGATCG; Ectopic overexpression of WdStuA in W. dermatitidis. The site-specific, integrative expression vector pYEX303 was used for the expression of WdStuA in the nonessential WpKPS1 genomic locus of W. dermatitidis as previously described (52, 53). Briefly, pYEX303 contains the glaA promoter and terminator, the hph gene, a WpKPS1 targeting sequence, an Escherichia coli replication origin sequence, and a Cm resistance marker. Because WpKPS1 encodes a specific enzyme in the melanin biosynthesis pathway, the disruption of which makes black W. dermatitidis white without otherwise affecting its growth rate or morphology, numerous transformants having integrations at the same locus and in the same orientation are easily obtained for comparisons; the resulting mutants thus are not affected by random gene disruptions that might in turn adversely affect the construct. The WdStuA targeting sequence by NarI prior to transformation of E. coli with pGEM-T Easy vector (Promega, Inc., Madison, WI) with the genomic DNA sequence. Pu- lencia, CA) with the genomic DNA sequence.
spectrophotometer (NanoDrop Technologies), the RNA quality was determined by RNA gel electrophoresis. Reverse transcription reactions were then performed with 15 μg RNA, amino allyl-modified dUTP, oligo(dT), and SuperScript II reverse transcriptase. The cDNA of the expression strain was labeled with fluorescent dye Cy5 (red, channel 2, 635 nm), and cDNA of the vector alone strain was labeled with Cy3 (green, channel 1, 532 nm). After the two labeled samples were simultaneously denatured, they were then hybridized to the same glass slide at 65°C overnight. The slides were then washed and scanned with a GenePix 4000B scanner (Axon, Union City, CA), and the resulting files were loaded to the Longhorn Microarray Database for normalization (15). Three biological repeats were carried out. Undetected spots on the array were flagged and excluded from the analysis. Spots with sum of the mean intensities of both channels (532 and 635) of more than 150 were accepted. Only genes with regulation levels of more than 1.4-fold and greater than 80% were subjected to further analysis.

Nucleotide sequence accession number. The sequence of WdSTUA was submitted to the GenBank database. The accession number is AY445507.

RESULTS

Isolation, characterization, and expression of WdSTUA. Using degenerate primers with designs based on conserved sequences in the DNA-binding domains of APSES factors of A. nidulans (AnStuAp), P. marneffei (PmStuAp), and N. crassa (Asm1p), we amplified by PCR a 386-bp gene fragment encoding a similar domain from W. dermatitidis DNA. This PCR product was then used as a probe to determine by Southern analysis that the fragment amplified was from a gene present as a single copy in the W. dermatitidis genome (data not shown) and to screen a cosmid library to isolate the full-length gene. Sequence analysis of 3,683 bp of one cosmide insert showed it contained the entire open reading frame (ORF) from bp 1153 to 3197 (see Fig. S1A in the supplemental material). Comparison of the cDNA sequence amplified by RT-PCR with the corresponding genomic sequence confirmed the presence of the predicted three introns of 52, 55, and 54 bp (bp 1415 to 1466, 1692 to 1746, and 1880 to 1933). Analysis of the ORF showed that it encoded a putative protein of 627 amino acids, with an APSES DNA-binding domain consisting of residues 157 to 234 (see Fig. S1B in the supplemental material). The analysis also showed that the predicted protein shared significant identity along its whole sequence with the APSES transcription factors AnStuAp (48%), AlStuAp (50%), PmStuAp (48%), and NcAsm1p (45%) from the pizzicomycteous filamentous fungi A. nidulans, A. fumigatus, P. marneffei, and N. crassa, respectively (see Fig. SIC in the supplemental material). Hence, the cloned gene from W. dermatitidis was named WdSTUA and its encoded protein WdStuAp. However, few similarities outside the APSES domains were noted between WdStuAp and Phd1p (residues 123 to 238 in WdStuAp, 63% identity) or SoK2p (residues 132 to 243 in WdStuAp, 68% identity) of the hemiascomycetous yeast S. cerevisiae, although a number of additional identities were found in EfG1p (residues 22 to 422 in WdStuAp, 35% identity) of its dimorphic relative C. albicans. Northern analysis of RNA from wild-type W. dermatitidis cells cultured in YPDB medium for 24 h at both 25°C and 37°C showed that WdSTUA was expressed at similar levels under both conditions (see Fig. S2 in the supplemental material).

WdSTUA is not an essential gene, but its deletion alters colony morphology at 37°C. To help reveal the functions of WdSTUA, its entire coding region, except for a 100-bp sequence upstream of the stop codon, was deleted from the W. dermatitidis genome by a one-step gene replacement strategy. Southern analysis, PCR, and RT-PCR showed that about 10% of the hygromycin B-resistant transformants had an hph insertion at the WdSTUA locus (see Fig. S3 in the supplemental material). Because a variety of preliminary studies revealed that the phenotypes of all the WdstuaΔ mutants were identical, most subsequent detailed comparisons with the wild type were carried out with the WdstuaΔIA mutant.

The deletion of WdSTUA produced no phenotypic differences in growth rate or gross colony morphology between the wild type and the WdstuaΔIA mutant cultured at 25°C on YPDA medium (Fig. 1A). However, because 37°C is the infection temperature W. dermatitidis encounters when introduced into the human body, the growth on YPDA of the WdSTUA deletion mutant was also compared with that of the wild-type strain at the higher temperature. The results revealed that, while the growth patterns of the wild type and the WdstuaΔIA mutant at 37°C were again generally the same, the morphology of WdstuaΔIA colonies was obviously distinct from that of the wild type (Fig. 1A). Specifically the WdstuaΔIA strain produced colonies with surfaces that were considerably more convoluted than those of the wild type, although the colonies of both were darker and more convoluted than the colonies produced at 25°C. In addition, the morphotypes making up the colonies of the WdstuaΔIA strain were two times more likely to be hypha-like, instead of yeast-like, than those of the wild-type colonies (data not shown).

Furthermore, when both strains were grown in YPDB medium at 37°C, the hyphae of the WdstuaΔIA strain were more aggregated than those of the wild type (Fig. 1B). This aggregation was particularly evident after the liquid cultures were allowed to be stationary: in this case, the WdstuaΔIA strain formed sediment at the bottom of a test tube more quickly than the wild type (data not shown).

The WdSTUA deletion reduces filamentation and consequently condiation on nitrogen-poor, hypha-inducing agar media. Because STUA homologs in some filamentous fungi are involved in condiation, we evaluated the effects of the WdSTUA deletion on this process in W. dermatitidis. On PDA, a well-known nitrogen poor, hypha- and conidium-inducing medium, the wild type and WdstuaΔIA mutant both increased the diameters of their initial central yeast colonies equally (Fig. 2). However, at 25°C, the colonies of the wild type formed many long branching hyphae at the colony edges, whereas WdstuaΔIA colonies produced considerably fewer hyphae, which were shorter (Fig. 2A). On CMDA and CMA, two other nitrogen-poor, hypha- and conidium-inducing media, the WdstuaΔIA strain showed similar filamentation defects (data not shown). In contrast, on Sabouraud dextrose agar, a relatively nitrogen-rich medium for molds, neither the wild type nor the WdstuaΔIA strain formed equivalent filamentous growth (data not shown). Therefore, we tested the effects of the WdSTUA deletion on a SLADA (50 μM NH4+) medium. As on PDA, CMA, and CMDA at 25°C, the wild type produced colonies with many hyphae, whereas the WdstuaΔIA colonies exhibited considerably fewer hyphae (data not shown). When the ammonium concentration of the SLADA was increased 10-fold, hyphal growth was largely repressed, suggesting that nitrogen limitation was a critical factor in the induction of filamentous growth (data not shown). When cultured at 37°C on PDA (Fig. 2B), CMA, CMDA, and SLADA
(data not shown), WdstuaΔ1A colonies again produced fewer hyphae than did the wild type but considerably more than at 25°C (Fig. 2A), suggesting that 37°C suppressed the inhibition of filamentation caused by the WdSTUA deletion.

PDA slide culture observations showed that, at 25°C, the wild type, as expected, produced mostly long, thin aerial moniliiform and true hyphae (Fig. 3). Along these hyphae, numerous yeast-like cells were produced at septation regions. After 2 weeks of incubation at 25°C, the wild type was also observed to produce clusters of terminal conidia from relatively undifferentiated conidiophores in the manner characteristic of W. dermatitidis. In contrast, the WdstuaΔ1A strain was strongly repressed in aerial hyphal growth and conidiation (Fig. 3). Critical observation of the few hyphae produced by the WdstuaΔ1A mutant cultured in this manner showed they were obviously shorter, appeared to be pseudohyphae, and were largely covered with yeast cells. Thus, without WdStuAp, aerial hyphal development was dramatically reduced in W. dermatitidis, which consequently inhibited conidiation.

The WdstuaΔ1A mutant is defective in invasive hyphal growth. Hyphal growth in vitro and in vivo often includes both aerial hyphae and invasive hyphae. Because the WdSTUA deletion produced hyphal growth defects and invasive growth is sometimes important for fungal penetration of human tissues, the wild type and WdstuaΔ1A mutant were examined in vitro for the ability to produce invasive growth (growth that penetrates agar media). After 8 days, the yeast colonies of the mutant strain produced at 25°C on the surface of PDA plate medium containing 2% agar had formed very few hyphae of any type, whereas the wild type developed luxurious filamentous growth around and over the surface of the central yeast colonies (Fig. 4A). After the surface growth of the colonies was washed away, comparisons revealed that the wild-type strain had invaded the agar medium considerably more than had the WdstuaΔ1A mutant (Fig. 4B and C). Microscopic examination showed that the invasive growth of the wild type consisted of both yeast and long true hyphae, whereas that of the WdstuaΔ1A mutant consisted mostly of yeast (Fig. 4D). These results indicated that the WdstuaΔ1A mutant was defective in invasive hyphal growth.

Deletion of WdSTUA in the Hf1 strain reduces hyphal growth. The Hf1 strain of W. dermatitidis is a ts hyphal-form
mutant that produces yeast cells in the manner of the wild type in most rich media at 25°C and generates many more hyphae than the wild type under the same conditions at 37°C (50, 53). To further investigate the effects of WdSTUA on filamentation, several Hf1 WdstuaΔ mutant strains were made by the one-step gene replacement of WdSTUA in the Hf1 background. Because subsequent studies revealed that the phenotypes of these mutants were identical, only the results of the phenotypic comparisons with the Hf1 WdstuaΔ mutant are described. On YPDA medium at 37°C, the colony surfaces of Hf1 were more convoluted and were not particularly affected by the deletion of WdSTUA (Fig. 5A). However, culture of the strains on PDA medium at 25°C showed that the deletion of WdSTUA obviously reduced hyphal growth in Hf1 (Fig. 5B). Nonetheless, the deletion of WdSTUA did not completely inhibit hypha production by Hf1, suggesting that WdSTUA influenced only one hyphal developmental pathway among a number that may be triggered in Hf1. Slide culture observations showed that both Hf1 and Hf1 WdstuaΔ produced conidia on PDA at 25°C, although the conidiophores of Hf1 were longer and more abundant than those produced by the Hf1 WdstuaΔ mutant (Fig. 5C). These results demonstrated that the Hf1 WdstuaΔ strain retained the ability to produce conidia.

**Ectopic overexpression of WdSTUA in W. dermatitidis represses hyphal growth.** A previously developed and well-tested color-selectable and site-specific integrative transformation system for the ectopic overexpression of genes in *W. dermatitidis* was used (52, 53). When WdSTUA was overexpressed in

![WT and WdstuaΔ1A at 25°C and 37°C](image1)

**FIG. 2.** The WdSTUA deletion reduced *W. dermatitidis* filamentous growth on PDA at 25°C. The wild-type (WT) and WdstuaΔ1A strains were streaked on PDA and incubated at 25°C (A) and 37°C (B). The filamentous growth at the colony edges was visualized with a compound light microscope, and the photomicrographs were taken after 4 days of incubation. Scale bar, 0.2 mm (applicable to all colonies in the figure).

![WT and WdstuaΔ1A](image2)

**FIG. 3.** The WdSTUA deletion repressed aerial hyphal growth and consequently conidiogenesis. The wild-type (WT) and WdstuaΔ1A strains were inoculated on PDA in slide cultures and incubated at 25°C. Growth at the edge of the medium and protruding into the air space between the slide and coverslip was visualized with a compound light microscope and photographed after 2 weeks of incubation. Scale bar, 10 μm (applicable to all the growth in both photomicrographs). The arrow points to a cluster of conidia produced at the terminus of a conidiogenous hypha.
this manner in the WdSTUA deletion background and cultured at 37°C on the glaA induction medium YPMA (data not shown) and on YPDA (Fig. 6A), the convolutions in the colony surfaces of the WdSTUA deletion mutant (Wdpks1Δ WdstuaΔ1) were eliminated. Microscopic examination of the morphotypes making up the colonies showed that those overexpressing WdSTUA in the WdSTUA deletion strain background (Wdpks1Δ WdstuaΔ WdSTUAExct-1) as well as in the presence of the wild-type WdSTUA gene (Wdpks1Δ WdSTUAWt WdSTUAExct-1) were the least hyphal among the strains tested (data not shown). Moreover, on PDA at 37°C, hyphal growth was also most inhibited in the same two overexpression strains among the strains tested (Fig. 6B). In this case, the WdSTUA deletion mutant (Wdpks1Δ WdstuaΔ1) produced fewer filaments at the colony periphery than the wild type, and the two overexpression strains produced the least number of filaments. This
inhibition of morphotype transition by WdSTUA overexpression in the absence of wild-type WdSTUA and in the presence of wild-type WdSTUA was also observed when the strains were cultured on PDA at 25°C (Fig. 6C). Moreover, the overexpression of WdSTUA in the Hf1 strain also repressed the convoluted colony surface growth on YPDA at 37°C and hyphal growth on PDA at 37°C and 25°C (data not shown). Confirmation that the WdSTUA RNA levels were increased in the ectopically overexpressed strains was obtained by Northern analyses of cells cultured in PDB and YPDB at 25°C and 37°C (see Fig. S4 in the supplemental material), which showed that wild-type endogenous WdSTUA produced a transcript of about 3 kb, whereas the overexpressed WdSTUA under the control of the glaA promoter-terminator produced a transcript of about 2 kb, due to the reduced sizes of the 5′ and 3′ untranslated regions. The results from these experiments showed that the overexpression of WdSTUA in W. dermatitidis strongly repressed filamentous growth. In contrast, the ectopic expression of only the amino-terminal half of WdStuAp had no effect (data not shown), suggesting that full-length WdStuAp is required for its function in W. dermatitidis.

**Heterologous expression of the cDNA of WdSTUA in S. cerevisiae.** Because the APSES transcription factors Phdlp of *S. cerevisiae* and Efglp of *C. albicans* both induce pseudohyphal growth when overexpressed in *S. cerevisiae* (4, 35), we tested whether WdSTUA might also induce pseudohyphal growth in *S. cerevisiae*. For this, the WdSTUA cDNA was cloned into the *S. cerevisiae* expression vector pYES2 and transformed into the MR12 strain, which contains a FLO11 promoter-lacZ reporter construct (35). Compared with MR12 carrying the pYES2 vector without an insert, MR12 containing pYES2-WdSTUA induced pseudohyphal growth and the formation of rougher and more firmly attached colonies on SLARA medium at 30°C (see Fig. S5 in the supplemental material). Because FLO11, which encodes a cell wall glycosylphosphatidylinositol protein, is required for such pseudohyphal growth in *S. cerevisiae* (11), we also carried out β-galactosidase assays, which confirmed that the FLO11-lacZ activity was also more induced in the pYES2-WdSTUA strain than in the pYES2 strain (1.3 versus 0.17 Miller units). Microarray analysis subsequently showed that a number of other genes were significantly influenced by WdSTUA (see Tables S1 and S2 in the supplemental material), including PGU1, which has been reported to be up-regulated in the microarray assays of the *S. cerevisiae* filamentous pathway (3, 21). In contrast, the heterologous expression of the amino-terminal half of WdSTUA in *S. cerevisiae* did not induce pseudohyphal growth.
WdStuAp did not show these effects (data not shown). Collectively, these results indicated that the expression of WdSTUA was able to activate the pseudohyphal growth pathway in S. cerevisiae.

**DISCUSSION**

Known or suspected Ascomycota species are often divided somewhat arbitrarily into two groups, those that are called molds or filamentous fungi and those called yeasts. Fungi in the first group must generally reproduce vegetatively by fragmentation or by the production of asexual spores called conidia, whereas those in the second group tend to be single-cell fungi that grow reproductively by budding or fission. However, many species in both groups are vegetatively dimorphic or even polymorphic and thus able to express vegetative growth in more than one manner. For example, although the predominant morphotype of *W. dermatitidis* is a yeast cell, it is in actuality a filamentous, conidiogenous mold (44). Although a number of environmental factors that trigger the transition of the yeast morphotype of *W. dermatitidis* to one of its alternate morphotypes have been identified, nothing is known about the roles played by any transcription factor in controlling those transitions.

After the WdSTUA gene of *W. dermatitidis* was identified and cloned by a degenerate PCR method, our analyses showed that its APSES DNA-binding domain was well conserved, implying it evolved early among fungi and has remained relatively unchanged since. Similar to its homologs in other filamentous, conidiogenous fungi (2, 26, 30), WdSTUA has three introns near and at the region encoding the 78-amino-acid APSES domain. Multiple alignment of WdStuAp with orthologs of closely related filamentous fungi showed several conserved sequence fragments (see Fig. S1C in the supplemental material): first, residues 122 to 231, a region that includes the APSES domain; second, residues 308 to 321, a region rich in proline, serine, and threonine; third, residues 359 to 371, a second region also rich in proline, serine, and threonine; fourth, residues 577 to 581, a cluster of charged amino acids; and fifth, residues 598 to 601, a region of all positive amino acids that contains a predicted nuclear localization signal. We speculate that these sequences play important roles in WdStuAp interactions with other proteins and in regulation. In contrast with the finding that PmSTUA transcripts are detected only in conidiogenous cells (2), our Northern analysis showed that WdSTUA was expressed at similar levels during vegetative growth in YPDB at 25°C and 37°C (see Fig. S2 in the supplemental material), as well as when cells were cultured on PDA for 6 days (data not shown). Therefore, the expression patterns for WdSTUA were more like those of the FoSTUA and AnSTUA transcripts, which are also expressed during the periods of vegetative growth and conidiation (25, 30).

A functional characterization of WdStuAp was possible because the deletion of WdSTUA from the *W. dermatitidis* genome was not a lethal event. This allowed for the production of numerous WdstuaΔ knockout mutants, not only in the wild-type strain but also in a strain defective in melanin biosynthesis (Wdpks1Δ-I strain) and in a ts, so-called hyphal-form mutant strain (Hf1). In spite of these different genetic backgrounds, all of the mutants that were derived having a WdSTUA deletion exhibited the same general characteristics, with the expected exceptions that the Wdpks1Δ WdstuaΔ double mutants were albino and the Hf1 WdstuaΔ double mutants tended to produce more hyphae. Unfortunately, for unknown reasons, our
attempts to disrupt WdSTUA in our ts Mc3 (Wddez2) mutant, which converts to sclerotic cells and sclerotic bodies that can undergo slow fission at 37°C, were unsuccessful. Therefore, we investigated whether the WdSTUA deletion affected the production of sclerotic bodies and their ability to proliferate by slow fission when induced by pH 2.5 (50). In the manner of the wild type, the WdstuaΔ strain still produced normal numbers of sclerotic cells, planate cells, and sclerotic bodies, with the last retaining the ability to undergo slow fission (data not shown). This is in agreement with a previous report that yeast growth by fission in P. marneffei is not affected by PmSTUA deletion (2).

The initial observation suggesting that deletion of WdSTUA might affect morphotype transitions between yeast and hyphae in W. dermatitidis was our finding that the WdstuaΔ deletion strains produced colonies with more convoluted surfaces than those of the wild type during culture on richYPD medium at 37°C (Fig. 1). Colonies of the WdstuaΔ mutants were also found to be less convoluted than those of the ts Hf1 strain at 37°C (Fig. 5A), suggesting that WdstuAp is associated with partial expression of this trait. Among ascomycetous yeast species, such correlations between colony morphology changes and morphotype changes, such as those associated with white-opaque colony morphology switching of the WO-1 strain, are best known in C. albicans (39). EFG1 deletion strains of this pathogen display opaque colonies that contain elongated yeast (41), and it is well documented that Efg1p is required for a subset of characteristics associated with this colony switching event (42). In S. cerevisiae, convoluted colony morphology is similarly reported to be related to the polarized growth of yeast cells and to cell wall adhesin proteins (49).

WdstuaΔ is required not only for smooth yeast colonial growth on YPD at 37°C, but also for vigorous aerial and invasive hyphal growth on PDA at 25°C (Fig. 2, 3, and 4). For aerial and invasive hyphal growth to occur, new cell wall proteins are often required. For example, hydrophobins are cell wall proteins reported to be necessary for aerial hyphal growth, which serve as activators to overcome surface tensions and to provide hydrophobic surfaces on aerial hyphae to prevent desiccation (8). In S. cerevisiae, FLO11 is required for pseudohyphal growth on the colony periphery and cell adhesion to substrates (48). And in C. albicans, many cell wall genes have been identified as hyphal phase specific, required for biofilm formation (18, 29). We speculate that WdstuAp may also activate similar cell wall proteins. In addition, WdstuAp appears to be part of a network of pathways needed to produce hyphae during development. For example, the repression of hyphal growth in the WdstuaΔ mutant at 25°C on PDA was suppressed by 37°C (Fig. 2). Also, WdstuA deletion in the ts hyphal-form mutant strain Hf1 still permitted hyphal production to some degree even at 25°C (Fig. 5B). Possibly the elevation of the temperature of culture of W. dermatitidis and mutations in Hf1 stimulated other parallel pathways to suppress the WdstuaΔ phenotype.

Overexpression of WdstuaΔ in W. dermatitidis under the control of the glaA promoter repressed filamentous growth (Fig. 6). These results are in general agreement with those obtained with C. albicans, which documented that EFG1 overexpression causes a switch from the opaque-phase phenotype to the white-phase phenotype (41), represses true hyphal growth, and induces pseudohyphal development (43, 46). After the initiation of hyphal growth in C. albicans, EFG1 expression is immediately repressed at the beginning of the filamentation process. We suspect that, when WdstuA is not under the control of its own promoter, the inhibition of its expression after the initiation of filamentation also does not occur. Consequently, the hyphal morphotypes are not induced. Furthermore, because filamentous growth was more strongly repressed by the overexpression of WdstuA than by its deletion, we additionally suspect that WdstuA overexpression may affect other pathways that repress filamentous growth. It is interesting to note that, in contrast to the repressive effects of overexpression of WdstuA in W. dermatitidis, the overexpression of WdstuA, in the manner of PHDI and EFG1, induced pseudohyphal growth in S. cerevisiae (see Fig. S5 in the supplemental material) (10, 35).

In summary, this study determined that WdstuAp is an important regulator of yeast-hyphal transitions in W. dermatitidis. Our results document for the first time among conidigenous fungi that this APSES transcription factor can act both as a positive and negative regulator. Our evidence for this conclusion is that WdstuA overexpression strongly repressed W. dermatitidis filamentous growth and that, at the wild-type WdstuA expression level, WdstuAp negatively regulated filamentous growth on rich media at 37°C and positively regulated filamentous growth on nitrogen-poor media at 25°C. We anticipate that further exploration of the mechanism of WdstuAp function in this fungus will help us better understand the complex mechanisms controlling yeast and hyphal transitions and ultimately their contribution to fungal pathogenesis.

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