Saccharomyces cerevisiae S288C Has a Mutation in *FLO8*, a Gene Required for Filamentous Growth

Haoping Liu,* Cora Ann Styles† and Gerald R. Fink†

**Department of Biological Chemistry, College of Medicine, University of California, Irvine, California 92717 and †Whitehead Institute for Biomedical Research, Massachusetts Institute of Technology, Cambridge, Massachusetts 02142*

Manuscript received May 15, 1996
Accepted for publication August 8, 1996

ABSTRACT

Diploid strains of baker's yeast *Saccharomyces cerevisiae* can grow in a cellular yeast form or in filaments called pseudohyphae. This dimorphic transition from yeast to pseudohyphae is induced by starvation for nitrogen. Not all laboratory strains are capable of this dimorphic switch; many grow only in the yeast form and fail to form pseudohyphae when starved for nitrogen. Analysis of the standard laboratory strain S288C shows that this defect in dimorphism results from a nonsense mutation in the *FLO8* gene. This defect in *FLO8* blocks pseudohyphal growth in diploids, haploid invasive growth, and flocculation. Since feral strains of *S. cerevisiae* are dimorphic and have a functional *FLO8* gene, we suggest that the *flo8* mutation was selected during laboratory cultivation.

SACCHAROMYCES cerevisiae is a dimorphic fungus capable of interconverting between a unicellular yeast form and a multicellular filamentous form called a pseudohypha (HANSEN 1886; GIMENO *et al.* 1992). Pseudohyphae are induced in *MAT***a**/α diploid cells by starvation for nitrogen in the presence of a fermentable carbon source (GIMENO *et al.* 1992). Pseudohyphal cells exhibit at least five distinctive characteristics: They have an elongated cell shape, fail to separate after cell division, divide in a unipolar manner (the first and subsequent buds emerge from the free ends), invade beneath the surface of the agar, and have a distinct cell cycle that appears to lack the extended G1 phase typical of yeast form cells (KRON *et al.* 1994).

Elements of the yeast mating signal transduction pathway Ste20p(PAK), Ste11p(MEKK), Ste7p(MEK), and the transcription factor Ste12p are required for pseudohyphal growth as well as for mating (LIU *et al.* 1993). Mutations in the genes encoding these proteins of the mitogen-activated protein (MAP) kinase cascade block the formation of filaments. Neither of the MAP kinases, Fus3p or Kss1p, appears to be required for pseudohyphal growth because the *fus3kss1* double mutant forms normal pseudohyphae under conditions of nitrogen starvation. Recently, it has been shown that this filamentation kinase cascade is activated by the RAS2 gene (MOSCH *et al.* 1996). A number of other genes have been reported to affect pseudohyphal growth including *SHR3* (LJUNGDAHL *et al.* 1992), *GRR1* (BARRAL *et al.* 1995; BARRAL and MANN 1995), *PHD1-7* (GIMENO and FINK 1994), *ELM1* (BLACKETER *et al.* 1993), and *PPS1* (BLACKETER *et al.* 1994). However, the relationship of these genes to the kinase cascade has not yet been elucidated. The MAP kinase cascade appears to control development in other fungi as well; Cph1p, a Ste12p homologue has been implicated in the regulation of hyphal growth in *Candida albicans*, the most common fungal pathogen in humans (LIU *et al.* 1994). Similarly, Fuz7p, a Ste7p homologue, is involved in filamentation in *Ustilago maytis* (BANUETT and HERSKOWITZ 1994).

Haploid strains of Saccharomyces exhibit an invasive growth behavior with many similarities to pseudohyphal development, including filament formation and agar penetration. The same components of the MAP kinase cascade necessary for diploid pseudohyphal development (*STE20, STE11, STE7,* and *STE12*) are also required for haploid invasive growth (ROBERTS and FINK 1994). Despite the similarities between diploid pseudohyphae and haploid filamentous growth, there are several differences. Haploids invade well on rich YPD medium whereas diploids do not. Moreover, diploids but not haploids form pseudohyphae under conditions of nitrogen starvation.

Although many feral strains of Saccharomyces are capable of forming filaments, a number of laboratory strains dubbed "wild type" are incapable of forming pseudohyphae or of haploid invasion. In this report we examine the genetic basis for this variation between different laboratory strains. The genetic and molecular analysis of a strain that can form pseudohyphae (Σ1278b) with one that can not (S288C) shows that S288C contains a "naturally occurring mutation" in the *FLO8* gene, which prevents both pseudohyphal development and haploid invasion.

MATERIALS AND METHODS

Media and yeast strains: Standard yeast media were prepared and yeast genetic manipulations were performed as

Corresponding author: Gerald R. Fink, Whitehead Institute for Biomedical Research, Nine Cambridge Center, Cambridge, MA 02142.

TABLE 1

Yeast strains used in this study

Yeast strains	Genotype	Background and source
CGx68	**a**/α	Σ1278b, GIMENO *et al.* (1992)
CG25	**a** *ura3-52 shr3-102*	Σ1278b, GIMENO *et al.* (1992)
CG41	α *ura3-52 shr3-102*	Σ1278b, GIMENO *et al.* (1992)
CGx52	**a**/α *ura3-52/ura3-52 shr3Δ1::URA3/shr3Δ1::URA3*	Σ1278b, LJUNGDAHL *et al.* (1992)
L4973	α *ura3-52 ade2 lys2 shr3Δ1::URA3 flo8-1*	S288C, *shr3Δ1::URA3* transformant in AA255 RUDOLPH *et al.* (1989)
F762	**a** *trp1Δ1 ura3-52 flo8-1*	S288C, gift from JIM THOMAS
10362-6A	**a** *shr3Δ1::URA3 ura3-52 ade2 flo8-1*	S288C, ascospore of L4973 × F762
10362-3A	α *shr3Δ1::URA3 ura3-52 flo8-1*	S288C, ascospore of L4973 × F762
L5809	**a**/α *shr3Δ1::URA3/shr3Δ1::URA3 flo8-1/flo8-1 ura3-52/ura3-52 ade2/ADE2*	10362-6A × 10362-3A
X10375	**a**/α *shr3Δ1::URA3/shr3-102 ura3-52/ura3-52 ade2/ADE2 flo8-1/FLO8*	10362-6A × CG25
10375-6B	α *ura3-52 shr3-102 flo8-1*	S288C and Σ1278b, ascospore of X10375
10415-8D	**a** *ura3-52 shr3-102 flo8-1*	S288C and Σ1278b
L5281	**a** *ura3-52 shr3-102 flo8-1::URA3::FLO8*	10415-8D transformed with pHL11
X10397	**a**/α *ura3-52/ura3-52 shr3-102/shr3-102 flo8-1/FLO8*	10375-6B × CG25
10397-9B	**a** *flo8-1 shr3-102 ura3-52*	S288C and Σ1278b, ascospore of X10397
10397-7D	α *flo8-1 shr3-102 ura3-52*	S288C and Σ1278b, ascospore of X10397
L5107	**a**/α *flo8-1/flo8-1 shr3-102/shr3-102 ura3/ura3-52*	10397-9B × 10397-7D
L5810	**a**/α *flo8-1/flo8-1 shr3-102/shr3-102 ura3/ura3-52 (pRS316)*	L5107 + pRS316
L5811	**a**/α *flo8-1/flo8-1 shr3-102/shr3-102 ura3/ura3-52 (pHL1)*	L5107 + pHL1
L5812	**a**/α *flo8-1/flo8-1 shr3-102/shr3-102 ura3/ura3-52 (pHL36)*	L5107 + pHL36
FY3	**a** *ura3-52 flo8-1 GAL*$^+$	S288C, WINSTON *et al.* (1995)
FY2	α *ura3-52 flo8-1 GAL*$^+$	S288C, WINSTON *et al.* (1995)
L5302	**a** *flo8-1::URA3::FLO8 ura3-52 GAL+*	S288C
L5304	α *flo8-1::URA3::FLO8 ura3-52 GAL+*	S288C
L5813	**a**/α *ura3-52/ura3-52 flo8-1/flo8-1 GAL+*	FY3 × FY2
L5814	**a**/α *ura3-52/ura3-52 flo8-1/flo8-1 GAL+ (URA3)*	L5813+ pRS316
L5306	**a**/α *flo8-1::URA3::FLO8 ura3-52/flo8-1::URA3::FLO8 ura3-52*	L5302 × L5304
10459-4B	α *flo8-1::URA3::FLO8 ura3-52 his3Δ200*	S288C
16-1	**a** *spr6::HIS3::SPR6 his3 trp1 leu2 ura3 ilv2 met6 ade1(or ade2)*	KALLAL *et al.* (1990)
10480-5C	**a** *ura3-52*	Σ1278b
10480-5D	α *ura3-52*	Σ1278b
L5366	**a**/α *ura3-52/ura3-52*	Σ1278b, 10480-5C × 10480-5D
L5816	**a** *flo8-2 ura3-52*	Σ1278b
L5817	α *flo8-2 ura3-52*	Σ1278b
L5818	**a**/α *flo8-2/flo8-2 ura3-52/ura3-52*	L5816 × L5817
L5819	**a**/α *ura3-52/ura3-52 + (FLO8/URA3/2μ)*	L5366 + pHL135
DA1rho^{0}XJJM113	**a**/α *lys2/LYS2 ura3/URA3 ade2/ADE2* [*cox2-10*]	MULERO and FOX (1994), MULLER *et al.* (1984)
D273-10B/A1	α *met6*	SHERMAN; CORUZZI *et al.* (1978)
A364A	**a** *ade1 ade2 gal1 his7 lys2 tyr1 ura1*	HARTWELL.
L5682	**a**/α *ura3-1/ura3-1 can1-100/can1-100 eu2-3,112/LEU2 his3Δ200/HIS3 trp1-1/TRP1*	W303, F1575 × EY698, ELION *et al.* (1991a)
EY699	**a** *can1-100 ura3-1 leu2-3,112 his3-11.15 ade2-1 trp1-1*	W303, ELION *et al.* (1991a)
EM93	**a**/α	S288C progenitor, MORTIMER and JOHNSTON (1986)
Fleischmann's yeast	**a**/α	Specialty Brands, San Francisco, CA.
Red Star dry yeast	**a**/α	Universal Foods Corp., Milwaukee, WI.
Beer2	**a**/α	JAIME CONDE, Seville, Spain

described (SHERMAN *et al.* 1986). SLAD medium (synthetic low ammonia dextrose medium) was prepared as described (GIMENO *et al.* 1992).

The yeast strains used in this study are listed in Table 1. All Σ strains were derived from Σ1278b (also known as MB1000) and MB758-5B (BRANDRISS and MAGASANIK 1979; SIDDIQUI and BRANDRISS 1988). As documented in RESULTS, the pseudohyphal defect in S288C results from a mutation in the *FLO8* gene. The gene in our lab was originally designated *PHD10* before recent identification of this sequence as that of *FLO8* in the database. To avoid confusion, the *FLO8* acronym is used in all gene designations. The scoring of pseudohyphal growth in crosses was complicated by the segregation of modifiers, which sometimes made it difficult to distinguish *FLO8* from *flo8*. To aid in the scoring of tetrads (in the cross between S288C and Σ1278b) we used strains that carried the *shr3* mutation, an enhancer of pseudohyphal growth [either a spontaneous mutation *shr3-102* or an *in vitro*-constructed deletion *shr3Δ1::URA3* (GIMENO *et al.* 1992; LJUNGDAHL *et al.* 1992)]. Two different S288C strain backgrounds were used in this study. In the initial crosses between Σ1278b and S288C, we used a S288C strain (10362-6A) that carries a *shr3* mutation. 10362-6A was one of the ascospore progeny from a cross between L4973 and F762 (a gift from JIM THOMAS). As described in the RESULTS, 10362-6A differs from Σ1278b by confounding mutations in addition to *flo8* and cannot be restored

TABLE 2

Plasmids used in this study

Plasmid	Description	Source or reference
B3258	Σ yeast genomic library on pRS316	This study
pHL1	4.3-kb insert with *FLO8* on pRS316	This study
pHL36	>6-kb insert with *FLO8* on pRS316	This study
pHL11	*FLO8/YIP/URA3*	This study
pHL129	*flo8::hisGURA3KanRhisG*	This study
pHL130	*FLO8::myc3/URA3/CEN*	This study
pHL138	*FLO8::myc3/URA3/2μ*	This study
pHL132	*flo8::LacZ/URA3/2μ*	This study
pHL135	*FLO8/URA3/2μ*	This study
pCT3	S288C yeast genomic library	THOMPSON *et al.* (1993)
YEp357R	*LacZ/URA3/2μ*	MYERS *et al.* (1986)
pKB241	Triple myc epitope in pUC19	DANIEL KORNITZER and STEVE KRON
pSE1076	*hisGURA3Kan^{R}hisG*	STEVE ELLEDGE
pRS316	*URA3/CEN*	SIKORSKI and HIETER (1989)
pRS306	*URA3/YIP*	SIKORSKI and HIETER (1989)
pRS202	*URA3/2μ*	PHIL HIETER, unpublished data

to pseudohyphal growth simply by transformation with *FLO8*. However, ascospore derivatives from a Σ1278b × 10362-6A, which segregate only *flo8*, can be complemented by *FLO8* on a plasmid. After we obtained the *FLO8* gene on a DNA plasmid, we used another set of S288C strains (FY3 and FY2), which do not seem to have these confounding mutations and can be transformed to Phd$^+$ by *FLO8*.

We also examined other laboratory strains, some industrial strains, and EM93, a progenitor of S288C (MORTIMER and JOHNSTON 1986) to determine the presence of a *flo8* mutation and the ability to form filaments.

Construction of a Σ yeast genomic library: Genomic DNA was prepared from a Σ strain CGx68 and partially digested with *Sau3A*. DNA fragments were then size-fractionated over a gel filtration column (a prepacked G50 column from BRL) to collect fragments larger than 4 kb. The *Sau3A* ends were partially filled in with dATP and dGTP and ligated to dCTP- and dTTP-filled *Sal*I ends in the *URA3/CEN* plasmid pRS316 (SIKORSKI and HIETER 1989). Over 75,000 independent *Escherichia coli* transformants were obtained. Almost all the plasmids in the library have inserts, two-thirds of which carry inserts of 4–8 kb, and one-third of which have inserts of 2–4 kb.

Cloning of *FLO8* by complementation in a *flo8/flo8* diploid strain: Our Σ yeast genomic library was transformed into a diploid *flo8/flo8* strain (L5107) yielding ~9000 Ura$^+$ transformants. These Ura$^+$ transformants were collected, titered, and replated onto SLAD plates at a colony density of ~100–200 per plate. After 4–5 days of growth at 30°, ~7500 colonies were visually screened for pseudohyphal growth under a dissecting microscope. One clone (pHL1) from the screen complemented the *flo8/flo8* pseudohyphal growth defect of L5107 very well. In a second experiment, ~100,000 transformants were obtained on SC −Ura plates. These were pooled and replated onto SLAD plates at ~1000 per plate. We visually screened about 700,000 total colonies on SLAD medium, and isolated one clone (pHL36) that fully complemented the Phd$^-$ phenotype in L5107. Nine transformants that failed to complement L5107 fully were also isolated. Restriction digestions indicate that pHL1 and pHL36 contain overlapping DNA fragments, and the other nine isolates all carry another gene.

Plasmid constructions: Plasmids constructed in this study are listed in Table 2. pHL1 and pHL36 are the two original *FLO8* isolates from our Σ yeast genomic library. pHL1 has 4.8-kb insert and pHL36 has an ~6-kb insert. The pHL1 and pHL36 have an overlap of ~3 kb.

The *FLO8::URA3*/YIP plasmid (pHL11) was constructed by placing the insert from pHL1 (a *Xho*I-*Xba*I fragment) into the *Xho*I-*Xba*I site in the polylinker of pRS306 (SIKORSKI and HIETER 1989). For integrative transformation at *FLO8* the plasmid was linearized with *Bgl*II enzyme.

The *FLO8::LacZ* plasmid (pHL132) was constructed by cloning the *Xba*I-*Kpn*I fragment from pHL1 into a *LacZ/URA3/2μ* plasmid, YEp357R (MYERS *et al.* 1986). The *Xba*I site is in the polylinker and the *Kpn*I site is in *FLO8* coding region. This fragment contains 5′ flanking sequence and part of the coding sequence of *FLO8* fused in frame to the *LacZ* gene at the *Kpn*I site.

The *FLO8/URA3/2μ* plasmid (pHL135) was constructed by cloning the insert from pHL1 (a *Sac*I-*Xho*I fragment) into an *URA3/2μ* plasmid pRS202 (at the *Sac*I-*Xho*I site of the polylinker).

The *FLO8::myc3/URA3/CEN* plasmid (pHL130) was constructed by inserting a *Bam*HI triple myc (myc3) fragment from B2768 (a gift from Drs. D. KORNITZER and S. KRON) at the *Bgl*II site of the *FLO8* in pHL1. The orientation of the insertion and in-frame fusion of the myc3 in *FLO8* was confirmed by sequencing. A *FLO8::myc3/URA3/2μ* plasmid (pHL138) was created by subcloning the insert from pHL130 (a *Sac*I-*Xho*I fragment) into pRS202, a *URA3/2μ* plasmid.

A deletion of *FLO8* called *flo8-2* on plasmid pHL129 was constructed in two steps. First, the *Bst*EII to *Sna*BI region of the *FLO8* in pHL1 plasmid was replaced by the *Bam*HI-*Bgl*II fragment of *hisGURA3KanRhisG* from pSE1076 (a gift from Dr. STEVE ELLEDGE) by blunt-end ligation and subsequent selection of transformants for both ampicillin and kanamycin resistance. After isolating the Amp^{r}Kan^r transformants and the corresponding plasmid, we cut out the entire *flo8::hisG-URA3KanRhisG::flo8* region with *Sac*I and *Kpn*I and cloned it into a bluescript plasmid to form plasmid pHL129.

DNA sequence analysis of *FLO8*: Nested deletions (from the *Sac*I direction only) of the insert in pHL36 were generated by digestion with *Exo*III (HENIKOFF 1984) and single-strand nucleotide sequence was determined by the dideoxy chain termination method (SANGER *et al.* 1977). Based on this sequence, the appropriate primers were synthesized to sequence the complementary strand. Protein homology searches were performed via the World Wide Web by using

the BLAST Search Page at National Center for Biotechnology Information (ALTSCHUL *et al.* 1990). The GenBank accession number for *FLO8* is U51431.

DNA manipulations of *FLO8*: To integrate *FLO8* at the homologous chromosomal site, we cut pHL11 with *Bgl*II to linearize the plasmid at the *FLO8* coding region and then transformed yeast Ura$^-$ strains. Ura$^+$ yeast transformants were selected and integration by gene replacement was confirmed by Southern analysis. A strain carrying a null allele of *FLO8* was constructed by digesting plasmid pHL129 carrying *flo8-2* with *Kpn*I and *Sac*I to cut out the *flo8::hisGURA3kan^{R}hisG* fragment and then transforming Σ *FLO8* strains with the digested plasmid. Ura$^+$ transformants were first screened by PCR reactions for loss of the *FLO8* band and the putative *flo8-2* strains were confirmed by Southern blot analysis.

The *flo8* mutant allele present in S288C was cloned by gap repair. The autonomously replicating plasmid pHL1, containing *FLO8*, was digested with *Bst*EII and the digested DNA fragments were separated on an agarose gel from which the larger DNA fragment (containing gapped *FLO8* on the vector) was eluted. *Bst*EII, which cuts twice in *FLO8* gene and has no sites in the vector, liberates a segment of DNA that spans the region of the presumed mutation in the S288C *flo8* gene (see Figure 2). The purified DNA fragment was used to transform an S288C *flo8-1/flo8-1* diploid strain (L5107). Ura$^+$ transformants were tested for their ability to form pseudohyphae on SLAD medium. All Ura$^+$ transformants with gapped pHL1 gave Phd$^-$ colonies. DNA was extracted from the Ura$^+$ Phd$^-$ transformants and transformed into *E. coli* to amplify the plasmid for DNA sequencing. Control experiments in which a plasmid with an intact *FLO8* gene on the plasmid was used to transform a *flo8/flo8* diploid gave Phd$^+$ transformants.

Photomicroscopy: Photographs of colonies were taken under bright field (GIMENO *et al.* 1992). Indirect immunofluorescence was carried out using standard procedures for yeast (PRINGLE *et al.* 1991). Formaldehyde was added to fresh early log cultures to a final concentration of 3.7%, cultures were incubated at room temperature for 30 min. Cells were then collected and resuspended in 3.7% formaldehyde, 0.1 M potassium phosphate buffer (pH 6.5), incubated for 1 hr at room temperature, washed three times with spheroplasting solution (1.2 M sorbitol, 0.1 M potassium phosphate pH 7.5) and resuspended in 1 ml of the same solution plus 25 mM β-mercaptoethanol and 25 μg/ml zymolyase. After 30 min of digestion at 30°, spheroplasts were collected and loaded onto poly-L-lysine coated multi-well slides. Excess cells were aspirated away. Spheroplasts on the slide were immersed in cold methanol for 5 min at −20° and rewetted with phosphate-buffered saline (PBS) solution containing 1 mg/ml bovine serum albumin (BSA). Cells were then covered with 15 μl primary anti-myc mouse monoclonal 9E10 (1:100 diluted into the PBS/BSA solution) and incubated in a moisture chamber for 1 hr. The second antibody was an DTAF-conjugated anti-mouse IgG (1:50 dilution). Antibodies were washed away after each incubation. 4′,6-diamidino-2-phenylindole (DAPI) (1 μg/ml) was incubated with the cells before loading the mounting solution.

RESULTS

S288C strains have a mutation that blocks pseudohyphal formation: Haploid strains (Mat**a** and Matα) from the S288C and Σ1278b (Σ) backgrounds were mated in all combinations to form diploids, and these were subsequently analyzed for their ability to make pseudohyphae on SLAD medium. Diploids obtained from Σ × Σ matings form abundant pseudohyphae (Phd$^+$), whereas those from a S288C × S288C mating fail to form pseudohyphae (Phd$^-$) (Figure 1, A and B). In all matings between a Σ haploid and a S288C haploid, the diploids made pseudohyphae (Phd$^+$, Figure 1C). One of the crosses (X10375) between a strain from the Σ background (CG25) and one from the S288C background (10362-6A) was examined by tetrad analysis to determine whether the difference between Σ and S288C resulted from a mutation in a single gene. In each tetrad the ability of an ascospore to form pseudohyphae was assayed by mating it to a strain from the S288C background as a tester (10362-6A, 10362-3A) and determining the Phd phenotype of the resulting diploid. Tetrads derived from the Σ × S288C cross (X10375) gave strictly 2:2 segregation for all known markers. Each tetrad segregated two Phd$^-$ spores, diploids formed between these and an S288C strain failed to form any pseudohyphae. However, the pseudohyphal phenotype of the other two spores varied considerably, occasionally, one of the two formed florid pseudohyphae; more often, they produced very few pseudohyphae. These observations indicate that this cross was segregating a mutation in a single gene that blocks pseudohyphal formation, but was also segregating additional mutations that modify the level of pseudohyphal

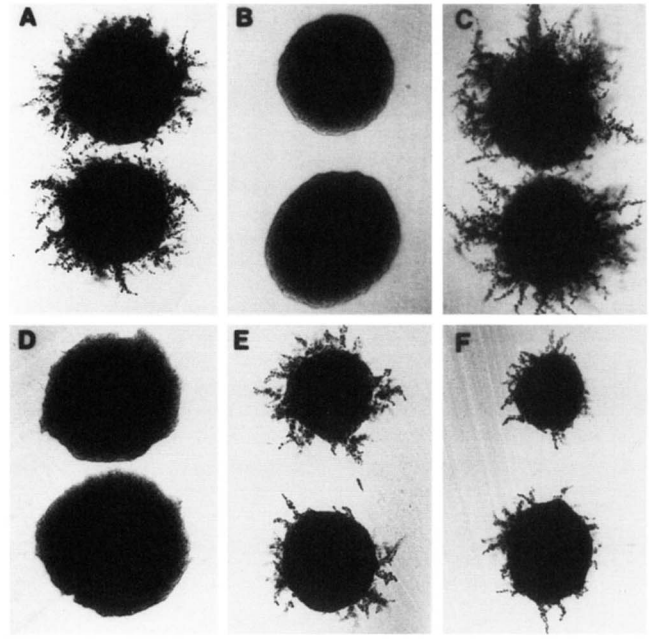

FIGURE 1.—S288C strains have a mutation (*flo8-1*) in *FLO8*, a gene necessary for filamentous growth. (A) A Σ *FLO8/FLO8* diploid strain (CGx52). (B) A S288C *flo8-1/flo8-1* diploid strain (10362-6A × 3A). (C) A diploid formed by a mating between Σ *FLO8* and S288C *flo8-1* (X10375). (D) A *flo8-1/flo8-1* diploid strain formed between progeny from the cross X10375 (L5810). (E) The *flo8-1/flo8-1* diploid strain in D transformed with *FLO8* in plasmid pHL1: (L5811). (F) The *flo8-1/flo8-1* diploid strain transformed with *FLO8* in plasmid pHL36: (L5812). Yeast cells were streaked out on SLAD medium and colonies were photographed after growth for 4 days at 30°.

growth. The effects of these additional mutations on pseudohyphal growth were subtle and variable, precluding their isolation and identification by genetic analysis. However, the mutation with the strongest Phd$^-$ phenotype could be separated from other mutations by backcrosses.

To obtain strains segregating the single strong pseudohyphal mutation we backcrossed a Phd$^-$ spore (10375-6B) from this cross (X10375) to a Σ strain (CG25) to produce diploid X10397. The cross X10397 yielded tetrads with a clear 2 Phd$^+$:2 Phd$^-$ segregation and little variation among the Phd$^+$ spores. The gene required for pseudohyphal formation that is segregating in this cross was provisionally designated *PHD10*.

Cross X10397 produced a convenient set of haploid *phd10* testers (10397-7D, 10397-9B). Matings between these *phd10* tester strains and any other *phd10* strains fail to produce pseudohyphae (Phd$^-$). Using these testers, we found that strains from the Σ1278b background are all *PHD10*, whereas those from the S288C background are all *phd10*. As noted previously and in MATERIALS AND METHODS, many S288C derivatives contain additional mutations that mute the ability of S288C *PHD10* strains to form pseudohyphae in diploids formed in matings with either Σ or S288C *PHD10* strains.

***PHD10* is *FLO8*:** A *URA3* centromere-based plasmid library was constructed from a Σ strain (see MATERIALS AND METHODS) and used to clone a functional copy of *PHD10*. The Σ genomic library was transformed into a diploid *phd10/phd10* strain L5107, and the transformants were screened visually on SLAD medium for clones that complemented the Phd$^-$ defect. Restriction digests of the 11 plasmids isolated by this visual assay indicated that they represent two genes. One of these, deemed the *PHD10* gene, represented by plasmids pHL1 and pHL36, complemented the Phd$^-$ phenotype fully (Figure 1, E and F). The other nine isolates represent another gene, which did not complement fully and only stimulated surface pseudohyphal growth in a *phd10/phd10* diploid. Subsequent gene integration and genetic analysis showed that the second gene is not linked to the *phd10* mutation, and therefore it was not studied further.

To determine whether the putative *PHD10* clones (pHL1 and pHL36) actually contained the *PHD10* gene, we first integrated the cloned DNA segment together with the *URA3* gene (pHL11), into a site of *PHD10* homology in a haploid strain (10415-8D) by transformation (MATERIALS AND METHODS). The *URA3* gene functions as a selectable marker that tags the integrated region. Southern analysis confirmed that the integrations occurred by homologous recombination. One Ura$^+$ transformant (L5281) was mated to a *phd10* strain (10397-7D) and the resulting diploid was fully capable of forming pseudohyphae. Therefore, the integrated DNA segment (pHL11) in single copy is capable of complementing the Phd$^-$ defect of *phd10* strains. The diploid strain was dissected and all 16 spores from four tetrads were analyzed for their ability to form pseudohyphae in diploids formed in matings with *phd10* tester strains 10397-7D or 10397-9B. In all four tetrads, pseudohyphal growth segregated 2 Phd$^+$:2 Phd$^-$ with Phd$^+$ completely linked to Ura$^+$. Thus, the sequence that conferred the Phd$^+$ phenotype segregated as a single gene and was linked to the *URA3* sequence that was introduced by integrative transformation. The Ura$^+$ transformant (L5281) was also mated to a *PHD10* strain (CG41). The resulting diploid was dissected and 12 tetrads were analyzed for their ability to form pseudohyphae in diploids formed by mating with *phd10* tester strains (10397-7D or 10397-9B). All the meiotic progeny from the diploid strain were Phd$^+$. This result shows that plasmid pHL11 integrated at the *PHD10* locus. From the ability of pHL11 to suppress the functional defect of *phd10* strains and its ability to integrate by homology at the *PHD10* locus we conclude that we have cloned the Σ1278b *PHD10* gene.

To map the *PHD10* gene, we first hybridized a *PHD10* probe to a panel of phage lambda clones that covers 82% of the yeast genome (RILES *et al.* 1993). *PHD10* hybridized with clones 2650 and 6052 from chromosome *V* on the blot, locating the gene to the region of overlap between these two clones. The positions of clone 2650 and 6052 suggested the following order for genes in this region: *TRP1, RAD51, PHD10, BEM2* and *SPR6*. This position of *PHD10* was confirmed by meiotic mapping. Tetrad analysis of a three point cross between yeast strains 16-1 and 10459-4B place *PHD10* between *met6* and *spr6* about 0.9 cM from *spr6*.

The DNA segment shared by both *PHD10* complementing plasmids pHL1 and pHL36 was sequenced. One large open reading frame (ORF) with 799 amino acid residues was identified. The putative amino acid sequence did not reveal any informative motifs. There is a stretch of glutamine residues that is present in many transcription factors, which accounts for the fact that several yeast transcription factors have the highest similarity to Phd10p from our BLAST search (Gal1p, Pho2p, TBP and Ssn6p). After submission of this paper, this sequence we were calling *PHD10* appeared in Genbank as *FLO8*, a putative transcriptional activator of *FLO1* (KOBAYASHI *et al.* 1996). Although previous work had mislocated *FLO8* on chromosome *VIII* (TEUNISSEN *et al.* 1995), *FLO8* and *PHD10* are the same gene, which is located on chromosome *V*. Therefore, we will refer to this gene as *FLO8* and its mutations as *flo8*. All the strain designations in the tables and figures follow this assignment.

The S288C *flo8-1* mutant allele: The SGD sequence of *FLO8*, derived from S288C, differs by 1 bp in the coding region from our sequence of *FLO8* obtained from Σ (Figure 2). The existence of this base-pair change in S288C strains was confirmed by cloning and

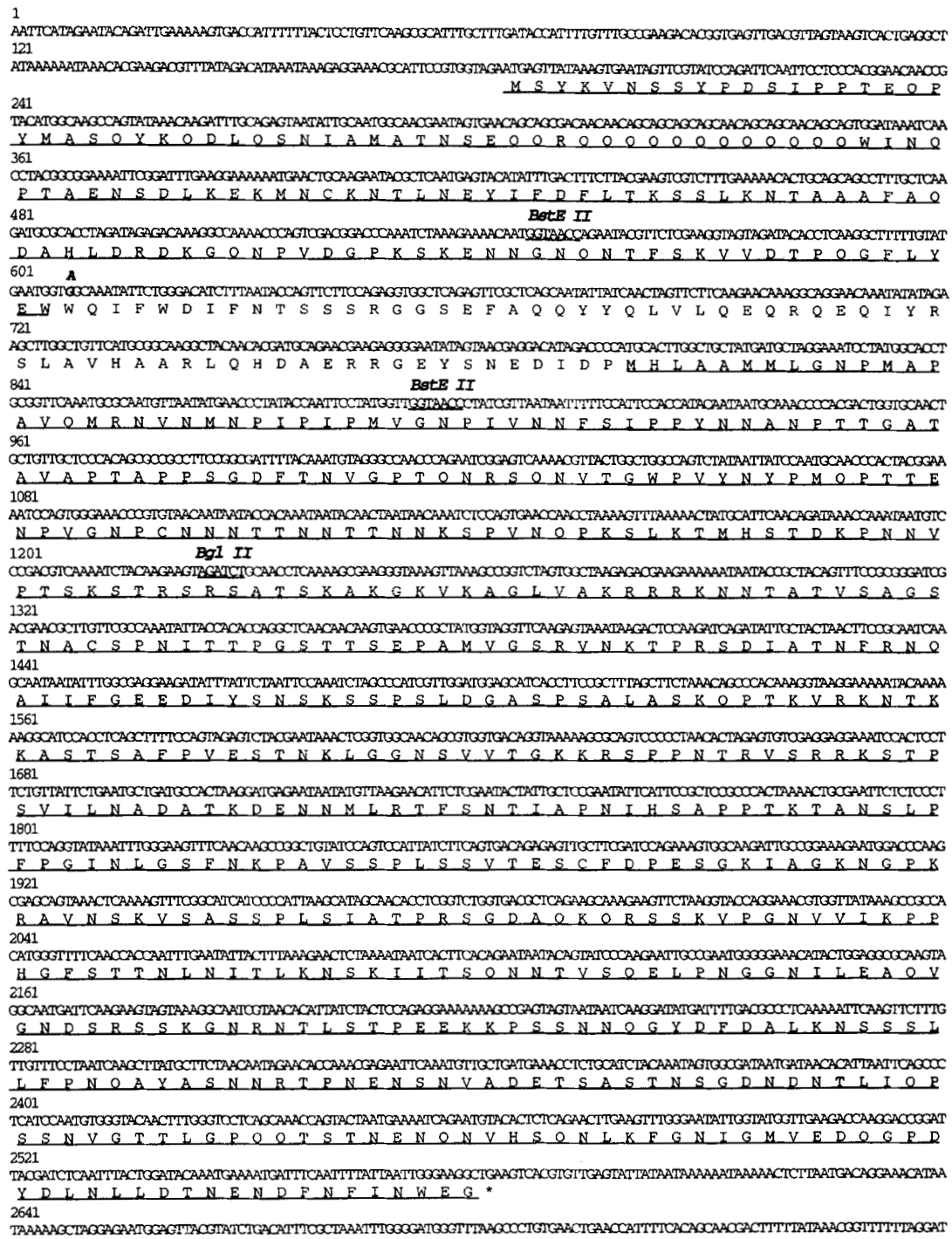

FIGURE 2.—DNA sequences of *FLO8* from Σ strains and *flo8-1* of S288C strains. The *FLO8* DNA sequence from Σ strains and its deduced protein sequence are shown. The change at position 608 from A to G in the S288C strains (*flo8-1* allele) is indicated. The underlined protein segments represent the expected ORFs from S288C strains. The region between two *Bst*EII sites was removed to clone the *flo8-1* mutation by gap repair. The myc-tag is inserted at the *Bgl*II site of *FLO8*.

sequencing the *flo8* region from S288C strains. In one approach, we used the Σ *FLO8* gene to probe a S288C yeast genomic library and cloned the S288C *flo8* gene (MATERIALS AND METHODS). In an alternative approach, we cloned the S288C mutation by gap repair. *S. cerevisiae* replaces a gap on a plasmid with its corresponding chromosomal DNA by homologous recombination. The Σ *FLO8* region corresponding to the mutation in plasmid pHL1 was deleted by *Bst*EII digestion (see Figure 2) and the gapped *FLO8* on this plasmid was transformed into an S288C strain identified as *flo8/flo8* (L5107). All transformants of the *Bst*EII-digested pHL1 showed a Phd$^-$ phenotype, whereas control transformants of undigested pHL1 gave a Phd$^+$ phenotype. This result indi-

cates that the gap-repaired plasmid had replaced the gapped region with the corresponding chromosomal DNA that carries a *flo8* mutation. Sequence comparison among the cloned S288C *flo8* gene, gap-repaired *flo8,* and the Σ *FLO8* confirmed that S288C *flo8* and Σ *FLO8* indeed differ by only 1 bp. S288C *flo8,* now called *flo8-1,* has one base change from G to A, resulting in a stop codon in the coding sequence (see Figure 2). Therefore, S288C strains probably produce a truncated inactive Flo8p (Figure 2). Our finding of a nonsense mutation in S288C agrees with our genetic data indicating that S288C has a mutation in the *FLO8* gene that blocks filamentous growth.

Northern analysis indicates that the *FLO8* gene is expressed in both haploid cells and diploid cells grown in SC medium (not shown). The same result was obtained with a *FLO8-LacZ* construct, which showed roughly equivalent activity in both haploid and diploid cells (not shown).

***FLO8* is required for pseudohyphal growth in both Σ and S288C strains:** The *FLO8* gene was deleted in Σ strains 10480-5C and 10480-5D using plasmid pHL129 to replace the resident *FLO8* gene with deletion *flo8-2* (creating strains L5816 and L5817, respectively). The presence of the deletion was confirmed by Southern analysis. The deletion *flo8-2* has 85% of the *FLO8* coding region replaced by a *hisGURA3KanRhisG* insertion, resulting in a null mutation. Σ strains carrying this null mutation have no obvious growth defect on rich media. On SLAD medium, diploid *flo8-2/flo8-2* strains are defective in pseudohyphal development (Figure 3, A and B); they fail to invade or form filaments.

Expression of *FLO8* from a high copy plasmid (pHL135) in Σ diploids enhances filament formation on SLAD medium (Figure 3C). This enhancement is limited to this nitrogen starvation medium. No enhancement was observed on media with standard levels of nitrogen. By comparison, *PHD1* and *CPH1,* genes whose overexpression enhances pseudohyphal growth, stimulate filament formation even on media containing standard nitrogen concentrations (GIMENO and FINK 1994; LIU *et al.* 1994).

We also introduced a single copy of the *FLO8* gene into an S288C *flo8-1* strain background and found that this functional *FLO8* gene introduced by transformation is sufficient to suppress the pseudohyphal defect. The experiment was executed by transforming *FLO8* (pHL11) into haploid *flo8-1* strains FY3 and FY2 yielding transformants L5302 and L5304, respectively. Diploids (*e.g.,* L5306) from the mating between the transformed strains L5302 and L5304 are Phd^+, whereas a diploid (L5813) from the mating between the untransformed FY3 and FY2 is Phd^- (Figure 3, E and F). Diploids formed by mating transformed strains by the Phd^- parents are also Phd^+. These results show that *flo8-1* is the mutation in the S288C strain responsible for the defect in pseudohyphal growth.

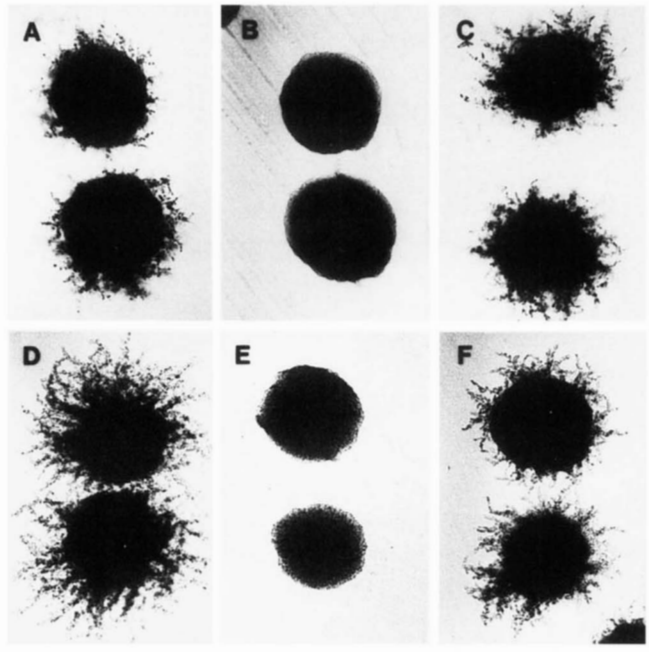

FIGURE 3.—*FLO8* is necessary for pseudohyphal development in diploids. (A) A Σ *FLO8/FLO8* strain (L5366). (B) A Σ *flo8-2/flo8-2* strain (L5818+pRS202). (C) A Σ *FLO8/FLO8* strain carrying *FLO8* on a high copy plasmid (L5366+pHL135). (D) EM93. (E) S288C *flo8-1/flo8-1* strain L5813. (F) S288C *FLO8/FLO8* diploid strain created by integrative transformation of *FLO8* (L5306). Yeast cells were streaked out on SLAD medium for single colonies. A–C were grown for 3 days before photography; D–F were grown for 4 days.

The *flo8* mutation also affects haploid invasion and flocculation: Previous studies have shown that haploids of Saccharomyces are capable of invading the agar (ROBERTS and FINK 1994). Though the haploid invasion and diploid pseudohyphal pathways have many features in common, haploid invasion is different from diploid invasion because haploid cells invade on rich YPD medium and poorly on SLAD, whereas diploids invade well on SLAD and poorly on YPD. We examined the Σ *FLO8* and *flo8* strains to determine whether *flo8* was required for haploid invasion. A haploid Σ *FLO8* strain invades well, whereas an isogenic Σ strain carrying the null allele of *FLO8* (*flo8*-2) is unable to invade (Figure 4). This phenotype is completely linked to *flo8-2* in crosses, suggesting that *FLO8* in Σ is required both for diploid pseudohyphal growth and haploid invasion.

Although haploid *flo8* strains from the S288C background also fail to invade on YPD, *FLO8* transformants of S288C do not completely restore a vigorous haploid invasion phenotype. A S288C haploid strain (FY3) and its *FLO8* transformants (L5302) were analyzed on YPD. The S288C::*FLO8* transformants were only partially restored for haploid invasive growth, despite the fact that the corresponding S288C::*FLO8* diploid (L5306) had completely normal filamentous growth on SLAD (Figure 3F). S288C must carry mutations in addition to *flo8* that affect haploid invasive growth, but not diploid pseudohyphal growth. This result further supports the

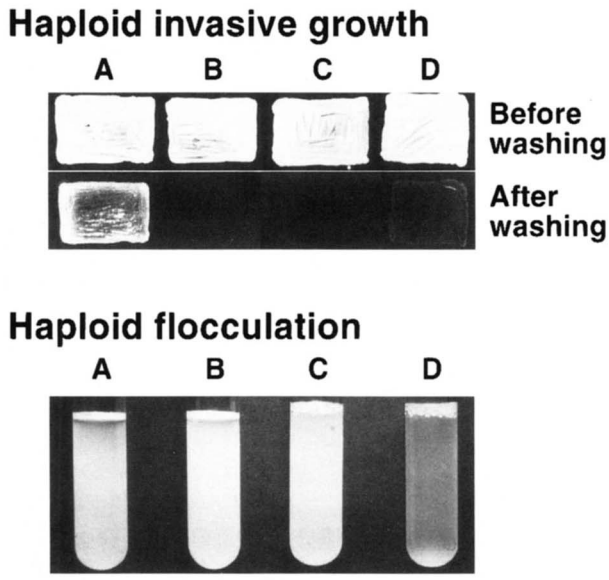

FIGURE 4.—*FLO8* is required for invasive growth and flocculation in haploids. (A) A Σ *FLO8* strain (10480-5C). (B) A Σ *flo8-2* strain (L5816). (C) A S288C *flo8-1* strain (FY3). (D) A S288C::*FLO8* strain (L5302). The top panel shows the invasive growth of haploid cells on solid YPD medium. Strains were patched and grown on YPD medium for 3 days at 30°. The plate was then washed with running tap water to rinse away cells on the agar surface. Pictures were taken before and after washing. The bottom panel shows the flocculation of different strains in liquid YPD medium. Cells were grown in YPD to saturation, agitated on a vortex mixer, allowed to settle for 10 min and then photographed. The S288C::*FLO8* culture (D) is more transparent because flocculent cells settle rapidly. The Σ *FLO8* strain is only slightly more flocculent than the Σ *flo8-2* strain, and this subtle difference is not apparent in the photograph.

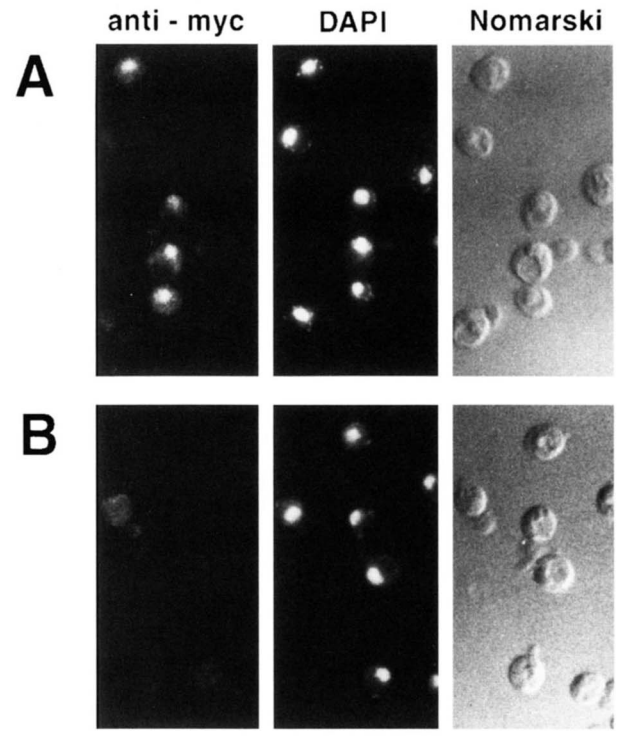

FIGURE 5.—Nuclear localization of the myc-tagged Flo8p. (A) A *flo8-2/flo8-2* diploid strain carrying the myc-tagged Flo8p (L5818+pHL138). (B) The same *flo8/flo8* diploid strain carrying untagged Flo8p (L5818+pHL135).

idea that although haploid invasive growth and diploid pseudohyphal growth share common features, they are not identical developmental events. Some mutations may affect one process without affecting the other.

FLO8 haploids in the S288C background flocculate and settle more rapidly in liquid media than *flo8* haploids (Figure 4, C and D). This flocculation phenotype cosegregates with *FLO8* in S288C strains. The flocculation in *FLO8* strains is a consequence of an especially avid aggregation of the cells because the aggregated cells can be dispersed by chelating out the calcium (STRATFORD 1989; TAYLOR and ORTON 1975); addition of 4 mM EDTA disperses the cell aggregates, and subsequent addition of Ca^{++} leads to reaggregation. However, this flocculation phenotype is not as pronounced in Σ*FLO8* haploid strains (Figure 4, A and B), indicating the existence of other differences between S288C and Σ that affect the flocculation phenotype. Thus, with respect to both haploid invasion and flocculation, the magnitude of the difference between *FLO8* and *flo8* strains depends upon the genetic background (S288C or Σ).

Flo8p is localized to the nucleus: An epitope-tagged version of Flo8p was localized by immunofluorescence microscopy. A triple myc epitope tag from pKB241 was inserted in frame with Flo8p (Figure 2) and *FLO8::myc3* was expressed from its own promoter on a high copy yeast vector (details in MATERIALS AND METHODS). This construct partially complements the Phd^- phenotypes: *FLO8::myc3* completely restores haploid invasive growth to *flo8* (L5816) cells; however, it only partially complements the pseudohyphal growth defect in diploid *flo8/flo8* (L5818). The diploid *flo8/flo8* strain carrying *FLO8::myc3* was subjected to indirect immunofluorescence microscopy. myc-tagged Flo8p colocalized with the DAPI staining, suggesting that Flo8p is localized to the nucleus (Figure 5A). This nuclear localization was present in cells observed at all phases of yeast cell cycle. A control strain overexpressing nontagged *FLO8* showed only a hazy background level of staining throughout the cell (Figure 5B).

Pseudohyphal growth in frequently used laboratory strains We tested frequently used haploid laboratory strains for their ability to form pseudohyphae in diploids formed by mating with the Σ haploid *flo8* tester strains 10397-7D and 10397-9B. Diploids formed between a haploid strain D273-10B/A1 obtained from Dr. F. SHERMAN (used frequently in mitochondrial studies) and our *flo8* tester form pseudohyphae on SLAD medium. This result suggests that the D273-10B/A1 strains have a functional copy of the *FLO8* gene. Moreover, a diploid from that strain background, DA1rho°XJJM113, forms pseudohyphae on SLAD showing that respira-

tion-deficient strains are fully competent to undergo the morphological switch. Other studies differ in their assessment of the filamentation behavior of D273-10B strains; one concludes that D273-10B haploid strains are capable of forming filaments (WRIGHT *et al.* 1993), whereas others found strains from this background incapable of forming pseudohyphae (BLACKETER *et al.* 1993, 1994). The discrepancy could mean that mutations such as *flo8* were introduced upon subculturing in one case and not in the other. Another haploid strain, A364A from L. HARTWELL (used extensively in cell division cycle studies) when mated to our *flo8* tester also produces diploids that filament under nitrogen starvation. On the other hand, W303 diploid strains (L5682) or diploids from a mating between a W303 haploid strain (EY699) and the *flo8* tester are all Phd$^-$. This result suggests that W303 like S288C contains a nonfunctional *FLO8* gene. Transformation of *FLO8* into a W303 diploid strain did not rescue the Phd$^-$ phenotype, suggesting that W303 has mutations in both the *FLO8* gene and at least one other gene essential for pseudohyphal growth (result not shown).

We also tested the correlation between diploid pseudohyphal growth and haploid invasive growth in these laboratory strains. As summarized in Table 3, Σ1278b strains and the A364A strains are competent in both haploid invasion and diploid pseudohyphal growth, whereas S288C strains and W303 strains are deficient in both. However, D273-10B/A1 fails to manifest haploid invasive growth on YPD medium, even though the diploid strain of the same background forms filaments on SLAD. This result emphasizes the difference between haploid invasive growth and filamentous growth, D273-10B/A1 haploids contain a mutation(s) that interferes with haploid invasive growth, but this mutation does not affect diploid filament formation.

TABLE 3

Comparison of haploid invasive growth and diploid filamentous growth in various strain backgrounds

Strains	Haploid invasion	Pseudohyphal growth
Haploids		
Σ1278b	+	+++
S288C	−	−
D273-10B/A1 (Sherman)	−	++
A364A (Hartwell)	+	+++
W303-1A	−	−
Diploids or polyploids		
EM93	NA	++++
Fleischmann's yeast	NA	−
Red Star dry yeast	NA	++++
Beer 2	NA	−

The +, pseudohyphal growth was observed. The number of +s indicates the relative extent of pseudohypal growth. −, no pseudohyphal growth was observed. The + for haploid invasion means that the strain invaded the agar as determined by the haploid invasion assay (RESULTS). NA, not applicable. For the haploid strains pseudohyphal growth was determined in diploids, made in each instance by mating the relevant haploid by a S288C *flo8* strain.

Pseudohyphal formation in feral and industrial strains: EM93 is a feral strain isolated in 1936 from a rotting fig in Merced, California (MORTIMER and JOHNSTON 1986). This strain is the ancestor of S288C and contributes most of the genome in S288C. EM93 is a heterothallic diploid and develops florid pseudohyphae on standard nitrogen starvation medium (Figure 3D). The spores from seven EM93 tetrads were mated to *flo8* tester strains to determine whether they could form pseudohyphae in diploids. In each tetrad all four spores were capable of forming pseudohyphae with the *flo8* tester, indicating that EM93 was homozygous for *FLO8* and was not the origin of the *flo8* mutation in S288C. In addition to EM93, many strains isolated from AIDS patients are dimorphic, making elaborate pseudohyphae upon nitrogen starvation. One of these extensively analyzed by MCCUSKER (MCCUSKER *et al.* 1994a,b) is a diploid.

Industrial strains are not strictly comparable either to laboratory or feral strains although both the laboratory and industrial strains are presumed to be derived from the feral strains. One difficulty in comparing these strains of different origin is that the industrial strains are often polyploid and of uncertain chromosome constitution. Nevertheless, several of them were analyzed for pseudohyphal formation on nitrogen starvation medium. Red Star baking yeast made dramatic filaments similar to those of EM93. However, Fleischmann's yeast, another baking strain, fails to make filaments when starved for nitrogen. A brewing yeast strain from the brewery in Seville, Spain also fails to make filaments.

DISCUSSION

***FLO8* is required for pseudohyphal formation in diploids, and invasive growth and flocculation in haploids:** Several lines of evidence indicate that a functional copy of *FLO8* is necessary for filamentous growth in diploids. First a *flo8* mutation in S288C strains blocks the pseudohyphal growth of S288C diploids. Introduction of a single copy of Σ-derived *FLO8* into a S288C *flo8* strain restores the Phd$^+$ phenotype. Second, deletion of the *FLO8* gene in Σ strains renders them Phd$^-$. Moreover, overproduction of *FLO8* in Σ diploids promotes more vigorous pseudohyphal growth.

FLO8 is also involved in two haploid specific processes: invasive growth and flocculation. Invasive growth is defined operationally by washing cells off the agar surface of petri plates; strains that are competent to invade leave a visible residue after the wash, whereas those that fail to invade do not (ROBERTS and FINK 1994). Although haploid invasive growth requires many of the same genes as diploid pseudohyphal growth, including elements of the pheromone response pathway

and *FLO8*, the two processes have a number of differences. First, haploid invasive growth is limited to rich media and does not occur on SLAD. By contrast, diploids invade much more poorly than haploids on rich medium. Second, the haploid cells on rich medium can be removed from the agar surface by rubbing with a finger, whereas diploid filaments on low nitrogen medium have penetrated far beneath the agar and cannot be rubbed off.

Both the invasive growth on rich medium and flocculation are under the control of *FLO8*. Since these are haploid phenotypes that are not manifest in diploids, both may be regulated by **a**1-α2 repression. Although a functional *FLO8* gene is required for both processes, a comparison of Σ *FLO8* and S288C::*FLO8* haploid strains indicates that the extent of invasive growth does not correlate with that of flocculation, the Σ *FLO8* strain is more invasive but less flocculent than S288C::*FLO8*.

The function of Flo8p: The *FLO8* gene was recently reported to be the putative transcriptional activator for *FLO1* (KOBAYASHI *et al.* 1996). *FLO1* and *FLO5* are dominant flocculation genes that encode cell wall-associated proteins that share 96% identity in protein sequence (TEUNISSEN *et al.* 1993, 1995a; BIDARD *et al.* 1995). Our observation that Flo8p is localized to the nucleus and is required for flocculation in S288C is consistent with its reported role as a transcriptional activator of *FLO1*. Furthermore, the finding that *FLO8* is also required for diploid filamentous growth and haploid invasion suggests the existence of other genes under Flo8p regulation. Ssn6p and Tup1p(Sp12p) unlike Flo8p are negative regulators of flocculation (TEUNISSEN *et al.* 1995b); haploid strains containing the *ssn6* or *tup1* mutations are more flocculent than *SSN6 TUP1* strains. Ssn6p-Tup1p, which acts as a complex in transcriptional repression (KELEHER *et al.* 1992), could play a direct role in repressing *FLO1* transcription.

Many laboratory and industrial strains are defective in pseudohyphal formation: A number of laboratory strains (S288C and W303) have a recessive mutation in the *FLO8* gene that blocks haploid invasive growth and diploid pseudohyphal growth. Where did the *flo8* mutation arise? Presumably, the *flo8* mutation was selected passively by yeast geneticists who found the properties of the *FLO8* haploids a nuisance for genetic and biochemical analysis. The diploid progenitor of S288C is EM93, which is homozygous for a functional *FLO8* gene. Its haploid progeny are extremely flocculent making them difficult to transfer upon replica-plating. Moreover, in liquid cultures, these flocculent cells do not stay in suspension, making it difficult to measure their growth rate or to obtain colonies that arise from a single cell upon plating on solid medium. It is likely that in the decades since yeast was first domesticated the *flo8* mutation was selected by yeast geneticists who picked nonflocculent colonies that replica-plate well on solid medium and stay in suspension in liquid.

Laboratory strains of Saccharomyces contain many naturally occurring mutations in addition to *flo8*. S288C is known to have a number of mutations including *gal2*, *suc2*, *mal*, and *flo1* (MORTIMER and JOHNSTON 1986). The *suc2* mutation is the result of an amber codon in the coding sequence of the *SUC2* gene (CARLSON *et al.* 1981; GOZALBO and HOHMANN 1989). The *mal* and *flo1* mutations are the consequence of deletions of repeated sequence close to telomeres (MORTIMER and JOHNSTON 1986). S288C also has a mutation in the *KSS1* gene (ELION *et al.* 1991a,b). Another example of naturally occurring mutations is *SSD1* in W303 strains (SUTTON *et al.* 1991; COSTIGAN *et al.* 1992; STETTLER *et al.* 1993; KIKUCHI *et al.* 1994). There is also variation in the copy number and location of the *RTM1* gene in different *S. cerevisiae* strains (NESS and AIGLE 1995). Some strains are completely devoid of the *RTM1* sequence. The difference between *RTM1* or *rtm1* strains appears to be a consequence of deletions caused by rearrangements between repeated telomeric sequences.

With the completion of the sequence of the entire yeast genome, the presence of other naturally occurring mutations may become recognized. One way to identify these mutations is to search for pairs of ORFs transcribed from the same DNA strand that are interrupted by a nonsense codon or short insertion sequence. Our survey of ORFs on yeast chromosome *III* have identified at least three pairs of ORFs that are separated by a very short DNA segment, including: YCL061c/YCL060c, YCR086w/YCR087w, and YCR099c/YCR100c. These short interruptions could represent genes that are nonfunctional in S288C, but functional in other strain backgrounds.

We also find that many industrial strains do not form pseudohyphae. Most industrial strains are diploid or of higher ploidy and should be capable of filament formation on SLAD medium. Yet, many do not form filaments. Presumably, the filament formation and aggregation properties of pseudohyphal cells were not desirable for the growth in a fermentor and were selected against when these brewing and baking strains were developed. It would be interesting to know whether these strains also have a defective *FLO8* gene.

Many feral strains of Saccharomyces are filamentous: A number of Saccharomyces strains that have been isolated directly from their natural habitat (fruits or vegetables) and analyzed without extensive laboratory culture appear to be dimorphic diploids. EM93 the progenitor of S288C was isolated from a rotting fig in Merced, California. Recently, strains of *S. cerevisiae* have been isolated from immunocompromised humans. Many of these strains are diploid and filamentous (MCCUSKER *et al.* 1994a,b; C. A. STYLES and G. R. FINK, unpublished results). We suspect that dimorphism, the ability of Saccharomyces to switch between its yeast and filamentous form, contributes to its ability to survive in both plant and animal tissue. The earliest recorded

observation of filamentous growth in feral strains of Saccharomyces was made by EMIL CHRISTIAN HANSEN (HANSEN 1886).

> . . . I have shown that "veil formation" commonly occurs with a variety of microorganisms, bacteria as well as fungi.
>
> We have observed this with the Saccharomyces as well, and learned that the cells in the veils of old cultures develop a more elongated form, and more complex colonies; filiform cells were formed, resembling bacteria.
>
> (translated by Dr. DANIEL KORNITZER)

It is probably significant that the unusual filaments observed by HANSEN formed in cultures that were incubated over long periods of time. Presumably, the cells in these cultures were starved for nutrients such as nitrogen.

We thank C. GIMENO and MARY CLANCY for sharing strains and information, J. LOEB for critical reading of this manuscript, and members of the FINK laboratory for helpful discussions. This work was supported by a Jane Coffin Childs Fellowship (H.L.), a Bristol-Myers Squibb Award in Infectious Disease, and National Institutes of Health grant GM402661 (G.R.F.). G.R.F. is an American Cancer Society Professor of Genetics.

LITERATURE CITED

ALTSCHUL, S. F., W. GISH, W. MILLER, E. W. MYERS and D. J. LIPMAN, 1990 Basic local alignment search tool. J. Mol. Biol. **215:** 403–410.

BANUETT, F., and I. HERSKOWITZ, 1994 Identification of Fuz7, a Ustilago maydis MEK/MAPKK homolog required for a-locus-dependent and -independent steps in the fungal life cycle. Genes Dev. **8:** 1367–1378.

BARRAL, Y., and C. MANN, 1995 G1 cyclin degradation and cell differentiation in *Saccharomyces cerevisiae.* C. R. Acad. Sci. **318:** 43–50.

BARRAL, Y., S. JENTSCH and C. MANN, 1995 G1 cyclin turnover and nutrient uptake are controlled by a common pathway in yeast. Genes Dev. **9:** 399–409.

BIDARD, F., M. BONY, B. BLONDIN, S. DEQUIN and P. BARRE, 1995 The *Saccharomyces cerevisiae* FLO1 flocculation gene encodes for a cell surface protein. Yeast **11:** 809–822.

BLACKETER, M. J., C. M. KOEHLER, S. G. COATS, A. M. MYERS and P. MADAULE, 1993 Regulation of dimorphism in *Saccharomyces cerevisiae:* involvement of the novel protein kinase homolog Elm1p and protein phosphatase 2A. Mol. Cell. Biol. **13:** 5567–5581.

BLACKETER, M. J., P. MADAULE and A. M. MYERS, 1994 The *Saccharomyces cerevisiae* mutation elm4-1 facilitates pseudohyphal differentiation and interacts with a deficiency in phosphoribosylpyrophosphate synthase activity to cause constitutive pseudohyphal growth. Mol. Cell. Biol. **14:** 4671–4681.

BRANDRISS, M. C., and B. MAGASANIK, 1979 Genetics and physiology of proline utilization in *Saccharomyces cerevisiae.* J. Bacteriol. **143:** 1403–1410.

CARLSON, M., B. C. OSMOND and D. BOTSTEIN, 1981 Genetic evidence for a SUC gene in yeast. Genetics **98:** 41–54.

CHRISTIANSON, T. W., R. S. SIKORSKI, M. DANTE, J. H. SHERO and P. HIETER, 1992 Multifunctional yeast high-copy-number shuttle vectors. Gene **110:** 119–122.

CORUZZI, G., M. K. TREMBATH and A. TZAGOLOFF, 1978 Assembly of the mitochondrial membrane system: mutations in the pho2 locus of the mitochondrial genome of *Saccharomyces cerevisiae.* Eur. J. Biochem. **92:** 279–287.

COSTIGAN, C., S. GEHRUNG and M. SNYDER, 1992 A synthetic lethal screen identifies SLK1, a novel protein kinase homolog implicated in yeast cell morphogenesis and cell growth. Mol. Cell. Biol. **12:** 1162–1178.

ELION, E. A., J. A. BRILL and G. R. FINK, 1991a Functional redundancy in the yeast cell cycle: FUS3 and KSS1 have both overlapping and unique functions. Cold Spring Harbor Symp. Quant. Biol. **56:** 41–49.

ELION, E. A., J. A. BRILL and G. R. FINK, 1991b FUS3 represses CLN1 and CLN2 and in concert with KSS1 promotes signal transduction. Proc. Natl. Acad. Sci. USA **88:** 9392–9396.

GIMENO, C. J., and G. R. FINK, 1994 Induction of pseudohyphal growth by overexpression of PHD1, a *Saccharomyces cerevisiae* gene related to transcriptional regulators of fungal development. Mol. Cell. Biol. **14:** 2100–2112.

GIMENO, C. J., P. O. LJUNGDAHL, C. A. STYLES and G. R. FINK, 1992 Unipolar cell divisions in the yeast S. cerevisiae lead to filamentous growth: regulation by starvation and RAS. Cell **68:** 1077–1090.

GOZALBO, D., and S. HOHMANN, 1989 The naturally occurring silent invertase structural gene suc2 zero contains an amber stop codon that is occasionally read through. Mol. Gen. Genet. **216:** 511–516.

HANSEN, E. C., 1886 *Recherches sur la physiologie et al morphologie des ferments alcooliques.* C. R. Trav. Lab.

HENIKOFF, S., 1984 Unidirectional digestion with exonuclease III creates targeted breakpoints for DNA sequencing. Gene **28:** 351–359.

KALLAL, L. A., M. BHATTACHARYYA, S. N. GROVE, R. F. IANNACONE, T. A. PUGH *et al.,* 1990 Functional analysis of the sporulation-specific SPR6 gene of *Saccharomyces cerevisiae.* Curr. Genet. **18:** 293–301.

KELEHER, C. A., M. J. REDD, J. SCHULTZ, M. CARLSON and A. D. JOHNSON, 1992 Ssn6-Tup1 is a general repressor of transcription in yeast. Cell **68:** 709–719.

KIKUCHI, Y., Y. OKA, M. KOBAYASHI, Y. UESONO, A. TOH-E *et al.,* 1994 A new yeast gene, HTR1, required for growth at high temperature, is needed for recovery from mating pheromone-induced G1 arrest. Mol. Gen. Genet. **245:** 107–116.

KOBAYASHI, O., H. SUDA, T. OHTANI and H. SONE, 1996 Molecular cloning and analysis of the dominant flocculation gene FLO8 from Saccharomyces cerevisiae. Mol. Gen. Genet. **251:** 707–715.

KRON, S. J., C. A. STYLES and G. R. FINK, 1994 Symmetric cell division in pseudohyphae of the yeast *Saccharomyces cerevisiae.* Mol. Biol. Cell **5:** 1003–1022.

LIU, H., C. A. STYLES and G. R. FINK, 1993 Elements of the yeast pheromone response pathway required for filamentous growth of diploids. Science **262:** 1741–1744.

LIU, H., J. KOHLER and G. R. FINK, 1994 Suppression of hyphal formation in Candida albicans by mutation of a STE12 homolog. Science **266:** 1723–1726.

LJUNGDAHL, P. O., C. J. GIMENO, C. A. STYLES and G. R. FINK, 1992 SHR3: a novel component of the secretory pathway specifically required for localization of amino acid permeases in yeast. Cell **71:** 463–478.

MCCUSKER, J. H., K. V. CLEMONS, D. A. STEVENS and R. W. DAVIS, 1994a Genetic characterization of pathogenic *Saccharomyces cerevisiae* isolates. Genetics **136:** 1261–1269.

MCCUSKER, J. H., K. V. CLEMONS, D. A. STEVENS and R. W. DAVIS, 1994b *Saccharomyces cerevisiae* virulence phenotype as determined with CD-1 mice is associated with the ability to grow at 42 degrees C and form pseudohyphae. Infect. Immun. **62:** 5447–5455.

MORTIMER, R. K., and J. R. JOHNSTON, 1986 Genealogy of principal strains of the yeast genetic stock center. Genetics **113:** 35–43.

MÖSCH, H. U., R. ROBERTS and G. R. FINK, 1996 Ras2 signals via the Cdc42/Ste20/MAPK module to induce filamentous growth in *Saccharomyces cerevisiae.* Proc. Natl. Acad. Sci. USA **93:** 5352–5356.

MULERO, J. J., and T. D. FOX, 1994 Reduced but accurate translation from a mutant AUA initiation codon in the mitochondrial COX2 mRNA of *Saccharomyces cerevisiae.* Mol. Gen. Genet. **242:** 383–390.

MULLER, P. P., M. K. REIF, S. ZONGHOU, C. SENGSTAG, T. L. MASON *et al.,* 1984 A nuclear mutation that post-transcriptionally blocks accumulation of a yeast mitochondrial gene product can be suppressed by a mitochondrial gene rearrangement. J. Mol. Biol. **175:** 431–452.

MYERS, A. M., A. TZAGOLOFF, D. M. KINNEY and C. J. LUSTY, 1986 Yeast shuttle and integrative vectors with multiple cloning sites suitable for construction of lacZ fusions. Gene **45:** 299–310.

NESS, F., and M. AIGLE, 1995 RTM1: a member of a new family of telomeric repeated genes in yeast. Genetics **140:** 945–956.

PRINGLE, J. R., A. E. M. ADAMS, D. G. DRUBIN and B. K. HAARER, 1991 *Immunofluorescence Methods for Yeast.* Academic Press, Inc., London.

RILES, L., J. E. DUTCHIK, A. BAKTHA, B. K. MCCAULEY, E. C. THAYER *et al.,* 1993 Physical maps of the six smallest chromosomes of *Saccharomyces cerevisiae* at a resolution of 2.6 kilobase pairs. Genetics **134:** 81–150.

ROBERTS, R. L., and G. R. FINK, 1994 Elements of a single MAP kinase cascade in *Saccharomyces cerevisiae* mediate two developmental programs in the same cell type: mating and invasive growth. Genes Dev. **8:** 2974–2985.

RUDOLPH, H. K., A. ANTEBI, G. R. FINK, C. M. BUCKLEY, T. E. DORMAN *et al.,* 1989 The yeast secretory pathway is perturbed by mutations in PMR1, a member of a Ca2+ ATPase family. Cell **58:** 133–145.

SANGER, F., S. NICKLEN and A. R. COULSON, 1977 DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74:** 5463–5467.

SHERMAN, F., G. R. FINK and J. B. HICKS, 1986 *Methods in Yeast Genetics.* Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

SIDDIQUI, A. H., and M. C. BRANDRISS, 1988 A regulatory region responsible for proline-specific induction of the yeast *PUT2* gene as adjacent to its TATA box. Mol. Cell. Biol. **8:** 4634–4641.

SIKORSKI, R. S., and P. HIETER, 1989 A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in *Saccharomyces cerevisiae.* Genetics **122:** 19–27.

STETTLER, S., N. CHIANNILKULCHAI, S. HERMANN-LE DENMAT, D. LALO, F. LACROUTE *et al.,* 1993 A general suppressor of RNA polymerase I, II and III mutations in *Saccharomyces cerevisiae.* Mol. Gen. Genet. **239:** 169–176.

STRATFORD, M., 1989 Yeast flocculation: calcium specificity. Yeast **5:** 487–496.

SUTTON, A., D. IMMANUEL and K. T. ARNDT, 1991 The SIT4 protein phosphatase functions in late G1 for progression into S phase. Mol. Cell. Biol. **11:** 2133–2148.

TAYLOR, N. W., and W. L. ORTON, 1975 Calcium in flocculence of *Saccharomyces cerevisiae.* J. Instit. Brew. **81:** 53–57.

TEUNISSEN, A. W., E. HOLUB, J. VAN DER HUCHT, J. A. VAN DEN BERG and H. Y. STEENSMA, 1993 Sequence of the open reading frame of the FLO1 gene from *Saccharomyces cerevisiae.* Yeast **9:** 423–427.

TEUNISSEN, A. W., J. A. VAN DEN BERG and H. Y. STEENSMA, 1995a Localization of the dominant flocculation genes FLO5 and FLO8 of *Saccharomyces cerevisiae.* Yeast **11:** 735–745.

TEUNISSEN, A. W., J. A. VAN DEN BERG and H. Y. STEENSMA, 1995b Transcriptional regulation of flocculation genes in *Saccharomyces cerevisiae.* Yeast **11:** 435–446.

THOMPSON, C. M., A. J. KOLESKE, D. M. CHAO and R. A. YOUNG, 1993 A multisubunit complex associated with the RNA polymerase II CTD and TATA-binding protein in yeast. Cell **73:** 1361–1375.

WINSTON, F., C. DOLLARD and S. L. RICUPERO-HOVASSE, 1995 Construction of a set of convenient *Saccharomyces cerevisiae* strains that are isogenic to S288C. Yeast **11:** 53–55.

WRIGHT, R. M., T. REPINE and J. R. REPINE, 1993 Reversible pseudohyphal growth in haploid Saccharomyces cerevisiae is an aerobic process. Curr. Genet. **23:** 388–391.

Communicating editor: A. G. HINNEBUSCH