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HOY1, a Homeo Gene Required for Hyphal Formation in *Yarrowia lipolytica*

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The dimorphic fungus *Yarrowia lipolytica* grows to form hyphae either in rich media or in media with GlcNAc as a carbon source. A visual screening, called FIL (filamentation minus), for *Y. lipolytica* yeast growth mutants has been developed. The FIL screen was used to identify three *Y. lipolytica* genes that abolish hypha formation in all media assayed. *Y. lipolytica HOY1*, a gene whose deletion prevents the yeast-hypha transition both in liquid and solid media, was characterized. *HOY1* is predicted to encode a 509-amino-acid protein with a homeodomain homologous to that found in the chicken *Hox4.8* gene. Analysis of the protein predicts a nuclear location. These observations suggest that Hoy1p may function as a transcriptional regulatory protein. In disrupted strains, reintroduction of *HOY1* restored the capacity for hypha formation. Northern blot hybridization revealed the *HOY1* transcript to be approximately 1.6 kb. Expression of this gene was detected when *Y. lipolytica* grew as a budding yeast, but an increase in its expression was observed by 1 h after cells had been induced to form hyphae. The possible functions of *HOY1* in hyphal growth and the uses of the FIL screen to identify morphogenetic regulatory genes from heterologous organisms are discussed.

The yeast-to-hypha morphological transition (dimorphism) is typical of many pathogenic fungi (62). The dimorphic transition is a freely reversible process that can be induced by changes in many parameters (56). Much attention has been focused on *Candida albicans* as a model for analyzing dimorphism because it is the most frequently isolated fungal pathogen in humans. However, although several groups have reported the isolation of mutants which persist in either the yeast (10) or hyphal (21, 30) form, analysis of these mutants is hampered by the difficulties inherent to genetic manipulations of this asexual diploid organism.

Two approaches have been successfully used to carry out the analysis of dimorphism. With one of them, differential hybridization screening (7), several genes that are differentially expressed during morphogenesis have been isolated in *C. albicans* (i.e., *ECE1*, expressed in association with cell elongation, and *PHR1*, which is differentially regulated in response to the pH of the growth medium). Deletion of *PHR1* results in a pH-conditional defect in morphogenesis (59).

The second approach consists of cloning *Candida* homologs of *Saccharomyces cerevisiae* genes known to regulate pseudofilamentous growth. Under conditions of nutrient limitation (i.e., nitrogen starvation), *S. cerevisiae* undergoes a dimorphic transition to growth of pseudohyphae, linear chains of elongated cells in which the daughters remain attached to the mothers (23, 24). In this context, several *C. albicans* genes that are members of a *Candida* map kinase (MAPK) cascade, i.e., *CPH1* (40) or *ACPR* (43), *HST7* (11), and *CST20* (36, 39), have been isolated by complementation of the corresponding *Saccharomyces* mutants, i.e., *STE12*, *STE7*, and *STE20*, respectively (11, 36, 39, 40, 43). When *C. albicans* strains heterozygous and homozygous for null alleles of those genes were constructed, defects in hyphal formation were detected. For example, the *CPH1* gene (*cph1*/*cph1*) shows suppressed hyphal formation on solid medium but not in liquid medium (40), and all mutant strains still form hyphae in response to serum (36, 39).

Homeobox-containing genes have been found in many species (15). In fungi, homeobox-containing genes occupy a key position in the determination of the mating type in both budding (1, 46) and fission (33) yeast cells and in the determination of filamentous versus yeast-like growth forms in *Ustilago maydis* (60); however, a gene, *PHO2*, with a homeoregion, which activates transcription of the regulated acid phosphatase gene *PHO5* and possibly a phosphate permease, has also been reported for *S. cerevisiae* (9, 61).

Y. lipolytica is a dimorphic heterothallic yeast (3) that is unusual in both the structure of its genes coding for rRNA (17) and in its relationship to other yeasts (2). *Y. lipolytica* is amenable to genetic analysis (48), and DNA-mediated integration (12, 20) and autonomous transformation systems (18) have been developed. Mutations in the *SEC14* gene (42) and deletion of *XPR6* (16) have strong effects on the yeast-to-hypha transition. However, it is not known whether the proteins encoded by these genes are directly involved in the regulation of this transition. To gain insight into the developmental switch, we have developed an easy screen to obtain morphological mutants unable to form hyphae. The screening is based on the simple system for inducing the yeast-hypha transition reported previously by us (54). In the present work, we describe characterization of the *HOY1* gene. *HOY1* encodes a putative nuclear protein with a homeodomain which is differentially regulated during the yeast-to-hypha transition. Deletion of *HOY1* results in a defect in morphogenesis.

MATERIALS AND METHODS

Yeast strains, media, and microbiological techniques. The yeast strains used in the experiments are listed in Table 1. Our prototrophic standard strain, *Y. lipolytica* SA-1, was selected for its excellent ability to form mycelia in *N*-acetylglucosamine after sporulation of a diploid strain obtained from crossing CX 39-74B (*MatB trp1*) with CX 39-72C (*MatA ura1*) (48). Cells were grown in YED (1%

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TABLE 1. Yeast strains used in this study

Strain	Genotype	Reference, source, or derivation
W29	*MatA*	C. Gaillardin[a]
SA-1	*MatB*	A. Dominguez
21401-1	*MatA leu2-35 lys8-11*	C. Gaillardin
21501-13	*MatA leu2-35 lys5-12 ura2-21*	C. Gaillardin
21603-3	*MatB leu2-35 lys1-13*	C. Gaillardin
JM12	*MatB leu2-35 lys5-12 ura3-18*	C. Gaillardin
INAG33122	*MatB leu2-35 lys2-5 xpr2 ade1*	C. Gaillardin
P01a	*MatA leu2-270 ura3-302*	C. Gaillardin
JC8	*MatB filA*	This work
JC8-1	*MatB arg filA*	This work
JC20	*MatB filA*	This work
JC20-6	*MatB leu filA*	This work
JC90	*MatB trp filB*	This work
JC97	*MatB lys filC*	This work
JC80-1	*MatB leu2-35 arg filA*	This work
JC90-14	*MatA leu2-35 filB*	This work
JC97-1	*MatB leu2-35 lys filC*	This work
JC2113-1	*MatA leu2-35 lys5-12 ura2-21 Δhoy1::LEU2*	This work
JC33-1	*MatB leu2-35 lys2-5 xpr2 ade1 Δhoy1::LEU2*	This work
ADO16	*MatA leu2-270 ura3-302 Δhoy1::LEU2*	This work

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yeast extract, 1% glucose) or in appropriately supplemented minimal medium (MM; 0.67% yeast nitrogen base, 1% glucose). The induction of the yeast-hypha transition was carried out as described previously (54). Cells were grown on MM buffered with citric acid-sodium citrate (50 mM, pH 6.0) with glucose as a carbon source until they reached the exponential phase. They were then centrifuged, washed, and resuspended in the same medium (control cultures, growth in the yeast form) or in the same medium with *N*-acetylglucosamine at 1% as a carbon source (induction of the yeast-hypha transition). The media and procedures used for mating, sporulation, and transformation of *Y. lipolytica* have already been described (4, 67).

S. cerevisiae media and genetic manipulations were prepared and performed by standard methods described elsewhere (63).

Mutagenesis was carried out with ethyl methanesulfonate, as reported previously (38).

The *Escherichia coli* strains used for transformation and amplification of recombinant DNA were DH5α [*supE44 ΔlacU169*(ϕ80 *lacZΔM15*)*hsdR17 recA1 endA1 gyrA96 thi-1 relA1*] (25); MC1061 [*hsdR mcrB araD139* Δ(*araABC-leu*) 7679 Δ*lacX74 galU galK rpsL thi*] (45), and MV1190 {Δ(*lac-proAB*) *thi supE* Δ(*srl-recA*)*306*::Tn*10*(*tet*^{r}) [*F'*::*traD36 proAB lacI*^{q}*Z ΔM15*]; Bio-Rad}. *E. coli* strains were grown on LB broth.

Complementation test. After transformation of *Y. lipolytica*, cells were plated onto selective MM containing no leucine. The Leu$^+$ MM transformants obtained were replica plated onto YED to screen for Fil$^+$ transformants.

Plasmid. The *HOY1* gene was isolated as a 5.2-kb insert from a DNA library constructed from *Y. lipolytica* W29 partially digested with *Sau*3A and cloned in the *Bam*HI site of the pINA62 vector (Fig. 1) (67). Subclones were constructed by internal deletions within the 5.2-kb insert present in pJCT3 (Fig. 1).

DNA manipulations. Restriction enzyme digestion and DNA ligations were performed as described in the recommendations of the manufacturer. Isolation of plasmid DNA from *E. coli* and nick translation were performed by standard procedures (55). DNA fragments for use as probes were labelled by random priming with digoxigenin-dUTP. Southern blotting, hybridization, and immunological detections were carried out as described in the instructions of the manufacturer (Boehringer).

Sequence analysis of the *HOY1* locus. A cloned DNA restriction fragment harboring the *HOY1* gene was isolated from plasmid pJCT3 as a 2.3-kb *Bam*HI-*Bam*HI fragment. This fragment was subcloned into the *Bam*HI sites of pBluescript plasmids (SK$^+$ and KS$^+$; Stratagene), and a nested set of closely spaced deletions was created with exonuclease III. Templates were sequenced on both strands with Sequenase enzyme (Boehringer) in dideoxynucleotide chain termination sequencing reactions (58). The products of the sequencing reactions were resolved on buffer gradient polyacrylamide-urea sequencing gels (6) and exposed to Kodak XAR-5 X-ray film.

RNA preparations and Northern analysis. RNA was prepared from exponentially growing cultures on YED by the method of Percival-Smith and Segall (51). Poly(A)$^+$ RNA was isolated by affinity chromatography on oligo(dT)-cellulose. Prehybridization and hybridization were performed as described by Sambrook et al. (55). In all Northern analysis experiments, the RNA concentration was normalized by hybridization with the *LEU2* gene from *Y. lipolytica.*

DNA and protein sequences were analyzed with the DNASIS, PROSIS (Pharmacia-LKB; Hitachi), and PSORT (version 6.3; WWW) programs. The amino acid sequence of *HOY1* was compared with the SWISS-PROT data bank by use of the FASTA program (50). Alignments of protein sequences were done with CLUSTAL programs (27).

Nucleotide sequence accession number. The sequence data reported here have been assigned EMBL accession number Z34956.

RESULTS

Isolation and characterization of morphological mutants. Normal Fil$^+$ strains of *Y. lipolytica* (Fig. 2A) form rough-surfaced colonies on YED similar to those described for *C. albicans* for the irregular wrinkle type (64). Cells of *Y. lipolytica* SA-1 in the exponential phase (growing on YED) were mutagenized with ethyl methanesulfonate at a survival rate of 9% and plated onto YED agar. After 5 days of incubation at 28°C, 8,000 colonies were analyzed by visual screening, and 25 putative smooth nonfilamentous colonies were detected. However, only seven showed a clear phenotype that was maintained through the multiple propagations (Fig. 2B). The smooth colonies exhibited an unmottled, or unwrinkled, surface, with no aerial mycelia. Cells from smooth colonies were observed only in the budding form of growth. The frequency of mutants was 8.7×10^{-4}, similar to those described for *Y. lipolytica* by other authors (18, 48).

The effects of the culture medium (YED or MM) and carbon sources (Glc or GlcNAc) on the growth and induction of the yeast-mycelium transition were analyzed in all seven mutants selected (Fig. 2B and D). One of them, JC3, showed a strong tendency to revert to the mycelial form. JC105 and JC109 displayed a lower growth rate than that of the wild type. Accordingly, these three mutants were discarded for further study. The other four mutants had similar growth rates to the wild type and were unable to form mycelia under any of the conditions tested. JC90 was auxotrophic for tryptophan and JC97 was auxotrophic for lysine. The other two mutants were prototrophic and were subjected to another round of mutagenesis. Two auxotrophic mutants, JC20-6 and JC8-1, were selected.

Segregation of Fil$^-$ mutations. The Fil$^-$ strains JC 20-6, JC 8-1, JC90, and JC97 were crossed with *Y. lipolytica* 21501-13 and 21401-1. The mutants formed stable diploids with both strains. All the diploids grew in the mycelial form, indicating that the Fil$^-$ mutations were recessive.

Diploids were sporulated on CSM medium (4). Since tetrad analysis is quite difficult for *Y. lipolytica* (67), segregation of the Fil$^-$ phenotype was analyzed in random spores. The results shown in Table 2 indicate a 2:2 pattern of segregation.

Complementation analysis. The four Fil$^-$ mutants were analyzed by genetic complementation. Table 3 shows the results of this analysis. The results point to the existence of three complementation groups.

The *Y. lipolytica* gene bank constructed in plasmid pINA62 contains the *LEU2* gene of this yeast as a selectable marker. The unique sites *Apa*I and *Not*I were localized outside the *LEU2* gene (Fig. 1). Since the *Not*I sites are scattered at low frequencies in the yeast genome, for increased efficiency (67), we decided to linearize the gene bank by *Not*I digestion prior to the transformation experiments.

We transformed all the Fil$^-$ mutants with the gene bank, selected for Leu$^+$ colonies, and, after replica plating the colonies on YED, searched for rough colonies. A total of 18,279 Leu$^+$ colonies of JC80-1 (*filA*), 4,361 of JC90-14 (*filB*), and 1,930 of JC97-1 (*filC*) were analyzed, and only one stable trans-

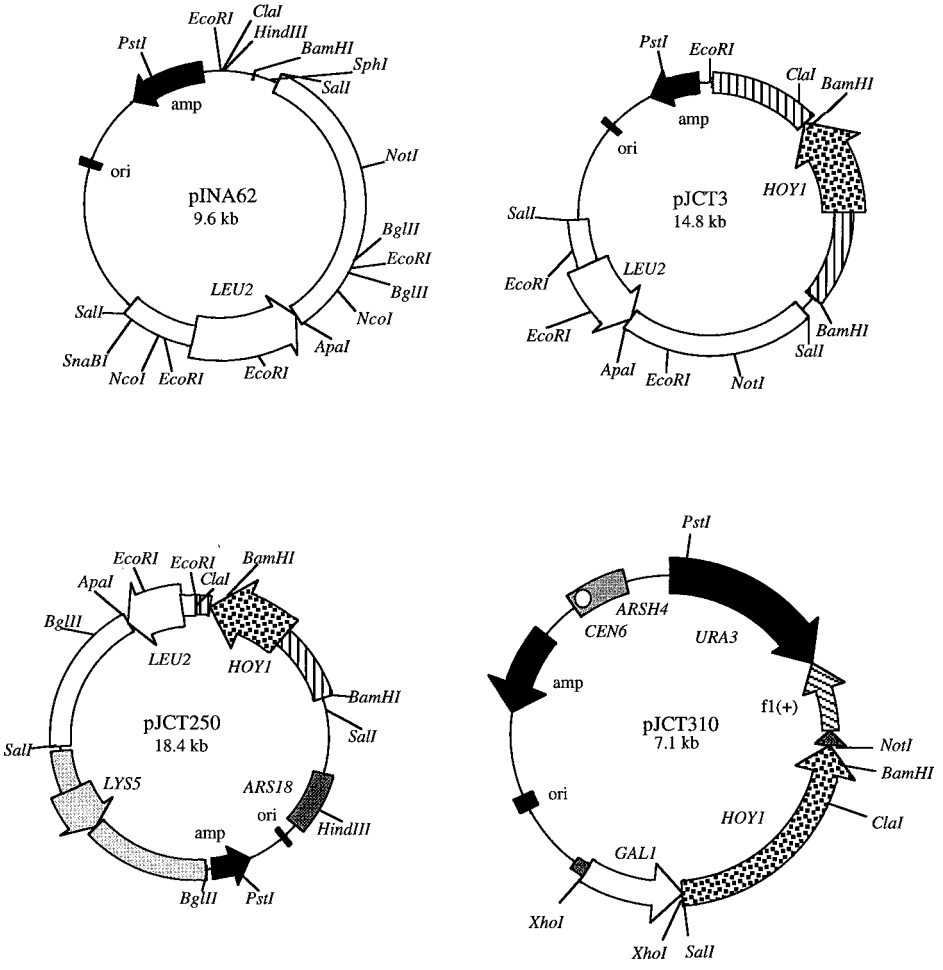


FIG. 1. Restriction maps of the plasmids used in this work. pINA62 contains a 5.2-kb *Sal*I fragment carrying the *LEU2* of *Y. lipolytica* in pBR322. The gene library was constructed at the *Bam*HI site of this plasmid (66). pJCT3 is a plasmid rescued with the whole insert from the JC110-1 transformant. pJCT250 is an autonomous plasmid used for the complementation of the Δ*hoy1*::*LEU2* strains and for overexpression of *HOY1*. pJCT310 is a plasmid constructed for the expression of *HOY1* in *S. cerevisiae* under the *GAL1* promoter.

formant, called JC110-1 (obtained from JC80-1), with a rough phenotype similar on YED to that of the wild type was found.

Isolation of the *HOY1* gene. To test whether homologous integration occurs at the Leu^+ locus, as has been described previously (12, 67), and to obtain a physical map of the inserted DNA, we digested the DNAs of JC80-1 and JC110-1. After electrophoresis and Southern transfer, these DNAs were hybridized with a *LEU2* probe. This probe hybridizes in the wild type after transformation with pINA62 with a *Bam*HI-*Bam*HI fragment of 11 kb or with a *Sph*I-*Sph*I fragment of 8 kb (see reference 67). The results indicate that the unique *Bam*HI or *Sph*I *LEU2*-specific chromosomal bands disappeared in the *HOY1* transformant, and, hence, that integration of the transforming plasmid at the *LEU2* locus had occurred (data not shown). The size of the plasmid was estimated to be about 15 kb. We rescued the *HOY1* sequence from the DNA integrated at the *LEU2* locus by a complete *Not*I cut of the total DNA of this transformant, followed by dilution, ligation, and transformation of *E. coli* DH5α and selection for ampicillin resistance. A resistant clone called pJCT3, which has only one *Not*I site over its entire 14.8-kb sequence, was isolated. The restriction map of the pJCT3 was established (Fig. 1). Since the restriction data obtained were in accordance with the molecular weights observed on the Southern blots (data not shown), we concluded that this plasmid had not undergone sequence rearrangements upon being subcloned into *E. coli.*

Nucleotide sequence of the *HOY1* gene and analysis of its flanking sequences. The segment of pJCT3 that contains *HOY1* was defined by subcloning fragments of the yeast segment into pINA62, transforming these constructs into JC80-1, and assaying their ability to undergo the yeast-hypha transition. The smallest segment that produced the morphological transition (a *Bam*HI-*Bam*HI 3.2-kb fragment) was sequenced after subcloning in pBluescript KS^+ plasmid. An open reading frame was found, but no termination codon was detected and the sequence was extended. Finally, from 3,657 nucleotides sequenced, an open reading frame of 1,527 bp (Fig. 3), lacking introns and coding for a protein of 509 amino acids, was detected.

The first ATG codon of the *HOY1* open reading frame has the conserved adenine residue at position −3 that is present in most of the *Y. lipolytica* genes (13a) and in 75% of *S. cerevisiae* initiator ATG codons (14). The region flanking the *HOY1* gene shows a possible TATA box at position −227 (Fig. 3, upstream from the ATG codon). TATA boxes have been found in yeasts at positions ranging from −30 to −300 upstream from the translation start codon ATG (8, 34). The existence of a CAA (T/G) box is believed to be important for transcription. It is

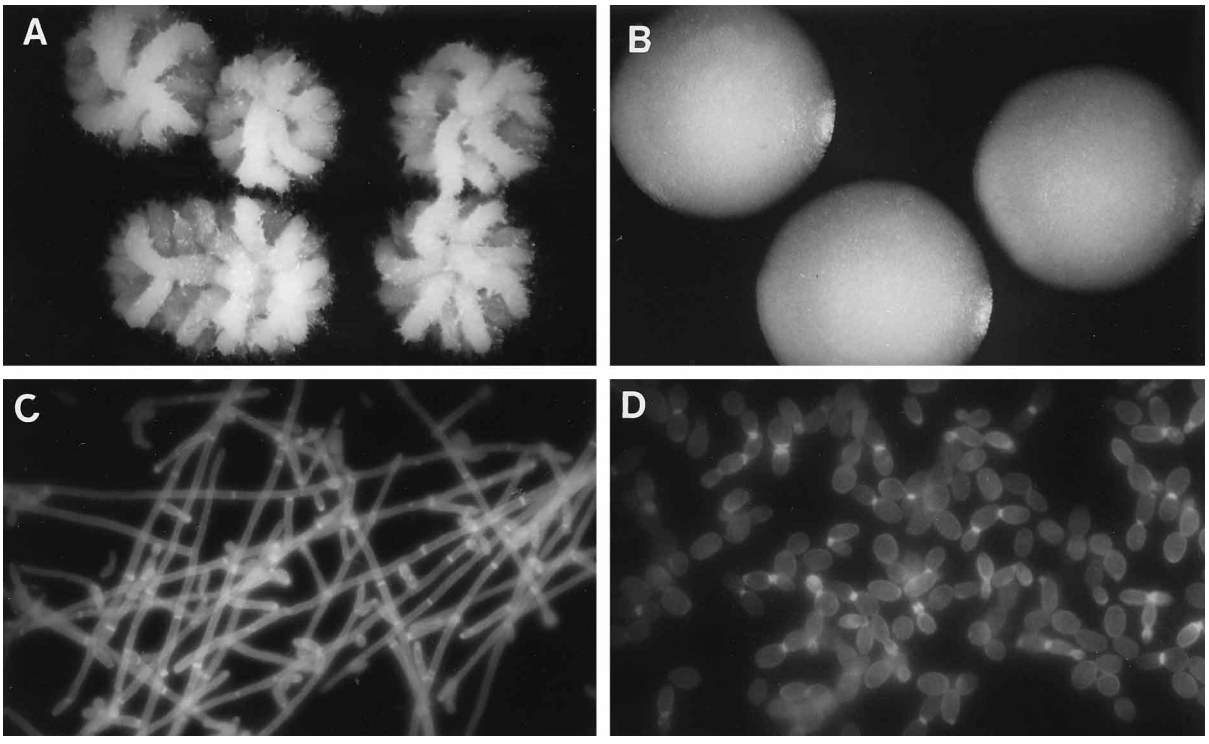


FIG. 2. (A and B) Morphology of the parental *Y. lipolytica* Fil$^+$ strain, SA-1 (A), and the Fil$^-$ mutant JC80-1 (B). SA-1 and its derivative JC80-1 were streaked for single cells on YED and grown at 28°C for 5 days. Representative colonies were photographed. A similar aspect of the JC80-1 phenotype (filA) was obtained in the Fil$^-$ complementation groups JC90-14 (filB) and JC97-1 (filC). (C and D) Cell morphology after 14 h of growth in liquid minimal medium (MM with GlcNAc) for the induction of the yeast-hypha transition for strain SA-1 (C) and strain JC80-1 (D). The same aspect (growth in the yeast form) was observed for all the Fil$^-$ strains. Also, the yeast morphology (D) was obtained with all the strains grown on liquid minimal medium with glucose as a carbon source.

normally located upstream from the TATA box. One sequence confirming this consensus was found upstream from the ORF at position −283 (Fig. 3).

Four putative targets for the general control of amino acid biosynthesis (29) were found in the 5′ sequence at positions −686, −447, −189, and −157 (Fig. 3). Two of them (−686 and −157) have the canonical sequence TGACTC, and the other two contain the highly conserved pentanucleotide sequence TGACT. Similar sequences were found upstream from the *LYS5* (66) and *LYS1* genes of *Y. lipolytica* (52), and previous reports have shown that both *LYS1* and *LYS5* are under general amino acid control (19). The meaning of these sequences on the *HOY1* promoter is unknown.

Also, four copies of the pentanucleotide CCCCT (named thermal stress responsive element C_4T [35]) were found in the *HOY1* promoter at positions −486(−), −98(−), −87(+), and −16(+). In the 3′ region, typical terminator consensus sequences, i.e., TAA, TTT, and TAGT (68), were found, in common with other *Y. lipolytica* genes (57, 66).

TABLE 2. Meiotic segregation by random spores of Fil$^-$ mutants in crosses with the wild type

Cross	Total no. of colonies	% Rough colonies (Fil$^+$)	% Smooth colonies (Fil$^-$)
JC20-6 × 21501-13	1,176	48.9	51.1
JC8-1 × 21501-13	1,368	49.4	50.2
JC90 × 21501-13	1,246	45.7	54.3
JC97 × 21501-13	933	50.6	49.4

Hoy1p is related to homeobox genes. The deduced protein contains 509 amino acids corresponding to a molecular size of 55,920 Da, with an isoelectric point of 6.14. According to Bennetzen and Hall (5), the codon bias value is 0.42, which corresponds to an intermediate protein abundance. Hydropathy analysis of the product inferred from the *HOY1* nucleotide sequence, based on the calculations of Kyte and Doolittle (37), reveals a slightly hydrophilic polypeptide (data not shown).

Further examination of the polypeptide sequence was carried out with the PSORT program (see Materials and Methods) to determine the possible location of Hoy1p in the cell. The results obtained, in agreement with the proposal of Rob-

TABLE 3. Complementation analysis of four Fil$^-$ mutants of *Y. lipolytica*[a]

Strain	Phenotype when mated pairwise with:			
	JC26-6	JC80-1	JC90-14	JC97-1
JC20-6	S	S	R	R
JC80-1	S	S	R	R
JC90-14	R	R	S	R
JC97-1	R	R	R	S
AD16	S	S	R	R
JC2113-1	S	S	R	R

[a] Pairwise matings were done between all *MatA* and *MatB* haploid strains derived from random spore analysis (Table 2). The resulting diploid strains were scored for complementation or noncomplementation of hyphal formation. The mutations present in strains in noncomplementation pairs were placed in the same complementation group. S and R, smooth and rough phenotypes, respectively.

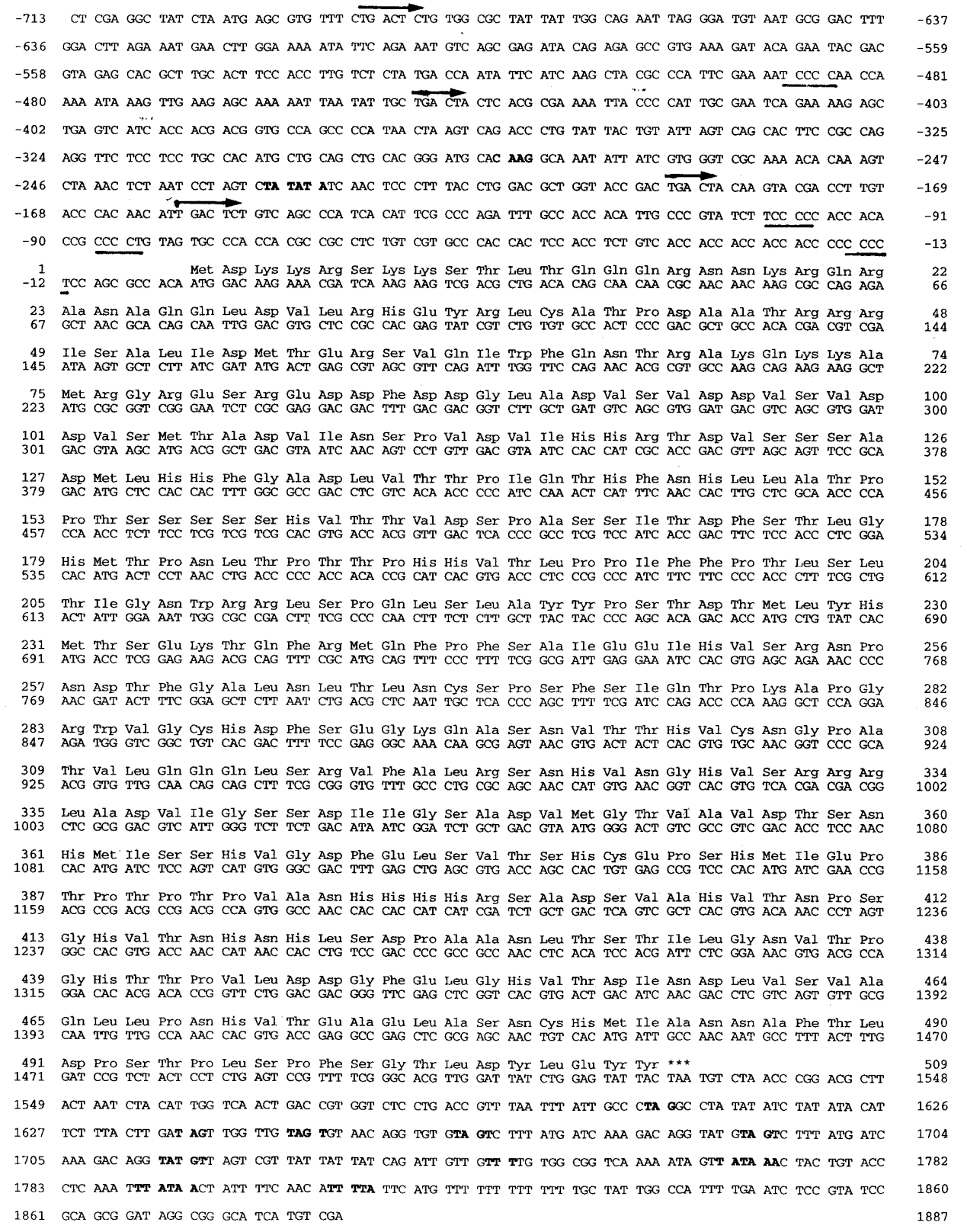


FIG. 3. Nucleotide sequence of the yeast *Y. lipolytica HOY1* gene region isolated from wild-type strain W29(pJCT3). The sequence of one DNA strand and the deduced amino acid sequence for the protein are shown. Nucleotides are numbered from the 5′ end of the sequenced fragment to the 3′ end. Amino acids are numbered from the first putative ATG in the large open reading frame at the left of the figure. A possible TATA box is indicated by bold type upstream from the ATG codon at position −227. A sequence confirming the existence of a CAA(T/G) box is indicated in bold type upstream of the open reading frame at position −283. Four putative targets for the general control of amino acid biosynthesis were found in the 5′ sequence and are indicated by arrows. Underlining indicates four copies of the pentanucleotide CCCT found in the *HOY1* promoter.

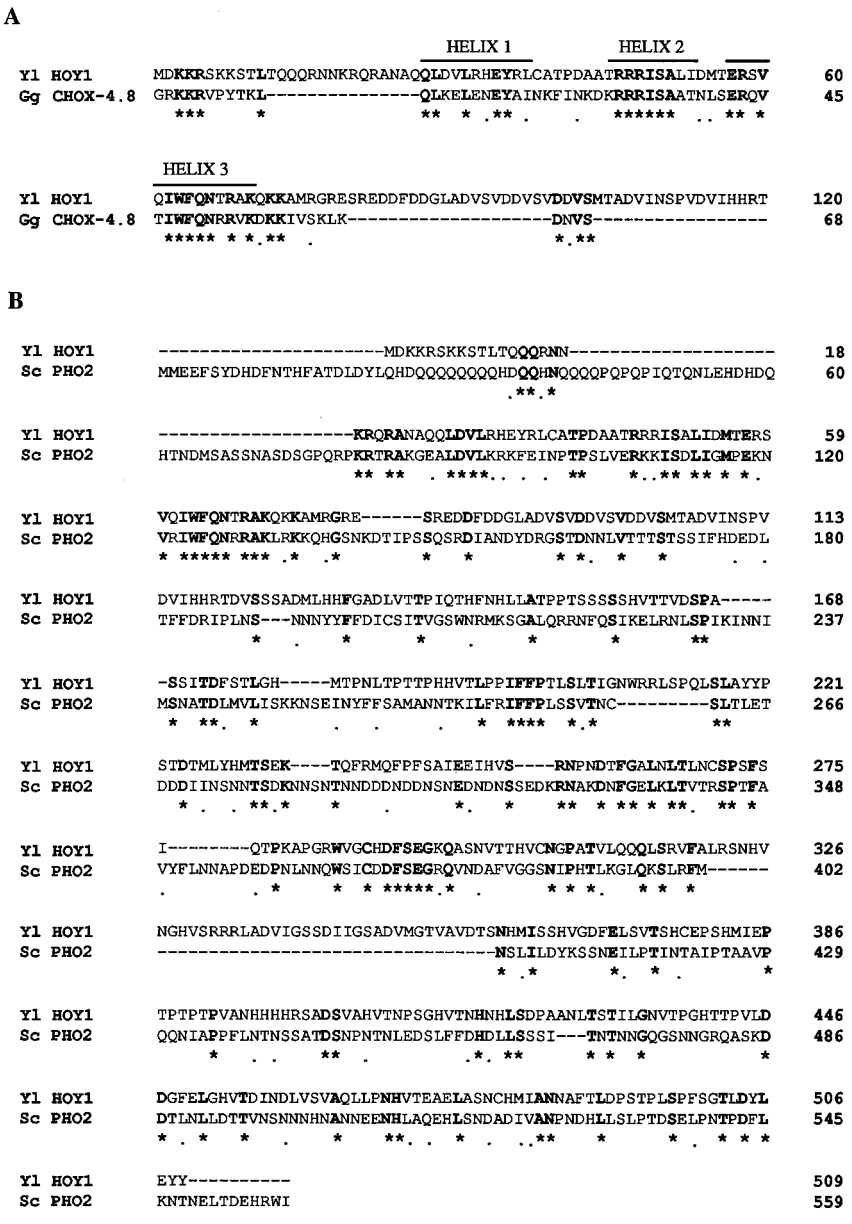


FIG. 4. Sequence comparison of the homeodomain of *Y. lipolytica Hoy1* (Yl HOY1) with the homeodomain of the chicken Hox4.8p (Gg CHOX-4.8) (A) and with the Pho2p transcriptional activator of *S. cerevisiae* (Sc PHO2) (B). Identical residues (stars) and conservative amino acid substitutions (dots) are indicated; dashes represent gaps introduced to optimize alignments.

bins et al. for *Xenopus* nucleoplasmin (53), indicate the presence of two very clear nuclear targeting signals at positions 3 ($KRX_{10}RNNKR_9$) and 6 ($KKX_{10}KRQRA$) and point to the location of the enzyme in the nucleus.

Protein sequence analysis showed that Hoy1p has a homeobox at its N terminus. A potential DNA binding site is conferred by the 60-amino-acid homeodomain, which consists of a flexible stretch of nine residues, referred to as the N-terminal arm, followed by three α-helices (15). All three appear in the *HOY1*-predicted protein (Fig. 4A) corresponding to the most conserved region of the C-terminal helix 3, as occurs with all the homeoboxes described to date (15). The best overall homology between the Hoy1p homeodomain and those described in the data bank is with the chicken *Hox4.8* homeoregion (31), where 25 of 60 amino acids are identical. In addition, the same region of Hoy1p is similar to several genes containing homeodomains (*Antp*, *en*, *ftz* [15], *bE2* [22], and *MAT* α*2* [1], etc.) (data not shown). At the total amino acid sequence level, Hoy1p is most closely related to the *S. cerevisiae* transcriptional activator Pho2p (Fig. 4B). *PHO2* is a positive regulator of the repressible acid phosphatase encoded by *PHO5* (61), which also contains a homeodomain (9). However, similar to those of the chicken and the mouse, the *HOY1* homeodomain is located nearer to the N-terminal position (positions 27 to 74, of a total of over 501 amino acids) than the *PHO2* homeodomain (positions 88 to 134, of a total of 559 amino acids). *PHO2* also contains a stretch of glutamine residues (14 of 18 amino acids) upstream from the homeobox, designated the M or *opa* repeat, that is found in products that contain several developmental control genes such as *Notch*, *en*, *Antp*, *bcd*, and *Dfd* (15). Only

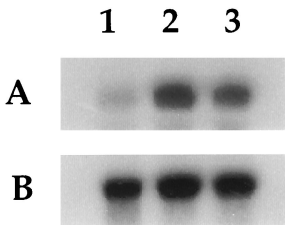


FIG. 5. Northern blot analysis of *Y. lipolytica HOY1* mRNA levels as a function of the yeast-hypha induction time. Total RNA was isolated from cells grown under induction conditions (cells in the exponential phase growing on glucose, in the yeast form, were collected, washed, and resuspended in the MM with GlcNAc as a carbon source). Samples of 15 μg of RNA taken after 0, 1, and 7 h (lanes 1, 2, and 3, respectively) after the yeast-hypha transition were hybridized with a *HOY1* fragment as a probe (A) or with a *LEU2* gene fragment (B).

three glutamine residues were detected in Hoy1p. Whether such differences are meaningful remains to be elucidated.

Analysis of the hybridization patterns of restriction fragments from different *Y. lipolytica* strains. DNA from four different strains of *Y. lipolytica* (SA-1, W29, 21501-13, and INAG33122) was digested with *Bam*HI, *Sal*I, or *Sal*I-*Sph*I, and the fragments obtained were hybridized with a radiolabeled *Sal*I-*Cla*I fragment of 0.7 kb. The results indicate the existence of a single band and demonstrate that the restriction patterns of all four strains were identical (data not shown and reference 65).

Transcription of the *HOY1* gene and expression during the dimorphic transition. *Y. lipolytica* SA-1 was grown in MM until the exponential phase was reached with glucose as a carbon source. The cells were then transferred to fresh medium under the same conditions (control) or to GlcNAc-containing medium (for inducing the yeast-hypha transition). At the times indicated, aliquots were recovered and growth and cell morphology were recorded. In the medium containing GlcNAc, the yeast-to-hypha transition was initially observed after 7 h, and germination tubes reached a significant length after 10 h (54). By contrast, cells kept under control conditions remained yeast-like. RNA was extracted at the same incubation times and then assayed by Northern analysis. Northern blotting with the 0.7-kb *Bcl*I-*Sna*BI fragment as a probe revealed a single band of about 1.6 kb. This indicates that the *Bcl*I-*Sna*BI fragment does not include another transcriptional unit. RNA was also obtained from a deleted strain (see below), and the RNA band disappeared, indicating correct gene disruption. Figure 5A also shows that an increase of between two and three times in the amount of *HOY1* transcript occurs during the dimorphic transition. mRNA levels remained constant during yeast-like growth. To control the integrity and the amount of RNA isolated under the different conditions, the same blot was hybridized to a fragment containing the *Y. lipolytica LEU2* gene (Fig. 5B).

Integrative disruption of the *HOY1* gene elicits the yeast morphology. To check the phenotype of a *HOY1* null mutant in *Y. lipolytica*, gene disruption was performed. The *LEU2* gene of *Y. lipolytica* was isolated as a *Sal*I-*Sal*I fragment from pINA62 (Fig. 1) and was inserted into the *HOY1* gene between the *Sal*I-*Sal*I sites (see Fig. 6A). This replaced 1,043 bp of the *HOY1* gene open reading frame with a 5.2-kb fragment containing the entire *LEU2* gene. The construct was digested with *Bam*HI-*Kpn*I to release the entire *LEU2* gene (Fig. 6A). This linear molecule was used to transform *Y. lipolytica* 21501-13 and INAG33122 to leucine prototrophy. Of 9 and 68 Leu^+ transformants obtained from strains 21501-13 and INAG33122, respectively, 3 and 3 exhibit a clear smooth phenotype in all the media tested (MM or YEPD with Glc or GlcNac as a carbon source in both solid and liquid media). By Southern blotting followed by hybridization, we checked that the Leu^+ transformants indeed resulted from replacement of the chromosomal *HOY1* gene by the disrupted *LEU2* construct (Fig. 6B and C). Transformation of the disrupted strain with a *LYS5* autonomously replicating plasmid carrying *HOY1* (plasmid pJCT250) (Fig. 1) restored the rough phenotype. To further confirm these results, plasmid segregation experiments were carried out as described previously (56). In all cases (50 transformants selected randomly), loss of the Lys^+ character was accompanied by loss of the rough phenotype. The deleted strains JC2113-1 and ADO16 were crossed with *filA* strains (Table 1). Diploids were unable to form hyphae in normal inducing culture conditions (Table 3). Sporulation of the diploids shows a 4:0 segregation (smooth-rough colonies; only 2 of the 613 colonies analyzed were rough). Both results indicate that the *HOY1* gene corresponds to the original *filA* mutation.

***HOY1* enhanced hyphal growth.** To examine the effect of *HOY1* on filamentous growth, we transformed two wild-type and two mutant (*filB* and *filC*) *Y. lipolytica* strains (21501-13, P01a, JC90-14, and JC97-1, respectively) (Table 1) with the autonomous replicative plasmid pJCT250 (Fig. 1). We selected six Leu^+ transformants from each strain and examined growth in liquid and solid media (in MM, YED with glucose, or both containing GlcNAc as a carbon source; for induction of the yeast-to-hypha transition). In all the media assayed, *filB* and *filC* mutants transformed with pJCT250 gave rise to pseudohyphae which resembled those formed in *C. albicans* (Fig. 7A and B).

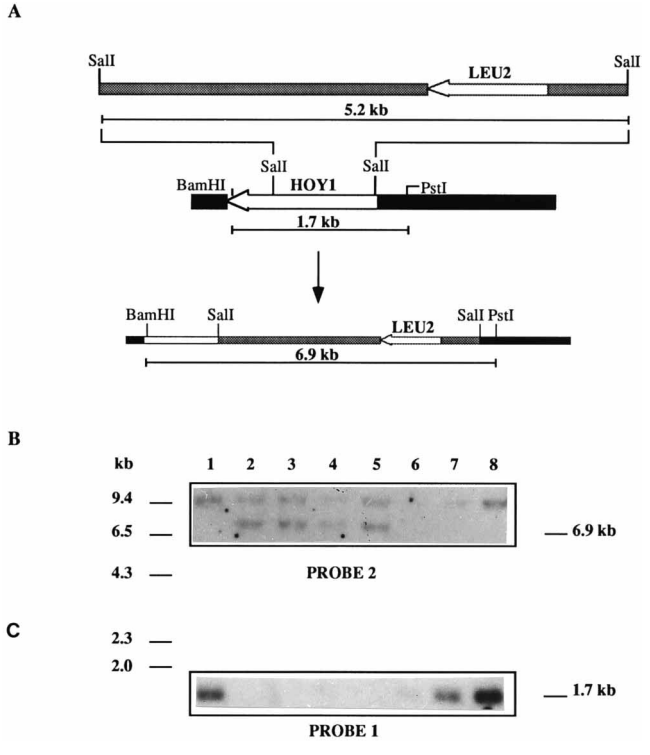
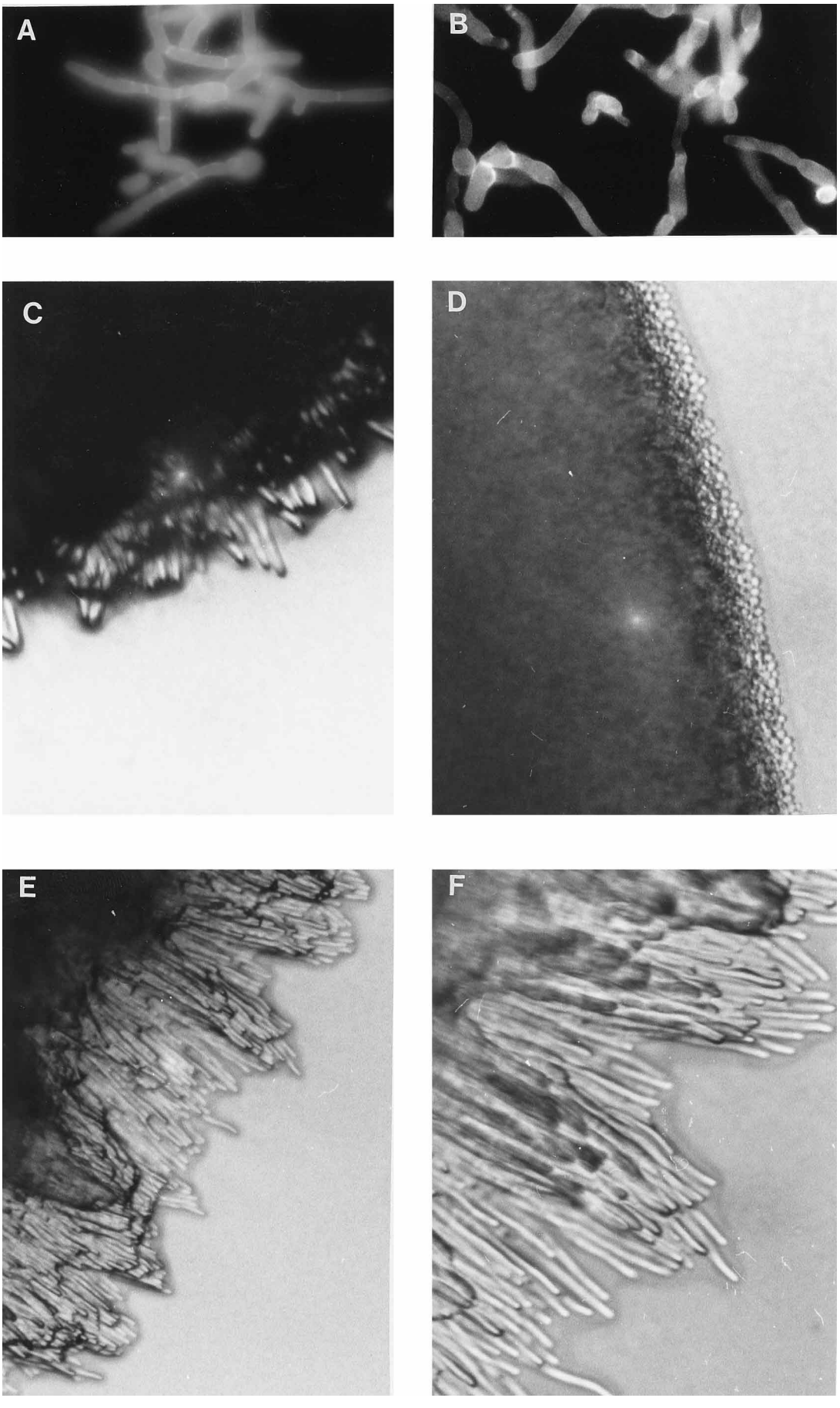


FIG. 6. Deletion substitution in the *HOY1* region. (A) Restriction maps of the wild-type *HOY1* locus and the locus at which 65% of the *HOY1* gene has been replaced by the *Sal*I-*Sal*I 5.2-kb fragment containing the *LEU2* gene; (B) Southern blot of total DNA from the deleted *HOY1* strain. Lanes: 1, wild-type DNA, strain SA-1; 2 to 5, disrupted strains 21501-13, 21401-1, INAG33122, and JC80-1, respectively, digested with *Bam*HI-*Pst*I; 6, strain W29; 7, strain 21501-13; 8, strain 21401-1. The probes were the 1-kb *Sal*I-*Sal*I fragment of the *HOY1* gene (probe 1) and the 5-2.kb *Sal*I-*Sal*I fragment of the *LEU2* gene (probe 2).


A
B
C
D
E
F

Pseudohyphal growth occurred in a more uniform way and was faster in all the media containing GlcNAc. Plasmid segregation experiments indicated that loss of the Leu^+ character was accompanied by loss of the capacity for pseudohyphal formation.

Overexpression of *HOY1* in the wild-type strains enhanced hyphal formation in all the media assayed. Again, hyphal formation was quicker and more vigorous in media with GlcNAc as a carbon source. In liquid medium, the differences were quantified with difficulty. Hyphal formation appeared retarded and hyphae appeared to be smaller in the transformant strain under noninducing conditions. The increase in hyphal formation appeared clearly in solid medium both in YED and in MM with GlcNAc as a carbon source. Figure 7 shows the behavior of the wild-type strain 21501-13, the transformed strains 21501-13 and JCT250, and the disrupted strain JC2113-1 (Δ*hoy1*::*LEU2*). The *HOY1* overexpression strains formed larger, well-defined hyphae (Fig. 7E and F), whereas the wild-type strain did not (Fig. 7C) and the deleted strain did not produce hyphae at all (Fig. 7D).

Expression of *Y. lipolytica HOY1* in *S. cerevisiae*. When starved for nitrogen, diploid strains of *S. cerevisiae* switch from growth of the yeast form to growth of pseudohyphae (24). To check whether *HOY1* is able to trigger the morphogenetic switch, a *Bam*HI-*Bam*HI fragment of 3.2 kb was inserted in the *Bam*HI site of plasmids YEp352 (32) and YCp50 (28) (data not shown and reference 65).

We transformed *S. cerevisiae* CG25, CG41, CGX19, and CG67 with the four plasmids, and the Ura^+ transformants were induced to form pseudohyphae (24). Only strains CGX19 and CG67 were able to form pseudophyphae, in agreement with results reported previously (24). No differences were found in the behaviors of the *S. cerevisiae* transformants carrying the plasmids containing the *HOY1* gene. It is possible that the *Y. lipolytica HOY1* gene might not be expressed in *S. cerevisiae* under the control of its own promoter. To circumvent this problem, we cloned the *HOY1* gene under the control of the *S. cerevisiae GAL1* promoter.

By designing specific oligonucleotides and using PCR amplification (65), we cloned a 1.5-kb band containing the entire *HOY1* gene that was inserted in the *Xho*I-*Not*I sites of pRS316-*GAL1* (41), giving rise to pJC310 (Fig. 1). After transformation of the previously described *S. cerevisiae* strains, we selected the Ura^+ transformants and grew all of them in either liquid or solid medium with glucose and galactose as carbon sources. No pseudohyphal formation was found in any of the cases assayed.

DISCUSSION

FIL screen as a method to identify developmental pathways. The FIL (filamentation minus) screen described in this work is a convenient method for identifying any gene involved in the yeast-hypha transition in *Y. lipolytica* because chemical and UV mutagenesis are standard procedures in many laboratories. Also, the FIL screen permits easy screening of a large number of transformants.

Y. lipolytica offers two main advantages compared to *S. cerevisiae* for analyzing the yeast-hypha transition. The first is that *Y. lipolytica* forms true septate hypha. The second is that *Y. lipolytica* is genetically closer to fungi than *S. cerevisiae*, as can be deduced by its rRNA (2) and by its codon bias (13a).

Moreover, some of the genes controlling development in *Y. lipolytica* are likely to be conserved in other fungi, plants, and animals, and the FIL screen could be used to identify these. Also, the same types of screening described for the morphological analysis of *S. cerevisiae* pseudohyphal formation (23) or transposon mutagenesis (47) can be carried out with *Y. lipolytica.*

***HOY1* is related to transcriptional factors.** With the FIL screen, we have been able to isolate several mutants of *Y. lipolytica* which grow perfectly only in the yeast form in all the media assayed. We have characterized at least three complementation groups (several more were obtained in a second screening and are currently being analyzed [52a]). By complementation of one of them, we isolated a gene, *HOY1*, which differs in several characteristics from the genes related to the yeast-hypha transition previously isolated in other yeast species (i.e., *S. cerevisiae* and *C. albicans*). From the structural point of view, although *HOY1* contains a homeobox in its sequence, it does not present relevant homology either with the fungal genes involved in mating type functions (which also contain homeobox regions), with the genes involved in signal transduction (26), or with the genes controlling development in *Aspergillus* (44). A higher degree of homology was detected between *HOY1* and the *S. cerevisiae* regulatory gene *PHO2* (9, 61). One effect of *HOY1* in phosphate metabolism cannot be ruled out. Thus, the filamentation defect of *hoy1* mutants would be a secondary metabolism defect. However, since no direct function in the transmission of P_i signals has been described for *PHO2* (49), we are not in favor of such a hypothesis. In *S. cerevisiae*, Pho2p, also known as Bas2p or Grf10p, activates the transcription of numerous other genes, including *HIS4*, *TRP4*, *CYC1*, and genes for adenine biosynthesis. We are currently carrying out experiments to elucidate the effect of Pho2p on pseudohyphal formation in *S. cerevisiae.*

Along its whole sequence, *HOY1* has characteristics similar to other *Y. lipolytica* genes, and only in its promoter are two aspects remarkable. One of them is the existence of several signals for general control of amino acid biosynthesis. Until now, in *Y. lipolytica*, we have been unable to establish a clear relationship between nitrogen starvation and filamentous growth, as has been described for *S. cerevisiae* (24). However, we cannot exclude this possibility, and, hence, the possible involvement of the TCACTC sequences in gene function is currently being investigated by directed mutagenesis.

The second interesting point is the existence of the pentanucleotide CCCCT, an essential component in stress response. It has been reported that any unfavorable circumstance that adversely affects growth can be understood as stress. Thus, the possibility exists that, at least partially, the yeast-hypha transition could be induced or regulated by a stress response. Experiments to test this hypothesis are also currently under way.

The results of Southern hybridization of genomic DNA digested with three restriction enzymes in four different strains of *Y. lipolytica* point to the existence of a single *HOY1* gene, whose context appears to be relatively constant in the species.

Northern blotting revealed a single transcript hybridization, and an increase in the amount of mRNA between two- and

FIG. 7. Cell morphology after 14 h of growth in liquid minimal medium (MM with GlcNAc) for the induction of the yeast-hypha transition for *Y. lipolytica* JC90-14 (*filB*) (A) and JC97-1 (*filC*) transformed with pJCT250 (B) wild-type strain 21501-13 (C), JC2113-1 (Δ*hoy1*) (D), and 21501-13 transformed with plasmid pJCT1250 (E and F). Cells were placed in solid MM with GlcNac as a carbon source for 26 h at 28°C and then photographed at ×10 magnification (except for panel G, at ×20 magnification) with phase optics.

threefold was observed during the yeast-hypha transition. Since deletion of *HOY1* is not lethal, our interpretation of the data is that in *Y. lipolytica*, hyphal formation is sensitive to the dosage of the *HOY1* gene.

The Δ*hoy1* strains displayed suppressed hyphal formation in comparison to *HOY1* strains, both in solid medium (YED) and in medium designed to induce hyphal formation (liquid MM with GlcNAc as a carbon source). The morphology of the colonies and of the cells in liquid medium (with either glucose or GlcNAc as a carbon source) of all the disrupted strains was similar to that obtained with the mutant strain JC80-1 (Fig. 1B and D). The flaw in hyphal formation is unlikely to result from a growth defect because all the strains grew with approximately equivalent doubling times. Several experiments indicated that defective hyphal formation was a direct consequence of the loss of *HOY1* function. (i) Four independent strains showed the same defect in hyphal formation. (ii) Reintroduction of a wild-type *HOY1* gene by integrative (with pJCT3 [Fig. 1]) or autonomous (with pJCT250 [Fig. 1]) transformation restored the ability of Δ*hoy1* strains to form hyphae. Because Δ*hoy1* strains were defective in hyphal formation in all media tested, our results indicate that *HOY1* is an essential gene in the morphological transition. We transformed wild-type strains and the *filB* and *filC Y. lipolytica* mutants with the only replicative vector (containing the *HOY1* gene) functional in this yeast and present at 1 to 3 copies per cell (3). The resulting phenotypes of this moderate *HOY1* overexpression enhanced pseudohyphal growth in the Fil$^-$ mutants and hyphal formation in the wild-type in all the media tested. Pseudohyphal formation is unusual in *Y. lipolytica*, and more experiments are needed to further clarify this behavior. The induction of hyphal formation in the wild-type strains is consistent with the hypothesis that *HOY1* is a transcription factor, and the phenotypes may be explained by assuming that *HOY1* would activate genes responsible for the hyphal growth program.

Hox-4 homeobox genes are coordinately expressed during mouse limb and chicken wing development in striking temporal and spatial patterns. Furthermore, local application of retinoic acid, a putative endogenous morphogen, induces de novo transcription of *Hox-4* genes (13, 31). Our results suggest that a similar hitherto-unknown mechanism may be involved in the yeast-hypha transition in fungi, this being the first time that a gene similar to the homeobox genes in higher eukaryotes and involved in development has been described for fungi. Alternatively, the possibility exists that *HOY1* might interact with homologs to the *STE7* or *STE12* genes of *S. cerevisiae* in *Y. lipolytica* in such a way that the yeast-hypha transition would be triggered by some input signal(s), as it is in *S. cerevisiae* (26). Experiments to test both hypotheses are currently under way.

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REFERENCES

1. **Astell, C. R., L. Ahlstron-Jonasson, and M. Smith.** 1981. The sequence of the DNAs coding for the mating type of loci of *Saccharomyces cerevisiae*. Cell **27:**15–23.
2. **Brans, S. M., D. J. Lane, M. L. Sogin, C. Bibeau, and W. G. Weisburg.** 1991. Evolutionary relationships among pathogenic *Candida* species and relatives. J. Bacteriol. **173:**2250–2255.
3. **Barth, G., and C. Gaillardin.** 1996. *Yarrowia lipolytica*, p. 313–388. *In* K. Wolf (ed.), Non-conventional yeasts in biotechnology. Springer-Verlag, Berlin, Germany.
4. **Barth, G., and H. Weber.** 1986. Improvement of sporulation in the yeast *Yarrowia lipolytica*. Antonie Leeuwenhoek J. Microbiol. Serol. **51:**167–177.
5. **Bennetzen, J. L., and B. D. Hall.** 1982. Codon selection in yeast. J. Biol. Chem. **257:**3026–3031.
6. **Biggin, M. D., T. J. Gibson, and G. F. Hong.** 1983. Buffer gradient gels and ^{35}S label as an aid to rapid DNA sequence termination. Proc. Natl. Acad. Sci. USA **80:**3963–3965.
7. **Birse, C. E., M. Y. Irwin, W. A. Fonzi, and S. P. Shypherd.** 1993. Cloning and characterization of *ECE1*, a gene expressed in association with cell elongation of the dimorphic pathogen *Candida albicans*. Infect. Immun. **61:**3648–3655.
8. **Breathnach, R., and P. Chambon.** 1981. Organization and expression of eukaryotic split genes coding for proteins. Annu. Rev. Biochem. **50:**349–383.
9. **Bürglin, T. R.** 1988. The yeast regulatory gene *PHO2* encodes a homeo box. Cell **53:**339–340.
10. **Canno, R. D.** 1980. Isolation of a mycelial mutant of *Candida albicans*. J. Gen. Microbiol. **132:**2405–2407.
11. **Clark, K. L., P. J. F. Feldmann, D. Dingard, R. Larocque, A. J. P. Brown, M. G. Lee, D. Y. Thomas, and M. Whiteway.** 1995. Constitutive activation of the *Saccharomyces cerevisiae* mating response pathway by a MAP kinase kinase from *Candida albicans*. Mol. Gen. Genet. **249:**609–612.
12. **Davidow, L. S., D. Apostolakos, M. M. O'Donnell, A. R. Proctor, D. M. Ogrydziak, R. A. Wing, I. Stasko, and J. R. DeZeeuw.** 1985. Integrative transformation of the yeast *Yarrowia lipolytica*. Curr. Genet. **10:**39–48.
13. **Dollé, P., J. C. Izpisúa-Belmonte, H. Falkenstein, A. Renucci, and D. Duboule.** 1989. Co-ordinate expression of the murine *Hox-5* complex homeobox-containing genes during limb pattern formation. Nature (London) **342:**767–772.

13a. **Dominguez, A.** Unpublished data.

14. **Donahue, T. F., and A. M. Cigan.** 1990. Sequence and structure requirements for efficient translation in yeast. Methods Enzymol. **185:**366–372.
15. **Duboule, D.** 1994. Guide book to the homeobox genes. Oxford University Press, Oxford, United Kingdom.
16. **Enderlein, C. S., and D. M. Ogrydziak.** 1994. Cloning, nucleotide sequence and functions of *XPR6*, which codes for a dibasic processing endoprotease from the yeast *Yarrowia lipolytica*. Yeast **10:**67–79.
17. **Fournier, P., C. Gaillardin, M. A. Persuy, J. Klootwijk, and H. van Heerikuizen.** 1986. Heterogeneity in the ribosomal family of the yeast *Yarrowia lipolytica*: genomic organization and segregation studies. Gene **42:**273–282.
18. **Fournier, P., L. Guayaneux, M. Chasles, and C. Gaillardin.** 1991. Scarcity of *ars* sequences isolated in a morphogenesis mutant of the yeast *Yarrowia lipolytica*. Yeast **7:**25–36.
19. **Gaillardin, C., L. Poirier, A. M. Ribet, and H. Heslot.** 1979. General and lysine-specific control of saccharopine dehydrogenase levels in the yeast *Saccharomycopsis lipolytica*. Biochimie **61:**473–482.
20. **Gaillardin, C., A. M. Ribet, and H. Heslot.** 1985. Integrative transformation of the yeast *Yarrowia lipolytica*. Curr. Genet. **11:**369–375.
21. **Gil, C., R. Pomes, and C. Nombela.** 1990. Isolation and characterization of *Candida albicans* morphological mutants derepressed for the formation of filamentous hypha-type structures. J. Bacteriol. **172:**2384–2391.
22. **Gillisen, B., J. Bergemann, C. Sandmann, B. Schoeer, M. Bölker, and M. Kahmann.** 1992. A two-component regulatory system for self/non-self recognition in *Ustilago maydis*. Cell **68:**647–657.
23. **Gimeno, C. J., and G. R. Fink.** 1994. Induction of pseudohyphal growth by over expression of *PHD1*, a *Saccharomyces cerevisiae* gene related to transcriptional regulators of fungal development. Mol. Cell. Biol. **14:**2100–2110.
24. **Gimeno, C. J., P. O. Ljundahl, C. A. Styles, and G. R. Fink.** 1992. Unipolar cell divisions in the yeast *Saccharomyces cerevisiae* lead to filamentous growth: regulation by starvation and RAS. Cell **68:**1077–1093.
25. **Hanahan, D.** 1983. Studies on transformation of *Escherichia coli* with plasmids. J. Mol. Biol. **166:**557–580.
26. **Herskowitz, H.** 1995. MAP kinase pathways in yeast: for mating and more. Cell **80:**187–197.
27. **Higgins, D. G., and P. M. Sharp.** 1988. Clustal: a package for performing multiple sequence alignments on a microcomputer. Gene **73:**237–244.
28. **Hill, J., A. Myers, T. Koerner, and A. Tagaloff.** 1986. Yeast/*E. coli* shuttle vectors with multiple unique restriction sites. Yeast **2:**163–167.
29. **Hinnebusch, A. G.** 1990. Transcriptional and translational regulation of gene expression in the general control of amino acid biosynthesis in *Saccharomyces cerevisiae*. Prog. Nucleic Acid Res. Mol. Biol. **38:**195–240.
30. **Hubbard, M. J., P. Markie, and R. T. Poulter.** 1986. Isolation and morphological characterization of a mycelial mutant of *Candida albicans*. J. Bacteriol. **165:**61–65.
31. **Izpisúa-Belmonte, J. C., C. Tickle, P. Dollé, L. Wolpert, and D. Duboule.** 1991. Expression of the homeobox *Hox-4* genes and the specification of position in chick wing development. Nature (London) **350:**585–589.

32. **Johnston, M., and R. W. Davis.** 1984. Sequences that regulate the divergent *GAL1-GAL10* promoters in *Saccharomyces cerevisiae* Mol. Cell. Biol. **4:** 1440–1448.
33. **Kelly, M., J. Burke, M. Smith, A. Klar, and D. Beach.** 1988. Four mating-type genes control sexual differentiation in the fission yeast. EMBO J. **7:** 1537–1547.
34. **Kim, S., J. Mellor, A. J. Kingsman, and S. M. Kingsman.** 1986. Multiple control elements in the *TRP1* promoter of *Saccharomyces cerevisiae* Mol. Cell. Biol. **6:**4251–4258.
35. **Kobayashi, N., and K. McEntee.** 1993. Identification of cis and trans components of a novel heat shock stress regulatory pathway in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **13:**248–256.
36. **Köhler, J. A., and G. R. Fink.** 1996. *Candida albicans* strains heterozygous and homozygous for mutations in mitogen-activated protein kinase signalling components have defects in hyphal development. Proc. Natl. Acad. Sci. USA **93:**13223–13228.
37. **Kyte, J., and R. F. Doolittle.** 1982. A simple method for displaying the hydropathy of a protein. J. Mol. Biol. **157:**105–132.
38. **Lawrence, C.** 1991. Classical mutagenesis techniques, p. 237–279. *In* C. Guthrie and G. R. Fink (ed.), Methods in enzymology: guide to yeast genetics and molecular biology. Academic Press, Inc., San Diego, Calif.
39. **Leberer, E., D. Harcus, I. D. Broadbent, K. L. Clark, D. Dingard, K. Ziegelbauer, A. Schmidt, N. A. Gow, A. J. P. Brown, and D. Y. Thomas.** 1996. Signal transduction through homologs of the ste20p and ste7p protein kinases can trigger hyphal formation in the pathogenic fungus *Candida albicans*. Proc. Nat. Acad. Sci. USA **93:**13217–13222.
40. **Liu, H., J. Köhler, and G. R. Fink.** 1994. Suppression of hyphal formation in *Candida albicans* by mutation of a *STE12* homolog. Science **266:**1723–1726.
41. **Liu, H., J. Krizek, and A. Bretscher.** 1992. Construction of a *GAL1*-regulated yeast cDNA expression library and its application to the identification of genes whose overexpression causes lethality in yeast. Genetics **132:**665–673.
42. **Löpez, M. C., J. M. Nicaud, H. B. Skinner, C. Vergnolle, J. C. Kader, V. A. Bankaitis, and C. Gaillardin.** 1994. A phosphatidylinositol/phosphatidylcholine transfer protein is required for the differentiation of the dimorphic yeast *Yarrowia lipolytica* from the yeast to the mycelial form. J. Cell Biol. **124:**113–127.
43. **Malathi, K., K. Ganesan, and A. Datta.** 1994. Identification of a putative transcription factor in *Candida albicans* that can complement the mating defect of *Saccharomyces cerevisiae ste12* mutants. J. Biol. Chem. **269:**22945–22951.
44. **Marhoul, J. F., and T. H. Adams.** 1996. *Aspergillus fabM* encodes an essential product that is related to poly(A)-binding proteins and activates development when overexpressed. Genetics **144:**1463–1470.
45. **Meissner, P. S., W. P. Sisk, and M. L. Berman.** 1987. Bacteriophage λ cloning system for the construction of directional cDNA libraries. Proc. Natl. Acad. Sci. USA **84:**4171–4175.
46. **Miller, A. M.** 1984. The yeast *Mata1* gene contains two introns. EMBO J. **3:**1061–1065.
47. **Mösch, H.-U., and G. R. Fink.** 1997. Dissection of filamentous growth by transposon mutagenesis in *Saccharomyces cerevisiae*. Genetics **145:**671–684.
48. **Ogrydziak, D. M., J. Bassel, R. Contopoulo, and R. K. Mortimer.** 1978. Development of genetic techniques and the genetic map of the yeast *Saccharomycopsis lipolytica*. Mol. Gen. Genet. **163:**229–239.
49. **Oshima, Y., N. Ogaba, and S. Harashima.** 1996. Regulation of phosphate synthesis in *Saccharomyces cerevisiae*—a review. Gene **179:**171–177.
50. **Pearson, W. R., and D. J. Lipman.** 1988. Improved tools for biological sequence comparison. Proc. Natl. Acad. Sci. USA **85:**2444–2448.
51. **Percival-Smith, A., and J. Segall.** 1984. Isolation of DNA sequences preferentially expressed during sporulation in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **6:**2443–2451.
52. **Pérez-Campo, F. M., J. M. Nicaud, C. Gaillardin, and A. Domínguez.** 1996. Cloning and sequencing of the *LYS1* gene encoding homocitrate synthase in the yeast *Yarrowia lipolytica*. Yeast **12:**1459–1469.

52a. **Pérez-Campo, F.M., and A. Dominguez.** Unpublished data.

53. **Robbins, J., S. M. Dilworth, R. A. Laskey, and C. Dingwall.** 1991. Two interdependent basic domains in nucleoplasmin nuclear targeting sequence: identification of a class of bipartite nuclear targeting sequence. Cell **64:**615–623.
54. **Rodrĭguez, C., and A. Domínguez.** 1984. The growth characteristics of *Saccharomyces lipolytica*: morphology and induction of mycelial formation. Can. J. Microbiol. **30:**605–612.
55. **Sambrook, J. E., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
56. **San Blas, G., and F. San Blas.** 1984. Molecular aspects of fungal dimorphism. Crit. Rev. Microbiol. **11:**101–127.
57. **Sánchez, M., M. Prado, F. J. Iglesias, and A. Domínguez.** 1995. Cloning and sequencing of the *URA5* gene from the yeast *Yarrowia lipolytica*. Yeast **11:**425–433.
58. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitor. Proc. Natl. Acad. Sci. USA **74:**5463–5467.
59. **Saporito-Irwin, S. M., C. E. Brise, P. S. Shypherd, and W. A. Fonzi.** 1995. *PHR1*, a pH-regulated gene of *Candida albicans*, is required for morphogenesis. Mol. Cell. Biol. **15:**601–613.
60. **Schulz, B., F. Banuet, M. Dahi, R. Schlesinger, W. Schäfer, T. Martin, I. Herskowitz, and R. Kahmann.** 1990. The b alleles of *Ustilago maydis*, whose combination program pathogenic development, code for polypeptides containing a homeodomain-related motif. Cell **60:**925–306.
61. **Senstag, H., and A. Hinnen.** 1987. The sequence of the *Saccharomyces cerevisiae* gene *PHO2* codes for a regulatory protein with unusual amino acid composition. Nucleic Acids Res. **15:**233–246.
62. **Sheperd, M. G.** 1988. Morphogenetic transformation of fungi. Curr. Top. Med. Mycol. **2:**278–304.
63. **Sherman, F., G. R. Fink, and J. Hicks.** 1986. Methods in yeast genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
64. **Slutsky, B., J. Buffo, and D. R. Soll.** 1985. High-frequency switching of colony morphology in *Candida albicans*. Science **230:**666–669.
65. **Torres-Guzzmán, J. C.** 1994. Aislamiento y caracterización del gen *HOY1* involucrado en la transición levadura-micelio de *Yarrowia lipolytica*. Ph.D. thesis. Universidad de Salamanca, Salamanca, Spain.
66. **Xuan, J. W., P. Fournier, N. Declerek, M. Chasles, and C. Gaillardin.** 1990. Overlapping reading frames at the *LYS5* locus in the yeast *Yarrowia lipolytica*. Mol. Cell. Biol. **10:**4795–4806.
67. **Xuan, J. W., P. Founier, and C. Gaillardin.** 1988. Cloning of the *LYS5* gene encoding saccharopine dehydrogenase from the yeast *Yarrowia lipolytica* by target integration. Curr. Genet. **14:**15–21.
68. **Zaret, K. S., and F. Sherman.** 1982. DNA sequence required for efficient transcription termination in yeast. Cell **28:**265–273.