

## The *Candida albicans* *HYRI* Gene, Which Is Activated in Response to Hyphal Development, Belongs to a Gene Family Encoding Yeast Cell Wall Proteins

DAVID A. BAILEY, PASCALE J. F. FELDMANN,<sup>†</sup> MARY BOVEY, NEIL A. R. GOW,  
AND ALISTAIR J. P. BROWN\*

Department of Molecular and Cell Biology, Institute of Medical Sciences, University of Aberdeen,  
Aberdeen AB25 2ZD, United Kingdom

Received 17 May 1996/Accepted 15 July 1996

**A hyphally regulated gene (*HYRI*) from the dimorphic human pathogenic fungus *Candida albicans* was isolated and characterized. Northern (RNA) analyses showed that the *HYRI* mRNA was induced specifically in response to hyphal development when morphogenesis was stimulated by serum addition and temperature elevation, increases in both culture pH and temperature, or *N*-acetylglucosamine addition. The *HYRI* gene sequence revealed a 937-codon open reading frame capable of encoding a protein with an N-terminal signal sequence, a C-terminal glycosylphosphatidylinositol-anchoring domain, 17 potential N glycosylation sites, and a large domain rich in serine and threonine (51% of 230 residues). These features are observed in many yeast cell wall proteins, but no homologs are present in the databases. In addition, Hyr1p contained a second domain rich in glycine, serine, and asparagine (79% of 239 residues). The *HYRI* locus in *C. albicans* CAI4 was disrupted by “Ura-blasting,” but the resulting homozygous  $\Delta$ *hyr1*/ $\Delta$ *hyr1* null mutant displayed no obvious morphological phenotype. The growth rates for yeast cells and hyphae and the kinetics of germ tube formation in the null mutant were unaffected. Aberrant expression of *HYRI* in yeast cells, when an *ADHI-HYRI* fusion was used, did not stimulate hyphal formation in *C. albicans* or pseudohyphal growth in *Saccharomyces cerevisiae*. *HYRI* appears to encode a nonessential component of the hyphal cell wall.**

*Candida albicans* is a major fungal pathogen in humans (44, 45). Most frequently it causes superficial, irritating infections of the oral and urogenital tracts. However, serious deep-seated or systemic infections can develop, particularly in immunocompromised individuals.

A number of factors are thought to promote the virulence of *C. albicans*. Many of these factors relate to properties of the *C. albicans* cell surface, for example, the ability to adhere to host tissues (7, 8, 27) and the immunomodulatory effects of various cell wall components (40, 72). Another potential virulence factor is the ability to undergo a morphological transition from a budding yeast to a hyphal form, but this has not been established unambiguously (12, 39, 48, 57, 60, 61). Changes in the *C. albicans* cell surface accompany the morphological transition (9, 15, 16, 31, 38, 64), and hence morphogenesis is intimately linked with other virulence factors such as adherence. Processes germane to the regulation of the yeast-to-hypha transition are therefore important in establishing the role of this transition in the pathogenicity of *C. albicans*.

Several factors influenced our experimental approach. First, classical genetic approaches were inappropriate because *C. albicans* is asexual and diploid (55). Second, nonstandard usage of the CTG codon in *C. albicans* (52, 53, 78) precluded the use of standard reporter genes, and until recently (62), no sensitive reporter genes for *C. albicans* were available. Hence,

we attempted to identify *C. albicans* genes which are regulated specifically in response to morphogenesis. Using various approaches, we identified numerous genes that are regulated during morphogenesis, including *HSP90*, *TEF3*, *ADHI*, *PYKI*, *RP10*, and *GFAI* (59, 65–67, 69). However, in all cases we showed that these genes were responding to changing growth conditions rather than to morphogenesis. Nevertheless, we did identify a morphogenetically regulated sequence via a serendipitous route. This *C. albicans* cDNA, which was a false positive in an immunological screen for  $\beta$ -subunits of heterotrimeric G proteins (18), was used as a probe in Northern (RNA) analyses of yeast and hyphal RNAs as part of a routine screen for morphogenetically regulated sequences. The corresponding mRNA was not detected in yeast cells but was abundant in hyphae. Hence, the gene was called *HYRI* (for hyphally regulated).

This paper describes the isolation and characterization of the *HYRI* gene. We show that *HYRI* is expressed specifically during hyphal development and that it appears to encode a nonessential component of the hyphal cell wall.

### MATERIALS AND METHODS

**Strains and culture conditions.** The following *C. albicans* (Robin) Berkhout strains were used: ATCC 10261 (58), 3153A (Public Health Laboratory Services Mycology Reference Laboratory, Department of Microbiology, University of Leeds, Leeds, United Kingdom), and CAI4 ( $\Delta$ *ura3::imm434*/ $\Delta$ *ura3::imm434*) and its wild-type parent, SC5314 (21). *Saccharomyces cerevisiae* W303-1B (*MAT $\alpha$*  *ade2 his3 leu2 trp1 ura3*) was used (71). *Escherichia coli* DH5 $\alpha$ F' [K-12  $\Delta$ (*lac ZYA-argF*)<sub>U169</sub> *supE44 thi1 recA1 endA1 hisR17 gyrA relA1* ( $\phi$ 80lacZ $\Delta$ M15)F'] was used for the manipulation of cDNA and genomic clones.

The dimorphic transition was induced under three different sets of experimental conditions. (i) *C. albicans* was grown in the yeast form to late exponential growth phase (optical density at 600 nm, about 0.6) in YPD (2% glucose, 2% bacteriological peptone, 1% yeast extract) at 25°C with shaking at 200 rpm. To induce hyphal growth, 10 ml of this culture was used to inoculate 100 ml of YPD containing 10% bovine calf serum at 37°C (approximately  $2 \times 10^7$  cells per ml). Samples were removed at various times up to 120 min. Control cultures were

\* Corresponding author. Mailing address: Department of Molecular and Cell Biology, Institute of Medical Science, University of Aberdeen, Aberdeen AB25 2ZD, United Kingdom. Phone: 44-1224-273188. Fax: 44-1224-273144. Electronic mail address: al.brown@abdn.ac.uk.

<sup>†</sup> Present address: Institute of Cancer Research, Royal Cancer Hospital, Chester Beaty Laboratories, London SW3 6JB, United Kingdom.

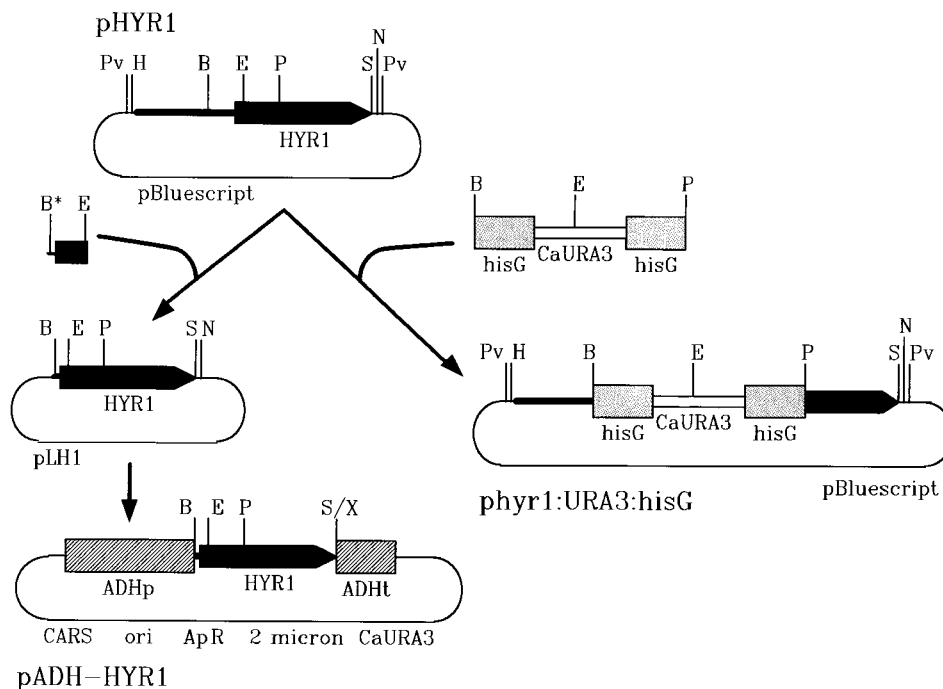


FIG. 1. Construction of *HYR1* plasmids for gene disruption and expression. The plasmid pHYR1 contains the entire *HYR1* coding region and 1.3 kbp of 5' sequences cloned into pBluescript. The *hisG::URA3::hisG* cassette from pMB-7 was cloned between the *Bgl*II and *Pst*I sites in pHYR1 to generate phyr1:URA3:hisG, which was used to make a *C. albicans*  $\Delta$ *hyr1* null mutant (see Materials and Methods). The *ADHI-HYR1* fusion was created essentially in two steps. First, the 2.7-kbp *Eco*RI-*Not*I fragment from pHYR1 (containing most of the *HYR1* coding region), and a 0.1-kbp PCR product with an artificial 5' *Bgl*II site and a natural 3' *Eco*RI site (representing the 5' end of the *HYR1* coding region) were cloned in a three-way ligation into pLH1. Second, the resulting *Bgl*II-*Not*I fragment containing the complete coding region was subcloned into the *C. albicans* expression vector YPB-ADHPt to generate pADH-HYR1. This expression plasmid carries the *HYR1* coding region under the control of the *C. albicans ADHI* promoter and terminator as well as *C. albicans* and *S. cerevisiae* replication origins (CAARS and 2 $\mu$ m) and the *C. albicans URA3* gene. Solid arrow-like boxes, *HYR1*; stippled boxes, *hisG* sequences; white rectangle, *URA3*; hatched boxes, *ADHI* promoter (ADHP) and terminator (ADHT); thin lines, backbone vector sequences. Abbreviations for restriction enzymes: B, *Bgl*II (B\*, artificial *Bgl*II site); E, *Eco*RI; H, *Hind*III; N, *Not*I; P, *Pst*I; Pv, *Pvu*II; S, *Sal*I; X, *Xho*I. Drawings are not to scale.

grown in fresh YPD at 25°C without serum, 25°C with serum, or 37°C without serum. (ii) *C. albicans* was grown in the yeast form to late exponential growth phase in Soll's medium at 25°C and pH 4.5 with shaking at 200 rpm. Hyphal growth was induced by inoculating 10 ml of this culture into 100 ml of Soll's medium at 37°C and pH 6.5 (approximately  $2 \times 10^7$  cell per ml) (61), and samples were removed for analysis at various times up to 180 min. Control cultures were grown in Soll's medium at 25°C and pH 4.5, 25°C and pH 6.5, or 37°C and pH 4.5. Soll's medium refers to the defined medium of Lee et al. (34) as modified by Buffo et al. (6). (iii) Hyphal growth was induced with *N*-acetylglucosamine as described previously (41), and samples were removed for RNA analysis at various times thereafter. Cell numbers and morphology were monitored by light microscopy with an improved Neubauer hemocytometer.

**Isolating the *HYR1* gene.** The genomic library, which was derived from *C. albicans* ATCC 10261, was provided by M. Payton (Glaxo Institute for Molecular Biology, Geneva, Switzerland). The library was constructed by inserting DNA partially digested with *Sau*3A into YCp50 (58). Colony hybridization (49) was performed with the 1.4-kbp *Hind*III fragment from the *HYR1* cDNA as a probe following radiolabelling by random priming (17).

**DNA sequencing.** Dideoxy DNA sequencing (50) was performed with synthetic oligonucleotide primers (Oswel, Southampton, United Kingdom). Double-stranded DNA templates were sequenced with Sequenase (Amersham International, Little Chalfont, United Kingdom) and <sup>35</sup>S-dATP according to the manufacturers' instructions. DNA sequences were analyzed with the Genetics Computer Group programs (14) on the SERC computer at the Daresbury Laboratory.

**Preparation and analysis of nucleic acids.** Genomic DNA (26) and total RNA (5, 36) were prepared from *C. albicans* and *S. cerevisiae* as described previously. Southern blotting (51) and Northern analysis (42) were performed with radiolabelled probes prepared by random priming (17) the purified 1.4-kbp *Hind*III fragment from the *HYR1* coding region. For Northern blotting, yields of total RNA were measured by spectrophotometry at 260 nm, and approximately equal amounts of RNA were loaded in each lane. Following electrophoresis on formaldehyde-containing gels, the RNA was transferred to nylon membranes by vacuum blotting, and hybridizations were performed under conditions of probe excess (42). To date, there has been no report of a reasonably abundant *C.*

*albicans* mRNA that remains at sufficiently constant levels during the dimorphic transition to use as an internal loading control on Northern blots (3, 11, 59, 65–67, 69). Therefore, mRNA levels were measured relative to those of the rRNAs by loading equal amounts of total RNA in each lane of the Northern gels (3, 11, 59, 65–67, 69).

**RNA 5' mapping.** The 5' end of the *HYR1* mRNA was mapped by primer extension using standard procedures (49). Briefly, 400 ng of oligonucleotide primer (5'-TGAATCCACCACGGTC-3'; Oswel) was end labelled with [ $\gamma$ -<sup>32</sup>P]ATP (35). Labelled primer (200 ng) was precipitated with 160  $\mu$ g of *C. albicans* RNA prepared from hyphal cells. After denaturing and annealing this mixture, primer extension was performed in a total volume of 20  $\mu$ l with 48 U of avian myeloblastosis virus reverse transcriptase (Boehringer Mannheim UK Ltd., Lewes, East Sussex, United Kingdom). A sequencing reaction was performed with the same primer and electrophoresed alongside the primer extension reaction on a 6% urea-polyacrylamide gel.

**Disruption of *HYR1*.** The *hisG::URA3::hisG* "Ura-blaster" cassette from pMB7 (21) was isolated by digestion with *Bgl*II and *Pst*I and used to replace the 1.4-kbp *Bgl*II-*Pst*I fragment in pHYR1 to create phyr1:URA3:hisG (Fig. 1). The 9.6-kb  $\Delta$ *hyr1::hisG::URA3::hisG* insert was released from pBluescript by digestion with *Pvu*II and used to transform *C. albicans* CA14 (33). Late logarithmic cells (50 ml) were harvested, spheroplasted with  $\beta$ -glucuronidase (type H2; Sigma, St. Louis, Mo.), and transformed with approximately 5  $\mu$ g of DNA. Cells were plated onto SD medium [2% glucose, 0.17% yeast nitrogen base without amino acids, 0.5% (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>] containing 1 M sorbitol and lacking uridine. Approximately three to four transformants per  $\mu$ g of DNA were visible after incubation at 30°C for 3 days. Primary transformants were replated onto SD medium lacking uridine and then patched onto minimal medium containing uridine (50  $\mu$ g/ml) and 5'-fluoroorotic acid (FOA) (1 mg/ml). FOA-resistant colonies were used for a second round of transformation with the  $\Delta$ *hyr1::hisG::URA3::hisG* fragment to disrupt the second *HYR1* allele. Cells were subjected to Southern analysis at each stage of this process.

**Overexpression of *HYR1*.** The 5' end of the *HYR1* coding region was PCR amplified with primers designed to introduce a *Bgl*II site at position -13 with respect to the first nucleotide of the coding region. The primer sequences were 5'-CAATTTCAGATCTATAACAACATGAAGTGG-3' (the *Bgl*II site is un-

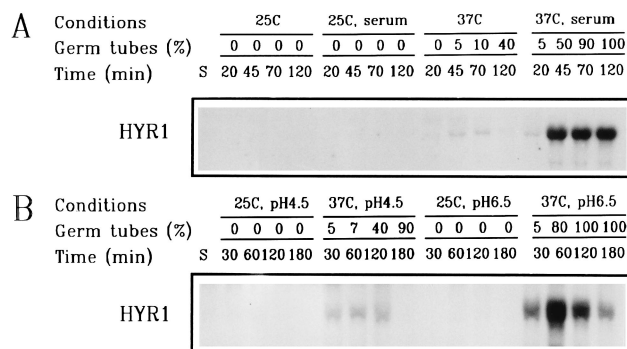


FIG. 2. The *HYR1* mRNA is induced in response to morphogenesis. The yeast-to-hypha transition was induced in *C. albicans* 3153A with different stimuli. Northern analysis of the *HYR1* mRNA was performed at various times thereafter with approximately 20  $\mu$ g of RNA in each lane, and the proportion of cells forming germ tubes was determined by light microscopy. (A) An overnight starter culture grown in YPD at 25°C (S), YPD containing serum at 25°C (25C, serum), YPD at 37°C (37C), or YPD containing serum at 37°C (37C, serum). (B) An overnight starter culture grown in Soll's medium at pH 4.5 and 25°C (S) was used to inoculate fresh Soll's medium either at pH 4.5 and 25°C (25C, pH 4.5), pH 4.5 and 37°C (37C, pH 4.5), pH 6.5 and 25°C (25C, pH 6.5), or pH 6.5 and 37°C (37C, pH 6.5).

derlined and the initiation codon is shown in italics) and 5'-CCTTGATTGTA GCTGG-3'. The PCR product was digested with *Bgl*II and *Eco*RI to release a 100-bp fragment which was mixed with the 2.7-kbp *Eco*RI-*Not*I fragment from pHYR1 and ligated into the vector pLH1 (Amersham International) (Fig. 1). After being subjected to resequencing to check its integrity, this construct was digested with *Bgl*II and *Sal*I and the 2.8-kbp *HYR1* coding region was cloned between the *Bgl*II and *Xho*I sites in the *C. albicans* expression vector YPB-ADHpt to generate pADH-HYR1 (Fig. 1). YPB-ADHpt carries the *C. albicans ADHI* promoter and terminator regions (3), the *C. albicans URA3* marker, and *C. albicans* (CAARS) and *S. cerevisiae* (2 $\mu$ m) replication origins (2a). pADH-HYR1 was transformed into *C. albicans* CAI4 (33) and *S. cerevisiae* (30).

**Nucleotide sequence accession number.** The *HYR1* sequence was submitted to the EMBL database and given accession number Z50123.

## RESULTS

### The *C. albicans HYR1* gene is morphogenetically regulated.

In an effort to identify genes that are regulated specifically in response to the yeast-to-hypha transition, we routinely analyzed the expression of newly isolated *C. albicans* clones. As part of this process, we performed Northern analyses of yeast and hyphal RNAs using a cDNA clone isolated as a false positive in an immunological screen for  $\beta$ -subunits of *C. albicans* trimeric G proteins (18). This *C. albicans* cDNA detected an approximately 3-kb-long mRNA which was strongly induced when the yeast-to-hypha transition was induced by a combination of serum addition and temperature elevation (Fig. 2A), by increasing both the pH and temperature of the cultures (Fig. 2B), or by *N*-acetylglucosamine addition (not shown). The presence of this mRNA correlated with the formation of germ tubes rather than the induction conditions themselves. For example, weak induction of *HYR1* correlated with the slow emergence of germ tubes at pH 4.5 and 37°C (Fig. 2B). Therefore, this mRNA was induced specifically in response to hyphal growth, and hence the corresponding locus was named *HYR1* (for hyphally regulated).

**Isolation and sequencing of *HYR1*.** The *HYR1* cDNA contained only 2.6 kb of the 3-kb mRNA. Hence, the cDNA was used to isolate the gene from a *C. albicans* genomic library. The sequences of the *HYR1* cDNA (derived from a *C. albicans* 3153A library [68]) and gene (derived from a *C. albicans* 10261 library [58]) were identical over their 2.2-kb overlap (Fig. 3). However, the *HYR1* genomic clones were truncated, lacking 400 bp at the 3' end. Hence, the complete *HYR1* open reading

frame was PCR amplified from *C. albicans* ATCC 10261 with primers corresponding to the 5' and 3' untranslated regions. The sequence of this PCR product confirmed that the 3' end of the *HYR1* gene in ATCC 10261 was identical to that in 3153A. The 3' *Pst*I-*Sal*I fragment from the PCR product was ligated to the 5' *Hind*III-*Pst*I fragment from the genomic clone to reconstruct the full-length *HYR1* gene in plasmid pHYR1 (Fig. 1).

The *HYR1* sequence revealed a 2,810-bp open reading frame capable of encoding a 937-amino-acid protein with a predicted molecular mass of 94.1 kDa (Fig. 3). No homologs were identified in the databases. Computer analyses of the predicted amino acid sequence suggested the existence of an N-terminal signal sequence. Also the presence of a hydrophobic C-terminal tail preceded by consensus cleavage sites was consistent with the attachment of a glycosylphosphatidylinositol (GPI) anchor (19, 73). A large hydrophilic domain (residues 346 to 576) was rich in serine and threonine (51% of the residues). A second domain (residues 577 to 816) was rich in asparagine, serine, and glycine (79% of residues). The protein sequence carried 17 potential N-glycosylation sites, the last of which is unlikely to be used if the protein is GPI anchored (Fig. 3).

**Mapping the *HYR1* transcription unit.** The transcriptional start sites on the *HYR1* gene were mapped by primer extension (Fig. 4). The major transcriptional start site was at -60 with respect to the predicted translational start codon which represented the 5'-proximal ATG on the *HYR1* mRNA (Fig. 3). A TATA-like sequence (4, 14) existed 27 bp upstream from the major transcriptional start site. Analyses of the cDNA sequence revealed that 3' polyadenylation occurred between nucleotides 2999 and 3000. It was not clear whether the last A residue was part of the primary transcript or added posttranscriptionally as part of the poly(A) tail (Fig. 3). The length of the transcription unit (2,935 bp) was consistent with the length of the mRNA observed on Northern blots (about 3 kb).

**A *C. albicans*  $\Delta$ *hyr1*/ $\Delta$ *hyr1* mutant displays no obvious phenotype.** A *C. albicans*  $\Delta$ *hyr1*/ $\Delta$ *hyr1* mutant was constructed with a view to establishing the function of the *HYR1* gene. We exploited the Ura-blasting procedure (21, 51), which was first developed for sequential gene disruptions in *S. cerevisiae* (1). The plasmid p $\text{hyr1}::\text{URA3}::\text{hisG}$  was created essentially by inserting the *hisG*::*URA3*::*hisG* cassette from pMB-7 between the *Bgl*II and *Pst*I sites in the *HYR1* gene (Fig. 1), thereby deleting 290 bp of the 5' region and 1.1 kbp of the coding region, and leaving about 1 kbp of 5' sequence and 2 kbp of 3' sequence flanking the *hisG*::*URA3*::*hisG* cassette for targeted integration into the *HYR1* locus. No upstream open reading frames were affected by this insertion (not shown). The 9.8-kbp *Pvu*II fragment carrying the  $\Delta$ *hyr1*::*hisG*::*URA3*::*hisG* cassette was transformed into *C. albicans* CAI4, selecting for Ura<sup>+</sup> transformants. Following Southern analysis to confirm the correct disruption of the first *HYR1* allele, spontaneous Ura<sup>-</sup> segregants were selected on the basis of their resistance to FOA. Southern analysis confirmed the loss of the *URA3* gene from the  $\Delta$ *hyr1* allele via intrachromosomal recombination between the tandem *hisG* repeats (Fig. 5). To disrupt the second *HYR1* allele, this *HYR1*/ $\Delta$ *hyr1*::*hisG* mutant was transformed with the  $\Delta$ *hyr1*::*hisG*::*URA3*::*hisG* cassette and Ura<sup>+</sup> transformants were selected. FOA-resistant, Ura<sup>-</sup> segregants were selected once again, and the construction of a *C. albicans*  $\Delta$ *hyr1*/ $\Delta$ *hyr1* mutant was confirmed by Southern analysis (Fig. 5). This also confirmed the existence of a single *HYR1* locus in *C. albicans*.

The phenotype of the *C. albicans*  $\Delta$ *hyr1*/ $\Delta$ *hyr1* mutant was compared with that of its isogenic parent, CAI4. The mutant showed normal doubling times in minimal (2.1 h) and rich (1.2 h) media, no change in the kinetics of germ tube formation

1	CTCCAGTAAACCAAAATCAATCAAGATTCTAATGATTCTATAAATACTCCAAAGTATCCAGTTTGTGAAACCAATTTGGGCATCATTCTT	90
91	CTATTATCAACTACAATTTCAAGGGTAATAACAACATGAAAGTGGTATCAAACCTTTATATTCACAATTTTGCTTACTCTCAACCTCAGTG	180
1	M K V V S N F I F T I L L T L N L S A	19
181	CTSCATTAGAAAGTTGTCAAGCAGAATTGACCGTGGTAAATCAAAGTTCCATGGTGAATGTTAAAGTTCACTCGGTGCTACTTGGG	270
20	A L E V V T S R I D R G G I Q G F H G D V K V H S G A T W A	49
271	CTATTCTGGTACTACTCTTTGTTCCCTTTTGGTGGGTAGAAAGTTGAGAAAGGTGCTCTTTATTATTAAGCTGGACAAATGGTCTG	360
50	I L G T T L C S F P F G G L E V E K G A S L F I K L D N G P V	79
361	TCTTAGCTCTTAATGTCCTTTATCAACTTTAGTTAGACCAGTTATAAATAAGTGGTATTTCATTAATCTAAATCTCTACAAGTT	450
80	L A L N V A L S T L V R P V I N N G V I S L N S K S S T S F	109
451	TTTCAAATTTGACATTTGGTGGATCTTCATTCACATAATGGTGAATTTATCTTGATTTCTCGGGTCTTTAAAGTACAGCCTATC	540
110	S N F D I G G S S F T N N G E I Y L D S S G L V K S T A Y Q	139
541	TTTATGCAGTGAATGGACTAATAATGGGTTAATTTGTTCTTATCAAATCAAAAAGCTGCTGTAATTTGCTTTTGGTACTGCTTATC	630
140	Y A R E W T N N G L I V A Y Q N Q K A A G N I A F G T A Y Q	169
631	AAACCATCACTAATAATGGCCAAATTTGTTTGGTCATCAAGACTTTGTTCCAGCTACAAAATCAAAGTACTGGTGGTGTACTGCTG	720
170	T I T N N G Q I C L R H Q Q D F V P A T K I K G T G C V T A D	199
721	ATGAGACACATGGATAAAGTGGTAACTTATCACTTGAACCTACTCATAATTTTACTTGAAGATAGTAAATCGTCTTTGA	810
200	E D T W I K L G N T I L S V E P T H N F Y L K D S K S S L I	229
811	TTGTTCACTGCTTCAAGCAACCAACATTTACTGTCCATGGTTTGGTAATGGTAATAACTTGGTTGACTCTCCATTAACTGGAA	900
230	V H A V S S N Q T F T V H G F G N G N K L G L T L P L T G N	259
901	ATAGAGATCAITCCGTTTGAATACTACCTGACACTGGTATTTTACAATTAAGAGCTGATGCCCTCCACAATATTTAAAATTTGGTA	990
260	R D H F R F E Y P D T G I L Q L R A D A L P Q Y F K I G K	289
991	AAGTTATGATCTAAGTTGTTAGAATTTGTTAATCTCGGTGGTCTCAAAAATGCTGTTACTTATGATGGTCTGCTTCAACCAAGAAA	1080
290	G Y A D S K L F R I V N L R G L K N A V T Y D G P V P N N E I	319
1081	TCCAGCTGTTTGGTATACCATGTACCAATGGACCATCTGCACCAGAAAGTGAATCTGATTTGAATCTCCTACTACATCATCAATG	1170
320	F A V C L I P C T N G P S A P E S E S D L N T P T T S S I E	349
1171	AAACAGCTCATAAGTTCTGCTGCACTGAATCTAGTGGTTAGTGAATCTAGTCTCGACTTGAAGCTTCACTTCACTCTCTAT	1260
350	T S S Y S S A A T E S S V V S E S S S A V D S L T S S S L S	379
1261	CATCAAATCTGAATCTAGCGATGTTGTTCTAGTACCACCAACATTAATCATCTTCCACTGCTATTGAACTACTATGAACCTCAGAAA	1350
380	S K S E S S D V V S S T T N I E S S S T A I E T T M N S E S	409
1351	GTTCACTGATGCTGGTTCAAGTTCTATTAGTCAATCCGAATCATCTCCACCGCCATCACTTCACTTTCAGAACTAGTTCATCCGAAT	1440
410	S T D A G S S S I S Q S E S S S T A I T S S S E T S S S E S	439
1441	CTATGCTGCTTCACTACTGATCAAACTCTTCTATTGAAACTGACTCAGGTATTGTTAGCCAATCTGAATCATCTAGCAATGCAT	1530
440	M S A S S T T A S N T S I E T D S G I V S Q S E S S S N A L	469
1531	TATCATCACTGAACAAGTATCACATCAAGTCTGGTCAATCTACTATTTACGTCAACCTGACTGTTACATCACTATTACTTCTCTGTG	1620
470	S T S E Q S I T S S P G Q S T I Y V N L T V T S T S C D	499
1621	ATGAAAATAAATGACTGAAGATGTTGCACTATCTCACCAGTTCATGCTGATTTGGTTCACAACTGGTGATATTCCAA	1710
500	E N K C T E D V V T I F T T V P C S T D C V P T T G D I P M	529
1711	TGCTACTTCTACACACAAGAAGTGTACCTCCATTTACTAATCTGATGAAGTCAAGTGTTCACAAGATGTTGATGACATACACTA	1800
530	S T S Y T Q R T V T S T I T N C D E V S C S Q D V V T Y T T	559
1801	CTAATGTTCCACATACCAGTAGATGCTACAACACTACTACTAGTACTGGTGGTATAAATCAACTGGAGGTAATGAAAGTGGTT	1890
560	N V P H T T V D A T T T T T T S T G G D N S T G G N E S G S	589
1891	CTAACCTGGTCCAGGCAATGGTCTACTGAAGTTCAGGAATGGTTCAGGAGCTGGTCCCAATGAAGGTTCTCAATCTGGTCTTAATA	1980
590	N H G P P G N G S T E G S G N G S G A G S N E G S G S G P N N	619
1981	ACGGTCTGGTCTGGTCTGAAGTGGATCTAACAATGGTCTGGTCTGATTCGGCTCTAATAATGGTCTGGTCTGGTCTTAATA	2070
620	G S G S G S E G G S N N G S G S G S D S G S N N G S G S G S N N	649
2071	ATGTTCCGGTCCGGTTCAGTCAAGGTTCTGAAGTGGTCTGGTTCAAATGAAGGATCACAACTCCGGTTCGGTCTCAGCTGCTC	2160
650	G S G S G S T E G S E G G S G S N E G S Q S G S G S Q P G P	679
2161	CTAATGAAGTTCGAAGTGGTCTGGTTCAAATGAAGGCTTCAACATGGTCCCAATGAAGGATCTGGTCTGGTCTGGTCCGGCT	2250
680	N E G S E G G S G S N E G S N H G S N E G S G S G S G S	709
2251	CTAATGAAGTTCGGTTCAGGCTCAACATCCGGCTCAGGCTCAGCTCAACATCCGGTCTGAATCTGGTCTCAACATGGTTCCAATG	2340
710	N N G S G S G S Q S G S G S G S Q S G S E S G S N S G S N E	739
2341	AAGTTCTAACCCAGTCTGGTACCGATCAACGAAGGTTCAAGTCAAGTCTCGGAATGGTCTGAAGTGGTCTGGACAAGTT	2430
740	G S N P G A G N G S N E G S G Q G S G N G S E A G S G G T	769
2431	CTGGTCTAACCAAGGTTCTGGAAGTGGTCAACGATGGCTCTGGTCTGGATCTAATCAAGTTCCAATCCAGGCTGGTCTGGT	2520
770	G P N N G S G S G H N D G S G S G S N Q G S N P G A G S G S	799
2521	CTGGTCCGAAAGTGGTCTAAGCAGGTTCAACTCTGGTCTAATGAAGTGGTCAAGCAGACTCTAATGAAGTTCACACTGAAT	2610
800	G S E S G S K A G S H S G S N E G A K T D S I E G P H T E S	829
2611	CTAAACCTGGTTCAAATACCGGTGCTCACACTGATGCTACTGCTCACTGGTAACTCTGTTGCTAATCCAGTTACTACTCCACAGAAAGTG	2700
830	K P G P N T G A H T D A T V T G N S V A N P V T T S T E S D	859
2701	ACACCACATTTCAAGTACTGTTCAATCACTAGTTATATGACTGGCTTTGATGGAAAACCTAAACCATTTCAACATGTTGATGTTATTC	2790
860	T T I S V T V S I T S Y M T G F D G K P K P F T T V D V I P	889
2791	CAGTACCACATTTCTATGCCATCAACACAAGTCACTCATCTAGTGGTCCAACTATTGATACTAATGAAATGGTTCATCTATTGTTA	2880
890	V P H S M P S N T T D S S S S V P T I D T N E N G S S I V T	919
2881	CTGGTGGTAAATCCATTTGTTGTTAATGTTCCATGGTGTATTCATGATGAGTGAAGCTCTATTGTCATGTTTATCTT	2970
920	G K S I L P G L I V S M V L F M	937
2971	ATAGTTGTTTTCGCTGACTGATGTTTCA	3000

FIG. 3. Nucleotide and predicted amino acid sequences of the *C. albicans* *HYR1* gene. Regarding the nucleotide sequence, the positions of the major transcriptional start site at nucleotide 65 (see Fig. 4) and the 3' cleavage and polyadenylation sites at nucleotides 2999 and 3000 are marked with crosses above the sequence. A TATA-like sequence at nucleotides 38 to 42 is indicated by double underlining. The 2.2-kbp cDNA sequence is identical to the 3' end of the gene sequence. With respect to the predicted amino acid sequence, the serine- and threonine-rich domain is shown in boldface type, whereas the glycine-, serine-, and asparagine-rich domain is shown in italics (see text). The sequence NEGS, which is repeated seven times in the latter domain, is underlined. Potential N glycosylation sites are represented by diamonds below each target asparagine residue. The predicted cleavage site for the N-terminal signal sequence is illustrated with an arrow below the site. The most likely cleavage site for the C-terminal GPI-anchoring domain is shown by the large arrow between residues 913 and 914, and a second possible cleavage site is indicated by the small arrow between residues 902 and 903.

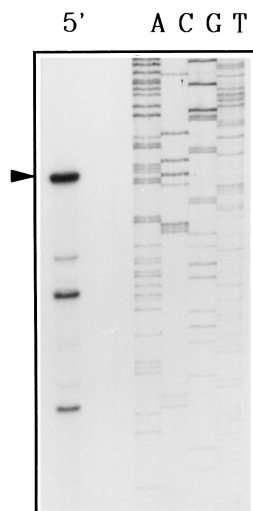


FIG. 4. The *HYR1* transcriptional start site. The transcriptional start site was mapped by primer extension (lane 5') and electrophoresed alongside an *HYR1* sequencing reaction (lanes A, C, G, and T) performed with the same primer. The position of the major start site on the *HYR1* sequence (Fig. 3) is shown (arrowhead).

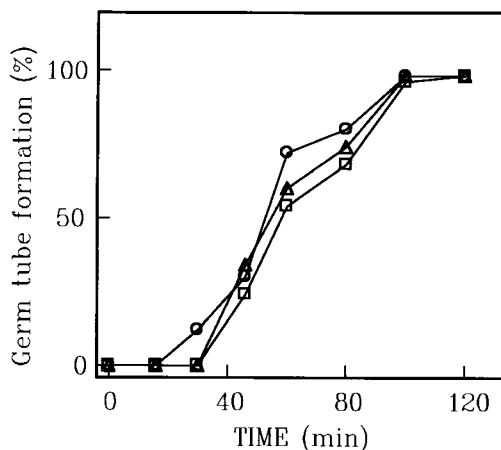


FIG. 6. Kinetics of germ tube formation in *C. albicans* *HYR1* mutants. The wild-type parental strain (SC5314) (squares), the *hyr1* null mutant ( $\Delta hyr1::hisG/\Delta hyr1::hisG$ ) (circles), and the pADH-HYR1 transformant (triangles) were grown overnight in YPD at 25°C and then inoculated at the same cell density into YPD at 37°C containing 10% serum. The proportion of cells forming germ tubes was determined by light microscopy at various times thereafter (errors were less than 5%).

(Fig. 6), and no significant difference in its sensitivity to caffeine or calcofluor white. Microscopic analyses revealed no abnormal cell morphologies for this mutant compared with CAI4.

**Forced expression of *HYR1* in yeast cells of *C. albicans* and *S. cerevisiae*.** Since no obvious phenotype was observed upon disruption of *HYR1*, we tested the effects of expressing this hypha-specific gene in the yeast form of *C. albicans*. To achieve this, the *HYR1* coding region was amplified by PCR, resequenced, and cloned into the *C. albicans* expression vector YPB-ADH1pt to create pADH-HYR1 (Fig. 1). pADH-HYR1 was transformed into *C. albicans* CAI4, and Northern analysis confirmed the aberrant expression of *HYR1* during the growth of the yeast form (Fig. 7). The level of *HYR1* expression in

yeast cells transformed with pADH-HYR1 was roughly equivalent to that observed upon induction of hyphal growth in untransformed cells. This did not affect the growth rate of the transformed yeast cells, their sensitivity to caffeine or calcofluor white, or their cell morphology. Germ tube formation in these transformants was not observed under conditions which favored the growth of the yeast form.

The plasmid pADH-HYR1 was also transformed into *S. cerevisiae* W303-1B. Northern analysis revealed high levels of the *ADH1-HYR1* mRNA (Fig. 7), consistent with a high plasmid copy number, generated by the 2  $\mu$ m sequences in the vector and the efficient expression of the *C. albicans* *ADH1* gene in *S. cerevisiae* (3). Again, no obvious phenotype in the

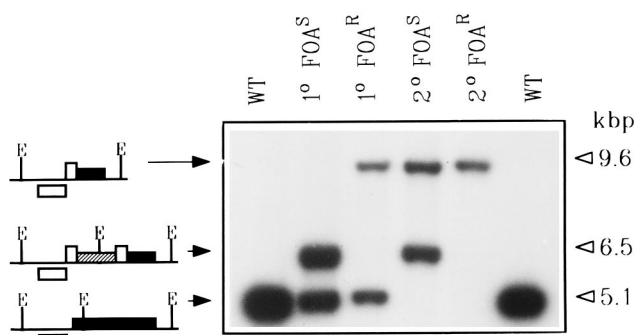


FIG. 5. Disruption of the *C. albicans* *HYR1* locus. Southern analysis was performed on *EcoRI* digested DNA from the parental strain CAI4 (lanes WT), a first-round *Ura3*<sup>+</sup> transformant (*HYR1* $\Delta hyr1::hisG::URA3::hisG$ ) (lane 1° FOA<sup>S</sup>), a first-round FOA-resistant segregant (*HYR1* $\Delta hyr1::hisG$ ) (lane 1° FOA<sup>R</sup>), a second-round *Ura3*<sup>+</sup> transformant ( $\Delta hyr1::hisG::URA3::hisG/\Delta hyr1::hisG$ ) (lane 2° FOA<sup>S</sup>), and a second-round FOA-resistant segregant ( $\Delta hyr1::hisG/\Delta hyr1::hisG$ ) (lane 2° FOA<sup>R</sup>). The blot was probed with the radiolabelled 5' *HindIII*-*BglII* fragment from pHYR1 (Fig. 1). The lengths of the *EcoRI* fragments detected are shown to the right of the gel. Cartoons representing wild-type and disrupted loci are shown opposite the corresponding *EcoRI* fragments, on the left. Black boxes, *HYR1* coding region; horizontal lines, *HYR1* 5' and 3' regions; boxes below cartoons, *HYR1* probe; white rectangles, *hisG* sequences; hatched rectangle, *CaURA3* sequence; E, *EcoRI* site.

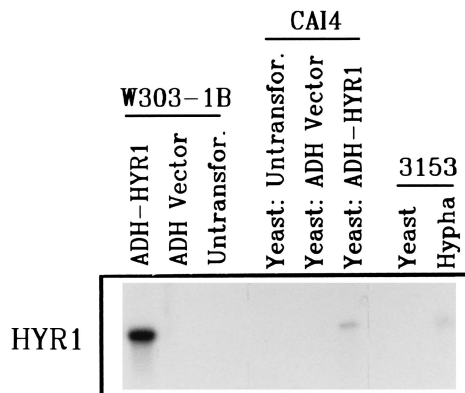


FIG. 7. *HYR1* mRNA levels in *C. albicans* and *S. cerevisiae* transformed with pADH-HYR1. Northern analysis of the *HYR1* mRNA was performed on approximately 5  $\mu$ g of RNA: *S. cerevisiae* W303-1B transformed with pADH-HYR1 (ADH-HYR1), W303-1B transformed with the empty *ADH1* expression vector (ADH Vector), untransformed W303-1B (Untransfor.), untransformed *C. albicans* CAI4 growing in the yeast form (Yeast: Untransfor.), CAI4 transformed with the empty *ADH1* expression vector and growing in the yeast form (Yeast: ADH Vector), CAI4 transformed with pADH-HYR1 and growing in the yeast form (Yeast: ADH-HYR1), and *C. albicans* 3153A growing in the yeast form (yeast) and the hyphal form (Hypha). All lanes in this composite figure were taken from the same Northern gel; an underexposed autoradiograph was used because of the high *HYR1* mRNA levels in *S. cerevisiae*.

pADH-HYR1 transformants was observed. *HYR1* expression had no significant effect upon their growth rate, did not force the growth of pseudohyphae (11, 23), and did not promote increased self-agglutination of yeast cells.

## DISCUSSION

We isolated and characterized the *HYR1* gene because it is one of only three morphogenetically regulated *C. albicans* genes characterized to date. We define these as genes that have been shown to be expressed specifically in response to the yeast-to-hypha transition. *HYR1* fulfils this criterion, the mRNA being detected only in cultures forming germ tubes and expression being independent of the method used to induce hyphal growth (Fig. 2). A recent report describes *HWP1* which encodes a hypha-specific cell surface protein that differs from *Hyr1p* in several respects (63). For example, *Hwp1p* contains numerous proline- and glutamate-rich repeats and does not appear to be N glycosylated. The expression of *ECE1*, which encodes a nonessential intracellular protein of unknown function, correlates with the elongation of hyphal cells, irrespective of the induction conditions (4).

Other *C. albicans* genes are induced during hyphal development, but only under certain specific conditions. These include the pH-responsive gene *PHR1* (54), the serum-induced genes *SAP2*, *SAP4*, *SAP5*, and *SAP6*, encoding secretory aspartyl proteinases (29), and the *ALSI* gene, which is induced in the defined medium RPMI (28). The expression of many other *C. albicans* genes fluctuates in response to the inducing conditions during the yeast-to-hypha transition rather than to morphogenesis itself. These genes encode proteins involved in wide-ranging functions such as glycolysis (*ADH1*, *PYK1*, *GPM1*, and *PGK1* [67]), protein synthesis and folding (*TEF3*, *RP10*, and *HSP90* [65, 66, 69]), cell wall biosynthesis (*CHS1*, *CHS2*, *CHS3*, and *GFA1* [10, 24, 59]), and formation of the cytoskeleton (*ACT1* [13, 66]). We know of only one *C. albicans* mRNA (*HST7*) that remains at relatively constant levels during the yeast-to-hypha transition. This low-abundance mRNA encodes a mitogen-activated protein kinase homolog probably involved in intracellular signalling (11). These findings support the conclusion, based on a two-dimensional gel analysis of proteins from the yeast and hyphal cells, that only a small number of *C. albicans* proteins are form specific (20).

The predicted amino acid sequence of *Hyr1p* has provided some clues about its possible function. Although no homologs exist in the databases, *Hyr1p* shares several features with known yeast cell wall proteins, namely, a putative N-terminal signal sequence, a putative C-terminal GPI-anchoring domain, and a serine- and threonine-rich domain (Fig. 8). The *S. cerevisiae* proteins *Gas1p* (43, 74), *Flo1p* (70), *Agα1p* (37, 79), *Aga1p* (47), and *Cwp1p*, *Cwp2p*, and *Tip1p* (77) all contain these features, as does *Phr1p* from *C. albicans* (54). All of these proteins reside at the cell surface.

*Gas1p* and *Phr1p* are structural (43, 74) and functional (75) homologs. *C. albicans*  $\Delta$ *phr1*/ $\Delta$ *phr1* mutants display reduced virulence (22) as well as defects in the formation of germ tubes and yeast buds: enlarged, rounded, and multibudded cells are formed at the restrictive pH (54). This conditional phenotype is observed at the alkaline pHs at which *PHR1* is normally expressed. Deletion of *GAS1* yields similar, if unconditional, morphological defects in *S. cerevisiae* (46) which may arise indirectly through the aberrant targeting or processing of other proteins (43). Significantly, *GAS1* has been shown to encode an abundant N-glycosylated and O-glycosylated cell surface protein (76), the function of which is dependent on its C-terminal GPI-anchoring domain (43).

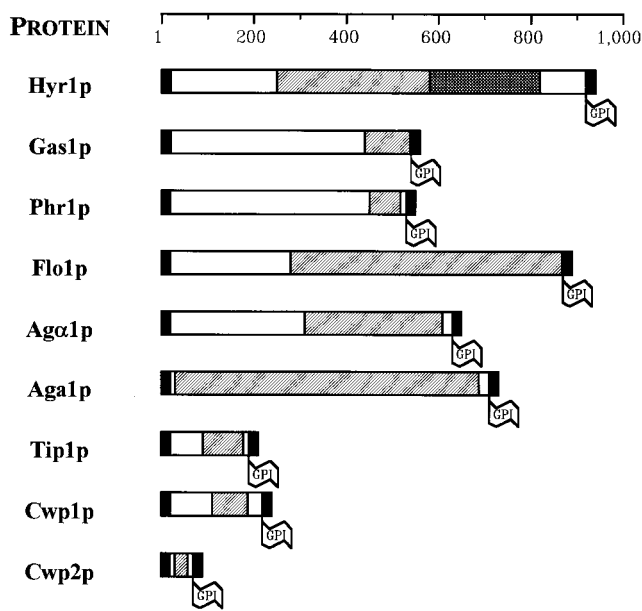


FIG. 8. *Hyr1p* belongs to a family of yeast cell surface proteins containing N-terminal signal sequences, C-terminal GPI-anchoring domains, and serine- and threonine-rich regions. Each protein sequence is represented diagrammatically by a rectangle (approximately to scale [see scale, in amino acid residues, above constructs]; the N terminus is oriented to the left). Signal sequences are represented by the solid boxes at the N termini, and GPI-anchoring domains are illustrated at the C termini as solid boxes with the flag (GPI) below. Serine- and threonine-rich regions are shown as hatched boxes. More than 40% of the residues in these domains are serine or threonine. *Hyr1p* contains a second region (shaded box) rich in asparagine, serine, and glycine (79% of residues) (Fig. 3). The proteins shown are discussed in references as follows: *Hyr1p*, this work; *Gas1p*, Nuoffer et al. (43); *Phr1p*, Saporito-Irwin et al. (54); *Flo1p*, Teunissen et al. (70); *Agα1p*, Lipke et al. (37); *Aga1p*, Roy et al. (47); and *Tip1p*, *Cwp1p*, and *Cwp2p*, Van Der Vaart et al. (77).

*S. cerevisiae* *Cwp1p*, *Cwp2p*, and *Tip1p* contain the three main features associated with the family of cell wall proteins shown in Fig. 8, and all three proteins have been shown to correspond to specific glucanase-extractable cell wall proteins (77). Disruption of the *CWPI*, *CWP2*, or *TIP1* gene generates cell wall phenotypes of various intensities, with the *cwp2* mutant showing a significant decrease in the thickness of the electron-dense mannoprotein layer of the yeast cell wall (77).

*Flo1p* is thought to reside at the *S. cerevisiae* cell surface, stimulating flocculation (or self-aggregation). This protein carries an N-terminal signal sequence and a GPI-anchoring domain and is serine and threonine rich (70). Several repeated motifs appear in the amino acid sequence, one of which might be associated with *FLO1* deletions that affect the ability of host cells to flocculate.

The C-terminal region of *S. cerevisiae*  $\alpha$ -agglutinin (encoded by the *AGA1* gene [37]) can direct a heterologous protein to the yeast cell wall (56). Significantly, this C-terminal region is serine and threonine rich and contains a GPI-anchoring domain (37, 79). *S. cerevisiae* *Aga1p* carries analogous domains (47). This protein appears to be required for the cell surface attachment of  $\alpha$ -agglutinin since both  $\alpha$ -agglutinin and the  $\alpha$ -agglutinin binding subunit are still secreted by *aga1* mutants. Hence, *AGA1* is thought to encode a core subunit of  $\alpha$ -agglutinin, located at the cell surface (47). A related gene in *C. albicans* has been identified (28). This gene (*ALSI*) appears to encode a secreted, GPI-anchored, serine- and threonine-rich protein which might play a role in the adherence of this asexual yeast to host tissue. However, since no supportive biochemical

or genetic data regarding its cell surface location are available (28), Als1p has not been included in Fig. 8. Nevertheless, it seems likely that Als1p, like Hyr1p, might belong to this family of cell surface glycoproteins.

Hyr1p displays the key features of the protein family shown in Fig. 8, including the serine- and threonine-rich domain which is probably O glycosylated. However, Hyr1p does not show significant sequence similarity to any members of this protein family, and Hyr1p differs from them with respect to the additional asparagine-, glycine-, and serine-rich domain near its C terminus. This domain contains seven copies of the repeated sequence asparagine-glutamate-glycine-serine (NEGS), the significance of which is not known, and it includes numerous potential sites for N glycosylation (Fig. 8). We speculate, therefore, that *HYR1* encodes a hypha-specific cell surface glycoprotein. By analogy with other well-characterized yeast cell wall proteins, Hyr1p might enter the secretory pathway where it might become decorated by N glycosylation and O glycosylation (32) and attached to the membrane via a GPI anchor (19, 73).

The lack of obvious phenotypes for the *HYR1* mutants is not inconsistent with the idea that Hyr1p is a structural component of the hyphal cell wall. The lack of an obvious phenotype for the *C. albicans*  $\Delta$ *hyr1*/ $\Delta$ *hyr1* mutant can be explained if Hyr1p provides a nonessential function for the construction of hyphal cell walls, or if, like *CHS2* (25), *HYR1* provides a function overlapping with that of some other cell wall component. Similarly, the aberrant expression of a structural protein (when the *ADH1-HYR1* fusion was used) was unlikely to force hyphal development under conditions which favor the growth of *C. albicans* yeast cells. Finally, the fact that the *ADH1-HYR1* fusion did not affect the self-agglutination of *C. albicans* or *S. cerevisiae* yeast cells suggests that Hyr1p is distinct from a previously isolated *C. albicans* genomic fragment which can promote the self-aggregation of *S. cerevisiae* cells (2).

Whatever the function of *HYR1*, the tight regulation of this gene in response to the yeast-to-hypha transition provides an important handle on morphogenetic regulation in *C. albicans*. Hence, the focus is now on the dissection of the *HYR1* promoter and the identification of the regulatory factors which mediate its transcriptional activation.

#### ACKNOWLEDGMENTS

We are grateful to M. Payton for the *C. albicans* genomic library and to W. Fonzi and S. Scherer for plasmids. We thank Rolf Swoboda for providing the *C. albicans* RNAs and Michael Ferguson and Mark Egerton for helpful discussions.

This work was supported by the Biotechnology and Biological Research Council, United Kingdom (grant 031097) and the Wellcome Trust (grant 039643). N.A.R.G. has been awarded a Royal Society of Edinburgh/Caledonian Research Foundation Fellowship, and A.J.P.B. has been awarded an Aberdeen University Research Fellowship.

#### REFERENCES

- Alani, E., L. Cao, and N. Kleckner. 1987. A method for gene disruption that allows repeated use of *URA3* selection in the construction of multiply disrupted yeast strains. *Genetics* **116**:541-545.
- Barki, M., Y. Koltin, M. Yanko, A. Tamarkin, and M. Rosenberg. 1993. Isolation of a *Candida albicans* DNA sequence conferring adhesion and aggregation on *Saccharomyces cerevisiae*. *J. Bacteriol.* **175**:5683-5689.
- Bertram, G., I. D. Broadbent, P. J. F. Feldman, S. Black, and A. J. P. Brown. Unpublished data.
- Bertram, G., R. K. Swoboda, N. A. R. Gow, G. W. Gooday, and A. J. P. Brown. 1996. Structure and regulation of the *Candida albicans* *ADH1* gene encoding an immunogenic alcohol dehydrogenase. *Yeast* **12**:115-128.
- Birse, C. E., M. Y. Irwin, W. A. Fonzi, and P. S. Sypherd. 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.
- Brown, A. J. P. 1994. RNA extraction and mRNA quantitation in *Candida albicans*. In B. Maresca and G. S. Kobayashi (ed.), *Molecular biology of pathogenic fungi: a laboratory manual*, p. 127-134. Telos Press, New York.
- Buffo, J., M. A. Herman, and D. R. Soll. 1984. A characterization of pH regulated dimorphism in *Candida albicans*. *Mycopathologia* **86**:21-30.
- Calderone, R. A. 1993. Recognition between *Candida albicans* and host cells. *Trends Microbiol.* **1**:55-58.
- Calderone, R. A., and P. C. Braun. 1991. Adherence and receptor relationships of *Candida albicans*. *Microbiol. Rev.* **55**:1-20.
- Casanova, M., J. L. Lopez-Ribot, J. P. Martinez, and R. Sentandreu. 1992. Characterization of cell wall proteins from yeast and mycelial cells of *Candida albicans* by labelling with biotin: comparison with other techniques. *Infect. Immun.* **60**:4898-4906.
- Chen-Wu, J. L., J. Zwicker, A. R. Bowen, and P. W. Robbins. 1992. Expression of chitin synthase genes during yeast and hyphal growth phases of *Candida albicans*. *Mol. Microbiol.* **6**:497-502.
- Clark, K. L., P. J. F. Feldmann, D. Dignard, 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**:609-621.
- Cutler, J. E. 1991. Putative virulence factors of *Candida albicans*. *Annu. Rev. Microbiol.* **45**:187-218.
- Delbruck, S., and J. F. Ernst. 1993. Morphogenesis-independent regulation of actin transcript levels in the pathogenic yeast *Candida albicans*. *Mol. Microbiol.* **10**:859-866.
- Devereux, J., P. Haeberli, and O. Smithies. 1984. A comprehensive set of sequence analysis programs for the VAX. *Nucleic Acids Res.* **12**:387-395.
- Elorza, M., V. A. Murgui, and R. Sentandreu. 1985. Dimorphism in *Candida albicans*: contribution of mannoproteins to the architecture of yeast and mycelial cells. *J. Gen. Microbiol.* **131**:2209-2216.
- Elorza, M., V. A. Murgui, and R. Sentandreu. 1988. Wall mannoproteins of the yeast and mycelial cells of *Candida albicans*: nature of the glycosidic bonds and polydispersity of their mannan moieties. *J. Gen. Microbiol.* **134**:2393-2403.
- Feinberg, A. P., and B. Vogelstein. 1983. A technique for radiolabelling DNA restriction endonuclease fragments to high specific activity. *Anal. Biochem.* **132**:6-13.
- Feldmann, P. J. F. 1994. Isolation of genes involved in signal transduction in *Candida albicans*. Ph.D. thesis. University of Aberdeen, Aberdeen, Scotland.
- Ferguson, M. A. J. 1988. Cell surface anchoring of proteins via glycosylphosphatidylinositol structures. *Annu. Rev. Biochem.* **57**:285-320.
- Finney, R., C. J. Langtimm, and D. R. Soll. 1985. The programs of protein synthesis accompanying the establishment of alternative phenotypes in *Candida albicans*. *Mycopathologia* **91**:3-15.
- Fonzi, W. A., and M. Y. Irwin. 1993. Isogenic strain construction and gene mapping in *Candida albicans*. *Genetics* **134**:717-728.
- Ghannoum, M. A., B. Spellberg, S. M. Saporito-Irwin, and W. A. Fonzi. 1995. Reduced virulence of *Candida albicans* *PHR1* mutants. *Infect. Immun.* **63**:4528-4530.
- Gimeno, C. J., P. O. Ljungdahl, 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**:1077-1090.
- Gow, N. A. R., B. Hube, D. A. Schofield, C. Munro, R. K. Swoboda, G. Bertram, C. Westwater, I. Broadbent, R. J. Smith, G. W. Gooday, and A. J. P. Brown. 1995. Genes associated with dimorphism and virulence of *Candida albicans*. *Can. J. Bot.* **73**:S335-S342.
- Gow, N. A. R., P. W. Robbins, J. W. Lester, A. J. P. Brown, W. A. Fonzi, T. Chapman, and O. S. Kinsman. 1994. A hyphal specific chitin synthase gene (*CHS2*) is not essential for growth, dimorphism or virulence of *Candida albicans*. *Proc. Natl. Acad. Sci. USA* **91**:6216-6220.
- Hoffman, C. S., and F. Winston. 1987. A ten minute DNA preparation from yeast efficiently releases autonomous plasmids for transformation of *Escherichia coli*. *Gene* **57**:267-272.
- Hostetter, M. K. 1994. Interactions of *Candida albicans* with eukaryotic cells. *ASM News* **60**:370-374.
- Hoyer, L. L., S. Scherer, A. R. Shatzman, and G. P. Livi. 1995. *Candida albicans* ALS1: domains related to a *Saccharomyces cerevisiae* sexual agglutinin separated by a repeating motif. *Mol. Microbiol.* **15**:39-54.
- Hube, B., M. Monod, D. A. Schofield, A. J. P. Brown, and N. A. R. Gow. 1994. Transcriptional regulation of six members of the gene family encoding secretory aspartyl proteinases in *Candida albicans*. *Mol. Microbiol.* **14**:87-99.
- Ito, H., Y. Fukuda, K. Murata, and A. Kimura. 1983. Transformation of intact yeast cells treated with alkali cations. *J. Bacteriol.* **153**:163-168.
- Kapteyn, J. C., R. C. Montijn, G. J. P. Dijkgraaf, and F. M. Klis. 1994. Identification of  $\beta$ -1,6-glucosylated cell wall proteins in yeast and hyphal forms of *Candida albicans*. *Eur. J. Cell Biol.* **65**:402-407.
- Klis, F. M. 1994. Cell wall assembly in yeast. *Yeast* **10**:851-869.
- Kurtz, M. B., M. W. Cortelou, and D. R. Kirsch. 1986. Integrative transformation of *Candida albicans*, using a cloned *Candida ADE2* gene. *Mol. Cell. Biol.* **6**:142-149.
- Lee, K. L., H. R. Buckler, and C. C. Campbell. 1975. An amino acid liquid synthetic medium for the development of mycelial and yeast forms of *Candida albicans*. *J. Gen. Microbiol.* **86**:21-30.

- didia albicans*. Sabouraudia 13:148–153.
35. Lillehaug, J. R., and K. Kleppe. 1975. Effect of salts and polyamines on T4 polynucleotide kinase. Biochemistry 14:1225–1230.
  36. Lindquist, S. 1981. Regulation of protein synthesis during heat shock. Nature (London) 293:311–314.
  37. Lipke, P. N., D. Wojciechowicz, and J. Kurjan. 1989. *AGα1* is the structural gene for the *Saccharomyces cerevisiae* α-agglutinin, a cell surface glycoprotein involved in cell-cell interactions during mating. Mol. Cell. Biol. 9:3155–3165.
  38. Lopez-Ribot, J. L., M. Casanova, J. P. Martinez, and R. Sentandreu. 1991. Characterization of cell wall proteins of yeast and hydrophobic mycelial cells of *Candida albicans*. Infect. Immun. 59:2324–2332.
  39. Martin, M. V., G. T. Craig, and D. J. Lamb. 1984. An investigation of the role of true hypha production in the pathogenesis of experimental oral candidosis. J. Med. Vet. Mycol. 22:471–476.
  40. Matthews, R. C. 1994. Pathogenicity determinants of *Candida albicans*: potential targets for immunotherapy? Microbiology 140:1505–1511.
  41. Mattia, E., G. Carruba, L. Angiotella, and A. Cassone. 1982. Induction of germ tube formation by *N*-acetyl-D-glucosamine in *Candida albicans*: uptake of inducer and germination response. J. Bacteriol. 152:555–562.
  42. Moore, P. A., F. A. Sgliooco, R. M. C. Wood, and A. J. P. Brown. 1991. Yeast glycolytic mRNAs are differentially regulated. Mol. Cell. Biol. 11:5330–5337.
  43. Nuoffer, C., P. Jenő, A. Conzelmann, and H. Riezman. 1991. Determinants for glycopospholipid anchoring of the *Saccharomyces cerevisiae* *GAS1* protein to the plasma membrane. Mol. Cell. Biol. 11:27–37.
  44. Odds, F. C. 1988. *Candida* and candidosis: a review and bibliography, 2nd ed. Balliere Tindall, London.
  45. Odds, F. C. 1994. *Candida* species and virulence. ASM News 60:313–318.
  46. Popolo, L., M. Vai, E. Gatti, S. Porello, P. Bonfante, R. Balestrini, and L. Alberghina. 1993. Physiological analysis of mutants indicates involvement of the *Saccharomyces cerevisiae* glycoposphatidylinositol-anchored protein gp115 in morphogenesis and cell separation. J. Bacteriol. 175:1879–1885.
  47. Roy, A., C. F. Lu, D. L. Marykwas, P. N. Lipke, and J. Kurjan. 1991. The *AGA1* product is involved in cell surface attachment of the *Saccharomyces cerevisiae* cell adhesion glycoprotein α-agglutinin. Mol. Cell. Biol. 11:4196–4206.
  48. Ryley, J. F., and N. G. Ryley. 1990. *Candida albicans*—do mycelia matter? J. Med. Vet. Mycol. 28:225–239.
  49. Sambrook, J., E. F. Fritsch, and T. Maniatis. 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
  50. Sanger, F., S. Nicklen, and A. R. Coulson. 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA 74:5463–5467.
  51. Santiago, T. C., I. J. Purvis, A. J. E. Bettany, and A. J. P. Brown. 1986. The relationship between mRNA stability and length in *Saccharomyces cerevisiae*. Nucleic Acids Res. 14:8347–8360.
  52. Santos, M. A. S., G. Keith, and M. F. Tuite. 1993. Non-standard translational events in *Candida albicans* mediated by an unusual seryl-tRNA with a 5'-CAG-3' (leucine) anticodon. EMBO J. 12:607–616.
  53. Santos, M. A. S., and M. F. Tuite. 1995. The CUG codon is decoded *in vivo* as serine and not leucine in *Candida albicans*. Nucleic Acids Res. 23:1481–1486.
  54. Saporito-Irwin, S. M., C. E. Birse, P. S. Sypherd, and W. A. Fonzi. 1995. *PHR1*, a pH-regulated gene of *Candida albicans*, is required for morphogenesis. Mol. Cell. Biol. 15:601–613.
  55. Scherer, S., and P. T. Magee. 1990. Genetics of *Candida albicans*. Microbiol. Rev. 54:226–241.
  56. Schreuder, M. P., S. Brekelmans, H. Van den Ende, and F. M. Klis. 1993. Targeting of a heterologous protein to the cell wall of *Saccharomyces cerevisiae*. Yeast 9:399–409.
  57. Sherwood, J., N. A. R. Gow, G. W. Gooday, D. W. Gregory, and D. Marshall. 1992. Contact sensing in *Candida albicans*: a possible aid to epithelial penetration. J. Med. Vet. Mycol. 30:461–469.
  58. Smith, D. J., M. Cooper, M. DeTiani, C. Losberger, and M. A. Payton. 1992. The *Candida albicans* *PMM1* gene encoding phosphomannomutase complements a *Saccharomyces cerevisiae* *sec53-6* mutation. Curr. Genet. 22:501–503.
  59. Smith, R. J., S. Milewsky, A. J. P. Brown, and G. W. Gooday. 1996. Isolation and characterization of the *GFA1* gene encoding the glutamine:fructose-6-phosphate amidotransferase of *Candida albicans*. J. Bacteriol. 178:2320–2327.
  60. Sobel, J. D., G. Muller, and H. R. Buckley. 1984. Critical role of germ tube formation in the pathogenesis of candidal vaginitis. Infect. Immun. 44:576–580.
  61. Soll, D. R. 1986. The regulation of cellular differentiation in the dimorphic yeast *Candida albicans*. Bioessays 5:5–11.
  62. Srikantha, T., A. Klapach, W. W. Lorenz, L. K. Tsai, L. A. Laughlin, J. A. Gorman, and D. R. Soll. 1996. The sea pansy *Renilla reniformis* luciferase serves as a sensitive bioluminescent reporter for differential gene expression in *Candida albicans*. J. Bacteriol. 178:121–129.
  63. Staab, J. F., C. A. Ferrer, and P. Sundstrom. 1996. Developmental expression of a tandemly repeated, proline and glutamine-rich amino acid motif on hyphal surfaces of *Candida albicans*. J. Biol. Chem. 271:6298–6305.
  64. Sundstrom, P. M., M. R. Tam, E. J. Nichols, and G. E. Kenny. 1988. Antigenic differences in the surface mannoproteins of *Candida albicans* as revealed by monoclonal antibodies. Infect. Immun. 56:601–606.
  65. Swoboda, R. K., G. Bertram, S. Budge, G. W. Gooday, N. A. R. Gow, and A. J. P. Brown. 1995. Structure and regulation of the *HSP90* gene from the pathogenic fungus *Candida albicans*. Infect. Immun. 63:4506–4514.
  66. Swoboda, R. K., G. Bertram, D. R. Colthurst, M. F. Tuite, N. A. R. Gow, G. W. Gooday, and A. J. P. Brown. 1994. Regulation of the gene encoding translation elongation factor 3 during growth and morphogenesis in *Candida albicans*. Microbiology 140:2611–2616.
  67. Swoboda, R. K., G. Bertram, S. Delbruck, J. F. Ernst, N. A. R. Gow, G. W. Gooday, and A. J. P. Brown. 1994. Fluctuations in glycolytic mRNA levels during morphogenesis in *Candida albicans* reflect underlying changes in growth and not a response to cellular dimorphism. Mol. Microbiol. 13:663–672.
  68. Swoboda, R. K., G. Bertram, H. Hollander, D. Greenspan, J. S. Greenspan, N. A. R. Gow, G. W. Gooday, and A. J. P. Brown. 1993. Glycolytic enzymes of *Candida albicans* are nonubiquitous immunogens during candidiasis. Infect. Immun. 61:4263–4271.
  69. Swoboda, R. K., I. D. Broadbent, G. Bertram, S. Budge, G. W. Gooday, N. A. R. Gow, and A. J. P. Brown. 1995. Structure and regulation of a *Candida albicans* *RP10* gene which encodes an immunogenic protein homologous to *Saccharomyces cerevisiae* ribosomal protein 10. J. Bacteriol. 177:1239–1246.
  70. Teunissen, A. W. R. H., E. Holub, J. Van Der Hucht, J. A. Van Den Berg, and H. Y. Steensma. 1993. Sequence of the open reading frame of the *FL01* gene from *Saccharomyces cerevisiae*. Yeast 9:423–427.
  71. Thomas, B. J., and R. Rothstein. 1989. Elevated recombination rates in transcriptionally active DNA. Cell 56:619–630.
  72. Torosantucci, A., M. Boccanera, I. Casalnuovo, G. Pellegrini, and A. Cassone. 1990. Differences in the antigenic expression of immunomodulatory mannoprotein constituents on yeast and mycelial forms of *Candida albicans*. J. Gen. Microbiol. 136:1421–1428.
  73. Udenfriend, S., and K. Kodukula. 1995. How glycosylphosphatidylinositol-anchored membrane proteins are made. Annu. Rev. Biochem. 64:563–591.
  74. Vai, M., E. Gatti, E. Lacana, L. Popolo, and L. Alberghina. 1991. Isolation and deduced amino acid sequence of the gene encoding gp115, a yeast glycopospholipid-anchored protein containing a serine-rich region. J. Biol. Chem. 266:12242–12248.
  75. Vai, M., and L. Popolo. 1995. GPI-anchored proteins and cell wall organization, p. 176–187. In J.-P. Latge, R. Calderone, and H. Yamaguchi (ed.), Proceedings of Meeting on Morphogenesis and Metabolism in Opportunistic Fungal Pathogens. Poitiers, France.
  76. Vai, M., L. Popolo, R. Grandori, E. Lacana, and L. Alberghina. 1990. The cell cycle modulated glycoprotein PG115 is one of the major yeast proteins containing glycoposphatidylinositol. Biochim. Biophys. Acta 1038:277–285.
  77. Van Der Vaart, J. M., L. H. P. Caro, J. W. Chapman, F. M. Klis, and C. T. Verrips. 1995. Identification of three mannoproteins in the cell wall of *Saccharomyces cerevisiae*. J. Bacteriol. 177:3104–3110.
  78. White, T. C., L. E. Andrews, D. Maltby, and N. Agabian. 1995. The “universal” leucine codon CTG in the secreted aspartyl proteinase 1 (*SAP1*) gene of *Candida albicans* encodes a serine *in vivo*. J. Bacteriol. 177:2953–2955.
  79. Wojciechowicz, D., C.-F. Lu, J. Kurjan, and P. N. Lipke. 1993. Cell surface anchorage and ligand-binding domains of the *Saccharomyces cerevisiae* cell adhesion protein α-agglutinin, a member of the immunoglobulin superfamily. Mol. Cell. Biol. 13:2554–2563.