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PRR1, a Homolog of *Aspergillus nidulans palF*, Controls pH-Dependent Gene Expression and Filamentation in *Candida albicans*

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The pH of the environment has been implicated in controlling the yeast-hypha transition and pathogenesis of *Candida albicans*. Several *C. albicans* genes, including *PHR1* and *PHR2*, are pH dependent in their expression. To investigate the mechanism of pH-dependent expression, we have cloned and characterized *PRR1* (for pH response regulator). *PRR1* is homologous to *palF*, a component of the pH response pathway in *Aspergillus nidulans*. Expression of *PRR1* was itself pH dependent, being maximal at acid pH but reduced severalfold at alkaline pH. In a *prr1* null mutant the alkaline-induced expression of *PHR1* was completely abolished. Conversely, expression of *PHR2* was no longer repressed at alkaline pH. A *prr1* null mutant exhibited no morphological abnormalities at either pH; however, it lost the ability to form hyphae on medium 199 and on 10% serum plates. The ability to filament on serum was not restored by forced expression of *PHR1*, indicating that additional *PRR1*-dependent genes are required for hyphal development. These developmental genes appear to be distinct from those controlled by the developmental regulator *EFG1*, since the *EFG1*-dependent gene *HWP1* was expressed normally in the *prr1* null mutant. We conclude that *PRR1* encodes a component of the pH-dependent response pathway in *C. albicans* and that this pathway regulates the expression of multiple components of hyphal development.

Candida albicans is an opportunistic fungal pathogen of humans. It is the most frequent agent of superficial mucocutaneous infections, and, in immunocompromised hosts, it can cause life-threatening infections (38). The ability of this organism to cause infection depends, in part, on its ability to respond to changes in the pH of the environment (10). The pH response in *C. albicans* involves the differential expression of at least three genes, *PHR1*, *PHR2*, and *PRA1* (34, 42, 44). *PHR1* is an alkaline-expressed gene. It is highly expressed at between pH 7.0 and 8.0 but is not expressed at detectable levels at below pH 5.5 (42). *PHR2* is an acid-expressed gene with an inverse pattern of expression (34). Mutations in *PHR1* or *PHR2* result in pH-conditional defects in growth, morphogenesis, and virulence (10, 34, 42). The significance of these genes and the pH response in the biology of *C. albicans* has prompted an investigation of the mechanisms controlling these responses.

The control of pH-dependent gene expression has been most extensively studied with *Aspergillus nidulans*. Seven genes critical to this regulation have been identified. These include six *pal* genes, *palA*, *-B*, *-C*, *-F*, *-H*, and *-I*, and *pacC* (2, 9, 11, 12, 31, 37, 48). *pacC*, the terminal component of this regulatory pathway, encodes a transcription factor containing a zinc finger motif that directly induces expression of alkaline-expressed genes and indirectly represses acid-expressed genes (43, 48). The *pacC* protein, PacC, is synthesized in an inactive form that is proteolytically activated at alkaline pH (39). Proteolysis of PacC is dependent upon the *pal* genes, although their role in this process is unknown (2, 11, 12, 37, 39).

The available data suggests that this response pathway is conserved, at least in part, in other fungi. In *Yarrowia lipolytica* a *pacC* homolog has been identified and shown to control alkaline-induced gene expression (27). In addition, four *pal*-like genes have been defined by mutation analysis (27). In *Saccharomyces cerevisiae* the *pacC* homolog, *RIM101*, was initially identified as controlling meiosis and haploid invasiveness (29, 46). More recently *RIM101* and the yeast homology of *palB*, *CPL1*, have been implicated in a pH-dependent growth response of yeast (17). Genes homologous to *palA* and *palI* are also present in *S. cerevisiae* (11, 37). *C. albicans* sequences homologous to *palA* and *pacC* were recently reported (49). Null mutations in these genes affected morphological development, but it is not known if they affected the pH response (49).

To investigate the regulation of pH-dependent gene expression in *C. albicans*, we have isolated and characterized *PRR1* (for pH response regulator), the *C. albicans* homolog of *palF*. Expression of *PRR1* was pH dependent, and mutants lacking *PRR1* were defective in pH-dependent regulation of gene expression. *PHR1* was no longer induced at alkaline pH, and *PHR2* was no longer repressed. Thus, *PRR1* is a component of the pH response pathway in *C. albicans*. In addition, mutation of *PRR1* resulted in a medium-conditional loss of hyphal development. This defect was not related to the altered expression of *PHR1*, suggesting that this pathway controls development-specific functions. This control was shown to be independent of the *EFG1*-dependent regulation of hyphal development.

MATERIALS AND METHODS

Strains and growth conditions. The *C. albicans* strains utilized in this study and their genotypes are listed in Table 1. The strains were routinely cultured on either YPD (1% yeast extract, 2% Bacto Peptone, 2% glucose) or YNB (2% glucose, 0.67% Difco yeast nitrogen base) at 30°C. The effects of culture pH were assessed in medium 199 containing Earle's salts and glutamine but lacking sodium bicarbonate (Gibco-BRL) and containing 155 mM Tris or 150 mM HEPES adjusted to pH 7.5 or 4.0. To induce germ tube formation in liquid culture, yeast cells were inoculated at a density of 6×10^6 cells/ml into medium 199 (pH 7.5), Spider medium (30), the medium of Lee et al. (28), or 10% fetal calf serum (Difco) and incubated at 37°C with vigorous agitation on a rotary shaker. These same media were solidified with 2% agar to assay filamentation. Solidified media were spotted with 10^6 cells in 5 μl of water and incubated at 37°C for 3 to 6 days. To test for invasive hyphal growth, the surface of the plates was washed with sterile distilled water (20). In all assays, stationary-phase cells grown at 25°C in YPD were used as the inoculum. Media were supplemented

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TABLE 1. *C. albicans* strains used in this study

Strain	Parental strain	Genotype	Source or reference
SC5314		Clinical isolate	19
CAI4		*Δura3::imm434/Δura3::imm434*	16
CAI12	CAI4	*Δura3::imm434/URA3*	This work
CAPM1	CAI4	*Δprr1::hisG-URA3-hisG/PRR1 Δura3::imm434*/Δura3::imm434	This work
CAPM2	CAPM1	*Δprr1::hisG/PRR1 Δura3::imm434/Δura3::imm434*	This work
CAPM3	CAPM2	*Δprr1::hisG/Δprr1::hisG-URA3-hisG Δura3::imm434/Δura3::imm434*	This work
CAPM4	CAPM3	*Δprr1::hisG/Δprr1::hisG Δura3::imm434/Δura3::imm434*	This work
CAPM5	CAPM4	*PRR1*-pUC18-*URA3-Δprr1::hisG/Δprr1::hisG Δura3::imm434/Δura3::imm434*	This work
CAPM6	CAPM4	*Δprr1::hisG/Δprr1::hisG* TEF1pr::*PHR1::URA3 Δura3::imm434/Δura3::imm434*	This work
CAR4	CAR26	*Δprr2::hisG/Δprr2::hisG* TEF1pr::*PHR1::URA3 Δura3::imm434/Δura3::imm434*	This work

with uridine (25 μg/ml) when necessary. Ura$^-$ auxotrophs were selected on medium containing 5′-fluoro-orotic acid (5′-FOA) as described previously (6, 16). Cell and colony morphologies were assessed by light microscopy.

Identification and cloning of *PRR1*. Sequence data for *C. albicans* was obtained from the Stanford DNA Sequencing and Technology Center website (45a). A BLASTN search of the *C. albicans* genome sequences was conducted by using the sequence of *A. nidulans palF* as a query (1, 32). This identified a partial sequence related to *palF*, and the corresponding gene was named *PRR1* (for pH response regulator). This region was amplified by PCR with genomic DNA from SC5314 as a template and the primers 5′-AGTGATGATTTGGTTGCTTG-3′ and 5′-CGGATTTGGGATAGGTTC-3′. After 33 cycles consisting of incubations at 95°C for 60 s, 48°C for 45 s, and 72°C for 120 s, the amplified product was gel purified with the Geneclean II Kit (Bio 101 Inc.) and used as a probe for hybridization screening of a *C. albicans* λGEM-12 genomic library (5). About 25,000 phage plaques were blotted onto nylon filters (Hybond N+; Amersham) and hybridized with digoxigenin-labeled probe. The probe was labeled and hybridization was detected with the DIG DNA Labeling and Detection Kit (Boehringer Mannheim Biochemicals). Positive plaques were purified and characterized by restriction endonuclease mapping. A 6-kb *Bam*HI DNA fragment, which hybridized to the PCR product, was gel purified and subcloned into pUC18 to generate plasmid pAP1.

DNA sequence analysis. Nucleotide sequencing was performed by PCR with the dRhodamine terminator cycle sequencing Ready Reaction kit with AmpliTaq DNA polymerase, FS (Perkin-Elmer), and custom-synthesized oligonucleotide primers. Nucleotide and protein sequence analyses were conducted with DNA Strider (33) and LaserGene (DNASTAR). Homology searches of the nonredundant GenBank database were done by using the BLAST algorithm (1). Multiple-sequence alignments were constructed by using Clustal W 1.6 (47).

Strain constructions. To construct a *prr1* null mutant, a 2,475-bp *Eco*RI fragment was isolated from pAP1 and subcloned into the *Eco*RI site of pUC18 to generate plasmid pAP2. This fragment contains 1,069 bp of sequence upstream of the coding region and all but the last 456 bp of the coding region. A 1,074-bp region flanked by *Nsi*I-*Bgl*II sites was deleted from pAP2 and replaced with a 3.8-kb *Bgl*II-*Pst*I fragment from plasmid pMB-7 containing *hisG-URA3-hisG* (16). The resulting plasmid, pAP3, was digested with *Ban*II, releasing a 4,428-bp DNA fragment containing the *hisG-URA3-hisG* fragment flanked by 478 bp of *PRR1* sequence on the 5′ end and 94 bp on the 3′ end. Approximately 5 μg of transforming DNA was used to sequentially disrupt both *PRR1* alleles in strain CAI4 by previously published methods (16) except that transformation was performed by the lithium acetate method (18).

To obtain a reconstituted strain, a 2.5-kb *Sst*I-*Kpn*I fragment from plasmid pAP1 was purified and ligated into the *Sst*I-*Kpn*I sites of plasmid pSMS-44 (42), which contains the *Eco*RV-*Xba*I fragment of *URA3*. The resulting plasmid, designated pAP4, was linearized at the unique *Afl*II site 832 bp upstream of *PRR1* and used to transform a Ura$^-$ *Δprr1* strain.

To restore *PHR1* expression in the *prr1* null mutant, plasmid pSMS-43 (42), containing the *C. albicans* EF-1α promoter driving expression of the *PHR1* gene, was linearized at the unique *Hpa*I site within the *URA3* sequences and used to transform strain CAPM4 to Ura$^+$, generating strain CAPM6. An analogous construct was obtained in a *prr2* null mutant, CAR26 (41). Constitutive expression of *PHR1* was verified for both strains by Northern blot analysis. The integration events in all transformants were characterized by Southern blot analysis.

The control strain CAI12 was constructed by transformation of strain CAI4 with a *Pst*I-*Bgl*II fragment containing *URA3* (26) to restore one allele at the *URA3* locus.

Southern blot analysis. Genomic DNA for Southern analysis was prepared as previously described (25). About 5 μg of genomic DNA was digested to completion with *Bam*HI or double digested with *Nsi*I and *Bgl*II and electrophoresed through a 0.8% agarose gel. The fractionated DNA was transferred to positively charged nylon membranes (Hybond N+; Amersham), and the membranes were fixed by UV irradiation. The blots were prehybridized and hybridized in 1× phosphate buffer solution (0.5 M sodium phosphate [pH 7.2], 5% sodium dodecyl sulfate, 10 mM EDTA) at 65°C. A 1.7-kb *Nsi*I fragment of plasmid pAP1 was labeled by random priming with the DNA Ready-to-Go Labeling Beads (−dCTP) Kit and [α-^{32}P]dCTP (Amersham Pharmacia Biotech Inc.) and used as a probe. Following hybridization, the filters were washed three times for 15 min each in 0.1× phosphate buffer and exposed to X-ray film, using intensifying screens, at −70°C overnight.

Northern blot analysis. To obtain RNA samples, cells were grown to stationary phase in YPD at 25°C and used to inoculate 300 ml of medium 199, pH 7.5 or 4.0, at a density of 6×10^6 cells/ml. The cultures were incubated with vigorous agitation at 25°C for 2 h. Yeast cells were collected by centrifugation at 4,000 × *g* for 10 min and washed with ice-cold sterile distilled water. Total RNA was prepared by selective precipitation with LiCl as previously described (40). Samples containing 10 μg of RNA were fractionated by electrophoresis in agarose gels containing MOPS (morpholinepropanesulfonic acid) and formaldehyde essentially as described previously (7) except that 2.2% formaldehyde was included in the running buffer. The gels were blotted and hybridized as described for Southern blot analysis. The blots were hybridized with the 867-bp PCR fragment containing part of the *PRR1* open reading frame, a 1,063-bp *Aat*II-*Nde*I fragment of *PHR1* (42), a 1,257-bp *Bam*HI-*Nhe*I fragment of *PHR2* (34), or a 4.3-kb *Eco*RI fragment of *HWP1* (45). The blots were subsequently stripped in boiling 0.1% sodium dodecyl sulfate and hybridized with a 1.9-kb *Sal*I fragment of *ACT1* DNA. Hybridization intensity was quantified by using a model 445SI PhosphorImager (Molecular Dynamics) and ImageQuant software and normalized to *ACT1* mRNA. All Northern data was confirmed with at least two independent RNA preparations. Transcript sizes were approximated by comparison with rRNA.

RESULTS

Identification and cloning of the *palF* ortholog. Several *C. albicans* genes are differentially expressed in relation to the ambient pH of the culture medium (34, 42, 44). The response to pH affects in vitro dimorphism and is critical to virulence (3, 8, 10, 14). To investigate the mechanism(s) that controls the pH response in *C. albicans*, we searched for homologs of the genes that control pH responsiveness in *A. nidulans* (48). By using the nucleotide sequence of *palF* (32) in a BLASTN search of the *C. albicans* genome sequences, a short homologous sequence of 867 nucleotides was identified. This fragment encoded a putative peptide 27% identical and 44% similar to PalF, the *palF* protein. A genomic clone was isolated, and the nucleotide sequence of the corresponding region was determined. Within the 3,336 bp of sequence obtained, a single extended open reading frame of 1,863 bp was identified. The predicted 621-amino-acid protein was 24% identical to PalF (Fig. 1), and the identity was distributed along the entire lengths of the proteins. On the basis of this homology and the potential role of the gene in the pH response, the gene was designated *PRR1* (for pH response regulator). A BLASTP search of the nonredundant GenBank database revealed no other related genes except for two adjacent open reading frames in the *S. cerevisiae* genome, YGLO46w (accession no. Z72568) and YGLO45w (accession no. Z72567), as previously noted for *palF* (32) (Fig. 1). No functional motifs were identified in a search of the BLOCKS (21, 22) and PROSITE (4) databases. However, a potential nuclear localization signal,

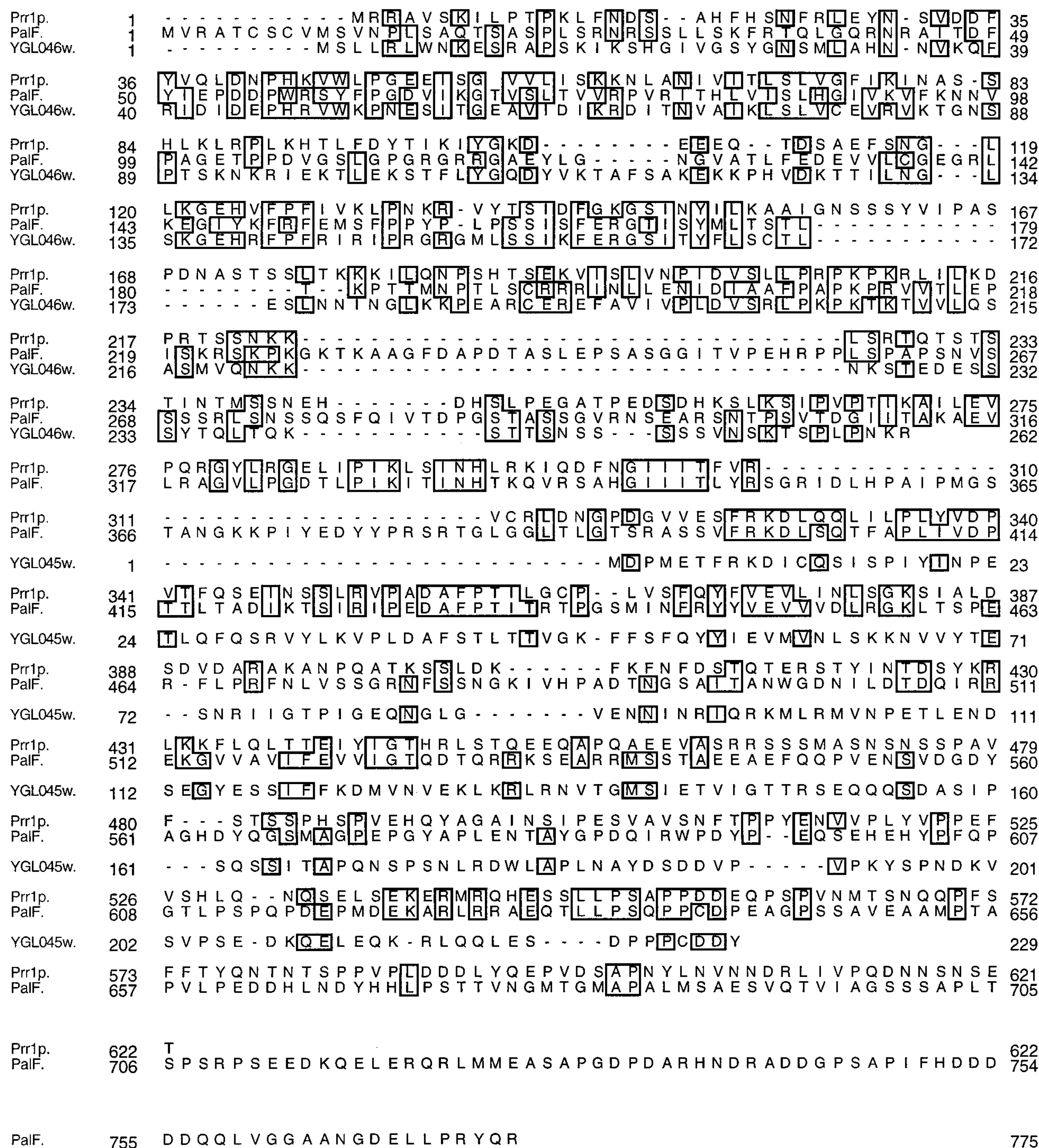

FIG. 1. Alignment of the amino acid sequences of Prr1p, PalF, YGL045w, and YGL046w. Identical residues are boxed.

PRPKPKR, located between positions 205 and 211 (23), was identified by PSORT II (35).

Transcription of *PRR1*. Northern analysis was performed to define the conditions under which *PRR1* is expressed. Because of the potential role of *PRR1* in the pH response, the effect of ambient pH on *PRR1* expression was examined. The probe hybridized with a single transcript of approximately 2 kb, consistent with the size of the predicted open reading frame (Fig. 2). The intensity of this band was maximal at pH 4.0 and was reduced about threefold at pH 7.5 when normalized against actin mRNA. Thus, expression of *PRR1* was pH dependent and elevated at acidic pH. The pH dependence of *PRR1* expression was further confirmed by the effect of *PRR2* mutations on *PRR1* expression, as discussed in the accompanying paper (41).

***PRR1* is required for pH-dependent gene expression.** Recessive mutations in *palF* mimic the effect of growth at acid pH by blocking the induction of alkaline-expressed genes and preventing the repression of acid-expressed genes under alkaline growth conditions (9, 48). If *PRR1* is a functional homolog of *palF*, then a *prr1* null mutant should have a similar phenotype. To test this possibility, *PRR1* mutants were constructed (16),

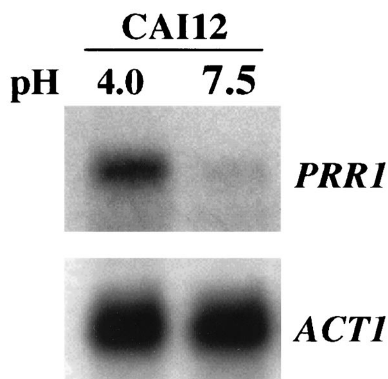

FIG. 2. Expression of *PRR1* as a function of ambient pH. RNA was prepared from strain CAI12 cultured at pH 4.0 or 7.5. A Northern blot of these samples was hybridized with either *PRR1* or *ACT1* DNA as indicated.

and expression of *PHR1*, an alkaline-expressed gene, and *PHR2*, an acid-expressed gene, was examined in the mutants.

The mutants were constructed by targeted gene replacement. The cells were transformed with a deletion-and-replacement construct in which a 1,074-bp segment of *PRR1* was removed and replaced with a *hisG-URA3-hisG* cassette (Fig. 3). Southern blot analysis of a representative Ura$^+$ transformant, strain CAPM1, demonstrated the presence of a 1.7-kb *Nsi*I-*Bgl*II band characteristic of the wild-type *PRR1* and of the predicted 5.5-kb band representing the disrupted allele (Fig. 3). After 5′-FOA selection, the latter band was reduced to 2.8 kb, as shown in the representative heterozygous mutant CAPM2 (Fig. 3). Transformation of CAPM2 yielded a homozygous null mutant, CAPM3, in which the 1.7-kb fragment was absent and replaced by the 5.5-kb fragment, indicative of the replacement event (Fig. 3). Northern analysis confirmed the absence of a *PRR1* transcript in the null mutant CAPM3 (data not shown).

A wild-type *PRR1* allele was introduced into a *prr1* null mutant by targeted integration of plasmid pAP4 in strain CAPM4. Strain CAPM4 is a Ura$^-$ strain derived from CAPM3 by 5′-FOA selection. Southern blot analysis of *Bam*HI-digested genomic DNA revealed one hybridizing band of about 8.5 kb in the control strain, CAI12. DNA from the transformed strain, CAPM5, showed this band as well as an approximately 14-kb band corresponding to the reconstructed allele (Fig. 3). Expression of *PRR1* in the revertant was confirmed by Northern analysis (data not shown).

The effect of *PRR1* mutation on pH-dependent gene expression was examined by Northern blot analysis. The various mutants were cultured at either acidic pH or alkaline pH, and RNA from these cells was hybridized with either *PHR1* (42) or *PHR2* (34). In the control strain CAI12, the expected expression pattern of *PHR1* was observed (Fig. 4). *PHR1* was abundant in cells grown at pH 7.5 but undetectable in cells grown at pH 4.0. The same pattern was exhibited by strain CAPM1, which lacks one allele of *PRR1*. In contrast, *PHR1* was not expressed in the homozygous null mutant, CAPM3, cultured at alkaline pH (Fig. 4). Alkaline-induced expression was restored upon introduction of a wild-type allele of *PRR1* (Fig. 4). Hybridization with *PHR2* revealed the expected expression pattern in CAI12 and an identical pattern in the heterozygous mutant CAPM1 (Fig. 4). In the homozygous null mutant, CAPM3, however, *PHR2* was highly expressed at both acid and alkaline pH (Fig. 4). Repression at alkaline pH was restored upon reintroduction of *PRR1* (Fig. 4). These results demonstrate that *PRR1*, like its *palF* homolog, regulates the pH response and is required for induction of alkaline-expressed genes and repression of acid-expressed genes.

Effect of *PRR1* mutations on growth and hyphal development. Ambient pH has a dramatic effect on hyphal development in vitro; the process is inhibited at acid pH and induced at neutral to alkaline pH (3, 8, 14). Having established the role of *PRR1* in the pH response, its role in determining cell morphology and hyphal development was examined. The null mutant exhibited a typical yeast morphology when grown in a variety of liquid media at 25°C, under acidic or alkaline conditions. However, a high percentage of multibudded cells was

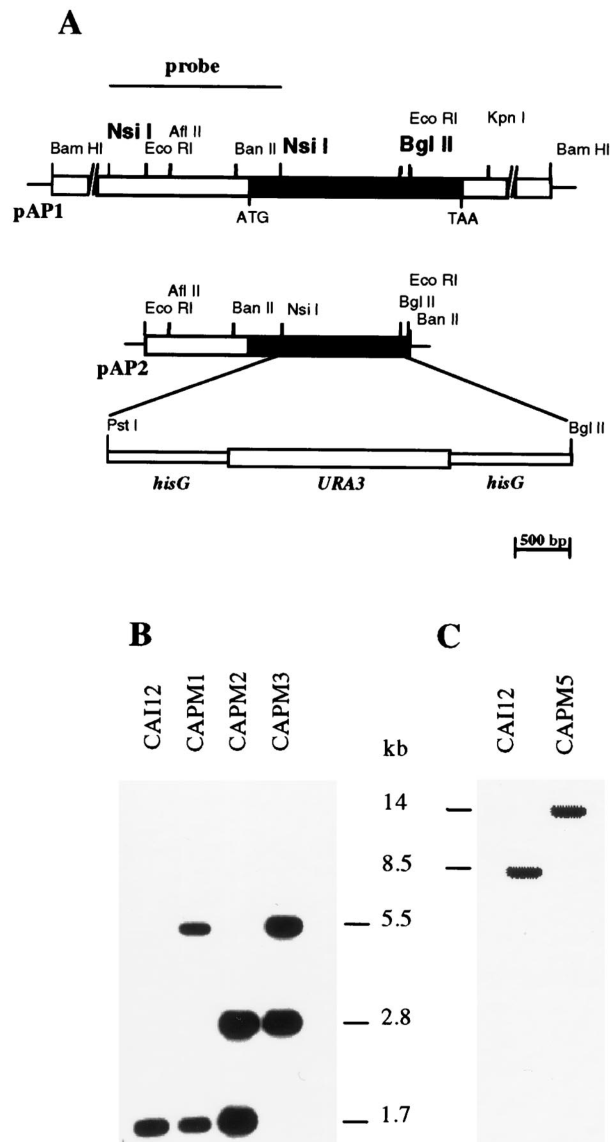

FIG. 3. Construction of *PRR1* mutants. (A) Partial restriction map of the 6-kb *Bam*HI genomic fragment containing the *PRR1* gene in plasmid pAP1 and the subcloned *Eco*RI fragment in plasmid pAP2. The black bar indicates the coding region of *PRR1*. The region replaced by the *URA3* cassette is also indicated. The overlined region of pAP1 was used as a hybridization probe in the Southern blot show in panel B. The *Bgl*II and *Nsi*I recognition sites relevant to the Southern blot analysis are indicated in boldface. (B) Genomic DNAs from the parental strain CAI12 and representative *PRR1* mutants CAPM1, CAPM2, and CAPM3 were double digested with *Nsi*I and *Bgl*II and used in Southern blot analysis. (C) Genomic DNAs from CAI12 and one representative revertant strain, CAPM5, were digested with *Bam*HI.

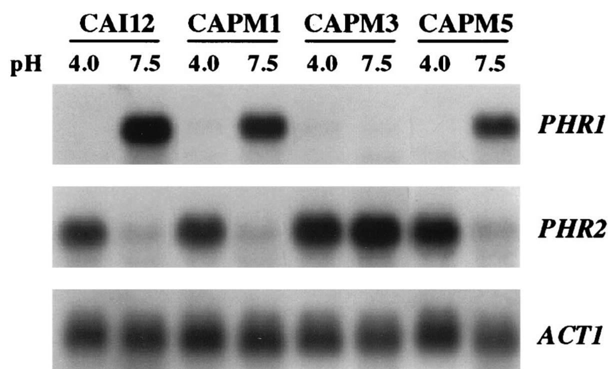

FIG. 4. Effect of *PRR1* mutations on *PHR1* and *PHR2* expression. Total RNAs were isolated from the indicated strains cultured at either pH 4.0 or 7.5. A Northern blot of the samples was prepared and sequentially hybridized with of *PHR1*, *PHR2*, and *ACT1* DNAs.

observed when the Δ*prr1* mutant was grown until stationary phase in YPD at 25°C.

No differences were observed between CAI12 and the *prr1* null mutant in the frequency or rate of germ tube formation when inoculated into Spider medium, the medium of Lee et al. (28), or 10% serum and incubated at 37°C (data not shown). However, the null mutant formed no germ tubes in medium 199 buffered at pH 7.5 (Fig. 5A). Instead, chains of typical yeast cells were formed. This defect was not evident in either heterozygous mutant, CAPM1 or CAPM5, and was verified with three independent null mutants.

Mutation of *PRR1* had a more significant consequence for hyphal development on agar-solidified media (Fig. 5A). On 10% serum plates the heterozygous mutant CAPM1 exhibited a modest reduction in filamentation in comparison with the control strain CAI12. However, no hyphae were observed emanating from colonies of the null mutant CAPM3. Upon reintroduction of the gene, as in strain CAPM5, filamentation was restored to the same extent as observed for the heterozygous disruptant. Filamentation was similarly absent from the null mutant cultured on medium 199, pH 7.5, plates. In contrast, filamentation was greatly reduced, but not eliminated, when the null mutant was cultured on either Spider medium or the medium of Lee et al. (28). Again, the null phenotype was largely reversed in the revertant strain. The same null phenotype was observed in three independent mutants.

The null mutant also exhibited medium-conditional defects in invasion of the agar below the colony dome. Invasiveness was observed on Spider medium, the medium of Lee et al. (28), and even medium 199, despite the absence of filamentation. However, the mutant was not invasive on 10% serum (data not shown).

Mutation of *palF* imparts a pH-conditional growth defect. The mutants are unable to grow at alkaline pH (9, 13, 48). The *prr1* null mutant grew equally well at either pH 4.0 or 7.0, although the colonies appeared to be slightly smaller than those of the control strain (Fig. 6). This was observed for all three independent mutants.

***PRR1* controls development-specific functions.** The absence of hyphal development in the *prr1* null mutant may reflect its role in controlling development-specific functions or may simply reflect the absence or reduction of *PHR1* expression. The cellular morphology of the mutant in medium 199 at pH 7.5 (Fig. 5A) is similar to that of a *phr1* null mutant at pH 7.0 (42). To distinguish between these possibilities, expression of *PHR1* was restored in the Δ*prr1* mutant by placing *PHR1* under the control of a constitutive, non-pH-regulated promoter. As shown in Fig. 5B, the constitutive expression of *PHR1* in this strain, CAPM6, did not restore the ability to form hyphae on 10% serum plates. However, on Spider medium and medium 199, filamentation was enhanced compared to that of the null mutant but did not match that of the control strain CAI12. Thus, the absence of *PHR1* partially limits hyphal development under some conditions, but *PRR1* apparently controls additional functions required for filamentation.

Because the function of *PRR1* is unknown, it is conceivable that it has a dual function, one within the pH response pathway and the other within a development-specific pathway. To test this possibility, *PHR1* was constitutively expressed in a Prr1$^+$ Prr2$^-$ background. *PRR2* is a second component of the pH response pathway that is homologous to *Aspergillus pacC* (41). *pacC* encodes the terminal component of the pH response pathway, a zinc finger transcription factor (48). A *prr2* null mutant is phenotypically similar to a *prr1* null mutant in altered expression of pH-dependent genes and in its failure to filament on serum and medium 199 (41). As seen with the *prr1* null mutant, constitutive expression of *PHR1* partially restored filamentation on medium 199 but failed to restore filamentation on 10% serum (data not shown). This suggests that *PRR1* acts via the pH response pathway in its control of hyphal development.

***PRR1* control of development is independent of *EFG1*.** Multiple regulatory genes influence the process of hyphal development. We recently identified *HWP1* as a downstream target of one of these regulators, *EFG1* (45). *EFG1* is required to induce *HWP1* expression, and *HWP1* is required for normal filamentation. To determine if the pH response pathway, as defined by *PRR1*, controls filamentation via *EFG1*, the effect of *PRR1* mutations on *HWP1* expression was examined. The control strain CAI12 and the *prr1* null mutant CAPM3 were cultured in medium 199, either at pH 4.0 and 25°C or at pH 7.5 and 37°C. These conditions repress and strongly induce expression of *HWP1*, respectively (45). RNA from these cells was examined by Northern blot analysis with *HWP1* as a probe. As shown in Fig. 7, expression of *HWP1* was induced in the absence of *PRR1*, indicating that the pH response pathway is not required for the expression of *EFG1*-dependent genes.

DISCUSSION

Previous studies established that several genes in *C. albicans* are differentially expressed in relation to the ambient pH of the culture medium (34, 42, 44). Furthermore, this pH response appears to be essential for virulence (10). The regulation of pH-dependent gene expression has been extensively studied with *A. nidulans* and entails a set of six *pal* genes which are required for the alkaline-induced, proteolytic activation of the *pacC*-encoded transcription factor (2, 9, 11, 12, 31, 37). This mechanism of the pH response is conserved, at least in part, in *Y. lipolytica* and *S. cerevisiae* (17, 27). To determine if pH-dependent gene expression was similarly regulated in *C. albicans*, we cloned and characterized *PRR1*, the *C. albicans* homolog of *palF*.

The *C. albicans* homolog, *PRR1*, was homologous to *palF* along its entire length. No motifs that would provide clues to the biochemical function of this protein were identified in Prr1p. However, a potential nuclear localization site is present in Prr1p. Although this sequence was not conserved in PalF, PalF does contain other potential nuclear localization signals, suggesting that these proteins may function within the nucleus. Unlike *palA*, *palB*, and *palI*, for which homologs have been identified in other species, no additional full-length homologs

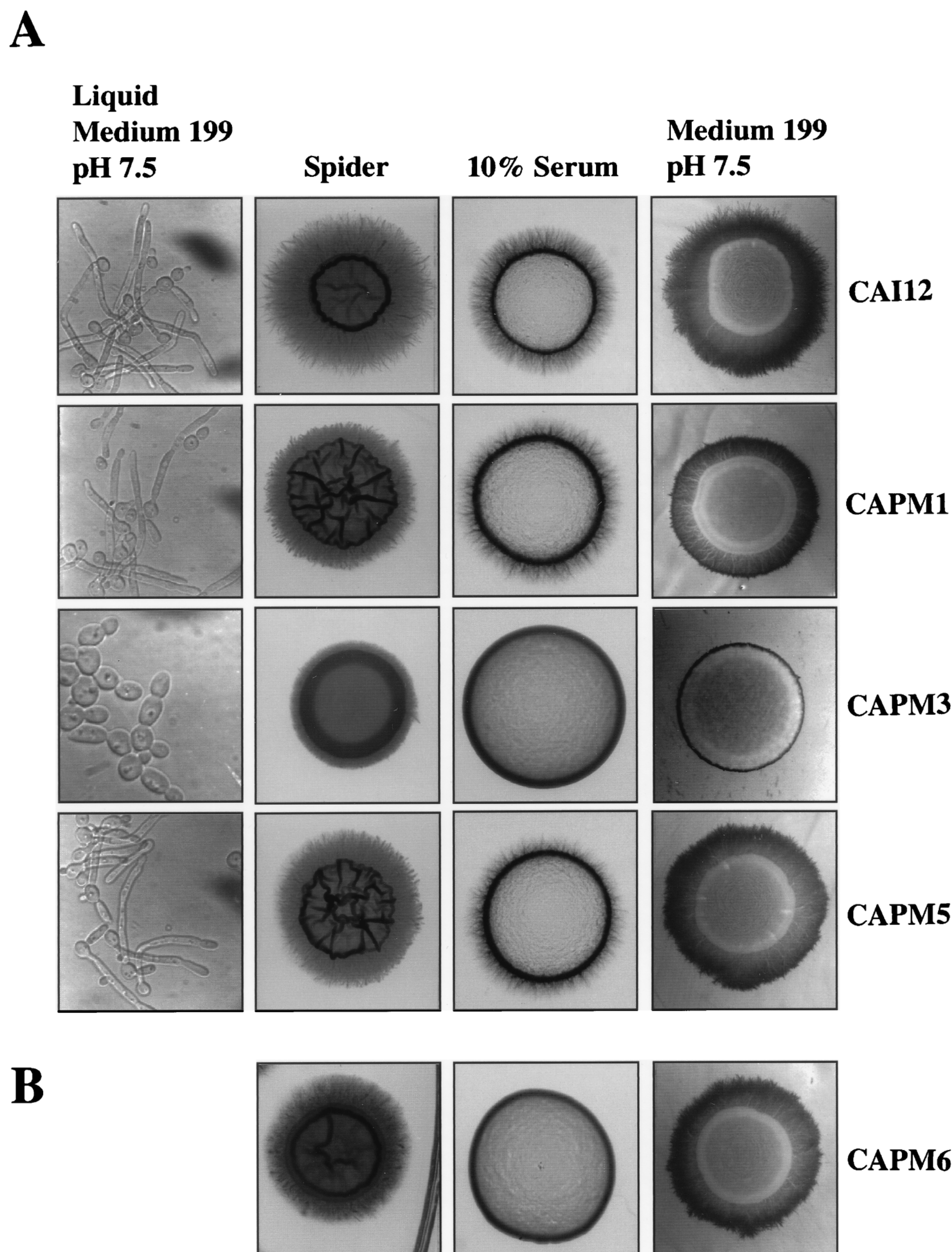

FIG. 5. Effect of *PRR1* mutations on hyphal development. (A) Photomicrographs of CAI12 and the *PRR1* mutants induced to form germ tubes in medium 199, pH 7.5, and to filament on agar-solidified medium. (B) Filamentation of a *prr1* null mutant containing a constitutively expressed *PHR1* allele.

of *palF* were identified. As previously reported (32), two adjacent open reading frames related to the amino and carboxy termini of PalF were identified in the *S. cerevisiae* genome sequences. The available expression data suggests that these are independently expressed (24) and not simply the result of a sequencing error. This may indicate that PalF and Prr1p are bifunctional proteins and that these functions have became separated in yeast.

Although the biochemical function of Prr1p is unclear, Prr1p was clearly demonstrated to play a role in the pH response of *C. albicans. PHR1* and *PHR2* are alkaline-expressed and acid-expressed genes, respectively (34, 42). The balanced, reciprocal expression pattern of these genes suggested a coordinated control via a common regulatory mechanism (34). Indeed, expression of both genes was affected in a homozygous *prr1* null mutant. Expression of *PHR1* was no longer induced at alkaline pH, and expression of *PHR2* was no longer repressed. This parallels the effect of *palF* mutations on pH-dependent gene expression in *A. nidulans* and indicates that the pH response pathway has been conserved between these species.

PRR1 not only is a component of the pH response pathway but is itself regulated by this response. Expression was substantially reduced at alkaline pH. This observation was somewhat surprising, assuming that Prr1p, like PalF, is required at alkaline pH to activate the terminal transcription factor. However, as shown in related studies (41), the alkaline repression of *PRR1* likely reflects a feedback loop to prevent runaway expression of *PRR2*, the homolog of *pacC*. It is not known if this

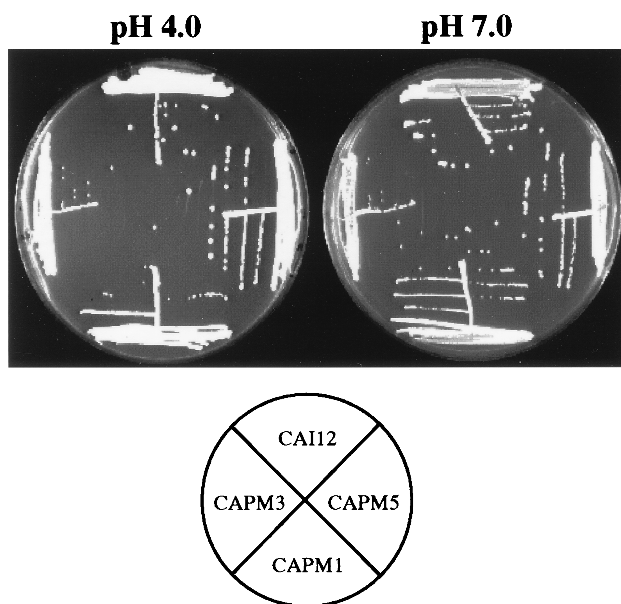

FIG. 6. Influence of pH on growth of *prr1* null mutants. Cells of all indicated strains were streaked on YNB agar adjusted to pH 4.0 or 7.0 and incubated for 3 days at 30°C.

is a conserved regulatory feature, since the expression of *palF* in relation to ambient pH has not been reported. However, pH does not affect the expression of *palA*, *-B*, *-C*, *-H*, or *-I* (11, 12, 36, 37).

In addition to controlling expression of *PHR1* and *PHR2*, which encode proteins involved in cell wall biosynthesis (15), *PRR1* also controls development-specific functions. *prr1* null mutants exhibited medium-conditional defects in germ tube induction and filamentation on agar-solidified media. The mutants lost the ability to form germ tubes in medium 199, but not in other media. Filamentation was blocked on 10% serum and on medium 199 and was partially reduced on Spider medium or the medium of Lee et al. (28). These defects were not due to differential acidification of the media by the mutant, since the media were strongly buffered and the pH did not change over the course of the experiments. Nor were they due to the absence of *PHR1* expression in the mutant. This was directly demonstrated by forced expression of *PHR1* in a *prr1* null mutant, which failed to restore filamentation on 10% serum.

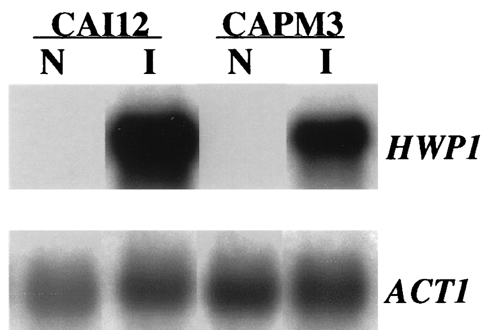

FIG. 7. Effect of *PRR1* mutation on *HWP1* expression. Total RNA was extracted from either CAI12 or the *prr1* null mutant CAPM3 cultured under noninducing (lanes N) or hypha-inducing (lanes I) conditions. A Northern blot was prepared and probed with *HWP1* and *ACT1* (as a control).

This suggests that the mutant fails to express functions required for hyphal development.

Constitutive expression of *PHR1* did restore some measure of filamentation on medium 199. This was not expected, because *PHR2* is constitutively expressed in the *PRR1* mutant and Phr2p is functionally homologous to Phr1p (34). In fact, unlike a *phr1* null mutant (42), the *PRR1* mutants exhibited a normal yeast morphology at alkaline pH despite the absence of *PHR1* expression. This presumably is due to the expressed Phr2p substituting for Phr1p. However, the ability of the constitutive *PHR1* allele to partially restore filamentation suggests that there may be important functional distinctions between Phr1p and Phr2p. Alternatively, the level of *PHR2* expression may simply be inadequate for hyphal development under these conditions. This presupposes that different levels of these proteins are needed for yeast versus hyphal wall synthesis.

It remains to be determined how the pH response pathway is integrated with other signal pathways that control differentiation in *C. albicans*. However, in the absence of *PRR1*, the *EFG1*-dependent expression of *HWP1* was still noted under the conditions tested. This suggests that the pH response pathway is distinct from the *EFG1*-dependent signaling pathway and probably acts on a separate set of morphogenic functions. Finally, it should be noted that the role of *PRR1* in development is consistent with, and provides a partial mechanistic explanation for, the longstanding observation that ambient pH strongly influences hyphal development in vitro (3, 8, 14).

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