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The pH of the Host Niche Controls Gene Expression in and Virulence of *Candida albicans*

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Little is known of the biological attributes conferring pathogenicity on the opportunistic fungal pathogen *Candida albicans*. Infection by this pathogen, as for bacterial pathogens, may rely upon environmental signals within the host niche to regulate the expression of virulence determinants. To determine if *C. albicans* responds to the pH of the host niche, we tested the virulence of strains with mutations in either of two pH-regulated genes, *PHR1* and *PHR2*. In vitro, *PHR1* is expressed when the ambient pH is at 5.5 or higher and deletion of the gene results in growth and morphological defects at neutral to alkaline pHs. Conversely, *PHR2* is expressed at an ambient pH below 5.5, and the growth and morphology of the null mutant is compromised below this pH. A *PHR1* null mutant was avirulent in a mouse model of systemic infection but uncompromised in its ability to cause vaginal infection in rats. Since systemic pH is near neutrality and vaginal pH is around 4.5, the virulence phenotype paralleled the pH dependence of the in vitro phenotypes. The virulence phenotype of a *PHR2* null mutant was the inverse. The mutant was virulent in a systemic-infection model but avirulent in a vaginal-infection model. Heterozygous mutants exhibited partial reductions in their pathogenic potential, suggesting a gene dosage effect. Unexpectedly, deletion of *PHR2* did not prevent hyphal development in vaginal tissue, suggesting that it is not essential for hyphal development in this host niche. The results suggest that the pH of the infection site regulates the expression of genes essential to survival within that niche. This implies that the study of environmentally regulated genes may provide a rationale for understanding the pathobiology of *C. albicans*.

Because *Candida albicans* is an important agent of opportunistic fungal infections, considerable effort has been directed toward elucidating the biological features that contribute to its ability to cause disease. To date, relatively few attributes have been defined or put forth as potentially contributing to its virulence (12). These include secretory hydrolases, both proteinases (19) and phospholipase (14), as well as the organism's dimorphic ability and property of phenotypic switching (17, 21, 29, 30). A limitation in identifying virulence attributes is the empirical approach generally employed. Some factors have been studied because of their significance in other pathogenic microbes or because they represent distinctive features of the organism. A more rational approach to elucidating the pathobiology of *C. albicans* would be of substantial benefit.

Studies of bacterial pathogens have shown that many of the genes required for virulence are regulated in response to environmental signals indigenous to the host niche (18). These signals include temperature, pH, osmotic pressure, iron concentration, and calcium ion concentrations (18). Recognition of this control scheme suggests that studying an organism's response to such signals could reveal much about the mechanisms of adaptation and survival within the host niche (18). *C. albicans* causes a broad range of infections in diverse host niches, and one or more of these environmental signals may be of significance in regulating the virulence traits of this fungus. Our prior identification of candidal genes regulated in response to the pH of the growth environment (20, 26) is of particular interest from this perspective and also because pH influences the expression of some putative virulence factors, such as the aspartyl proteinases (2, 31).

We initially identified a gene designated *PHR1* which encoded a putative cell surface glycoprotein anchored to the membrane by glycosylphosphatidylinositol (26). This gene was expressed at high levels when the pH of the growth medium was above 5.5, but levels were undetectable below this pH. *PHR2*, a functional homolog of *PHR1*, was subsequently identified (20). *PHR2* was expressed in an inverse pattern: it was expressed at high levels below pH 5, but not at a pH above 6 (20). Both genes encode a function required for in vitro morphogenesis of *C. albicans*. Deletion of *PHR1* results in the inability to form a normal yeast or hyphal morphology at an alkaline pH, but not at an acidic pH, which mirrors the expression pattern of the gene (26). Conversely, deletion of *PHR2* results in a morphogenic defect which is expressed at an acidic pH (20). In addition to the morphogenic defect, both mutants exhibit altered growth rates at the restrictive pH (20). More recently, we have identified a third pH-regulated gene, *PRA1*, which is also involved in morphogenesis (27).

The converse expression pattern of *PHR1* and *PHR2* and the pH-dependent phenotypes associated with their loss provide an opportunity to ask whether the pH of the host niche is a relevant environmental signal governing the virulence of *C. albicans*. We previously demonstrated that the virulence of *PHR1* null mutants was severely compromised in a mouse model of systemic infection (10). This was the response predicted if pH was a relevant signal, since the phenotypic defects of the mutant are expressed in vitro at pH values near that of mammalian systemic pH. An untested prediction was that mutations in *PHR1* would have no consequence in an acidic host

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

Strain	Parent	Genotype	Reference
SC5314		Clinical isolate	11
CAI-10	CAF-3	*URA3*/Δ*ura3*::*imm434*	9
CAS-5	CAF-3	*PHR1*/Δ*phr1*::*hisG-URA3-hisG* Δ*ura3*::*imm434*/Δ*ura3*::*imm434*	26
CAS-10	CAS-8	Δ*phr1*::*hisG*/Δ*phr1 URA3*/Δ*ura3*::*imm434*	26
CAS-11	CAS-8	Δ*phr1*::*hisG*/*PHR1*-pUC18-*URA3*-Δ*phr1* Δ*ura3*::*imm434*/Δ*ura3*::*imm434*	26
CFM-0	CAF-3	*PHR2*/Δ*phr2*::*hisG-URA3-hisG* Δ*ura3*::*imm434*/Δ*ura3*::*imm434*	20
CFM-2	CFM-1	Δ*phr2*::*hisG*/Δ*phr2*::*hisG-URA3-hisG* Δ*ura3*::*imm434*/Δ*ura3*::*imm434*	20
CFM-3	CFM-2	*PHR2*/Δ*phr2*::*hisG-URA3-hisG* Δ*ura3*::*imm434*/Δ*ura3*::*imm434*	20

environment. In this study we have compared the effects of mutations in *PHR1* or *PHR2* on virulence in a rat vaginitis model, which provides an acidic host niche, with their effects in a mouse model of systemic infection, which provides a host niche with a slightly alkaline pH. Predictions related to the virulence of these mutants were fully substantiated. *PHR1* mutants lost the ability to cause systemic disease but were virulent in vaginal infections. Conversely, *PHR2* mutants lost the ability to cause vaginal infection but were fully capable of causing systemic infection. The results indicate that the pH of the host niche is a significant environmental signal in determining the biological response and survival of *C. albicans* during infection.

MATERIALS AND METHODS

Strains and growth conditions. The *C. albicans* strains used and their genotypes are listed in Table 1. YPD medium (1% yeast extract, 2% peptone, 2% dextrose) containing 150 mM HEPES was used to culture the strains prior to inoculation into animals. The medium was adjusted to pH 7 for the growth of strains CFM-0, CFM-2, and CFM-3. This is a permissive pH for the growth of the *PHR2* null mutant, CFM-2. The medium was adjusted to pH 5 for the growth of strains CAS-5, CAS-10, and CAS-11. This pH is permissive for the growth of CAS-10 containing the Δ*phr1* mutation. The control strains SC5314 and CAI-10 were grown either at pH 7, for comparison with the *PHR2* mutants, or at pH 5, for comparison with the *PHR1* mutants. The cultures were grown to stationary phase at 28°C on a gyratory shaker at 200 rpm. Sabouraud dextrose agar (Difco) containing chloramphenicol (50 mg/ml) was used for the enumeration of CFU from infected kidneys and vaginal fluid. The plates were adjusted to pH 7.0 for recovery of *PHR2* mutants and to pH 4.5 for recovery of *PHR1* mutants.

Systemic infection of mice. Stationary-phase yeast cells were harvested from the YPD cultures by centrifugation at 1,500 × *g*. The cells were washed with sterile distilled water, suspended in physiological saline solution, and counted in a hemacytometer. Following quantitation, the cells were adjusted to a density of 7.5×10^6/ml. The cell suspension, 0.2 ml, containing 1.5×10^6 cells, was injected into the lateral tail veins of inbred male CD2F1 mice (18 to 21 g; Charles River, Calco, Italy). Each test strain was injected into 8 mice (wild type and *PHR1* mutants) or 10 mice (wild type and *PHR2* mutants), which were observed daily postinfection. The experimental end points were the overall mortality on day 30 after challenge (expressed as percent survival) and the median survival time (MST), expressed in days.

Quantitation of tissue fungal burden. The fungal burden in the kidneys of infected mice was examined 1, 2, and 5 days postinfection. Each candidal strain was injected intravenously into three mice for each time point at which fungal burden was examined. Mice were sacrificed by cervical dislocation, and the kidneys were aseptically removed, weighed, and homogenized in 5 ml of sterile saline. The homogenates were serially diluted and plated on Sabouraud dextrose agar. After 48 h of incubation at 28°C, the colonies were counted, and counts were expressed as CFU per gram of tissue.

Vaginal infection of rats. Ovariectomized female Wistar rats (80 to 100 g; Charles River) were maintained in pseudoestrus by subcutaneous injection with 0.5 mg of estradiol benzoate (Benzatrone; Samil, Rome, Italy) at 2-day intervals for the entire duration of the experiments. Six days after the first estradiol treatment, the animals were inoculated intravaginally with 0.1 ml of a cell suspension containing 10^7 yeast cells. The cell suspension was prepared as described for the systemic infection of mice. The inoculum was dispensed into the vaginal cavity through a syringe equipped with a multipurpose calibrated tip (Combitip; Pool Bioanalysis International, Milan, Italy). Two independent experiments were carried out, and in each experiment, each candidal strain was inoculated into five rats.

The kinetics of vaginal infection was followed by enumerating the CFU present in the vaginal fluid. A 1-μl sample was harvested at intervals from the vaginal cavity by using a calibrated plastic loop (Disponoic; Pool Bioanalysis International) and was streaked onto Sabouraud dextrose agar supplemented with chloramphenicol (50 μg/ml). After 48 h of incubation at 28°C, the colonies were counted.

Genotypes of isolates recovered from infected animals. The genotypes of isolates recovered from infected tissue were examined by Southern blot analysis. Four random isolates of each strain recovered from vaginal samples taken 2 days following infection and five isolates from day 9 samples were examined. For kidney samples, four or five random isolates from samples recovered 2 or 5 days after infection were genotyped. Genomic DNA was prepared from each isolate and characterized by Southern blot analysis using either *PHR1* or *PHR2* DNA as the hybridization probe. Genomic DNA isolation and Southern blot analysis were performed as previously described (20, 26).

Microscopic and histological examination of tissue samples. Vaginal fluids were stained with periodic acid-Schiff (PAS) reagent for microscopic examination. Mouse organs were fixed in 10% (vol/vol) formalin, and sections of paraffin-embedded tissues were examined after treatment with PAS and Van Gieson stains. Details of these procedures have been given elsewhere (2, 5).

Statistical analysis. Statistical comparisons were performed by the Mann-Whitney U test for comparisons of MST and by Fisher's exact test for comparisons of end point mortality. For the differences in vaginal CFU, the Mann-Whitney U test and Student's *t* test were used.

RESULTS

***PHR2* is not required for systemic infection of mice.** The blood pH of mice is approximately 7.3. When *C. albicans* is grown at this pH in vitro, *PHR1* is expressed at high levels and expression of *PHR2* is undetectable (20, 26). Accordingly, *PHR1* null mutants exhibit growth and morphological defects at alkaline pHs, while *PHR2* null mutants are phenotypically normal at these pHs. Hence, in contrast to the dramatic effect of *PHR1* mutations (10), loss of *PHR2* was expected to have little or no effect on the ability of the mutant strain to establish systemic infection. This hypothesis was tested by intravenous infection of CD2F1 mice with the various mutants.

As a control, we first established that CD2F1 mice yielded results similar to those previously obtained with BALB/c mice (10). Intravenous injection of 1.5×10^6 cells of the clinical isolate SC5314 resulted in 100% mortality within 3 days after challenge (Fig. 1a). Identical results were obtained with strain CAI-10, which is a heterozygous Urd^+ derivative of strain CAF-3, the parental strain of all the PHR mutants (20, 26) (data not shown). There was a dramatic difference between animals injected with the wild-type strain, SC5314 (100% mortality), and those challenged with the *PHR1* null mutant (0% mortality) (Fig. 1a). This confirmed previous results demonstrating the avirulence of this mutant (10). Interestingly, the two mutants that were heterozygous for *PHR1*, the parental strain CAS-5 and the revertant strain CAS-11, produced similar survival curves, but the extent of mortality was half that of the wild-type strain (Fig. 1a), suggesting that a single allele is insufficient for full expression of the pathogenic potential of these strains. All of these differences, as well as the differences in MST, between any two groups of animals were statistically significant ($P < 0.05$ or $P < 0.01$ with the two-tailed test, depending on the specific comparison). An independent repetition of the experiment yielded essentially the same results.

The requirement for *PHR2* was tested by infection with strain CFM-2, which contains a homozygous deletion of this gene. Infection with this mutant yielded results comparable to those for the wild-type strain, 100% mortality within 4 days (Fig. 1b). Infection with the heterozygous parent strain, CFM-0, or the heterozygous revertant of CFM-2, strain CFM-3, also resulted in 100 and 80% mortality, respectively,

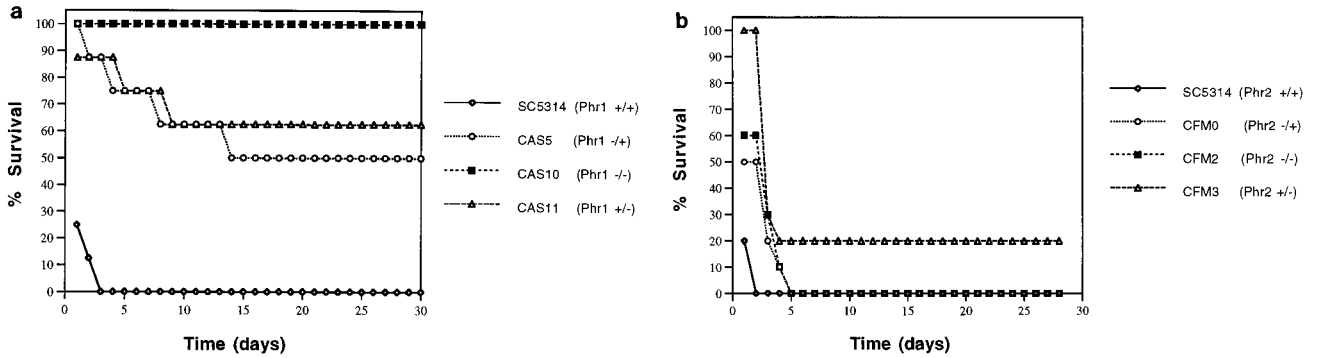

FIG. 1. Survival of CD2F1 mice following intravenous challenge with *C. albicans*. Results from independent experiments comparing the wild-type strain SC5314 with *PHR1* mutants and *PHR2* mutants are shown. The results shown in each panel are from one of two independent experiments.

within the 1st week (Fig. 1b). There were no statistically significant differences in the end point mortality. Similar results were obtained in a second independent repetition of the experiment. Small (1- to 2-day) differences were observed between the MST of the mice inoculated with the null mutant or the revertant and that of the animals injected with SC5314, but these differences either were statistically insignificant or were not observed in the independent repetition of the experiment.

The mortality results were mirrored in the kidney fungal burdens of infected mice. The fungal burdens in animals infected with SC5314 and in animals infected with any of the *PHR2* mutants were comparable (Table 2). In contrast, infection with the *PHR1* mutants resulted in substantially lower (>1 log unit) counts (Table 2). Histological sections of kidneys from infected mice showed that the *PHR2* mutants exhibited normal hyphal development in this tissue (Fig. 2), further demonstrating the negligible consequences of these mutations at the systemic pH.

As a control, the genotypes at the mutant loci of isolates recovered from the kidneys were examined by Southern blot analysis. In all cases, the genotypes of the recovered strains were identical to those of cells in the initial inoculum (data not shown). Thus, by all criteria examined, the loss of *PHR2* had a minimal effect, if any, on the ability of the strain to establish systemic infection, whereas the loss of *PHR1* had a marked influence, as previously established (10) and confirmed here.

***PHR2*, but not *PHR1*, is required for rat vaginitis.** Rat vaginal pH is approximately 4.5; thus, the rat vagina provides a host niche with a pH significantly different from that of blood. Thus, the requirement for *PHR1* and *PHR2* in vaginal infections was predicted to be the opposite of that demonstrated for systemic infection. This prediction was tested in two independent experiments with identical results. The results of one of these experiments are shown in Fig. 3.

Inoculation with the wild-type strain SC5314 resulted in a sustained vaginal infection during the 1st week, with a gradual decline in the fungal burden over the ensuing 3 weeks (Fig. 3a). The kinetics of clearance with this strain was very similar to that reported for other vaginopathic strains (4, 5). As predicted, loss of either one or both alleles of *PHR1* was without effect; infection with either the null mutant or the heterozygous control strains resulted in rates of clearance identical to that for the wild-type strain (Fig. 3a). The control strain CAI-10 gave similar results (data not shown). No statistically significant differences were detected with the Mann-Whitney U test or Student's *t* test between any two groups of rats, at any time point during infection.

Microscopic examination of vaginal scrapings taken from rats infected with strain CAS-5, CAS-10, or CAS-11 showed the typical yeast forms 1 h after challenge (Fig. 4), but 2 days after challenge, each of these strains developed hyphal filaments which persisted until at least day 7 (Fig. 4). Identical morphologies were observed with either SC5314 or CAI-10 (data not shown). Thus, the morphological aberrations characteristic of the *PHR1* null mutant when it is grown at an alkaline pH were not evident. Multiple isolates recovered from these infected animals were genotyped and found to contain the expected mutation(s) at the *PHR1* locus, verifying that the infections were due to propagation of the mutants. Together, these data demonstrated that *PHR1* is not required for vaginal infection or proper morphogenesis in an acidic host niche.

In contrast to the *PHR1* mutants, deletion of *PHR2* had a dramatic effect on the kinetics of vaginal clearing. Within 24 h of infection, the fungal burden with the *PHR2* null mutant, CFM-2, was reduced to half that with the clinical isolate, SC5314 (Fig. 3b). This rapid rate of clearance continued over the next 6 days. The fungal burden at day 7 was lower than that seen 4 weeks after infection with the wild-type strain, SC5314. Infection with either of the heterozygous control strains, CFM-0 or CFM-3, resulted in much higher fungal burdens and a more prolonged course of infection than infection with the null mutant. Nonetheless, these strains reproducibly showed slightly, but statistically significantly, lower fungal burdens and slightly more rapid clearance than those observed with homozygous $Phr2^{+}$ strains. There were highly significant differences ($P < 0.01$ with the two-tailed Mann-Whitney U test) at all time points between the fungal burdens of rats challenged with SC5314, CFM-0, or CFM-3 and those of rats receiving the null mutant, CFM-2. Statistically significant differences ($P < 0.01$ with the two-tailed Mann-Whitney U test) were also noted

TABLE 2. *Candida* counts in infected kidneys

Strain	Genotype	Tissue count (10^4 CFU/g ± SE) on:		
		Day 1[a]	Day 2	Day 5
SC5314	*PHR1*/*PHR1* *PHR2*/*PHR2*	64.2 ± 1.9	56.6 ± 3.5	15.3 ± 2.1
CAS-5	Δ*phr1*/*PHR1*	5.65 ± 0.85[b]	27.25 ± 2.0[b]	4.65 ± 2.5
CAS-10	Δ*phr1*/Δ*phr1*	2.25 ± 0.4[b]	2.17 ± 0.48[b]	4.5 ± 0.75
CAS-11	*PHR1*/Δ*phr1*	4.73 ± 0.78[b]	26 ± 1.9[b]	6.87 ± 3.4
CFM-0	Δ*phr2*/*PHR2*	77.5 ± 3.5	58.0 ± 2.8	11.9 ± 4.2
CFM-2	Δ*phr2*/Δ*phr2*	110 ± 11.1	84.1 ± 3.7	23.9 ± 5.7
CFM-3	*PHR2*/Δ*phr2*	73.7 ± 3.1	20.4 ± 5.1[b]	14.5 ± 4.4

[a] Days postinfection.
[b] $P < 0.01$ by two-tailed Student's *t* test.

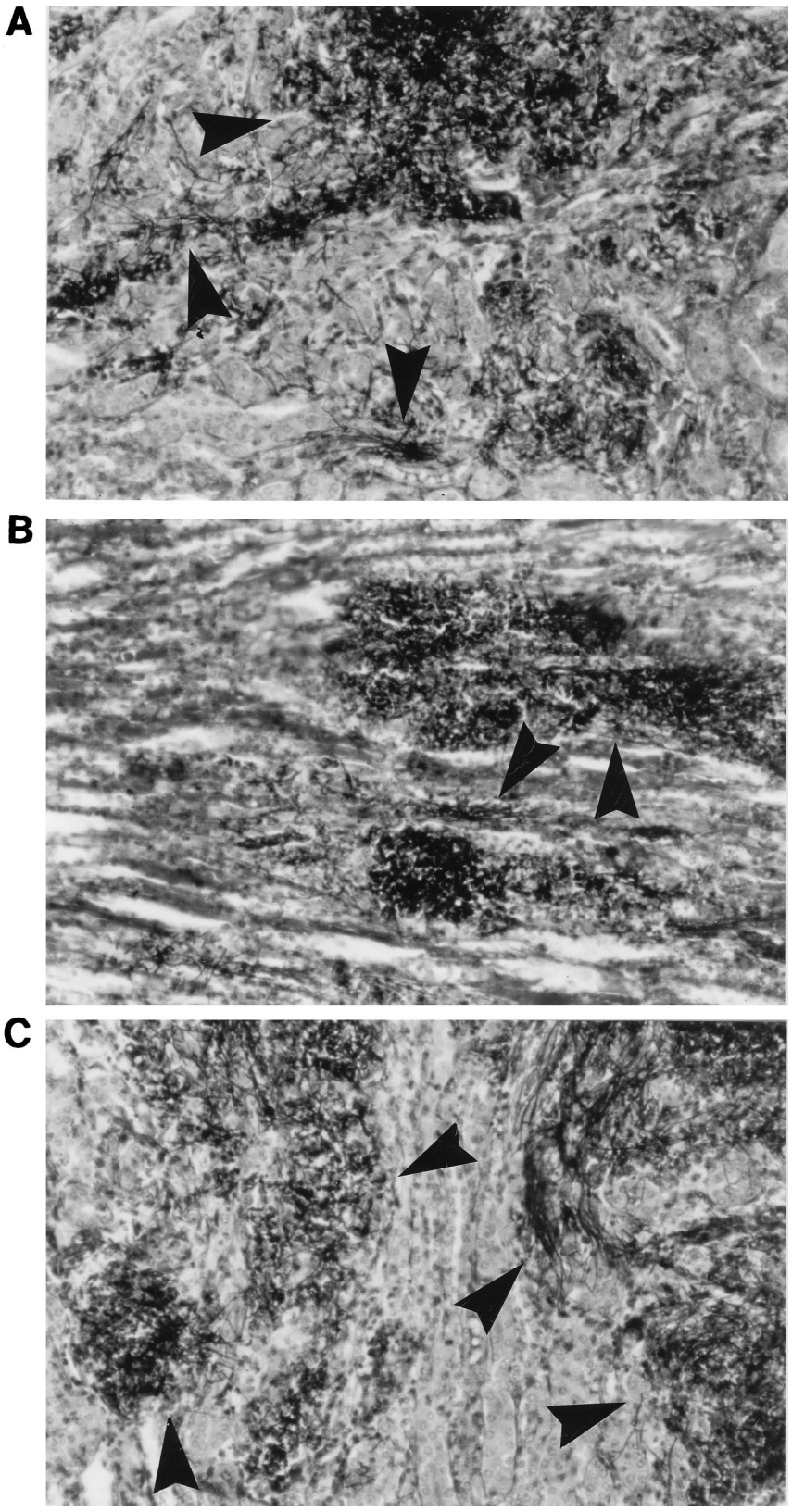

FIG. 2. PAS-Van Gieson-stained sections of kidneys of CD2F1 mice 5 days after challenge with strain CFM-0 (A), CFM-2 (B), or CFM-3 (C). Arrows indicate locations of hyphal forms. Extensive hyphal invasion of the organ was observed in all sections. Magnification, ×315.

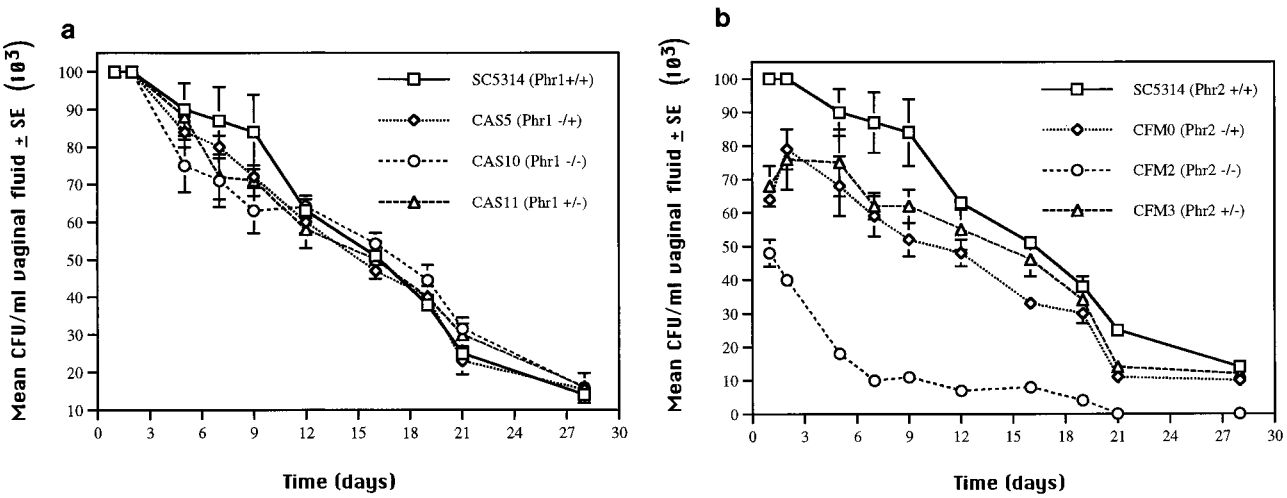

FIG. 3. *C. albicans* count during vaginal infection of ovariectomized, pseudoestrus rats. The animals were inoculated on day 0 with the wild-type control, SC5314, or the indicated *PHR1* mutant (a) or with the wild-type strain or the indicated *PHR2* mutant (b). Error bars, standard errors of the means.

between the fungal burdens of rats challenged with SC5314 and those of rats challenged with either heterozygous strain, CFM-0 or CFM-3, during the 1st week of infection. No statistically significant difference, at any time point, was observed between the two heterozygous strains. Thus, there was a clear requirement for *PHR2* in establishing and maintaining vaginal infection, and the loss of even one allele had a demonstrable effect.

Microscopic examination of vaginal scrapings taken from the rats infected with the *PHR2* mutants was performed to determine if the in vitro morphological aberrations characteristic of the null mutant were evident in vivo. The heterozygous mutants CFM-0 and CFM-3 showed the expected cell morphologies. One hour after inoculation the cells had a yeast morphology, and extensive hyphal growth was evident 2 days postinfection (Fig. 5). However, unexpected results were observed with samples from rats infected with the null mutant. Whereas a *PHR2* null mutation results in an aberrant cell morphology at an acidic pH in vitro (20), apparently normal hyphae were present in the vaginal samples (Fig. 5). This was not due to a contaminant strain, since Southern blot analysis of isolates recovered from these rats demonstrated the presence of the *PHR2* deletion (data not shown). Thus, while *PHR2* is required for vaginal infection, ostensibly this requirement is not related to hyphal development.

DISCUSSION

The question posed in this study was whether or not the pH of the host niche acts as an environmental signal that regulates gene expression in and virulence of *Candida albicans*. This question was addressed by using mutants that exhibit pH-dependent phenotypes as biological probes. There were two pertinent observations in this regard. One was that a *PHR1* null mutant was avirulent when tested in a mouse model of systemic candidiasis but was indistinguishable from a wild-type strain in its ability to cause vaginal disease. The other was that a *PHR2* null mutant was inversely affected. It was avirulent in a rat model of vaginal candidiasis but uncompromised in its ability to cause systemic infection in mice. Several controls were incorporated into these studies to verify that these phenotypes were direct consequences of the mutations in *PHR1* and *PHR2*. Reintroduction of a wild-type allele into the respective mutant reverted the phenotype to that of the parental heterozygote, demonstrating the correspondence between genotype and phenotype. In addition, the genotypes of isolates recovered from infected animals were determined to demonstrate that the infections resulted from the inoculum of mutant cells and not from endogenous wild-type strains or cross-contamination from other animals.

The key concept in interpreting these results is that *PHR1* and *PHR2* encode structurally homologous proteins that are functionally interchangeable (20). Forced expression of *PHR1* at an acidic pH restores normal growth and morphogenesis to a *PHR2* null mutant, and conversely, forced expression of *PHR2* at an alkaline pH complements a deletion of *PHR1* (20). Thus, a *PHR1* null mutant grown at an alkaline pH has essentially the same biochemical defect as a *PHR2* null mutant grown at an acidic pH. Recognizing this equivalence of function, the avirulence of the *PHR1* null mutant in systemic infection implies that *PHR2* is not expressed at all or is not expressed to a significant extent under these conditions. If it were, it would complement the deletion of *PHR1*, and the cells would be phenotypically normal. By the same reasoning, *PHR1* is not expressed during vaginal infection; if it were, it would complement the *PHR2* deletion and the cells would exhibit normal virulence. One caveat to this interpretation is the possibility that, in addition to their shared activity, either Phr1p or Phr2p has an additional function, not shared by the other, that specifically affects virulence. While we cannot exclude this possibility, there is certainly no evidence for it. Thus, we conclude that these genes are differentially expressed in vivo, *PHR1* is expressed during systemic infection but not during vaginal infection, and *PHR2* has the inverse pattern of expression.

What factor(s) governs the differential expression of these genes in vivo? Previous work established that expression of *PHR1* and *PHR2* in vitro is regulated in response to the ambient pH of the growth environment, independent of temperature, nutritional factors, or morphology (20, 26). The in vivo expression patterns exhibited a similar correlation with pH. The systemic pH of mice is 7.3, while the vaginal cavity in rats has a pH of 4.5. Thus, *PHR1* was expressed at a near-neutral pH in the systemic niche but not at pH 4.5 in the vaginal niche. In vitro, the same pH dependence is observed; *PHR1* expression occurs only when the ambient pH is 5.5 or higher (26). Conversely, *PHR2* was expressed in the acidic environment of

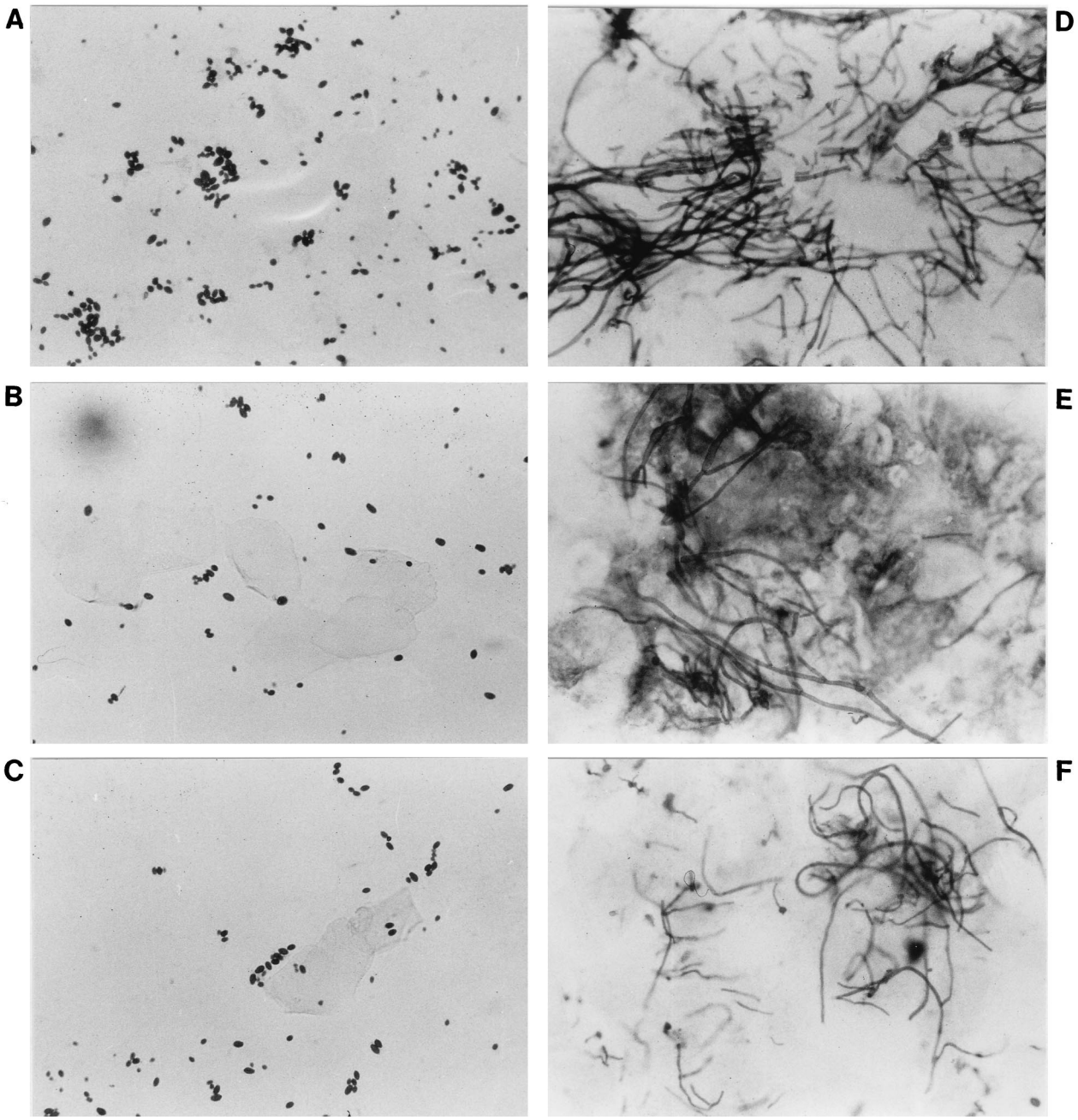

FIG. 4. Photomicrographs of vaginal scrapings. (A through C) Samples of strains CAS-5, CAS-10, and CAS-11, respectively, taken 1 h postinfection. (D through F) Samples of strains CAS-5, CAS-10, and CAS-11, respectively, taken 2 days postinfection. The vaginal smears were stained by the PAS-Van Gieson method. Note the qualitatively similar hyphal development of all strains after 2 days of infection. Magnification, ×192.

the vaginal niche but not in the slightly alkaline environment of the blood. This parallels the in vitro pH-dependent pattern in which *PHR2* is expressed at a pH of 5.0 or lower (20). Therefore, the simplest interpretation of the in vivo expression pattern is that the pH-dependent regulation demonstrated in vitro is operative within the host niche. However, we cannot exclude the possibility that factors other than pH influence the expression of these genes. There are numerous differences between the two animal models. They differed in species, sex of the animals, and pharmacological treatment with estrogen. Even if these differences are eliminated, the systemic and vaginal environments are grossly different. However, there is no reason to believe that the pH-dependent regulation seen in vitro would become inoperative in vivo, and no regulatory influences other than pH need be hypothesized to explain the results.

While the results clearly demonstrated the requirement of *PHR1* and *PHR2* for systemic and vaginal infections, respectively, we can only speculate as to why the null mutants are avirulent. Given the common biochemical defect of the mutants, avirulence in the systemic and vaginal models may likely have similar bases. The precise biochemical function of the encoded proteins has not been defined. In *Saccharomyces cer-*

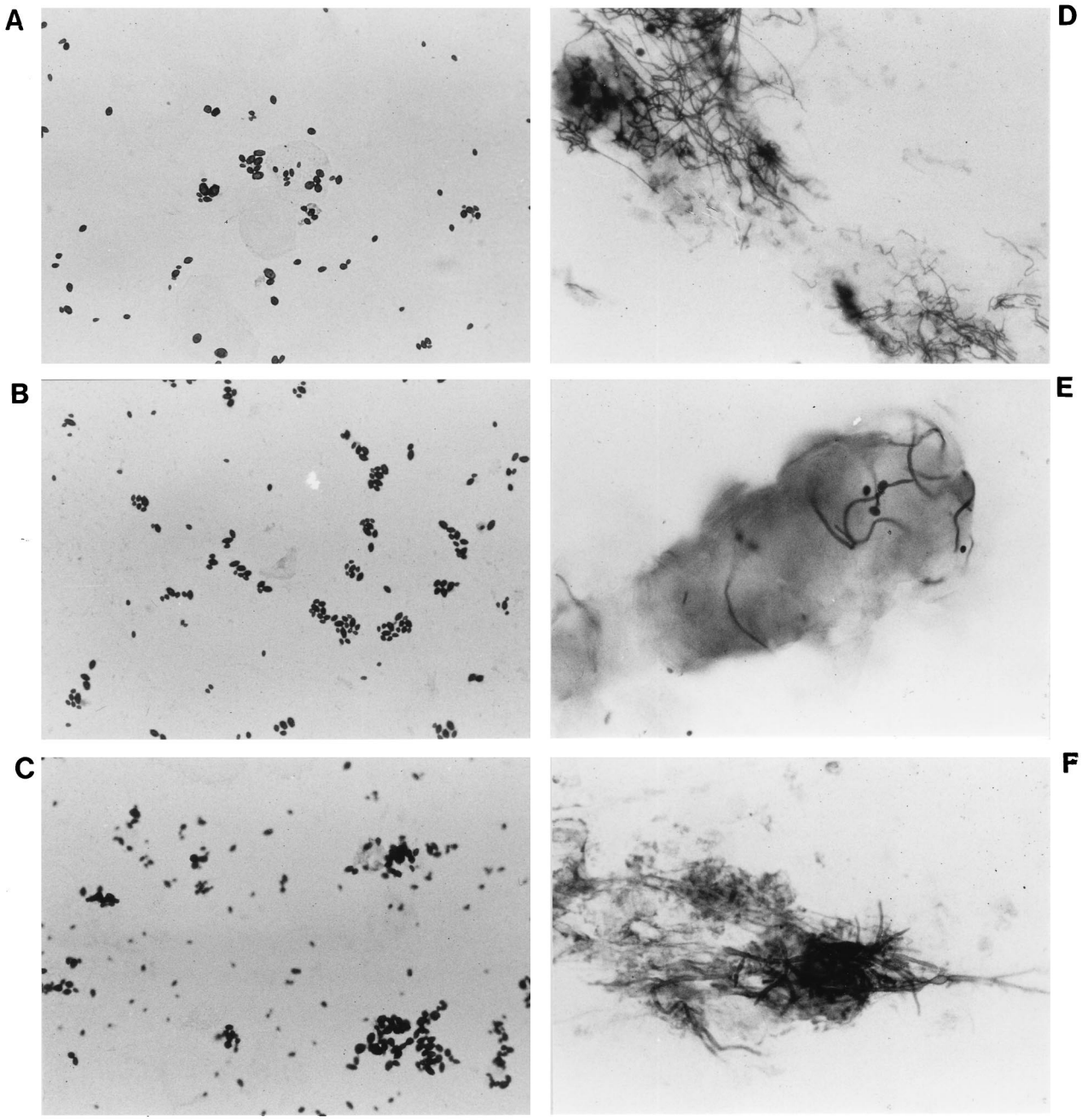

FIG. 5. Photomicrographs of vaginal scrapings. (A through C) Samples of strains CFM-0, CFM-2, and CFM-3, respectively, taken 1 h postinfection. (D through F) Samples of strains CFM-0, CFM-2, and CFM-3, respectively, taken 2 days postinfection. The vaginal smears were stained by the PAS-Van Gieson method. Note the qualitatively similar hyphal development of all three strains. Magnification, ×192.

evisiae, deletion of the *PHR1*/*PHR2* homolog *GAS1*/*GGP1 CWH52* results in multiple phenotypic consequences, including alterations in the structure of the cell wall (15, 23–25). Although differing from *S. cerevisiae*, *PHR1* and *PHR2* exhibit similar pH-dependent defects in cell wall organization (8). It is not clear if these are direct or indirect consequences of the mutations or whether they directly impact virulence. However, one secondary consequence which is likely to have a significant impact on virulence is the severely compromised growth rates of the mutants. The doubling time of a strain lacking both *PHR1* and *PHR2* is five times that of a wild-type strain (20). At a systemic pH the *PHR1* null mutant is phenotypically Phr1$^-$ Phr2$^-$, since *PHR2* is not expressed at this pH. Similarly, the *PHR2* null mutant in the acidic environment of the vagina is the phenotypic equivalent of the double mutant, since *PHR1* is not expressed. Thus, the in vivo growth of these null mutants should be greatly reduced relative to that of a wild-type strain. This gross reduction in growth rate may be incompatible with the establishment or maintenance of infection in either host niche. Alternatively, or in addition, the mutations may indirectly affect the expression of virulence-specific attributes. For instance, the secretory aspartyl proteinases (SAPs) have been

implicated in the vaginopathic potential of *C. albicans* and *Candida parapsilosis* (5, 7). Of the multiple SAP genes in *C. albicans* (19), *SAP2* is known to require acidic pH for expression and is expressed during vaginal infection (7). Moreover, recent genetic analysis has shown this gene to be essential for such infections (6). The cell surface defects in the *PHR2* mutant may indirectly compromise *SAP2* expression, leading to reduced virulence. A less likely explanation is that *PHR1* and *PHR2* encode multifunctional proteins and that, in addition to their common activity, Phr1p has a secondary function specific to systemic virulence and Phr2p has a secondary activity specific to vaginal infection. If the avirulence is due simply to the altered growth rate, then the virulence patterns of the mutants can be predicted for any animal model based on the pH of the host niche. In this regard it should be noted that a *PHR2* null mutant, unlike a *PHR1* null mutant, is defective in gastric colonization of mice, as would be predicted based on pH alone (6).

In addition to the differential avirulence of *PHR1* and *PHR2* mutants, there were several other interesting observations. One was an apparent gene dosage effect. Both mutants that were heterozygous for a deletion of *PHR1*, the parent and the revertant of the null strain, caused only 50% of the mortality associated with the homozygous $Phr1^+$ strains, SC5314 and CAI-10. Similarly, heterozygous *PHR2* mutants showed a reproducible reduction in vaginal virulence, although it was not as dramatic. While these mutants are also heterozygous for *URA3* by virtue of the construction methods, and while *URA3* is required for virulence (16, 28), this does not account for the observed attenuation, since the control strain, CAI-10, is also heterozygous for *URA3* and exhibited full virulence in both animal models. Genetic instability of the *URA3* marker also seems an unlikely explanation. Although *URA3* is flanked by direct repeats in these strains and this can cause recombinational loss of the marker (9), any virulence effects of this instability would be manifested equally in all strains, and this was not observed. We cannot discount the possibility that secondary mutations were inadvertently introduced during construction of the strains and that these resulted in the attenuation of the heterozygous strains. However, if this did occur, it had to have occurred independently in the *PHR1* and *PHR2* mutant lines. This follows from the observation that the virulence of the parental strain of both mutant lineages was indistinguishable from that of a wild-type strain. It might also be noted that the *PHR1* revertant, strain CAS-11, showed no attenuation when systemic infection was tested in BALB/c mice (10), suggesting that these apparent dosage effects may not be evident in all host strains.

An unexpected observation was that histological examination of vaginal tissue demonstrated the presence of morphologically normal hyphae in animals infected with the *PHR2* null mutant. This would seem to contradict the foregoing arguments that *PHR1* was not expressed in this niche. However, these hyphal forms were exceedingly few in number and not characteristic of the vast majority of the cells. Their presence might indicate limited microenvironments within the vaginal tissue with a pH commensurate with *PHR1* expression and, thus, with limited hypha formation. We also cannot rule out the possibility that these represent a few endogenous or exogenous wild-type cells. Although the genotypes of a number of isolates recovered from these infections were determined, the screening was not extensive enough to detect infrequent members of the population.

The primary conclusion from these studies is that pH appears to be an environmental signal governing differential gene expression and virulence. A corollary of this conclusion is that the ability of *C. albicans* to inhabit diverse host niches is mediated by adaptations specific to the niche and that these adaptations are effected, at least in part, by differential gene expression. This was already implicit in previous work with *C. albicans* CA-2, an echinocandin-resistant mutant (4). This strain was unable to form germ tubes in vitro and was avirulent in systemic infection models, yet it was competent in establishing vaginal infections and developing a hyphal morphology in this host niche (4). However, the nature of the mutation(s) in this strain is not defined, and in view of the chemical mutagenesis used, it is likely that multiple mutations were introduced. In contrast, this study used well-defined mutants, and the expression patterns of the mutated genes had been previously established.

A broader implication of these results is that environmental cues in the host niche may be of similar significance to fungal pathogens as to bacterial pathogens, serving to regulate expression of the genetic potential needed to survive within that niche. The response to pH is not likely to be limited to these two genes, since in vitro expression of other candidal genes is modulated by ambient pH (27, 31). It is also unlikely that pH is the only environmental signal relevant to candidal infection. Temperature is another parameter with significant effects on candidal biology, affecting dimorphism (22), surface properties (13), virulence (1), and gene expression (3). This suggests a new avenue and rationale to dissecting the biological basis of candidal virulence. By analyzing the organism's response to environmental signals intrinsic to the host niche, we may learn much about the basis of its pathobiology.

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