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Hypha Formation in the White-Opaque Transition of *Candida albicans*

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Cells of *Candida albicans* strain WO-1 and related strains switch frequently and reversibly between a white-colony-forming unit (white phase) and a gray-colony-forming unit (opaque phase). Cells in the budding white phase exhibit the usual smooth round phenotype observed in other *C. albicans* strains, but cells in the budding opaque phase exhibit a unique elongate shape with surface pimples or protrusions. In this study, it was demonstrated that opaque cells formed hyphae at low to negligible levels in suspension cultures but could be induced to form hyphae at high levels when anchored to the chamber wall of a perfusion chamber or to a monolayer of human skin epithelial cells. Variability in the proportion of hyphae formed between experiments appeared to be due to variability between individual opaque clones. The hyphae formed by opaque cells were morphologically identical to hyphae formed by white cells (i.e., they were devoid of pimples or protrusions and exhibited the same shape and septal locations). They also did not stain with an opaque-specific antiserum which differentially stained opaque budding cells in a punctate fashion. However, when stimulated to form buds, opaque hyphae formed opaque-shaped daughter buds, demonstrating that although they are morphologically similar to hyphae formed by white cells, they are genetically opaque.

Cells of *Candida albicans* strain WO-1 can switch back and forth at high frequency between two major phenotypes, white and opaque (1, 12, 14, 18, 22). In the white phenotype, cells form smooth white colonies (13); in the opaque phenotype, cells form wider, flatter gray colonies (1, 14). The white-opaque transition affects not only colony phenotype but cellular phenotype as well (1, 14). White budding cells are oval to round in shape and bud with the same pattern as does the standard laboratory strain 3153A (5). In marked contrast, opaque cells are elongate and bean shaped, exhibit roughly twice the volume and mass as do white cells, and contain a large vacuole (1, 14). The budding pattern is similar to that of white cells but sometimes includes bipolar budding, a condition rarely observed in white cell cultures (14). Opaque cells also differ from white cells in wall morphology, exhibiting unique bumps, or pimples, evident in scanning electron micrographs (1) and possessing a punctate-staining opaque-specific surface antigen which may be related to the pimple (1). White and opaque cells contain roughly the same amount of DNA (14).

Using the method of pH-regulated dimorphism (4, 17), we originally reported that although white budding cells could be induced to form hyphae by dilution of stationary-phase cells into fresh medium at 37°C, pH 6.7, opaque cells were uninducible (14). When stationary-phase cells in the opaque phase were diluted into fresh medium at 37°C, pH 6.7, they formed elongate buds which expanded into the bean-shaped cells characteristic of budding opaque cells rather than forming hyphae (14). It was noted at that time (14) that if the opaque phase of WO-1 and related strains was involved in pathogenesis, one would have expected opaque cells to form tissue-penetrating hyphae. Strains exhibiting the white-opaque transition have been isolated from both systemic and vaginal infections (14, 21). In this report, we demonstrate that opaque cells can indeed be induced to form hyphae when immobilized on the surface of a perfusion chamber or on the surface of a monolayer of human skin epithelial cells. Surprisingly, the hyphae formed by opaque cells are devoid of the wall pimples unique to budding opaque cells and do not exhibit punctate staining with opaque-specific antiserum. These results are discussed in terms of developmental gene regulation and switching.

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MATERIALS AND METHODS

Maintenance of strain WO-1. *C. albicans* strain WO-1 was originally isolated from the blood and lungs of a bone marrow transplant patient at the University of Iowa Hospitals (14). Stock cultures were maintained on agar slants (19, 23). For experimental purposes, cells from a stock culture were clonally plated on amino acid-rich nutrient agar containing the ingredients of Lee et al. (6) supplemented with arginine and zinc sulfate (2). A white colony was picked for cells in the white phase, and an opaque colony was picked for cells in the opaque phase (see Results for a description of white and opaque colony discrimination; 1, 14). For suspension cultures, cells were inoculated into 125-ml plastic Erlenmeyer flasks containing defined medium (2) and rotated at 200 rpm in a Gyrotory water bath shaker (model G76; New Brunswick Scientific Co., Inc., Edison, N.J.) at 25°C.

pH-regulated dimorphism. The methods for pH-regulated dimorphism have been presented in detail in previous publications (e.g., 4, 8). In brief, cells were grown in suspension in defined medium (2). After 24 h in stationary phase, cells were lightly sonicated and inoculated at a final concentration of 5×10^6 to 1×10^7 cells per ml into 25 ml of fresh medium prewarmed to 37°C that had been previously adjusted to pH 4.5 (conditions conducive to bud formation) or 6.7 (conditions conducive to hypha formation). Cultures were then sampled at time intervals and scored for the percentage of cells with buds or hyphae.

Sykes-Moore chamber experiments. The Sykes-Moore chamber and accessory equipment used in *Candida* perfusion experiments have been described previously (20). In brief, 1.5×10^7 cells per ml from a stationary-phase culture (described above) were released into fresh defined nutrient medium adjusted to pH 6.7 and prewarmed to 37°C. The cells were immediately placed on one of the walls of the chamber, which was either uncoated or coated by preincubation for 2 min with 0.1% polylysine at room temperature or for 2 h in 20 μg of human plasma fibronectin per ml (Bethesda Research Laboratories, Inc., Gaithersburg, Md.) at 37°C. The opposing uncoated chamber wall was immediately set in place, and defined nutrient medium at 37°C, pH 6.7, was continuously perfused through the chamber at a rate of 0.4 to 0.5 ml/min. The temperature of the chamber was controlled by an air current incubator (Sage model 299; Orion Research, Inc., Cambridge, Mass.) and monitored with a digital thermometer (model BAT-12; Sensortek, Clifton, N.J.) inserted into the chamber interior. The medium was incubated in a 37°C water bath and perfused through the chamber by a peristaltic pump (Polystaltic; Buchler Instruments Div., Nuclear-Chicago Corp., Fort Lee, N.J.) attached to the outflow tube. Cells in the chamber were viewed through a Nikon compound microscope.

Epithelial cell-*Candida* cultures. Suspensions of human epidermal cells were prepared by incubating small (1 by 3 mm) pieces of adult human leg skin overnight in a cold solution of trypsin (24) and then plating and growing the cells in vitro in the media and by the methods described in detail by Rheinwald and Green (11). Epidermal cells from the second passage were removed from culture flasks with 0.2% trypsin and then plated and grown to near confluence on untreated glass cover slips (25 mm). Tissue culture medium was removed, and cover slips with cells were gently rinsed with the defined nutrient medium for *Candida* cultures. Then 1.5 ml of stationary-phase opaque cells at 1.5×10^7 cells per ml in defined nutrient medium adjusted to pH 6.7, 37°C, was placed on the cover slips resting in 60-by-15-mm petri dishes. The dishes were then incubated at 37°C for 7 h. In control cultures, cover slips without cells were used. Photographs were taken through a Zeiss ICM 405 inverted microscope, using T-Max 100 film (Eastman Kodak Co., Rochester, N.Y.).

Scanning election microscopy. Cells on Sykes-Moore cover slips were fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2) overnight (4°C). Cells were then postfixed in 1% OsO_4 as described by Anderson and Soll (1) except that cells were not treated with 6% thiocarbohydrazide and a second 1% OsO_4 fixation. Cells were sputter coated with gold palladium to reduce charging and viewed with a Hitachi 5-570 scanning electron microscope.

Indirect immunofluorescent staining. Cells on cover slips were fixed in 4% formaldehyde and stored at 4°C. Antiserum preparation and indirect immunofluorescence were performed as described by Anderson and Soll (1). In brief, antiserum prepared against opaque budding cells was diluted 1:10 and absorbed three times with white cells forming hyphae. Absorbed antiserum (20 μl) was incubated with the fixed opaque hyphal cells for 1 h at 25°C, and the cells were then washed three times in phosphate-buffered saline plus bovine serum albumin. Washed cells were incubated for 1 h at 25°C with 20 μl of fluorescein-conjugated, affinity-purified goat anti-rabbit immunoglobulin G diluted 1:25 in phosphate-buffered saline plus bovine serum albumin. Treated cells were washed four times in phosphate-buffered saline plus bovine serum albumin, and the cover slip was placed on a drop of Gelvatol containing 100 mg of 1,4-diazabicyclo(2.2.2)octane per ml. Cells were viewed with a Zeiss ICM 405 inverted microscope equipped for epifluorescence microscopy, using Zeiss filter set 17. Photographs were made on Kodak T-Max 400 film.

RESULTS

White-opaque transition. When cells of strain WO-1 are removed from a storage streak on agar and plated at low density on fresh nutrient agar, the majority of colonies exhibit a creamy white phenotype (white) and a minority exhibit a larger, flatter gray phenotype (opaque). If cells from a white colony are in turn plated, the majority of colonies are white and the minority are opaque. The frequency of opaque colonies in this latter plating varies from roughly 10^{-2} to 10^{-4}. If cells from an opaque colony are in turn plated, the majority of colonies are opaque and the minority are white. The frequency of white colonies in this plating varies from roughly 10^{-2} to 10^{-3}. In both cases, minor colony sectors of the alternative colony phenotype are discernible, especially when phloxine B is added to the agar (phloxine B preferentially stains opaque cells; 1).

Outgrowth under the regimen of pH-regulated dimorphism. To stimulate hypha formation in standard laboratory strains of *C. albicans* (e.g., 3153A) under the regimen of pH-regulated dimorphism (4, 17), cells are grown at 25°C in amino acid-rich medium (2, 6) to stationary phase. After 24 h in stationary phase, the unbudded singlets, which accumulate as a result of zinc starvation (2, 16), are diluted into fresh medium at 37°C, pH 6.7. After an average lag period of 130 to 140 min, 3153A cells evaginate synchronously, and each evagination elongates into a hypha. If stationary-phase cells are diluted into fresh medium at 37°C, pH 4.5, cells evaginate after roughly the same average lag period, but each evagination expands into an ovoid yeast cell. This regimen of pH-regulated dimorphism was applied to cells of the alternative phases of the white-opaque transition of strain WO-1.

At stationary phase in liquid medium at 25°C, the majority of white cells accumulated as unbudded round cells (Fig. 1A). When diluted into fresh medium at 37°C, pH 6.7, white cells evaginated semisynchronously, with an average evagination time of approximately 135 min and maximum evagination of roughly 80% (Fig. 2A). Each evagination elongated into a hypha (Fig. 1E). When diluted into fresh medium at 37°C, pH 4.5, white cells evaginated with an average evagination time of approximately 185 min and maximum evagination of roughly 80% (Fig. 2B). In contrast to results with standard laboratory strain 3153A (4, 17), a significant proportion of evaginations (between 30 and 80% in independent experiments) again elongated into hyphae (Fig. 1C); the remaining evaginations expanded into ovoid buds.

At stationary phase at 25°C, the majority of opaque cells accumulated as unbudded elongate cells exhibiting the standard opaque phenotype (Fig. 1B). When diluted into fresh medium at 37°C, pH 6.7, opaque cells evaginated semisynchronously, with an average evagination time of approximately 145 min and maximum evagination of roughly 80% (Fig. 2A). Each evagination expanded into an elongate cell with the unique opaque-cell morphology (Fig. 1F). When diluted into fresh medium at 37°C, pH 4.5, opaque cells evaginated with an average evagination time of approximately 150 min and maximum evagination of roughly 80% (Fig. 2B). Each evagination again expanded into an elongate opaque cell (Fig. 1D). These results demonstrate that under the regimen of pH-regulated dimorphism, cells in the white

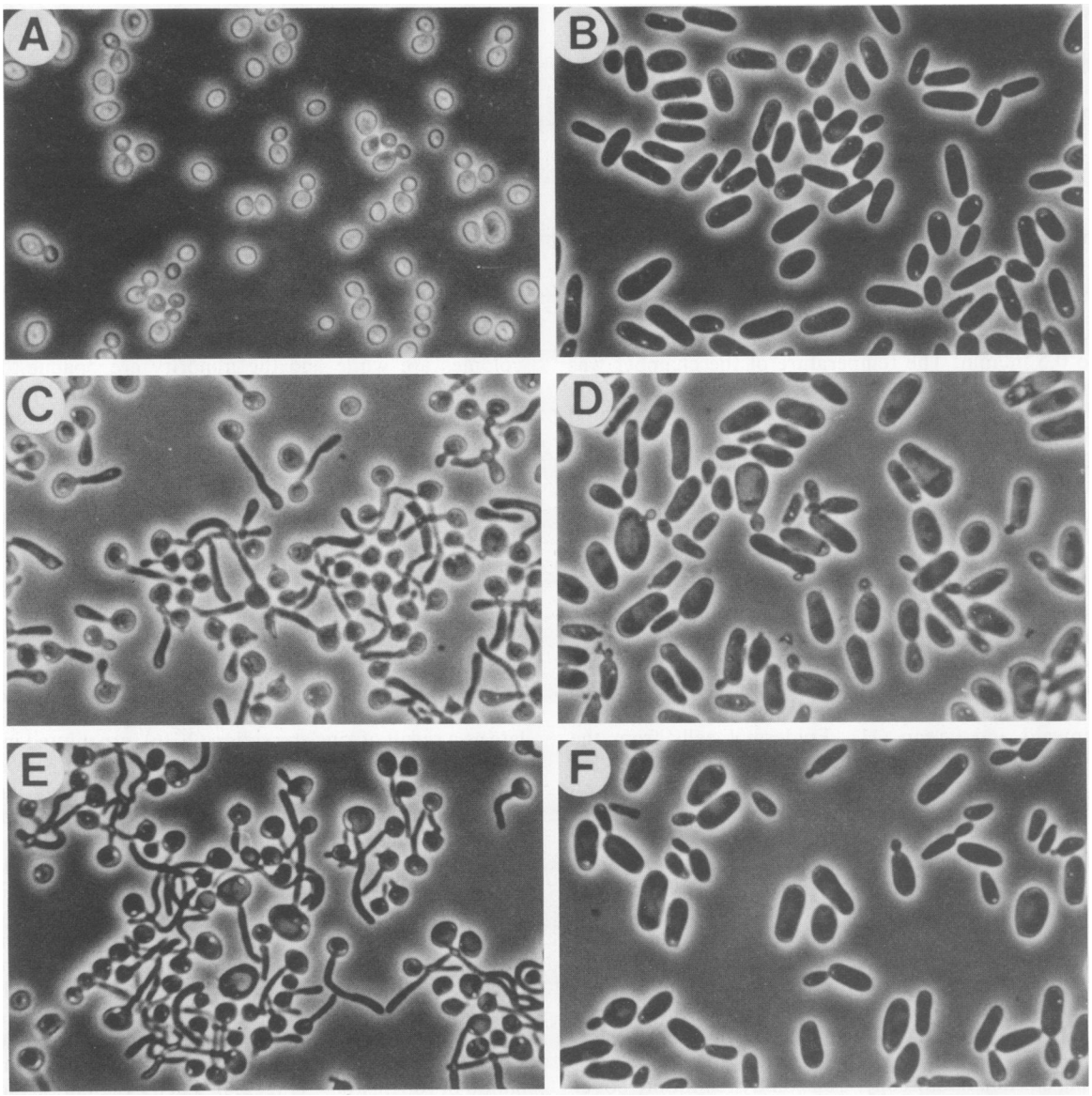

FIG. 1. Cellular phenotypes of stationary-phase cultures and daughter cells generated under the regimen of pH-regulated dimorphism for white and opaque cells. (A) White cells from a stationary-phase liquid culture; (B) opaque cells from a stationary-phase liquid culture; (C) hypha formation by stationary-phase white cells 180 min after dilution into fresh nutrient medium, 37°C, pH 4.5 (note that under this regimen, white cells still make hyphae rather than buds, in contrast to laboratory strain 3153A); (D) bud formation by stationary-phase opaque cells 180 min after dilution into fresh nutrient medium, 37°C, pH 4.5; (E) hypha formation by stationary-phase white cells 180 min after dilution into fresh nutrient medium, 37°C, pH 6.8; (F) bud formation by stationary-phase opaque cells 180 min after dilution into fresh nutrient medium, 37°C, pH 6.8. Magnification, ×563.

phase form hyphae at both high and low pH, but cells in the opaque phase form opaque-shaped cells in the budding phase at both pHs.

Formation of hyphae by opaque cells. When stationary-phase cells of laboratory strain 3153A were inoculated into a Sykes-Moore chamber with polylysine-coated walls and perfused with fresh medium at 37°C, pH 6.7, they evaginated after an average lag period of 130 to 150 min, and each evagination elongated into a hypha (5). When stationary-phase cells of strain WO-1 in the white phase were inoculated into a chamber and perfused with medium at 37°C, pH 6.7, they also formed hyphae (Fig. 3D). To our surprise, when stationary-phase cells in the opaque phase were inoculated into a chamber and perfused with medium, a proportion of the cells formed elongate hyphae (Fig. 3A to C). Although initial experiments suggested that hypha induction in opaque cells was facilitated by aeration or the nature of the treated surface, repeat experiments demonstrated that the proportion and variability of hypha formation were relatively independent of aeration or the nature of the surface coat (uncoated, fibronectin coated, or polylysine coated) of the glass Sykes-Moore chamber wall (Table 1).

The extreme variability in hypha formation between experiments (see standard deviations in Table 1) appeared to be a result of clonal variability. Variability within a clone appeared to be less than variability between clones (Table 2). This point is demonstrated by an analysis of variance. The mean square between the clones in Table 2 was 2,785, and

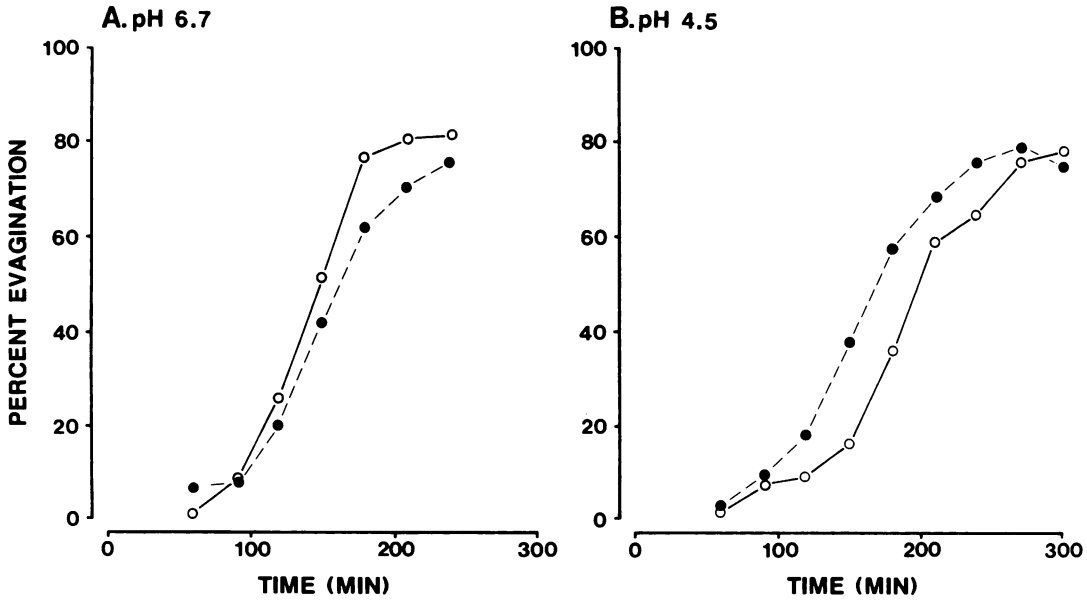

FIG. 2. Evagination kinetics of white (○)- and opaque (●)-phase cells released into fresh nutrient medium (37°C) at pH 6.7 (A) or 4.5 (B). The phenotypes of mature daughter cells of white cells were hypha in both cases, and those of opaque cells were buds in both cases. Micrographs of the cell populations after 180 min are presented in Fig. 1C through F.

the mean square within the clones was 361. By using an *F* test, the clones were found to be significantly different at a *P* value of 0.0001.

Evidence that opaque cells form hyphae in association with epithelial cells. When stationary-phase cells with the opaque phenotype were incubated on glass cover slips in growth medium, pH 6.7, in small petri dishes at 37°C, they formed buds with the opaque phenotype (Fig. 4A). In contrast, when opaque cells were incubated on glass cover slips supporting a monolayer of epithelial cells in *Candida* growth medium,

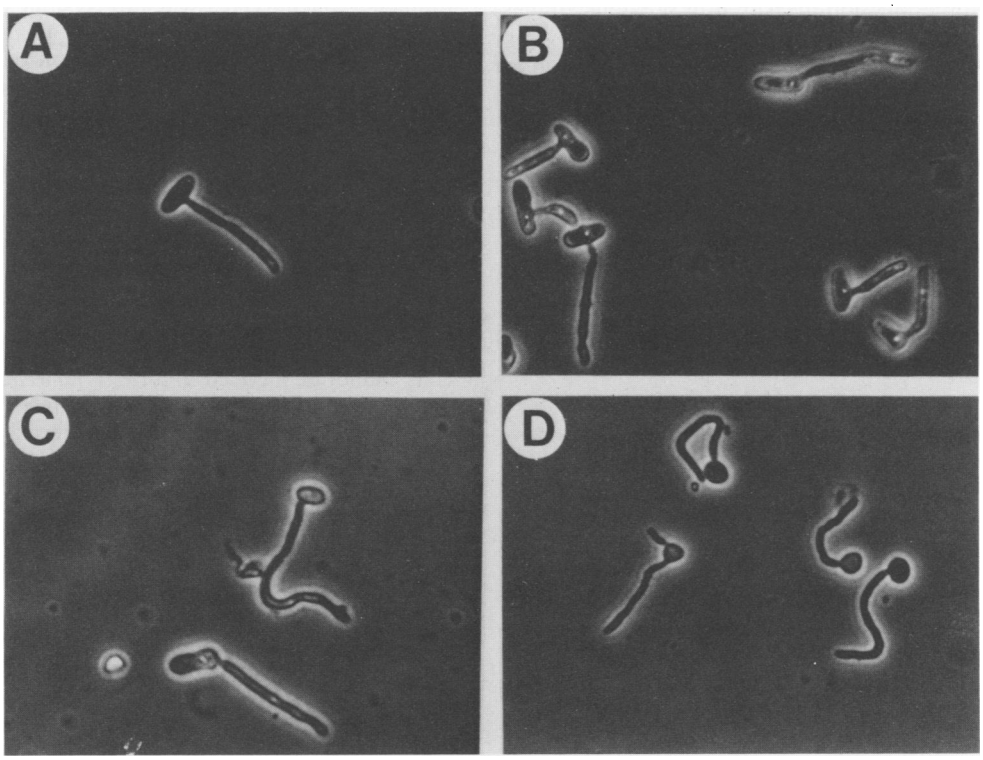

FIG. 3. Examples of hypha formation by opaque cells in Sykes-Moore chambers. (A through C) Opaque-cell hypha formation; (D) white-cell hypha formation. Note the difference in mother cell morphology between opaque and white but the similarity of hypha morphology. Magnification, ×563.

TABLE 1. Hypha formation in opaque-cell populations in a Sykes-Moore chamber

Exptl conditions[a]	No. of expts	No. of clones	% Hyphae (mean ± SD)[b]
A	15	13	32 ± 25
B	19	18	42 ± 31
C	13	11	22 ± 22
D	3	3	33 ± 26

[a] A, Wall treated with polylysine, perfusion medium unaerated; B, wall treated with fibronectin, perfusion medium unaerated; C, wall untreated, medium unaerated; D, perfusion medium aerated, wall treated with polylysine in one experiment, treated with fibronectin in one experiment, and untreated in one experiment.

[b] Calculated as the percentage of daughter cells exhibiting the hypha phenotype rather than the bud phenotype. The proportion of the population that evaginated varied between 50 and 98%. Therefore, the percent buds formed in the population was 100 minus percent hyphae. Chamber cultures were scored between 180 and 240 min.

pH 6.7 (see Materials and Methods for details), in small petri dishes at 37°C, they formed hyphae (Fig. 4B).

Surface morphology of opaque-cell hyphae. Opaque cells with the budding phenotype exhibit not only a unique asymmetric bean shape but also a unique surface morphology when viewed by scanning electron microscopy (1). Over the entire surface of each mature budding cell, either pimples (Fig. 5A and C) or small protrusions (Fig. 5B and D) are evident. This surface morphology contrasts dramatically with the smooth surface of buds and hyphae of cells in the white phase (1).

The hyphae formed by opaque cells were largely devoid of pimples or protrusions (Fig. 5) and appeared in all respects similar to the hyphae formed by white cells. Septal rings were evident along several of the hyphae at distances from the mother cell-tube junction measured in previous studies by Calcofluor staining (9).

Absence of the opaque-cell antigen on opaque-cell hyphae. The punctate pattern of pimples and protrusions on the opaque-cell wall is paralleled by a punctate staining pattern with an antiserum generated against opaque cells and absorbed with hypha-forming white cells (1). This absorbed antiserum does not stain white budding cells or white hypha-forming cells (1). Figure 6 shows examples of hypha-forming opaque cells stained with this absorbed antiserum by indirect immunofluorescence (see Materials and Methods). It should be noted that these cells had not been permeabilized; therefore, staining was confined to the wall. When one compares fluorescent micrographs (Fig. 6A, C, and E) with phase-contrast micrographs (Fig. 6B, D, and F), it is immediately evident that although all mother cells exhibited intense punctate staining, their hyphae did not.

TABLE 2. Comparison of variability of hypha formation within clones with variability between clones

Clone no.	% Hyphae	
	Per expt[a]	Mean[b] ± SD
1	61, 85	73 ± 17
2	42, 2, 78, 64	47 ± 33
3	44, 81, 78	68 ± 21
4	15, 36, 50	34 ± 18
5	6, 12, 15	11 ± 5
6	87, 65, 53, 90	74 ± 18
7	50, 24, 7, 0	20 ± 22
8	0, 8, 8, 3	5 ± 4
9	0, 0, 4	1 ± 2

[a] Calculated from the proportion of cells that evaginated.

[b] Calculated from experiments performed on each clone.

Evidence that opaque-cell hyphae are genetically opaque. The preceding results demonstrate not only that opaque cells can form hyphae, but that the hyphae are morphologically indistinguishable from those formed by white cells. In addition, an antigen in the wall of opaque budding cells stained in a punctate fashion by an opaque-specific antiserum is not detectable in the walls of opaque-cell hyphae. Since hyphae are compartmentalized and each compartment contains a nucleus, the question arises as to the genetic state of the hypha compartments (i.e., have they reverted to the white genotype?). To answer this question, opaque cells were induced to form hyphae in Sykes-Moore chambers by perfusion with nutrient medium, pH 6.7, at 37°C for 240 min, conditions conducive to hypha formation, and then were perfused with nutrient medium, pH 4.5, at 25°C, conditions conducive to bud formation (4). After an additional 240 min, hyphae had formed lateral and apical buds with the unique opaque-cell morphology (Fig. 7). Therefore, although morphologically distinct opaque and white mother cells generated hyphae with identical phenotypes, the opaque hyphae were still in the opaque phase.

DISCUSSION

C. albicans and related species possess two levels of phenotypic regulation. At the basic level of cellular differentiation, each of these dimorphic species is able to differentiate between a budding growth form and a hyphal growth form (10, 17), and environmental cues can be used in vitro to initiate this phenotypic transition in either direction (10, 15, 17). Recently, it was demonstrated that a second high-frequency phenotypic switching system was superimposed on the basic dimorphic transition (1, 13, 14, 18). In contrast to the dimorphic transition, which can be induced in every cell and which no doubt involves the developmental modulation of gene expression by the environment, switching occurs at a far lower frequency (10^{-2} to 10^{-4}) and very likely involves reversible changes in the genome which in turn affect gene expression (18). In the white-opaque transition, it is clear that the switch from the white to the opaque phase involves fundamental changes in the expression of the budding phenotype (1) and thus provides us with an excellent situation for investigating the interactions between the two systems of phenotypic regulation.

In this report, we have demonstrated that although opaque cells cannot be induced to form hyphae in suspension under the regimen of pH-regulated dimorphism (14), they can be induced to form hyphae when adhering to the wall of a Sykes-Moore chamber. It appears that at least in a chamber, neither aeration nor coating of the surface with polylysine or fibronectin has a significant effect on the proportion of cells which form hyphae when anchored to the chamber wall. Statistically significant variability was observed between opaque clones. Some clones exhibited low hypha formation (e.g., 0 to 4% in clone 9), whereas others exhibited high levels of hypha formation (e.g., 53 to 90% in clone 6). Although all opaque clones formed colonies with the same gray phenotype and exhibited the same unique opaque-cell phenotype, they were probably not identical. The highly mobile, moderately repetitive DNA sequence Ca7 is repeated 20 to 30 times in the genome and exhibits extraordinary mobility in strain WO-1 (1, 21). Opaque clones isolated in parallel or in sequence after very short periods of time

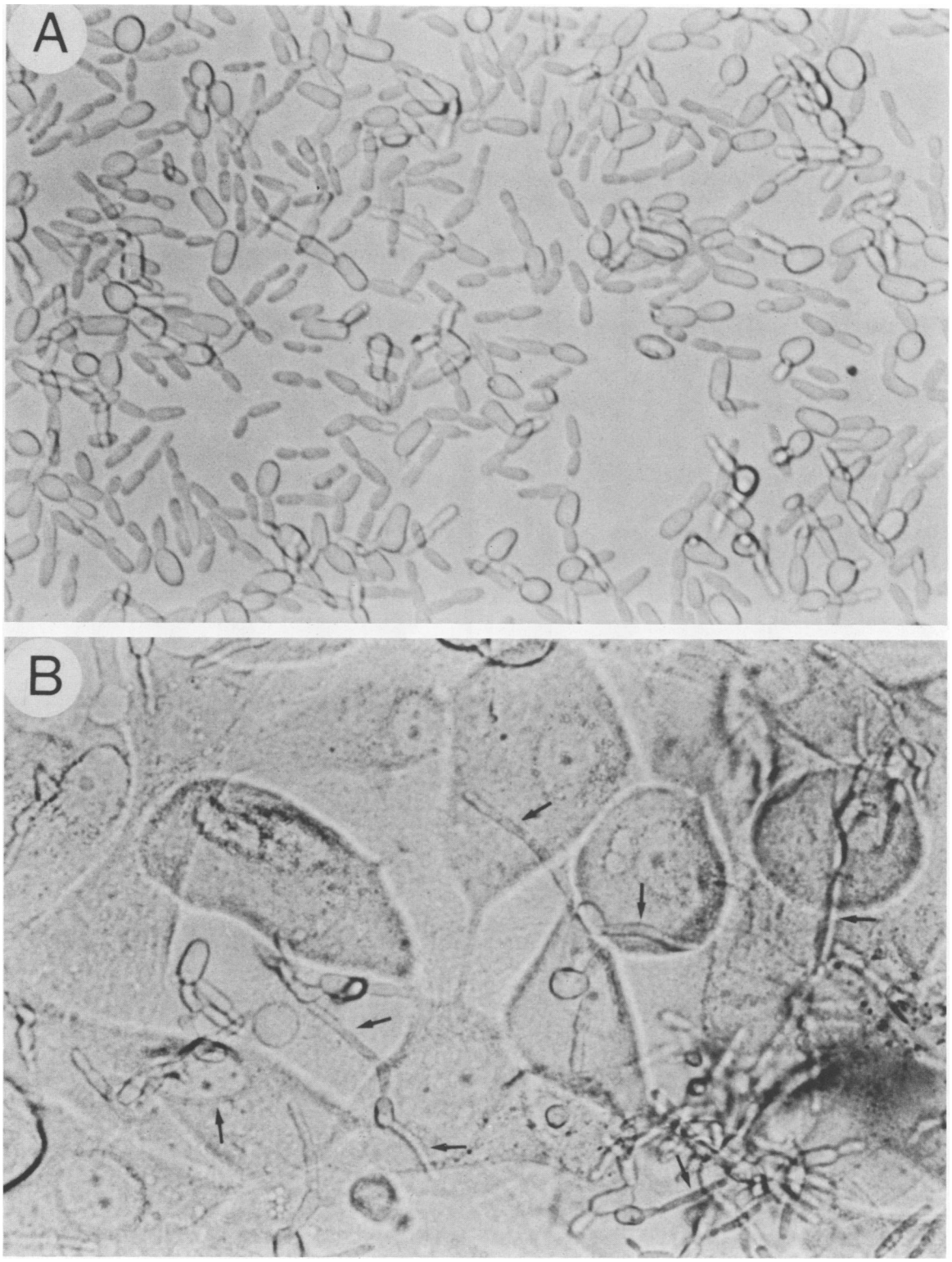

FIG. 4. Stimulation by epithelial cells of hypha formation by opaque cells. (A) Opaque cells incubated on a glass cover slip in nutrient medium at 37°C, pH 6.7; (B) opaque cells inoculated onto a sheet of skin epithelium and incubated in the same medium as used for panel A. Arrows point to long hyphae. Magnification, ×563.

exhibit differences in Southern hybridization patterns with Ca7 (21; D. R. Soll, unpublished observations; J. Hicks, M. J. McEachern, E. P. Bulgac, J. Schmid, and D. R. Soll, submitted for publication), which demonstrates that opaque clones are not genetically identical. The variability in hypha formation between opaque clones could very well be the result of frequent Ca7 transposition or rearrangement.

Conditions conducive to hypha formation in opaque cells

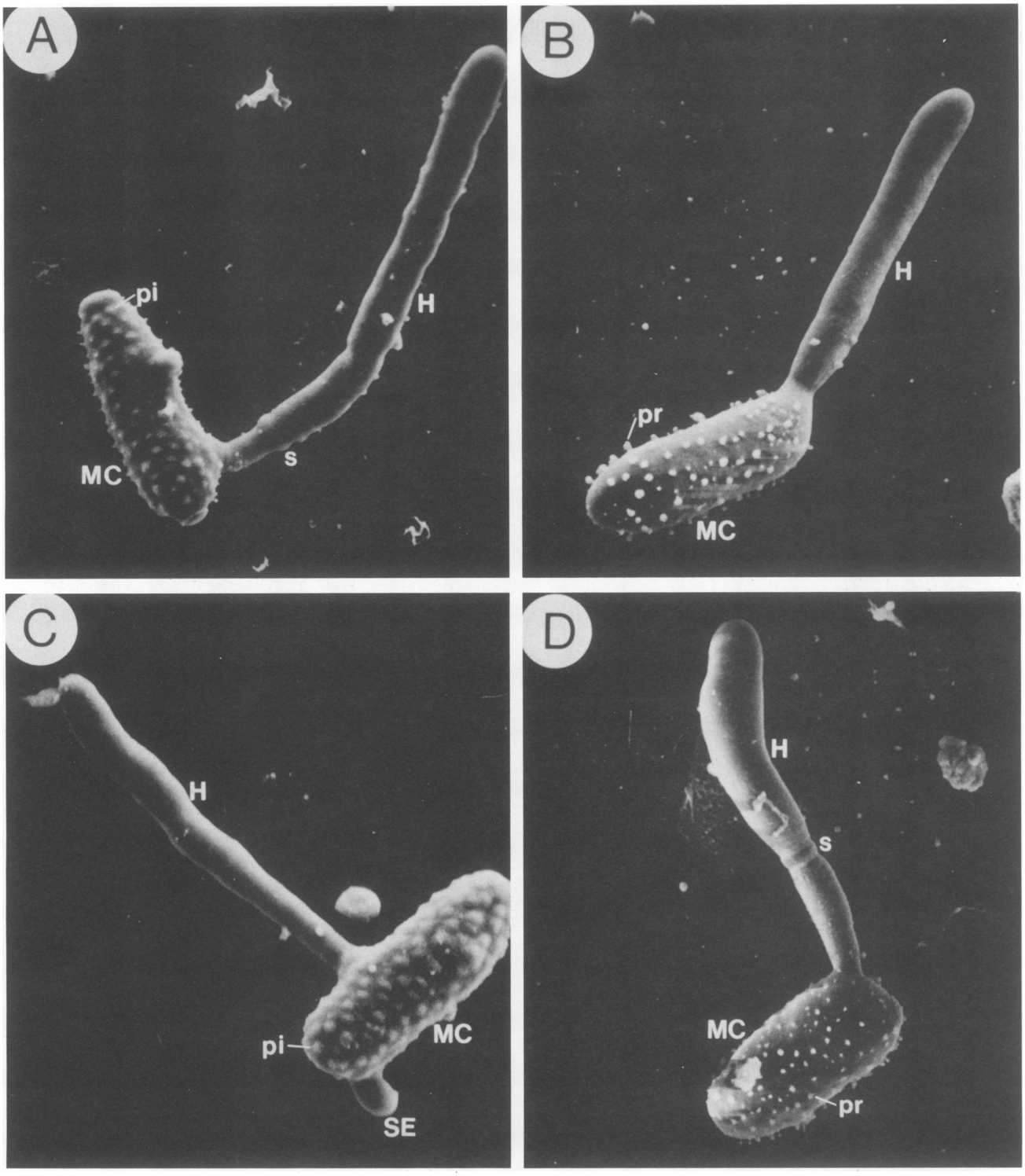

FIG. 5. Scanning electron micrographs of opaque cells which have formed hyphae in a Sykes-Moore chamber. Note that mother cells (MC) exhibit the unique phenotype of opaque budding cells with pimples (pi) or protrusions (pr) on their surfaces. Opaque hyphae (H) are relatively smooth. SE, Secondary evagination; S, septal ring. Magnification: A, B, and D, ×5,760; C, ×6,720.

appear to include either a cell-substrate interaction or anchoring, conditions which will support but which are not necessary for white-cell hypha formation. Differences in the environmental constraints on the bud-hypha transition have also been demonstrated between the switch phenotypes in the switching system in strain 3153A (B. Slutsky, Ph.D. thesis, University of Iowa, Iowa City, 1986), which suggests that this is a general characteristic of switching systems. Cell-substrate interaction leading to hypha formation by opaque cells was pronounced when the substrate consisted of a sheet of human skin epithelium in nonperfused culture dishes. In the epithelial cell experiments, control opaque

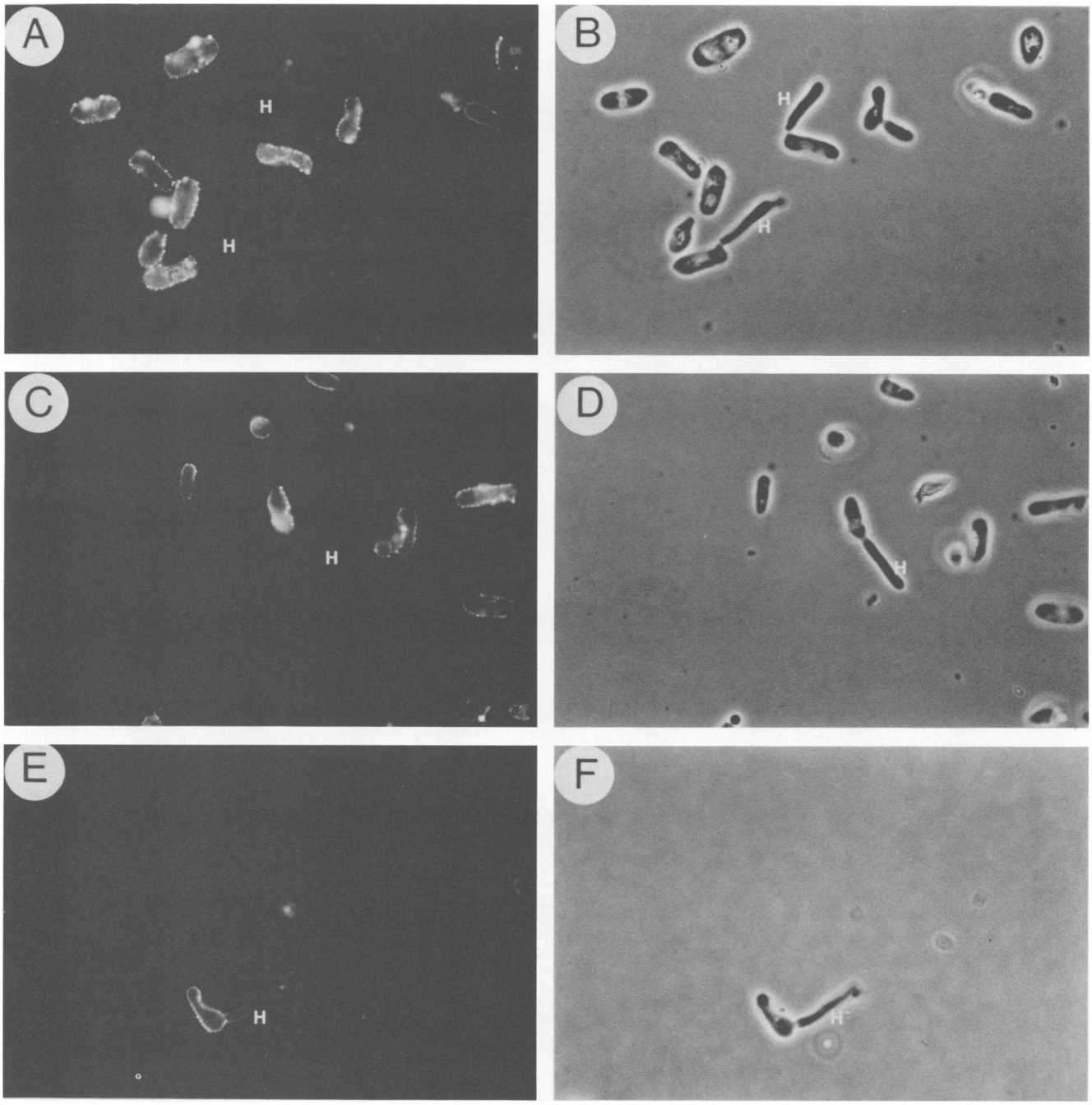

FIG. 6. Indirect immunofluorescence staining of opaque cells forming hyphae with an antiserum against opaque budding cells. The antiserum was generated in rabbits against opaque budding cells and then absorbed three times with white cells that had formed hyphae (see Materials and Methods). Stationary-phase opaque cells were placed in a Sykes-Moore chamber and perfused with fresh nutrient medium, 37°C, pH 6.7. (A, C, and E) Opaque cells forming hyphae (H) immunostained with absorbed serum (note particulate staining of mother cells and lack of staining of hyphae); (B, D, and F) phase-contrast micrographs of immunostained preparations shown in panels A, C, and E, respectively. Magnification, ×968.

cells cultured under the same conditions in the absence of epithelial cells formed few hyphae, which suggests that epithelial cells either conditioned the microenvironment or provided a surface signal for hypha formation. The latter possibility is intriguing for two reasons. First, the unique pimples and protrusions on opaque cells (1) could represent complex adhesion receptor systems for anchoring budding cells, which in turn is necessary in hypha stimulation. The absence of pimples and protrusions on hyphae may facilitate penetration of tissue. Second, J. Hicks and co-workers (Hicks, personal communication) recently cloned the gene for G protein in *C. albicans*. This protein exhibits homology with the G protein of *Saccharomyces cerevisiae* and suggests that membrane receptors exist in *C. albicans*. The possible role of the complex pimple as a receptor is now under intensive investigation in our laboratory.

Perhaps the most unexpected result of this study is the morphology of the opaque hyphae. The shape, smooth

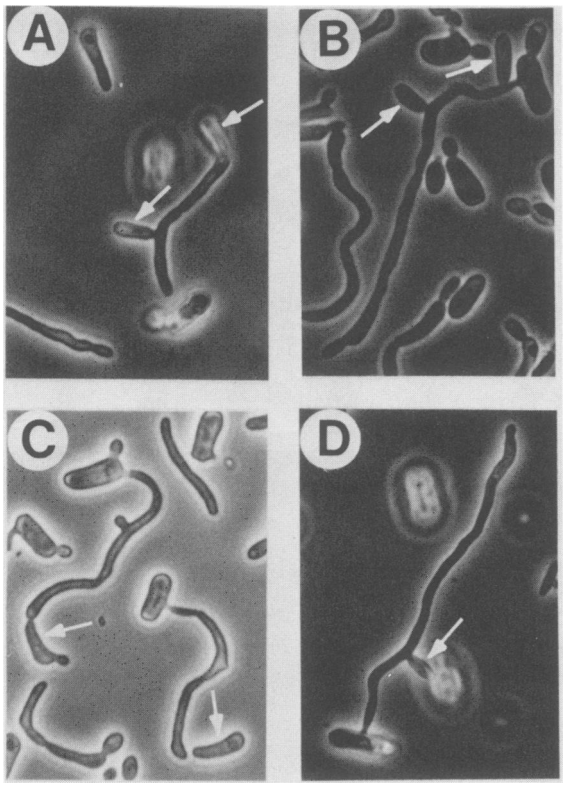

FIG. 7. Unique opaque phenotype of daughter cells formed on opaque hyphae. Stationary-phase opaque cells were placed in a Sykes-Moore chamber and perfused with fresh nutrient medium, 37°C, pH 6.7, for 240 min to induce hypha formation. The medium was then shifted to 25°C, pH 4.5, to induce bud formation. Micrographs of budding hyphae were taken 240 min after the shift. Arrows point to new buds, which exhibit the unique opaque-cell phenotype. Magnification, ×563.

surface (absence of pimples or protrusions), and septum positions of the hyphae are similar to those of white hyphae. However, when induced to change from the hyphal to the budding growth form, the cells generate bean-shaped opaque cells. Therefore, they remain genetically opaque. These results have very interesting ramifications for the effect of switching on gene expression. Although the genesis of buds and hyphae involves a majority of common mechanisms and gene products (17), it is likely that differential gene expression plays an integral, if not fundamental, role (3, 7, 17). One can therefore assume that there are a limited number of genes differentially expressed between the bud and hyphal growth forms (17). When a cell of strain WO-1 switches to the opaque phase, the budding phenotype is altered in a dramatic fashion, including changes in virtually all aspects of wall and cytoplasmic morphology (1, 14). Therefore, the switch to the opaque phase may effect repression of one or more standard bud-specific genes, partial expression of hypha-specific genes (a hypha-specific antigen is expressed in opaque budding cells; 1), expression of opaque-specific genes (an opaque-specific antigen is expressed in opaque budding cells; 1), or all of these possibilities. In contrast, the switch to the opaque phase has no overt effect on hyphal morphology, which suggests that the switch has no effect on the repertoire of genes differentially expressed in the hyphal growth form. This discussion points to the need for investigations into the role of differential gene expression in the bud-hypha transition, a surprisingly neglected area of research, and into the effects of switching on bud-hypha gene regulation.

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