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Ultrastructure and Antigenicity of the Unique Cell Wall Pimple of the *Candida* Opaque Phenotype

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Cells of *Candida albicans* WO-1 switch frequently and reversibly between two colony-forming phenotypes, white and opaque. In the white form, budding cells appear similar to those of most other strains of *C. albicans*, but in the opaque form, budding cells are larger, are bean shaped, and possess pimples on the wall. These pimples exhibit a unique and complex morphology. With scanning electron microscopy, a central pit can be discerned, and in many cases, a bleb can be observed emerging from the pimple center. With transmission electron microscopy, channels are evident in some pimples and vesicles are apparent under the pimple in the cytoplasm, in the actual wall of the pimple, or emerging from the tip of the pimple. A large vacuole predominates in the opaque-cell cytoplasm. This vacuole is usually filled with spaghettilike membranous material and in a minority of cases is filled with vesicles, many of which exhibit a relatively uniform size. An antiserum to opaque cells recognizes three opaque-cell-specific antigens with molecular masses of approximately 14.5, 21, and 31 kilodaltons (kDa). Absorption with nonpermeabilized opaque cells demonstrated that only the 14.5-kDa antigen is on the cell surface; indirect immunogold labeling demonstrated that it is localized in or on the pimple. The possibility is suggested that the vacuole of opaque cells is the origin of membrane-bound vesicles which traverse the wall through specialized pimple structures and emerge from the pimple with an intact outer double membrane, a unique phenomenon in yeast cells. The opaque-cell-specific 14.5-kDa antigen either is in the pimple channel or is a component of the emerging vesicle. The functions of the unique opaque-cell pimple and emerging vesicle are not known.

Candida albicans and related species are capable of switching at high frequencies between a number of general phenotypes distinguishable by colony morphology (18, 20, 21, 24). There are a number of different switching systems in the species *C. albicans* and *Candida tropicalis*, which differ from one another in phenotypic repertoire (18, 20, 21, 26, 27). Thus far, all of the systems tested share characteristics of high- and low-frequency modes of switching, heritability, reversibility, a limited number of phenotypes, and stimulation by low doses of UV irradiation (24, 25). One of these switching systems, the white-opaque transition, involves a dramatic change not only in colony morphology but also in the basic phenotypes of cells in the budding growth form (1, 2, 19, 21). In the white phase, cells produce smooth white colony domes in all respects similar to those produced by most other strains of *C. albicans* (2, 21). However, at a frequency of approximately 10^{-5}, white cells generate a wider, flatter, grey colony dome referred to as the opaque phase. When opaque cells are plated, they form white colonies at frequencies as high as 10^{-1} (2, 21; D. R. Soll, J. Anderson, and M. Bergen, *in* M. Prasad, ed., *Candida albicans: Cellular and Molecular Biology*, in press).

White-phase budding cells are indistinguishable from budding cells of most other strains of *C. albicans*. They are round to slightly ellipsoidal and form buds which grow into mature round cells. The cell surfaces are relatively smooth, except for bud scar ridges. In contrast, opaque-phase budding cells are elongate or bean shaped and are roughly three times the volume and twice the mass of white cells on average but contain approximately the same amount of DNA as do white cells (21). More remarkably, opaque cells differ from white cells in both cytoplasmic composition and cell wall morphology (2, 21). Opaque cells contain unusually large and sometimes multiple vacuoles (21) as well as unique pimples on the mature cell wall (1, 2). In addition, it has been demonstrated that opaque cells possess one or more opaque-cell-specific antigens distributed in the cell wall in a punctate fashion similar to pimple distribution (1, 2).

We present here scanning electron microscopy (SEM) and transmission electron microscopy (TEM) evidence that these unique opaque-cell pimples possess a complex morphology and may represent a mechanism for moving materials, including membrane-bound vesicles, through the cell wall. In addition, it is demonstrated that there are three opaque-cell-specific antigens differentially identified by an antiserum generated against opaque cells. Two of these antigens (molecular masses, 21 and 31 kilodaltons [kDa]) are localized intracellularly, but the third antigen (molecular mass, approximately 14.5 kDa) is located on the cell surface. Immunogold labeling demonstrated that this opaque-cell-specific 14.5-kDa antigen is localized on the opaque-cell wall pimple.

MATERIALS AND METHODS

Growth and maintenance of stock cultures. *C. albicans* WO-1 was isolated from the blood and lungs of an immunosuppressed bone marrow transplant patient at the University of Iowa Hospitals and Clinics (21). This strain has been maintained on agar slants for 4 years with no loss in its capacity to switch from white to opaque or opaque to white. For experimental purposes, cells were cloned from stock slants onto agar plates containing the medium described by Lee et al. (17) supplemented with arginine and zinc (3). Cells were removed from white or opaque colonies (2, 21) and inoculated directly into 125-ml Erlenmeyer flasks containing 25 ml of supplemented Lee medium (17). These cultures were rotated at 200 rpm in a Gyrotory water bath shaker (model G76; New Brunswick Scientific Co., Inc., Edison,

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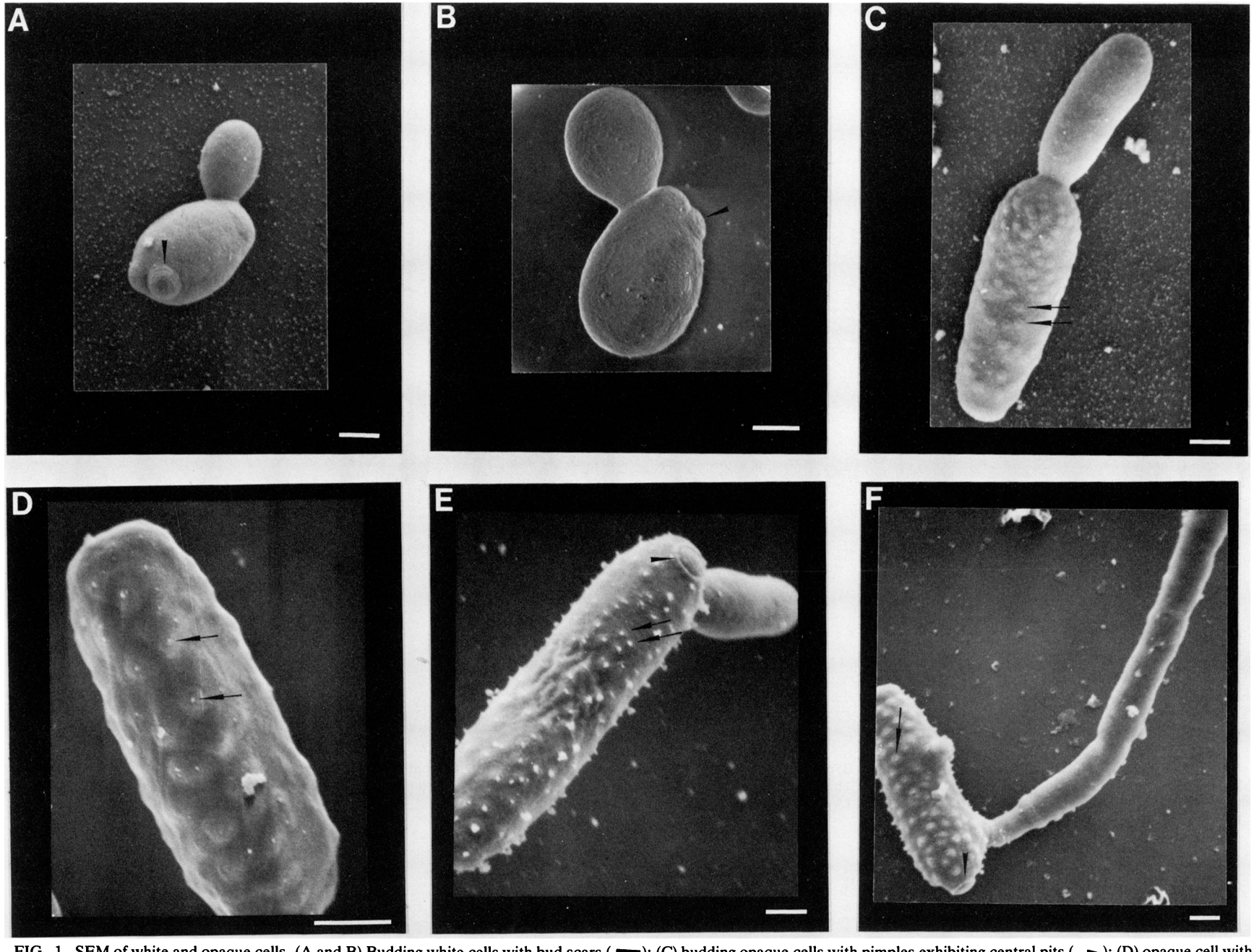


FIG. 1. SEM of white and opaque cells. (A and B) Budding white cells with bud scars (➡); (C) budding opaque cells with pimples exhibiting central pits (→); (D) opaque cell with small blebs (→) emerging from pimples; (E) budding opaque cell (➡, bud scar) with larger blebs (→) emerging from pimples; (F) hypha-forming opaque cell with pimples on mother cell but smooth-walled hypha (→, pimple with small central bleb; ➡, bud scar). Bars = 1 μm.

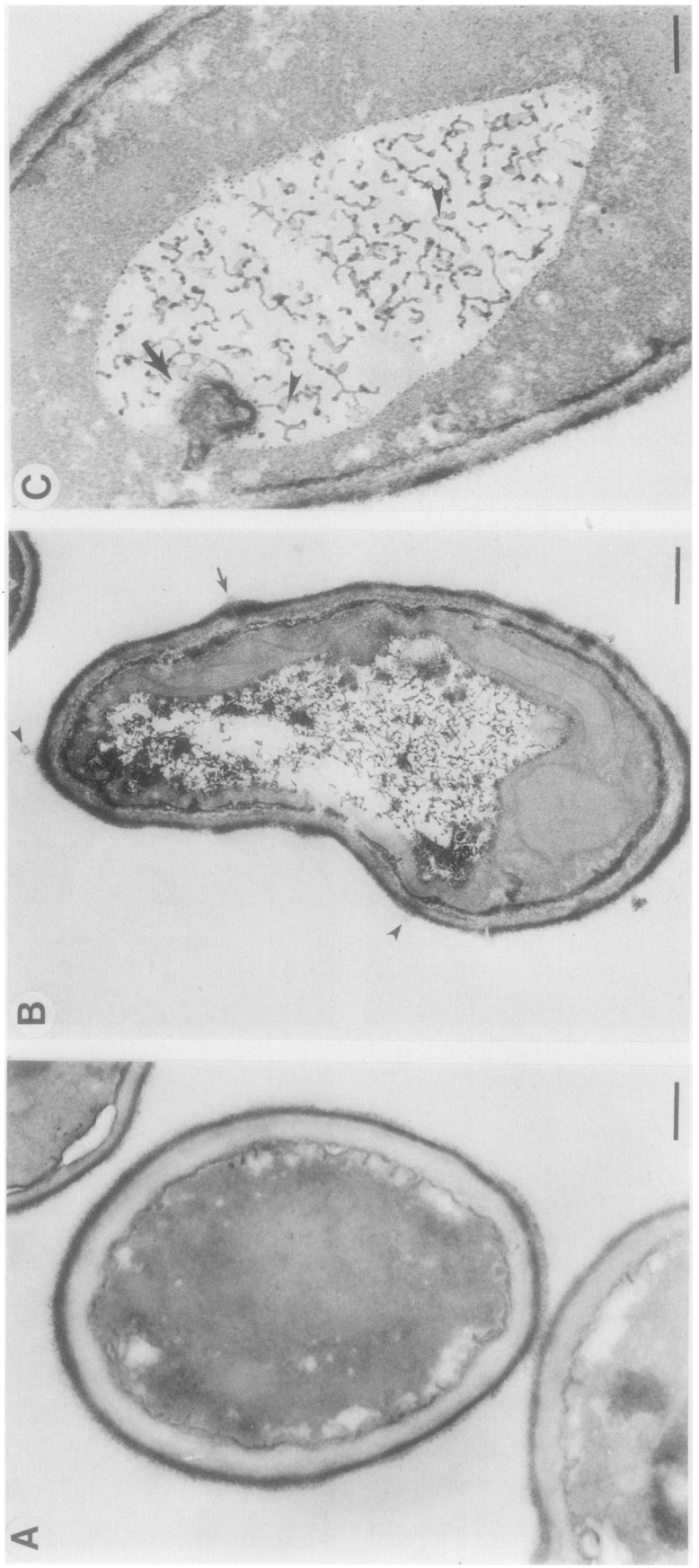


FIG. 2. TEM of white and opaque cells. (A) White cell; (B) opaque cell with pimples (▲ , vesicles or blebs emerging from wall; —▲, pimple with prominent central pore and emerging bleb) and large vacuole containing spaghettilike material; (C) high magnification of spaghettilike material sometimes appearing membranous (▲) or vesiculated (—▲, mushroom-shaped structure penetrating the vacuole lumen). Bars = 0.33 μm (A), 0.55 μm (B), and 0.21 μm (C).

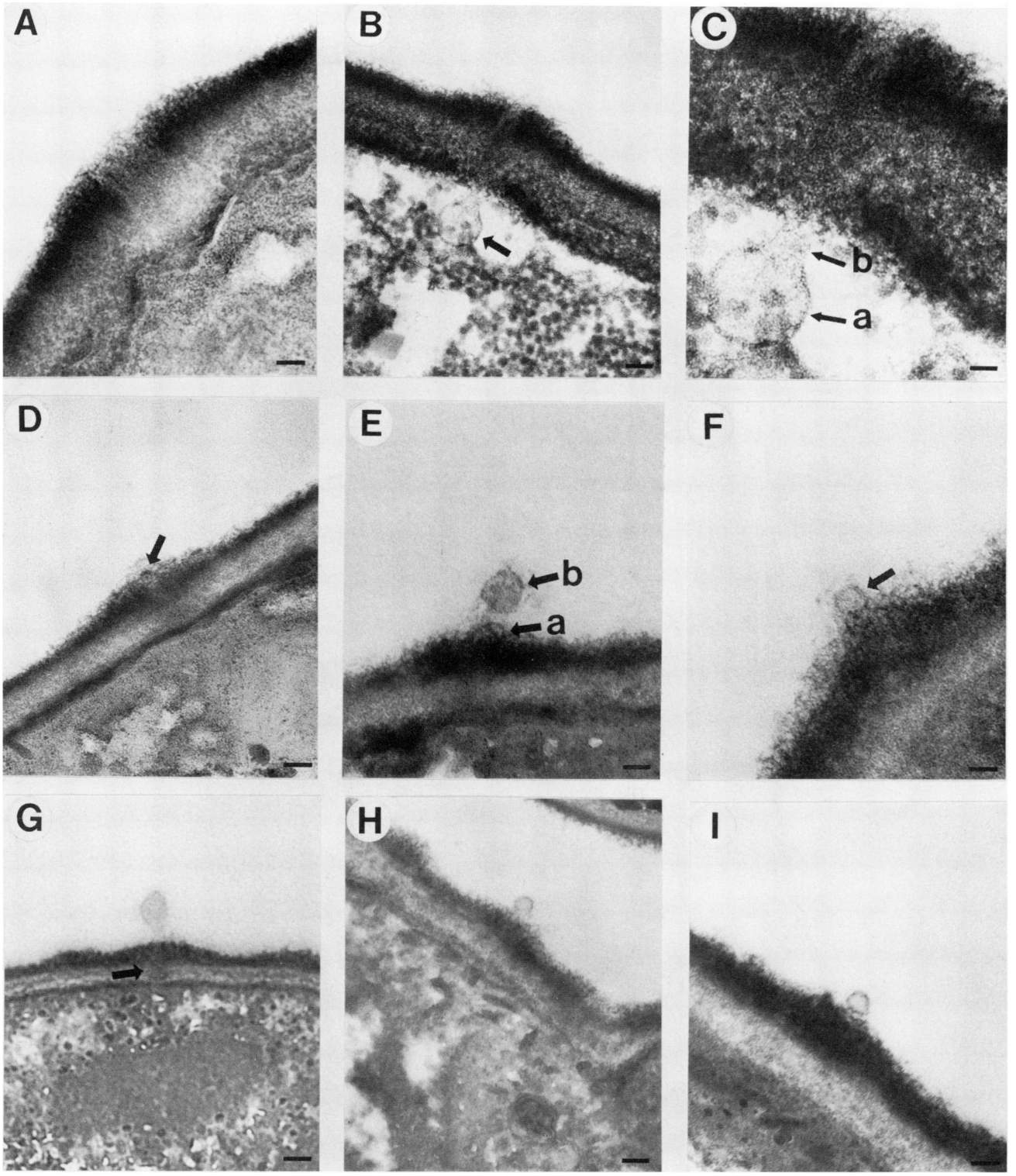


FIG. 3. Vesicles associated with opaque-cell pimples. (A) Region of wall in a pimple which exhibits altered consistency. Such wall incongruities, when observed, are associated with pimples. (B) Flask-shaped vesicle (➨) under a pimple, with the neck penetrating the wall; (C) higher magnification of the pimple-associated flask-shaped vesicle in panel B (a, vesicle; b, neck); (D) cross section of a bleb (➨) emerging from the center of a pimple; (E) emerging bleb (a) and emerged bleb enclosed by a discernible double membrane (b); (F) emerging bleb (➨) enclosed by discernible double membrane; (G) emerging bleb with rootlike structure in wall (➨); (H and I) viruslike particles with base in wall. Bars = 0.04 μm (A), 0.08 μm (B), 0.03 μm (C), 0.05 μm (D), 0.05 μm (E), 0.04 μm (F), 0.11 μm (G), 0.11 μm (H), and 0.07 μm (I).

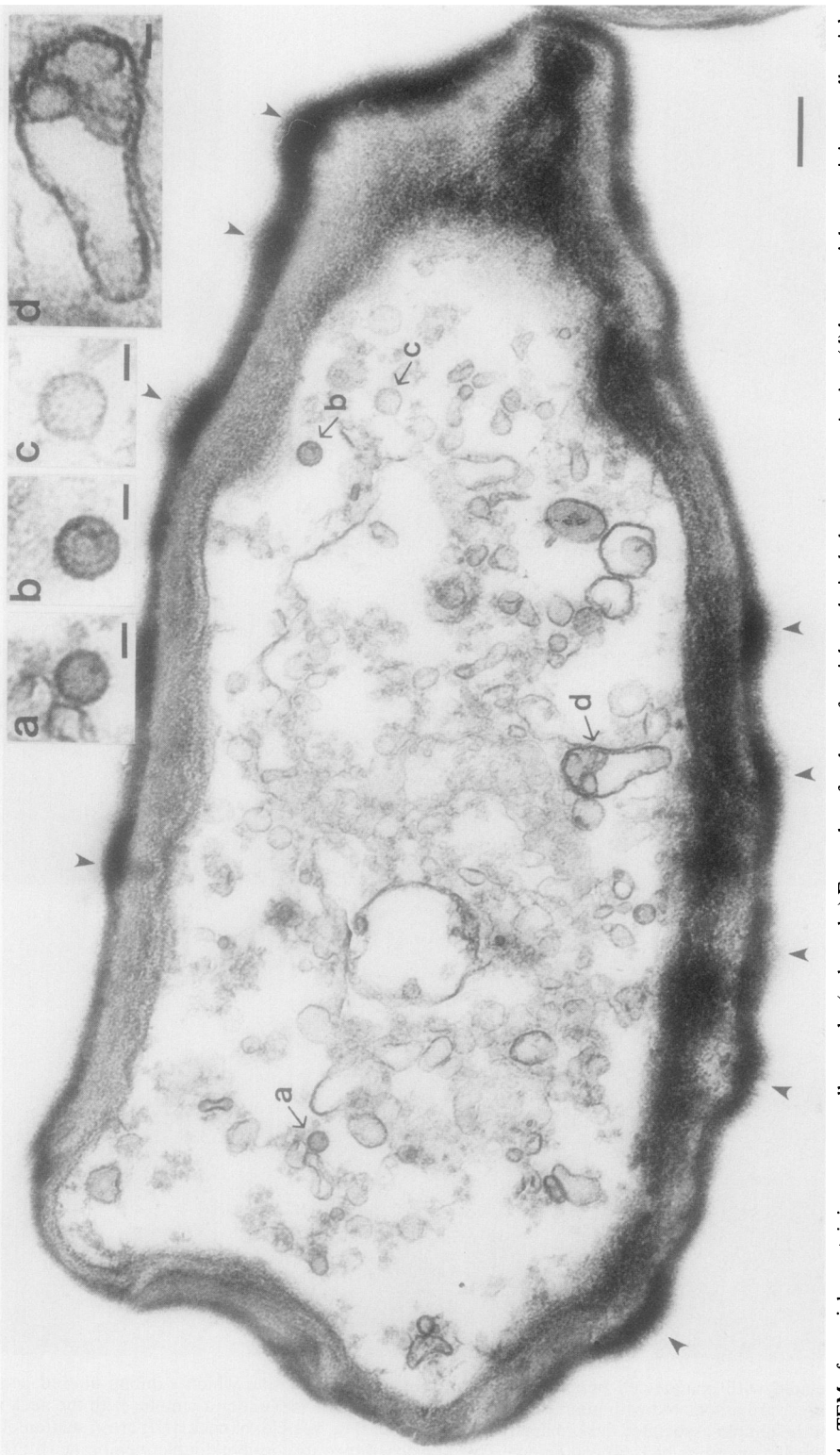


FIG. 4. TEM of a vesicle-containing opaque-cell vacuole. (a, b, and c) Examples of a class of vesicles relatively homogenous in size; (d) large vesicle containing small vesicles. Symbol: ▲, wall pimples. Bar = 1.0 μm; inset bars = 0.08 μm.

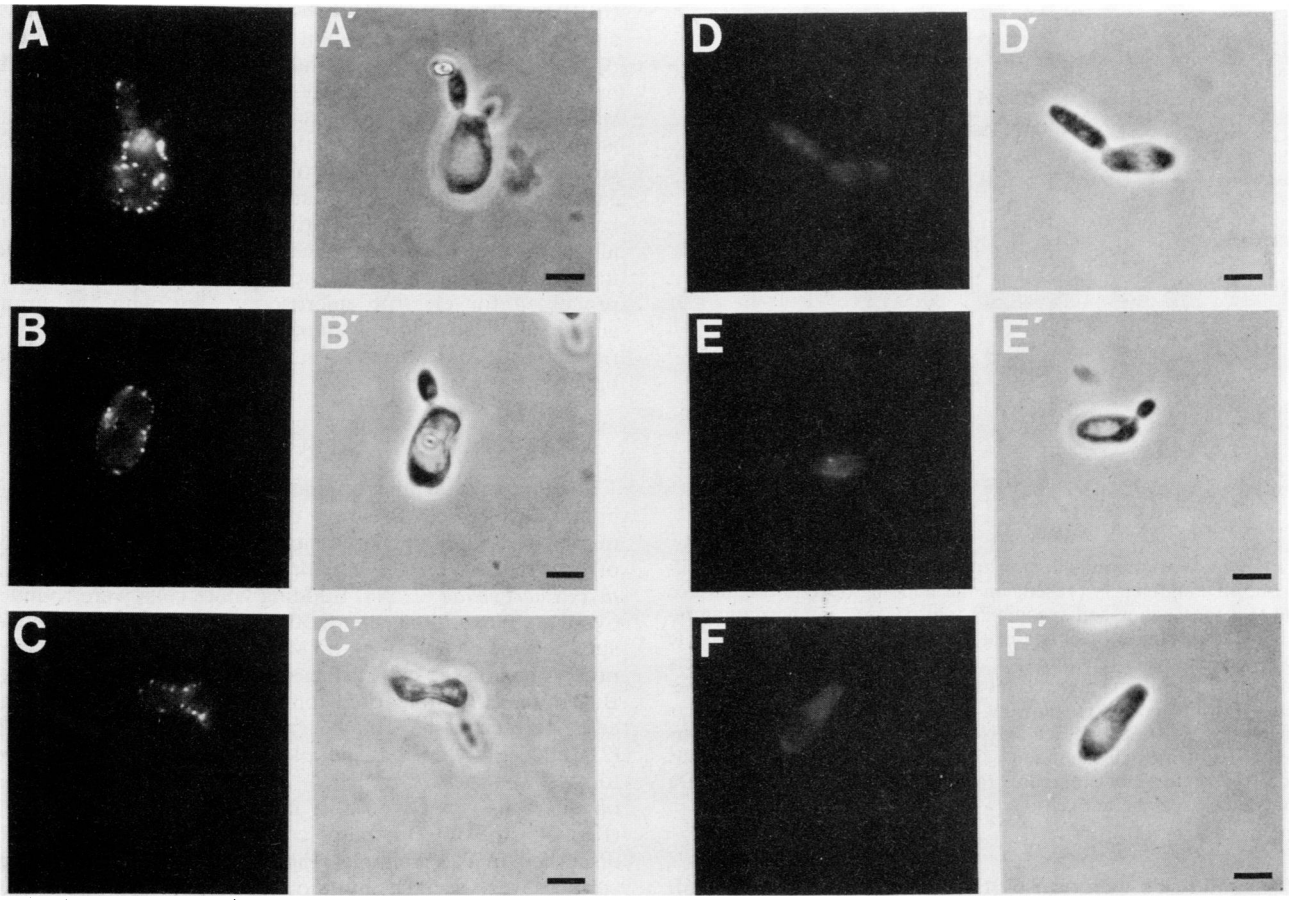


FIG. 5. Punctate staining of the walls of opaque cells by indirect immunofluorescence with an antiserum against opaque cells. (A through C) Examples of punctate staining of nonpermeabilized cells with the anti-opaque-cell antiserum after absorption with white cells; (A' through C') phase-contrast micrographs of the immunostained cells in panels A through C; (D through F) examples of the lack of staining of the cell surface with antiserum against opaque cells absorbed first with white cells and then with opaque cells; (D' through F') phase-contrast micrographs of the immunostained cells in panels D through F. Bars = 4.7 μm.

N.J.) at 25°C. Serial transfers were performed no more than three times, and the population was monitored for the proportions of white and opaque cells by phase-contrast microscopy at ×400.

SEM. SEM samples containing 10^9 cells were harvested from either the late log phase or the early stationary phase of growth by filtration (2) or centrifugation and washed twice with double-distilled water. Washed cells were suspended in 5 ml of 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.2) for at least 1 h and then placed on either glass cover slips or silicon wafers coated with poly-L-lysine. Cells were postfixed for 2 h in 1% osmium tetroxide in 0.1 M sodium cacodylate buffer (pH 7.2), gradually dehydrated in ethanol, and dried in a CPD 750 critical-point dryer (Emscope Laboratories, Ltd., Ashford, England) (2). After being dried, the samples were mounted on aluminum stubs and coated with gold palladium in an SC500 sputter coater (Emscope). Cells were viewed with a Hitachi S-570 SEM.

TEM. TEM samples containing 10^9 cells were washed twice in double-distilled water and then fixed in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer (or 0.1 M phosphate buffer) (pH 7.2) for at least 1 h. Cells were then postfixed in 1% osmium tetroxide–1.5% potassium ferrocyanide (0.1 M sodium cacodylate buffer [pH 7.2]) for 1 to 2 h. The cells were washed with 0.1 M sodium cacodylate buffer (pH 7.2), stained with 2.5% uranyl acetate in distilled water for 20 min, dehydrated in acetone, and embedded in Spurr epoxy resin for 2 days. Embedded cells were sectioned with a Reichart Ultracut E microtome (Reichert-Hung, Inc., Buffalo, N.Y.) and mounted on either uncoated or Formvar-coated 400-mesh grids. The sections were stained with 5% uranyl acetate in distilled water for 10 min and then stained with 0.5% lead citrate in distilled water for 8 min. Sectioned cells were viewed with a Hitachi H700 TEM.

Western blots (immunoblots), antiserum absorption, and indirect immunofluorescence staining. A sample (2×10^8 cells) was washed and extracted by a modification of the method of Finney et al. (11). In brief, cell pellets were suspended in 80 μl of buffer (50 mM Tris hydrochloride [pH 6.8], 5 mM $MgCl_2$, 50 μg of pancreatic RNase per ml) and 8 μl of β-mercaptoethanol in a tube (5 by 50 mm). Glasperlin beads were added to the tube until the bead level was just below the liquid surface. Samples were treated with a series of 20-s vortex pulses interspersed with 40-s periods of cooling in an ice bath until 90 to 95% of the cells were lysed. The sample was removed from the beads by washing the beads three times with 135 μl of buffer containing 50 mM Tris hydrochloride (pH 6.8) and 7.5% β-mercaptoethanol. A sample of the combined washes was assayed for protein by a microprotein assay (Bio-Rad Laboratories, Richmond, Calif.). The sample was adjusted to 1.4% sodium dodecyl sulfate and boiled for 4 min. Sodium dodecyl sulfate-poly-

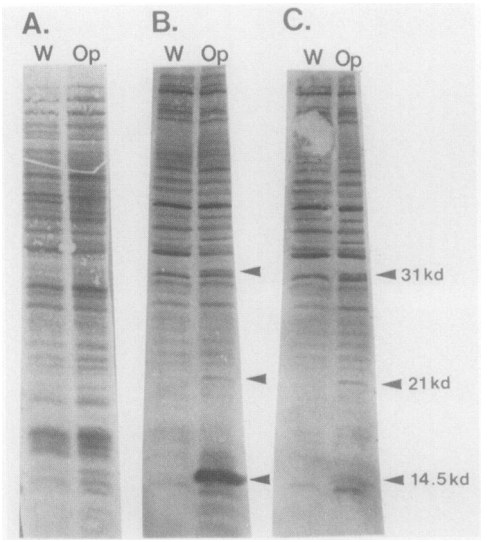


FIG. 6. Western blots demonstrating that the opaque-cell-specific 14.5-kDa antigen is present on the budding-opaque-cell surface and that the opaque-cell-specific 21- and 31-kDa antigens are intracellular. (A) Amido black-stained blots of white (W)- and opaque (Op)-cell extracts; (B) blots of white- and opaque-cell extracts stained with anti-opaque-cell antiserum absorbed with nonpermeabilized white cells; (C) blots of white- and opaque-cell extracts stained with anti-opaque-cell antiserum absorbed first with nonpermeabilized white cells and then with nonpermeabilized opaque cells (note the removal of antibodies to only one antigen, the 14.5-kDa opaque-cell-specific antigen).

acrylamide gels were run by the methods of Laemmli (16). Proteins were blotted to Immobilon PVDF (polyvinylidene difluoride) transfer membranes (Millipore Corp., Bedford, Mass.) in a TE50 electroblotter (Hoeffer Scientific Instruments, San Francisco, Calif.) with a transfer buffer containing 25 mM Tris hydrochloride (pH 8.3), 192 mM glycine, and 20% methanol. Blotted proteins were stained with 0.1% amido black in 45% methanol and 10% acetic acid and then destained with 90% methanol containing 2% acetic acid.

Blots were immunostained by the Protoblot alkaline phosphatase system (Promega Biotec, Madison, Wis.), in which the transfer membrane was blocked in TBST (10 mM Tris hydrochloride [pH 8.0], 150 mM NaCl, 0.05% Tween 20) containing 1% bovine serum albumin for 1.5 h at 25°C. The primary antibody in the initial incubation (1.5 h at 25°C) was a 1:10 dilution of rabbit antiserum generated against opaque cells (1, 2) absorbed either with budding white cells once or with budding white cells once and then budding opaque cells three times. Unbound antibody was removed by three washes in TBST solution. A 1:7,500 dilution of anti-rabbit immunoglobulin G–alkaline phosphatase conjugate was employed in the second incubation (30 min at 25°C). After being washed, blots were treated with a color development solution containing 0.033% Nitro Blue Tetrazolium and 0.0165% 5-bromo-4-chloro-3-indolyl phosphate in alkaline phosphatase buffer (100 mM Tris hydrochloride [pH 9.5], 100 mM NaCl, 5 mM $MgCl_2$). The reaction was stopped after 5 to 10 min by washing the blots with distilled water. Blots were photographed with a Pentax camera (60 by 70 mm) and Kodak T-max 100 film. Indirect immunofluorescence staining was performed by methods previously described (2).

Immunogold labeling for TEM. For TEM, a sample containing 10^8 cells was heat fixed in phosphate-buffered saline (PBS) for 10 min at 68°C (2) and then pelleted. The pellet was suspended in 10 μl of rabbit antiserum generated against opaque cells (1, 2) which had been absorbed once with budding white cells and incubated for 1 h at 25°C. Cells were washed twice in PBS and once in PBS plus 1% bovine serum albumin and were suspended in goat anti-rabbit immunoglobulin G conjugated with colloidal gold (diameter, 15 nm; Janssen Life Sciences Products, Piscataway, N.J.) which had been diluted twofold with PBS plus 1% bovine serum albumin. After 1 h at 25°C, the cells were washed, suspended in 2.5% glutaraldehyde in cacodylate buffer (pH 7.2), and processed for electron microscopy, but with the uranyl acetate and lead citrate staining steps omitted. A control was run with PBS–1% bovine serum albumin in place of absorbed opaque-cell antiserum.

RESULTS

Opaque cells exhibit a uniquely pimpled surface. When budding white cells of strain WO-1 were examined by light microscopy, they appeared similar to budding cells of most other strains of *C. albicans* or diploid strains of *Saccharomyces cerevisiae* (2, 21). Budding white cells were round to ovoid and exhibited a smooth or consistently roughened surface when viewed by SEM (Fig. 1A and B). The only protrusions on the cell surfaces were bud scars (Fig. 1A and B, arrowheads). In marked contrast, mature budding opaque cells were bean shaped and larger than budding white cells (21) and exhibited wall pimples, which appeared in three forms when viewed by SEM. First, the surface of budding opaque cells was covered by mounds of roughly equal size (Fig. 1C and D). In many cases, a central pit could be discerned in each pimple (Fig. 1C, arrows). Second, the surface was covered by mounds which exhibited small central blebs (Fig. 1D, arrows). In some cases, the surface was covered by blebs without prominent mounds. Third, the surface was covered by larger blebs, usually extending from the centers of mounds (Fig. 1E).

Although pimples in one of the three forms were obvious on most mature budding opaque cells when examined by SEM, they were absent on opaque-cell buds which had not attained half to two-thirds of their final volume (Fig. 1C and E) (2) and on hyphae formed by opaque cells (Fig. 1F) (1). They were also absent on the bud scars of mature opaque cells (Fig. 1E and F, arrowhead).

The pimples on the SEM profiles of six cells were counted, and the number was multiplied by 2 to obtain the approximate number on the entire cell surface. The average number of pimples per cell was 141, and the standard deviation was ±39. To account for variability in cell sizes, the number of pimples per cell was divided by the estimated surface area of each cell. The average number of pimples was 1.33/μm^2, and the standard deviation was ±0.33.

Opaque-cell pimples and opaque-cell vacuole viewed by TEM. When thin sections of mature budding white cells were scrutinized by TEM, there were no suggestions of pimples, channels, or pits in the cell walls (Fig. 2A). Wall diameters were relatively constant around cell profiles, except at the positions of bud scars. In contrast, the walls of the majority of mature budding opaque cells exhibited pimple thickenings, as well as pits, channels, and small blebs (Fig. 2B). The wall diameter was highly variable and increased to form mounds which most likely represent the pimples observed by SEM. The consistency of the wall regions at the presumptive sites of pimples appeared altered, with electron-dense material in some cases on the outer

pimple edge. In select profiles, channels traversing the pimple could be observed (Fig. 2B, arrow), and blebs were apparent at pimple surfaces (Fig. 2B, arrow and arrowheads). In many cases, the texture of the inner wall of the pimple region appeared less electron dense than lateral, interpimple wall regions did (Fig. 3A). In a limited number of pimple profiles, a flask-shaped vesicle (Fig. 3B, arrow) was observed just under the pimple in the cytoplasmic cortex, with the narrow neck of the vesicle penetrating the pimple (Fig. 3C).

In a number of profiles, vesicular structures were observed embedded in the wall pimple (Fig. 3D and E) or emerging from the pimple (Fig. 3E, F, and G). When the best vesicle profiles were image intensified, double membranes were discernible on their outer boundaries. In some cases, the emerging vesicles appeared to be flask shaped, with the narrow neck of the vesicle penetrating the pimple (Fig. 3G, arrow) in an orientation opposite to that of the cytoplasmic vesicle shown in Fig. 3B and C. In a number of cases, the vesicles appeared bacteriophagelike in morphology, with taillike structures penetrating the electron-dense pimple surface (Fig. 3H and I). The mean diameter of seven emerging or emerged particles was calculated to be 62 nm (standard deviation, ±13.7). The diameter of the cortical vesicle underlying the pimple shown in Fig. 3B and C was 129 nm, twice the average diameter of emerging vesicles.

The TEM profiles of opaque cells were unique not only in the presence of pimples with complex morphologies and associated vesicles in the cell wall but also in the presence of a large vacuole (Fig. 2B and C). In most profiles, these vacuoles contained strands which appeared membranous at a higher power (Fig. 2C). In a limited number of vacuole profiles, a mushroom-shaped structure studded with small particles penetrated the vacuole lumen (Fig. 2C). A limited number of vacuoles were filled with vesicles rather than membranous strands (Fig. 4). Some of the larger vesicles contained smaller vesicles (e.g., Fig. 4d), while many of the smaller vesicles were electron dense and relatively homogeneous in size (e.g., Fig. 4a, b, and c). The mean diameter of the vesicles in Fig. 4a, b, and c was 146 nm (standard deviation, ±16.5), which is similar to the diameter of the vesicle under the pimple shown in Fig. 3B and C but roughly twice the diameter of most of the vesicles observed on pimple surfaces.

Localization of an opaque-cell-specific antigen in the pimple. The antiserum from a rabbit immunized with opaque cells which was absorbed with nonpermeabilized white cells selectively stained the surface of nonpermeabilized opaque cells in a punctate fashion (Fig. 5A to C), indicating that the antiserum contained opaque-cell-specific antibodies which identified one or more antigens in the pimple. Amido black staining of blots of white- and opaque-cell extracts revealed no obvious differences in the major stained proteins (Fig. 6A). However, when Western blots of white- and opaque-cell extracts were stained with antiserum absorbed with nonpermeabilized budding white cells, three bands evident in opaque-cell blots were missing in white-cell blots (Fig. 6B). One very intense band was positioned at 14.5 kDa, and two minor bands were positioned at 21 and 31 kDa. To test which of these opaque-cell-specific bands might be associated with the punctate staining of the opaque-cell surface, the white-cell-absorbed antiserum was absorbed with nonpermeabilized opaque cells and then used to stain Western blots of white- and opaque-cell extracts (Fig. 6C). The 14.5-kDa band was reduced by more than 90%, but the 21- and 31-kDa bands remained intact. As a control for the removal of antibodies after opaque-cell absorption, the antiserum was tested for opaque-cell staining after absorption. No cell surface staining was apparent (Fig. 5D to F), demonstrating that all antibodies to the opaque-cell surface had been removed. These results demonstrate that the 21- and 31-kDa antigens are absent from and the 14.5-kDa antigen is present on the cell surface. Western blots were also performed on white cells forming hyphae. The 14.5-kDa antigen was absent (data not shown), demonstrating further that the appearance of the 14.5-kDa antigen is regulated by switching and not by dimorphic transition.

To test whether the 14.5-kDa antigen is in fact associated with pimples, opaque cells were treated with white-cell-absorbed antiserum and then with goat anti-rabbit antibody conjugated to colloidal gold. After being stained, cells were examined by TEM for the localization of surface staining (Fig. 7). In almost all cases, gold particles were localized at the apices of pimples, demonstrating that the opaque-cell-specific 14.5-kDa antigen is localized in pimples on the surfaces of opaque cells.

DISCUSSION

The molecular basis of the white-opaque transition in *C. albicans* is still not known, although it has been proposed (5, 22) that high frequencies, reversible genetic rearrangements, or transposition will eventually be demonstrated, as they have been in other switching systems (7–9, 12, 14). Whatever the mechanism, the white-opaque switch affects not only colony phenotype and cellular morphology (1, 2, 21, 24) but also gene expression. Two-dimensional polyacrylamide gel electrophoresis analysis of pulse-labeled polypeptides demonstrated that at least one major protein is differentially synthesized in budding white cells and that at least two major proteins are differentially synthesized in opaque cells (Soll et al., in press). In addition, we have demonstrated here that antiserum generated against opaque cells distinguishes three opaque-cell-specific antigens. However, the most dramatic aspects of the white-opaque transition are the ultrastructural consequences of switching to the opaque phenotype. The ultrastructure of budding white cells is in all respects similar to that of budding cells of other standard strains of *C. albicans* (23, 24). In contrast, the ultrastructure of opaque cells appears in most respects to be different. The wall is uneven, with pimples which contain central pits which in many cases contain emerging blebs or vesicles. The pimple surface, or apex, contains a 14.5-kDa antigen which is opaque specific. In addition, opaque cells possess very large vacuoles containing membranous strands. It should be realized that with each heritable switch from white to opaque, these unusual opaque-cell-specific phentoypic characteristics are acquired, and with each switch back to white, they are lost.

Opaque-cell pimple. It is not clear what the opaque-cell pimple represents. A pimple is composed of a localized thickening of wall material. If this were the extent of pimple morphology, one could simply dismiss it as an abnormality due to uneven wall deposition. However, pimple morphology is too intricate for so simple and trivial an explanation. We have demonstrated that when the pimple is viewed by SEM, pits or small blebs can be visualized in pimple centers. In some cases, the blebs emanating from the central pit are quite large. The absence of any such pimples, pits, or blebs on the surfaces of budding white cells of strain WO-1 or budding cells of other strains carefully scrutinized by SEM and TEM suggests that the presence of pimples on opaque

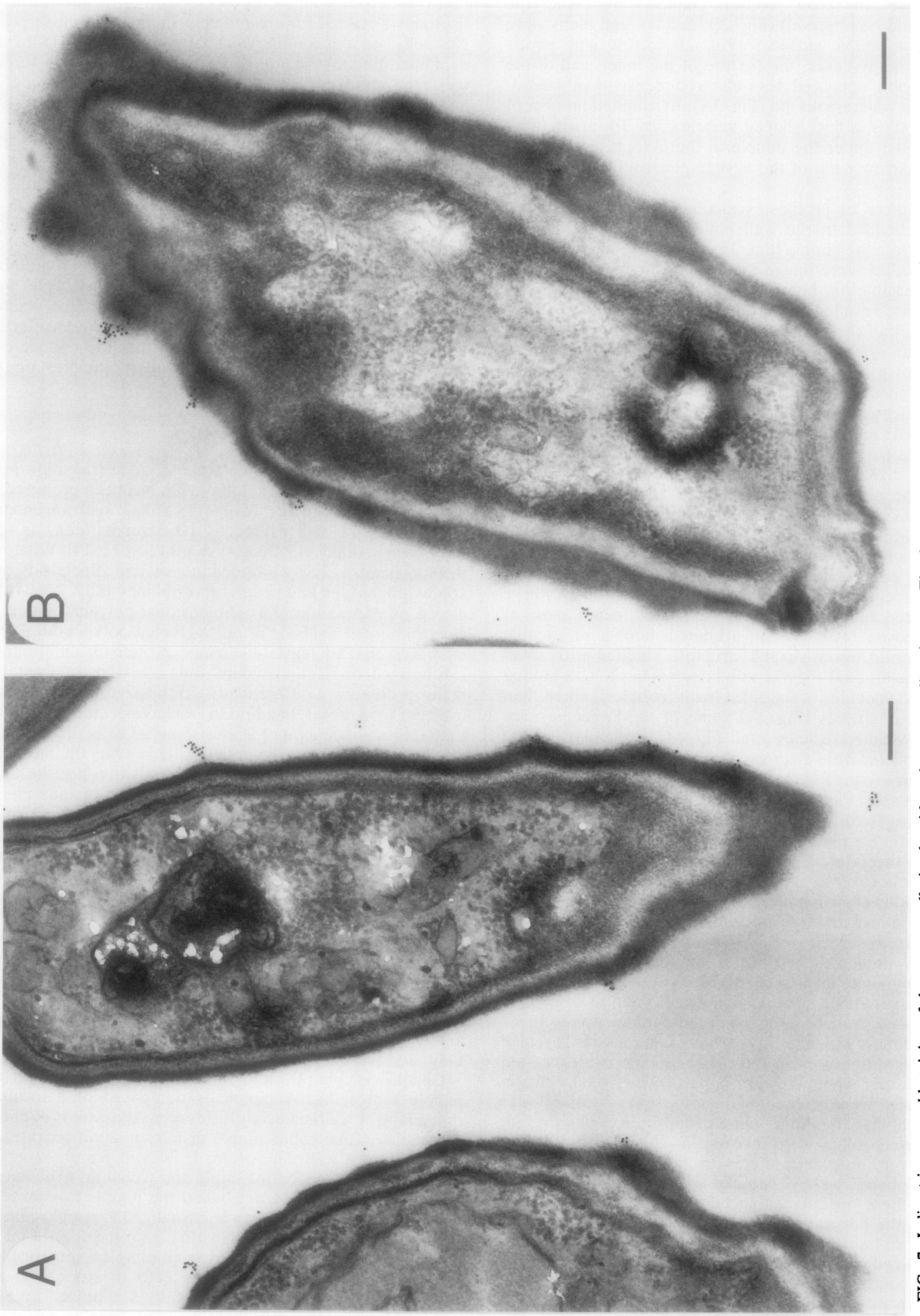
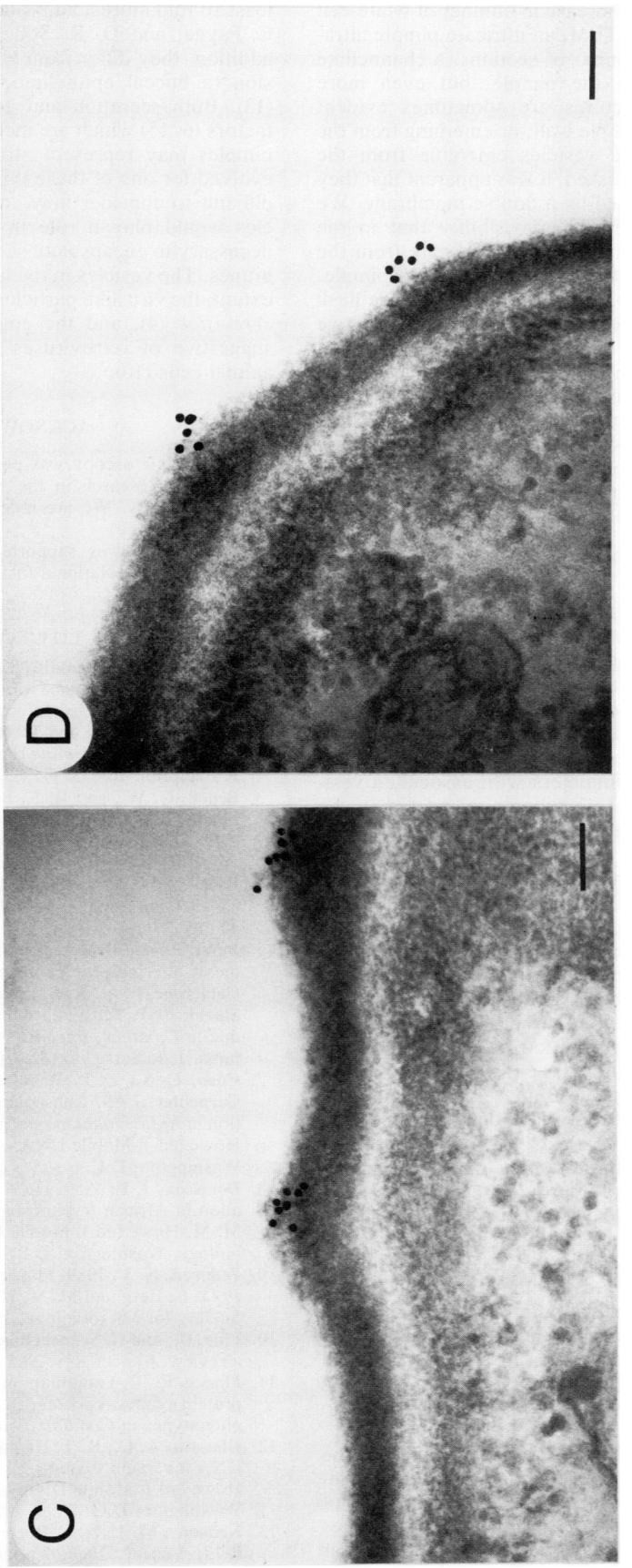


FIG. 7. Indirect immunogold staining of the opaque-cell pimple with anti-opaque-cell antiserum. Fixed, nonpermeabilized cells were treated first with anti-opaque-cell antiserum absorbed with white cells and then with goat anti-rabbit antibody conjugated to colloidal gold, and they were finally processed for TEM. (A and B) TEMs of whole cells demonstrating localized gold labeling at the apices of pimples; (C and D) increased magnification of pimple staining. Bars = 0.4 μm (A and B), 0.15 μm (C), and 0.06 μm (D).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cells is not due simply to an increase in number of white-cell structures. When viewed by TEM, an intricate pimple ultrastructure is evident. In a number of sections, a channellike structure is visible through the pimple, but even more surprisingly, vesicular structures are sometimes evident under pimples, within the pimple wall, or emerging from the apex of pimples. When the vesicles emerging from the pimple apex were image processed, it was apparent that they were at least partially enclosed by a double membrane. We are therefore confronted with the possibility that in the opaque cell, membrane-enclosed vesicles migrate from the cytoplasm to the cell surface through channels in the pimple. In many TEM profiles, pimple-associated vesicles are flask shaped, with the stem traversing the wall. As far as we know, this phenomenon has not been previously described for any other *Candida* strain or for related yeasts, such as *Saccharomyces* spp. In the case of the psychrophilic yeast *Leucosporidium scottii*, protrusions that cover the cell surface have been observed by SEM (29, 30). However, when viewed by TEM (28), these protrusions do not appear to be associated with pimple structures, and the thickness of the wall is quite constant around the cell. In addition, the protrusions on the surface of *L. scottii* cover bud scar surfaces, which are free of protrusions in opaque cells of *C. albicans*. It is also not clear whether the *L. scottii* protrusions are membrane bound. A comparative analysis of *L. scottii* and *C. albicans* protrusions appears to be warranted.

Unusual opaque-cell vacuole. The budding opaque cell is on average three times the volume and twice the mass of a budding white cell (21). A portion of this increased size is due to one or more large vacuoles in the cell interior (21). Interestingly, most opaque-cell vacuoles are filled with membranous strands of various diameters with associated vesicles. In some cases, unusual protuberances rooted in the vacuole wall and covered with small particles stick out into the vacuole lumen. They are usually covered with minivesicles and are of unknown function. More interestingly, in a few cases, vacuoles are filled with vesicles. These vesicles are of various sizes, but some apparently filled vesicles appear phenotypically homogenous, with diameters of approximately 146 nm. It is possible that the spaghettilike material in the majority of opaque vacuoles represents either collapsed vesicles or precursors to vesicles. It is also tempting to speculate that these intravacuole vesicles are precursors to the vesicles emerging from the pimples, but there is no evidence supporting this suggestion.

Opaque-cell-specific antigens. We have demonstrated here that an antiserum generated against budding opaque cells contains antibodies against three opaque-cell-specific antigens with molecular masses of approximately 14.5, 21, and 31 kDa. When nonpermeabilized opaque cells are used to absorb antibodies from this antiserum, the antibodies to the 14.5-kDa antigen are removed, but the antibodies to the 21- and 31-kDa antigens remain. This demonstrates that the 21- and 31-kDa antigens are absent from the cell surface but that the 14.5-kDa antigen is present. Immunogold staining demonstrated that the 14.5-kDa antigen is localized on the cell surface at the site of the pimples. These results exclude the 21- and 31-kDa antigens from the cell surface, but they do not exclude the 14.5-kDa antigen from the cell interior.

Function of the opaque-cell pimple. The presence of the opaque-cell-specific 14.5-kDa antigen in the opaque-cell pimple suggests that the pimple is not simply an aberrant form of a structure also present in white cells. However, it provides no clue to the possible function of opaque cells or their specialized pimples. Interestingly, opaque cells secrete at least 10-fold more acid protease than do white cells (T. Ray, C. Payne, and D. R. Soll, manuscript in preparation). In addition, they differ from budding white cells in their adhesion to buccal epithelium, cohesion, and hydrophobicity (13). Both secretion and adhesion are potential virulence factors (6, 15) which are mediated through the cell wall, and pimples may represent structures which have selectively evolved for one of these types of processes. However, it is difficult to consider how emerging, membrane-bound vesicles would play a role in adhesion or why it would be necessary to encapsulate secretion products in double membranes. The vesicles in the large vacuoles resemble, to some extent, the viruslike particles containing the Ty element of *S. cerevisiae* (4), and the emerging vesicles are remarkably suggestive of retroviruses blebbing from the surfaces of animal cells (10).

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LITERATURE CITED

1. **Anderson, J., L. Cundiff, B. Schnars, M. Gao, I. Mackenzie, and D. R. Soll.** 1989. Hypha formation in the white-opaque transition of *Candida albicans*. Infect. Immun. **57**:458–467.
2. **Anderson, J. M., and D. R. Soll.** 1987. Unique phenotype of opaque cells in the white-opaque transition of *Candida albicans*. J. Bacteriol. **169**:5579–5588.
3. **Bedell, G. W., and D. R. Soll.** 1979. Effects of low concentrations of zinc on the growth and dimorphism of *Candida albicans*: evidence for zinc-resistant and -sensitive pathways for mycelium formation. Infect. Immun. **26**:348–354.
4. **Boeke, J. D., D. J. Garfinkel, C. A. Styles, and G. R. Fink.** 1985. Ty elements transpose through an RNA intermediate. Cell **40**:491–500.
5. **Borst, P., and D. R. Greaves.** 1987. Programmed gene rearrangements altering gene expression. Science **235**:658–667.
6. **Calderone, R. A., R. L. Cihlar, D. L. Lee, K. Hoberg, and W. M. Shield.** 1985. Yeast adhesion in the pathogenesis of endocarditis due to *Candida albicans*: studies with adherence-negative mutants. J. Infect. Dis. **152**:710–715.
7. **Coen, E. S., T. P. Robbins, J. Almeida, A. Hudson, and R. Carpenter.** 1989. Consequences and mechanisms of transposition in *Antirrhinum majus*, p. 413–436. *In* D. E. Berg and M. M. Howe (ed.), Mobile DNA. American Society for Microbiology, Washington, D.C.
8. **Donelson, J. E.** 1989. DNA rearrangements and antigenic variation in African trypanosomes, p. 763–781. *In* D. E. Berg and M. M. Howe (ed.), Mobile DNA. American Society for Microbiology, Washington, D.C.
9. **Fedoroff, N. V.** 1989. Maize transposable elements, p. 375–411. *In* D. E. Berg and M. M. Howe (ed.), Mobile DNA. American Society for Microbiology, Washington, D.C.
10. **Fine, D., and G. Schochetman.** 1978. Type D primate retrovirus: a review. Cancer Res. **38**:3123–3139.
11. **Finney, R., C. Langtimm, and D. R. Soll.** 1985. The programs of protein synthesis accompanying the establishment of alternative phenotypes in *Candida albicans*. Mycopathologia **91**:3–15.
12. **Glasgow, A. C., K. T. Hughes, and M. I. Simon.** 1989. Bacterial DNA inversion systems, p. 637–659. *In* D. E. Berg and M. M. Howe (ed.), Mobile DNA. American Society for Microbiology, Washington, D.C.
13. **Kennedy, M. J., A. L. Rogers, L. A. Hanselman, D. R. Soll, and R. J. Yancey.** 1988. Variation in adhesion and cell surface hydrophobicity in *Candida albicans* white and opaque pheno-

types. Mycopathologia **102**:149–156.
14. **Klar, A. J. S.** 1989. The interconversion of yeast mating type: *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*, p. 671–691. *In* D. E. Berg and M. M. Howe (ed.), Mobile DNA. American Society for Microbiology, Washington, D.C.
15. **Kwon-Chung, K. J., D. Lehman, C. Good, and P. T. Magee.** 1985. Genetic evidence for role of extracellular proteinase in virulence of *Candida albicans*. Infect. Immun. **49**:571–575.
16. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
17. **Lee, K. L., H. R. Buckley, and C. C. Campbell.** 1975. An amino acid liquid synthetic medium for development of mycelial and yeast forms of *Candida albicans*. Sabouraudia **13**:148–153.
18. **Pomes, R., C. Gil, and C. Nombela.** 1985. Genetic analysis of *Candida albicans* morphological mutants. J. Gen. Microbiol. **131**:2107–2113.
19. **Rikkerink, E. H. A., B. B. Magee, and P. T. Magee.** 1988. Opaque-white phenotype transition: a programmed morphological transition in *Candida albicans*. J. Bacteriol. **170**:895–899.
20. **Slutsky, B., J. Buffo, and D. R. Soll.** 1985. High frequency switching of colony morphology in *Candida albicans*. Science **230**:666–669.
21. **Slutsky, B., M. Staebell, J. Anderson, L. Risen, M. Pfaller, and D. R. Soll.** 1987. "White-opaque transition": a second high-frequency switching system in *Candida albicans*. J. Bacteriol. **169**:189–197.
22. **Soll, D. R.** 1984. The cell cycle and commitment to alternate cell fates in *Candida albicans*, p. 143–162. *In* P. Nurse and E. Streiblova (ed.), The microbial cell cycle. CRC Press, Inc., Boca Raton, Fla.
23. **Soll, D. R.** 1986. The regulation of cellular differentiation in the dimorphic yeast *Candida albicans*. Bioessays **5**:5–11.
24. **Soll, D. R.** 1989. High-frequency switching in *Candida albicans*, p. 791–798. *In* D. E. Berg and M. M. Howe (ed.), Mobile DNA. American Society for Microbiology, Washington, D.C.
25. **Soll, D. R., and B. Kraft.** 1988. A comparison of high frequency switching in *Candida albicans* and *Dictyostelium discoideum*. Dev. Genet. **9**:615–628.
26. **Soll, D. R., C. J. Langtimm, J. McDowell, J. Hicks, and R. Galask.** 1987. High-frequency switching in *Candida* strains isolated from vaginitis patients. J. Clin. Microbiol. **25**:1611–1622.
27. **Soll, D. R., M. Staebell, C. Langtimm, M. Pfaller, J. Hicks, and T. V. G. Rao.** 1988. Multiple *Candida* strains in the course of a single systemic infection. J. Clin. Microbiol. **26**:1448–1459.
28. **Srivastava, K. C., and D. G. Smith.** 1974. Electron microscopy of a psychrophilic yeast, *Candida gelida*. Micron **5**:191–199.
29. **Watson, K.** 1987. Temperature relations, p. 41–71. *In* A. H. Rose and J. S. Harrison (ed.), The yeasts, vol. 2. Academic Press, Inc., Orlando, Fla.
30. **Watson, K., and H. Arthur.** 1977. Cell surface topography of *Candida* and *Leucosporidium* yeasts as revealed by scanning electron microscopy. J. Bacteriol. **130**:312–317.