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Heterogeneous Surface Distribution of the Fibrinogen-Binding Protein on *Candida albicans*

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As detected by indirect immunofluorescence and confocal microscopy, fibrinogen binding was heterogeneously distributed on the surface of *Candida albicans*. A low level of binding was generally observed homogeneously distributed on some yeast and most hyphal extensions of germ tubes. However, on most hyphal extensions, there were randomly distributed areas of increased expression, as revealed by patches of greater fluorescence intensity.

Candida albicans is a dimorphic fungus which may be present in humans either as a commensal or as an agent of infection in the compromised host. The cell wall surface of the fungus as a mediator of interactions requiring contact between microbe and host has been the focus of a number of studies. The cell wall surface is composed primarily of glucan and chitin polysaccharides and mannan present as mannoprotein (6, 10). Several surface determinants, mostly mannoproteins, have been identified, and their expression has been examined by binding of antibodies, lectins, and host proteins as detected by indirect immunofluorescence, binding of radioactive ligand, and transmission electron microscopy (6, 10). Several of these determinants detected by fluorescence microscopy are reported to be variably expressed, depending on the morphology, phenotypic switch state, growth state, or growth condition of the organism (1, 2, 6, 10). In addition to the population or form distribution just noted, some reports have noted the apparent homogeneous or heterogeneous distribution of determinants on individual organisms (1, 2, 5, 7, 9, 11–13, 17, 18). The distribution of fibrinogen-binding protein was reported to be primarily on germ tubes and hyphae, with occasional expression by emerging buds, and generally homogeneous on young hyphae, as determined by indirect immunofluorescence (3, 4, 18). Binding of fibrinogen detected by scanning electron microscopy or transmission electron microscopy on thin sections showed a heterogeneous distribution (3, 19). More recently, Casanova et al. (8) identified a 58-kDa protein (mp58) as having receptor-like activity for fibrinogen but not other ligands such as laminin, fibronectin, and type IV collagen. Antibody to this protein showed a heterogeneous distribution of the determinant in the laboratory strain studied, ATCC 26555. In this study, we have examined the surface distribution of the fibrinogen-binding mannoprotein in greater detail, using serial sections obtained with fluorescence confocal microscopy.

The laboratory strain *C. albicans* 3153A was grown in the defined medium of Lee et. al (15). Germ tubes were induced at 37°C from stationary-phase yeast cells which were grown at 24°C, harvested, and resuspended in water at 4°C for 48 h. Germ tubes were washed twice in phosphate buffered saline (PBS; 0.015 M phosphate [pH 7.4], 0.15 M NaCl), resuspended in PBS containing 1 mM $CaCl_2$, 1 mM $MgCl_2$, and 4 mg of human fibrinogen (Sigma Chemical Co., St. Louis, Mo.) per ml, and incubated at 37°C for 45 min. Cells were washed and incubated with a 1:10 dilution of fluorescein isothiocyanate (FITC)-conjugated goat antifibrinogen (Cappel, Organon-Teknika Corp., Durham, N.C.) at 37°C for 45 min. Cells were washed and examined by microscopy. The proportion of fluorescent cells with homogeneous and heterogeneous distribution was determined by fluorescence microscopy, using a Nikon Labophot microscope equipped for epifluorescence. Cells incubated only with the FITC-conjugated second antibody showed no detectable fluorescence. The proportions of germ tubes expressing the fibrinogen-binding mannoprotein with homogeneous and heterogeneous distributions were 3 and 91%, respectively, in two experiments, with a minimum of 98 organisms counted for each determination.

Serial sections from fluorescent cells were obtained with an Olympus LSM GB200 laser scanning confocal microscope, using a 488 argon ion laser (Olympus Corp. Precision Instruments Division, Lake Success, N.Y.). The gain settings, etc., were optimized for each image. Serial sections, *xy* plane, were obtained at 0.5-μm intervals along the *z* axis. Three-dimensional reconstructions were obtained by the resident software. The heterogeneous distribution observed with fluorescence microscopy was confirmed by fluorescence confocal microscopy (Fig. 1). Single focal plane sections through the length or long axis of the organism (Fig. 1A to E) showed that the fibrinogen-binding protein was generally distributed over the surface of hyphal extensions at low density, with areas or patches of greater density. No pattern of the frequency, size, or location of the areas of increased density was readily discernible. Serial sections through a single organism showed the changes in the density of fibrinogen-binding mannoprotein with greater clarity (Fig. 1F to K). Three-dimensional reconstructions obtained from the serial sections resemble the images obtained with regular fluorescence microscopy (Fig. 1L). *xz* cross sections along the *y* axis were obtained by resident software (Fig. 2). These sections, through the width rather than the length of the hyphal extension, clearly demonstrated that patches varied along the *y* axis. A small percentage of germ tubes and a few parent cells appeared to express the determinant homogeneously on the surface. This was confirmed in serial sections (Fig. 3A to H) and three-dimensional reconstructions (Fig. 3I to J).

The distribution of the fibrinogen-binding protein was also examined by using antibody (8) to the receptor (Fig. 4). Germ

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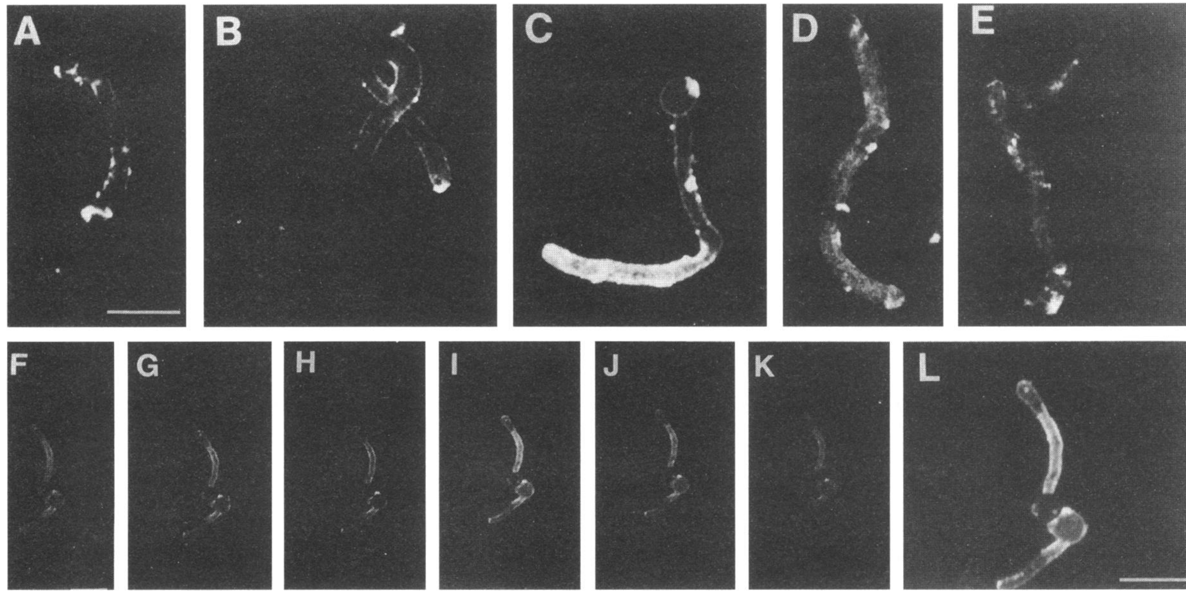

FIG. 1. Heterogeneous distribution of binding of fibrinogen to *C. albicans* as revealed by fluorescence confocal microscopy. Single focal plane sections of different cells (A to E), serial sections at 0.5-μm intervals through part of a cell (F to K), and a three-dimensional reconstruction of the whole cell (L) were obtained by confocal microscopy, with associated software, of cells binding human fibrinogen detected with an FITC-conjugated goat antifibrinogen antibody. The bar marker represents 10 μm.

tubes were incubated sequentially with a 1:50 dilution of rabbit anti-mp58 antiserum and FITC-conjugated goat anti-rabbit immunoglobulin (Boehringer Mannheim, Indianapolis, Ind.) essentially as previously described (8). No fluorescence was detected with FITC-conjugated second antibody alone. Heterogeneous distribution of the determinant was evident in single focal plane sections (Fig. 4) and in serial sections and three-dimensional reconstructions (not shown) of cells reacting with antibody. The observation of heterogeneous distribution obtained with both fibrinogen and mp58 antiserum and the

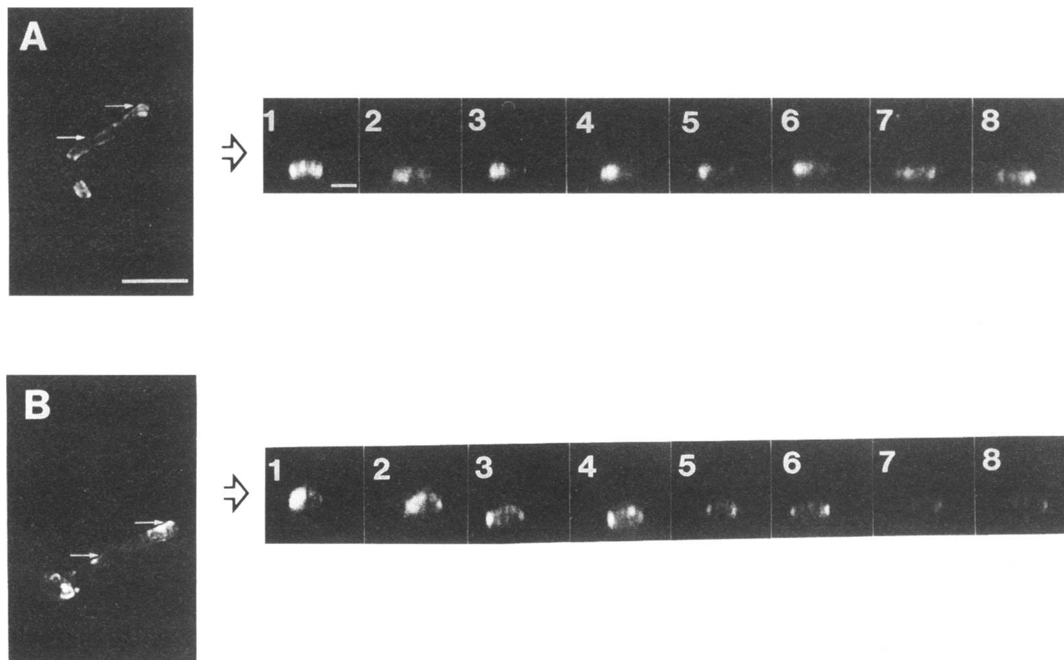

FIG. 2. Fibrinogen binding viewed in the *xz* plane. A three-dimensional reconstruction of each of two cells was obtained from serial sections in the *xy* plane (A and B) along the *z* axis as in Fig. 1. The same cells were rescanned, and software was used to generate an image from which *xz* plane sections (1 to 8) were generated through the width of the hyphal extension from top (1) to bottom (8), which begin and end approximately as indicated by the arrows in panels A and B. Bar markers represent 10 μm in panels A and B and 2 μm in panels 1 to 8.

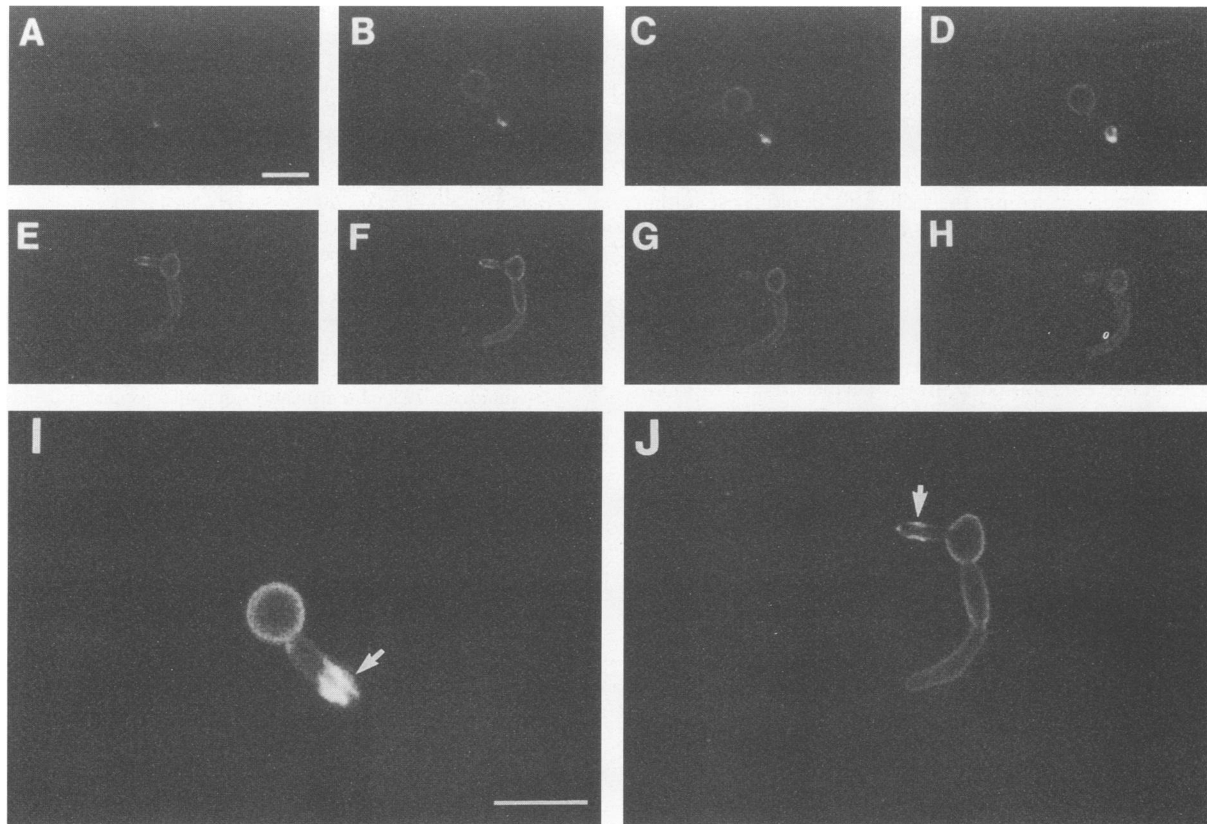

FIG. 3. Homogeneous distribution of binding of fibrinogen to *C. albicans*, as revealed by fluorescence confocal microscopy. Three-dimensional reconstructions from serial sections were obtained for two cells which showed homogeneous distribution over the parent cell (I and J) of binding human fibrinogen detected with an FITC-conjugated goat antifibrinogen antibody. A sequence of serial sections obtained at 0.5-μm intervals for the cell in panel I is shown in panels A to D, and an analogous sequence for the cell in panel B is shown in panels E to H. In panels I and J, arrows indicate an area of increased intensity on a structure that appeared to be a short germ tube. The bar marker represents 10 μm.

presence of some cells with homogeneous distribution of the fibrinogen-binding protein suggest that the heterogeneity observed is not attributable to artifacts of preparation or reagent clumping.

The receptor was distributed over the entire surface of the cell expressing the determinant. However, there were areas in which increased fluorescence intensity indicated binding of either fibrinogen or antibody to a localized increase in the

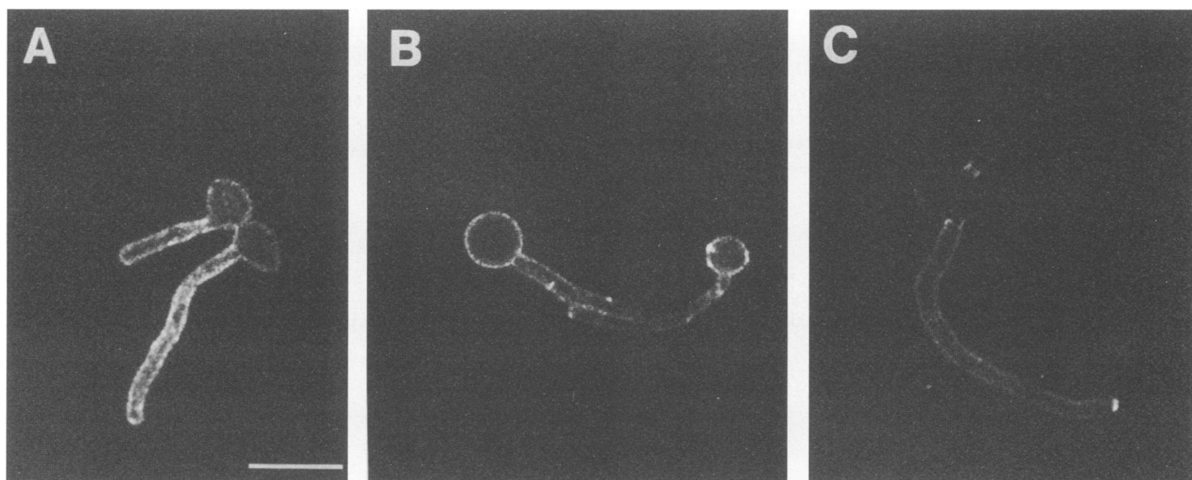

FIG. 4. Binding of anti-mp58 antibody to germ tubes. Single focal plane sections of different cells were obtained by confocal microscopy, with associated software, of cells binding anti-mp58 rabbit antiserum and detected with an FITC-conjugated goat anti-rabbit antibody. The bar marker represents 10 μm.

surface density of the fibrinogen-binding determinant mp58. The heterogeneous distribution revealed by the serial sections obtained by confocal microscopy supported the patchiness observed with regular fluorescence microscopy but provided more detail about the heterogeneous distribution in the cell wall. Previous studies with transmission electron microscopy did not use serial sections (3, 19). This asymmetrical distribution of the receptor within the cell wall could arise either during synthesis of the cell wall by asymmetrical delivery of a mannoprotein to the site of the growing wall, during maturation of the cell wall, or as a result of rearrangement of the receptor in the cell wall as a result of binding of fibrinogen or antibody in a change similar to the capping phenomena observed with plasma membranes in response to binding of various reagents, e.g., antibody, to the surface. The fixation of the cell with formaldehyde prior to reaction with antibody does not affect the heterogeneous distribution of mp58 (8), and thus the latter is not a likely possibility. Another protein, HMWM260, which is suggested to play a structural role in the cell wall, is expressed preferentially and homogeneously on the surface of germ tubes, as shown by indirect immunofluorescence (7, 9). Germ tube formation is inhibited and the protein shows an asymmetrical distribution when germ tube formation is induced in the presence of Fab fragments recognizing HMWM260 (9); in this case, presumably interaction of the Fab fragment with the determinant inhibits its normal deposition in the wall. The homogeneous distribution pattern is consistent with the predicted behavior of a protein postulated to be integral to the structure of the germ tube cell wall. Hydrophobic properties of mp58 (16) could promote self-association leading to areas of increased density. Clustering or asymmetric distribution of cell wall mannoproteins such as the fibrinogen-binding mp58 does not appear to be reflected in the physical surface topography of yeast cells in the typical ovoid phenotype and germ tubes, since scanning electron microscopy does not show any similar structural perturbations not associated with bud scars (1, 2, 14) (data not shown). The binding of *C. albicans* to host proteins such as fibrinogen or components of the extracellular matrix such as laminin, fibronectin, or collagen type IV has been postulated to contribute to the pathogenesis of infection. Clustering of receptors for such ligands could increase the security of the interaction at the point of contact between the microbe and host.

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