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# Expression of the fibrinogen binding mannoprotein and the laminin receptor of *Candida albicans* in vitro and in infected tissues

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**Abstract**

We have previously reported a 37 kDa laminin-binding protein (p37) and a 58 kDa fibrinogen-binding mannoprotein (mp58) on the surface of *Candida albicans*. A few yeast cells expressed both functional receptors at the surface while germ tubes expressed a functional mp58 fibrinogen but not a functional p37 laminin receptor. These receptors were heterogeneously dispersed at the surface as shown by binding of rabbit antiserum to mp58 (PAb anti-mp58) and antiserum to the human high affinity laminin receptor. In this report we have used a dual fluorescence technique to determine if the two receptors colocalize, perhaps as part of a receptor complex. Fibrinogen was used as a probe for mp58 and polyclonal antiserum generated to the p37 (PAb anti-p37) was used as a probe for the 37 kDa laminin-binding protein. Both receptors were heterogeneously distributed, but the receptors were not colocalized as the areas of concentration of each receptor were different. Immunohistochemical analysis of tissue sections from patients with disseminated and superficial candidiasis with PAb anti-p37 and PAb anti-mp58 revealed that both receptors were also expressed in infected tissues. The patterns of morphological expression were similar to the in vitro patterns detected by immunofluorescence.

*Keywords:* *Candida albicans*; Cell wall; Surface receptors

## 1. Introduction

*Candida albicans* is both a commensal and an agent of opportunistic disease. Depending on the underlying host defect, *C. albicans* is able to produce different clinical manifestations, ranging from superficial or mucocutaneous candidiasis to more serious life-threatening systemic or disseminated disease [1]. Adhesion of fungal cells to host cells and tissues is considered to be an essential prerequisite in the establishment of infection and can lead to metastatic sites of disease. Interactions with the host are mediated through complementary molecules on both the surface of the fungus and host cells and

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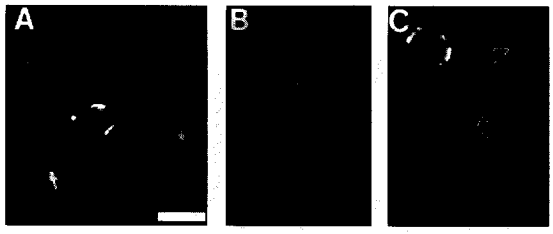


Fig. 1. Surface distribution of p37 detected by PAb anti-p37. The reactivity of blastospores (A and B) and blastospores bearing germ-tubes (C) with PAb anti-p37 was examined by IIF. Arrows in A and B indicate blastospores exhibiting undetectable or weak fluorescence. Open arrows in C indicate mycelial filaments exhibiting undetectable or weak fluorescence while some non-germinating blastospores present in the preparations were strongly fluorescent. Microscopic images were obtained and processed as described in Section 2. Bar is 5 μm.

tissues [2]. Fungal molecules that play a role in these interactions have been designated with the term 'adhesins' and the large repertoire of adhesins displayed by this fungus may be a reflection of the variety of sites that it can invade in the host [2–4]. Some of these adhesins may represent the same molecule with different biological activities [5,6], whereas others are different biological entities [7]. Also the existence of different adhesins with affinity for the same ligand has been postulated [8,9].

We have previously reported the presence on the cell surface of *C. albicans* of a 37 kDa laminin-binding protein (p37) with homology to the human high affinity laminin receptor [9], and a 58 kDa fibrinogen-binding mannoprotein (mp58) which specifically interacts with human fibrinogen [10]. Both components were present in the cell wall materials solubilized with β-mercaptoethanol (βME extract) from the surface of intact cells from both *C. albicans* morphologies (blastospores and blastospores bearing germ-tubes). However, p37 appeared to be functional (as measured by its ability to bind laminin in a ligand affinity blot) exclusively in the material extracted from blastospores. These two receptor-like components were heterogeneously distributed on the surface of *C. albicans* cells, as determined by immunofluorescence analysis [9,10] and confirmed by confocal microscopy [11]. Cell surface receptors may be present as multi-subunit complexes arising from the association of different components [7,12], and thus the similarity in the distribution patterns of these two *C. albicans* receptors may be the result of being part of the same multi-subunit receptor complex.

In the present report we have examined the expression of these two receptors in vitro and in vivo. Antibody prepared to the purified p37 (PAb anti-p37) and fibrinogen have been used to detect the expression of p37 and mp58 respectively. A double labeling indirect immunofluorescence assay (IIF) was used to investigate the in vitro surface expression and possible colocalization of these binding proteins. Immunohistochemical methods were used to examine the expression of these two adhesins on fungal cells found in tissues from patients with candidiasis.

## 2. Materials and methods

### 2.1. Organism and culture conditions

*C. albicans* 3153 A was maintained by subculturing on plates containing Sabouraud-dextrose medium. It was propagated as blastospores (yeast cells) or blastospores bearing germ-tubes (also referred to as mycelium) in the medium of Lee et al. [14], as described previously [6].

### 2.2. Indirect immunofluorescence assay

A polyclonal antibody generated against the candidal 37 kDa laminin-binding protein (PAb anti-p37) [9,13] was used for IIF. *C. albicans* cells were washed twice in cold phosphate buffered saline (PBS) and resuspended in a 1:10 dilution of the antiserum in PBS containing 1% bovine serum albumin (PBSB). After incubation for 2 h at 37°C with gentle agitation in a gyratory incubator shaker, the cells were washed 4 times with PBS and resuspended in Texas Red-conjugated goat anti-rabbit immunoglobulin (EY Labs. Inc., San Mateo, CA) diluted 1:10 in PBSB. After a 1 h incubation at 37°C, the cells were washed and resuspended in PBS. Samples in which incubation with PAb anti-p37 was omitted were used as negative controls.

For dual labeling with PAb anti-p37 and fibrinogen, *C. albicans* blastospores were washed twice in PBS and resuspended in PBS containing PAb anti-p37 diluted 1:10, 4 mg/ml of fibrinogen essentially

plasminogen free (Sigma Chemical Co., St. Louis, MO), 1 mM $MgCl_2$, and 1 mM $CaCl_2$. After incubation for 2 h at 37°C with gentle agitation in a gyratory incubator shaker, the cells were washed 4 times with PBS and resuspended in a mixture of fluorescein isothiocyanate (FITC) conjugated goat anti-human fibrinogen (Cappel Organon Teknika Corp.) and Texas Red-conjugated goat anti-rabbit immunoglobulin (EY Labs. Inc.) each diluted 1:10 in PBSB. After a 1 h incubation at 37°C, the cells were washed and resuspended in PBS. Samples in which either or both incubation with fibrinogen and PAb anti-p37 were omitted were used as negative controls.

Drops (10 μl) from the different samples were placed in wells of a microslide and examined. Digital images were obtained with a cooled (−45°C) CCD camera (Star 1 camera and Star1/Macintosh Image Acquisition Software, Photometrics Ltd., Tucson, AZ). The digital image was converted to a TIFF file and processed for brightness and contrast (Adobe Photoshop, Adobe Systems, Inc., Mountainview, CA), scaled (imgworks, Silicon Graphics Inc., Mountainview, CA) and printed (Codonics printer, Codonics Inc., Middleburg, OH).

### 2.3. Immunohistochemistry

Formalin-fixed, paraffin-embedded tissue samples from patients with disseminated and superficial candidiasis were obtained through autopsy (three cases) or biopsy (two cases) respectively. 5 μm thick tissue sections were immunostained according to the avidin-biotin immunoperoxidase technique described by Hsu et al. [15]. Endogenous peroxidase activity was blocked in deparaffinated sections by exposing the tissues to 0.3% $H_2O_2$ in absolute methanol for 30 min. The slides were incubated sequentially with 2% normal goat serum for 20 min, with PAbs anti-mp58 or anti-p37 for 45 min with biotinylated goat anti-rabbit immunoglobulins (Vector, Burlingame, CA), and with avidin-biotin-peroxidase complex (Vector, Burlingame, CA) for 45 min. The reaction was developed in 0.5 mg/ml of 3,3′-diaminobenzidine tetrahydrochloride (Sigma) and 3 μl/ml of 3% $H_2O_2$ in 50 mM Tris, pH 7.6. Finally the slides were counterstained with hematoxylin. All incubations were performed at room temperature. Antibody dilutions were 1:200 for PAb anti-mp58, 1:50 for PAb anti-p37, and 1:200 for the biotinylated antibody. PBS was used for all washes between steps and for antibody dilutions. Control slides were made by replacing the primary antibody with PBS.

## 3. Results

### 3.1. Immunofluorescence patterns using PAb anti-p37

We have generated a polyclonal antibody against the *C. albicans* 37 kDa laminin-binding protein [9,13]. When this antiserum was used in an IIF assay the patterns of fluorescence observed were very similar to what we observed in our original report using antibodies against the human-high affinity laminin receptor [9]. As seen in Fig. 1, reactivity was found mainly on the surface of blastospores. Lower intensity fluorescence levels or no detectable fluorescence were observed along the surface of hyphal filaments (Fig. 1, panel C, open arrows) and on the surface of other blastospores present in the preparations (Fig. 1, panels A and B, arrows).

### 3.2. Dual labeling fluorescence assay

We have previously described the heterogeneous distribution of the 58 kDa fibrinogen-binding mannoprotein (mp58) and the 37 kDa laminin-binding protein on the surface of *C. albicans* [9,10]. PAb anti-p37 and binding of fibrinogen were used simultaneously as probes in a dual label fluorescence assay to assess the relative topological localization of the p37 and mp58 respectively. As described for the individual components, a patchy distribution of fluorescence was observed for both probes (Fig. 2). However, the areas of maximum concentration of each receptor were different, indicating that these two molecules were not colocalized as a potential receptor complex. Moreover, binding of one probe did not seem to affect simultaneous binding of the other, thus showing no competition or even steric effect between both probes. In one experiment, from a total of 157 fluorescent cells counted, 86.0% showed a fluorescence pattern with no colocalization of both probes, colocalization was observed for 10.8% of the cells, and 3.2% displayed a more confluent fluorescence pattern.

### 3.3. Immunohistochemistry

In human infected tissues, immunoreactivity of *Candida* organisms was detected using polyclonal antibodies against both candidal receptor-like proteins. Most yeast and mycelial cells in superficial and disseminated candidiasis were strongly positive when PAb anti-mp58 was used as a probe (Fig. 3, panels A and B). On the other hand, using PAb anti-p37 as a probe, immunostaining was essentially confined to less than 50% of the blastospores (Fig. 3, panels C and D) whereas hyphal extensions were mostly negative or showed only weak reactivity. No immunostaining of fungal elements was present in control sections (Fig. 3, panels E and F).

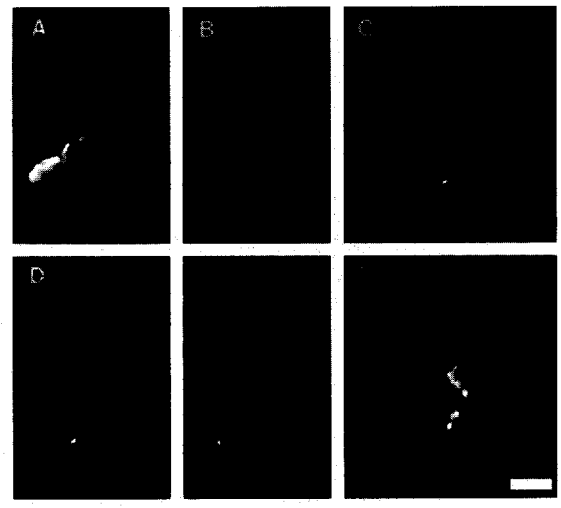


Fig. 2. Dual labeling of *C. albicans* cells with fibrinogen and PAb anti-p37. Blastospores were incubated simultaneously with fibrinogen and PAb anti-p37 to determine expression of mp58 and p37 respectively. The location of binding of each probe was determined by IIF using FITC-conjugated goat anti-fibrinogen antibody (A–C) and Texas Red-conjugated goat anti-rabbit antibody (D–F). Three fields are shown (field 1, A and D; field 2, B and E; field 3, C and F). Microscopic images were obtained and processed as described in Section 2. Bar is 5 μm.

## 4. Discussion

Binding of *C. albicans* cells to serum factors (e.g., fibrinogen) and extracellular matrix (ECM) components (e.g., laminin) may represent a virulence factor [16], since adhesion of fungal cells to host cells and tissues is an essential prerequisite in the establishment of infection. We have previously identified a 37 kDa laminin-binding protein (p37) and a 58 kDa fibrinogen-binding mannoprotein on the surface of *C. albicans* [9,10]. We have also studied the expression of both receptor-like components by morphological phases of the fungus, and reported their heterogeneous distribution at the surface [9–11]. Clustering of receptor molecules may confer on the fungal cells an advantage in their interaction with host ligands [11]. Both blastospores and blastospores bearing germ-tubes appeared to express these adhesins; however, only blastospores seemed to have a functional p37 [9]. Both components clearly appeared as two different moieties in the βME cell wall extracts that had been separated under denaturing conditions [9,10]. Nevertheless, the similarity in the topological localization on the surface of *C. albicans* cells for both receptors, especially in the case of blastospores in which probes for both mp58 and p37 showed a patchy distribution, raised the possibility that these two components were part of a multisubunit receptor complex. In the present report we show that the areas of maximum concentration of each receptor are different. Thus these two adhesins did not colocalize as part of the same receptor complex. Moreover, binding of one reagent did not affect simultaneous binding of the other. Similar results were found in experiments in which cells were sequentially incubated with each probe, independently of the order used (not shown), thus showing no competition or even an steric hindrance effect between both probes.

Previous reports have demonstrated expression of hydrophobic cell wall proteins and the receptor for C3d of *C. albicans* during pathogenesis [17,18]. In order to assess the in vivo expression of the p37 and mp58 by *C. albicans* cells present in infected tissues, polyclonal antibodies preparation raised against each of the purified receptors (PAb anti-p37 and PAb anti-mp58) [10,13] were used as probes in immunohistochemistry experiments. Both receptors were expressed in fungal cells present in necropsy sections from patients with disseminated candidiasis and biopsy samples from patients suffering from superficial infection (Fig. 3). The in vivo patterns of morphological expression were similar to the

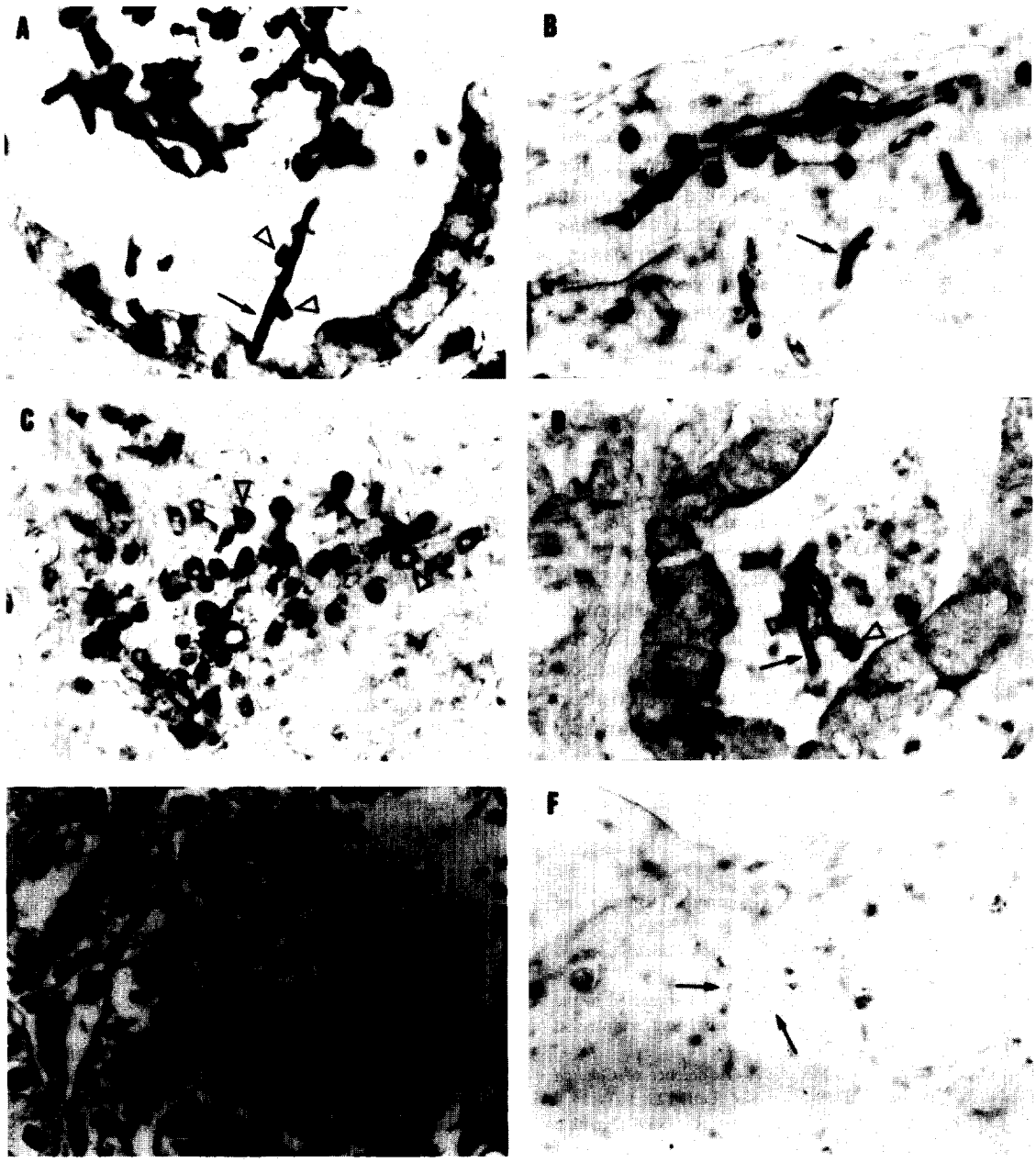


Fig. 3. Immunohistochemical detection of *C. albicans* fibrinogen and laminin receptors in human tissues. Tissue sections from autopsy and biopsy material from patients with candidiasis were examined for expression of the two receptors as described in Section 2. Strong reactivity with anti-mp58 antibody was observed in blastospores (open triangles) and mycelial filaments (arrow) in a collecting duct of the kidney (A) and in superficial candidiasis of the tongue (B). Heterogeneous reactivity with PAb anti-p37 was observed with blastospores (C, triangles) while mycelial filaments were essentially non-reactive (D, black arrows) using PAb anti-p37. No reactivity was found in negative control sections with (E) or without (F) counterstaining. Arrows in F indicate unstained fungal elements. Magnification ×1000.

in vitro patterns detected for cells growing under laboratory conditions [9,10,13].

In summary, in the present study we present evidence that the 37 kDa laminin-binding protein (p37) and the 58 kDa fibrinogen-binding mannoprotein on the surface of *C. albicans* (mp58) are separate moieties and do not colocalize as part of a multisubunit complex. These receptors are expressed by *C. albicans* cells in vivo in infected tissues from patients suffering from candidiasis. Their in vivo expression supports their potential to play a role during pathogenesis.

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