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Cloning of a cDNA fragment encoding part of the protein moiety of the 58-kDa fibrinogen-binding mannoprotein of *Candida albicans*

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Abstract

Immunoscreening of a *Candida albicans* expression library with antibodies against the 58 kDa fibrinogen-binding mannoprotein (mp58) of the fungus resulted in the isolation of clones encoding the protein moiety of this molecule. Sequence of the 0.9 kb cDNA of one of the clones selected for further analysis, revealed an open reading frame coding for 292 amino acids, which displays sequence similarity to proteins belonging to a family of immunodominant antigens of *Aspergillus* spp. The gene corresponding to this cDNA was named *FBP1* (fibrinogen-binding protein). These results represent the first report on the identification of *C. albicans* genes encoding surface receptors for host proteins.

Keywords: *Candida albicans*; Fibrinogen-binding; Virulence factor; Cloning

1. Introduction

Candida albicans is a dimorphic fungus that is both a commensal and opportunistic pathogen of man [1]. Adhesion of the fungus to host cells and tissues is the initial step leading to establishment of infection, and thus considered as a potential virulence factor [2]. These binding mechanisms are mediated by complementary molecules at both the surface of the fungus and the host. In this context, several categories of receptor-like molecules (also designated with the term adhesins) have been identified on the surface of *C. albicans*, including molecules with integrin- and lectin-like activities, along with species in which the sugar moieties (most commonly known as mannan) seem to play an essential role in the interactions [3].

We have identified and characterized a 58-kDa fibrinogen-binding mannoprotein (mp58) on the surface of *C. albicans* [4]. Mp58 appears to be a major component in cell wall extracts of both morphological phases of the fungus and displays potent antigenic properties. It is both N- and O-glycosylated and probably ubiquitinated [4,5], and O-linked oli-

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gosaccharides could be involved in the interaction of mp58 with fibrinogen [4]. Confocal microscopy studies revealed that mp58 is heterogeneously distributed along the surface of the fungus [6]. It is different from other surface adhesins such as laminin receptors [7,8], entactin receptors [9], and receptors binding complement factors [10], and it is highly hydrophobic [11]. This moiety is expressed in all *C. albicans* strains tested, including collection strains and fresh clinical isolates [12]. Moreover, it is expressed in fungal cells infecting tissues [4,8]. These observations seem to suggest an important and active role for mp58 during the interaction between *C. albicans* and the host, and possibly in the pathogenesis of candidiasis.

Here we present the cloning and characterization of a cDNA clone encoding the protein moiety of mp58, for which we have taken advantage of its antigenic characteristics. The clone was identified by screening a *C. albicans* cDNA library in λgt11 with monospecific antibodies generated against purified mp58. Results presented here represent the first report in the literature on the identification of *C. albicans* genes encoding cell surface receptors for host proteins.

2. Materials and methods

2.1. Yeast strain and growth conditions

C. albicans ATCC 26555 was employed throughout this study. The organism was propagated as blastoconidia or blastoconidia bearing germ tubes as described previously [4].

2.2. Immunoscreening of a C. albicans cDNA library

A *C. albicans* cDNA library constructed in the expression vector λgt11d (Promega) [13] was screened with monospecific antibodies generated against the purified 58-kDa fibrinogen-binding candidal mannoprotein (PAb anti-mp58) following standard procedures [14]. The phage DNA from the selected immunoreactive clone was purified, the cDNA amplified by the polymerase chain reaction (PCR) using λgt11 forward and reverse primers, and ligated to the pGEM-T vector (Promega). Lysogens from the selected clone were prepared in *E. coli* Y1089 and lysates were obtained after heat induction in the presence of isopropyl β-D-thiogalactoside, as previously described [13,14].

2.3. Electrophoresis and Western blotting analysis

Protein and glycoprotein components of the walls were released from intact *C. albicans* cells grown under conditions that induce formation of germ tubes by sequential treatment with β-mercaptoethanol (βME) and Zymolyase as described previously [4]. The total sugar content in the extracts was determined colorimetrically with mannose as a standard [15]. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) under denaturing conditions was performed basically as described before [4] using slab 5–15% gradient gels. Proteins in polyacrylamide gels were visualized by staining with Coomassie blue dye. Electrophoretic transfer of separated proteins to nitrocellulose paper and immunodetection either with a polyclonal antibody towards mp58 (PAb anti-mp58) or with affinity-purified antibodies against the fusion protein produced by the cDNA clone were carried out following the procedure reported previously [4]. The affinity-purified antibodies were obtained by standard methods [14,16] from polyclonal antisera generated against β-mercaptoethanol and Zymolyase cell wall extracts of *C. albicans* [17].

2.4. Southern blotting and DNA sequencing

Preparation of chromosomal DNA from *C. albicans* ATCC 26555 and WO-1 strains, digestion with restriction enzymes, agarose gel electrophoresis and transfer to Hybond-*N* filters (Amersham) were carried out according to standard procedures [14]. Labelling of DNA probes and hybridizations were performed using the digoxigenin (DIG) nucleic acid labelling and detection kits (Boehringer-Mannheim). The cDNA fragment subcloned in pGEM-T was sequenced using the λgt11 forward and reverse primers. Synthetic oligonucleotides deduced from the sequence were also used as primers. Sequencing reactions were performed using the Taq DyeDeoxy Terminator Sequencing Kit protocol (Applied Biosystems Inc.) and the nucleotide sequences were de-

termined in an Applied Biosystems automated DNA sequencer model 373A.

2.5. Nucleotide sequence accession number

The sequence has been deposited in GenBank under accession number CAU83997.

3. Results

.1. Isolation of clones encoding mp58

Screening of the *C. albicans* cDNA library with PAb anti-mp58 led to the identification of several immunoreactive clones. Of these, one was selected for further analysis. Lysogens from this clone were analyzed in order to confirm that the immunoreactivity was due to the expression of the fusion protein (not shown). Also, to verify that the identified clone corresponds in fact to a sequence specifying the protein portion of *C. albicans* mp58, affinity-purified antibody to the fusion protein produced by the cDNA clone was used as a probe in immunoblots of cell wall extracts of the fungus containing a complex mixture of proteinaceous components. As shown in Fig. 1, these experiments demonstrated that this antibody preparation recognized specifically the homologous material (mp58) among all other cell wall components present in the extracts. The cDNA of this clone was amplified by PCR, and the resulting 0.9-kb fragment subcloned in pGEM-T for further analysis.

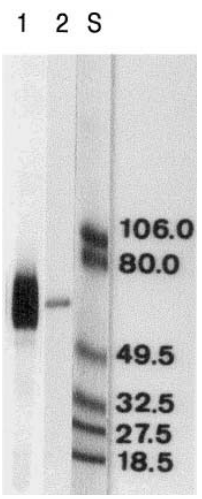

Fig. 1. Immunoblot analysis of βME (lane 1) and Zymolyase (lane 2) extracts from germ tubes of *C. albicans* using as a probe the affinity-purified antibody against the fusion protein produced by the cDNA clone. Each lane was loaded with 100 μg (expressed as total sugar content) of the respective extract, containing a complex mixture of protein and mannoprotein components. Molecular masses (in kDa) of protein standards run in parallel (lane S) are indicated.

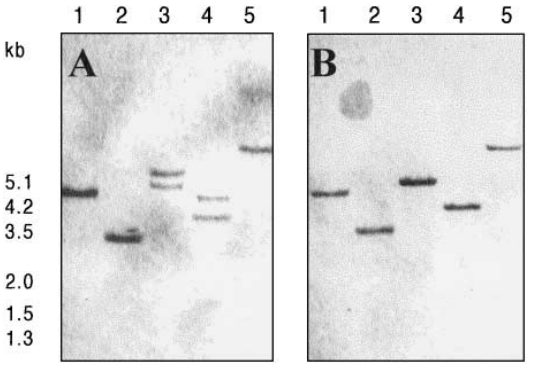

Fig. 2. Southern blot analysis of chromosomal DNA obtained from *C. albicans* strains ATCC 26555 (panel A) and WO-1 (panel B). Each lane contains 12 μg of DNA digested with *Xba*I (lane 1), *Xba*I and *Hin*dIII (lane 2), *Hin*dIII (lane 3), *Hin*dIII and *Bam*HI (lane 4) and *Bam*HI (lane 5); the 0.9-kb cDNA fragment of the selected clone was used as a probe. Molecular mass markers (λ DNA digested with *Eco*RI and *Hin*dIII) are indicated.

.2. Southern blotting

C. albicans chromosomal DNA from strain ATCC 26555 was digested with different enzymes or combinations of enzymes, and after electrophoretic separation, it was transferred to a nylon membrane and Southern blot analysis performed with the 0.9-kb fragment from the immunoreactive clone (Fig. 2). The probe hybridized with one single fragment when the DNA from *C. albicans* ATCC 26555 was digested with *Xba*I, *Hin*dIII/*Xba*I, and *Bam*HI, and two fragments were detected with *Hin*dIII and *Hin*dIII/*Bam*HI (Fig. 2A). Since the probe does not contain internal target sites for the restriction enzymes used, this result suggests that there is one gene ho-

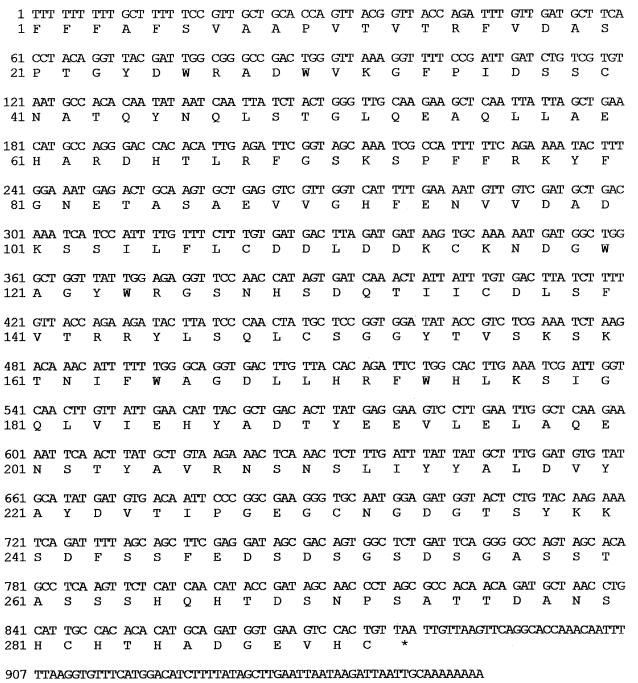

Fig. 3. Nucleotide sequence of the 0.9-kb cDNA fragment corresponding to *C. albicans FBP1*. The cDNA sequence between the adaptors used to construct the library is shown. The predicted amino acid sequence of the encoded protein is also shown.

mologous to the cDNA probe in the *C. albicans* genome, although one *Hin*dIII site is missing in one of the alleles. This was confirmed when chromosomal DNA from another strain (*C. albicans* WO-1) was digested with the same enzymes and probed with the cDNA, since only one single fragment was detected in all five cases (Fig. 2B). The largest fragments detected in *C. albicans* ATCC 26555 with *Hin*dIII and *Hin*dIII/*Bam*HI digestion were not found in *C. albicans* WO-1.

3.3. Sequence analysis

The 0.9-kb cDNA insert was subcloned into pGEM-T, and sequenced using the λgt11 forward and reverse primers, and primers consisting of synthetic oligonucleotides derived from the oligonucleotide sequence. The gene corresponding to the cDNA clone has been named *FBP1* (for fibrinogen-binding protein). Fig. 3 shows the nucleotide sequence and the deduced sequence for the 292 amino acids corresponding to the longest open reading frame, which lacks the 5′ end of the coding region. A search of non-redundant protein databases using the BLAST algorithm [18] revealed sequence similarity to proteins belonging to a family of immunodominant antigens (ASPND1 and AspfII) from *Aspergillus nidulans* and *A. fumigatus* [19,20]. The identity of the mp58 sequence deduced from the cDNA clone (292 aa) and the *A. nidulans* ASPND1 antigen (277 aa) is 47.3% (131 of 292 aa), and similarity is 72.9% when amino acid substitutions are included (213 of 292 aa) (Fig. 4). The four potential N-glycosylation sites (Asn-X-Ser/Thr) and the eight cysteine residues present in the ASPND1 antigen are conserved in the *C. albicans* mp58 species (Fig. 4). The mp58 also contains a serine-rich region of potential O-glycosylation close to the C-terminus similarly to the AspfII antigen [19,20]. In addition the mp58 sequence is almost identical to a pH-regulated antigen (*PRA1*; accession number CAU84261) of *C. albicans* cell wall [21]. Also, more restricted homology was found with the *S. cerevisiae* protein encoded by *YOL154*, of unknown function.

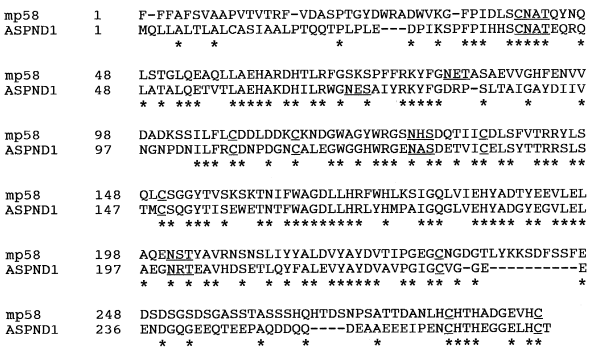

Fig. 4. Comparison of the amino acid sequences of the protein encoded by the *C. albicans FBP1* cDNA and the *A. nidulans* ASPND1 antigen. Sequences are aligned to maximize identity (asterisks indicate identical amino acids). Putative glycosylation sites and conserved cysteines are underlined.

4. Discussion

Mp58 on the surface of *C albicans* displays potent adhesive and antigenic functions [4], and based on these important biological characteristics mp58 may represent an important virulence factor during the pathogenesis of candidiasis. Here we present the isolation of a cDNA clone encoding mp58, for which we have taken advantage of the antigenic characteristics of this molecule. We have used monospecific antibody generated against the purified molecule (PAb anti-mp58) to screen a *C. albicans* expression library in λgt11, thus bypassing the necessity for sequence and/or function homology with *S. cerevisiae* genes. The same approach has been successfully used before for the molecular cloning of the secretory acid proteinase of *C. albicans*, also a putative virulence factor [22]. Of the several immunoreactive clones detected, one was selected for further characterization. Confirmation of the cloning of mp58 was provided by the fact that affinity-purified antibody prepared to the fusion protein produced by the clone recognized with high specificity the homologous moiety (mp58) in immunoblots of *C. albicans* cell wall extracts containing complex mixtures of proteinaceous components. Southern blot analysis of chromosomal DNA revealed that the gene homologous to the probe is present in a single copy in the *C. albicans* genome, although in the case of the ATCC 26555 strain, both alleles differ in one *Hin*dIII site. The 0.9-kb cDNA insert was sequenced. The gene corresponding to the cDNA clone has been named *FBP1*. Interestingly, the deduced amino acid sequence shows similarity (including a high degree of conservation of cysteine residues and potential N-glycosylation sites) with a family of immunodominant antigens expressed by members of the genus *Aspergillus* [19,20]. The fact that the mp58 sequence is almost identical to a *C. albicans* pH-regulated antigen [21] suggests that they may correspond to the same gene. Since we have previously found that expression of mp58 depends on culture conditions [16], pH changes may possibly also exert a regulatory effect on mp58 production.

In summary, results presented here represent the first report on the identification of sequences coding for *C. albicans* adhesins, and should provide the basis for future experiments aimed at increasing our understanding of the roles played by mp58 in the pathogenesis of candidiasis. The relatedness of immunodominant antigens present in *Aspergillus* spp. and the *C. albicans* mp58 moiety suggest the existence of a family of antigens involved in pathogenesis, which are conserved among fungal species.

Acknowledgments

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