Inactivation of CaMIT1 Inhibits Candida albicans Phospholipomannan β-Mannosylation, Reduces Virulence, and Alters Cell Wall Protein β-Mannosylation*

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Studies on Candida albicans phospholipomannan have suggested a novel biosynthetic pathway for yeast glycosphingolipids. This pathway is thought to diverge from the usual pathway at the mannosino-isitol-phospho-deramidase (MIPC) step. To confirm this hypothesis, a C. albicans gene homologue for the Saccharomyces cerevisiae SUR1 gene was identified and named MIT1 as it coded for GDP-mannoseinositol-phospho-deramidase mannose transferase. Two copies of this gene were disrupted. Western blots of cell extracts revealed that strain mit1Δ contained no PLM. Thin layer chromatography and mass spectrometry confirmed that mit1Δ did not synthesize MIPC, demonstrating a role of MIT1 in the mannosylation of C. albicans IPCs. As MIT1 disruption prevented downstream β-1,2 mannosylation, mit1Δ represents a new C. albicans mutant affected in the expression of these specific virulence attributes, which act as adhesins/immunomodulators. mit1Δ was less virulent during both the acute and chronic phases of systemic infection in mice (75 and 50% reduction in mortality, respectively). In vitro, mit1Δ was not able to escape macrophage lysis through down-regulation of the ERK1/2 phosphorylation pathway previously shown to be triggered by PLM. Phenotypic analysis also revealed pleiotropic effects of MIT1 disruption. The most striking observation was a reduced β-mannosylation of phosphopipidomannan. Increased β-mannosylation of mannanoproteins was observed under growth conditions that prevented the association of β-oligomannosides with phosphopipidomannan, but not with PLM. This suggests that C. albicans has strong regulatory mechanisms associating β-oligomannosides with different cell wall carrier molecules. These mechanisms and the impact of the different presentations of β-oligomannosides on the host response need to be defined.

A series of studies has established that specific oligomannose sequences synthesized by Candida albicans are among the virulence attributes of this important opportunistic pathogen. These oligomannosides are linked through β-1,2 bonds that confer a unique spatial conformation (1) recognized by innate and acquired immunity of the host. In contrast to the ubiquitous α-linked mannoside residues, β-1,2 oligomannosides induce protective antibodies (2), do not bind to C-lectins but to galectin-3 (3), trigger macrophages to produce TNF-α (4), and inhibit mouse gut colonization (5). In C. albicans, homopolymers of β-1,2 oligomannosides have been shown to be associated by phosphodiester bridges with phosphopipidomannan (PPM),1 commonly termed mannan, and to correspond to the acid-labile fraction of this molecule (6). Western blots of C. albicans cell wall extracts show that β-oligomannoside epitopes co-localize with α-oligomannoside epitopes on mannosylated proteins (7) and are electively expressed on low molecular weight antigens that have been characterized as phospholipomannan (PLM) (8). Besides PPM, PLM is the second C. albicans molecule in which the presence of β-1,2 oligomannosides has been demonstrated chemically (9). PLM appears as a member of the cell membrane MIPC family from which it diverges by the addition of mannosene and phosphate linking long linear chains of β-mannosides (10). This large polysaccharide moiety confers hydrophilic properties on the molecules that allow it to diffuse into the cell wall (11). PLM is shed by C. albicans in contact with host cells (12) and, through β-oligomannosides of its polysaccharide moiety, induces TNF-α synthesis through a toll-like receptor-2-NFκB activation pathway (13). The same molecule is also at the center of the host cell response, but the mechanisms for this have not yet been elucidated. Finely tuned mechanisms induced by C. albicans PLM and involving the ERK pathway are among the non-adaptative events (14) but can transform Saccharomyces cerevisiae into a macrophage-surviving yeast through the induction of macrophage apoptosis (15).

In parallel, studies on β-1,2 oligomannoside expression in C. albicans have shown that the association of these sequences with different carrier molecules such as PPM, mannanoproteins, and PLM is dependent on growth conditions like pH (16) and temperature likely to be encountered in host tissue (17). At low pH and high temperature, cell wall β-mannosides are not as-

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1 The abbreviations used are: PPM, phosphopipidomannan; PLM, phospholipomannan; IPC, inositol-phospho-deramidase; MIPC, mannanase IPC; MIP, mannose-di-inositol-phospho-deramidase; ERK, extracellular signal-regulated kinase; MEK, mitogen-activated protein kinase/extracellular signal-regulated kinase kinase.
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associated with proteins but almost exclusively with PLM. Depending on the co-receptors involved, these differences in presentation could lead the same molecules to trigger different immune pathways.

Despite the recognized role of β-mannosides in C. albicans virulence, the enzymes responsible for the synthesis of β-mannosides and/or the genes encoding them have not yet been identified. In this study, elucidation of the structure of PLM and complete sequencing of the S. cerevisiae and C. albicans genomes enabled the identification of a candidate gene in the MIPC pathway, an analogue of SUR1 in S. cerevisiae (18), which was named MIT1 in C. albicans. Inactivation of this gene prevented PLM β-mannosylation. The constructed mutants exhibited normal growth and morphotypes but displayed reduced virulence. It was interesting to note that PPM β-mannosylation was altered in the mutant at neutral pH, whereas at low pH the mutant compensated for the absence of β-mannosides in PPM and PLM by β-mannosylating cell wall mannoproteins. These results suggest that the expression of β-1,2 oligomannosides, which are among the virulence attributes of C. albicans, is closely regulated in this species.

**EXPERIMENTAL PROCEDURES**

**Strains and Media—**The C. albicans strains used in this study are listed in Table I. Strains were grown on YPD-uridine medium (1% yeast extract, 2% peptone, 2% dextrose, 20 mg/l uridine) at 37 °C for 16 h. S. cerevisiae strain Su1 was included as a reference strain for the study of yeast-macrophage interactions (19). Synthetic dextrose was prepared as described previously (20). When required, 5-μlurorotic acid was added to synthetic dextrose medium at a concentration of 1 g/liter. The bacterial strain Escherichia coli XL1-blue (Stratagene, La Jolla, CA) was used for propagation of all plasmids. All procedures for manipulating DNA were performed as described previously (21).

**−** The two coding regions of MIT1 (MIT1-5/H11032) were amplified by PCR using the primer couples MIT1-5/H11032 (AGATCTGGATCCGGTTGTCATCATCTTCCTCGT), MIT1-3/H11032 (GACAC-TGGAAACAGTTGGTGTTGTTGGG) and MIT1-3/H11032 (A-GATCCTGGATCCTTGTTCATCCTCCTTG). MIT1-3’ (GACAC-GATTCTTCCATGGCATC), respectively. Amplified fragments were then cloned in a pGEM-T Easy cloning vector (Promega), resulting in plasmids pAL35 and pAL36, respectively. The BglIII-SphI fragment of MIT1 was then cloned at the BglIII-SphI sites of the plasmid pAL35 to give plasmid pAL39. Finally, plasmid pAL41 was obtained by inserting the fragment of the plasmid pAL39 carrying the hisG-URA3-hisG gene at the BglII-BamHI sites of pAL39. The mouse macrophage-like cell line, J747 (ECACC 85011428), which was derived from a tumor in a female BALB/c mouse, was cultured at 37 °C in an atmosphere containing 5% CO2 in Dulbecco’s modified Eagle’s medium supplemented with 10% heat-inactivated fetal calf serum (Valbiotech, Paris, France), 5 mM l-glutamine, 100 μg/ml streptomycin, and 50 μg/ml penicillin. Before each experiment, cells were gently scraped with a rubber policeman, distributed into 12-well culture plates at a concentration of 105 cells/well, and incubated for 18 h to allow adherence. Plated cells were incubated with yeasts at a concentration of 20 yeast cells/ml. Yeasts were washed with Dulbecco’s modified Eagle’s medium, and endocytosed yeasts were re-exposed to the same concentration of yeast cells, with sterile fetal calf serum, for 60 min. The supernatants were collected, acidified with concentrated HCl, and assayed for hemolysis.

**PPM—** Purification of the PPM was performed from strain CAI4 by the URA blaster method (22). The two copies of the hisG gene at the BglII-BamHI sites of pAL39 were replaced with the YPD-uridine specific probe (Promega), resulting in plasmid pAL40. The YPD-uridine specific probe was isolated from plasmid pAL40 by digestion with the restriction enzymes BglII or BamHI. The YPD-uridine specific probe was then inserted into the BglII site of plasmid pAL39. The resulting plasmid was used for propagation of plasmid pAL41. Plasmid pAL41 was obtained by inserting the yeast-specific probe into the BglII site of plasmid pAL39. The resulting plasmid was used for propagation of plasmid pAL41. Plasmid pAL41 was obtained by inserting the yeast-specific probe into the BglII site of plasmid pAL39. The resulting plasmid was used for propagation of plasmid pAL41.

**RESULTS**

**Determination of the Yeast-Specific Probe—** The yeast-specific probe was isolated from plasmid pAL40 by digestion with the restriction enzymes BglII or BamHI. The yeast-specific probe was then inserted into the BglII site of plasmid pAL39. The resulting plasmid was used for propagation of plasmid pAL41. Plasmid pAL41 was obtained by inserting the yeast-specific probe into the BglII site of plasmid pAL39. The resulting plasmid was used for propagation of plasmid pAL41.

**PPM—** Purification of the PPM was performed from strain CAI4 by the URA blaster method (22). The two copies of the hisG gene at the BglII-BamHI sites of pAL39 were replaced with the YPD-uridine specific probe (Promega), resulting in plasmid pAL40. The YPD-uridine specific probe was isolated from plasmid pAL40 by digestion with the restriction enzymes BglII or BamHI. The YPD-uridine specific probe was then inserted into the BglII site of plasmid pAL39. The resulting plasmid was used for propagation of plasmid pAL41. Plasmid pAL41 was obtained by inserting the yeast-specific probe into the BglII site of plasmid pAL39. The resulting plasmid was used for propagation of plasmid pAL41.
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RESULTS

Identification of C. albicans MIT1 as the Potent Homologue of S. cerevisiae SUR1 Responsible for Mannosylation of the IPC Core, and Construction of mit1Δ and Revertant Strains—Complete elucidation of the C. albicans PLM structure (10) strongly suggested that it was a member of the MIPC family, from which it differs by the presence of a second phosphate group and extensive mannosylation (Fig. 1, A and B). Among the genes involved in the S. cerevisiae MIPC biosynthetic pathway, SUR1 is responsible for mannosylation of the IPC core (18), and its inactivation in S. cerevisiae results in viable mutants (18). It was considered that biosynthesis of PLM in C. albicans uses the MIPC biosynthetic pathway described in S. cerevisiae and that inactivation of the C. albicans homologue of SUR1 could lead to C. albicans viable mutants defective in PLM biosynthesis (Fig. 1C). A SUR1 homologue was identified in the C. albicans genome whose open reading frame is referred to as PF04488 (Table I). A complemented strain was also constructed by reintroduction of a wild copy of the MIT1 gene into the mit1Δ::hisGΔ::hisG strain. Each strain was tested by Southern blotting to confirm correct integration of the cassette (Fig. 3B). All studies were performed on strains having one copy of the URA3 gene.

Deletion of MIT1 Has No Effect on C. albicans Growth and Morphogenesis but Induces Increased Sensitivity to Some Agents Used to Detect Cell Wall Defects—When the growth of each strain in YPD or Sabouraud’s broth was monitored, no significant difference was observed among the wild-type, revertant, or null strains (data not shown). Similarly, no difference was observed when strains were incubated in media inducing either yeast-hyphal transition or chlamydospore formation. All strains were able to produce hyphae and chlamydospores (Fig. 4). Therefore, deletion of MIT1 had no obvious microscopic effect on growth or morphogenesis.

When strains were incubated on medium containing increasing concentrations of calcium, calcofluor white, and SDS, the mit1Δ strain was more sensitive to calcium and SDS than the wild-type and revertant strains (Fig. 5, A and C) but was...
equally resistant to calcofluor white (Fig. 5B). These results suggest a plasma membrane or cell wall structural defect in the mutant that could not be observed by microscopic analysis.

**MIT1 Is Involved in the PLM Biosynthetic Pathway—**

The effect of MIT1 deletion on expression of \( \alpha \)H9251-1,2 oligomannoside epitopes was analyzed on Western blots stained with an anti-\( \alpha \)H9251-1,2 oligomannoside monoclonal antibody (Fig. 6). Whole-cell extracts of strains grown at pH 6.0 (Fig. 6A) displayed \( \beta \)-1,2 oligomannoside epitopes on mannoproteins, but no PLM was apparent in the \( \text{mit1}^- \) strain (arrow). PPM extracted from \( \text{mit1}^- \) grown under the same conditions still displayed \( \alpha \)-oligo- mannosides epitopes (data not shown) but revealed a strong decrease in \( \beta \)-1,2 oligomannoside epitope expression (Fig. 6C). When strains were grown at pH 2.0, a growth condition known...
to reduce PPM β-mannosylation, all strains displayed a strong reduction in PPM β-1,2 oligomannoside expression compared with growth at pH 6.0 (Fig. 6D). This reduction in expression of β-1,2 oligomannoside epitopes was also observed on mannoproteins but was less pronounced in mit1Δ (Fig. 6B), suggesting that in the absence of PLM and under conditions where PPM is poorly mannosylated a compensatory mechanism promotes additional mannosylation of mannoproteins. These results strongly suggest that in C. albicans all processes of β-mannosylation are related and are under the control of a global regulatory mechanism.

**MIT1 Is Involved in the Conversion of IPC into MIPC—** Under the solvent and staining conditions used for TLC of sphingolipid extracts, PLM remained at the origin with other insoluble components, and two spots corresponding, respectively, to M(IP)_2C and MIPC were observed (Fig. 7A). TLC of extracts from all strains showed that mit1Δ (lane 2) did not express MIPC or M(IP)_2C. These results were confirmed by mass spectrometry of sphingolipid extracts. Strain CAF2–1 displayed (M-H) molecular-related ions revealing the presence of IPC, MIPC, and M(IP)_2C (Fig. 7B, panel 1). As observed for M(IP)_2C from strain VW32 (10), each of these sphingolipids displayed a series of five peaks arising from variability in their ceramide moiety, which combines C18 or C20 phytosphingosine with C24-C26 mono- or dihydroxylated fatty acids. The major peak resulted from linkage of the OH C24 fatty acid with C20 phytosphingosine. The mit1Δ strain still displayed IPC peaks but, as expected from TLC analysis, was devoid of MIPC and M(IP)_2C (Fig. 7B, panel 2), which were recovered in the complemented strain (panel 3). These results confirm that MIT1 is needed for the conversion of IPC to MIPC.

**The mit1Δ Mutant Is Less Virulent in a Mouse Systemic Model of Infection—** To determine whether MIT1 is involved in virulence, the mortality induced in a murine model of hematogenously disseminated candidiasis was determined (Fig. 8). All mice infected with the wild-type or revertant strains died within 12 days postinjection. At this time, 65% of mice infected with mit1Δ were still alive. Fifty percent were fully resistant to infection within 1 month postinjection. These differences in virulence were also obvious during the initial acute phase of infection (1–4 days) when 70% of mice infected with CAF2–1 died compared with 10% of those infected with mit1Δ.

**Deletion of MIT1 Reduces C. albicans Survival in Macrophages and Alters Macrophase Transduction Pathways—** A significant difference was observed in the number of colony-forming units of mit1Δ and wild-type strains recovered after coculture with J774 cells, demonstrating that the mutation resulted in increased sensitivity of yeasts to macrophase lysis (Fig. 9A).

Parallel analysis of macrophase transduction pathways concerned ERK1/2 phosphorylation (Fig. 9B), which has previously been shown to be an early triggering step in macrophase apoptosis (15). The results confirmed the down-regulation of ERK1/2 phosphorylation in C. albicans strain CAF2–1 (lane 2) demonstrated previously in C. albicans strain VW32 (or its PLM) compared with S. cerevisiae strain Su1 (lane 2). However, in contrast to CAF2–1, depletion of MIT1 resulted in increased ERK1/2 phosphorylation (lane 4), leading to signals similar to those induced by S. cerevisiae.

**DISCUSSION**

A large number of concordant studies have shown that sequences of β-1,2 oligomannosides synthesized by C. albicans contribute specifically to Candida-host relationships and may be among the C. albicans virulence attributes. They act as adhesins for human enterocytes (34) and can prevent colonization of the mouse gut by C. albicans (5). They also bind to macrophages (3, 35), stimulating these cells to produce mediators or effectors of the immune response (4, 36). They elicit the production of specific antibodies that are protective in rodent models of systemic or vaginal candidiasis (37, 38). All these conclusions about the biological effects of β-1,2 oligomannosides have been drawn using oligomannosides derived from PPM or its synthetic analogues. Additional evidence to prove that these residues are involved in C. albicans virulence could be obtained by producing mutant strains depleted in β-1,2 oligomannosides.

Although β-1,2 mannosyltransferase activity has been described in C. albicans (39), the corresponding gene has not yet been identified. As an alternative approach, this study aimed at depleting β-1,2 oligomannosides from a C. albicans molecule that expresses them electively. This molecule was C. albicans PLM, whose structure has recently been described (10). Like the β-1,2 oligomannosides of its polysaccharide moiety, PLM induces TNF-α secretion from cells of macrophase lineage (40), which requires toll-like receptor-2 (13). PLM has also been shown to induce apoptosis of macrophages (15) that have ingested yeasts via upstream modulation of the ERK pathway (19). Through the construction of this mutant, which did not express β-1,2 oligomannosides on PLM, interrelated questions about C. albicans virulence and MIPC and β-mannosylation pathways were addressed.

Western blots of whole-cell extracts of the mit1Δ mutant showed that PLM could no longer be detected with an anti-β-1,2 oligomannoside antibody. This demonstrated that MIT1 is required upstream for the presence of β-1,2 oligomannoside epitopes in PLM. TLC revealed that the absence of PLM reactivity on Western blots could arise from a lack of MIPC. Mass spectrometry confirmed these results and showed that the biosynthetic pathway was interrupted upstream between IPC and MIPC. Despite slight differences between S. cerevisiae and C. albicans phytosphingosine and fatty acid chains already observed with another C. albicans strain, these results confirm that the C. albicans gene MIT1, like its homologue SUR1 in S. cerevisiae (18), is responsible for the addition of mannose to inositol of IPCs. As expected, inactivation of MIT1 prevented further addition of inositol phosphate leading to the synthesis of M(IP)_2C. This inactivation also prevented the addition of mannose phosphate, the first step in the extensive mannosylation of sphingolipids deduced from complete elucidation of the
PLM structure. The present results showing the absence of PLM synthesis following disruption of MIT1 confirm that PLM is derived from the MIPC pathway.

In C. albicans, M(IP)2C and PLM appear as two end products of the sphingolipid pathway and only diverge after the MIPC step. MIT1 therefore encodes a protein that is responsible for mannosylation of IPC, the last biosynthesis step common to these two components that have highly different targets in the cell, mainly in the plasma membrane or cell wall, according to their hydrophobic/hydrophilic properties. These assertions are in agreement with mass spectrometry analysis that revealed the same heterogeneity of their ceramide moiety (10) and a similar effect of growth temperature on ceramide moiety composition, mainly a shift of the major phytosphingosine from C18 to C20 when the temperature increases from 28 to 37 °C (10).

mit1/H9004 is the first C. albicans strain totally affected in the mit1/H9252-mannosylation of a glycoconjugate. The effects of this deletion on phenotype and, more particularly, on virulence were also analyzed. Morphogenesis was not affected by the deletion, including the yeast-to-hyphal transition considered to be a

**FIG. 5.** MIT1 deletion affects Ca2+ and SDS sensitivity of C. albicans cells. Serial 10-fold dilutions of strains were inoculated on YPD agar medium containing different concentrations of either CaCl2 (A), calcofluor white (B), or sodium dodecyl sulfate (C). Results were observed after 24 h of incubation at 37 °C. Lanes 1, CAF2–1 (MIT1/MIT1); lanes 2, C41.11 (MIT1/mit1-Δ:hisG-URA3-hisG); lanes 3, S11.A14 (mit1-Δ:hisG-URA3-hisGlmit1-Δ:hisG), and lanes 4, AS1 (mit1-Δ:hisGlmit1-Δ:hisG + MIT1-URA3).

**FIG. 6.** MIT1 deletion affects the synthesis of phospholipomannan and β-1,2 mannosylation of C. albicans glycoconjugates. Western blots of whole-cell extracts (A and B) or phosphopeptidomannan (C and D) from strains grown at pH 6.0 (A and C) or pH 2.0 (B and D) stained with anti β-1,2 oligomannoside monoclonal antibody, 5B2. Lanes 1, CAF2–1 (MIT1/MIT1); lanes 2, C41.11 (MIT1/mit1-Δ:hisG-URA3-hisG); lanes 3, S11.A14 (mit1-Δ:hisG-URA3-hisGlmit1-Δ:hisG), and lanes 4, AS1 (mit1-Δ:hisGlmit1-Δ:hisG + MIT1-URA3).
**FIG. 7.** *MIT1* deletion inhibits the synthesis of MIPC and M(IP)2 C. Cell pellets were obtained from strains grown on Sabouraud dextrose agar for 16 h at 37 °C. Sphingolipids were then extracted by incubation of the cell pellets in ethanol/water/diethyl ether/pyridine/ammonia (15/9/5/1/0.018) for 1 h at 60 °C. A, thin layer chromatography performed on silica gel plates using a chloroform/methanol/ammonia 4.2 N (9/7/2) solvent system and revealed with orcinol. Lane 1, CAF2–1 (*MIT1/MIT1*); lane 2, S11.A14 (*mit1−Δ:hisG-URA3-hisG/mit1−Δ:hisG); lane 3, AS1 (*mit1−Δ:hisG/mit1−Δ:hisG + MIT1-URA3). B, electrospray mass spectrum showing (M-H) molecular-related ions. Each labeled sphingolipid displays a series of peaks that arise from the heterogeneity of the ceramide moiety.

**FIG. 8.** Virulence assay. Cumulative mortality of mice injected with $2 \times 10^5$ yeasts. CAF2–1 (*MIT1/MIT1*) (circles), S11.A14 (*mit1−Δ:hisG-URA3-hisG/mit1−Δ:hisG) (triangles), AS1 (*mit1−Δ:hisG/mit1−Δ:hisG + MIT1-URA3) (squares).
The overall effect on the reinforcement of macrophage candicidal activity of pathogenic trait of *C. albicans*. However, as observed for *sur1Δ* in *S. cerevisiae* (18), the mutant was more sensitive to calcium. This result, suggesting a plasma membrane or cell wall defect, was confirmed by increased sensitivity of the strain to SDS. Such an increased sensitivity is commonly observed in *C. albicans* mutants, including genes that are not directly related to the synthesis of cell wall components, because of the complexity of the cellular machinery leading to fungal cell wall biogenesis. However, the absence of sensitivity to calcium suggests that an increase in chitin content, a common compensation mechanism triggered by cell wall defects (41), is not triggered in response to the mit1Δ cell wall defect. Transcriptional analysis would be interesting to determine whether these results in differences in the set of genes that are co-regulated in a large number of mutants with cell wall defects (42).

Despite the unaffected growth rate in vitro, the mit1Δ strain was less virulent in a murine model of systemic candidiasis. Fifty percent of mice survived inoculation by mit1Δ compared with 0% with CAF2–1. This clear difference in pathogenic potential during the chronic phase was also obvious during the acute early phase of infection when PLM has previously been shown to be responsible for acute mortality correlating with high levels of TNF-α (43). Among the effects of *MIT1* deletion is the absence of *MIP2* that could play a role in reduction of virulence. However, among the biological activities specific to *C. albicans* PLM that can be deleterious to the host is its ability to promote yeast survival after endocytosis through modulation of the MEK-ERK pathway (19). The current study shows that mit1Δ was more sensitive to macrophage lysis and was not able to modulate phosphorylation of mitogen-activated protein kinase of the MEK-ERK-p90 pathway. This relation between ERK1/2 phosphorylation and an efficient cell response toward *C. albicans* has been confirmed in other models (44). Like PD98059 (45), the addition of PLM interferes with specific activation of this pathway. One explanation for PLM-induced yeast survival is the induction of mitochondrial apoptosis (15) initiated by p90 dephosphorylation (46). Interestingly, among the molecules that can trigger such an apoptotic effect are yeast phytosphingosines (47), which are lipid moieties of *C. albicans* PLM (10). It is possible that the mit1Δ cell wall defects may render this mutant more sensitive to phagocytosis. However, it is clear that its inability to produce PLM and, therefore, to alter the ERK1/2 phosphorylation pathway probably contributes to the reinforcement of macrophage candidicidal activity.

This study also revealed that disruption of *MIT1* had a overall effect on β-mannosylation of *C. albicans* mannoproteins. This was first demonstrated for PPM extracted from strains grown at pH 6.0. To assess the extent of these modifications, the cells were grown under less favorable conditions for the synthesis of β-oligomannoside epitopes. This consisted of growth at pH 2.0 where β-1,2 oligomannosides are known to be associated with PLM, but not with PPM (9, 16). Under these conditions, none of the strains appeared to contain β-1,2 oligomannoside epitopes in PPM, but mit1Δ compensated for the absence of β-1,2 oligomannosides in PLM by an increase in β-mannosylation of mannosproteins. This observation raises three important interrelated issues about the regulation and function of β-1,2 oligomannosides and their carrier molecules in *C. albicans* and its host. The first is the need for chemical evidence of the presence of β-1,2 oligomannosides in mannoproteins. At the moment, the presence of β-1,2 oligomannosides has only been chemically proven in PPM and PLM (6, 8). It is obvious from a large number of studies where blots were probed with anti-β-1,2 oligomannoside monoclonal antibodies that, besides PPM and PLM, and depending on the growth conditions, β-1,2 oligomannoside epitopes are also expressed on a wide range of cell wall proteins where they generally co-localize with α-oligomannoside epitopes (7–9, 26). In parallel, several *C. albicans* cell wall proteins have been shown to play an important role in pathogenesis or cell wall organization/remodeling. Most of these proteins display glycosylation sites but the chemical nature of the mannos oligomers attached is unknown, contrasting with recent studies indicating that cell wall protein post-translational modifications caused by variable glycosylation can control transcription (48). The second issue is the observation of β-1,2 oligomannoside epitope overexpression on mannoproteins at pH 2.0 in the absence of PLM. This strongly suggests overall regulation of β-1,2 oligosaccharide expression at the cell level. The third issue is the impact of modulation of β-1,2 oligomannoside expression on the host response because the signal induced depends on the β-1,2 oligomannoside carrier molecule and co-receptors involved (49). Using this mutant and others, glycogenic analysis of cell wall molecules, with a special focus on β-1,2 oligomannosides, is in progress. This will complement current proteomic and genomic analyses designed to study *C. albicans* virulence attributes and their regulation under conditions encountered in the host.

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