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Phosphatidylinositol 3-kinase VPS34 of *Candida albicans* is involved in filamentous growth, secretion of aspartic proteases, and intracellular detoxification

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Abstract

The phosphatidylinositol (PI) 3-kinase Vps34p of *Candida albicans* influences vesicular intracellular transport, filamentous growth and virulence. To get a clearer understanding how these phenomena are connected, we analysed hyphal growth in a matrix under microaerophilic conditions at low temperature, the detoxification of metal ions and antifungal drugs, the secretion of aspartic proteinases (Saps), as well as expression of adhesion-associated proteins of the *C. albicans vps34* null mutant strain. The hyphal growth in a matrix, which is repressed in the wild-type strain by Efg1p, was derepressed in the mutant. *CZF1*, which encodes an activator of hyphal growth in a matrix, was up-regulated in the mutant. In addition, *CZF1* expression was pH-dependent in the wild-type. Expression of *EFG1* was not changed. Examination of Saps secretion showed a reduction in the *vps34* null mutant. Determination of sensitivity against metal ions and antimycotic drugs revealed defects in detoxification. Expression studies indicated that the *vps34* mutant reacts to the phenotypical defects with an up-regulation of genes involved in these processes, including the aspartyl proteinases *SAP2* and *SAP9*, adhesion proteins *ALS1* and *HWP1*, and the ABC transporters *CDR1* and *HST6*. We also found an increased expression of the PI 4-kinase *LSB6* indicating a complex feed-back mechanism for the compensation of the multiple defects arising from the lack of the PI3-kinase *VPS34*.

Keywords: *Candida albicans*; Phosphatidylinositol 3-kinase; Vps34p; Hyphal growth; Saps; Detoxification

1. Introduction

Candida albicans is the major fungal pathogen in humans, which can cause life-threatening diseases in immunocompromised patients and a variety of mucosal infections in healthy individuals [1]. Present evidence suggests that the virulence of *C. albicans* is dependent

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on several properties, including the ability of the yeast to switch between different morphogenetic forms, host epithelial and endothelial cell recognition and adhesion, as well as to secrete proteinases and phospholipases [2,3]. A number of virulence factors of *C. albicans* have been characterised. However, the mechanisms that enable the opportunistic fungus to become pathogenic have not been revealed yet.

In higher eukaryotic organisms phosphoinositide-based signal transduction mechanisms play an important role in the mediation of cellular responses to extracellular signals. Phosphatidylinositol 3-kinases (PI3-kinases) phosphorylate the 3′OH position of the inositol ring of phosphoinositides, generating the second messengers PtdIns(3)P, PtdIns(3,4)P_2, and PtdIns(3,4,5)P_3. PI3-kinases were shown to be involved in a wide variety of cellular processes including mitogenesis, protection from apoptosis, growth factor receptor downregulation, stimulation of glucose-uptake, endocytosis, actin cytoskeleton rearrangement, and intracellular protein/membrane trafficking [4,5]. Other phospholipids like PtdIns(4,5)P_2 and PtdIns(3,5)P_2 have been implicated in exocytosis, membrane trafficking, and osmotic-stress responses [4,6].

In the yeast *Saccharomyces cerevisiae* the only PI3-kinase activity is represented by the gene product of *VPS34* (vacuolar protein sorting) [7]. The PI3-kinase Vps34p of *C. albicans* regulates virulence and vesicular protein transport. The *C. albicans vps34* null mutant is unable to form hyphae on different solid media, shows significantly delayed yeast-to-hyphae transition in liquid media, and is hypersensitive to different stress conditions. The low electron transparency of the vacuoles of the mutant is indicative for a defective proton transport. In addition, the *vps34* null mutant is avirulent in the mouse model of systemic candidiasis [8–10].

Here, we report further characterisation of the phosphatidylinositol 3-kinase *vps 34* null mutant, with respect to regulation of hyphal growth, expression of surface and secreted proteins required for pathogenic host interaction, and sensitivity against antimycotics and metal ions. After a shift to low-temperature embedded conditions the vps34 null mutant shows increased hyphal growth which is suppressed at ambient temperature. Furthermore, secretion of aspartyl proteinases is decreased, while sensitivity against antimycotics and metal ions is increased in the null mutant. Analytical comparative RT-PCR showed that genes involved in the pathways are differentially regulated in the *vps34* null mutant.

2. Materials and methods

2.1. Strains and growth conditions

Strains used in this study are listed in Table 1. 10 ml YPD [2% (w/v) dextrose, 2% (w/v) peptone, 1% (w/v) yeast extract] was inoculated and incubated for 15 h at 30 °C. This culture was added to 200 ml YPD medium and incubated for 2 h at 30 °C. Cells were pelleted, frozen in liquid nitrogen and kept at −80 °C until RNA isolation. For the pH experiment, 10 ml of Sabouraud medium [2% (w/v) glucose, 1% (w/v) peptone (casein), pH 6.0] containing 100 mM TRIS was inoculated and incubated for 15 h at 30 °C. The culture was used to inoculate 150 ml of Sabouraud medium containing 50 mM Tris (pH 11.0) with 10^6 cells. The culture was divided into three aliquots of 50 ml followed by titration to pH 4.0, 6.0 and 8.0 with citric acid and grown at 30 °C for 4 h. Cells were pelleted, frozen in liquid nitrogen and kept at −80 °C until RNA isolation.

2.2. Hyphal induction within agar matrix

For filamentous growth within agar matrix *C. albicans* strains were grown overnight in YPD and diluted to a cell concentration of 5×10^6 cells ml^{-1}. After growth of 4 h at 30 °C, cells were diluted to 10^3 cells ml^{-1}. 100 μl of the diluted cells was mixed with YPD agar (YPD supplemented with 2% agar) and plated. After 48, 53, 58, and 120 h of incubation at 23 °C colonies were examined microscopically and the percentage of filamentous colonies was plotted as a function of time.

2.3. RNA preparation

Each cell pellet was resuspended in 1 ml RNase-free water, dropped into liquid nitrogen and milled in a mortar until fine powder was obtained. Cell powder was resuspended in lysis buffer (Quiagen, Hilden, Germany), samples were centrifuged at 4000 rpm for 5 min and supernatant was used for RNA isolation. RNA was isolated using the RNeasy isolation kit (Quiagen) according to the manufacturer's protocol. Samples were treated with DNases (Rosch, Mannheim, Germany) and RNA Clean-up protocol (Qiagen) was performed. RNA samples dissolved in RNase-free water were stored at −20 °C.

Table 1
Strains used in this study

C. albicans strains	Genotype or description	Refs.
SC5314	Wild-type	[34]
CAV1	Δ*vps*34::*hisG-URA*3-*hisG*/*VPS*34Δ*ura*3::*imm*434Δ*ura*3::*imm*434	[9]
CAV3	Δ*vps*34::*hisG*/Δ*vps*34::*hisG-URA*3-*hisG*Δ*ura*3::*imm*434Δ*ura*3::*imm*434	[9]
CAV5	Δ*vps*34::*hisG*/*VPS*34::*URA*3Δ*ura*3::*imm*434Δ*ura*3::*imm*434	[9]