Carbon source induced yeast-to-hypha transition in Candida albicans is dependent on the presence of amino acids and on the G-protein-coupled receptor Gpr1

M.M. Maidan, J.M. Thevelein and P. Van Dijck

Department of Molecular Microbiology, Flanders Interuniversity Institute for Biotechnology (VIB) and Laboratory of Molecular Cell Biology, Katholieke Universiteit Leuven, Kasteelpark Arenberg 31, B-3001 Leuven-Heverlee, Flanders, Belgium

Abstract

Yeast-to-hypha transition in Candida albicans can be induced by a wide variety of factors, including specific nutrients. We have started to investigate the mechanism by which some of these nutrients may be sensed. The G-protein-coupled receptor Gpr1 is required for yeast-to-hypha transition on various solid hypha-inducing media. Recently we have shown induction of Gpr1 internalization by specific amino acids, e.g. methionine. This suggests a possible role for methionine as a ligand of CaGpr1. Here we show that there is a big variation in methionine-induced hypha formation depending on the type of carbon source present in the medium. In addition high glucose concentrations repress hypha formation whereas a concentration of 0.1%, which mimics the glucose concentration present in the bloodstream, results in maximal hypha formation. Hence, it remains unclear whether Gpr1 senses sugars, as in Saccharomyces cerevisiae, or specific amino acids like methionine.

Introduction

Candida albicans is the most prevalent opportunistic fungal pathogen in humans causing various forms of candidiasis ranging from superficial mucosal infections to life-threatening systemic diseases, predominantly in patients with a compromised immune system [1]. It is a pleiomorphic organism, undergoing reversible morphogenetic transitions between budding yeast, pseudohyphal and hyphal growth forms. This ability to change morphology is a very important virulence factor [2,3]. The yeast-to-hypha transition of C. albicans can be triggered in vitro by a wide variety of factors, including specific carbohydrates or amino acids, salts, pH changes, temperature increases, starvation, serum and growth within a matrix [4]. The signal transduction pathways, including the cAMP-PKA (protein kinase A) and mitogen-activated protein kinase (MAPK) pathways, that are triggered by these factors have been studied extensively but the mechanisms of sensing for the different triggers are far less understood compared with the situation in Saccharomyces cerevisiae.

Both haploid and diploid S. cerevisiae cells can change their morphology under conditions of nutrient limitation. Nitrogen limitation causes diploid cells to form pseudohyphae whereas glucose limitation causes haploid cells to undergo invasive growth [5]. Both pseudohyphal formation and invasive growth are regulated by the activity of the cAMP/PKA and MAPK pathways. The sensing part of the cAMP/PKA pathway consists of the G-protein-coupled receptor Gpr1, the phospholipase C Plc1 and the G protein Gpa2 [6,7]. Deletion of either gene results in a defect in morphogenesis upon starvation conditions. Stimulation of cAMP synthesis by glucose and sucrose is dependent on Gpr1 and Gpa2 [8,9]. Recently we have used the substituted cysteine accessibility method (SCAM) to determine the binding sites of the putative sugar ligands of Gpr1. We have obtained strong evidence that glucose and sucrose, but no other sugars, are able to interact with the transmembrane domain VI of Gpr1 to induce rapid activation of adenylate cyclase [10].

Two different sensing systems in C. albicans have been studied recently. The first one is the C. albicans homologue of the S. cerevisiae Ssy1 amino acid sensor. Like its Ssy1 homologue in S. cerevisiae, CaCsy1 amino acid sensor is important for the induction of genes encoding amino acid permeases. Deletion of this gene results in a defect in filamentation in serum- and amino acid-based media [11]. The requirement of functional amino acid transporters for hyphal morphogenesis induction (and virulence) was confirmed by studying the deletion phenotype of the CSH3 endoplasmic reticulum (ER) packaging chaperone encoding gene [12]. The second sensing system involves CaGpr1. Recently we and others have shown that, similar to the situation in S. cerevisiae, CaGpr1 functions upstream of the cAMP/PKA pathway in C. albicans ([13], and M.M. Maidan, L. De Rop, J. Serneels, S. Exler, S. Rupp, H. Tournu, J.M. Thevelein and...
P. Van Dijck, unpublished work). In addition we showed that CaGpr1 is required for methionine-induced yeast-to-hyphae transition on solid medium (M.M. Maidan, L. De Rop, J. Serneels, S. Exler, S. Rupp, H. Tournu, J.M. Thevelein and P. Van Dijck, unpublished work). Here we show that apart from glucose, other sugars are also able to support this methionine-induced transition. In addition we show that, in the presence of methionine, low concentrations of glucose induce morphogenesis whereas it is repressed by high concentrations of glucose.

Materials and methods
The wild-type SC5314 and the LDR8 gpr1Δ/gpr1Δ strains were used in the experiments. The construction of the gpr1Δ/gpr1Δ strain has recently been described (M.M. Maidan, L. De Rop, J. Serneels, S. Exler, S. Rupp, H. Tournu, J.M. Thevelein and P. Van Dijck, unpublished work). To determine the ability to undergo the yeast-to-hypha transition, cells of both strains were grown overnight at 28°C with vigorous shaking in YPD (1% yeast extract, 2% bacto peptone and 2% glucose) medium. Ten to fifty cells were then incubated per plate for 6 days at 30°C. Different concentrations of glucose were added to synthetic medium [0.5% (NH₄)₂SO₄ and 0.17% YNB] containing 20 mg/l methionine. To study the effect of the different carbon sources, 0.2% of the carbon source was added to this medium. SLD medium is a synthetic medium containing 0.1% glucose.

Results and discussion
Pseudohyphae induction in S. cerevisiae is triggered by nitrogen starvation. As this phenotype depends on a functional Gpr1 protein, it has been claimed that Gpr1 functions as a nitrogen sensor [15]. However, as mentioned in the introduction we have recently demonstrated that only glucose and sucrose are able to activate the cAMP-PKA pathway through Gpr1 [10]. Also in C. albicans there seems to be a connection between both carbon and nitrogen availability and Gpr1. Recently, we have shown that addition of low concentrations of methionine to SLD medium results in hypha formation in the wild-type but not in the gpr1Δ/gpr1Δ mutant (M.M. Maidan, L. De Rop, J. Serneels, S. Exler, S. Rupp, H. Tournu, J.M. Thevelein and P. Van Dijck, unpublished work). These results suggest that CaGpr1 may be required directly or indirectly for the sensing of methionine. In this paper we have determined the morphology of wild-type and gpr1Δ/gpr1Δ strains in the presence of increasing concentrations of glucose. Minimal medium containing 20 mg/l methionine but no glucose allows growth of C. albicans but the colonies remain small and smooth (Figure 1A). Addition of 0.01% of glucose to the medium already results in a few hyphae. Up to the addition of 0.1% glucose, there is a strong increase in hyphal formation. Higher concentrations result in larger colonies with fewer hyphae. When 2% glucose is present in this medium, C. albicans forms smooth colonies. In all the conditions tested, however, gpr1Δ/gpr1Δ strains never formed hyphae (Figure 1A). These results indicate that glucose limitation (less than 1%) in the presence of methionine induces the yeast-to-hypha transition. In addition the glucose concentration dependent hypha formation in the wild-type but not in the gpr1Δ/gpr1Δ strain suggests a function of CaGpr1 in glucose sensing, similar to the situation in S. cerevisiae.
To determine whether in *C. albicans* glucose (or sucrose) sensing through Gpr1 is essential for the yeast-to-hypha transition we have repeated these experiments with different carbon sources (Figure 1B). Apart from glucose, fructose, maltose, sucrose and galactose are also able to induce the yeast-to-hypha transition in the wild-type strain but not in the *gpr1Δ/gpr1Δ* strain. Lactose, sorbitol and glycerol are unable to induce the yeast-to-hypha transition in this medium. Mannose induces very small hyphae. There seems to be a correlation between the ‘quality’ of the sugar and the ability to induce hypha formation. For the moment it is unclear why different sugars induce hypha formation in the wild-type but not in the *gpr1Δ/gpr1Δ* strain. One possibility is that CaGpr1 is required for proper expression of various sugar transporters, similar to the CsY1 sensor that is required for proper expression of amino acid transporters [11].

Although these results do not yet allow us to identify unequivocally the ligand(s) of CaGpr1 they do reveal an important role for micromolar concentrations of specific amino acids and millimolar concentrations of glucose or related sugars as triggers for hyphal morphogenesis either through CaGpr1 or at least in concert with a pathway controlled by CaGpr1.

This work was supported by Interuniversity Attraction Poles Network P5/30 and the Research Fund of the Katholieke Universiteit Leuven (Concerted Research Actions) to J.M.T., the Fund for Scientific Research-Flanders (G.0242.04) to P.V.D. and OSTC for a research fellowship for Central and Eastern Europe to M.M.M.

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Received 30 September 2004