Defective Morphogenesis in *MBP1* Null Mutant Strains of *Candida albicans* is Specific to Solid Media

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Introduction

Candida albicans is a pathogenic yeast capable of undergoing morphogenesis, which is the transition from yeast to hyphal morphologies. Morphogenesis is induced under a variety of environmental conditions such as limiting amounts of nitrogen, lack of a fermentable carbon source, neutral pH, and serum. These environmental stimuli are transmitted to the nucleus via several different signal transduction pathways resulting in the differential gene expression required for morphogenesis. We have previously cloned and partially characterized the *MBP1* gene of *C. albicans* and shown that null mutant strains that cannot synthesize the Mbp1 protein are defective in morphogenesis under nitrogen limiting conditions on solid media. To further characterize the function of the Mbp1 gene, the ability of wild-type and *MBP1* null mutant strains to undergo morphogenesis was assessed when grown in liquid media under conditions that should stimulate morphogenesis. No difference in germ tube formation was evident between wild-type and *MBP1* null mutant strains in all media tested, including nitrogen limiting media. We conclude that the Mbp1 protein is required for morphogenesis under nitrogen limiting conditions when grown on solid media, but the Mbp1 protein is not required for morphogenesis when *C. albicans* is grown in liquid media.

Materials and Methods

Fresh liquid cultures of yeast strains were made from colonies grown in Yeast Nitrogen Base broth. The cells were centrifuged in the YNB broth, washed with water, pelleted again, and resuspended in 5 ml of water. Yeast from fresh liquid culture were inoculated (with a volume sufficient to give 50-100 colonies/plate) on SLAD (0.17% yeast nitrogen base w/o amino acids and ammonium sulfate, 2% dextrose, 50 mM ammonium sulfate, pH 7.2), Spider (1% Nutrient broth, 1% Mannitol, 0.2% K_2HPO_4, pH 7.2), FBS (10% vol/vol Fetal Bovine Serum), and M199 (Medium 199 containing Earle's salts and glutamine, but lacking sodium bicarbonate, buffered with 155mM Tris-HCL at pH 7.5) agar plates and incubated at 37° C for 7 days prior to taking pictures. Liquid media (SLAD, Spider, FBS, and M199) were inoculated to give a final cell density of 5 X 10^6 cells/ml and incubated at 37° C for 4 hours, followed by preparation of microscopic wet mounts that were photographed.

Results

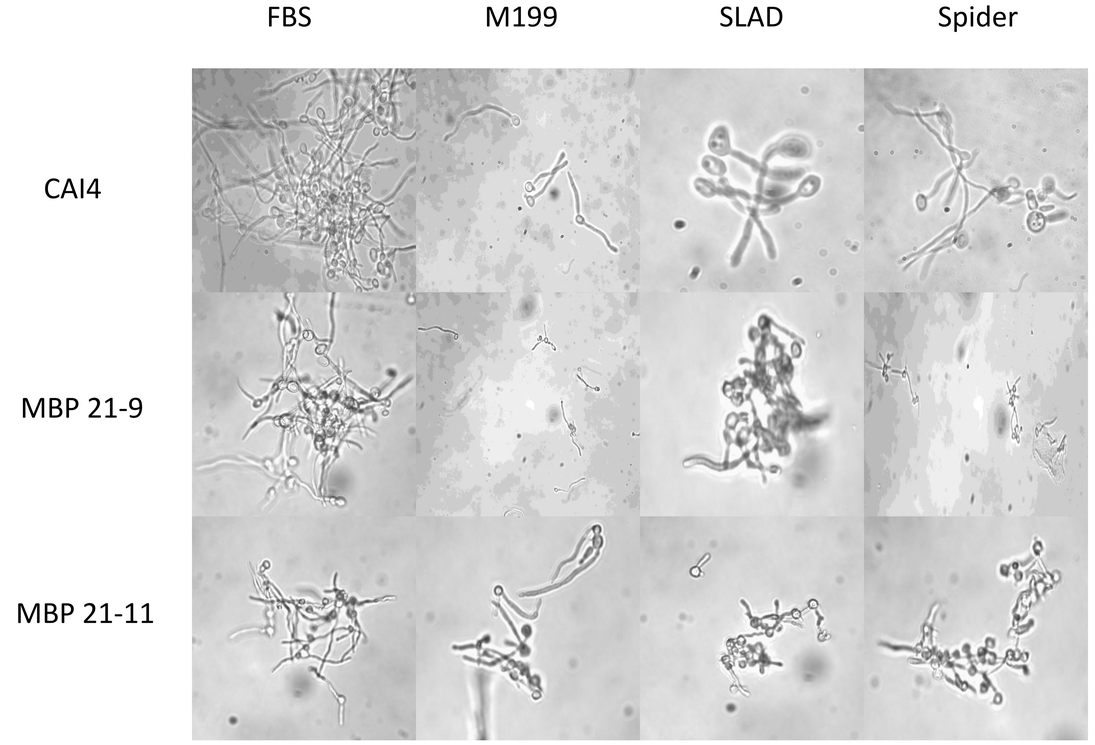

Figure 1. Broth cultures of CAI4 (wild-type strain), MBP 21-9, and MBP 21-11 null mutant strains inoculated in FBS, M199, SLAD, and Spider liquid medias. All strains underwent morphogenesis in the liquid media. Germ tube formation is evident in all medias.

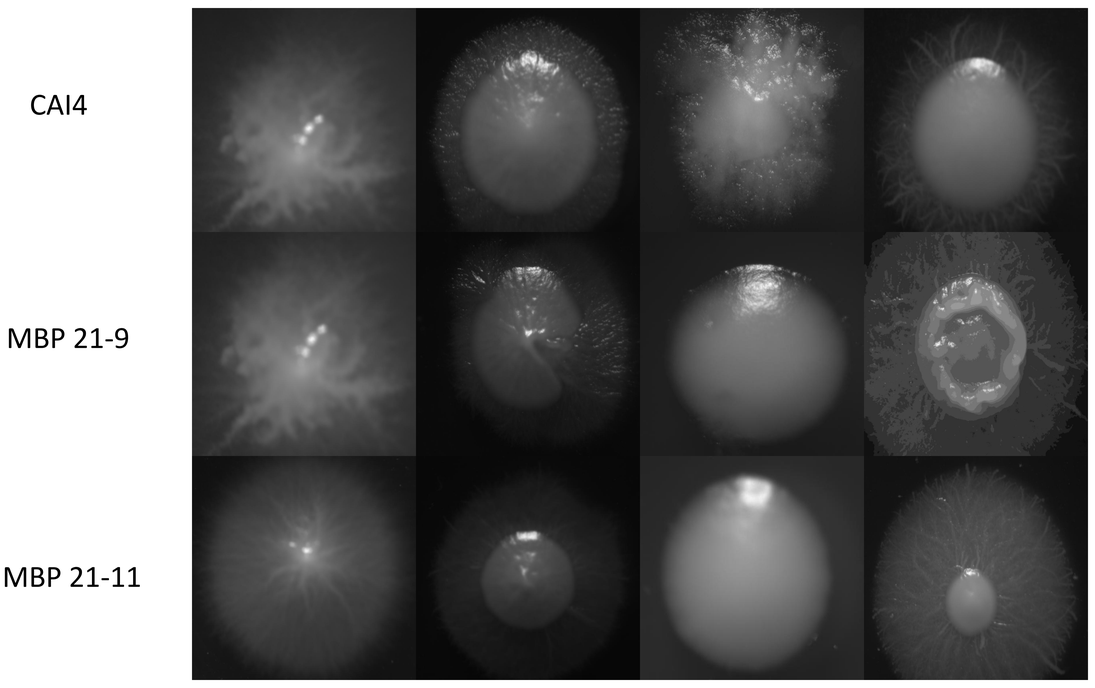

Figure 2. Plate cultures of CAI4 (wild-type strain), MBP 21-9, and MBP 21-11 null mutant strains inoculated on FBS, M199, SLAD and Spider medias. All strains underwent morphogenesis except the null mutant strains when inoculated on SLAD (nitrogen-limiting).

Table 1. *Candida albicans* strains used in this study.

Strain	Relevant Genotype	Source/ Reference
CAI4	Δ*ura3*::*imm434*/ Δ*ura3*::*imm434*	Fonzi and Irwin (1993)
MBP21-9 and MBP21-11	Δ*ura3*::*imm434*/ Δ*ura3*::*imm434* Δ*mbp1*::*hisG*/ Δ*mbp1*::*hisG-URA3-hisG*	This work

Discussion

Null mutant strains (MBP 21-9 and MBP 21-11) were unable to undergo morphogenesis when grown on solid media under nitrogen limiting conditions (SLAD). However, the null mutant strains were able to change morphology when grown in liquid cultures that were deficient in nitrogen. We conclude that MBP1 is required for morphogenesis on solid media, but not liquid media.

Acknowledgments

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