Expression Analysis of the *MBP1* Gene of *Candida albicans*

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Introduction:

Candida albicans is the most commonly isolated fungal pathogen in humans. *Candida albicans* is a dimorphic species, having both single-celled (the yeast form) and filamentous growth forms. Morphogenesis, the ability to transition from the yeast state to the filamentous morphology, has been identified as an important factor in causing systemic infections. Environmental factors that regulate morphological conversions include pH, temperature, the presence or absence of serum, and the availability of nitrogen. Information about these environmental factors are conveyed to the interior of the yeast cell through signal transduction pathways that activate the expression of genes involved in morphogenesis. The Mbp1 protein has a number of functional domains characteristic of a transcription factor and we have previously demonstrated that mutants lacking the protein are defective in morphogenesis when grown under nitrogen-limited conditions. Our research then set out to determine whether the Mbp1 protein is constitutively expressed or differentially expressed according to environmental signals, which would further elucidate the role Mbp1p plays in signal transduction pathways that regulate morphogenesis. Comparisons of *MBP1* expression were made by isolating total RNA content from *C. albicans* grown on different media types that induce morphogenesis (both solid and liquid forms of M199, Spider, FBS, and SLAD) and non-inducing media (YNB). Reverse transcriptase-PCR was used to detect and amplify any *MBP1* transcripts present in the RNA samples. Results indicated that Mbp1p is constitutively expressed. This suggests that Mbp1p may interact with another protein or proteins whose expression is regulated according to environmental clues.

Table 1: Environmental factors that regulate morphological conversion.

Factor	Yeast	Hyphae
pH	<7	>7
Temperature	<30C	>30C
Serum	Absent	Present
Nitrogen	Non-limiting	Limiting

Results:

Table 2: Relevant *C.albicans* strains used in this study.

Strain	Relevant Genotype	Source/Reference
SC5314	Wild Type	Fonzi and Irwin (1993)
MBP 21-11	Δ*ura3*::*imm434*/ Δ*ura3*::*imm434* Δ*mbp1*::*hisG*/ Δ*mbp1*::*hisG-URA3-hisG*	This work

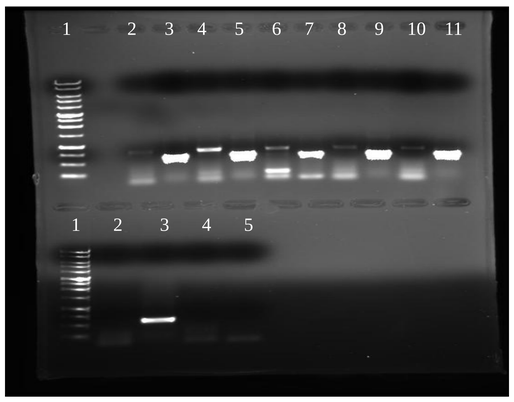

Figure 1: RT-PCR gel results of *C.albicans* grown on solid media types. The last 4 lanes in the bottom half of the gel are controls and represent PCR products from water controls and from the 21-11 *MBP1* null mutant strain (for which actin product should only be present). Bands present in water controls are indicative of primers and nucleotides left-over from RT-PCR reaction components. TOP: lane 1: molecular size markers, lane 2: *MBP1* product from YNB, lane 3: actin product from YNB, lane 4: *MBP1* product from FBS, lane 5: actin product from FBS, lane 6: *MBP1* product from SLAD, lane 7: actin product from SLAD, lane 8: *MBP1* product from Spider, lane 9: actin product from Spider, lane 10: *MBP1* product from M199, lane 11: actin product from M199. BOTTOM: lane 1: molecular size markers, lane 2: *MBP1* product of null mutant from SLAD, lane 3: actin product of null mutant from SLAD, lane 4: *MBP1* water control, lane 5: actin water control.

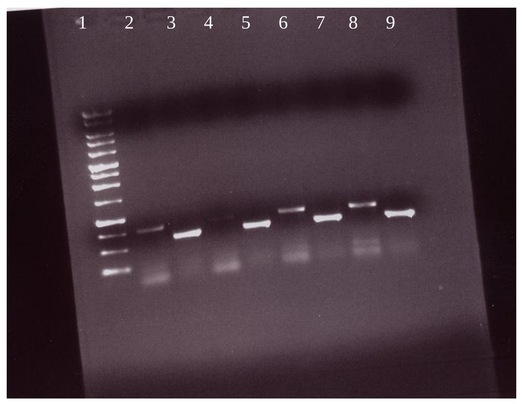

Figure 2: RT-PCR gel picture of wild type *C. albicans* grown in liquid media types. Lane 1: molecular size markers, lane 2: *MBP1* product from YNB, lane 3: actin product from YNB, lane 4: *MBP1* product from YNB at room temperature, lane 5: actin product from YNB at room temperature, lane 6: *MBP1* product from FBS, lane 7: actin product from FBS, lane 8: *MBP1* product from SLAD, lane 9: actin product from SLAD.

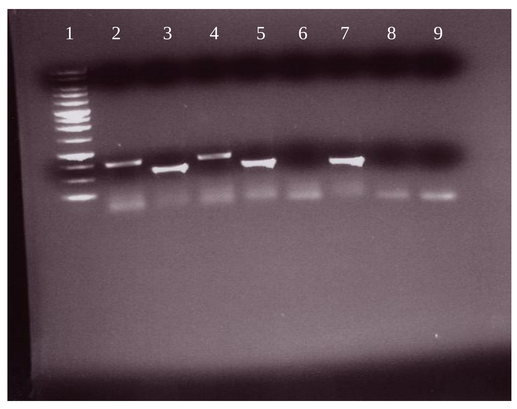

Figure 2: RT-PCR gel picture of *C. albicans* strains grown in morphogenesis-inducing liquid media. The last four lanes represent control PCR product from the 21-11 *MBP1* null mutant strain and water controls. Lane 1: molecular size markers, lane 2: *MBP1* product from Spider media, lane 3: actin product from Spider media, lane 4: *MBP1* product from M199, lane 5: actin product from M199, lane 6: null mutant *MBP1* product from SLAD, lane 7: null mutant actin product from SLAD, lane 8: water control for *MBP1*, lane 9: water control for actin transcripts.

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Methods:

1. Wild-type and 21-11 (*MBP1* null mutants) *C. albicans* were grown on solid and liquid types of both morphogenesis-inducing and non-inducing media:
   - 10% Fetal Bovine Serum
   - M199, pH 7.5
   - Spider (non-fermentable carbon source)
   - SLAD (nitrogen limiting)
   - YNB (non-inducing)
2. Total RNA content was isolated from each *C. albicans* population.
3. Two reverse transcriptase-PCR reactions were set up for each RNA sample, one using actin primers and one using *MBP1* primers. Actin transcripts were amplified in the RT-PCR to serve as a control, since actin is always expressed. The null mutants served as another control, since *MBP1* transcripts should be lacking in these RNA samples. Water controls were also run as a check for possible contamination in the RT-PCR reactions.
4. Gel electrophoresis was used to visualize RT-PCR reaction products and to compare amounts of *MBP1* transcripts present in the different RNA samples.

Discussion:

It appears that there are some temperature effects on the expression of *MBP1*. This can be seen when the *MBP1* PCR product bands of *C. albicans* grown on YNB at 35 C are compared to *C. albicans* grown on YNB at room temperature. The brightness of the band is much reduced in the sample that was grown at room temperature, indicating that higher temperatures increase the expression of the *MBP1* gene. In addition to this finding, *MBP1* expression also appeared to be higher in *C. albicans* grown on solid FBS media relative to the other solid media types. However, ignoring relative amounts and focusing on the fact that *MBP1* transcripts were present in all of the RNA samples, regardless of the media type, indicates that *MBP1* is constitutively expressed in *C. albicans*. If some *MBP1* transcript is always present in the yeast cells, then it appears that the Mbp1 protein interacts with another protein or proteins whose expression is/are regulated according nitrogen availability. Future research will use yeast two-hybrid analysis and co-immunoprecipitation to identify potential proteins with which Mbp1p interacts.

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