Localization of the *C. albicans* MBP1 Gene Product in *S. cerevisiae*

Aric Runzheimer Dr. Julie Anderson
Department of Biology
University of Wisconsin–Eau Claire

Abstract

The pathogenic yeast species, *Candida albicans*, is responsible for a number of opportunistic oral and genital infections, particularly those of the immunocompromised. Key to its pathogenicity is its ability to convert from yeast to hyphal form upon invasion of human tissue. Research suggests the *MBP1* gene product plays a role in this transition. This project continues work carried out by UWEC biology department faculty, Dr. Dan Herman and Dr. Julie Anderson, and former student, Greg Fischer. The *C. albicans MBP1* gene was expressed in the closely related, less pathogenic, *Saccharomyces cerevisiae*. The regulatory protein, Swi6, combines with the Mbp1 and Swi4 proteins to form the MBF and SBF complexes, respectively, which function as transcription factors. A *swi4¯/mbp1¯* double mutant generally results in lethality; however, viable strains were isolated, possibly indicating the presence of additional mutations. This project seeks to investigate the nature of these revertants. Key to this investigation is the cellular localization of the Mbp1 protein. If Mbp1 functions as a transcription factor, it should localize to the nucleus. Investigating the localization patterns of the *MBP1* gene product will further our understanding of the role it plays in the pathogenicity of *C. albicans*.

Introduction

- *C. albicans* becomes pathogenic in immunocompromised patients, such as HIV infected individuals, those undergoing chemotherapy, and marrow recipients.
- The U.S. Department of Health and Human Services estimates 75% of the female population has been afflicted with yeast infections due to *C. albicans*, and studies have associated *C. albicans* with over half of all dental cavity cases (Rozkiewicz et al. 2006).
- To become pathogenic, *C. albicans* converts from a unicellular state to a multicellular, filamentous form. The *MBP1* gene plays a major role in this morphogenesis (Herman, in progress).

A)

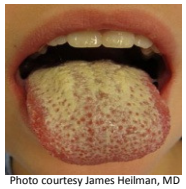

Photo courtesy James Heilman, MD

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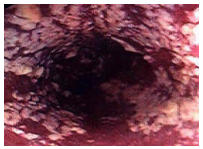


Photo courtesy tulasstory.wordpress.com

Figure 1. A) Oral and B) gastrointestinal Candidiasis

Previous Research

- The functional equivalence of *C. albicans* and *S. cerevisiae MBP1* was demonstrated by expressing *C. albicans MBP1* in *S. cerevisiae* (Fischer et al., unpublished results).
- Expression of C. albicans MBP1 suppressed the lethality of a *swi4¯/mbp1¯ S. cerevisiae* double mutant.

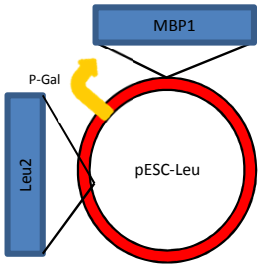
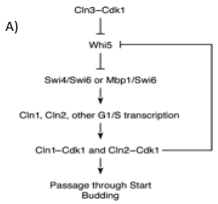


Figure 2. A pESC-Leu vector was created containing *C. albicans MBP1* under the control of a galactose induced promoter, allowing for selective expression. Leu2 served as a selectable marker to identify cells that had been transformed.

Function of MBP1 Gene Product

- In S. cerevisiae, the Mbp1 protein, in addition to Swi4, Swi6, and various cyclins and cyclin dependent kinases, promotes passage through the cell cycle (Figure 3A).
- Swi6 complexes with Mbp1 and Swi4 to from MBF and SBF, respectively, which activate expression of cyclin genes (Figures 3A and B).
- Functional *SWI4* compensates for a *MBP1* disruption, and vice versa, however; disruption of both is lethal (Koch et al. 1993).

A)



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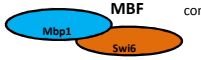
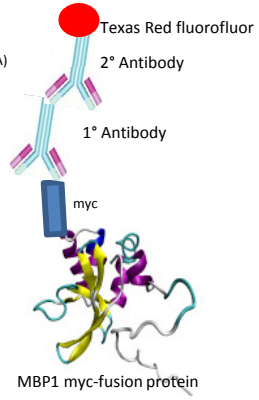


Figure 3. A) A diagram of the various proteins involved in progression through the cell cycle (Ubersax and Ferrell 2006). B) SBF complex. C) MBF complex

Indirect Immunofluorescence

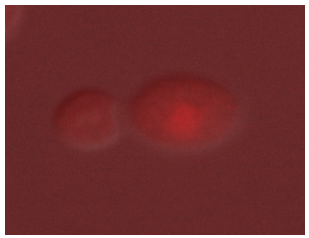
- As a transcription factor, *S. cerevisiae* MBF complex localizes in the nucleus
- Localization of *C. albicans* Mbp1 protein in *S. cerevisiae* nuclei further supports functional equivalence of the two genes.

A)



MBP1 myc-fusion protein

B)



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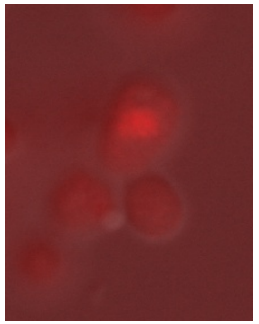

Figure 4. A) A Mbp1 fusion protein containing a myc epitope was created. Mouse anti-myc and goat anti-mouse antibodies were used as the primary and secondary antibodies, respectively. Attached to the secondary antibody was a Texas red fluorofluor, which fluoresces red when viewed under UV light. B) and C) Texas red fluorescence was concentrated in the nuclei of cells expressing *MBP1*, implying Mbp1 protein was concentrated in the nuclei of these cells.

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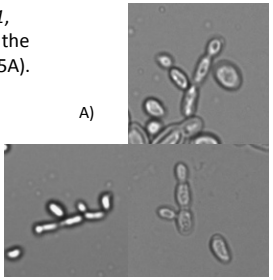
Previous Isolations

- Pseudo-hyphal growth was observed in *S. cerevisiae* cells expressing *C. albicans MBP1*, verifying the role *C. albicans MBP1* plays in the emergence of filamentous growth. (Figure 5A).
- Unexpectedly, six *swi4¯/mbp1¯ S. cerevisiae* mutants were isolated that were viable without apparent expression of *C. albicans MBP1.*
- Replica plating was used to determine if the implied additional mutation was chromosomal or plasmid dependent.
- Four of the six revertants (2-5) were definitively determined to be the result of a plasmid dependent mutation. (Figure 5B).

A)



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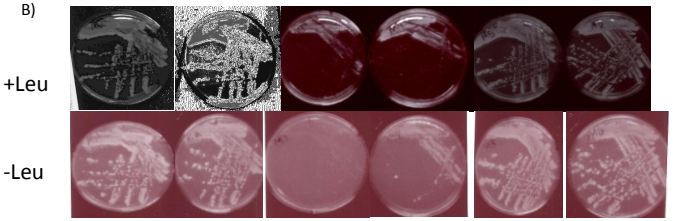

Figure 5. A) *S. cerevisiae* cells expressing *C. albicans MBP1* show signs of pseudo-hyphal growth. B) Cells were grown on glucose +/- leu. On +leu media, cells were allowed to lose the plasmid yet remain viable, whereas only cells retaining the plasmid are viable on -leu media. When streaked, revertants 1 and 6 produced colonies that were viable on +leu but not -leu, suggesting the mutation suppressed the double mutant lethality, despite loss of the plasmid. These colonies presumably possess chromosomal mutations and will require further analysis.

Future Research

- Further analysis of revertants may reveal a chromosomal mutation that suppresses the lethality of the *swi4¯/mbp1¯* genotype without *MBP1* expression.
- Quantifying expression of cyclin genes in *S. cerevisiae* expressing native or *C. albicans MBP1* could further our understanding of the homology between the genes.
- Comparing shifts in gene expression of *C. cerevisiae* as a result of *C. albicans MBP1* expression may further reveal the role *MBP1* plays in pathogenesis.
- Determining whether the pseudo-hyphal growth observed by *S. cerevisiae* is a direct result *C. albicans MBP1* expression, or if it can be induced by over-expression of native *MBP1*.

References

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