

Pathogenic Environmentally Dependent Antifungal Resistance Mechanism via +1 Programmed Ribosomal

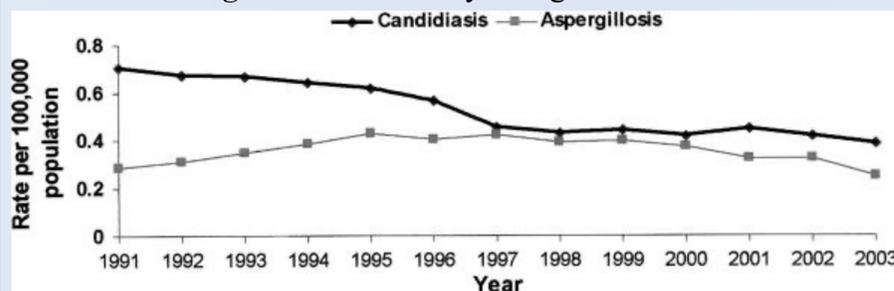
Frameshifting in *C. glabrata*

Maya Bussey, Department of Biology

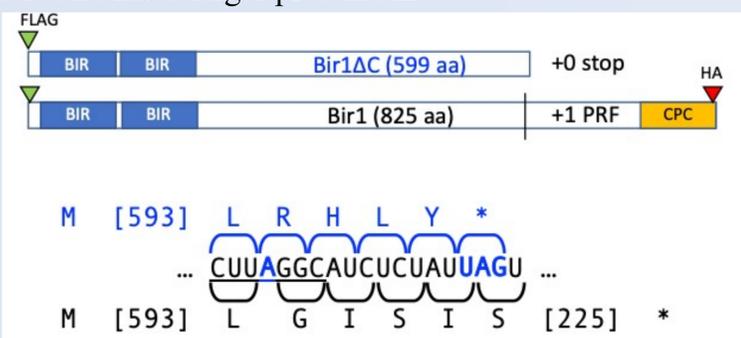
Mentor: Dr. Kyle Cunningham, Department of Biology

Background and Abstract

Candida glabrata is a pathogenic yeast that is the second leading cause of invasive candidiasis, fungal infections of the bloodstream, and is prevalent in nosocomial infections. Due to its natural resistance to fluconazole, the frontline antifungal, the incidence rate of *Candida glabrata* is steadily rising.¹



We are exploring a novel mechanism in *C. glabrata* and its close relatives that could relate antifungal resistance and chromosome instability as a response to environmental cues: Programmed +1 ribosomal frameshifting (PRF) in Bir1 and Ats1.² Bir1, a critical subunit of the chromosomal passenger complex, is the most promising gene to explain the connection between chromosome instability and antifungal resistance through programmed frameshifting because it is better understood with extensive past literature and has a larger protein size.³



1) Bir1

Approaches to study these genes:

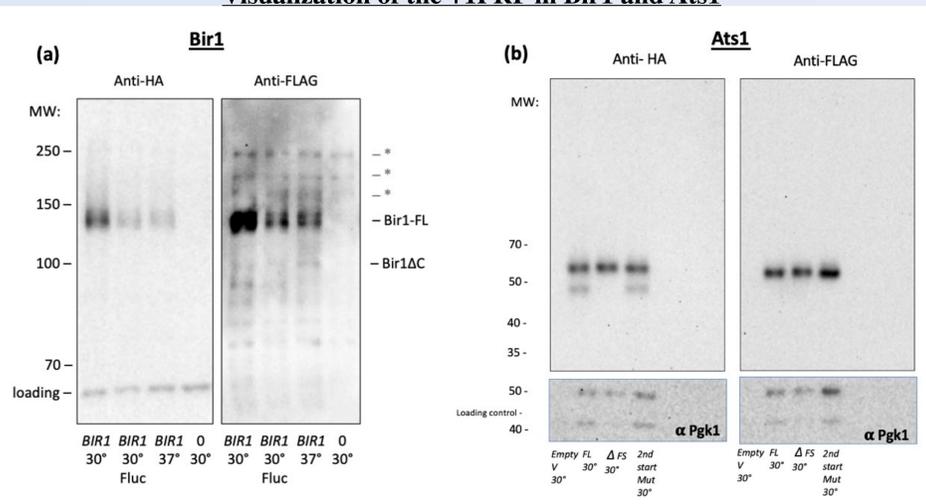
- 1) Visualize the Full Length Protein via Western Blot
- 2) Modify the frameshift with single-site directed mutations
- 3) Visualize the truncated protein if possible*
- 4) Explore beneficial phenotypes for growth/death for truncated protein overexpression *

2) Ats1

* Might not be possible due to gene size

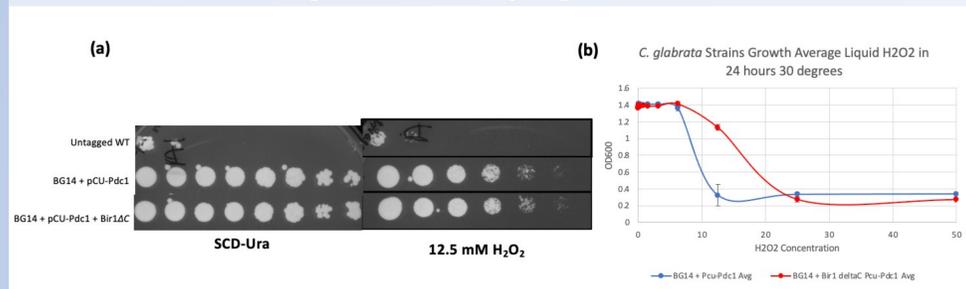
Results

Visualization of the +1PRF in Bir1 and Ats1



(a) Western blots for Bir1 performed on log phase *C. glabrata* cells with pCU-MET3-FLAG-BIR1-HA (lanes 1-3) or pCU-MET3 (lane 4) overnight in YNB dextrose medium at 30°C plus 16 μg/mL fluconazole, or 37°C (b) Western blots for Ats1 performed on log phase *C. glabrata* cells with pCU-PDC1-FLAG-ATS1-HA (lanes 2-4) or pCU-PDC1 (lane 1) overnight in SCD-URA medium at 30°C. Lane 3 plasmid has a single site mutation at the slippery site of the gene and only expresses the truncated (ΔFS) form. Lane 4 plasmid has a single-site mutation at a start site (atg) region downstream of the slippery site. All single-site mutations done using the Agilent QuikChange Lightning kit

Solid and Liquid Growth Assay Experiments with Bir1ΔC



(a) Solid media growth assay performed using the 'frogging' technique by pouring SCD-Ura +/- 12.5mM H₂O₂ where 1:3 serial dilutions of cells are transferred to the plates and grown for 36 hours (b) Liquid media growth assay performed with pipetting 1:2000 final dilutions of *C. glabrata* cells in a 2-fold serial dilution of H₂O₂ with a starting concentration of 100mM.

Interpretation & Conclusions

We were able to visualize the full-length proteins for both Bir1 and Ats1 using the plasmid cloning vectors pCU-MET3 and pCU-PDC1. The full length is only present in *C. glabrata* when the +1 ribosomal frameshift occurs which proves the presence of a functional +1 PRF in both genes in *C. glabrata*. For Bir1, the truncated protein was also able to be visualized which was an intriguing surprise, but there were many other degradation species that could be products of improperly regulated protein degradation. For Ats1, there is a second lighter band present around 50kD with only the HA C-terminus tag that could be an alternative protein transcript produced by a second translation start site that is downstream of the slippery site, but still in frame of the second half of the Ats1 protein transcript when +1 PRF occurs. However, when the suspected second start site was mutated the 50kD second band did not disappear as expected but did disappear when the "A" in the slippery site was mutated. Either the second site is from a third translation start site or the samples in lane 3 and lane 4 are in an incorrect order. Both theories will be tested in the future. The truncated form of Bir1 produced when the +1 PRF does not occur has a significant growth benefit than the vector control wildtype species in liquid media in H₂O₂ drug conditions supporting that +1 frameshifts in *C. glabrata* could increase environmental adaptability. Proper validations and more drug/environmental conditions will be tested.

Sources and Acknowledgements

- (1) Pfaller MA, Diekema DJ. Epidemiology of invasive candidiasis: a persistent public health problem. *Clin Microbiol Rev.* 2007;20(1):133-163. doi:10.1128/CMR.00029-06
- (2) Poláková S, Blume C, Zárate JA, et al. Formation of new chromosomes as a virulence mechanism in yeast *Candida glabrata*. *Proc Natl Acad Sci U S A.* 2009;106(8):2688-2693. doi:10.1073/pnas.0809793106
- (3) Walter D, Wissing S, Madeo F, Fahrenkrog B. *The inhibitor-of-apoptosis protein Bir1p protects against apoptosis in S. cerevisiae and is a substrate for the yeast homologue of Omi/HtrA2.* *J Cell Sci.* 2006;119(Pt 9):1843-51.

Special thank you to the people of Dr. Cunningham lab: Andrew Gale, Matthew Pavesic, Timothy Nickels, and Dr. Kyle Cunningham! Thank you as well to Dr. Natalie Strobach and the Woodrow Wilson Fellowship Program!