Effects of non-coding sequence deletion on RNA-binding protein levels and *Candida albicans* growth

Joanne Du, Class of 2024

Candida albicans is a fungus capable of infecting humans through the bloodstream and oral and vaginal cavities, which can be lethal in immunocompromised hosts. In *C. albicans*, Slr1 is an RNA-binding protein associated with growth and virulence that is notable for its unusually long intron, a sequence in its gene that does not code for protein production [1]. Introns are therefore removed from pre-mRNA, the molecules made from DNA for protein production. My research tested a two-step hypothesis: first, that deleting the *SLR1* intron would increase Slr1 protein levels; second, that this increased Slr1 protein production would adversely affect growth. This prediction was based on research conducted on Yra1, an RNA-binding protein with a similarly long intron, present in both *C. albicans* and the related model organism *Saccharomyces cerevisiae*, baker's yeast [2].

To test the significance of the intron to Slr1 protein production, I collaborated with lab-mate Foje-Geh Tendoh to construct *C. albicans* cells with and without the *SLR1* intron. In our designs of the *SLR1* DNA for the construction of these cells, we included DNA for green fluorescent protein (GFP), which allowed us to visualise the Slr1 proteins produced by our *C. albicans* cells. We first used the polymerase chain reaction and other molecular techniques to construct and verify GFP-tagged *SLR1* DNAs with and without the intron.

After introducing these DNAs to *C. albicans* cells, we extracted the proteins from both types of cells and performed a Western blot to compare the quantities of Slr1 produced. Although Slr1 quantities varied greatly among *C. albicans* cells without the *SLR1* intron, we found that all cultures without the intron produced notably more Slr1 protein than those with the intron, supporting the first step of our hypothesis.

However, the results of our assays assessing the impact of intron deletion and increased Slr1 levels on *C. albicans* growth were inconclusive. We performed a spotting assay to see how our different strains grew on agar plates, a growth curve assay to observe the strains' rate of growth in liquid growth medium, and a heat shock assay to determine whether the stress of increased temperatures affects the growth of cells with and without the *SLR1* intron. None of these assays showed a notable difference between the growth of *C. albicans* cells with and without the *SLR1* intron.

In summary, we produced evidence supporting part of our hypothesis: intron deletion increased Slr1 levels, but this deletion did not noticeably affect growth. These results suggest directions in which we might take future experiments. We could further investigate how intron deletion increased Slr1 protein production; while we inferred that we increased Slr1 protein levels by increasing *SLR1* mRNA levels with the intron deletion, employing RNA visualisation techniques to determine *SLR1* mRNA levels could verify this inference. Alternatively, we could insert a DNA sequence to ensure increased mRNA levels and observe protein levels. The interaction between a mutant Slr1 protein that is present at high levels and another *C. albicans* protein suggests that this protein might mitigate the expectedly damaging effect of Slr1 on cell growth. Repeating our experiments after having removed the candidate Slr1-interacting protein might explain the divergence of our results from our hypothesis. Researching mechanisms that control Slr1 levels and thereby *C. albicans* growth could be a precursor to optimising the treatment of *C. albicans* infections, and we hope that our results might contribute to this.

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References

1. Ariyachet, C., et al., *SR-like RNA-binding protein Slr1 affects Candida albicans filamentation and virulence.* Infection and immunity, 2013. **81**(4): p. 1267-1276.
2. Preker, P.J. and C. Guthrie, *Autoregulation of the mRNA export factor Yra1p requires inefficient splicing of its pre-mRNA.* Rna, 2006. **12**(6): p. 994-1006.