Hyper-Recombination in *Candida albicans*
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*Candida albicans* is the most common opportunistic fungal pathogen. As a commensal organism it can cause superficial infections in healthy people. Even more seriously it can cause life-threatening infections in humans with suppressed immune systems such as HIV, cancer or organ transplant patients. Few drugs are able to combat *C. albicans* infections and resistance to the few antifungal drugs that do exist is increasing.

*C. albicans* is a diploid, highly heterozygous organism that reproduces primarily by clonal division. To create genetic variation it relies mostly on recombination, mutation and to a lesser extent on a parasexual cycle. Preliminary studies have shown that loss of heterozygosity (LOH) in unstressed cells is mostly caused by break-induced replication (BIR) or single crossover (AF, unpublished). In addition, rates of LOH are higher in stressed cells and LOH mechanisms vary depending on the stress applied to the cells. Furthermore, these experiments have shown that double mutations are more common than single mutations in stressed cells than in unstressed cells (AF, unpublished).

Based on the observation that subpopulations in *C. albicans* exhibit double mutations, hypermutation may exist in *C. albicans* and play a role in adaptation to stresses such as the constantly changing environments within the host.

To test this hypothesis, during my summer research I used loss of heterozygosity (LOH) as a genetic tool to identify multiple recombination/mutation events in the genome. I wanted to know the frequency of multiple LOH events in different environments.

To study multiple LOH events in *C. albicans* a specific strain was used that is heterozygous for six marker genes, which are located on five of the eight chromosomes: GAL1 on chromosome 1, ADE2 and HIS1 on chromosome 3, HIS4 on chromosome 4, the mating type-like locus (MTL) on chromosome 5, and the URA3 gene on chromosome 7.

Single colonies were grown from freezer stock, transferred to cultures with rich medium and grown overnight. To determine the total cell count serial dilutions were spotted onto rich medium. Loss of the first marker, GAL1, was determined by selection on 2-deoxygalactose (2-DOG), a medium that allows only Gal+ cells to grow.

To select and/or screen for additional LOH events, Gal+ colonies were transferred to 96-well plates containing 50% glycerol. I spotted these cell suspensions onto selection/screening media using a multichannel pipettor. First, cells were screened for the Ura- phenotype on minimal media (MIN) containing histidine (His) and adenine (Ade). Only Ura+ cells grew and the number of Ura+ colonies can be determined by subtracting the number of Ura- colonies from the total number of colonies spotted. Similarly, screening for His- was done on MIN containing uridine (Uri) and Ade. Ade- colonies were apparent, as they were red or pink in color. Status of the MTL locus (heterozygous MTLa/MTLa or homozygous ‘a’ or ‘α’) was determined by MTL-specific PCR. Status of the HIS4 gene was determined by SNP-RFLP.

After identifying those with multiple LOH events I used SNP-RFLP analysis to determine the type of mechanism that caused these events. In doing this I was able to pinpoint which type of mechanism (e.g. gene crossover, BIR/single crossover, or whole chromosome loss) caused the LOH event.

My data supports the preliminary findings that in unstressed cells multiple mutations occur more frequently than single mutations (2.05*10-5 vs. 2.56*10-3), and this suggests a hyper-recombinant sub-population. Furthermore, the frequency of BIR or single crossover LOH events is the most common LOH mechanism among the multiple events. My results indicate that in *C. albicans* there is for more to understand about how they adapt to new environments within their host.

Faculty Mentor: Anja Forche
Funded by the Howard Hughes Medical Institute

References
