Analysis of loss of heterozygosity in *Candida albicans*

A. Forche, M. Weinzierl, D. Bruck, and J. Berman
University of Minnesota, Minneapolis, MN, USA

C. albicans is a highly heterozygous diploid organism that exhibits high levels of genetic and genomic variability among clinical isolates. We are interested in discovering and studying the mechanisms that lead to genetic and genomic variability, which may aid *C. albicans* to adapt to its many niches in the human host and in the transition from an opportunist to a pathogen. Although a parasexual cycle has been described, no meiotic process has been found and *C. albicans* is still thought of as a predominantly asexual organism that reproduces mainly by clonal division, thus preventing the use of classical genetics for studying genome plasticity. We measure the rates at which genetic variation arises (LOH fluctuation assays) and measure the types of events that accompany LOH (SNP arrays and SNP-RFLP analysis, together with CHEF karyotype gels). We determined basal rates of recombination in *C. albicans* by following loss of heterozygosity (LOH) in fluctuation assays. We used two counter-selectable genetic markers, *GAL1* and *URA3,* located on opposite alleles at the same locus on chromosome 1. Basal LOH rates were approximately 10^{-7} events/cell division for each marker, indicating that the assay is effective, that it is independent of the marker used and that both homologues of chromosome 1 undergo equal rates of LOH. To determine basal rates of LOH for the entire genome, we generated a library of strains with the *URA3* marker inserted into each chromosome arm. Fluctuation analyses yielded very similar LOH rates of ~10^{-7} events/cell division, suggesting that basal LOH rates are independent of chromosome size. The exception was on only one chromosome arm, where LOH rates were ~ 10^{-8} events/cell division at *LIP4* on Chr6R, likely due to the close proximity to the centromere (15kb away). We also compared LOH rates at other chromosomal positions such as telomeres and subtelomeric regions where LOH rates were three and two orders of magnitude higher, respectively, than LOH rates on chromosome arms. Using these LOH rates as the baseline for common laboratory growth conditions, we tested the hypothesis that stress causes increased genome instability. We measured LOH at seven chromosome arm positions on 4 different chromosomes during growth under stress conditions likely to be encountered in clinically relevant host environments: fluconazole (1ug/ml), hydrogen peroxide (0.4mM), high temperature (39 °C). LOH rates for fluconazole stress were elevated 2 orders of magnitude for all seven strains (compared to the no drug control), suggesting that fluconazole represents a genome-wide stress to the cells that causes increased whole-genome instability. Similarily, loss rates were 1 order of magnitude higher in all seven strains exposed to oxidative stress (hydrogen peroxide). Less dramatic effects were seen when high temperature (mimicking febrile conditions) was used as stressor. Two strains did not show an increase in loss rate while all other strains showed a 1 order of magnitude increase in LOH rate. This suggests that temperature stress affects *C. albicans* cells more locally than globally and it is not as detrimental as antifungal drugs. Taking advantage of the diploid assembly of the genome sequence, SNP microarrays were developed and used to study the extent and the mechanisms of LOH. Among the 21 strains that had undergone LOH on Chr1, 18 LOH events (86%) were caused by a single recombination event that then extended to the telomere, most likely due to a single cross-over coupled with co-segregation of similar alleles or by break-induced replication (BIR). Shorter gene conversion tracts and LOH of whole chromosomes were observed at low frequencies. We are currently analyzing the specific events that arose

when cells were exposed to stress, to see if the range of events is similar to, or different from, the range of events that occur during standard laboratory growth conditions.