HOMOLOGOUS RECOMBINATION AND GENOMIC INSTABILITY IN *Candida albicans*

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We have studied the role of Rad51 and Rad52 in the genomic stability of *C. albicans*. For this purpose, we have taken advantage of the heterozygosity of *HIS4* in the SC5314 lineage, where a SNP (G929T) causes the change $Gly^{310} \rightarrow$Val in the HIS4B domain with the concomitant inactivation of the protein (Gómez-Raja *et al*., 2008). We found that in *rad52*Δ/*rad52*Δ mutants the median frequency of His auxotrophs was ≥ 200 fold higher than in *RAD52/RAD52* strains. In a *rad52*Δ*/rad52*Δ background, His auxotrophy was caused by loss (52%) or truncation (48%) of the chromosome 4 homologue carrying the functional *HIS4* allele. Chromosome loss was followed by duplication of the remaining homologue whereas broken chromosomes were healed by *de novo* telomere addition. No translocations were detected. Some His^- strains showed an exceptional karyotype instability that affected several chromosomes. In fact, they underwent varying degrees of genome-wide homozygosis, including two strains that became completely homozygous for all the markers tested. Karyotype instability, when present, was abolished by reintroduction of *RAD52*. Point mutations were frequent within the *HIS4* ORF in both parental *rad52*His^+ and the His^- derivatives. His auxotrophs also showed a high frequency of mutation reversion independent of the restoration of Gly^{310} that could be only partially relieved by reintroduction of *RAD52*, suggesting the presence of secondary mutations. No His auxotrophs were found in ~20,000 colonies of a *rad59* null homozygous, an indication of a minor role for the *RAD52* homologue *RAD59* in the maintenance of genetic instability. In a *rad51*Δ*/rad51*Δ background, spontaneous LOH at the *HIS4* locus also occurred by chromosome loss (90%) and chromosome truncation (10%). Whereas *rad52* null homozygous strains were refractory to gene replacement using disruption cassettes carrying long flanking homology, *rad51–ΔΔ* strains could be effectively transformed with those cassettes. In addition, *rad51–ΔΔ* strains made heterozygous for a non essential gene of Chr2 *(SHE9/she9::hisG-URA3-hisG)* generated 5-FOA^R segregants at a rate similar to wt. About 67% of these segregants (79% for wild type) were characterized as *SHE/she9*::*hisG,* indicating that Rad51 is dispensable for single strand annealing (SSA). The rest (33%) carried exclusively the *SHE9* allele (11% for wild type); of these, about one half have lost one homologue and the other half carry both Chr2 homologues, one full and one truncated. Rad51 plays an important role in repair of DNA lesions caused by UV light (15 and 25 J/m^2), since the null homozygous mutant showed the same percentage of survivals (15%) than a *rad52–ΔΔ* counterpart (Ciudad *et al.,* 2004), suggesting that in *C. albicans* the UV lesions are processed through the Rad51-dependent HR pathway. In this assays, the *rad59* null homozygous strain behaved as the wild type, whereas the double mutant *rad59rad52* behaved as *rad52*. On the other hand, *rad51–ΔΔ* strains were significantly less sensitive to MMS than the *rad52* counterparts, all over the range of concentrations used (0.001 to 0.01%). This suggests that a large number of lesions caused by MMS are repaired using a Rad51-independent Rad52-dependent pathway. All these defects were reverted by the reintegration of *RAD51*. Again, in these assays, a *rad59* null strain behaved as the wild type, and the double mutant *rad59rad52* as *rad52*. These results reveal the existence of differences between the genome dynamics of *C. albicans* and *S. cerevisiae* that could be important for pathogenesis and disease development.