NUS National University of Singapore | Department of Biological Sciences Faculty of Science

SEMINAR

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Genomic Context Of Mitotic Copy Number Variation (CNV) In Yeast Diploids

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We optimized an assay for the genome-wide detection of CNVs in diploid S. cerevisiae cells. The method uses the SFA1 and CUP1 genes, which respectively confer gene dosage-dependent tolerance to formaldehyde (FA) and copper (Cu). Cells carrying a single chromosomal insertion of the SFA1-V208I-CUP1 gene-dosage reporter were plated on media containing FA and Cu, and individuals carrying a genome rearrangement resulting in two or more copies of the reporter were selected. We analyzed strains with insertions of the reporter at sites of interest on chromosomes (Chr) 4, 5, and 15. Differences in genomic context of the reporter resulted in a 15-fold range in CNV rates (0.16 to 2.45 x 10-6); and exposure to low doses of hydroxyurea (HU), camptothecin, methyl methanesulfonate, and ionizing radiation stimulated CNV rates by 3 to 17 fold. This assay can detect chromosomal rearrangements initiated by DNA lesions occurring anywhere in the genome, as long as the final outcome of repair involves amplification of the region containing the reporter. Most CNVs were formed by non-allelic homologous recombination (NAHR) between dispersed repeats, primarily Ty retrotransposon insertions, resulting in unbalanced translocations.

Unexpectedly, our analysis identified a robust bias for terminal deletions of the right arm of Chr7 associated with unbalanced translocations. This bias was observed at approximately constant strength across all three reporter sites, both spontaneously and after all four CNV-inducing conditions used. We conducted allelic recombination experiments to investigate the possibility of the presence of a fragile site on Chr7. The results disfavored this model, and indicated that the Chr7 bias applies to non-allelic rearrangements, but not to allelic interhomolog recombination. Finally, to independently validate the existence of a CNV bias at Chr7, we developed a genome rearrangement competition assay that does not rely on selection for FA+Cu resistance. In this case, a single homologous recombination donor substrate on Chr5 (URA-) can interact with any one of seven simultaneously available insertions of a recipient sequence (-RA3) dispersed across the diploid genome. NAHR between the donor and any one of the recipients generates a functional URA3 gene at the breakpoint of a chromosomal translocation. Independent clones containing such rearrangements were selected in uracil drop-out media. Using a custom multiplex PCR approach we determined that the -RA3 recipient inserted at the right arm of Chr7 was indeed the most frequent substrate used in the competition, and that HU-induced DNA replication stress quantitatively increased the overall NAHR rate, but did not change the Chr7 NAHR qualitative bias. Taken together, our results suggested that although Chr7R is not inherently more prone to DNA breakage than other regions, once a DNA lesion is formed there, it has a higher propensity to undergo inappropriate repair leading to a chromosomal rearrangement.

Artwork by Ann Nee, DBS, NUS