Neurospora exhibits the highest known non-viral mutation rate

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In the fungus *Neurospora crassa*, the *rid* (*RIP-defective*) and *dim-2* (*defective in methylation-2*) genes are required for the sexual stage-specific mutational process called RIP (repeat-induced point mutation). RIP occurs in the pre-meiotic dikaryotic cells called asci (singular, ascus), and induces multiple C to T transition mutations in any sizeable (>400 bp) DNA segment duplicated in the otherwise haploid nucleus[1]. An ascus forms following fertilization between hyphae of opposite mating types, and after karyogamy the resulting diploid nucleus divides by a meiosis and a mitosis to produce eight haploid nuclei that are partitioned into the eight progeny ascospores that develop per ascus. Conventionally, RIP studies have used either gene-sized duplications (<10 kb) created by transformation, or larger duplications (>100 kb) obtained by segregation in crosses heterozygous for certain translocation chromosomes. Now, Wang *et al*.[2] have shown that RIP can also occur in crosses lacking any conventional duplication. They compared RIP-proficient and RIP-deficient crosses (i.e. homozygous *rid*; *dim-2*) by examining the genome sequences of all four meiotic products and their parental strains. In RIP-proficient crosses, C to T mutations occurred at a frequency of 3.38×10^{-6} per bp per generation (i.e. about 137 mutations per genome) whereas in RIP-deficient crosses the rate was more than 50-fold lower ($\sim 4.7 \times 10^{-8}$ per bp per generation). A comparison with mutation rates in several other systems revealed the RIP-proficient crosses exhibited '... the highest known mutational rate... of any non-viral life'. Mutation rates per bp per generation in some other species were: *A. thaliana* 6.95×10^{-9}; *C. elegans* 1.45×10^{-9}; *D. melanogaster* 5.17×10^{-9}; *H. sapiens* 1.35×10^{-8}; *O. sativa* 7.1×10^{-9} and *S. cerevisiae* 2.63×10^{-10}. In *E. coli* it was 2.00×10^{-10} per division. Drake *et al*.[3] reported DNA viruses have mutation rates between 10^{-6} and 10^{-8} per bp per generation, and RNA viruses, 10^{-3} and 10^{-5} per base per generation.

Although the crosses had no conventional duplications, about 16% of the haploid *N. crassa* genome could be defined as 'duplicated' by the criterion that the sequences shared >65% identity over >100 bp alignable length. Notably, such duplicated segments accumulated 87% of the RIP mutations and another 3% were within 400 bp upstream or downstream of them. The C to T mutations showed additional hallmarks of RIP, such as 2 : 2 segregation and clustering within 1 kb of each other (in fact, >60% were within 100 bp of the nearest neighbour). In addition, they found that the clustering was of either C to T or G to A changes on the same DNA strand, but not both, supporting the notion that RIP acts on one strand at a time. RIP does not occur outside the sexual stage, and the mutation rate in vegetative mycelia over a comparable period as the sexual stage (20 days) was only about 6.03×10^{-10} per bp per mitosis, assuming 15 mitoses per day.

Two other significant findings were reported. First, the vast majority of RIP mutations tended to accumulate in non-coding sequences. Properties such as greater duplicate length, lower G : C content, and more 3D interactions imparted greater RIP-susceptibility to the duplicated DNA, and tended to exclude RIP from coding sequences, because coding sequences have shorter duplicate lengths, are G : C rich, and exhibit fewer 3D interactions. Evidently, RIP has evolved to reduce the resulting mutation burden. Despite this, coding sequence suffered 40-fold more mutations in RIP-proficient crosses than in RIP-deficient ones (respectively, 3.62 versus 0.085 mutations per genome). Second, ~60% of coding sequence mutations were non-C-to-T type. Thus, RIP-proficient crosses show an increase in non-classical RIP mutations in non-duplicated sequences.

These findings spark several questions: What would happen if one parent carried a conventional duplication? We previously showed that presence of a large duplication (>250 kb) in a cross enabled a small duplication (<5 kb) to escape RIP, possibly via titration of the RIP machinery[4]. Would the presence of a ~2 kb duplication act as a 'lightning rod' to draw most of the RIP mutations to itself, and thus spare the rest of the genome, or might it trigger an enhanced RIP response? Would non-coding sequences within a large Dp suffer more RIP mutations than the duplications defined in this paper? Are translesion DNA polymerases involved? We have studied RIP in backgrounds deficient for translesion polymerases[5], but our RIP assay, based on a gene's mutant phenotype, was not as sensitive as genome sequencing, given that we now know RIP tends to avoid coding sequences. These questions deserve to be investigated in future studies.

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