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. 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 ascosporas 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. 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^{-2} per bp per generation (i.e. about 137 mutations per genome) whereas in RIP-deficient crosses the rate was more than 50-fold lower (4.7 × 10^{-4} 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. reported DNA viruses have mutation rates between 10^{-6} and 10^{-8} per bp per generation, and RNA viruses, 10^{-3} and 10^{-7} 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. 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. 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, 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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