

An alternative explanation for the reduced repeat-induced point mutation (RIP) efficiency in a gene-sized duplication in *Neurospora crassa* crosses heterozygous for a larger chromosome segment duplication

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Recently, it was reported that the efficiency of repeat-induced point mutation (RIP) in a gene-sized duplication was reduced in *Neurospora* crosses heterozygous for another larger segmental duplication (Bhat and Kasbekar 2001 *Genetics* **157**: 1581-1590). Specifically, the authors showed that the frequency of RIP in the *erg-3* gene due to a 1.3 kb duplication was reduced, if the chromosome segment duplications *Dp(IIIR > [IR; IIR]) AR17*, *Dp(VIR > IIIR) OY329*, or *Dp(IVR > VII) S1229* were present in the same or the other parental nucleus of the premeiotic dikaryon. They have suggested that the larger segmental duplication acts as a sink and titrates the RIP machinery away from the smaller gene-sized duplication. However, there is an alternative and somewhat trivial explanation for their results.

Crosses heterozygous for large segmental duplications are characteristically barren, i.e., the perithecia produce very few viable ascospores (Raju and Perkins 1978 *Can. J. Genet. Cytol.* **20**:41-59). In contrast, crosses involving strains containing balanced rearrangements (i.e., without duplications) are generally fertile (Perkins and Barry 1977 *Adv. Genet.* **19**:133-285). Hence the presence of duplications is associated with the 'barren' phenotype. RIP, a process that detects and mutates duplications during the premeiotic phase of the sexual cycle (Selker 1990, *Annu. Rev. Genet.* **24**:597-613) offers a reasonable explanation for this phenomenon. Segmental duplications typically cover 10-50% of a chromosome and will include several essential genes. Selker proposed that the impaired fertility of such crosses might be due to inactivation of nucleus-limited or dosage-sensitive genes required for progression through the sexual cycle, by RIP (Selker 1990 *Annu. Rev. Genet.* **24**: 597-613). Alternatively, the defective products produced by genes mutated by RIP could be deleterious and inhibit perithecial development. Finally, alterations in the chromatin structure and / or methylation of large stretches of the genome could affect replication. This idea, which suggests RIP as the cause for barrenness in crosses involving segmental aneuploids is referred to as the 'RIP hypothesis'. Consistent with this hypothesis, cytological studies indicated that sexual development in these crosses is arrested at about the same stage, when RIP is known to occur or shortly thereafter (Raju and Perkins 1978 *Can. J. Genet. Cytol.* **20**: 41-59). The few ascospores generated in the barren crosses may be the ones, in which RIP either had not occurred or was very inefficient. In agreement with this possibility, a low frequency of mutants for loci covered by the duplication was observed, amongst the survivors (Perkins *et al.* 1997 *Genetics* **147**:125-136). In addition, molecular analysis of a few mutant alleles generated by RIP in a segmental duplication indicated a very low GC-to-AT conversion rate and lack of RIP-associated cytosine methylation, which are hallmarks of mild RIP. If such a strong selection for low RIP exists in the dikaryon containing a large segmental duplication, then it is not surprising to observe a decrease in the RIP efficiency in a gene-sized duplication present in the same dikaryon. The late ascospores have higher RIP efficiencies than the early ascospores (Singer *et al.* 1995 *FGN* **42**:74-75). If RIP is responsible for barrenness, then the early ascospores will have a clear advantage over the late ascospores for survival and this could represent a possible way to escape from the RIP machinery.

The titration model proposed (Bhat and Kasbekar 2001 *Genetics* **157**: 1581-1590) assumes that a larger duplication has a greater affinity/demand for the RIP machinery than a smaller duplication, which need not be true. Larger the size of the duplication, greater would be the ease of pairing. However, it is conceivable that following the pairing of duplications, a single molecule of the proposed processive enzyme (Selker 1990 *Annu. Rev. Genet.* **24**: 597-613) could mutagenize large stretches of duplicated DNA. A theoretical possibility of testing the 'titration hypothesis' would be to construct a strain having a large duplication, which does not cover any essential gene and test its effect on the RIP efficiency of a gene-sized duplication.

Although several lines of evidence support the 'RIP hypothesis', none of them are clinching. A rigorous test of the hypothesis has not been possible due to lack of any experimental tool to inhibit RIP. A mutant deficient in RIP has not yet been isolated. The hypothesis predicts that the suppression of RIP in crosses involving segmental aneuploids should lead to an increase in fertility.

Recently, RIP has been demonstrated to occur in another fungus, *Podospora anserina* albeit at a very low RIP efficiency (Hamann *et al.* 2000 *Mol. Gen. Genet.* **263**:1061-1069). This drawback could be 'transformed' into an advantage, if one identifies genes of *Neurospora*, which enhance the RIP efficiency in *Podospora*. Alternatively, one could isolate mutants that have increased RIP efficiency.

The *Neurospora* Genome project has provided the option of a 'candidate gene approach'. One of the models proposed for RIP involves a cytosine-methyl transferase (Selker 1990 *Annu. Rev. Genet.* **24**: 597-613). A BLAST search for this candidate gene in the *Neurospora* genome database (<http://www-genome.wi.mit.edu/annotation/fungi/neurospora/>) revealed the presence of two putative cytosine-methyl transferases (on contigs 1.498 and 1.827). Investigating their possible involvement in RIP could be worthwhile.