RIP tidings: Accelerating divergence of duplicated DNA sequences?

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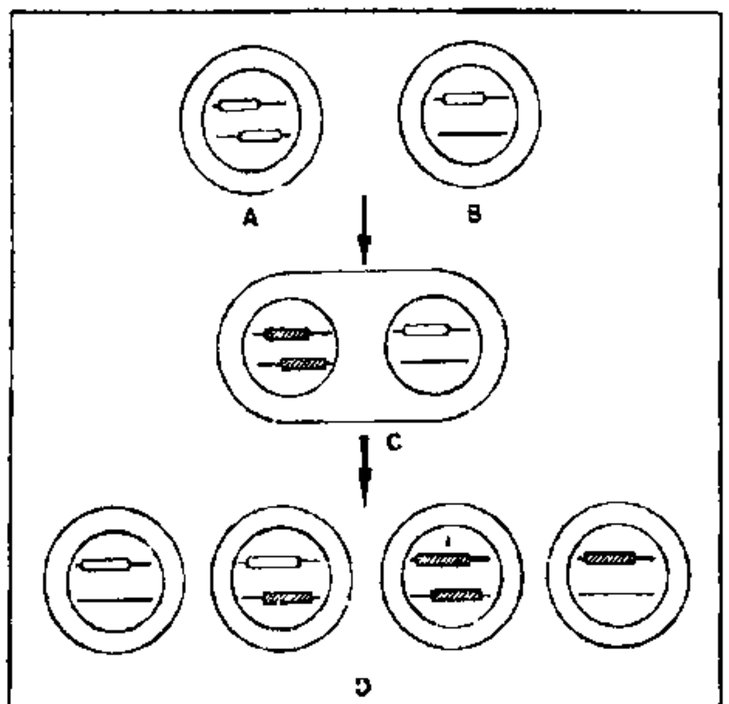
IN 1986 Mary Case reported[1] some baffling results with the filamentous fungus *Neurospora crassa*. She had transformed a *qa-2*$^-$ strain (lacking the enzyme catabolic dehydroquinase) with the *qa-2*$^+$ allele, which went on to integrate either at the *qa-2* locus or, as in the majority of cases, at unlinked (ectopic) chromosomal locations. All the transformants maintained their transformed phenotype (qa-2$^+$) through mitosis during vegetative growth. Unexpectedly, they appeared to lose it through meiosis. This happened even in self-crosses of the transformant strains. Classically, a self-cross is expected to produce only 4 0 (in this case, 4 *qa-2*$^+$: 0 *qa-2*$^-$) tetrads, but, incredibly, often in these crosses 0:4 (*qa-2*$^+$: *qa-2*$^-$) tetrads were obtained. In other words, the *qa-2*$^+$ allele had disappeared. Some crosses even displayed extreme perversity and yielded exclusively 0:4 tetrads. These results did not make sense in 1986. Moreover, the Case study was not unprecedented, similar results demonstrating unusual behaviour of transforming DNA in *Neurospora* had been reported at least thirteen years earlier[2]. Understanding came with a novel explanation: in the sexual phase preceding meiosis, duplicated DNA sequences (such as the *qa-2*$^+$ and *qa-2*$^-$ alleles) are subjected to a previously unknown genetic process, termed RIP (repeat-induced point mutation), which results in multiple G–C to A–T transition mutations[3] and consequently dismembers both the *qa-2* alleles.

In the sexual phase in *Neurospora*, fertilization produces a dikaryon, i e a cell with two nuclei The two haploid nuclei do not fuse until after about ten mitotic divisions of the dikaryotic cell RIP occurs in the duplicated DNA sequences in the haploid nuclei of the dikaryons. Duplications are identified, possibly by homologous pairing, and



RIP in an unlinked duplication A is a haploid nucleus containing a DNA sequence (open box) duplicated on another chromosome, B is a nucleus with that sequence present only at its normal location Fertilization (top arrow) produces a dikaryon C, with two haploid nuclei RIP occurs in the nucleus bearing the duplication and produces sequence divergence by converting G–C base pairs into A–T base pairs (hatched boxes) Fusion of the two haploid nuclei then transforms the dikaryotic cell into a diploid that immediately enters meiosis (lower arrow). D shows the four haploid nuclei that issue from meiosis A round of mitosis follows meiosis and the eight daughter nuclei get encapsuled in the eight spores of the ascus

cytosine residues in both copies of the duplication are methylated. The methylcytosine residues might then be targeted for deamination into thymine residues. This is one way in which C–G base pairs can be converted into T–G mismatches that can subsequently be repaired into T–A mutations. Alternatively, it is possible that it is the unmethylated C residues that get enzymatically deaminated to U, resulting in U–G intermediates that can be converted into T–A. In other words, the details relating cytosine methylation to the transition mutations are not yet established. RIP is highly efficient; approximately 10% of the G–C base pairs in a pair of unlinked duplicated DNA sequences were converted into A–T. RIP is also highly specific; single-copy sequences remain unscathed[4].

What happens when an essential gene is duplicated? Since both copies of the duplication get riddled with point mutations, the haploid nucleus bearing the duplication is bereft of a functional copy of the gene; the heterokaryon survives nevertheless, thanks to the presence of an intact single copy of the gene in the other nucleus. Karyogamy changes each dikaryotic cell into a diploid that immediately enters meiosis. Meiotic processes such as recombination and segregation then ensure that not all the information in the affected nucleus is lost to future generations. Additionally, gene conversion of one, or both, mutated copies could also potentially undo the effects of RIP. Can RIP be a last-ditch attempt to evolve new genes before meiotic scrambling?

A round of mitosis follows meiosis, and the eight haploid daughter nuclei end up in eight spores of the ascus. Thus, a single fertilization gives rise to many dikaryotic cells, each of which produces an eight-spored ascus; the collection of asci derived from a single fertilization is called the perithecium. Studies on the fate of the duplication in related asci suggest that RIP can occur at any of the mitotic divisions preceding karyogamy, and, judging from the patterns of mutations in related asci, that the same sequence can engage in multiple rounds of RIP[5]. A measure of RIP efficiency can be gleaned from the fact that, in a tandem duplication, the repeated sequences can acquire enough differences through two sexual cycles to have diverged sufficiently for them to be immune to further RIP in subsequent cycles[3].

A strikingly similar process of premeiotic inactivation has been observed[6] in another filamentous ascomycete, *Ascobolus immersus*. In this case, however, gene inactivation is spontaneously reversible after a number of mitotic divisions, and the rate at which such reversion occurs is increased upon growth in presence of 5-azacytidine, an agent that interferes with cytosine methylation. Although these studies do not rule out the possibility of mutations, it is conceivable that premeiotic inactivation in *Ascobolus* might only involve cytosine methylation.

Could it be that methylation and deamination are two distinct facets of RIP, with different relative frequencies in the two systems? Both would inactivate the gene but only in the former case would the inactivation be reversible. In a set of elegant experiments[6], the *Ascobolus* system has been used to show that pairing is required to identify duplications. Strains carrying two or three copies of a test gene were passed through the sexual cycle and the number of inactivated copies was determined. The results showed that two or three copies could be inactivated but it was never possible to inactivate just one copy. This finding indicated that the methylation machinery recognizes paired sequences and that, when three copies are present, one of the two copies which had been methylated in one round of inactivation can nonetheless go on to pair with the third copy in a subsequent round and thereby target it for inactivation. Similar results had been obtained and the same conclusions reached in earlier studies[4,7] in *Neurospora*.

There are, obviously some duplicated genes normally resident in the *Neurospora* and *Ascobolus* genomes, for instance the rRNA genes and transposons. How do they escape inactivation and RIP? Also, is there minimum length and sequence similarity required for RIP? Now that we know how RIP may occur, it becomes important to find out how it may not.

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