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Series

A cross-eyed geneticist's view
IV. Neurospora genes and inversions collude to cheat Mendel

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1. Introduction to *Spore killer* elements

The cell killing gene, *rfk-1* (*required for killing-1*) was found in Neurospora, skulking in the wings, sidled up against the right border of the *Spore killer-2* (*Sk-2*) element on chromosome 3R. Close by, but across the border, where chromosomes 3R of the *Sk-2* and Sk^S (Sk^S = *sensitive to Spore killing*) strains resume being collinear, lay the gene ncu06238. When *Spore killer* strains are crossed with the wild type (WT = Sk^S), four of the eight ascospores that form per ascus are killed and four survive. The survivors contain the *Spore killer* chromosome, and presumably the dead contain Sk^S. In the *Sk-2* × Sk^S cross, the *rfk-1* gene, borne on the *Sk-2* chromosome, does not have homologous sequences on the Sk^S chromosome to pair with in meiosis. Nevertheless, its proximity to the 'paired' ncu06238 alleles enables it to escape silencing by the RNAi-based MSUD (meiotic silencing by unpaired DNA) genome defense process. MSUD silences any gene not properly paired with its homologue during meiosis (Hammond 2017). When the *rfk-1*–ncu06238 distance was increased by inserting a 1.4 kbp transgene (*::hph*) immediately left of ncu06238, the killer gene became exposed to MSUD, and was rendered incapable of ascospore killing in the *Sk-2::hph* × Sk^S cross. On the contrary, if the transgenic sequence is made homozygous, as in the *Sk-2::hph* × Sk^S*::hph* cross, the *rfk-1* gene's proximity to paired sequences was restored, and it again could kill. Killing was also seen in the *Sk-2::hph* x Sk^S *Sad-2* cross, in which the semi-dominant *Sad-2* mutation suppresses MSUD. The apparently long-lived Rfk-1 protein kills cells, unless its accomplice, the Rsk^{Sk-2} protein, encoded by the rsk^{Sk-2} allele (*resistant to Spore killer – Sk-2*) also is present. The rsk^{Sk-2} allele is located near the left flank of the *Sk-2* element on the chromosome's left arm, playing the 'good cop' to *rfk-1*'s 'bad cop'. However don't be fooled by the more than 2.3 Mb separating rsk^{Sk-2} from *rfk-1*; they are genetically hand-in-glove, linked tightly by a 'dense set of inversions' (Svedberg *et al.* 2018), and inseparable by crossing over. The *rsk* allele on the Sk^S chromosome 3 does not protect against Rfk-1-induced killing; and is just another bystander like all other genes on this chromosome. *Spore killers* were discovered 40 years ago by Turner and Perkins (1979). More recently, the teams of Tom Hammond (Illinois State University), Patrick Shiu (University of Missouri) and Hanna Johannesson (Uppsala University) came together to solve this classical whodunit and uncovered the collusion between *rfk-1*, rsk^{Sk-2} and the inversions to subvert Mendel's Law of Segregation (Hammond *et al.* 2012; Harvey *et al.* 2014; Svedberg *et al.* 2018; Rhoades *et al.* 2019). This article summarizes their landmark achievement.

2. Copping the 'good' cop, Rsk^{Sk-2}

Hammond *et al.* (2012) did well to pay heed to the hint hidden like a Treasure Hunt clue in the 'Materials and Methods' and table 1 footnotes of Turner and Perkins (1979). In one place it said, 'FGSC 2222 is an *Sk-2*^{R} *Sk-3*^{S} *N. crassa* strain collected in Louisiana' and in the other, 'the *Sk-2*^{R} allele (…) was found in a wild-collected *N. crassa* strain from Louisiana (FGSC 2222)'. Turner and Perkins (1979) and Campbell and Turner (1987) showed that the Louisiana (LA) strain did not have the killer haplotype, and that its *Sk-2*^{R} allele, namely, rsk^{LA} could be mapped conventionally. Hammond *et al.* (2012) used the Neurospora gene knock-out library for fine-scale mapping. The mapping revealed that rsk^{LA} was the gene $ncu09151^{LA}$. Replacement of the standard Oak Ridge strain's $ncu09151^{OR}$

allele by *ncu09151*^{LA} conferred *Sk-2*-resistance to the OR chromosome 3. On the other hand, deletion of the *ncu09151*^{LA} allele from the LA strain resulted in ascus abortion in the LA *ncu09151*$^{\Delta LA}$ × *Sk-2* cross. The deletion caused unpairing and MSUD silencing of the *ncu09151*$^{Sk-2}$ allele, and the absence of Rsk^{Sk-2} protein and presence of Rfk-1 killer protein during early ascus development resulted in ascus abortion. In contrast, the Rsk^{Sk-2} protein is present throughout early ascus development in the *Sk-2* × *Sk*^{S} cross, and by neutralizing Rfk-1 it allows ascospores to develop. Following ascospore delimitation, Rsk^{Sk-2} protects only *Sk-2* ascospores, presumably because it is spore-autonomous, whereas the long half-life or non-spore-autonomous expression of Rfk-1 prevents *Sk*^{S} ascospores from maturing. The results suggested that *ncu09151*$^{Sk-2}$ is the *rsk*$^{Sk-2}$ gene that enables *Sk-2* to avoid being killed by its own killer, and that MSUD places constraints on its location within the *Sk-2* element. In other words, MSUD forces *rsk*$^{Sk-2}$ to be a 'good cop' and to march in step with *ncu09151*^{SkS}. Replacement of *ncu09151*^{OR} in OR by *ncu09151*$^{Sk-2}$ or *ncu09151*$^{Sk-3}$ prevented killing, respectively, in crosses with *Sk-2* or *Sk-3* strains, showing that the *rsk* allele determines whether a strain is resistant to *Sk-2* or *Sk-3* or sensitive to killing. Neither Rsk, nor its paralogous protein encoded by the nearby *ncu09148* gene, were essential for ascospore development.

3. … and the killer, Rfk-1

Ascus abortion in the *Sk-2 rsk*$^{\Delta}$ × *Sk*^{S} cross provided a strategy to select for *rfk-1* mutants. *Sk-2 rsk*$^{\Delta}$ conidia (asexual spores that also function as the male gamete) were mutagenized by UV-irradiation and used to fertilize a WT (*Sk*^{S}) mating partner. If any fertilizing conidia were mutated in *rfk-1*, ascus abortion would be abolished, and viable ascospores would be produced. Of 53 progeny examined, six turned out to be *rfk-1* mutants, and the *Sk-2 rsk*$^{\Delta}$ *rfk-1* × WT crosses did not show ascus abortion (Harvey *et al.* 2014). The remaining 47 were the rare (~0.1%) escapes from Rfk-1-induced killing in the *Sk-2 rsk*$^{\Delta}$ × *Sk*^{S} cross (which goes to show that chance favors the intrepid). The escapes had the parental *Sk-2 rsk*$^{\Delta}$ genotype and produced empty asci in crosses with WT. Next, cross-over *Sk-2 rfk-1* progeny from an *Sk-2 rsk*$^{\Delta}$ *rfk-1* × *Sk-2 sad-2*$^{\Delta}$ cross were mated with seven different *Sk-2 leu-1 hph* strains, bearing different *hph*-based insertions, including within the *Sk-2* element, and thus represented seven different three-point mapping crosses (i.e., *Sk-2 rfk-1* × *Sk-2 leu-1 hph*). These crosses, unlike *Sk-2 rfk-1* × *leu-1 hph*, are homozygous for the *Sk-2* inversions and hence can produce cross-over progeny. Mapping the cross-overs on an *Sk-2* genome assembly enabled the localization of *rfk-1* to a 45 kb interval that spanned the *Sk-2* right border (Harvey *et al.* 2014). Following this, different sub-intervals were replaced by an *hph* selectable marker, and the resulting deletion strains were crossed with *Sk*^{S}. Ascospore killing was eliminated when sequences were deleted from between the pseudogene ncu07838* (located left of the *Sk-2* right border) and the ncu06238 gene (located just right of the *Sk-2* right border), indicating that the intergenic region contained *rfk-1* (Rhoades *et al.* 2019). Finally, transformation experiments showed that a 1481 bp segment from within this region could confer the defining property of *rfk-1*$^{Sk-2}$, namely, the production of empty asci in MSUD-deficient heterozygous crosses lacking the *rsk*$^{Sk-2}$ allele. This property was not shared by the corresponding segment from the *rfk-1* mutants.

Additional experiments confirmed that the *rfk-1* gene does not overlap with *ncu06238*; is transcribed in *Sk-2* × *Sk*^{S} and *Sk-2* × *Sk-2*; contains four exons and three introns; is subject to sexual-stage-specific A-to-I mRNA editing that eliminates a stop codon upstream of the second intron; the edited mRNA encodes a 130 amino acid residue protein; and that *rfk-1* apparently evolved from a partial duplication of the gene ncu07086.

4. New questions

We still do not know how the Rfk-1 protein kills asci and ascospores, or how the Rsk^{Sk-2} protein prevents it from doing so. The toxin–antitoxin model is a good bet, in which Rfk-1 is the killer toxin, and Rsk^{Sk-2} is the antitoxin that neutralizes it. However, the *rsk* allele in several wild strains resistant to *Sk-3* but not *Sk-2* was more similar to those in *Sk-2*-resistant strains, and no polymorphisms unique for each resistance phenotype were found (Svedberg *et al.* 2018). This suggests that the specificity of *rsk*-based resistance to each *Spore killer* is also influenced by other genes. Svedberg *et al.* (2018) generated near-complete genome assemblies of the non-recombining *Sk-2* and *Sk-3* regions using PacBio long-read sequencing and found both contain a dense set of recombination-suppressing inversions in largely the same chromosomal region. However, all the inversions were unique and non-overlapping and apparently had ancient or separate origins. The ancestral gene order was not retained in any region and the inversions were interspersed with gene-poor and degenerate-transposable-element-rich regions. It was possible that the recombination block initially might not have been due to any inversions, that is, it was non-structural, of the kind seen in the *N. tetrasperma* mating type chromosomes, and that the inversions only subsequently evolved.

Another conundrum was that the *Sk-3* genome did not contain any *rfk-1*-like sequence, suggesting that different genes are responsible for killing in *Sk-3* and *Sk-2*. Of course, one can now pull-down the Rsk^{Sk-2} and Rfk-1 protein complexes and identify their interacting partners, and also determine their spatial and temporal occurrence.

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References

Campbell JL and Turner BC 1987 Recombination block in the Spore killer region of Neurospora. *Genome* **29** 129–135

Hammond TM 2017 Sixteen years of meiotic silencing by unpaired DNA. *Adv. Genet.* **97** 1–42

Hammond TM, Rehard DG, Xiao H and Shiu PKT 2012 Molecular dissection of *Neurospora* spore killer meiotic drive elements. *Proc. Natl. Acad. Sci. USA* **109** 12093–12098

Harvey AM, Rehard DG, Groskreutz KM, Kuntz DR, Sharp KJ, Shiu PKT and Hammond TM 2014 A critical component of meiotic drive in *Neurospora* is located near a chromosome rearrangement. *Genetics* **197** 1165–1174

Rhoades NA, Harvey AM, Samarajeewa DA, Svedberg J, Yusifov A, Abusharekh A, Manitchotpisit P, Brown DW, Sharp KJ, Rehard DG, Peters J, Ostolaza-Maldonado X, Stephenson J, Shiu PKT, Johannesson H and Hammond TM 2019 Identification of *rfk-1*, a meiotic driver undergoing RNA editing in Neurospora. *Genetics* **212** 93–110

Svedberg J, Hosseini S, Chen J, Vogan AA, Mozgova I, Hennig L, Manitchotpisit P, Abusharekh A, Hammond TM, Lascoux M and Johannesson H 2018 Convergent evolution of complex genomic rearrangements in two fungal meiotic drive elements. *Nat. Commun.* **9** 4242

Turner BC and Perkins DD 1979 Spore killer, a chromosomal factor in Neurospora that kills meiotic products not containing it. *Genetics* **93** 587–606