ECRONICON OPEN ACCESS

EC MICROBIOLOGY
Short Communication

Meiotic Silencing, Meiotic Drive and Chromosomal Rearrangements: An Interesting Triangle

Amrendra Kumar Ajay[1,3] and Parmit Kumar Singh[2,3]*

[1]*Brigham and Women's Hospital, Boston, Massachusetts, USA*
[2]*Dana Farber Cancer Institute, Boston, Massachusetts, USA*
[3]*Harvard Medical School, Boston, Massachusetts, USA*

***Corresponding Author**: Parmit Kumar Singh, Dana Farber Cancer Institute and Harvard Medical School, Boston, Massachusetts, USA.

Received: October 11, 2017; **Published:** October 31, 2017

Neurospora crassa is a haploid filamentous fungus that has two distinct genome defense processes during the sexual stage. These genome defense processes protect the genome of Neurospora from detrimental elements like transposons. Repeat induced point mutation (RIP) is a defense process that mutates any duplicated DNA > 400 bp by converting G to A and C to T [1]. RIP occurs during the dikaryon stage, where two parental nuclei divide independently in the same cytoplasm. Meiotic silencing by unpaired DNA (MSUD or meiotic silencing) is another genome defense process that occurs during meiosis [2,3]. MSUD is an RNAi based mechanism, causing silencing of unpaired genes and their paired homologues during meiosis [2,3]. Several genes (*sad-1*, *sad-2*, *sad-3*, *sad-4*, *sad-5*, *sad-6*, *sad-7*, *qip*, *dcl-2*, *sms-2* are important for meiotic silencing and deletions of these genes suppress meiotic silencing [2-8]. These deletions disrupt the normal pairing of wild type alleles (e.g., *sad-1*$^{+}$ and *sad-2*$^{+}$) during meiosis. Such un-pairing induces self-silencing of these suppressors by meiotic silencing itself, which results in suppression of meiotic silencing. This phenomenon suggests two different levels of meiotic silencing: one that suppresses the un-pairing of any gene, and another that suppresses un-pairing of genes involved in meiotic silencing. Recently, more genes (*sad-3, sad-4, sad-5, sad-6,* and *sad-7*) have been discovered, which are involved in meiotic silencing [7-10]. Homozygous crosses involving strong suppressors (e.g. *sad-2*) are barren in phenotype i.e., they make normal perithecia but produce no ascospores, whereas homozygous crosses involving weak suppressors of meiotic silencing (*sad-4* and *sad-5*) are fully fertile [10]. A suppressor except *sad-5* (i.e., *sad-2*, *sad-3* and *sad-4; sad-7* suppresses partially) suppresses the phenotype associated with the Round spore (*R*) locus in a cross that is heterozygous for the R locus [10]. Crosses that are heterozygous for the *R* locus produce round shaped ascospores instead of normal, spindle-shaped ascospores. It is assumed that the *R* locus has a deletion at the right arm of chromosome I (IR), thus the homologous region remains unpaired during meiosis. This un-pairing at the IR region induces meiotic silencing, which results round ascospores in a heterozygous cross.

Spore killers (*Sk*), examples of meiotic drive elements in Neurospora, also suppress meiotic silencing [11]. However, *Sk* does not suppress the phenotype that is associated with the *R* phenotype. Further, the nature of suppressor of meiotic silencing in *Sk* (*Mss*) is not known. Moreover, homozygous crosses for *Sk* are also homozygous for the suppressor *Mss.* Crosses that are homozygous for the *Mss* are fertile, suggesting that *Mss* might also be a weak suppressor, like *sad-4* and *sad-5*. The first wild isolated dominant suppressor of meiotic silencing in the Carrefour Mme. Gras (CMG) strain [12] is also a weak suppressor as the homozygous cross for the CMG is fertile. Further, the CMG is not a suppressor of the *R* phenotype. Because *sad-5* is fertile in a homozygous cross and does not suppress the *R* phenotype like the CMG and *Sk*, it suggests that suppressors of MSUD in *Sk* and in the CMG strain might be a protein that localizes in the nucleus, like *sad-5*. These three suppressors may play a role in recognizing of un-pairing or production of aberrant mRNA, which are then recognized by other suppressors at the perinuclear region [13] during mRNA export. Although *sad-4* suppresses the *R* phenotype, it produces viable ascospores in a homozygous cross like *Sk* and the CMG. Interestingly, *sad-4* localizes at the perinuclear region [10], suggesting that there is also a possibility that suppressors in *Sk* and the CMG strain might localize in the perinuclear region. However, insensitivity

of the *R* phenotype suggests that these suppressors in *Sk* and the CMG cannot localize in the same complex that contains *sad-2* and other perinuclear components of meiotic silencing.

It has been a dilemma since the discovery of meiotic silencing that what suppresses the suppressors of meiotic silencing [14]. Recent data on the localization of *sad-5* into the nucleus [10] suggest that there might be two types or parts of meiotic silencing: nuclear and cytoplasmic or perinuclear. Both are distinct in their functions. Components of nuclear meiotic silencing might sense un-pairing of small genes and genes involved in meiotic silencing at DNA level. However, components of cytoplasmic or perinuclear meiotic silencing might be involved in sensing the aberrant RNA produced by unpaired DNA. Components of nuclear meiotic silencing might be involved in self-silencing of dominant suppressors or silencing of the components of perinuclear meiotic silencing, if they are unpaired during meiosis. In this case, the components of nuclear meiotic silencing might act as the suppressor of suppressors. Weak suppressors that are localized inside the nucleus should be epistatic to the strong suppressors from the perinuclear complex i.e., weak suppressors in the CMG might suppress the self silencing of *sad-2* due to its un-pairing. Thus, in the background of the CMG, un-pairing of s*ad-2* might not be able to suppress the *R* phenotype. So, suppressor in the CMG strain might be epistatic to *sad-2*. Similar crosses in the *Sk* (or *sad-4* or *sad-5* or *sad-7*) background might reveal any epistatic interaction between weak (*Sk* or *sad-4* or *sad-5)* and dominant suppressors of meiotic silencing (*sad-2)*. If the hypothesis is true, *sad-5* should be epistatic to dominant suppressors (*sad-2)*. However, *sad-4* and *sad-7* might not show any such epistatic interactions.

In a heterozygous cross, *Sk* produces four viable black and four white spores, whereas homozygous cross for *Sk* produces all black viable ascospores. Crosses homozygous for the sensitive parent also produce eight black and viable ascospores. Moreover, heterozygous crosses involving *Sk* and a strain that is resistant for spore killing produces eight black ascospores. However, the resistant strain produces all eight black ascospores in a heterozygous cross with a sensitive strain, suggesting that killer and resistant genes are not allelic. It is assumed that *Sk* has several inversions that cause un-pairing within the region that contains *Sk*. Therefore, expression of genes within the inversions requires suppression of meiotic silencing.

Recently, the allele, *rsk,* which causes resistant to spore killing was identified [15]. It was observed that *rsk* is essential for the progression of meiosis in the presence of *Sk*. Thus, a heterozygous cross between *Sk* and the resistant strain that lacks *rsk* produces aborted asci with no ascospores instead of 4 white and 4 black ascospores. This is due to meiotic silencing induced by un-pairing at the *rsk* locus. If such cross is also heterozygous for *sad-2*, it produces viable spores, suggesting that the normal pairing of *rsk* is required during meiosis in a cross that is heterozygous for *Sk* also. In other words, the killer kills itself as there is no resistant proteins due to meiotic silencing due to un-pairing at the *rsk* locus.

Since un-pairing of *rsk* is resistant to *Mss* like *R*, this opens a new dimension for understanding the interaction between all known suppressors of meiotic silencing with respect to *Sk*. It suggests that un-pairing due to chromosomal rearrangements behaves differently than un-pairing by deletion of allele. Since meiotic silencing is an RNAi based silencing, the possibility that the whole recombination block consisting *Sk* protects genes residing inside the block from the action of *Mss* is low. In such case, it will be interesting to perform the cross (*Sk* x *rsk; sad-2)*, which was done by Hammond., *et al.* [15], in the CMG or *sad-5* background (*Sk* x *rsk; sad-5* and *Sk* x *rsk;* CMG*)* to understand whether un-pairing of *rsk* is resistant to the CMG or *sad-5*. In other words, whether such cross produces ascospores in the presence of weak suppressor of meiotic silencing like *sad-5* and CMG. In an alternative hypothesis, it is also possible that un-pairing at the *rsk* locus due to deletion of resistant allele causes pairing at the suppressor locus, *Mss* in a heterozygous cross due to the presence of several inversions that surround *Sk* allele. It could be tested easily by making a cross, which is heterozygous for GFP also, by putting an ectopic copy of GFP in one of the parental strain. If un-pairing at *rsk* locus causes pairing of *Mss*, there will be no expression of GFP due to activation of meiotic silencing. If there is no expression of GFP, it suggests that all dominant suppressors of meiotic silencing cause self-silencing by un-paring at the suppressor locus. Further, lack of GFP in such crosses might suggests that the first wild isolated suppressor of meiotic silencing in the CMG strain [12] might be a suppressor of meiotic silencing due to the presence of an inversion or chromosomal

rearrangement that causes un-pairing at the unidentified suppressor locus in the CMG. In fact, CMG contains putative duplication [16], suggesting some kind of link between chromosomal rearrangement and suppressor of meiotic silencing. Interestingly, CMG and *Sk* both are weak suppressors of meiotic silencing and contain chromosomal rearrangement. So, identification of more suppressors of meiotic silencing in the wild isolated population could reveal the importance of inversion or chromosomal rearrangements in meiotic silencing. In the opposite, it would be interesting to screen wild isolated population of Neurospora for the presence of inversions or chromosomal rearrangements, and then use these strains for testing the presence of meiotic silencing. Till today, we know that meiotic silencing acts on chromosomal rearrangements, but identification of suppressor of meiotic silencing amongst wild isolated strains that bear chromosomal rearrangements would suggest the role of rearrangements in meiotic silencing. Such findings will reveal another level of suppressor of suppressors. In past, such strains with putative chromosomal rearrangements have been identified (e.g., Sugartown, Golur etc) [17]. Interestingly, crosses that involve the CMG or meiotic silencing suppressors *sad-1* as a parent show loss of viability of RIP mutated (RIPed) progeny [12] after some time. Initial observation of this loss of viability of RIPed progeny led authors to identify the suppressor of meiotic silencing in the CMG strain. Since, Sugartown and Golur were also identified in the same screen for loss of viability of RIPed progeny, this suggests that chromosomal rearrangements (at least in Sugertown and Golur) might be coupled with suppressor of meiotic silencing to allow gene expression.

The common features between the CMG and *Sk* suppressors of meiotic silencing are that neither suppresses the round spore phenotype of the *R* strain and that both produce viable spores in a homozygous cross like *sad-4* and *sad-5*. It would be interesting to test the loss of viability of RIPed progeny from a cross that involves *Sk* and *sad5* as both are weak suppressors like CMG. Such observation of loss of viability of RIPed progeny in the presence of other weak suppressors might suggest a link between suppressor of meiotic silencing and meiotic drive. In fact, such observation might suggest that any suppressor of MSUD might be a killer or an example of meiotic drive. Therefore, even ascospores from a cross that involves *Sk* should also show such loss of viability that was observed among progeny of the CMG. Moreover, it might explain the loss of viability as the cost associated with suppression of meiotic silencing and not RIP. On the other hand, in the absence of such loss of viability, it might be interesting to look the viability of ascospores from a cross that is heterozygous for weak suppressors of MSUD like *Sk* and *sad-5* and in the presence of mutants like *sad-2* and *sad-3* for epistatic interaction. Moreover, It is very essential to study meiotic silencing in wild population like the CMG strain to understand the correlation of meiotic silencing, meiotic drive and chromosomal rearrangements.

Acknowledgment

We thank the Fellow Editorial Board, National Cancer Institute, National Institutes of Health for reading our manuscript and providing valuable suggestion in editing.

Author Contributions: A.K.A read and edited the manuscript. P.K.S developed the concept and wrote the article.

Bibliography

1. Aramayo R and EU Selker. "Neurospora crassa, a model system for epigenetics research". *Cold Spring Harbor Perspectives in Biology* 5.10 (2013): a017921.

2. Shiu PK., *et al*. "Meiotic silencing by unpaired DNA". *Cell* 107.7 (2001): 905-916.

3. Shiu PK and RL Metzenberg. "Meiotic silencing by unpaired DNA: properties, regulation and suppression". *Genetics* 161.4 (2002): 1483-1495.

4. Lee DW., *et al*. "An argonaute-like protein is required for meiotic silencing". *Genetics* 164.2 (2003): 821-828.

5. Shiu PK., *et al*. "SAD-2 is required for meiotic silencing by unpaired DNA and perinuclear localization of SAD-1 RNA-directed RNA polymerase". *Proceedings of the National Academy of Sciences of the United States of America* 103.7 (2006): 2243-2248.

6. Lee DW., *et al*. "QIP, a component of the vegetative RNA silencing pathway, is essential for meiosis and suppresses meiotic silencing in Neurospora crassa". *Genetics* 186.1 (2010): 127-133.

7. Decker LM., *et al*. "The Nuclear Cap-Binding Complex Mediates Meiotic Silencing by Unpaired DNA". *G3 (Bethesda)* 7.4 (2017): 1149-1155.

8. Samarajeewa DA., *et al*. "An RNA Recognition Motif-Containing Protein Functions in Meiotic Silencing by Unpaired DNA". *G3 (Bethesda)* 7.8 (2017): 2871-2882.

9. Hammond TM., *et al*. "SAD-3, a Putative Helicase Required for Meiotic Silencing by Unpaired DNA, Interacts with Other Components of the Silencing Machinery". *G3 (Bethesda)* 1.5 (2011): 369-376.

10. Hammond TM., *et al*. "Novel proteins required for meiotic silencing by unpaired DNA and siRNA generation in Neurospora crassa". *Genetics* 194.1 (2013): 91-100.

11. Raju NB., *et al*. "Neurospora spore killers Sk-2 and Sk-3 suppress meiotic silencing by unpaired DNA". *Genetics* 176.1 (2007): 43-52.

12. Kasbekar DP., *et al*. "Carrefour Mme. Gras: a wild-isolated Neurospora crassa strain that suppresses meiotic silencing by unpaired DNA and uncovers a novel ascospore stability defect". *Fungal Genetics and Biology* 48.6 (2011): 612-620.

13. Decker LM., *et al*. "Complex Formation of RNA Silencing Proteins in the Perinuclear Region of Neurospora crassa". *Genetics* 199.4 (2015): 1017-1021.

14. Kasbekar DP. "Sex and the single gene: meiotic silencing by unpaired DNA". *Journal of Biosciences* 27.7 (2002): 633-635.

15. Hammond TM., *et al*. "Molecular dissection of Neurospora Spore killer meiotic drive elements". *Proceedings of the National Academy of Sciences of the United States of America* 109.30 (2012): 12093-12098.

16. Ramakrishnan M., *et al*. "A factor in a wild isolated Neurospora crassa strain enables a chromosome segment duplication to suppress repeat-induced point mutation". *Journal of Biosciences* 36.5 (2011): 817-821.

17. Bhat A., *et al*. "Genetic analysis of wild-isolated Neurospora crassa strains identified as dominant suppressors of repeat-induced point mutation". *Genetics* 164.3 (2003): 947-961.

Volume 12 Issue 6 October 2017
© All rights reserved by Amrendra Kumar Ajay and Parmit Kumar Singh.