Commentary

Sex and the single gene: Meiotic silencing by unpaired DNA

1. Introduction

In the fungus *Neurospora crassa*, crosses made between normal sequence strains and some translocation strains can yield three kinds of progeny: normal sequence (like one parent), translocation sequence (like the other parent) and a novel class that is duplicated for the translocated segment. The duplication progeny arise when one component of the translocation segregates with a normal sequence chromosome; deletion progeny resulting from segregation of the complementary component are inviable (see figure 1). Regardless of the particular genomic segment duplicated (i.e. regardless of the translocation strain used in the parental cross), duplication strains in general show a characteristic barren phenotype when they, in turn, are crossed with non-duplication strains. Barren crosses produce plenty of perithecia (fruiting bodies) but yield only a few exceptional ascospores (haploid products of meiosis). A series of remarkable studies by Robert Metzenberg and colleagues (in particular, Rodolfo Aramayo and Patrick Shiu) has now revealed that the barren phenotype is caused by a newly discovered gene-silencing process called meiotic silencing by unpaired DNA (MSUD). MSUD occurs during meiosis and silences any gene that is unpaired and all genes homologous to it, including those that may themselves be paired. Large duplications can span many genes, including some that code for products required for meiosis and ascospore development. In crosses heterozygous for such duplications one copy of each such gene is unpaired so the silencing of all three copies by MSUD renders the

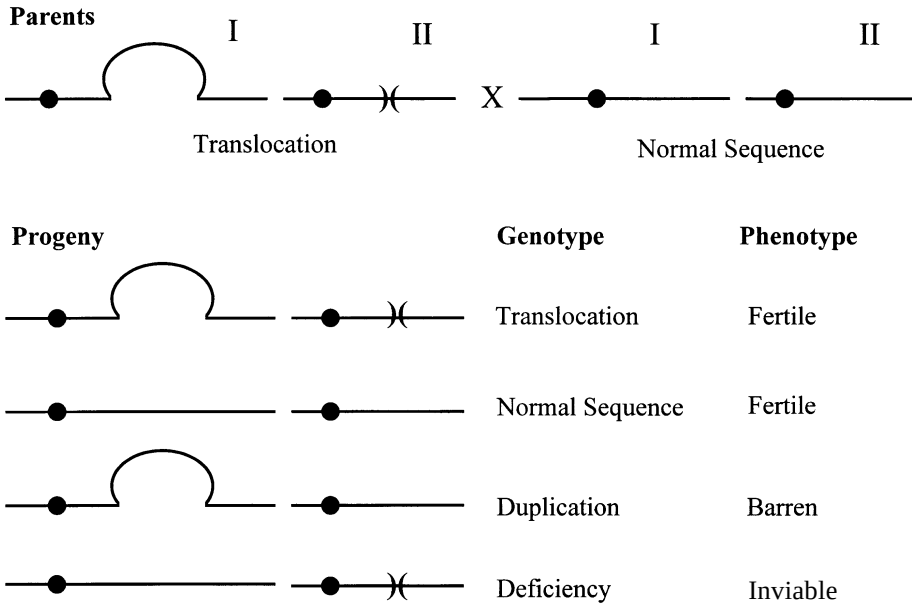

Figure 1. Generation of a duplication in a cross between a translocation and a normal sequence strain. Only two linkage groups are shown. The translocation indicated in this figure is of a segment of linkage group (LG) II, indicated by the inverse parentheses, that is inserted into LG I, which is indicated by the looping out.

cross barren. This commentary recounts the studies which led to the discovery of MSUD and of its involvement in the barren phenotype (Aramayo and Metzenberg 1996; Aramayo *et al* 1996; Shiu *et al* 2001; Shiu and Metzenberg 2002).

2. An ascus dominant phenotype

The story began with the identification, cloning and deletion of *Asm-1*, the Neurospora homologue of the *stunted A* (*stu A*) gene of *Aspergillus nidulans*. The Aspergillus StuA protein is a transcription factor required for the development of sexual reproductive structures (cleistothecia). If this role was evolutionarily conserved, then mutation of its Neurospora homologue also was expected to produce a sexual phenotype. Using low stringency hybridization with a *stu A* probe, Aramayo, Metzenberg and colleagues isolated a Neurospora homologue and used it to construct a deletion mutant by targeted gene replacement. The mutant, designated *Asm-1*$^{\Delta}$, fulfilled the prediction of a sexual development phenotype by failing to make female sexual structures (protoperithecia). Although the *Asm-1*$^{\Delta}$ mutant was female-sterile, it was fertile when crossed as a male but almost all ascospores produced in these crosses (including those that were genotypically wild type) were white, immature and inviable. Thus in addition to the defect in protoperithecial development, the deletion also conferred an ascus-dominant ascospore maturation defect (hence the designation *Asm-1*).

3. Ascus dominance and MSUD

Transformation of the *Asm-1*$^{\Delta}$ strain with the cloned *asm-1*$^{+}$ gene was able to restore normal protoperithecial development but failed to complement the ascus-dominant ascospore maturation defect. The complementing *asm-1*$^{+}$ sequence was integrated into the *his-3* locus; the possibility that it needed additional sequences to overcome the ascospore maturation defect was excluded when it was observed that ascospores could mature in crosses that were homozygous for the transgenic construct that is, in crosses where both parents carried identical *asm-1*$^{+}$ constructs at their *his-3* locus. In fact mature ascospores were produced even if one of the ectopic constructs was a non-functional frame shift mutant allele, *asm-1*^{fs}. In such *his-3* : : *asm-1*^{fs}; *Asm-1*$^{\Delta}$ × *his-3* : : *asm-1*$^{+}$; *Asm-1*$^{\Delta}$ crosses, the ascospores inheriting the ectopic *asm-1*$^{+}$ allele developed normally and only those receiving the ectopic *asm-1*^{fs} allele remained inviable. Thus ascospore maturation appeared to require that the complementing *asm-1*$^{+}$ allele be able to pair with homologous sequences during the diplophase. Subsequently Shiu, Metzenberg and colleagues (Aramayo *et al* 1996; Shiu *et al* 2001) showed that in fact the requirement was not that the *asm-1* alleles be paired, but that no allele remain unpaired. With hindsight it would appear that MSUD might have been discovered sooner had Aramayo and Metzenberg done the cross *his-3* : : *asm-1*^{fs}; *asm-1*$^{+}$ × *his-3*; *asm-1*$^{+}$. Although the ectopic *asm-1*^{fs} allele is non-functional, its remaining unpaired in this cross would have silenced even the paired endogenous *asm-1*$^{+}$ alleles and thereby established the essentials of the MSUD gene-silencing process.

Shiu, Metzenberg and colleagues then went on to construct ectopic integrations of genes whose products are known to be required during meiosis (e.g. *b*-tubulin, actin and the histones H3 and H4-1). Crosses heterozygous for these constructs were barren whereas homozygous crosses were fertile. In contrast, crosses in which the unpaired gene's product was known to not be required in meiosis (e.g. *pan-2*$^{+}$, *inl*$^{+}$) remained fully fertile. These results showed that MSUD can silence any gene that is unpaired but an ascus dominant phenotype results only if the product of the silenced gene is essential for meiosis or ascospore development.

4. A suppressor of MSUD

Shiu and Metzenberg (2002) isolated a UV-induced, semi-dominant suppressor of MSUD, designated *Sad-1*^{UV} (*Suppressor of ascus dominance 1*). Their selection/screen strategy rested on an inspired hunch by Aramayo and Metzenberg (1996) that another ascus dominant mutation, *Round spore* (*R*), also might be due to the MSUD gene-silencing process. First they did the cross *his-3* : : *asm-1*^{fs}; *asm-1*$^{+}$

× *Asm-1*$^{\Delta}$ in which almost all ascospores remain white and inviable because the only functional allele is unpaired. This imposes a selection for viable ascospores that are produced via a suppression of the ascospore maturation defect. In this way they obtained 166 viable segregants. Then they tested each of these segregants for ability to suppress the Round spore phenotype of *R*. The *Sad-1*^{UV} segregant was the only one that came through this selection/screen, the other 165 segregants represented the relatively large proportion of false positives coming through the selection step.

Crosses homozygous for *Sad-1*^{UV} turned out to be completely sterile. This made it easy to map the *Sad-1*^{UV} mutation to LG IL. Additionally, by selection for ability to complement the sterility of *Sad-1* homozygous crosses, Shiu and Metzenberg (2002) were able to identify *Sad-1*$^{+}$ clones from an LG I specific cosmid library. The nucleotide sequence of a subcloned *Sad-1*$^{+}$ fragment indicated that the encoded protein belonged to a family of RNA-directed RNA polymerases (RdRPs). RdRPs have been implicated in posttranscriptional gene silencing via targeted degradation of mRNAs. The sequence of the *Sad-1*^{UV} allele revealed a deletion of the 3′ half of the gene. The *Sad-1*$^{+}$ clone was used to construct a deletion mutant, called *Sad-1*$^{\Delta}$. Like *Sad-1*^{UV}, *Sad-1*$^{\Delta}$ also had a semi-dominant phenotype. Both alleles were able to suppress the barren phenotype of crosses between normal sequence and duplication strains thereby confirming that MSUD was responsible for the barrenness of duplication strains.

Sad-1^{+} alleles were also generated by RIP (repeat-induced point mutation) which is a mutational process that occurs during the premeiotic dikaryotic stage of the Neurospora sexual cycle and causes any duplicated DNA to undergo extensive G : C to A : T hypermutation (Selker 1990). Interestingly, some of the RIP-induced alleles were dominant whereas others were recessive; the dominant alleles were more severely altered by RIP mutations than the recessives. These findings indicated that a *Sad-1*$^{+}$ allele that is unpaired, either because its homologue is wholly or partially deleted or because its sequence is greatly altered by RIP, is itself subject to silencing.

5. In conclusion – a new riddle?

That *Sad-1* alleles themselves have to be paired for other unpaired genes to become silenced brings to mind Bertrand Russell's famous paradox. It has to do with a village barber who shaves all those who don't shave themselves: Who shaves the barber? One "solution", favoured by Shiu and Metzenberg, is to have the barber shave himself, but so incompletely as to create debate about whether he shaved or merely trimmed his beard. Another possibility is to sneak in an additional barber. Shiu and Metzenberg note that the Neurospora genome appears to encode another RdRP of unknown function. Might mutation of this unknown RdRP influence *Sad-1*$^{+}$ – dependent MSUD?

References

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