

Unifying homology effects

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Meiotic silencing by unpaired DNA is a new mechanism, related to other homology-dependent gene silencing phenomena, with implications not only for genome protection against invasive nucleic acids but for genome maintenance and speciation as well.

The ability of DNA sequences to pair with each other on the basis of sequence homology is well recognized, being the basis for homologous chromosome pairing during meiotic prophase. In the past decade, it has become clear that homology-based interactions between nucleic acids, both DNA and RNA, can be influential not only in accomplishing pairing and recombination during meiosis, but also in regulation of gene expression at other times in the cell cycle. These regulatory mechanisms—collectively called 'homology effects'¹—constitute a heterogeneous class of phenomena that are postulated to be mechanistically independent processes.

Homology effects can be triggered by the introduction of foreign nucleic acids, transgenes or double-stranded RNA molecules into cells, and result in a downregulation (silencing) of mRNA levels from the corresponding homologous endogenous genes. In other cases, as in paramutation and transvection phenomena, interactions between endogenous homologous alleles are required for gene silencing, or more generally, for proper regulation of gene expression. In a recent report in *Cell*, Patrick Shiu and colleagues² describe a new mechanism of meiotic transvection occurring in the fungus *Neurospora crassa*. They find that what has been called transvection unexpectedly shares features with other silencing phenomena, perhaps revealing a common ancestry.

Musical pairs. It has been proposed¹³ that repetitive DNA sequences could produce transcripts, often called aberrant RNAs (aRNAs), which might differ in some way from normal mRNAs. These aRNAs are converted by RNA-dependent RNA polymerases (RdRPs) into double-stranded RNA (dsRNA). Here, similar aRNAs are produced from unpaired DNA sequences during meiotic prophase. In *Neurospora*, two different RdRPs (*qde-1* and *sad-1*) are required for the two processes, suggesting that these two categories of aRNA may somehow differ from each other. Double-stranded RNA activates a universally conserved silencing pathway (green arrows) that has evolved to counteract viral infections. Short interfering RNAs (siRNAs) derived from the processing of dsRNA can work as guides to direct nuclease complexes to a target mRNA, leading to homologous RNA degradation.

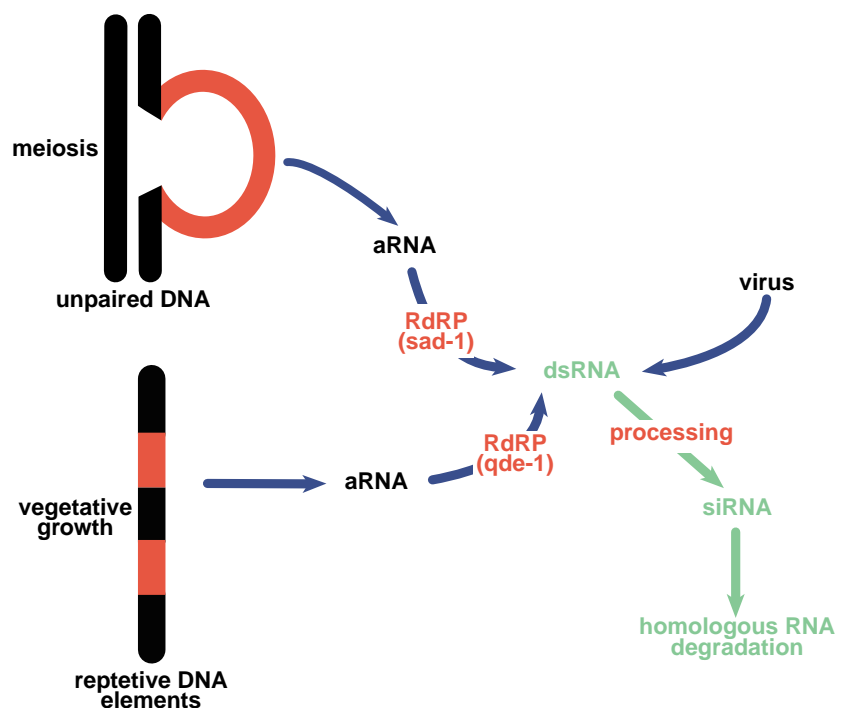
A single ancient origin

Neurospora crassa is haploid during vegetative growth, whereas in the sexual cycle, two haploid nuclei of opposite mating type fuse to produce a diploid cell, the zygote. The zygote immediately undergoes the two meiotic divisions, followed by one mitotic division, to produce haploid ascospores. Thus, the brief period after karyogamy and before meiosis is the only time during which *Neurospora* is diploid. It has been known for some time that certain genes in *Neurospora* exhibit 'meiotic transvection'³; in other words, the two alleles need to be paired for proper expression. In their study, Shiu *et al.*² found that proper expression during meiosis depends not only on the presence of paired alleles but also on the absence of any unpaired allele. Apparently, *Neurospora* can detect any DNA sequences unpaired during meiosis, and can silence both unpaired DNA (self-silencing) and all homologous DNA present (*trans-*

silencing). Based on these characteristics, which differ from transvection as it is observed in *Drosophila*, meiotic transvection has been renamed 'meiotic silencing by unpaired DNA' (MSUD). Shiu *et al.*² also discovered that MSUD is not restricted to a few genes as was previously believed, but that virtually the entire genome is subject to this process. A noteworthy finding is that a putative RNA-dependent RNA polymerase (RdRP) encoded by *sad-1* is necessary for MSUD. This suggests a common genetic basis for meiotic silencing and previously characterized post-transcriptional gene silencing mechanisms induced either by transgenes or by double-stranded RNA, as these also require RdRPs⁴⁻⁷.

Silencing the opposition

Post-transcriptional gene silencing is a mechanism by which supernumerary genes (multiple transgenic copies, for example) are detected and transgenic RNAs are con-



verted by RdRP to double-stranded RNA (dsRNA). This dsRNA is cleaved by a ribonuclease III-like activity⁸ into small RNAs, also called short interfering RNA (siRNA)⁹, which, in turn, guide an RNA-degrading complex in the hydrolysis of mRNA species homologous to the dsRNA (see figure)¹⁰. One of the major biological functions of post-transcriptional gene silencing is to protect cells from invading nucleic acids^{11,12}. The evolutionary origin of this mechanism is probably related to the development of the ability of a cell to recognize and deal with double-stranded RNA molecules. The presence of large dsRNA molecules in a cell signals the existence of an ongoing viral infection, because dsRNA is an intermediate produced during the life cycle of many viruses. It is conceivable that the first step was the evolution of enzymes capable of degrading dsRNA, and thus able to directly counteract replicating viruses.

A great increase in the efficiency of the viral defense mechanism would have resulted from the use of short RNAs derived from the degradation of dsRNA. These recognize, by base pairing, other RNA species that are homologous to the dsRNA and degrade them. The considerable advantage of this homology-dependent RNA degradation process is that, starting from a few dsRNA molecules, cells can generate a response directed against many single-stranded viral RNAs and arrest viruses early in their infection cycle. This mechanism could be particularly useful to prevent retroviruses, whose genomes are integrated into the genome, from successfully exiting their latent phase and completing their lytic cycle. The ability to control mobile DNA elements, such as transposons, by post-

transcriptional gene silencing can be considered a further exploitation of this mechanism. Cells can somehow detect RNA transcripts made from repetitive DNA sequences and use them as templates for RdRPs, producing dsRNA that can activate homology-mediated RNA degradation (see figure). It is noteworthy that when a repetitive sequence is detected, all related sequences, even those scattered around the genome, are inactivated *in trans*, preventing their expression and transposition.

Whereas gene-silencing phenomena studied so far seem to be activated by DNA segments present in supernumerary copies or by the abnormal RNA transcripts, Shiu *et al.*² describe a new silencing mechanism capable of examining the entire genome during the first meiotic prophase, identifying DNA segments present in any odd number of copies or in the wrong chromosomal location. As such, MSUD can be seen as an additional mechanism dedicated to the protection of cells against transposons, because it can detect and silence newly acquired sequences that are present as a single copy and thus unpaired during meiosis prophase. Although there is as yet no evidence that a similar process occurs in diploid organisms, it can be argued that meiotic silencing could be particularly effective in controlling mobile DNA elements that are frequently active in the animal germ line and are able to transpose at or near the time of meiosis.

Genome stability and speciation

Owing to its ability to scan the entire genome for unpaired DNA fragments, MSUD can be considered to be a mechanism committed to maintaining genome

integrity, with broader biological implications than a mere defense strategy against parasitic DNA molecules. In haploid organisms, rearrangements of the genome, such as duplications and translocations that occur during vegetative growth, can disrupt intimate pairing of several genes, leading to gene silencing and sterility during the sexual cycle. This would prevent most genomic rearrangements from being propagated. On the other hand, small changes in genome organization can escape MSUD and accumulate in the gene pool. Genome microheterogeneities, after accumulating in two genetically isolated populations, can be detected by MSUD during inter-population crosses, leading to infertility. Thus, MSUD could be a mechanism that promotes the reproductive isolation of species. The finding that mutations of *sad-1* confer fertility on otherwise barren interspecific crosses within the genus *Neurospora* is consistent with a role for MSUD in speciation. □

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