A fratricidal fungal prion

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Once again, 50 years after its discovery, the multifaceted het-s gene of Podospora anserina has surprised us. Long ago, it provided an important early example of heterokaryon incompatibility and cytoplasmic inheritance (1–4). In 1997, the product of allele het-s was shown to form a prion (infectious protein) that leads to cell death in incompatible combinations (5–7). Then, spore-killing meiotic drive factors were discovered in natural populations of Podospora (8). Ability to kill was correlated with heterokaryon incompatibility (9), and some of the killer strains resembled het-s in their effects on ascospore abortion. Now, in this issue of PNAS, Dalstra et al. (10) fit pieces of the jigsaw puzzle together, showing that the het-s prion acts as a spore killer in the sexual phase. It can thus be thought of as a selfish genetic element that promotes its maintenance by actively destroying meiotic products that bear the nonprion allele het-3.

Podospora is a filamentous ascomycete related to the orange mold Neurospora crassa. Several characteristics of P. anserina have been important for analysis of the het-s gene. Vegetative growth consists of multinucleate syncytial filaments that may be homokaryotic or heterokaryotic, with nuclei that are haploid. Mating occurs between two physiologically distinct mating types. In the sexual phase, all four products of each meiosis are retained together in an ascus containing large spores that are pigmented when viable but unpigmented when inviable. Detection of spore killing and the implication of prions were aided when inviable. Detection of spore killing pigmented when viable but unpigmented the sexual phase, all four products of the sexual cycle contains a heterozygous diploid fusion nucleus, which immediately undergoes meiosis. The haploid meiotic products then divide once before being enclosed in ascospores. Unlike its better known relative, Neurospora crassa, with its eight-spored ascus, most asci of P. anserina are four-spored. Nuclear divisions in the Podospora ascus are orchestrated to deliver one nucleus of each mating type into each of the four large binucleate ascospores. Thus, each spore produces a heterokaryotic germling that is self-fertile. Species of this type are commonly called pseudo-homothallic. Pseudohomothallism has evolved independently in many fungal taxa. Neurospora tetrasperma, Gelasinospora tetraspora, and Agaricus bisporus are perhaps the best-known examples (11). The P. anserina life cycle is described in ref. 12.

Choreography in the Podospora ascus depends on precisely programmed spindle alignment and nuclear movement, with the result that alleles at a heterozygous locus between the centromere and the first chiasma are homokaryotic in the four binucleate ascospores (S + S), (S + S), (s + s), and (s + s), whereas genes distal to a chiasma are heterokaryotic in all of the spores (S + s), (S + s), (S + s), and (S + s) (Fig. 1). The mating type locus is located far out in a chromosome arm, distal to a single chiasma that is formed in ~99% of the asci. Unlike mating type, het-s is located close in, where a proximal chiasma is formed in only 10% of asci. Critical evidence in the study by Dalstra et al. was provided by the 90% of ascus within which no chiasma occurred between het-s and the centromere.

The polymorphic het-s locus in P. anserina encodes a 30-kDa protein (13, 14). Two alleles, het-s and het-S, differ by 14 amino acid residues. These alleles originated from wild isolates and were identified as heterokaryon-incompatibility genes because a barrier (“barrage”) reaction occurred when homokaryotic het-s and het-S strains confronted one another on agar medium (1). Microscopic observation revealed that death occurs in the cells that result from hyphal fusion (2). A third, inactive allele, het-s*, was found that could form heterokaryons with both het-s and het-S (3).

Alleles het-s and het-S differed from other known heterokaryon-incompatibility genes in an important respect. Strains that are genetically het-s occur in two phentypic classes, [Het-s] and [Het-s*]. [Het-s] strains are heterokaryon incompatible with het-S, whereas [Het-s*] strains are not. When [Het-s] hyphae fuse with het-S hyphae, the heterokaryotic (het-S + het-s) cells die,

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whereas ([Het-s*] + het-S) combinations are fully compatible, and the cells that result from fusion do not die. When [Het-s] fuses with [Het-s*], the strains are heterokaryon compatible and the [Het-s*] strain is converted by invasive contagion to the active [Het-s] condition. The inactive [Het-s*] phenotype by itself is quite stable but [Het-s*] can revert to the active [Het-s] condition at a low frequency (2).

When a het-s strain of active type [Het-s] is used as female parent and is fertilized by [Het-s*], most of the het-s progeny are of the inactive [Het-s*] phenotype, which is heterokaryon compatible with both parents. Inactive [Het-s*] can also be recovered from protoplasted vegetative cells of [Het-s] (15). These observations all suggested that a cytoplasmic element is responsible for the difference between [Het-s] and [Het-s*].

Spore abortion in the sexual phase also indicates that a cytoplasmic element is responsible for the behavior of [Het-s] and that [Het-s*] is free of the element. In certain crosses of het-s × het-S, ascospores abort at high frequency (3). Abortion is elevated only when het-s is expressing the [Het-s] phenotype, and only when [Het-s] is the female parent. (Little or no cytoplasm from the male is transferred during fertilization.) Ascospore abortion is not elevated in crosses of [Het-s*] × het-s regardless of which strain is used as the female parent.

These observations led to the realization that the het-s product in [Het-s] strains has the properties expected of a prion, and studies of protein products were initiated (5, 6). The [Het-s*] → [Het-s] transition can be propagated as an infective agent in the absence of protein synthesis or nuclear migration. Proteins encoded by het-s and het-S were shown to interact to form heterodimers. The protein products of [Het-s] and [Het-s*] show differential sensitivity to proteinase K. Aggregates formed in vitro are infectious (16).

In sum, the following criteria for a prion are met: (i) the activity is transmitted through crosses maternally; (ii) it is contagious in vegetative cultures; and (iii) the condition is conditionally curable. Not only can active [Het-s] be converted to inactive [Het-s*], but the reverse can also occur, albeit at low frequency. (iv) Prion infectivity can be created de novo, in vitro, from recombinant protein. When fibers made in vitro from recombinant [Het-s*] protein are introduced into [Het-s*] fungal cells by using microprojectile bombardment, the [Het-s] prion emerges with a high frequency (16). het-s provides the only known example of a prion in fungi, other than those in Saccharomyces cerevisiae (reviewed in ref. 7).

Because the immediate products of meiosis survive and are readily observed, Podospora and other fungi offer favorable material for detecting and analyzing meiotic drive (17), also called segregation distortion or segregation ratio distortion. Drive is manifested in fungi by inviability of the sexually produced ascospores or basidiospores. Podospora provided the first description of meiotic drive in fungi, although it was not recognized as such at the time (18). Clear examples of segregation distortion have since been provided by the discovery of spore killers in Neurospora, Gibberella, Cochliobolus, and Magnaporthe (reviewed in refs. 19–21), and additional killer loci were found in natural populations of Podospora (8). These drive elements all were detected because they result in clearly defined patterns of ascospore abortion.

In Podospora, each large ascospore encloses nuclei that were derived from two different meiotic products. Binucleate ascospores from het-s × het-S are of three different genotypes: (het-s + het-s), (het-s + het-S), and (het-S + het-S). If the female parent was [Het-s], ascospores that are homokaryotic or heterokaryotic for the het-s allele survive, but spores that are homozygous for het-S abort. That is, [Het-s] acts as a spore killer. Killing requires that one of the parents be het-S. There is no killing when one of the participants is a null mutant, het-s*. Therefore, the het-S product appears to be actively involved.

Critical information is provided by patterns of ascospore abortion in individual ascii. With [Het-s] as female in a cross of [Het-s*] × het-S, half of the first-division ascii contain four viable black ascospores and half contain two that are black and two that are unpigmented and inviable (Fig. 1). Survivors in the ascii with two black and two aborted spores all carry the het-s allele. The spores that were killed must have been het-S. The het-s gene in its active state thus acts as a spore killer.

Killing of the sensitive het-s ascospores in Podospora bears a striking resemblance to the behavior of the Spore killer haplotypes Sk-2 and Sk-3 in N. crassa, where four spores in the eight-spored ascii are killed in crosses of killer × sensitive, and it is the killer ascospores that survive. In Podospora, just as in Neurospora, ascospores that carry only the sensitive allele are vulnerable to killing whereas heterokaryotic ascospores with both killer and sensitive nuclei survive; the sensitive nucleus is rescued if a killer nucleus is present in the same ascospore (19, 20). [Exceptional] heterokaryotic ascospores occur as the result of developmental anomalies in the ascii of N. crassa and as a regular feature of ascus development in the four-spored pseudohomothallic species N. tetrasperma (11).]

Dalstra et al. (10) have now shown that the het-s prion is sexually transmitted and that it acts as a spore killer in crosses with het-S strains, which are sensitive to killing. This behavior resembles that of the spore killer drive elements previously identified in wild-collected P. anserina strains. The authors also note that in a sample of ~100 strains from a local wild population, 50% were [Het-s] killers, 40% were sensitive to killing (het-S), and the remainder were [Het-s*].

Visual evidence confirming involvement of a prion was obtained by using ectopic het-s::GFP and het-S::GFP fusions in strains with a null allele at the resident locus. The het-s protein product is seen to exist either in diffuse form or as an amyloid aggregate. In ascii where killing occurs, aggregates of the het-s::GFP protein are present in the ascus cytoplasm and young aborting ascospores. The two surviving ascospores of such ascii are genotypically het-s. No killing occurs in spores that lack the prion. The prion is rarely transmitted in crosses with a null mutant as female and the [Het-s] killer as fertilizing parent.

Further evidence strengthening the prion hypothesis came from overexpression of het-s using a strong promoter. Under these conditions killing occurs with increased efficiency. Inactive [Het-s*] strains change to the active [Het-s] condition more frequently when het-s is overexpressed, and aggregates are then seen. Overexpression of het-S neutralizes [Het-s] activity in a dosage-dependent manner.

With het-s::GFP inserted ectopically, the het-s protein product appears only late in ascus development, at the time of spore formation. Dalstra et al. note that this is the timing that would be expected if expression of the gene is subject to meiotic silencing of unpaired DNA (MSUD), a sexual-phase silencing mechanism previously known only in N. crassa (22, 23). Experiments under way
will determine whether MSUD is responsible for the delay in expression.

Although all of the known fungal spore killers share common features, little is known about the molecular mechanisms underlying them. The mechanism may be quite different in different organisms and systems. Discovery that the het-s prion is a spore killer was novel and quite unexpected. Prions are clearly not involved in segregation distortion in Drosophila (24) or the t-complex in mice (25), the two meiotic drive systems best understood at the genomic, molecular, and cellular levels. No evidence exists that prions play a role in other known examples of drive in the fungi, nor does segregation distortion appear to be correlated with heterokaryon incompatibility in other fungi, or with the heterokaryon incompatibility that is specified by eight loci other than het-s in Podospora. The other fungal spore killers also differ from het-s in not showing a maternal effect in reciprocal crosses and in not being curable. Thus, it seems unlikely that prions will provide a general explanation for segregation distortion. More likely, prion formation is only one of many diverse molecular and cellular mechanisms that can result in meiotic drive. Similarly, it seems unlikely that het-s can serve as a general model for vegetative incompatibility.

The history of research with Podospora makes it clear that we do not depend solely on a few prestigious major model organisms for significant contributions of wide general interest. A wealth of biological information exists for species such as P. anserina, which are less well known but which may possess features empowering them to provide fundamental information that would be difficult to obtain from the better known species commonly used as models, each of which has its strengths and weaknesses.

The current Podospora study, and those that led up to it, testify to the foresight of Georges Rizet. This year marks the 60th anniversary of his thesis (26). As a student in occupied France during World War II, Rizet recognized the potential usefulness of P. anserina, worked out its intricate sexual behavior, and carried out the first genetic studies (26, 27). It was he who discovered het-s and described its novel features (1). During the decade after the war, Rizet and his colleague Boris Ephrussi taught genetics at the University of Paris-Sorbonne and contributed to the flowering of research in microbial genetics. After the two were awarded the first two chairs in genetics in France, Ephrussi moved to Gif, where his group concentrated on yeast, and Rizet went to the Faculty of Science in Orsay, where he and his associates continued working with filamentous fungi. In their hands, P. anserina, Ascomobolus immersus, and Sordaria macrospora were used in fundamental studies of vegetative incompatibility, senescence, recombination, meiosis, gene silencing, transposon-induced gene instability, and the structure and function of mating type genes (28). The current paper by Dalstra et al., together with other ongoing studies, continues to build on the foundation laid by Rizet.