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Commentary

A new prion controls fungal cell fusion incompatibility

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In solving a genetic puzzle posed by George Rizet in 1952 (1), Coustou, Deleu, Saupe, and Begueret report (2) evidence for the first prion (infectious protein) that carries out a normal function. It was studies of scrapie that gave rise to the prion concept, namely, that a normal cellular protein could change to an abnormal form (the prion form) that may be unable to carry out its normal function, but has acquired the ability to convert its normal form into this same abnormal (prion) form. This altered protein, by catalyzing, not its own synthesis, but its own alteration, becomes an infectious agent (a prion) if it can get from cell to cell or from individual to individual (reviewed in refs. 3–6).

Until now, all prions have seemed to cause diseases. Scrapie, Creutzfeldt–Jakob disease, Mad Cow disease, etc. in mammals are invariably lethal neurological diseases involving an altered form of PrP (prion protein), a nonessential cell surface protein (3–6). The non-Mendelian genetic element of *Saccharomyces cerevisiae*, [URE3] (for ureidosuccinate), is due to a prion change of Ure2p, a regulator of nitrogen metabolism, and results in slow-growing cells (7). [PSI], also a yeast prion, is due to an aggregating form of Sup35p, one of the translation termination proteins whose misfunction in cells carrying [PSI] results in abnormal read-through of translation termination codons (reviewed in ref. 7). This is clearly hazardous to one's health, but [PSI] strains seem healthy if no suppressor tRNA is around to read the translation termination codon as an amino acid. The [Het-s] prion of the filamentous fungus *Podospora anserina* causes cell death, but it is a purposeful cell death designed to limit the spread of fungal viruses by preventing cytoplasmic exchange between two colonies.

Podospora Heterokaryon Incompatibility

When two fungal colonies grow together the advancing cell processes (hyphae) of the two colonies may fuse (anastomose) to form cells (heterokaryons) with nuclei and cytoplasm from both parent colonies. The two colonies have, in effect, fused to form one interconnecting mat. This hyphal anastomosis or heterokaryon formation is genetically controlled in a different way from the sexual mating that the same two strains may be able to undergo. Whereas sexual mating requires different genotypes at a mating type locus, hyphal anastomosis requires identity at certain other loci, often several loci. In *Podospora anserina,* these genes are called *het* (for *het*erokaryon formation). One such locus, *het-s*, has alleles *het-s* and *het-S* (Table 1). Strains with the same allele can undergo hyphal anastomosis to form heterokaryons. But when *het-s* and *het-S* strains grow together, heterokaryon incompatibility is observed. In this case, the peripheral hyphae of the colonies fuse, but the fused hyphae die, the surrounding hyphae are unpigmented, and the line of dead cells between the two colonies acts as a barrier to the colonies growing together (Fig. 1, refs. 1, 8, and 9). The *het-S/s* locus encodes a protein of 289 amino acid residues, with *het-s* and *het-S* alleles differing at 14 residues (10, 11). Remarkably, a single amino acid difference between *het-s* and *het-S* is sufficient to produce incompatibility (12).

Sexual mating is probably a mechanism to shuffle the genetic cards, to generate variability. Therefore, mating with an identical strain makes no sense. Heterokaryon incompatibility is believed to be a mechanism to limit the spread of fungal viruses that spread from one colony to another by hyphal anastomosis. Only strains with identical *het* genes (which presumably already have the same viruses) can form heterokaryons. In filamentous fungi the spread of viruses is limited in sexual mating because germ cell formation often largely excludes the cytoplasm where the viruses are located (9).

Prions of Yeast Identified by Genetic Properties

[PSI] and [URE3] of yeast were proposed to be prions (13) based on three genetic properties that they share: (*i*) reversible curability—from strains cured of the genetic element could be isolated rare clones that had again acquired it spontaneously; (*ii*) overexpression of Sup35p or Ure2p, respectively, increased the frequency with which [PSI] and [URE3] arose; and (*iii*) Sup35p and Ure2p were necessary for propagation of [PSI] and [URE3], respectively, and yet *sup35* and *ure2* mutants had the same phenotypes as the presence of [PSI] and [URE3]. These are all properties expected of prions, but not of nucleic acid replicons like viruses or plasmids. This genetic evidence then was supported by finding that Ure2p is protease-resistant in [URE3] strains (14) and Sup35p is aggregated in [PSI] strains (15). [PSI] is eliminated by overexpression of the chaperone Hsp104, a finding that both supports the prion model for [PSI] and introduces a possible route for treatment of the lethal human disease (16). Recently, a [PSI] *in vitro* system reproducing the main *in vivo* features of [PSI] also has been reported (17).

[Het-s] Has the Properties of a Prion Form of the *het-s* Protein

The *het-s* mystery began in 1952 when Rizet reported that cells with genotype *het-s* could have either of two phenotypes. One, referred to now as [Het-s], shows the usual heterokaryon incompatibility with *het-S* colonies. The other, called [Het-s*], shows a neutral phenotype in that it can form heterokaryons with either *het-s* or *het-S* cells (Table 1). [Het-s] behaves genetically as a non-Mendelian (cytoplasmic) genetic element and [Het-s*] as its absence. Thus, heterokaryons formed between [Het-s] and [Het-s*] strains eventually become all [Het-s].

Mating *het-s* [Het-s] cells with *het-S* strains produced only *het-S* and *het-s* [Het-s*] meiotic segregants (a sort of curing; ref. 1). But these [Het-s*] segregants, when grown, gave rise to some [Het-s] segregants (reversible curing) (8). This is one of the genetic criteria for a prion. Coustou *et al.* (2) now have found that overproduction of the *het-s* protein increases the

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Table 1. How many het-s's are there?

Name	Meaning
het-s	The name of the locus and one of the alleles at that locus
het-S	The other allele at the *het*-s locus
[Het-s]	Genotype of cells with the *het*-s protein in the prion state
[Het-s*]	The absence of [Het-s], i.e., the genotype of cells with *het*-s protein not in the prion state
pHET-s	The prion form of the *het*-s protein
pHET-s*	The non-prion form of the *het*-s protein

frequency with which [Het-s*] strains become [Het-s] (acquire the putative prion). This is a second property expected of a prion. Propagation of the [Het-s] trait requires the *het-s* protein: *het-s*^{o} strains have the neutral phenotype and cannot propagate [Het-s] (2). Because the [Het-s] phenotype is opposite that of *het-s*^{o}, this result does not point to [Het-s] being a prion. If [Het-s] were a function of a plasmid whose propagation required the *het-s* protein, the same results would be found. But this result is not inconsistent with the prion model, if the prion form of the *het-s* protein does something positive to give a phenotype (not just by eliminating the normal form). Finally, the *het-s* protein is protease resistant in [Het-s] strains when compared with that from [Het-s*] strains (2). Although protease resistance is neither a necessary nor sufficient criterion of a prion change, it certainly argues that the *het-s* protein is altered in [Het-s] strains. Thus Coustou *et al.* (2) have made a strong case that [Het-s] represents a prion form of the *het-s* protein, fulfilling similar genetic and biochemical criteria to those supporting the view of the yeast [URE3] and [PSI] elements as prions.

Early Studies of [Het-s] in View of the Prion Interpretation

In light of the prion explanation of [Het-s], it is of interest to revisit the early studies of *het-s/S* heterokaryon incompatibility. In meiotic crosses of male *het-s* [Het-s] and female *het-s* [Het-s*], the segregants are all [Het-s*] (lacking the prion), but the progeny all carry the prion if the sexes are reversed, i.e., female *het-s* [Het-s] X male *het-s* [Het-s*] (1). This shows that the prion can pass through meiosis, but that it is restricted to the cytoplasm, almost none of which is included in the tiny male gametes (microconidia).

Surprisingly, crossing male or female *het-s* [Het-s] (carrying the prion) with *het-S* results in all genetically *het-s* segregants being [Het-s*] (lacking the prion). This means that the *het-S* protein cures the prion. Could the incorporation of the slightly different *het-S* protein into a *het-s* [Het-s] "crystal" poison crystal growth?

Why, in the incompatibility reaction, does the combination of *het-s* protein in the prion form and *het-S* protein lead to death of the fused hyphae? Why doesn't the *het-S* protein just poison crystal growth here? We can expect many interesting answers to these questions that may tell us important things about the way cells handle prions and incipient prions.

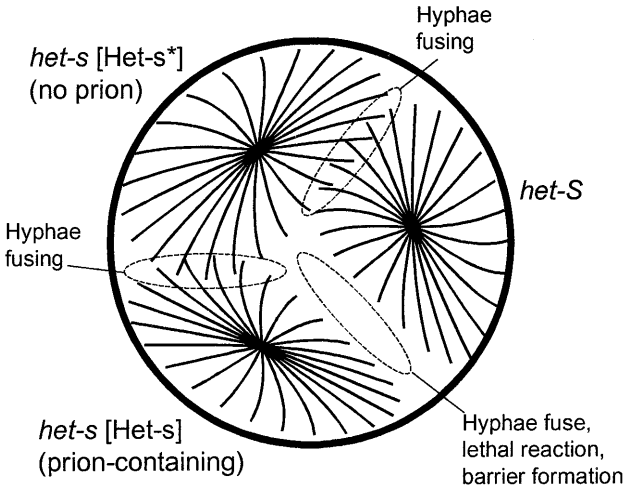

FIG. 1. Diagram of vegetative incompatibility in *Podospora*. Three strains were inoculated on a plate of growth media. Chromosomal alleles *het-s* and *het-S* and the presence ([Het-s]) or absence ([Het-s*]) of the prion form of the *het-s* protein are shown. After several days, the *het-s* [Het-s] strain and *het-S* strain show the incompatibility reaction, marked by death of fused hyphae and lack of pigmentation near the barrier.

Comparison of [Het-s] and Other Putative Prions (Table 2)

[Het-s] is like scrapie, and unlike [URE3] and [PSI], in that the prion form produces a phenotype by doing mischief, not by simply causing the absence of the active normal form of the *het-s* protein. The normal form of the protein is dispensable for growth, mating, and heterokaryon formation (10, 11).

Unlike all the other putative prions, [Het-s], the prion form of the *het-s* protein, is carrying out a normal fungal cell function. Heterokaryon incompatibility systems are widespread among filamentous fungi and usually are controlled by genetic loci showing none of the characteristics suggestive of prions. Is there an advantage to *Podospora* in using a prion to signal heterokaryon incompatibility? Because this is a purposeful cell death, and many viruses produce apoptosis in their host cells, could this heterokaryon incompatibility reaction be a form of fungal apoptosis?

The *het-s* protein has no evident similarity to other putative prion proteins. The prion domains of Ure2p and Sup35p are rich in asparagine and glutamine residues, but this is not true of either PrP or the *het-s* protein. Sup35p and PrP have similar octapeptide repeats, but these appear to be outside the prion domain of PrP and are not found in Ure2p or the *het-s* protein. Whether structural similarities will be found among the normal or prion forms of these proteins remains to be determined.

Conclusions

Have any of the putative prions been proven to be prions? There continues to be disagreement (e.g., refs. 3, 18, and 19),

Table 2. Comparison of putative prions

Prion	Species	Effect of prion form	Function of normal protein	Structural features
Scrapie, CJD...	Mammals	Ataxia, dementia, death	Unknown	Normal form: α helix Scrapie form: β sheet
[URE3]	*S. cerevisiae*	Loss of nitrogen regulation	Nitrogen regulation	Asparagine-rich prion domain
[PSI]	*S. cerevisiae*	Translation read-through	Translation termination	Asparagine-glutamine rich prion domain, octapeptide repeats
[Het-s]	*Podospora anserina*	Heterokaryon incompatibility	Unknown	??

CHD, Creutzfeldt–Jakob disease.

particularly in the case of scrapie, in part because of the practical difficulties of the animal systems. However, even for the yeast systems, where little rancor exists, the evidence is not conclusive. The exciting new results on the *Podospora* system widen the scope of application of the prion idea and again demonstrate the value of studying a wide variety of systems. The powerful genetic evidence for the yeast and fungal prions has, at least psychologically, complemented the biochemical evidence in the mammalian systems (which are not without some of their own genetic evidence; refs. 20 and 21), to bring wide acceptance to the prion concept.

Because of the hyphal anastomosis phenomenon, fungi should be quite susceptible to prions. When two fungal colonies grow together, if one is infected, the other will become so. In fact, the vegetative incompatibility systems may, in part, have evolved to block the spread of prions, as well as of fungal viruses and deleterious mitochondrial plasmids.

There are probably many prions in nature. The four cases described thus far were all described as phenomenon 25 to 250 years ago. With knowledge rapidly accumulating about prions, we can expect many new prions to be found in a more directed way. We also can thank the brilliant geneticists, George Rizet ([Het-s]), Brian Cox ([PSI]), and Francois Lacroute ([URE3]) for their pioneering work that has made possible the recent outbreak of prions.

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