Vegetative incompatibility in filamentous fungi: *Podospora* and *Neurospora* provide some clues

Sven J Saupe*, Corinne Clavé and Joël Bégueret

In filamentous fungi, vegetative cell fusion between genotypically distinct individuals leads to a cell-death reaction known as vegetative or heterokaryon incompatibility. Genes involved in this reaction have been characterised molecularly. We can now begin to get a better understanding of the mechanism and the biological significance of this intriguing phenomenon.

Addresses
Laboratoire de Génétique Moléculaire des Champignons, IBGC UMR CNRS 5095, 1 rue Camille St Saëns, 33077 Bordeaux Cedex, France
*e-mail: sven.saupe@ibgc.u-bordeaux2.fr

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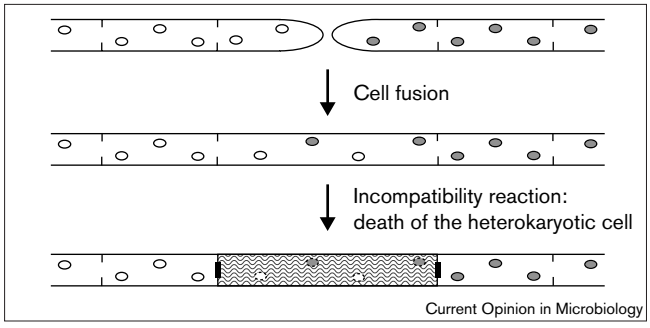
Introduction

Filamentous fungi, unlike most other organisms, are able to undergo spontaneous, vegetative cell fusion. When these fusions involve two distinct isolates, this leads to the formation of vegetative heterokaryons (cells containing two different nuclear types). Usually these heterokaryotic cells are unviable and are either rapidly destroyed by a lytic process or else severely inhibited in their growth (Figure 1). This phenomenon is referred to as vegetative, somatic or heterokaryon incompatibility [1–4].

Incompatibility is caused by genetic differences between the two individuals at particular loci, called *het* or *vic* loci (for *het*erokaryon or *v*egetative *inc*ompatibility loci).

In a given species, the number of *het* loci is high (usually around 10) but varies from one species to another. *het* genes can either define allelic or non-allelic systems. In allelic systems, which are the most frequent type, incompatibility is triggered by the co-expression of two incompatible alleles of the same locus. In non-allelic systems, incompatibility is due to the interaction of two genes that belong to distinct loci. Usually, two different alleles are found in wild-type isolates at a given *het* locus, although *het* loci with more than two alleles have been described [1–4].

Figure 1



Representation of the heterokaryon incompatibility reaction. Two hyphae from genotypically different individuals fuse, and the heterokaryotic cell is rapidly destroyed by a lytic process; septa are obturated, which prevents the lethal reaction spreading to adjacent homokaryotic cells.

Heterokaryon incompatibility is a widespread phenomenon in filamentous fungi, but its biological significance remains obscure. Heterokaryon incompatibility may constitute an allorecognition (nonself-recognition) system that preserves fungal individuality by preventing heterokaryon formation between unlike individuals, and by limiting the horizontal transfer of deleterious genetic elements like mycoviruses, senescence plasmids or transposons [5,6]. It has been shown, however, that such cytoplasmic elements can sometimes be transmitted horizontally, even between incompatible isolates (for example, see [7]). An alternative perspective is to consider that the existence of *het* genes (genes that are deleterious at the heteroallelic state) is an evolutionary accident [3]. In this hypothesis, *het* genes do not exist to limit heterokaryosis, but simply represent genes in which polymorphisms have accumulated in wild-type populations, leading to the appearance of allelic variants. When reunited in the same cytoplasm, the natural variants have a deleterious effect. In this hypothesis, heterokaryon incompatibility would be somewhat analogous to other phenomena in which heteroallelism leads to deleterious effects, such as inviability or sterility of hybrids.

We review recent studies on the characterisation of heterokaryon incompatibility genes, which to date has only been achieved in *Podospora anserina* and *Neurospora crassa*, as well as work on the characterisation of the vegetative incompatibility reaction per se.

het gene function is not restricted to incompatibility

Different *het* genes have been isolated in both *N. crassa* and *P. anserina* (Table 1). In a number of cases, it is clear that *het* genes do not only function to limit heterokaryon formation; they also have a cellular role. The best example is probably the *N. crassa* mating-type locus that functions as a heterokaryon incompatibility locus during the vegetative stage. The MAT A-1 and MAT a-1 mating polypeptides encode transcriptional regulators that specify different cell types during the sexual stage, but their co-expression in a vegetative cell is lethal [8,9]. The mating and incompatibility functions are apparently mediated by different mechanisms. Although the mating function of MAT a-1 relies on the DNA-binding activity, the DNA-binding domain is dispensable for the incompatibility function [10]. This is also illustrated by the

fact that incompatibility associated with mating type is suppressed by mutations in the *tol* (for tolerant) gene, but *tol* mutations do not disrupt the sexual cycle [11,12•]. Another example is the *het-c* locus of *P. anserina* — *het-c* is involved in the *het-c*/*het-e* and *het-c*/*het-d* non-allelic incompatibility systems, and encodes a putative glycolipid transfer protein [13]. This protein is required for production of sexual spores. Finally, the *un-24* gene recently identified at the *het-6* locus in *N. crassa* encodes an essential enzyme — the large subunit of type I ribonucleotide reductase [14•,15]. For the other cloned *het* genes, either no inactivation experiments have been performed yet, or no phenotype associated with the disruption has been observed [14•,16,17]. On the basis of the above examples, we think that it is likely that most *het* genes display a cellular function.

Different fungal species have at least partially different sets of *het* genes. For instance, the mating-type locus that functions as a *het* locus in *N. crassa* is not a *het* locus in numerous other ascomycetes (see references in [12•]), and the *P. anserina* homologue of the *N. crassa het-C* gene apparently does not correspond to one of the known *het* loci of *P. anserina* [18].

The products of the *het* genes cloned so far appear to be extremely diverse in terms of sequence, function and localization. They obviously do not belong to a single gene family. However, Smith *et al.* [14•] have recently identified a common sequence motif in a number of genes involved in heterokaryon incompatibility. This motif is found in HET-6 and TOL proteins from *N. crassa* and in the HET-E protein from *P. anserina* (Figure 2). The identification of this common motif is the first indication that the mechanism of incompatibility for different *het* genes might be similar within and also between species.

Polymorphism at *het* loci: neutral or selected?

Minor mutational events can generate incompatible gene-to-gene interactions. For instance, several different single amino-acid replacements in the *P. anserina het-S* gene product give rise to a protein of the antagonistic [Het-s] specificity [19,20•]. Similarly, single amino-acid substitutions in the HET-C protein of *P. anserina* can modify its specificity in incompatibility [21].

An amino-acid replacement in the *N. crassa* MAT a-1 protein abolishes the incompatibility function without affecting mating activity [8]. So, we can assume that the emergence of the incompatibility function of MAT a-1 has been achieved by a single amino-acid substitution. On the basis of these examples, it appears that, at least experimentally, a single point mutation is sufficient to turn a given locus into a *het* locus. This idea is also supported by the observation that in *P. anserina*, mutants that display lethal gene-to-gene interactions can be selected. The mutations occurred at loci that were not known as *het* loci in wild-type isolates [22]. In other words, new *het* genes can be created by mutational events.

Table 1

Cloned *het* genes of *N. crassa* and *P. anserina*.

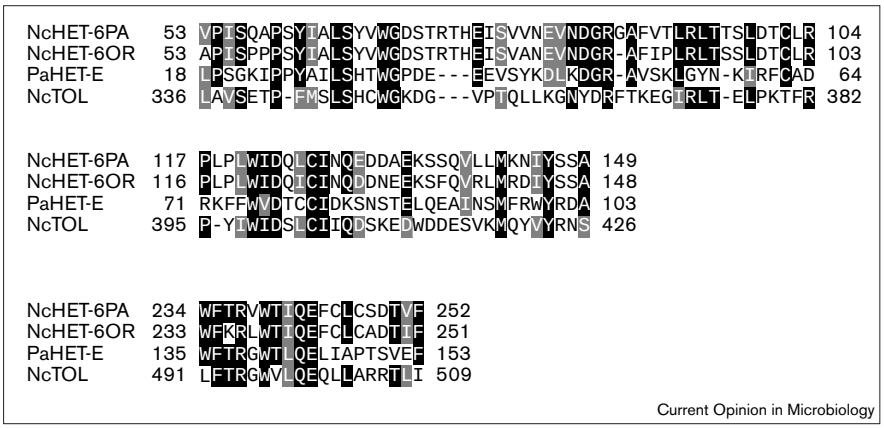
	Number of alleles	Size of encoded polypeptide	Protein feature	References
N. crassa				
mat A-1	/	293 aa	region of similarity to mat α1 of *Saccharomyces cerevisiae*	[8]
mat a-1	/	381 aa	HMG box	[9]
het-C	3	966 aa	signal peptide glycine-rich repeats	[17]
het-6	2	680 aa	region of similarity to TOL and HET-E	[14•]
un-24	2	929 aa	large subunit of type I ribonucleotide reductase	[15]
P. anserina				
het-s	3	289 aa	prion-like protein	[16,37]
het-c	4	208 aa	similarity to glycolipid transfer proteins	[13]
het-e	4	1056 aa	WD-40 repeats GTP-binding site region of similarity to TOL and HET-6	[38,39]

aa, amino acids.

Even if limited allelic difference can lead experimentally to incompatibility, in general, *het* alleles are characterised by a high degree of divergence. This is particularly striking for the *het-6* gene. The products of the two *het-6* alleles are only 68% identical, which is extremely low for two allelic forms of the same locus [14•]. This is also true to a lesser extent for the products of the three *het-C* alleles of *N. crassa*. Overall, they display 86% identity, but identity drops to less than 25% in the region defining allele specificity [23]. In a few examples, polymorphism at *het* loci appears to be correlated with the existence of genomic rearrangements in the region in which the *het* gene is located [14•,19]. These rearrangements might favour the accumulation of polymorphism by suppressing recombination at these loci.

To understand the biological significance of heterokaryon incompatibility, it is crucial to determine whether the polymorphism at *het* genes was generated and maintained under neutrality, or whether positive selection is acting on these loci to promote divergence, as it does on other self-/nonself-recognition systems. So far, the analyses that have been conducted on this point are limited and have provided contradictory results. Strong evidence that positive selection may be acting on *het* genes came from Wu *et al.*'s [24] analysis of *het-C* variability in *N. crassa* populations and in different related species. Their analysis suggested that *het-C* is under balancing selection. The three allelic types have equilibrated distribution in natural *N. crassa* populations. Most importantly, polymorphism at *het-C* has apparently been maintained over a very long period (about 36 million years) through several speciation events. Trans-species polymorphism is a characteristic that *het-C* shares with the

Figure 2



Sequence motifs common to several proteins controlling heterokaryon incompatibility. The amino-acid position is given for each sequence: the *N. crassa het-6*^{PA} allele product (NcHET-6PA), the *het-6*^{OR} allele product (NcHET-6OR), the *P. anserina* HET-E protein (PaHET-E) and the *N. crassa* TOL protein (NcTOL). Three blocks of higher similarity are shown. Residues that are identical in at least three of the four sequences are boxed in black, residues that are similar in at least three of the four sequences are boxed in grey. Alignment was performed using the CLUSTALW program [40] and the BOXSHADE server.

S self-incompatibility locus of flowering plants and the major histocompatibility complex loci. Conversely, a global survey by Milgroom and Cortesi [25••] of *het* allele distribution at six different loci in natural populations of *Cryphonectria parasitica* showed that, in most cases, allele distribution was very disequilibrated, which does not support the hypothesis that these loci are under balancing selection. Allele comparison at the *het-c* locus of *P. anserina* revealed rapid evolution at the amino-acid level, which also suggested that positive selection might be acting on this locus. However, Saupe [4] has proposed alternative hypotheses that might explain this rapid evolution without positive selection.

An oddity: a *het* gene encoding a fungal prion

The *het-s* locus of *P. anserina* exists as two incompatible alleles — *het-s* and *het-S*. This locus displays a characteristic feature that distinguishes it from all known *het* loci. Strains of the *het-s* genotype can display two distinct phenotypes: the neutral [Het-s*] phenotype ([Het-s*] strains are compatible with [Het-S] strains), and the reactive [Het-s] phenotype ([Het-s] strains are incompatible with [Het-S] strains) [26,27]. The [Het-s] character is transmitted as a non-Mendelian cytoplasmic element, and the [Het-s] phenotype is infectious. On anastomosis (vegetative cell fusion) with a [Het-s] strain, a neutral [Het-s*] strain rapidly acquires the [Het-s] phenotype. Coustou *et al.* [28] proposed that [Het-s] is a prion analogue. It shares the characteristics of the [PSI] and [URE3] yeast prions [29]. Namely, the [Het-s] character is transmitted through cytoplasmic contact, and its maintenance and propagation requires a functional *het-s* gene. Overexpression of the *het-s* gene strongly increases the frequency of *de novo* appearance of [Het-s] [28]. As for other prion proteins, transition to the prion state renders the *het-s* protein prone to aggregation and more resistant to proteinase K digestion ([28]; V Coustou, SJ Saupe, unpublished data). An amino-terminal 25-amino-acid polypeptide of HET-s is sufficient to allow prion propagation, as a strain expressing this peptide is capable of converting a [Het-s*] strain to the [Het-s] phenotype [20•].

For the yeast prions, transition to the prion state leads to a loss of function of the protein. In contrast, for [Het-s] the prion form is the reactive form of the protein that specifically interacts with HET-S to trigger incompatibility. In that way, transition to the prion state leads to a gain of function. This is true, however, only if the sole function of the HET-s protein is to limit heterokaryosis. It remains possible that HET-s has a cellular function, which is lost upon transition to the prion state.

Non-allelic incompatibility in *P. anserina*: the link with adaptation to starvation

Biochemical analyses have shown that in *P. anserina*, during the incompatibility reaction triggered by non-allelic systems (*het-r*/*het-v*, *het-c*/*het-e* and *het-c*/*het-d*), a number of degradative activities including laccases and proteases appear [30,31]. Moreover, a number of genes are specifically induced during this cell-death reaction and have been called *idi* genes (for *i*nduced *d*uring *i*ncompatibility). So far, three *idi* genes have been characterised [32]. They encode small polypeptides with signal sequences that are highly expressed during incompatibility. In addition, a protease specifically induced during the incompatibility reaction has recently been purified, and the corresponding gene, *psp*A, has been isolated. *psp*A encodes a subtilisin-like serine protease (C Clavé, M Paoletti, unpublished data). Its expression is not only induced during the incompatibility reaction, but also upon carbon and nitrogen starvation.

Similarly, expression of several *idi* genes is also induced upon carbon and/or nitrogen starvation (C Clavé, unpublished data). Inactivation of *psp*A leads to a number of developmental defects — production of aerial hyphae is impaired and the strain is female sterile. These results support a previous hypothesis suggesting that non-allelic vegetative incompatibility uses components of a cellular pathway that is involved in adaptation to starvation in order to provide nutrients required for the development of female reproductive structures [33]. This hypothesis was

based on the observation that mutations suppressing non-allelic incompatibility cause female sterility [34–36].

Conclusions

In spite of important progress achieved in the molecular characterisation of *het* genes, two main questions remain unanswered. How is molecular recognition of unlike *het* products achieved? And how is the associated cell-death reaction mediated? Moreover, the biological significance of this phenomenon remains an open issue as it has not been clearly established whether or not positive selection is promoting the accumulation of polymorphism in *het* genes. Recent work on incompatibility has, however, opened some interesting perspectives. Existence of a domain common to different genes involved in incompatibility suggests that there is a possible relation between different *het* systems, which might provide a global understanding of these systems. It is, however, unlikely that all *het* systems function in a similar manner. For instance, the *het-s* system might represent a particular situation in which the cell-death reaction is related to the prion-like properties of the *het-s* gene product.

Of particular interest is the work performed on non-allelic incompatibility in *P. anserina* that suggests that incompatibility represents an inappropriate activation of an existing cellular pathway controlling adaptation to starvation. This work indicates that the *het* genes and the cell-death pathway that they activate may not be specific to incompatibility. It remains to be established whether the appearance of the *het* function at these loci is accidental, or whether positive selection has recruited this pathway to limit heterokaryosis.

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This paper describes the molecular cloning and characterisation of the *het-6* region. Surprisingly, this study reveals that this region contains two distinct but tightly linked genes, each of which is capable of triggering a cell-death reaction when introduced in the antagonistic genetic background. One encodes a gene showing a region of similarity with HET-E from *P. anserina* and TOL from *N. crassa*, the second encodes the large subunit of type I ribonucleotide reductase [15]. Moreover, this work describes an unexpected genetical complexity, as it is proposed that these two incompatibility systems could in fact be of the non-allelic type.

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