A gene suppressing the allelic protoplasmic incompatibility specified by genes at five different loci in *Podospira anserina*

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Protoplasmic incompatibility (PI) in fungi is a phenomenon of immediate cell destruction resulting from the fusion of cells of unlike genotypes. In *Podospira anserina*, five loci contain genes determining PI as the result of allelic gene interactions. The present work shows that mutations of a gene called *modD* inhibit allelic PI irrespective of the locus responsible for the phenomenon. Other *modD* mutations show differential actions on the allelic interactions and on the expression of the two allelic incompatibility genes of the same locus. These results thus suggest that the *modD* gene product is involved in the trigger mechanism of allelic PI. The *modD* gene acts in differentiation: it has been previously shown to control proteolytic activities required for exit from stationary phase. There is thus a connection between this function and allelic PI. This leads to the suggestion that allelic incompatibility genes are involved in the control of stationary phase exit to promote differentiation.

Introduction

Protoplasmic incompatibility (PI) in fungi manifests itself as a process of cell destruction that immediately follows the fusion of hyphae of unlike genotypes (Garnjobst & Wilson, 1956). In *Neurospora crassa*, PI results from the interaction of the allelic genes of about ten identified loci, including that of the mating type (Mylyk, 1976; Perkins *et al.*, 1982). In *Podospira anserina*, PI was first described using the term 'barrage' due to a secondary effect of PI: the production of an unpigmented mycelial excrescence on the confluence area of mycelia cultured on solid media (Rizet, 1953; Rizet & Esser, 1953; Bernet, 1965). Five loci for allelic incompatibility and nine for non-allelic incompatibility have been identified in *P. anserina* (Fig. 1).

Several hypotheses have been proposed to explain the biological significance of PI and to account for its widespread occurrence in fungi. Due to its action on vegetative cells, PI has been interpreted as a mechanism protecting individual mycelial clones against cytoplasmic infections (Caten, 1972) or preventing exploitation of heterokaryons by nuclei that are non-adaptative in homokaryons (Hartl *et al.*, 1975). In *P. anserina*, protoperithecia and microconidia can display PI in their interaction and this leads to a general reduction of the

interfertility between wild-type isolates (Bernet, 1965, 1967). PI has hence been interpreted as a breeding system limiting the outbreeding supposed to result from the action of the mating type locus (Esser, 1971).

The selection, in this laboratory, of mutations inhibiting non-allelic PI has led to a radically different hypothesis. Genetic studies showed that the mutation of two genes (*modA* and *modB*) is required to suppress PI, irrespective of the non-allelic gene interaction (*C/D*, *C/E* ... or *R/V*) responsible for the phenomenon (Boucherie & Bernet, 1974). *modA modB* mutants do not produce aerial organs (protoperithecia and aerial hyphae), due to the action of the mutations on vegetative cells (Boucherie & Bernet, 1980). Cell death in fungi is a process of cell destruction associated with the development of aerial organs, especially proto- and perithecia. Work with *P. anserina* suggested that cell death is a requirement for the development of aerial organs by mycelium and is controlled by the *modA* and *modB* genes (Bernet, 1991). The action of *modA modB* mutations therefore established a connection between non-allelic PI and cell death. Accordingly, it was suggested that non-allelic PI results from the dysregulation (as a consequence of mutations in the *c* to *v* non-allelic incompatibility genes leading to the *C/D* ... to *R/V* lethal interactions) of degradative activities (especially proteases) that are developmentally controlled so as to transform stationary-phase cells into a source of nutrients for developing fruiting bodies (Bernet, 1992).

Abbreviation: PI, protoplasmic incompatibility.

Fig. 1. Allelic and non-allelic incompatibility genes in *P. anserina*. The five allelic loci (*b*, *q*, *s*, *z* and *v*) and five of the nonallelic incompatible loci (*c*, *d*, *e*, *r* and again the *v* locus) come from the study of 16 wild-type strains (Bernet, 1965, 1967). Non-allelic incompatibility loci *f*, *g*, *k* and *l* were identified following the selection of induced mutations in the reference strain (Delettre & Bernet, 1976). In non-allelic systems the genes encoding diffusible products are in the upper line (*V*, *C*, *G* and *L*) and those responsible for nondiffusible products (Bernet, 1965; Labarère *et al.*, 1974) in the lower line.

To date, allelic incompatibility has been mainly studied in *N. crassa*. A mutation (*tol*) has been identified that suppresses the PI resulting from the mating-type allele interaction (Newmeyer, 1970). This mutation did not help to interpret allelic PI as it has no phenotypic consequence in itself and no action on any allelic interaction other than that involving the mating-type alleles (Newmeyer, 1970; Perkins, 1988). There have been few studies of allelic PI in *P. anserina*. However, the *V* gene (a single cistron) is involved in both allelic (*V/V*₁) and non-allelic (*V/R*) gene interaction (Fig. 1), which suggests that there is a connection between allelic and non-allelic PI. Furthermore, both allelic and non-allelic PI were suppressed by β -phenylpyruvic acid, a competitive protease inhibitor (Geratz, 1965) which acts strongly *in vitro* on the proteolytic activities responsible for non-allelic PI (Delettre *et al.*, 1978).

Mutants of a gene, *modD*, have been identified by their interactions with mutants of the *modB* gene involved in the expression of non-allelic PI. The *modD* mutations inhibit differentiation. Studies of *modD* mutations suggested that they affect exit from the stationary phase, a step which is a prerequisite for the transition from the vegetative to the developmental phase (Labarère & Bernet, 1979a; Durrens *et al.*, 1979; Durrens & Bernet, 1982). Furthermore, comparative studies of wild-type and *modD* strains showed that exit from the stationary phase to initiate differentiation involves the action of proteolytic activities controlled by the *modD* gene. This has led therefore to the hypothesis of a possible connection between non-allelic PI and exit from the stationary phase (Labarère & Bernet, 1979b). There was no conclusive evidence for this notion, as non-allelic incompatibility genes had not been demonstrated to be involved in stationary phase exit or to act on the *modD* gene.

The present work shows that there is a connection between the action of the *modD* gene and allelic PI, as *modD* mutations suppress the expression of allelic incompatibility genes. Furthermore, the action of the *modD* gene on development suggests that the genes of allelic incompatibility loci are involved in differentiation by controlling exit from the stationary phase.

Methods

Organism. *Podospora anserina* is fundamentally a heterothallic ascomycete that reproduces by means of binucleate ascospores which give the heterokaryotic (+/−) self-fertile mycelia found in its natural environment (secondary homothallism: Ames, 1934). Due to the occasional production of uninucleate spores, it is possible to obtain the homokaryotic hermaphroditic self-sterile (+ or −) mycelia needed for genetic studies and for barrage and PI analysis.

The developmental products of vegetative mycelium and the action of *modD* mutations. Vegetative mycelium mainly grows by the activity of apical cells. The hyphal cells, after they have been committed to stationary phase (Durrens & Bernet, 1982), are the source of developmental products, which are: aerial hyphae (AH), distorted and slow-growing hyphae called secondary ramifications (SR) and, only when colony growth is stopped at the limit of the culture substrate, protoperithecia that support the female cycle up to spore production. Protoperithecia and perithecia develop outside the growth substrate. Mutations of the *modD* gene (Durrens & Bernet, 1982) suppress the production of all three hyphal cell derivatives (*hcd* phenotype).

Incompatibility genes. Sixteen wild-type strains were studied. Five loci involved in allelic incompatibility were identified. Each locus was polymorphic for two active genes (*B*₁/*B*₂, *s*/*S* ... *Z*₁/*Z*₂) with the exception of the *s* locus, which has a neutral allele *s*₀ found only in one wild-type strain (Bernet, 1965, 1967). Non-allelic incompatibility genes are designated by capital letters (*C*, *D* ...) when they are involved in lethal recombinant interactions (*C/D*, *R/V* ...) and by lower-case letters when in non-lethal combinations (*c/d* ... *f/g* ... *r/v*), irrespective of the wild-type strain from which they originated.

***modD* mutations.** Three mutations of the *modD* gene have been described. *modD-1* was isolated from a strain of complex genotype which was lethal due to a mutation inhibiting *modB* gene expression (Labarère & Bernet, 1979a). *modD-2* and *-3* were identified as suppressing a lethal *modB* mutation (Durrens *et al.*, 1979). The *modD* mutations were tested for dominance or recessiveness in the context of their screening (autolysis suppression): *ModD-1* is recessive; *modD-2* and *-3* are dominant. All *modD* mutations were outcrossed for the determination of their own phenotypic effects. All original *modD* mutations inhibit the production of hyphal cell derivatives and ascospore outgrowth (Table 1). The inhibitory action of the *modD-1* mutation on ascospore outgrowth and production of secondary ramifications (the only hyphal cell derivatives that develop inside the culture medium) is suppressed in media supplemented with β -phenylpyruvic acid (Labarère & Bernet, 1979a, b), a competitive proteinase inhibitor (Geratz, 1965; Barnett, 1967).

Reversions of *modD-2* and *modD-3* mutations. The procedure for screening for revertants of the dominant *modD* mutants has been described (Durrens, 1982). Growth of autolytic strains of *C E modD-2* or *-3* genotypes stops during development and most of cells lyse as a consequence of the *C/E* interaction. In *C E* strains, growing mutant sectors develop from viable cells that generally result from mutations of the *E* gene (*E/e* mutations suppressing the lethal interaction with the *C*

Table 1. *Ascospore outgrowth and the production of hyphal cell derivatives*

				Production of hyphal cell derivatives		
		Ascospore outgrowth		Secondary ramifications		
	Dominance/ recessiveness	Control	+ β -PPA* (0.03 M)	Aerial organs	Control	+ β -PPA* (0.04 M)
<i>modD</i> ⁺		+	—	+	+	—
<i>modD-1</i> †	Recessive	— (ascospore killed)	+	—	—	+
<i>modD-2</i> ‡	Dominant	+	—	—	—	—
<i>modD-3</i> }		(dormancy)				

* β -Phenylpyruvic acid.

† Labarère & Bernet (1979a, b).

‡ Durrens *et al.*, (1979); Durrens & Bernet (1982).

gene). Due to the inhibitory action of the *modD* mutations (inhibition of the growth renewal from stationary-phase cells), the *e* mutant sectors from the *C E modD* strains also contain, in most cases (80%), a reversion of the *modD* mutation. Phenotypically, the *modD* revertants are identified by the restoration of protoperithecium and aerial hyphae production (*hcd*⁺ phenotype). Each of the three *modD-2* (*-2*₁, *-2*₂, *-2*₃) and the two *modD-3* (*-3*₁, *-3*₂) revertants used in this work were crossed with the reference wild-type strain. The absence of strains of the *hcd* phenotype, among more than 200 fl strains examined for each cross, indicated that the reverse mutations mapped in, or close to, the *modD* locus.

Strain genotypes. The *modD* mutations (*-1*, *-2*, *-3*) were screened from the reference strain, which contains the active allelic incompatibility genes *B*₁, *Q*₁, *s*, *V* and *Z*₁. For barrage and PI analysis, the *modD* strains were confronted with a wild-type (*modD*⁺) strain differing from the reference strain by the presence of one of the antagonistic alleles, *B*₂, *Q*₂, *S*, *V*₁ or *Z*₂.

Determination of PI by barrage production or cell lysis. Implants from the strains to be confronted were deposited on solid medium at a distance of 3 cm, so that colony confluence occurred within 2 to 3 d. PI manifests itself by the production of an unpigmented mycelium excrescence called 'barrage' (Rizet, 1953). Barrage is due to the growth renewal of the cells situated behind the lysed cells at the tips of hyphae (Beisson-Schecroun, 1962). *modD* mutations (*-1*, *-2*, *-3*) suppress the production of a barrage when they are present in both matched strains by inhibition of the growth renewal of hyphal cells (Durrens & Bernet, 1982). Accordingly, no barrage is produced by strains containing *modD-1*, *-2* or *-3* mutations and microscopic examination in the confluence area of two such matched strains is thus necessary to detect PI. To facilitate this observation, the strains to be confronted were grown on a cellophane disk placed on culture medium to obtain a single monolayer of hyphae. Cells were examined less than 12 h after colony meeting. The PI reaction starts with the appearance of large vacuoles, continues with a violent agitation of newly formed cytoplasmic granules and terminates by protoplasm extrusion (Rizet & Schecroun, 1959; Bernet, 1965). These abnormal features are specific to PI, as the neighbouring healthy cells of the same age have dense and homogeneous cytoplasmic contents. It is not possible to assess the times of individual anastomoses (between 2 and 10 h) and thus to know whether the PI reaction is normal (cell destruction in 2–3 h), retarded or of weak intensity. When one of the matched strains is *modD*⁺ and the other *modD*, PI is accompanied by the production of a barrage (mycelial

excrescence by the *modD*⁺ strain) and is therefore easily detected. A *C/E* non-allelic incompatibility gene interaction, which is not suppressible by the *modD* mutation (Durrens *et al.*, 1979), was used as the positive control for the barrage test.

Results

Effect of the modD mutations on the expression of the allelic incompatibility genes of the reference strain

The reference strain with the *B*₁ *Q*₁ *s* *V* *Z*₁ genotype contained the *modD* mutations. All three original *modD* mutants and five revertants from the two dominant *modD* mutants (*modD-2*, *-3*) were confronted for the production of a barrage with wild-type (*modD*⁺) strains differing from the reference strain by the presence of the active allelic incompatibility genes *B*₂, *Q*₂, *S*, *V*₁ or *Z*₂ (see Fig. 1). In the absence of the *modD* mutations all allelic interactions tested (*B*₁/*B*₂, *Q*₁/*Q*₂, *s*/*S*, *V*/*V*₁, or *Z*₁/*Z*₂) will result in the production of a barrage morphologically similar to that produced by the non-allelic incompatibility gene interactions. The results, summarized in Table 2, show that there was no barrage in several of the strain confrontations involving allelic gene interactions. This contrasts with the non-allelic *C/E* barrage used as a control for barrage production. The dominant mutations *modD-2*, *-3* were more active in barrage suppression than the recessive *modD-1* mutation. In some strain confrontations, barrage was expressed by a thin and discontinuous mycelial excrescence (attenuated barrage). The *modD-2* mutation completely inhibited the barrage expressed by *B*₁, *Q*₁, *s* and *Z*₁ and partially inhibited that which involved the *V* gene.

The *modD-1* mutation apparently acted only on the *B*₁, *s* and *V* genes, and this resulted in the attenuation of the barrage expressed by these genes. Revertants from

Table 2. Barrage production between the reference strain *Ce B₁Q₁sVZ₁* containing a *modD* mutation and a wild-type strain (*modD*⁺) differing from the reference strain by the presence of the antagonistic allelic incompatibility gene *B₂*, *Q₂*, *S*, *V₁* or *Z₂* or the *E* non-allelic incompatibility gene

+, Barrage; a, barrage attenuated; −, barrage inhibited

<i>modD</i> mutation	<i>modD</i> ⁺ strain:					
	<i>B₂</i>	<i>Q₂</i>	<i>S</i>	<i>V₁</i>	<i>Z₂</i>	<i>CE</i> (<i>C/E</i> interaction)
<i>modD</i> ⁺	+	+	+	+	+	+
<i>modD-1</i>	a	+	a	a	+	+
-2	−	−	−	−	−	+
-3	−	a	−	−	−	+
-21	+	−	+	−	+	+
-22	+	−	+	+	+	+
-23	+	+	+	+	+	+
-31	−	+	+	+	+	+
-32	+	+	+	+	+	+

modD-2, -3 no longer suppressed barrages that were suppressed by the original mutants. It thus appears that reversion of *modD* towards normal production of hyphal cell derivatives (*Hcd*[−] versus *Hcd*⁺ phenotype) is accompanied by a concomitant restoration of barrage production.

Comparison of the effects of *modD* mutations on the expression of the two alleles of each allelic incompatibility locus

In the *modD-1* mutant and in *modD-2₁* and -2₂ revertants, the allelic incompatibility genes *B₁*, *Q₁*, *s*, *V* or *Z₁* of the reference strain were replaced by their antagonistic alleles *B₂*, *Q₂*, *S*, *V₁* or *Z₂*, respectively, by outcrossing. The five new recombinant genotypes for the *modD* mutants were thus *B₂ Q₁ s V Z₁ ...* to *B₁ Q₁ s V Z₂*. Each recombinant strain was matched to the wild-type (*modD*⁺) reference strain (*B₁ Q₁ s V Z₁*). Barrage production in each of these confrontations (for instance *modD*⁺ *B₁/modD B₂*) was compared (Table 3) with that previously found (*modD B₁/modD*⁺ *B₂*) with the same allelic incompatibility gene interaction (Table 2). The action of the *modD* mutations was not locus specific but was allele specific: the *modD-2₁* mutation inhibited expression of the *S* and *V* genes in the barrage production and not that of the corresponding alleles, *s* and *V₁*.

Effect of *modD* mutations on allelic incompatibility

To assess whether the absence of a barrage in *modD/modD*⁺ strain confrontations is due to a suppression of PI (and not to the inhibition of the barrage itself) cells were examined in the confluence area of strains containing identical *modD* mutations. In the absence of the *modD*

Table 3. Comparisons of the effects of three *modD* mutations on the expression in barrage production of the two alleles of each allelic incompatibility locus

+, Barrage; a, barrage attenuated; −, barrage inhibited. The effects of the *modD* mutations on *B₁*, *Q₁*, *s*, *V* and *Z₁* alleles come from the results of Table 2.

<i>modD</i> mutation	Allele pair:				
	<i>B₁ : B₂</i>	<i>Q₁ : Q₂</i>	<i>s : S</i>	<i>V : V₁</i>	<i>Z₁ : Z₂</i>
<i>modD-1</i>	a : a	++	a : a	a : a	++
<i>modD-21</i>	++	−−	+:−	−:+	++
<i>modD-22</i>	++	−−	++	+:a	++

Table 4. Presence of lysing and disintegrated cells in the confluence area of matched strains containing identical *modD* mutations and differing in a pair of allelic incompatibility genes

+, Lysed cells; −, no lysed cells.

Mutation	Allelic incompatibility gene interaction:				
	<i>B₁/B₂</i>	<i>Q₁/Q₂</i>	<i>s/S</i>	<i>V/V₁</i>	<i>Z₁/Z₂</i>
<i>modD-1</i>	−	+	−	−	+
<i>modD-2</i>	−	−	−	+	−
<i>modD-21</i>	+	−	−	−	+

mutations, it is known (Beisson-Schecroun, 1962; Bernet, 1965) that the confluence area of strains carrying antagonistic incompatibility genes (allelic or non-allelic) contains lysing or disintegrated cells. These dead cells are associated with anastomoses between hyphae from the opposite strains. This investigation (results in Table 4) showed that there were no dead cells in most of the

confluence areas despite the production of anastomoses. This showed that the suppression of allelic barrages in *modD*⁺/*modD* strain confrontations (Tables 2 and 3) was due to inhibition of PI. Furthermore, suppression of the expression of a single allele was sufficient to inhibit PI. The *modD-21* mutation inhibited *S/s* and *V/V₁* allelic PI (Table 4) due to its action on the *S* and *V* alleles alone (Table 3). *B₁/B₂*, *S/s* and *V/V₁* allelic PI were suppressed by the *modD-1* mutation. This suggests that the presence of an attenuated barrage in the *modD-1/modD*⁺ strain confrontations involving these interactions (Table 2) is due to the recessiveness of the mutation: in the *modD/modD*⁺ heterokaryotic cells that result from the fusion of *modD-1* and *modD*⁺ hyphae, the presence of the *modD*⁺ gene is thought to restore, at least partially, the action of the allelic incompatibility gene carried by the *modD-1* strain.

Discussion

Allelic PI is presumed to result from the interactions of proteins encoded by corresponding alleles (Rizet & Schecroun, 1959; Wilson *et al.*, 1961; Turcq *et al.*, 1991). This work shows that the *modD* gene product is involved in allelic PI. Indeed (Table 2), the dominant *modD-2*, *-3* mutations suppress barrage irrespective of the allelic gene interactions (*B₁/B₂* to *Z₁/Z₂*). This suppression thus establishes a significant difference between allelic and non-allelic PI (Durrens *et al.*, 1979; Durrens, 1982) since barrage production due to non-allelic gene interactions is not inhibited by the *modD* mutations. The general inhibition of allelic PI by the dominant mutations *modD-2* and *modD-3* suggests that the *modD* gene exerts a negative control on the phenomenon. Other *modD* mutations (*modD-1*, *-21-22* or *-31*) suppress only a part of the allelic barrages and the allelic interactions inhibited vary according to the *modD* mutations (Table 2). This leads to the presumption that the *modD* gene product does not act downstream of those of the allelic incompatibility genes in the general allelic PI pathway and is a common factor of the trigger mechanism of allelic PI. Allelic PI appears therefore to involve the action of the *modD* gene presumptively as the result of the complementary actions of the alleles of each incompatibility locus. Previous work showed that allelic PI (like non-allelic PI) is due to the expression of particular proteases (Delettre *et al.*, 1978). It may be deduced, from the suppression of allelic PI by *modD* mutations, that the *modD* gene is involved in the control (probably negative) of these proteases.

Previous work indicated that the *modD* gene controls differentiation presumptively by affecting exit from the stationary phase (Durrens & Bernet, 1982). It was also

shown that exit from the stationary phase (and ascospore germination) requires the action of proteolytic activities, the production of which depends on the *modD* gene (Labarère & Bernet, 1979a). Furthermore, it has been deduced, from the study of non-allelic PI (*C/D*, *F/G* ... *R/V* interactions), that the *modB* gene is responsible for a general repression of the proteolytic activities (and their associated catabolite activities) specific to non-allelic PI (Bégueret & Bernet, 1973; Boucherie *et al.*, 1976a). Consistent with this notion, a recessive mutation of *modB* results in an autolytic mutant (viable and growing only on a medium supplemented with β -phenylpyruvic acid) in which the protein synthesis specific to non-allelic PI (almost 50 polypeptides) is constitutive (Boucherie *et al.*, 1976b, 1981; Boucherie, 1983). *modD* mutations were screened either by action on a *modB* mutation (*modD-2*, *-3*) or the suppression of a mutation inhibiting *modB* (*modD-1*). This indicates therefore a connection between the actions of *modD* and *modB* genes (Labarère & Bernet, 1979a; Durrens *et al.*, 1979). The proteolytic activities associated with the action of the *modD* gene in stationary phase exit (and ascospore outgrowth) and in allelic incompatibility (the present work) are presumably those that are normally repressed by the *modB* gene. The *modB* gene therefore appears to be a common repressor for the lytic activities expressed by allelic and non-allelic PI. Accordingly, there is probably no difference in the biochemistry of allelic and non-allelic PI other than the trigger mechanism (allelic or non-allelic interaction).

The demonstration that *modA modB* double mutations (which together suppress non-allelic PI) inhibit cell death has led to the recognition of a connection between non-allelic PI and cell death (Bernet, 1991). Furthermore, it was shown that the genes of the *c-v* non-allelic incompatibility loci are also involved in the control of cell death via the additive, redundant and minor effects of the gene interactions *c/e* ... *f/g* ... *r/v* (homologous to the *C/E* ... *F/G* ... *R/V* lethal autolytic interactions). It has therefore been presumed that non-allelic PI is an artefact due to the dysregulation of the degradative activities (especially the proteases) evolved to transform the stationary-phase cells of fertile cultures into a source of nutrients for developing fruiting bodies (Bernet, 1992).

The specific suppression of allelic PI by *modD* mutations (this work) indicates a connection between allelic PI and exit from the stationary phase to promote differentiation. There is no demonstration to date that the genes of the allelic incompatibility loci are involved in the control of stationary phase exit via their action on the *modD* gene. However, indirect evidence for such involvement comes from the comparison of the original *modD-2*, *-3* mutants to their revertants (Table 2). Indeed, it is a general observation that normal and subnormal expression of *modD* alleles in differentiation correlates

Fig. 2. Hypothetical roles of the allelic and non-allelic incompatibility genes in the developmental cycle of *P. anserina* (for explanations see text). AH, aerial hyphae; SR, secondary ramifications.

with normal and subnormal expression of allelic PI. This supports the idea that allelic PI may be deviant expression of the function required to exit from the stationary phase. This suggests therefore that the genes of the allelic incompatibility loci might also be involved in this function. Since each allelic incompatibility gene can interact with the *modD* gene, it is possible that these genes exert redundant actions on the *modD* gene responsible for the control of the proteolytic activities required to disrupt stationary phase. Gene redundancy is assumed to be a general phenomenon in the control of complex developmental processes (Nasmyth, 1990).

A possible model for the postulated action of the allelic incompatibility genes of *P. anserina* is given by the genetics of the G1-cyclins of *Saccharomyces cerevisiae* (Reed, 1991). The G1-cyclin genes exert redundant functions, as the action of only one gene is sufficient to induce transition to the S phase. Gene redundancy is used to link exit from the G1 phase to several parameters of cell physiology (nutritional conditions, cell cycle phase, cell size, etc.). It is possible that the allelic incompatibility genes of *P. anserina* participate in the control of a phenomenon developmentally controlled to occur during stationary phase and programmed to affect the few vegetative cells that are transformed into the stem cells used for differentiation. The hypothetical model for the action of the allelic incompatibility genes in the developmental cycle of *P. anserina* is summarized in Fig. 2, together with an overview of a preceding

hypothesis on the action of the non-allelic incompatibility genes. Some features of the postulated actions of allelic and non-allelic incompatibility genes may be noted. (1) Incompatibility genes, whether allelic or non-allelic, control various functions in stationary-phase cells: exit from the stationary phase, entry into a developmental pathway (allelic incompatibility genes) or cell death (non-allelic incompatibility genes). In other words *P. anserina* PI (allelic or non-allelic) may be considered as the deviant expression of two stationary-phase functions that are mutually exclusive: differentiation and vegetative cell death. (2) Incompatibility genes are assumed to exert redundant regulatory functions: redundancy in the action of individual genes (allelic incompatibility genes) or redundancy of specific gene interactions leading to two complementary multigene systems (non-allelic incompatibility genes). (3) Allelic and non-allelic incompatibility genes act upstream from the regulatory genes *modA* and *modB* (for non-allelic incompatibility genes), *modD* and *modB* (allelic incompatibility genes) that are responsible for the transcriptional shifts associated with PI expression. Experiments are in progress to establish whether there is a direct link between *modD* and the allelic incompatibility genes outside their common actions on allelic PI. Furthermore, the *modB* and *modD* genes have been cloned and their nucleotide sequences will shortly be determined (J. Bégueret, personal communication).

In *P. anserina*, production of interfertility barriers and

fl lethal strains are the result of the actions of allelic and non-allelic incompatibility genes alone (Bernet, 1965, 1967). PI genes are thus potent factors that may restrict genetic exchange in the wild. This has led to the hypothesis that incompatibility genes have evolved to produce isolating mechanisms used to counterbalance the outbreeding action of the mating-type locus (Esser, 1971; Esser & Blaich, 1973). This presumptive function of the PI genes appears gratuitous. Indeed, it is known that wild-type isolates of *P. anserina* in their natural environment are scattered and self-fertile (Bernet *et al.*, 1960). Furthermore, potential intercrossing is limited, as *P. anserina* microconidia (the male gametes) are not airborne (Bernet, 1965). In Esser's hypothesis (1971), PI is thus suspected to have evolved for the control of functions that are already fulfilled by other genetic systems, in particular the secondary homothallism due to the normal production of $+/-$ heterokaryotic ascospores. However, the proposition that PI is an artefact leads to an apparent paradox. The incompatibility genes have redundant and minor (and consequently nearly undetectable) effects in their presumptive genuine functions (cell death or stationary-phase exit) and major effects in their deviant expressions (non-allelic or allelic PI) as the results of single mutations.

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