

## COMMENTARY

## Internuclear Recognition: A Possible Connection between Euscomycetes and Homobasidiomycetes

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Debuchy, R. 1999. Internuclear recognition: A possible connection between euscomycetes and homobasidiomycetes. *Fungal Genetics and Biology* 27, 218–223. In the heterothallic Euscomycete *Podospora anserina*, fertilization is followed by mitotic divisions of parental nuclei, resulting in a plurinucleate stage. Nuclei of opposite mating type then recognize one another and form dikaryons which undergo karyogamy and meiosis. The internuclear recognition is a characteristic feature of the sexual cycle of filamentous Euscomycetes and is controlled by mating-type genes. These genes encode transcription factors which have nucleus-limited expression. It is assumed that this characteristic allows nuclei of different mating type to express a pattern of specific proteins directly involved in internuclear recognition. As the molecular nature of these proteins is unknown, the exact mechanism of internuclear recognition remains elusive. Schuurs *et al.* (1998) have proposed that internuclear distance affects gene expression through a pheromone/receptor system in the Homobasidiomycete *Schizophyllum commune*. This model can be applied to internuclear recognition in *P. anserina* and can account for all data resulting from genetic analyses. © 1999 Academic Press

**Index Descriptors:** development; internuclear recognition; mating-type genes; pheromone/receptor system; plurinucleate cell; *Podospora anserina*.

Successful production of offspring in filamentous fungi depends on internuclear recognition, a process whereby nuclei contributed by parents of different mating type,

during cell fusion, coexist in a single cell, in a strict ratio of 1:1, prior to karyogamy and meiosis. In heterothallic filamentous Euscomycete fungi, such as *Podospora anserina*, the process of fertilization brings together a male nucleus into a female organ containing several nuclei of the opposite mating type (Fig. 1A). The male nucleus will undergo several mitotic divisions inside plurinucleate cells (the ascogonial cells) which also contain the female nuclei (Fig. 1B). Within the ascogonial cell, nuclei of opposite mating types recognize each other (Fig. 1C) and migrate to specialized hyphae (the ascogenous hyphae), where they divide mitotically, maintaining a strict ratio of 1:1 of each parental nucleus (Fig. 1D). Eventually pairs of nuclei fuse and meiosis ensues immediately (Fig. 1E), resulting in the expected Mendelian ratio of each mating-type allele in the progeny. A characteristic feature, and a major challenge in our understanding of the life cycle of these fungi, is the molecular mechanism by which these parental nuclei recognize each other as they migrate into the ascogenous hyphae. Internuclear recognition (IR) was recognized as early as 1956 by Papazian, who proposed that the male and female nuclei inside the female organ must have a different phenotype and predicted that mating-type genes of each nucleus determine its particular phenotype. I present here our current knowledge of IR control by mating-type genes in *P. anserina* and a model for the mechanism of IR.

Control of IR by Mating-Type Genes in *P. anserina*

Mating-type genes have been cloned in several filamentous ascomycetes, but their analysis has given little informa-



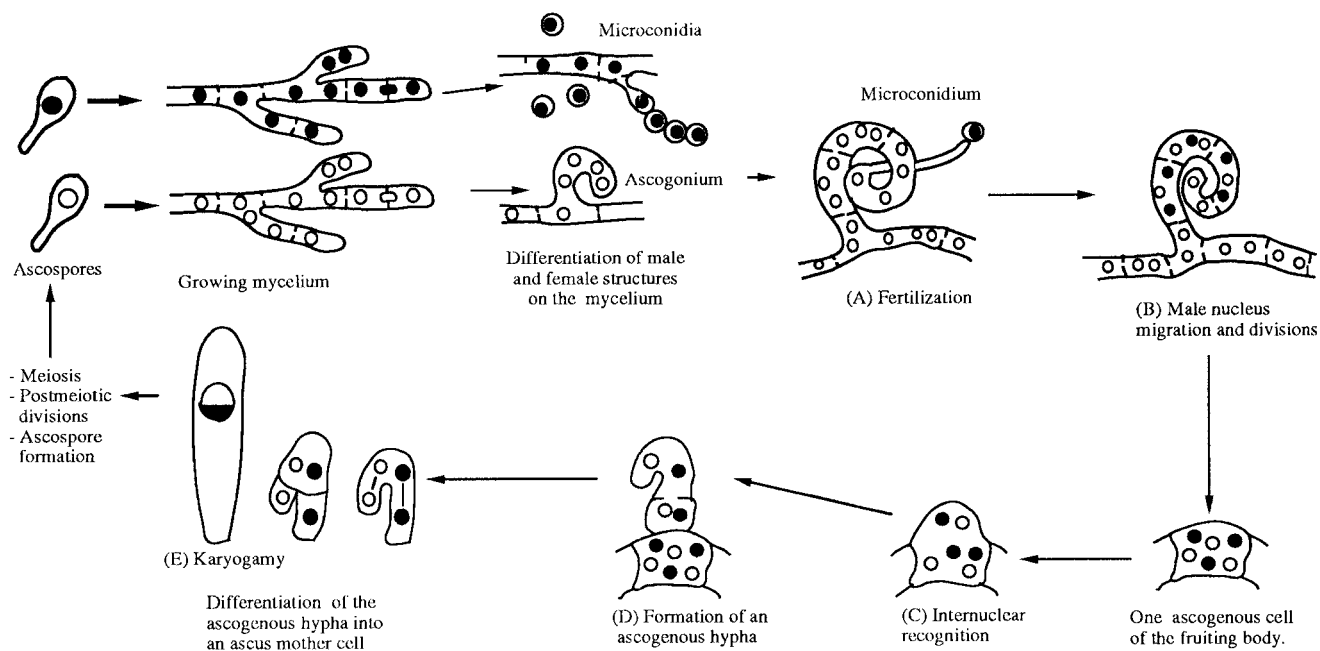


FIG. 1. Life cycle of *P. anserina*. Nuclei of different mating type are represented in black and white.

tion about the mechanism of IR, because these genes encode transcription factors and their regulatory targets are unknown (reviewed in Coppin *et al.*, 1997; Kronstad and Staben, 1997). In the heterothallic Ascomycete *P. anserina*, the *mat+* mating-type sequence contains one gene, *FPR1*, and the alternate *mat-* mating-type sequence contains three genes, *FMR1*, *SMR1*, and *SMR2* (Table 1). All these genes, except *SMR1*, encode regulatory proteins related to well-known transcription factors (Debuchy and Coppin, 1992; Debuchy *et al.*, 1993). It has been estab-

lished that the N-terminal portions of *FPR1* and *FMR1* are required for fertilization, probably because they control genes encoding pheromones and pheromone receptors (Debuchy and Coppin, 1992). Further analyses have characterized *FPR1* as the *mat+* gene involved in IR, while the *mat-* genes controlling IR are *FMR1* and *SMR2* (Zickler *et al.*, 1995; Arnaise *et al.*, 1997). Mutations in these genes (except in the region involved in fertilization) lead to aberrant nuclear migration into the ascogenous hyphae and non-Mendelian segregation of genetic markers in the progeny. This phenotype has been interpreted as resulting from internuclear misrecognition, which suggests that *FPR1*, *FMR1*, and *SMR2* control IR. The function of *SMR1* is not yet clear, but it has been demonstrated that it is not involved in fertilization and is not a bona fide mating-type gene (Arnaise *et al.*, 1997). Several lines of evidence suggest that it operates downstream of IR, at an initial stage of ascogenous hypha development. It has been conjectured that IR genes have a nucleus-limited expression. This feature allows *mat+* and *mat-* nuclei to have specific markers which help to discriminate between compatible and noncompatible nuclear associations within ascogonial cells (Zickler *et al.*, 1995). This hypothesis implies that IR gene products do not diffuse to adjacent nuclei. A genetic test based on internuclear complementation has confirmed that the products of *SMR2* and *FPR1*

TABLE 1  
Functions of the Mating-Type Genes in *Podospora anserina*

Developmental step <sup>a</sup>	<i>mat-</i> genes	<i>mat+</i> gene
(A) Fertilization	<i>FMR1</i>	<i>FPR1</i> <sup>b</sup>
(B) Male nucleus migration and divisions	?	?
(C) Internuclear recognition	<i>FMR1</i>	<i>SMR2</i> <sup>b</sup> , <i>FPR1</i> <sup>b</sup>
(D) Initial development of biparental ascogenous hyphae	<i>SMR1</i>	
(E) Karyogamy and following steps		

<sup>a</sup> The labeling letter preceding each step refers to Fig. 1.

<sup>b</sup> Genes with a nucleus limited expression.

have a nucleus-limited expression, while *FMR1* does not display this characteristic (Arnaise *et al.*, 1997). However, the yeast two-hybrid method indicated that *FMR1* interacts with *SMR2* (Debuchy and Coppin, unpublished data). It has been proposed that, in ascogonial cells, the interaction of *FMR1* with *SMR2* around *mat*<sup>-</sup> nuclei prevents the diffusion of *FMR1* to adjacent nuclei (Arnaise *et al.*, 1997). These data allow us to understand how mating-type genes control IR (Fig. 2), but the molecular basis of IR, namely which molecules signal to the cytoskeleton that two compatible nuclei are close and ready for the migration of the ascogenous hypha, is still a challenging missing link. Zickler *et al.* (1995) have proposed that IR is based on nuclear membrane-bound proteins, but the mechanism for discriminating between compatible and noncompatible nucleus associations has not been addressed. It is also possible that the circumstantial evidence which supports IR is in fact related to another function of mating-type genes. There may be no IR of the sort described and it can be hypothesized that selection for compatible associations of nuclei occurs in ascogenous hypha. This specialized hypha may fail to differentiate into crozier when two nuclei of like mating type are present. Uniparental ascogenous hypha would then abort or differentiate into paraphysis, a

sterile cell of the perithecium. However, a model presented recently by Schuurs *et al.* (1998) for differential gene expression in relation to nuclear positioning in the Homobasidiomycete *Schizophyllum commune* sheds light on a possible mechanism for signalling *mat*<sup>+</sup>/*mat*<sup>-</sup> nucleus associations in *P. anserina* and suggests experiments to test this model.

### Communication Between Nuclei Through the Pheromone-Receptor System in *S. commune*

Schuurs *et al.* (1998) have observed that positioning of nuclei in dikaryotic mycelium is correlated with differential gene expression in *S. commune*. A dikaryotic mycelium with distant nuclei has a monokaryotic type of gene expression, but the same strain with nuclei in close proximity is associated with a pattern of dikaryotic gene expression. The authors proposed that nuclear distance affects communication between nuclei through the pheromone-receptor system encoded by the *B* mating-type genes (Wendland *et al.*, 1995). They assume that pheromones and receptors are confined to a region of the cell

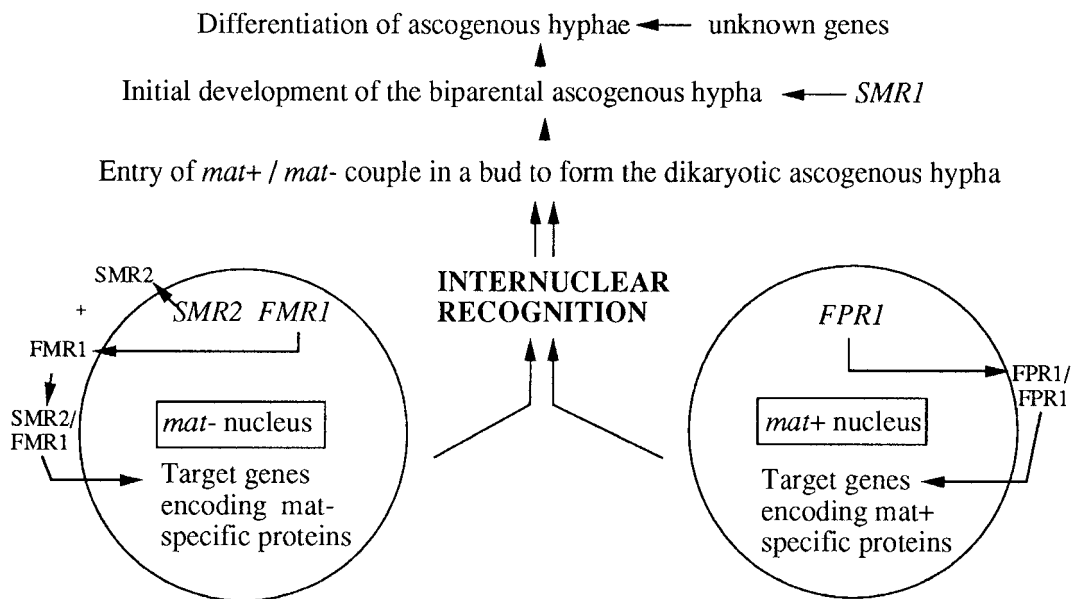


FIG. 2. Control of recognition between *mat*<sup>+</sup> and *mat*<sup>-</sup> nuclei by mating-type genes in ascogonial cells. The expression of genes controlling internuclear recognition is restricted to the immediate vicinity of each nucleus. *FMR1* does not have a nucleus-limited expression by itself but its diffusion to adjacent nuclei may be limited by *SMR2*, which has been demonstrated to interact with *FMR1* by the yeast two-hybrid method. The yeast two-hybrid method suggests also that *FPR1* makes homodimers. The cytoskeleton drives compatible nuclei into a bud which develops into a dikaryotic ascogenous hypha. *SMR1* is not nucleus-specific.

(the wall and the plasmalemma), under the control of a nucleus, thus defining the domain of a nucleus. Positioning of compatible nuclei at some distance would prevent any contact between compatible domains. Consequently, the interaction of pheromones with compatible receptors is not possible and results in a monokaryotic type of gene expression. When the nuclei are close enough to superimpose their specific domains, this permits binding of the pheromone to the responsive receptor and triggers the pheromone response which allows dikaryotic gene expression.

### The Nuclear Positioning Model and IR in *P. anserina*

The model proposed by Schuurs *et al.* (1998) for interaction of nuclei with different *B* alleles can be applied to IR inside the ascogonial cell, if one assumes that IR is also controlled by pheromone–receptor systems in *P. anserina*. As in *S. commune*, each nucleus is supposed to control a plasmalemma and cell wall domain for the localization of pheromones and receptors (Figs. 3A and 3B). The dynamic process of nuclear distribution inside the

ascogonial cell would determine the formation of nucleus associations and the successful formation of biparental ascogenous hyphae. Decreased distance between two nuclei of like mating type would have no developmental effect. Decreased distance between two nuclei of unlike mating type would lead to the overlap of their domain and to the interaction between pheromones and their responsive receptors. This interaction may induce a spatial cue, which triggers the reorganization of the cytoskeleton and of the secretory apparatus as described in yeast (for a review see Drubin and Nelson, 1996). These modifications would lead to the differentiation of the ascogenous hypha in *P. anserina* (Fig. 3C), while they result in cell fusion in yeast.

The N-terminus end of FMR1 and FPR1, which are likely to control the fertilization pheromone–receptor systems, may also control the pheromone–receptor systems required for IR. Frameshifts or disruptions in the C-terminus of FMR1 or FPR1, or in SMR2, indicate that these genes would have another function. Nuclei with such mutations become, indeed, able to migrate alone from the ascogonial cell into the ascogenous hypha and to yield a uniparental progeny (Zickler *et al.*, 1995; Arnaise *et al.*, 1997). This suggests that the spatial cue required for the

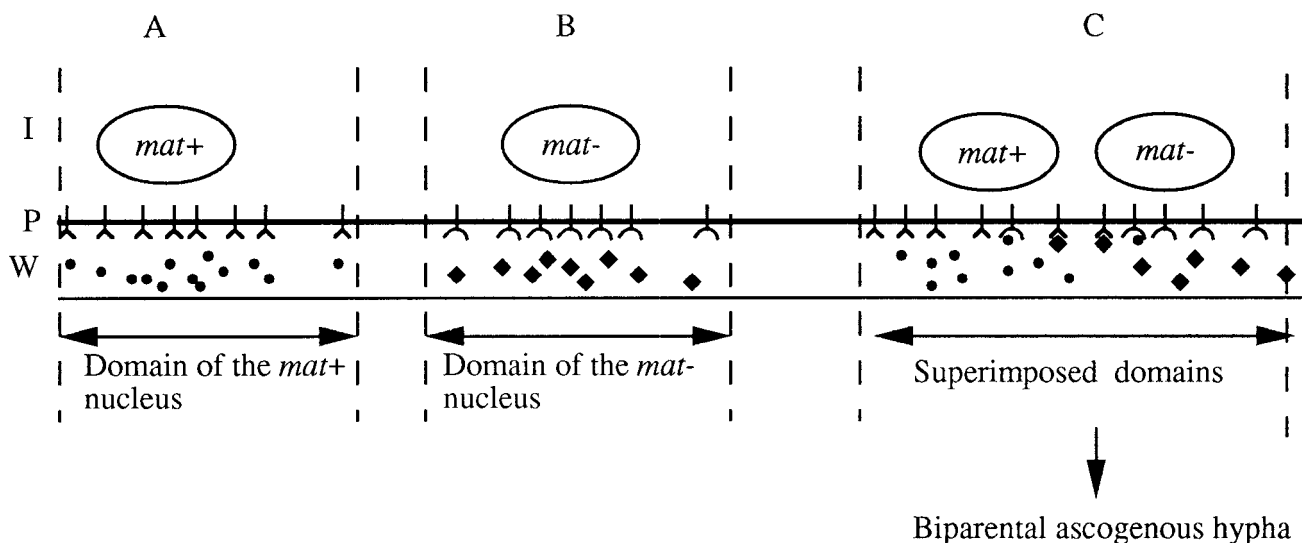


FIG. 3. Model for internuclear recognition in *P. anserina* based on the nuclear positioning model of *S. commune* (Schuurs *et al.*, 1998). I, inside of an ascogonial cell with wild-type *mat+* and *mat-* nuclei; P, plasmalemma; W, wall. (A) *mat+* nucleus with *mat-* pheromone receptors located in the plasmalemma and *mat+* pheromones secreted into the wall. Both proteins remain in the domain controlled by the *mat+* nucleus. (B) *mat-* nucleus with its specific cytoplasmic and wall domain. (C) Two compatible nuclei are in close proximity and their domains overlap. The pheromone receptors carried by one nucleus interact with the pheromones of the opposite nucleus and generate a spatial cue. The cue is then transduced from the cell wall to the interior of the cell (reviewed in Banuett, 1998), modifying the pattern of gene expression and protein activity. This leads to the reorganization of the cytoskeleton and the secretory apparatus (reviewed in Drubin and Nelson, 1996), which results eventually in the formation of an ascogenous hypha with two compatible nuclei.

formation of ascogenous hyphae is produced in the domain of the mutant nucleus, although no compatible nucleus is in close proximity. This may result from the diffusion, into the wall domain of the mutant nucleus, of the compatible pheromone from an adjacent but not superimposed compatible domain, because a system preventing this diffusion is defective in the domain of the mutant nucleus. We propose that SMR2 and the C-terminal end of FMR1 control the expression of a degradation system specific for the *mat+* pheromone, while the C-terminal end of FPR1 controls the expression of a degradation system specific for the *mat-* pheromone (Fig. 4). These degradation systems may help the nuclei to preserve their domains from contamination by compatible pheromones. These degradation systems are either downregulated when compatible nuclei superimpose their domain or their activity is low enough to degrade trace pheromone but not large amounts resulting from the fusion of domains. Proteases that cleave  $\alpha$  and  $\alpha$ -factors have been characterized in *Saccharomyces cerevisiae* (MacKay *et al.*, 1988; Marcus *et al.*, 1991) and may be similar to the pheromone degradation proteins hypothesized in *P. anserina*.

This model makes specific predictions about the proteins encoded by the target genes of mating-type proteins. The fertilization and developmental pheromone–receptor systems can be identical; however, fertilization requires that pheromones are secreted outside of the wall, whereas IR requires that pheromones are secreted in the wall. Another prediction of this model is that ectopic expression of a compatible pheromone or receptor gene in a nucleus should result in the interaction of pheromones with their responsive receptors in the nucleus domain, and in the formation of a uniparental progeny from the corresponding nucleus. A third prediction of this model is based on the observation that in yeast, the initial responses to pheromone include arrest of cell in the G1 phase of the cell cycle (for a review see Kurjan, 1992). A similar arrest can be postulated in *P. anserina* in response to the pheromone–receptor interaction which precludes to the formation of ascogenous hypha. The function of this growth arrest would be to synchronize the divisions of nuclei in this dikaryotic hypha. Several lines of evidence suggest that indeed a growth arrest occurs during the development of

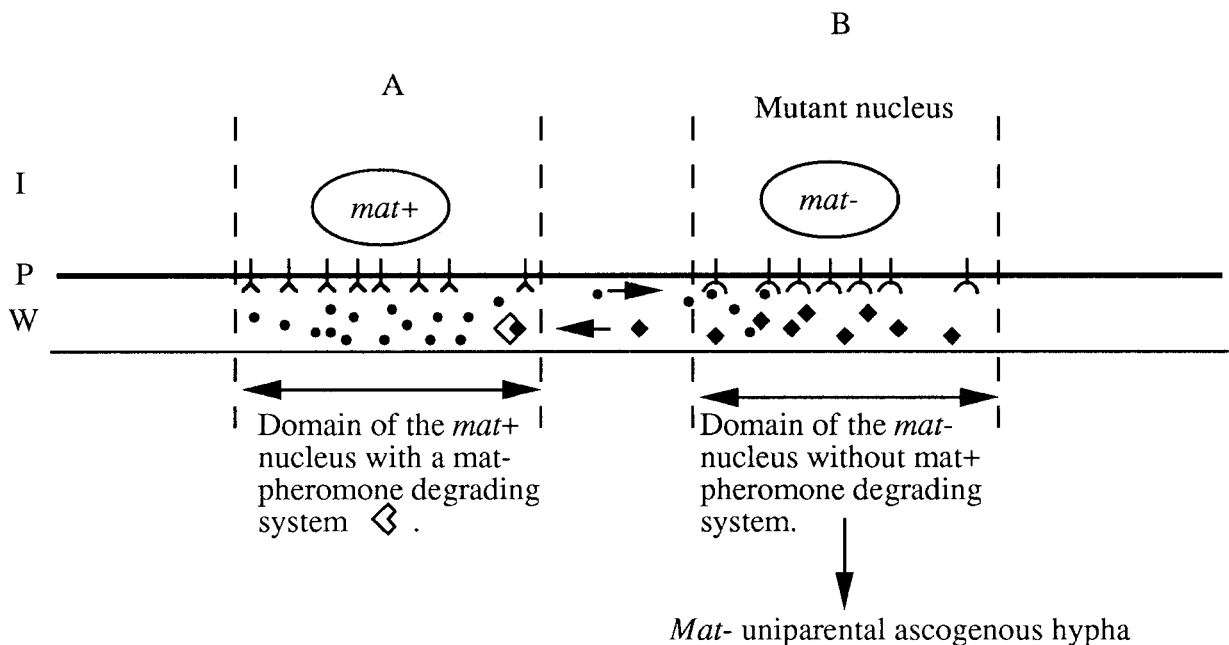


FIG. 4. Model for internuclear recognition in *P. anserina* in the case of a mutation in a gene involved in IR. I, P, W, the same as for Fig. 3 except that the ascogonial cell contains *mat-* mutant nuclei and wild-type *mat+* nuclei. (A) *mat+* wild-type nucleus. Its degradation system prevents the diffusion of the *mat-* pheromone into its wall domain. (B) *mat-* nucleus with a mutation in SMR2 or in the C-terminal part of FMR1. The degradation system is not active and *mat+* pheromones can diffuse into the cytoplasmic domain of the *mat-* nucleus. The *mat+* pheromones interact with *mat+* pheromone receptors. This generates a spatial cue which allows the formation of an ascogenous hypha with one *mat-* mutant nucleus. This ascogenous hypha can develop further and gives a *mat-* uniparental progeny.

the perithecium at the time of IR in *P. anserina* (Coppin and Debuchy, in preparation).

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