Differential expression of genes under control of the mating-type genes in the secondary mycelium of *Schizophyllum commune*

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The *Schizophyllum commune* SC3 gene, which encodes a hydrophobin that coats aerial hyphae, is expressed in both monokaryons and dikaryons. The dikaryons were formed by mating two monokaryons with different MATA and MATB genes, leading to activation of the MATA- and MATB-controlled pathways (MATA-on and MATB-on). In contrast to the monokaryons, the dikaryons also expressed other hydrophobin genes (SC1, SC4) as well as a gene (SC7) encoding a hydrophilic wall protein. None of these four genes was expressed in MATA-off MATB-on mycelia, indicating that MATB-on represses SC3 and that both MATA-on and MATB-on are required for activation of SC1, SC4 and SC7. In fruiting dikaryons, immunolabelling revealed that SC3p was produced by aerial hyphae but not by hyphae that constitute the fruit-body tissue. In contrast to aerial hyphae, the latter produced dikaryon-specific transcripts and secreted SC7p into the extracellular matrix of the tissue. This suggests that in the aerial hyphae of the dikaryon the MATB-on pathway was not effective (MATB-off). We observed that in these aerial hyphae the two nuclei were wider apart than in a typical dikaryon. Although other explanations are not ruled out, we tentatively propose that effective interaction of different MATB genes requires proximity of the two nuclei containing these genes, and that disruption of this binucleate state represents a novel mechanism of gene control for spatial cell differentiation in the secondary mycelium.

**Keywords:** *Schizophyllum commune*, mating-type genes, hydrophobin regulation, fruit-body formation

INTRODUCTION

Sexual morphogenesis in the homobasidiomycete *Schizophyllum commune* is primarily controlled by multi-allelic mating-type genes within the MATA and MATB loci (also called A- and B-incompatibility genes or incompatibility factors; Raper, 1966, 1988). Mating of monokaryons (one nucleus per hyphal compartment) that contain different MATA and MATB genes produces a dikaryon (with clamp connections and two closely associated nuclei per hyphal compartment). The monokaryon normally produces aerial hyphae only but the dikaryon also forms multicellular fruit bodies in which haploid nuclei fuse to form diploid nuclei that immediately undergo meiosis with the production of basidiospores.

Several MATA loci of *S. commune* have been cloned and sequenced (Specht *et al.*, 1992; Stankis *et al.*, 1992). These loci mostly contain allelic gene pairs with each of the two different genes in a pair containing a particular homeobox-like sequence, *HD1* or *HD2*. It appears that in a heterokaryon with different MATA genes (MATA+) the effective interaction (MATA-on) is between the product of an *HD1* gene in one nucleus and the product of an *HD2* gene in the other nucleus. Because of the presence of homeobox-like sequences these products are thought to be transcription factors that, in the effective combination, regulate downstream genes involved in sexual morphogenesis, as discussed by Casselton & Kües (1994). Less is known about the structure and operation of the MATB
loki. These loci appear to contain genes for pheromones and pheromone-receptors (Wendland & Kothe, 1994), and thus would resemble the a mating-type genes of the heterobasidiomycete Ustilago maydis ( Bölker et al., 1992).

Genes that are direct or indirect targets for regulation by the mating-type genes of S. commune have been cloned (Mulder & Wessels, 1986). The sequences of five of these, belonging to two different gene families and encoding abundant mRNAs, have been reported. The SC7/SC14 family encodes dikaryon-specific hydrophilic proteins with homology to pathogenesis-related proteins (PR1 proteins) of plants (Schuren et al., 1993). The SC1/SC3/SC4 gene family encodes cysteine-rich hydrophilic proteins (Schuren & Wessels, 1990) which we called hydrophobins (Wessels et al., 1991a, b). All five proteins contain signal sequences for secretion and the mature proteins encoded by SC7, SC3 and SC4 have been found in the culture medium and associated with cell walls in aerial structures. Except for the SC3 gene, which is also active in the monokaryon, these genes are expressed to high levels only in the dikaryon (MATA-on MATB-on) (Mulder & Wessels, 1986; Ruiters et al., 1988). They are all relatively inactive in young cultures that do, however, express the monokaryotic and dikaryotic cellular phenotypes. These genes are thus unlikely to play a role in the monokaryon–dikaryon transition. Their activation, as shown by nuclear run-on transcription assays (Schuren et al., 1993c), coincides with the formation of emergent structures, aerial hyphae in the monokaryon and aerial hyphae plus fruit-bodies in the dikaryon.

The SC3 hydrophobin self-assembles at the surface of aerial hyphae into a so-called rodlet layer that is highly insoluble and responsible for the high hydrophobicity of the hyphal surface (Wöstien et al., 1993, 1994a). Since SC3 is activated in both monokaryons (MATA-off MATB-off) and dikaryons (MATA-on MATB-on) it has been suggested (Wessels, 1992) that this gene is not regulated by the mating-type genes. However, in this paper we demonstrate that in MATA-off MATB-on mycelia, which form few aerial hyphae, the SC3 gene is down-regulated, suggesting that this may also be the case in MATA-on MATB-on hyphae. Using antibodies, we found that in the fruiting MATA- MATB- heterokaryon hyphae within fruit bodies produce the dikaryotic protein SC3p but not SC3p. On the other hand, aerial hyphae formed on this mycelium do produce SC3p, but the dikaryotic state appeared disrupted in the sense that the nuclei were wider apart than in typical dikaryotic hyphae and no clamp connections were formed. We suggest that in these aerial hyphae the MATB-on pathway is inoperative, despite the presence of different MATB genes, allowing for formation of SC3p but not SC7p.

**METHODS**

**Strains and culture conditions.** The following strains of S. commune were used in these experiments: the co-isogenic strains 4-39 (MATA<sup>4</sup> MATB<sup>4</sup>, CBS 341.81) and 4-40 (MATA<sup>4</sup> MATB<sup>4</sup>, CBS 340.81); a derived dikaryon; a heterokaryon derived from a hemi-compatible mating (MATA<sup>4</sup> MATB<sup>4</sup> × MATA<sup>4</sup> MATB<sup>4</sup>) and a co-isogenic mutant homokaryon with constitutive MATB activity (MATA<sup>4</sup> MATB<sup>B+</sup>). For RNA isolation, the mycelia were grown for 4 d at 24 °C in the light on minimal medium (Dons et al., 1979) solidified with 0.7% agar. For immunodetection of SC3p and SC7p, dikaryons were grown for 5 d from a central inoculum on 1.5% (w/v) agar minimal medium in a circulating air incubator in darkness at 24 °C and 95% relative humidity. The cultures were then transferred to the light and incubated for an additional 48 h to induce a ring of fruit-body primordia (Ruiters & Wessels, 1989). When analysing the secretion of SC3p and SC7p by submerged hyphae, the cultures were grown on perforated polycarbonate membranes (standard pore size 0.1 µm; Poretics) overlying the agar medium. After 5 d growth in the dark and 48 h in the light, the colonies were transferred to a new Petri dish and proteins were caught on a polivinyllidene difluoride (PVDF) membrane positioned under the polycarbonate membrane for 6 h (Wöstien et al., 1994a). For isolation of emergent hyphae, dikaryons were grown for 2 d from a mycelial homogenate mixed with 9 vols of 3.3% low-melting-point agarose layered on 3% agar minimal medium. The cultures were grown at 30 °C in the dark to stimulate formation of aerial hyphae and to suppress formation of fruit bodies.

**RNA isolation and Northern blot analysis.** Total RNA was isolated by the hot-phenol procedure (Wessels et al., 1987; Schuren et al., 1993b). For Northern blot analysis, the RNA was separated on agarose gels containing 2.2 M formaldehyde and blotted onto Hybond N+ membrane (Amersham) in 10× SSC (SSC is 150 mM sodium chloride, 15 mM sodium citrate, pH 7.0). Blots were hybridized at 65 °C according to the method of Church & Gilbert (1984) to <sup>32</sup>P-labeled cDNA clones (Mulder & Wessels, 1986; Ruiters et al., 1988).

**Isolation of emergent aerial hyphae and analysis of nuclear distribution.** Hyphae just emerging from a 3% low-melting-point agarose layer mixed with a mycelial homogenate, layered on 3% agar minimal medium, were isolated as follows. The culture was fixed for 2 h at room temperature under vacuum in freshly made 2.5% (v/v) paraformaldehyde and blotted onto Hybond N+ membrane (Amersham) in 10× SSC. The RNA was isolated by the hot-phenol procedure (Wessels et al., 1987; Schuren et al., 1993b). For Northern blot analysis, the RNA was separated on agarose gels containing 2.2 M formaldehyde and blotted onto Hybond N+ membrane (Amersham) in 10× SSC. Blots were hybridized at 65 °C according to the method of Church & Gilbert (1984) to <sup>32</sup>P-labeled cDNA clones (Mulder & Wessels, 1986; Ruiters et al., 1988).

**Immunodetection of SC3p and SC7p in aerial structures.** For immunodetection of SC3p and SC7p in differentiated aerial structures, a fruiting dikaryotic colony was fixed in 4% (w/v) paraformaldehyde in phosphate-buffered saline (PBS is 137 mM NaCl, 2.7 mM KCl, 1.6 mM NaH<sub>2</sub>PO<sub>4</sub>, 8.2 mM Na<sub>2</sub>HPO<sub>4</sub>, pH 7.6) according to the method of Ruiters & Wessels (1989). Sections (25–30 μm) were cut perpendicular to the surface on a freeze microtome and mounted on microscope slides coated with chrome-alum-gelatin. Sections were blocked overnight at room temperature in PBS containing 1% (v/v) BSA, 1% (w/v) polyvinylpyrrolidone (PVP), 0.5% normal goat serum, 0.05% Tween 20, 0.02% NaN<sub>3</sub> and 0.35 M NaCl. The antisera were used against purified SC3p (Wöstien et al., 1994a) and against a protein A–SC7p fusion protein produced in E. coli.
Cell differentiation in Schizopyllum commune (Schuren et al., 1993a). Prior to use, these antisera were purified by incubation with isolated hyphal walls of a bkn mutant as described by Wösten et al. (1994a), to prevent non-specific reactions with cell-wall components. The purified sera were diluted in blocking buffer (1:500 and 1:100 for anti-SC3p serum and anti-SC7p serum, respectively) and incubated with the sections at room temperature for 24 h. After four 5 min washings with PBS supplemented with 0.35 M NaCl, the sections were incubated for 2 h with goat-anti-rabbit antibodies conjugated with 5 nm gold particles (Zymed) diluted in blocking buffer according to the manufacturer's protocol. The sections were washed as above and the immunogold labelling was enhanced with silver (SilvEnhance-LM kit, Zymed).

Immunodetection of SC3p and SC7p secreted into the culture medium. PVDF membranes on which secreted proteins were caught were blocked for 2 h at room temperature with 5% skimmed milk and 1% PVP in PBS and then incubated for 1 h with antisera against SC3p (diluted 1:2000 in the blocking buffer) or SC7p (dilution 1:200). The immunoreaction was detected with goat-anti-rabbit IgG coupled with alkaline phosphatase and using BCIP and NBT as substrates (Harlow & Lane, 1988).

Electron microscopy. For low-temperature scanning electron microscopy, agar blocks (approximately 5 x 5 mm) containing fruit-body primordia were frozen in a mixture of solid and liquid nitrogen. Primordia were broken with a cooled knife within a Fisons E7400 cryo unit, etched for 4 min and coated with gold. Samples were examined at 20 kV in a Jeol JSM-35C scanning electron microscope. Immuno-electron microscopy of thin sections was performed according to Wösten et al. (1994a), except that primordia were fixed in 3% paraformaldehyde, 0.1 M cacodylate buffer pH 7.2 and embedded in Unicryl (BioCell).

RESULTS

SC3 is suppressed by the activity of the B mating-type genes

Mycelia of S. commune in which only the MATB-on pathway is operative, i.e. a MATA = MATB+ heterokaryon (common-MATA heterokaryon) and a MATA41 MATB-off homokaryon (in which a mutation constitutively activates the MATB-on pathway), produce few aerial hyphae (Fig. 1a); they have a so-called 'flat' phenotype (Papazian, 1950; Parag, 1962). Because of the known correlation between low SC3 expression and the absence of aerial hyphae (Wessels et al., 1991b), the level of SC3 mRNA in these mycelia was determined. Northern blot analysis (Fig. 1b) showed a high level of SC3 mRNA in the monokaryon (lane 1), but no hybridization signals were detected with RNA from the MATB-off homokaryon and the common-MATA heterokaryon (lanes 2 and 3), with similar amounts of RNA loaded on the gels (lanes 4-6). This suggests negative regulation of SC3 by the MATB-on pathway. Since a MATA+ MATB+ heterokaryon does produce SC3 mRNA with the formation of aerial hyphae (Mulder & Wessels, 1986; Ruiters et al., 1988), this indicates that in these mycelia either MATA-on counteracts the suppressive effect of the MATB-on pathway on SC3 activity, or that these mycelia contain specialized hyphae in which the MATB-on pathway is not operative.

Localization of the secretion of SC3p and SC7p by submerged hyphae

Submerged hyphae do not accumulate SC3p and SC7p in their cell walls but secrete them into the culture medium (Wessels et al., 1991a, b; Schuren et al., 1993a). The secretion of these proteins in a fruiting MATA+
**MA TB+** heterokaryon was localized by immunodetection on a PVDF membrane placed under the colony. Cultures were grown for 5 d in darkness and transferred to light to induce the formation of a ring of fruit-body primordia. The dikaryon-specific SC7p was primarily detected in the zone of leading hyphae and in the zone where fruit-body primordia were formed, whereas SC3p was hardly detected in the fruiting zone but it was secreted in the zone of leading hyphae and in the inner zone where aerial hyphae were being formed (Fig. 2). These results indicate heterogeneity of hyphae within the colony with respect to expression of the SC3 and SC7 genes.

**Immunolocalization of SC3p and SC7p in emergent structures**

Immunolocalization of SC3p and SC7p on transverse sections through a colony of a fruiting dikaryon (Fig. 3) shows that SC3p, but not SC7p, was present in individually growing aerial hyphae emerging from the substrate, and in hyphae covering the fruit body, and also in the hymenium. However, hyphae making up the inner tissue (plectenchyma) of the fruit body did not produce SC3p but did react with SC7p antibodies.

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**Fig. 2.** Immunolocalization of secretion of SC3p and SC7p at the colony level. A fruiting MA TB+ MA TB+ heterokaryon (a) secretes SC7p in the zone of fruit-body formation and the zone of peripheral hyphae (b), whereas SC3p is absent from the zone containing fruit-body primordia but is secreted in central parts of the colony forming aerial hyphae (c).

**Fig. 3.** Immunolocalization of SC7p (a, b) and SC3p (c, d) in fruit bodies (a, c) and aerial hyphae (b, d) of the MA TA+ MA TB+ heterokaryon. Bars, 100 μm.
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**Fig. 4.** (a) Low-temperature scanning electron micrograph of a broken fruit body showing the loose contact between aerial hyphae covering the fruit body and the extensive extracellular matrix surrounding and binding together the hyphae of the plectenchyma; bar, 100 μm. (b) Immunolocalization of SC7p in thin sections of the plectenchyma. The 15 nm gold particles indicate the presence of SC7p within the extracellular matrix (no signal was obtained with pre-immune serum or with the anti-SC3p serum); bar, 0.5 μm.

Fig. 4 (a) shows the morphology of a developing fruit body by cryo-scanning electron microscopy. Whereas the outside of the fruit body is covered by individually growing aerial hyphae that express *SC3*, the interior hyphae that form the plectenchyma and express *SC7* are held together by an extensive extracellular matrix. Fig. 4 (b) demonstrates that SC7p is a constituent of this extracellular matrix.

**Nuclear distribution in aerial hyphae**

Aerial hyphae of the *MATA*+ *MATB*+ heterokaryon were found to deviate from the normal dikaryotic morphology in that they mostly contained simple septa or pseudoclamps, i.e. clamp cells that have failed to fuse with the penultimate cell. This is particularly clear in the long hyphae that cover fruit-body primordia. These hyphae were divided into several compartments by simple septa. Since formation of clamp connections is closely associated with the dikaryotic state of the *MATA*+ *MATB*+ heterokaryon, we attempted to analyse the nuclear distribution. However, we were unable to stain nuclei in full-grown aerial hyphae. Either the DAPI stain did not penetrate these thick-walled hyphae or the hyphae had lost their nuclei. Giemsa or Feulgen staining was also unsuccessful. However, aerial hyphae just emerging from the substrate mycelium, isolated by embedding them in gelatin and peeling them off the surface, did contain stainable nuclei. When distances between the perimeters of stained nuclei were measured (see Fig. 5b), it was found that these aerial hyphae (cell lengths 92±2±35.5 μm) contained two nuclei positioned a considerable distance from each other (8.3±4.8 μm, minimum 14 μm, maximum 20.1 μm) in the 51 cells that were counted (Fig. 5b). This is in contrast to the dikaryotic hyphae in the substrate mycelium where the two nuclei occur in close proximity (distance 0.9±0.4 μm; Fig. 5a). For comparison we examined the nuclear distribution in a *MATA*+ *MATB*+ heterokaryon homozygous for the *f/f* mutation. In this mycelium, formation of all dikaryon-specific transcripts is suppressed, no fruit bodies are formed but *SC3* is highly expressed with abundant formation of aerial hyphae (Springer & Wessels, 1989). The mutation also prevents clamp formation by preventing the clamp cell fusing with the penultimate cell (Springer & Wessels, 1989). As a consequence, only the apical cell is binucleate, but all other cells are mononucleate with one nucleus caught in the unfused clamp cell (Fig. 5c).

**DISCUSSION**

Recently we showed that the *SC3* gene of *S. commune* codes for a hydrophobin that self-assembles at the surface of aerial hyphae forming a highly insoluble hydrophobic rodlet layer (Wosten *et al.*, 1993, 1994a). A targeted mutation in this gene (Wosten *et al.*, 1994b) reduces formation of aerial hyphae under certain conditions, indicating a primary role of SC3p in the formation of aerial hyphae (unpublished observations). The *f/f* mutation that inactivates all known hydrophobin genes, as well as other genes involved in emergent growth, suppresses formation of both aerial hyphae and fruit bodies (Wessels *et al.*, 1991b). Another instance in which few aerial hyphae are produced is when the *MATB*-on pathway is operating in the absence of the *MATA*-on pathway (Papazian, 1950; Parag, 1962), producing a so-called flat mycelium (Fig. 1a). In an earlier study (Ruiters *et al.*, 1988) we found that in one such mycelium, the *MATA*41 *MATB*con homo-karyon, *SC3* is not expressed. In the present paper we show that this is also the case in a *MATA*+ *MATB*+ heterokaryon, indicating that the *MATB*-on pathway negatively regulates the expression of *SC3* and thereby suppresses formation of aerial hyphae.
Fig. 5. Nuclear positions in a typical dikaryotic hypha (a) and a hypha (b) just emerging from the substrate to become aerial, both from the same \(\text{MAT}^+\ \text{MATB}^+\) heterokaryon. Distances between nuclei were measured as indicated in (b). Nuclear distribution in a \(\text{MAT}^+\ \text{MATB}^+\) heterokaryon homozygous for \(\text{fbf}\) is shown in (c). Note the binucleate apical cell at the top and the single nuclei caught in unfused clamp cells. Bar, 5 \(\mu\)m.

Notwithstanding the repressing effect of the \(\text{MATB}\)-on pathway on expression of \(\text{SC3}\), this gene is expressed in fruiting \(\text{MAT}^+\ \text{MATB}^+\) heterokaryons and \(\text{MAT}^+\text{on} \ \text{MATB}^+\text{on} \) homokaryons along with typical dikaryotic transcripts which, in addition, require the \(\text{MATA}\)-on pathway for their formation (Mulder & Wessels, 1986; Ruiters et al., 1988). One explanation for the (variable) expression of \(\text{SC3}\) in these mycelia is that the \(\text{MATA}\)-on pathway counteracts the effect of the \(\text{MATB}\)-on pathway on the activity of this gene. However, the results reported in this paper show that there is a spatial difference in the presence of \(\text{SC3}\) and the typically dikaryotic protein \(\text{SC7}\), both with respect to the sites of secretion into the medium and the presence in emergent structures; \(\text{SC3}\) was only detected in aerial hyphae and hymenium while the main tissue of the fruit body produces \(\text{SC7}\) but not \(\text{SC3}\). This agrees with the earlier observation that in fruit bodies all dikaryon-specific transcripts are abundant, but that the \(\text{SC3}\) transcript is low compared to other parts of the mycelium (Mulder & Wessels, 1986), indicating that with respect to mating-type gene regulation, cell differentiation within the \(\text{MAT}^+\ \text{MATB}^+\) mycelium has to be taken into account.

Normally, hyphae produced by the \(\text{MAT}^+\ \text{MATB}^+\) heterokaryon have the dikaryotic morphology, i.e. each cell contains two closely associated nuclei of opposite mating type and a clamp connection at each septum. Cytological evidence suggests that some structural material is holding the two juxtaposed nuclei together (Todd & Aylmore, 1985). However, in aerial hyphae that produce \(\text{SC3}\), we found that this binucleate state is disrupted and that nuclei occur at variable distances from each other while, during continued growth of these hyphae, clamp connections were rarely seen; pseudo-clamps and simple septa were abundant. This suggests that in truly dikaryotic hyphae, such as in the fruit-body plectenchyma, the \(\text{MATB}\)-on pathway, together with the \(\text{MATA}\)-on pathway, results in formation of dikaryon-specific transcripts, but that the effective \(\text{MATB}\)-on pathway results in inactivity of the \(\text{SC3}\) gene. We further suggest that in emerging aerial hyphae the \(\text{MATB}\)-on pathway is somehow switched off, resulting in activation of \(\text{SC3}\) and inactivation of genes producing dikaryon-specific transcripts.

How is the \(\text{MATB}\)-on pathway inactivated? The activity of \(\text{SC3}\) is correlated with the failure of clamp cells to fuse.
In aerial hyphae this may result from the fact that diffusible factors involved in fusion, possibly pheromones produced under direction of the $MATB$ genes, are unable to reach their targets in the air and therefore inactivation of the $MATB$-on pathway occurs. Why this should lead to a breakdown of the binucleate state in the aerial hyphae is unclear because nuclear conjugation is considered a function of the $MATA$-on pathway (Raper, 1966). In the $MATA \neq MATB \neq fbf/fbf$ heterokaryon, the absence of some gene product apparently blocks clamp-cell fusion. However, it is unlikely that this mutation directly interferes with the activity or the interaction of the $MATB$ genes since nuclear migration, another process regulated by the $MATB$ genes, normally occurs in matings of strains both carrying $fbf$. Another line of reasoning, which we prefer, concentrates on the significance of the binucleate state for proper interactions between mating-type genes. Even in many basidiomycete species that do not produce clamp connections, this binucleate state is a most characteristic feature of the secondary mycelium (Kühner, 1977; Ross et al., 1991). In both the incipient aerial hyphae and the $fbf$ mutant of $S. commune$ the binucleate state is disrupted and we hypothesize that this leads to a shut-down of the $MATB$-on pathway and expression in the secondary mycelium of a phenotype similar to that of the primary mycelium.

If disruption of the binucleate state is responsible for the expression of $SC3$, then this process must also regularly occur in hyphae of the $MATA \neq MATB \neq$ heterokaryon growing submerged because $SC3p$ is secreted under these conditions (Wessels et al., 1991a). As shown in Fig. 2, the $SC3p$-producing hyphae are not the same as those producing dikaryon-specific proteins. Microscopic observations show the regular occurrence of disruption of the binucleate state (data not shown), but correlation with $SC3p$ production has not yet been investigated. Why $SC3p$ is also produced by hymenial cells is unclear, but these cells obviously deviate from the binucleate state on their way to diploidy and meiosis.

It is difficult to say whether the disruption of the binucleate state also shuts down the $MATA$-on pathway. A diagnostic feature of this pathway is the occurrence of non-fused clamp cells (pseudoclamps) which were often observed in aerial hyphae. However, one would not expect a loss of interaction between $MATA$ genes to lead to an immediate cessation of the $MATA$-on phenotype because experimental de-dikaryotization has shown that pseudoclamp formation can continue for many cell divisions in derived homokaryons (Wessels et al., 1976).

Although this is the first time that disruption of the binucleate state has been related to developmental gene regulation, disruption of the binucleate state has previously been observed in the secondary mycelium of several basidiomycetes, leading to a phenotype called a pseudo-primary mycelium (Kühner, 1977). Gabriel (1967, cited in Kühner, 1977) found in $Panellus serotinus$ and $Phlebia radiata$ that the factor responsible for the transition to pseudo-primary mycelium is carbon dioxide accumulation. Interestingly, in the dikaryon of $S. commune$, increased carbon dioxide concentration leads to an increase in transcripts from the $SC3$ gene and to a decrease in transcripts of the dikaryon-expressed $SC$ genes, while aerial hyphae become more abundant and formation of fruit bodies is inhibited (Wessels et al., 1987). It would, therefore, not be surprising if in $S. commune$, too, carbon dioxide has an effect on the binucleate state of the secondary mycelium. Another example of disruption of the binucleate state was presented by Ross et al. (1991), who showed that an arginine-requiring mutant dikaryon of $Coprinus congregatus$ reversibly switched to the homokaryotic phenotype when deprived of exogenous arginine. The phenomenon of phenotypic switching on the basis of association–dissociation of nuclei in a heterokaryon, affecting interaction of mating-type genes, may thus be of general occurrence in basidiomycetes and may present a mechanism for regulation of cell differentiation unique to the fungi which mostly produce heterokaryons, rather than diploids, after mating. How nuclear association–dissociation is regulated and how it affects the interactions between different $MATB$ genes is unknown, but it may become clear once the mechanism of action of these genes is established.

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