The *thn* mutation of *Schizophyllum commune*, which suppresses formation of aerial hyphae, affects expression of the *Sc3* hydrophobin gene

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The spontaneous and recessive mutation *thn* in the basidiomycete *Schizophyllum commune* suppresses the formation of aerial hyphae in the monokaryon and, if present as a double dose, the formation of both aerial hyphae and fruit-bodies in the dikaryon. In the monokaryon, the mutation prevents accumulation of mRNA of the *Sc3* gene, and in the dikaryon it also prevents the accumulation of fruiting-specific mRNAs, including mRNAs of the *Sc1* and *Sc4* genes, which are homologous to the *Sc3* gene. These three genes code for hydrophobins, a family of small hydrophobic cysteine-rich proteins. In the *thn* monokaryon, the only detectable change in synthesized proteins is the disappearance of an abundant protein of apparent $M_r = 28$ K from the culture medium and from the cell walls. Protein sequencing shows that this is the product of the *Sc3* gene. The *Sc3* hydrophobin is present in the walls of aerial hyphae as a hot-SDS-insoluble complex. Submerged hyphae excrete large amounts of the hydrophobin into the medium.

Introduction

The *Sc3* gene of the basidiomycete *Schizophyllum commune* codes for a protein of 125 amino acids ($M_r = 12386$) which shares sequence homology with similarly sized products of two other genes, *Sc1* and *Sc4* (Schuren & Wessels, 1990). These proteins, which collectively we call hydrophobins because of their high content of hydrophobic amino acids, each have 8 cysteines conserved at identical positions and contain putative signal sequences for secretion. However, unlike the *Sc1* and *Sc4* genes, which are only expressed in dikaryons at the time of fruiting, the mRNA of the *Sc3* gene accumulates at high concentrations in both monokaryons and dikaryons at the time of formation of aerial hyphae (Mulder & Wessels, 1986). Whereas the mRNAs of the *Sc1* and *Sc4* genes also accumulate in developing fruit-bodies, the mRNA of the *Sc3* gene is more restricted to the vegetative mycelium (Mulder & Wessels, 1986; Ruiters & Wessels, 1989). However, the expression of the *Sc3* gene is variable and we have noticed lower expression where surface cultures failed to produce abundant aerial mycelium, such as in young cultures grown as a lawn from a mycelial homogenate, during heavy fruiting of similarly grown dikaryons, and in occasionally arising genetic variants of the monokaryons with a strongly reduced ability to form aerial hyphae. Such variants have earlier been described in *S. commune* (Schwalb & Miles,

1967) and were shown to be due to a frequently occurring spontaneous mutation, called *thin* (*thn*) (Raper & Miles, 1958). Here we report that the *THN* gene is required for the expression of the *Sc3* gene and for the expression of all known fruiting-specific genes in the dikaryon. In the monokaryon, the *thn* mutation leads to disappearance of the *Sc3* hydrophobin which is normally excreted into the culture medium and present as a hot-SDS-insoluble complex in the walls of aerial hyphae.

Methods

Strains, culture conditions, and labelling. The co-isogenic strains 4-39 (*A41 B41*, CBS 341.81) and 4-40 (*A43 B43*, CBS 340.81) of *S. commune*, the co-isogenic *thin* mutants (*thn*), and derived dikaryons were grown from a mycelial homogenate as a lawn on the surface of minimal medium (Dons *et al.*, 1979) in continuous light at 24 °C for 4 d. For RNA isolations, the mycelia were grown directly on medium solidified with 0.7% agar. For labelling experiments, mycelia were mostly grown for 3 d on water-permeable cellophane (330P, British Cellophane Ltd, Bridgewater, UK) overlaying 0.7% agar medium. The mycelia growing on the cellophane membrane (diameter 4.8 cm) were then detached and floated onto 4 ml liquid minimal medium containing 9.25 MBq ml⁻¹ [³⁵S]sulphate (specific activity 1000–1500 GBq mg⁻¹; MgCl₂ replacing MgSO₄ in the medium) and cultivation continued for 24 h. Alternatively, labelling was done with 400 kBq ml⁻¹ [¹⁴C]leucine (specific activity >11 GBq mmol⁻¹) or 400 kBq ml⁻¹ [¹⁴C]lysine (specific activity >11 GBq mmol⁻¹). To isolate submerged and aerial hyphae separately, the mycelium was grown on a nylon net (50 µm mesh) floating on liquid medium (Mulder & Wessels, 1986). Hyphae

protruding from the underside of the net were scraped off (submerged hyphae) and the remainder peeled off the net (aerial hyphae contaminated with submerged hyphae). Alternatively, the mycelium was grown on a perforated polycarbonate membrane (standard pore size 0.08 µm, Nuclepore) overlaying agar medium. After inoculation with a mycelial homogenate, a nylon net (50 µm mesh) was placed on top of the culture so that aerial hyphae could grow through the net. Scraping the surface of the net permitted isolation of a pure sample of aerial hyphae, the remainder containing submerged hyphae contaminated with some aerial hyphae. Placing a perforated polycarbonate membrane on top of the inoculum, instead of a nylon net, prevented formation of any aerial hyphae and permitted isolation of submerged mycelium only. All these cultures were labelled (277 kBq ml⁻¹ [³⁵S]sulphate in medium with 0.5 mg ml⁻¹ MgSO₄) during the whole period of growth. The isolated hyphal material was frozen in liquid nitrogen and stored at -70 °C.

Quantification of mRNAs. Total RNA was isolated, denatured, spotted onto Genescreen-plus membrane, and hybridized to ³²P-labelled cDNA clones (Mulder & Wessels, 1986; Ruiters *et al.*, 1988). Radioactivity was measured by liquid-scintillation counting. Hybridization to a clone of the gene for 18 S rRNA served to compare the amounts of total RNA spotted.

SDS-soluble proteins. To the culture medium used in brief labelling experiments, 1 vol. of the culture medium from a 4-d-old dikaryon was added for co-precipitation. The medium was then made up to 5% (w/v) in TCA, and precipitated proteins were collected after 1 h on ice. The precipitate was washed twice with 5% (v/v) TCA containing 10 mM-MgSO₄ in the case of labelling with [³⁵S]sulphate, and dissolved in SDS sample buffer [2% (w/v) SDS, 0.05 M-Tris/HCl, pH 6.8, 10% (v/v) glycerol, 0.001% bromophenol blue] by heating at 100 °C for 10 min. SDS-soluble mycelial proteins were obtained as follows: the mycelium was fragmented in an X-press (AB Biox, Göteborg, Sweden) at -25 °C, and the broken material was quickly thawed in extraction buffer (0.1 M-Tris/HCl, pH 8.0, 10 mM-MgSO₄, 1 mM-PMSF). To obtain total SDS-soluble proteins, the suspension was immediately brought to 5% TCA. The precipitate was washed twice with 5% TCA, 10 mM-MgSO₄, 1 mM-cysteine, 1 mM-methionine, then twice with cold acetone (-20 °C), and dried. Proteins were extracted with SDS sample buffer at 100 °C for 10 min. For cell-wall proteins, cell walls were prepared by centrifuging (1500 g) the broken mycelium in extraction buffer, and washing the pellet three times with extraction buffer and twice with water. The washed walls were freeze-dried and then extracted with SDS sample buffer at 100 °C for 10 min.

Dissolution of proteins with formic acid and oxidation with performic acid. Walls extracted with SDS, washed twice with water, and freeze-dried, were subsequently extracted with 98% formic acid [100 µl (mg dry wt)⁻¹] in a sonicating water bath (Sonicor Instruments, Farmingdale, NY, USA) for 1.5 h, keeping the temperature below 10 °C. After centrifugation, 2 vols performic acid were added to the supernatant, and oxidation was allowed to proceed on ice for 4 h to cleave disulphide bonds. Performic acid was prepared by mixing 1 vol. 30% (v/v) H₂O₂ with 9 vols 98% formic acid and was used after standing at room temperature for 1 h. Formic acid extracts, before and after oxidation with performic acid, were diluted 20 times with water, freeze-dried, and dissolved in SDS sample buffer. TCA-precipitated proteins from culture media were also dissolved in formic acid and oxidized with performic acid before being taken up in SDS sample buffer.

PAGE and protein sequencing. Electrophoresis was generally done in 12.5% (w/v) polyacrylamide gels according to Laemmli (1970). For autoradiography, gels were soaked in 1 M-sodium salicylate immediately after electrophoresis (Chamberlain, 1979), dried at 65 °C, and

exposed to pre-flashed film (Kodak Xomat AR). For amino-terminal protein sequencing, electrophoresis was done with the neutral buffer system MZE-3328IV of Jovin (1973) and gels were stored overnight prior to use to avoid amino-terminal blockage during electrophoresis (Moos *et al.*, 1988). Electrophoresis was done on PVDF membrane (Immobilon-P, Millipore) by semi-dry transfer (Multiphor II Electrophoresis System, Pharmacia) at 0.8 mA cm⁻² for 2 h. The blot was stained for 30 min in a fresh solution of 0.1% Coomassie Brilliant Blue R250 in 10% (v/v) acetic acid, 30% (v/v) methanol and destained in the same solvent for 3 h. Protein bands of interest, which were negatively stained by this procedure, were excised and amino acid sequencing was carried out with a pulse liquid sequencer on line connected to a PTH analyser (Applied Biosystems, Foster City, CA, USA).

Results

Genetics and morphology of the *thn* mutants

Over the years we have regularly encountered thin variants with few aerial hyphae, wavy submerged hyphae, and a pungent smell in the strains 4-39 (*A41B41*) and 4-40 (*A43B43*) of *S. commune*. Mostly the whole colony turned thin; rarely, a thin sector appeared in an otherwise normal-looking colony. For the purpose of the present investigation we isolated a few of these variants, both from strains 4-39 and 4-40. In crosses with the compatible wild-type strains bilateral dikaryotization occurred, though somewhat more slowly on the thin side, with the formation of aerial mycelium and fruit-bodies at both sides. In the monokaryotic progeny, a 1:1 segregation of the thin phenotype occurred, showing that thin behaves as a recessive Mendelian mutation (*thn*). In crosses between compatible independently isolated *thn* mutants, dikaryons always displayed the thin morphology and produced few fruit-bodies at the edge of the Petri dish only, or none at all. The *thn* mutants we isolated (about 10) thus all appear allelic, suggesting that the *THN* gene is a highly mutable locus. The mutation matches the phenotype described in detail by Schwalb & Miles (1967) in every respect, and probably corresponds to *thn-1*, mapped on linkage group II (Raper, 1988 and personal communication) but we did not perform a linkage analysis to confirm this. We did however establish that *thn* is not allelic to *fbf*, another frequently occurring recessive mutation which blocks fruiting, but not the formation of aerial hyphae (Springer & Wessels, 1989). In a *thn* × *fbf* cross, normal formation of aerial hyphae and fruit-bodies occurred on both sides of the mating.

When grown as colonies from a single inoculum, the *thn* monokaryons had a faster linear growth rate than their wild-type progenitors, had a more regular colony margin, and produced few aerial hyphae. With respect to the formation of aerial structures, the phenotypic expression of *thn* in monokaryons and dikaryons was best seen when mycelia were grown as a lawn from a

Fig. 1. Four-day-old cultures of *S. commune* without (a, c) and with (b, d) the *thn* mutation, grown as lawns from mycelial homogenates on minimal agar medium. (a) *A41 B41* (4-39); (b) *A41 B41 thn* (4-39-1); (c) *A41 B41* $\times$ *A43 B43*; and (d) *A41 B41 thn* $\times$ *A43 B43 thn*.

mycelial homogenate spread over the surface of an agar medium (Fig. 1). The analyses detailed below were made from such cultures. It is important to note that the mutation had no effect on synthetic capacities in general; the dry weights of the mycelia shown in Fig. 1 were approximately the same, and all incorporated [35 S] from sulphate into protein at about the same rate.

Expression of *Sc* genes

Cultures as shown in Fig. 1 were harvested and analysed for the presence of specific mRNAs by dot-blot analysis (Table 1). As expected from previous studies (Mulder & Wessels, 1986; Ruiters *et al.*, 1988), hybridizations detected only Sc3 hydrophobin mRNA in wild-type monokaryons, but the derived dikaryon also accumulated fruiting mRNAs, including the Sc1 and Sc4 hydrophobin mRNA. In the presence of the *thn* mutation, the concentration of Sc3 mRNA in the monokaryons dropped to very low levels, and a double dose of *thn* also prevented accumulation of the fruiting mRNAs in the dikaryon. In both wild-type mono-

Table 1. *Abundance of specific mRNAs in 4-d-old surface cultures of monokaryons and derived dikaryons in the absence and presence of the thn mutation*

Each value represents the mean of two cultures. Maximum variation between mRNA concentrations of duplicate cultures was 5% of the values indicated.

	10 ³ $\times$ Percentage of total RNA						
	Sc1	Sc2	Sc3	Sc4	Sc5	Sc6	Sc7
<i>A41B41</i>	ND	ND	23.5	ND	ND	ND	ND
<i>A41B41thn</i>	ND	ND	2.8	ND	ND	ND	ND
<i>A43B43</i>	1.4	ND	63.6	ND	ND	ND	ND
<i>A43B43thn</i>	ND	ND	ND	ND	ND	ND	ND
<i>A41B41</i> $\times$ <i>A43B43</i>	33.0	1.5	5.5	32.5	5.2	7.5	3.2
<i>A41B41thn</i> $\times$ <i>A43B43thn</i>	ND	ND	ND	ND	ND	ND	ND

ND, Not detected, i.e. an abundance of less than $0.5 \times 10^{-3}\%$.

karyons, the concentration of the Sc3 mRNA was high but somewhat variable (Table 1). This is probably due to the fact that the concentration of this mRNA peaks on about the fourth day of culture (Mulder & Wessels, 1986), and thus small differences in developmental age between

Fig. 2. SDS-PAGE autoradiographs of hot-SDS-extractable ^{35}S -labelled proteins in extracts obtained from (a) whole mycelium; (b) cell walls. Lanes: 1, wild-type monokaryon 4-39; 2, the derived *thn* mutant 4-39-1. Equal amounts of TCA-precipitable radioactive material were loaded on each gel.

cultures can result in large differences in the specific mRNA concentration. Also noteworthy is the low concentration of *Sc3* mRNA in the wild-type dikaryon. This correlated with the near complete absence of aerial hyphae in this culture (Fig. 1). The mRNA mass in these cultures has been estimated to amount to 3% of the total RNA mass (Hoge *et al.*, 1982). A concentration of the *Sc3* mRNA of 0.05% would thus indicate that this mRNA constitutes 1.5% of the total mRNA mass. In the wild-type monokaryon the *Sc3* gene could thus code for a very abundant protein which would be virtually absent in the *thn* mutant.

Differential synthesis of proteins by a wild-type monokaryon and a coisogenic thn mutant

The monokaryon (4-39) and a derived *thn* mutant (4-39-1) were grown for 4 d and labelled with [^{35}S]sulphate during the last day for harvest (see Methods). We used [^{35}S] label because of the high cysteine content of the putative *Sc3* protein but refrained from using [^{35}S]methionine because this amino acid is only present at the amino terminus of the putative signal sequence (Schuren & Wessels, 1990). Also, this amino acid is not transported into fruit-bodies (de Vries & Wessels, 1984). However, after labelling with [^{35}S]sulphate, hot-SDS

Fig. 3. SDS-PAGE autoradiographs of ^{35}S -labelled proteins in: (a) formic acid extracts from walls previously extracted with hot SDS and oxidized with performic acid before electrophoresis; (b) TCA precipitates from the culture media; (c) as (b) but oxidized with performic acid. Lanes: 1, wild-type monokaryon 4-39; 2, the derived *thn* mutant 4-39-1. For comparison, the amounts loaded per lane were obtained from samples of mycelia containing equal amounts of TCA-precipitable radioactive material.

extracts of broken mycelia failed to reveal significant differences between the strains after one-dimensional electrophoresis (Fig. 2a).

Because the amino acid sequence derived from the *Sc3* sequence (and from the *Sc1* and *Sc4* sequences) indicated the presence of a signal sequence for secretion (Schuren & Wessels, 1990), we examined the proteins excreted into the cell walls and into the medium during labelling. Whereas the medium of the wild-type monokaryon contained 3.2% of the counts incorporated into TCA-precipitable material, the washed cell walls of this monokaryon contained about 20% of the counts. Sixty percent of the labelled material could be removed from these walls by boiling in SDS buffer. When such SDS extracts made from cell walls of wild-type and *thn* monokaryon were compared by electrophoresis, no significant difference was seen (Fig. 2b). Subsequent cold formic acid extraction removed 80% of the residual radioactivity from the walls of the wild-type monokaryon. The extracted material was apparently of high molecular mass, because it did not penetrate into the SDS gels. However, after performic acid oxidation, this material resolved into a product (or a few closely spaced products) running at a position of approximately 28 K (Fig. 3a). As shown, very little of this product could be

found in the *thn* mutant. After taking up a TCA precipitate of medium proteins in SDS buffer, the wild-type monokaryon showed an apparently complex pattern of excreted proteins, but very few proteins were excreted into the medium by the *thn* mutant (Fig. 3*b*). However, after treating the TCA precipitate with formic and performic acid, the complex pattern seen in the medium of the wild-type monokaryon resolved into just one band (or a few closely spaced bands) at a position of 28 K which was not seen in the *thn* mutant (Fig. 3*c*).

The wall-bound 28 K protein occurs only in aerial hyphae

If the SDS-insoluble complex of 28 K subunits is essential for the occurrence of aerial hyphae, one would expect to find it in such hyphae. The wild-type monokaryon (4-39) was therefore grown from a mycelial homogenate on the surface of a nylon net floating on minimal liquid medium. After 4 d growth, pure sample of submerged hyphae was isolated by scraping the underside of the net. The rest of the mycelium was then harvested and categorized as 'aerial', although it also contained submerged hyphae. To avoid differential labelling of substrate hyphae and aerial hyphae, the former developing before the latter, [³⁵S]sulphate was present during the whole growth period. Using this separation of hyphal types, 91% of the TCA-precipitable counts were found in the 'aerial' mycelium, indicating heavy contamination with submerged mycelium. The pure submerged mycelium contained 6% of the counts while 3% of the counts were found in the culture medium. To make a comparison between cell walls of the 'aerial' and submerged mycelia, the cell walls used for extraction of aerial hyphae were derived from hyphal material with equal TCA-precipitable counts, in the case of the submerged mycelium including the proteins excreted into the medium. Fig. 4 shows that after SDS extraction, the walls of submerged hyphae contained very little of the 28 K protein; virtually all of this protein was found in the medium. On the other hand, in the walls of the 'aerial' hyphae the 28 K protein was present in a hot-SDS-insoluble form. However, this experiment did not exclude the possibility that the submerged hyphae present in the 'aerial' sample also contained the insolubilized 28 K protein, or contributed to the accumulation of the soluble form in the medium. To resolve this uncertainty, we labelled cultures growing on a perforated polycarbonate membrane and covered with a nylon net (see Methods). Aerial hyphae scraped off the surface of the net contained 34% of the mycelial protein, and the insoluble complex of 28 K protein was found in abundance in their walls. On a total protein basis, walls from the remaining part of the culture ('submerged') contained 2.5 times less of this complex. The presence of

Fig. 4. Presence of the complexed 28 K protein in the cell walls of aerial hyphae (a) and absence of this protein in cell walls of submerged hyphae (b) but which is excreted into the medium (c). The extracts applied to the gels a and (b plus c) were derived from material originally containing equal amounts of TCA-precipitable [³⁵S] counts.

the complex in this fraction could have been due to contamination with aerial hyphae. When a culture was covered with a perforated polycarbonate membrane, instead of a nylon net, no aerial hyphae could develop but submerged growth occurred unimpaired, as indicated by the amount of protein synthesized. On a total protein basis, the walls obtained from such submerged surface cultures contained 25 times less of the Sc3 hydrophobin complex than the walls of aerial hyphae.

Identity of the 28 K protein with the product of the Sc3 gene

The correlation between the presence and abundance of the Sc3 mRNA and the 28 K protein strongly suggests that this protein is the product of the Sc3 gene. Amino-terminal sequence analysis showed the sequence [(Gly)-Gly-His-Pro-Gly-X_{aa}-X_{aa}-X_{aa}-Pro-] as present in the deduced amino acid sequence after 24 amino acids of the signal peptide (Schuren & Wessels, 1990). The glycine doublet at the amino-terminal end was found in about 40% of the molecules. Further reading of the sequence was not possible, probably due to the presence of protein modifications. A second band visible (Fig. 4*c*) just below the 28 K band showed the same sequence and thus represents a differently modified form of the Sc3 protein. The exclusive presence of the Sc3 hydrophobin at the

Fig. 5. Differential labelling of the 28 K protein in the culture medium of the dikaryon 4-39 $\times$ 4-40 with [^{35}S]sulphate; (2) [^{14}C]lysine; and (3) [^{14}C]leucine.

position around 28 K was further substantiated by showing that a radioactive band at this position could be detected after labelling cultures with [^{35}S]sulphate and [^{14}C]leucine, but not with [^{14}C]lysine (Fig. 5), the latter amino acid being absent from the deduced sequence of the Sc3 protein (Schuren & Wessels, 1990). Fig. 5 shows results obtained with medium from the dikaryon to demonstrate the labelling by lysine of products of the *Sc4* gene (Wessels *et al.*, 1991).

Discussion

The thin mutation (*thn*) of *S. commune* has long been recognized, because it arises spontaneously at high frequency in the commonly used monokaryons, and has a distinct phenotype: suppression of the formation of aerial hyphae, the appearance of corkscrew-like or wavy hyphae in the substrate, and the suppression of both aerial hyphae and fruit-bodies in dikaryons when homozygous for *thn* (Schwalb & Miles, 1967, Raper, 1988). The nature of this mutation is unknown but because it sometimes appears to occur simultaneously in many nuclei it might involve an epigenetic modification of the gene, *e.g.* by methylation. However, we have been unable to reverse the phenotype by growing the thin mutant in the presence of 5-azacytidine (data not shown). Here we show that in the monokaryon the *thn* mutation prevents accumulation of the mRNA from the *Sc3* gene, and that in the dikaryon it also prevents the accumulation of the homologous *Sc1* and *Sc4* genes and

of other fruiting genes. These fruiting genes are under the additional control of the mating-type genes (Mulder & Wessels, 1986; Ruiters *et al.*, 1988) and the *FBF* gene (Springer & Wessels, 1989). The only control element found so far that affects the activity of the *Sc3* gene is the *THN* gene. However, it is important to note that the *Sc3* gene is also under developmental control. In young cultures the *Sc3* gene appears to be relatively silent; its mRNA only accumulates at the time of formation of aerial hyphae (Mulder & Wessels, 1986). When formation of aerial hyphae is prevented, as in shaken cultures, the *Sc3* mRNA nevertheless accumulates at the same time as in surface cultures (Wessels *et al.*, 1987).

The *Sc3* gene encodes a hydrophobin, a hydrophobic cysteine-rich protein of 125 amino acids including a signal peptide for secretion. The present results show that this protein is present in the medium and the hyphal walls as a protein subunit of apparent $M_r = 28$ K. Because the deduced M_r without signal peptide is 9830 and deglycosylation lowers the apparent M_r of the excreted protein to only about 20 K (results not shown), it is possible that the 28 K protein represents a dimer of two covalently linked glycosylated chains. We calculate that at the time of synchronous formation of aerial hyphae in the mycelial lawn about 5% of all protein synthesis is directed towards synthesis of this Sc3 hydrophobin.

As shown, both submerged hyphae and aerial hyphae excreted the Sc3 hydrophobin. However, submerged hyphae excreted the hydrophobin into the medium and retained little in their walls, whereas aerial hyphae accumulated all hydrophobin in their walls as a hot-SDS-insoluble complex. Surface-grown mycelia prevented from forming aerial hyphae by an overlaying perforated membrane accumulated very little of this complex in their walls. Also, hyphae grown in shaken culture excreted nearly all of the hydrophobin into the culture medium (data not shown).

The complex of Sc3 hydrophobin present in the walls of aerial hyphae could be extracted with formic acid and dissociated into 28 K subunits by cleavage and oxidation of any cystines with performic acid. Therefore, we originally attributed complex formation to the occurrence of intermolecular disulphide bonds. We have since found that extraction and dissociation of the complex can also be achieved by treatment with trifluoroacetic acid at room temperature (data not shown), indicating the importance of hydrophobic interactions in generating the insoluble complex in the walls of aerial hyphae.

Possibly, the insolubilized Sc3 hydrophobin forms the rodlet layer seen at the hyphal wall surface of *S. commune* (Wessels *et al.*, 1972). Such rodlets have generally been seen on the surface of spores, where they are believed to confer the hydrophobicity necessary for dispersal

(Beever & Dempsey, 1978); conidiospores without rodlets as found in a mutant of *Neurospora crassa* were easily wettable. The rodlet layer on the conidiospores of the wild-type *N. crassa* has been shown to consist of protein(s) with an amino acid composition (Beever *et al.*, 1979) which shows similarity to that of the hydrophobins of *S. commune* (Schuren & Wessels, 1990). Recently, the *rodA* gene from *Aspergillus nidulans*, implicated in the formation of conidial rodlets, was shown to code for a protein with an amino acid sequence clearly homologous to those of the *S. commune* hydrophobins (Stringer *et al.*, 1991). Apparently, this class of proteins occurs both in basidiomycetes and ascomycetes.

Assuming an essential role of the Sc3 hydrophobin in the generation of the hydrophobic aerial hyphae of *S. commune*, we can envisage the following sequence of events in the development of the mycelium. After inoculation, the hyphae first grow with minimal expression of the Sc3 gene, but after some growth has occurred, the gene is switched on by some unknown mechanism and its mRNA accumulates (Mulder & Wessels, 1986). The Sc3 hydrophobin is now excreted into the medium and we surmise that this potentiates the mycelium to form aerial hyphae. Hyphae emerging from the substrate would excrete the hydrophobin in their walls and thereby acquire a hydrophobic surface necessary for aerial growth. Direct evidence for this might be obtained by showing that disruption of the Sc3 gene prevents the emergence of aerial hyphae. Such experiments have not yet been successful because of the high incidence of the expected phenotype, due to the *thn* mutation, after transforming with a deleted Sc3 gene. Whether hydrophobic interactions between the Sc3 hydrophobin molecules are sufficient to create the insoluble hydrophobin complex in the wall of aerial hyphae, also requires further investigation. If the Sc3 hydrophobin is a determinant for aerial hyphae, the delay in expression of the Sc3 gene in young cultures may be important for the development of a substantial amount of submerged feeding mycelium before formation of aerial hyphae is possible. Early expression of the gene may be detrimental to development of the mycelium as a whole because aerial hyphae are unable to take up nutrients. On the other hand, failure to produce the Sc3 hydrophobin, as in the *thn* mutant, would lead to inability to produce aerial hyphae and could thus cause somewhat faster linear growth of submerged hyphae as observed.

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