15 Olsen, R.A. (1973) Triterpeneglycosides as inhibitors of fungal growth and metabolism. 6. The effect of aescin on fungi with reduced sterol contents, *Physiol. Plant.* 29, 145–149
16 Défago, G. and Kern, H. (1983) Induction of *Fusarium solani* mutants insensitive to tomatine, their pathogenicity and aggressiveness to tomato fruits and pea plants, *Physiol. Plant Pathol.* 22, 29–37
17 Crombie, W.M.L. *et al.* (1986) Pathogenicity of the take-all fungus to oats: its relationship to the concentration and detoxification of the four avenacins, *Phytochemistry* 25, 2075–2083
18 Bowyer, P. *et al.* (1995) Host range of a plant pathogenic fungus is determined by a saponin detoxifying enzyme, *Science* 267, 371–374
19 Turner, E.M. (1953) The nature of resistance of oats to the take-all fungus, *J. Exp. Bot.* 4, 264–271
20 Osbourn, A.E. *et al.* (1994) An oat species lacking avenacin is susceptible to infection by *Gaeumannomyces graminis* var. *tritici*, *Physiol. Mol. Plant Pathol.* 45, 457–467
21 Turner, E.M. (1961) An enzymic basis for pathogenic specificity in *Ophiobolus graminis*, *J. Exp. Bot.* 12, 169–175
22 Osbourn, A.E. *et al.* (1991) Partial characterization of avenacinase from *Gaeumannomyces graminis* var. *avenae*, *Physiol. Mol. Plant Pathol.* 38, 301–312
23 Bryan, G.T., Daniels, M.J. and Osbourn, A.E. (1995) Comparison of fungi within the *Gaeumannomyces graminis–Phialophora* complex by analysis of ribosomal DNA sequences, *Appl. Env. Microbiol.* 61, 681–689
24 Osbourn, A.E. *et al.* Fungal pathogens of oat roots and tomato leaves employ closely related enzymes to detoxify host plant saponins, *Mol. Plant-Microbe Int.* (in press)
25 Sandrock, R.W., DellaPenna, D. and VanEtten, H.D. Purification and characterization of β_2-tomatinase, an enzyme involved in the degradation of α-tomatine and isolation of the gene encoding β_2-tomatinase from *Septoria lycopersici*, *Mol. Plant-Microbe Int.* (in press)
26 Henrissat, B. (1991) A classification of glycosyl hydrolases based on amino acid sequence similarities, *Biochem. J.* 280, 309–316
27 Henrissat, B. and Bairoch, A. (1993) New families in the classification of glycosyl hydrolases based on amino acid sequence similarities, *Biochem. J.* 293, 781–788
28 Castle, L.A., Smith, K.D. and Morris, R.O. (1992) Cloning and sequencing of an *Agrobacterium tumefaciens* β-glucosidase gene involved in modifying a *vir*-inducing plant signal molecule, *J. Bact.* 174, 1478–1486
29 Lenman, M. *et al.* (1993) Characterization of a *Brassica napus* myrosinase pseudogene: myrosinases are members of the BGA family of β-glycosidases, *Plant Mol. Biol.* 21, 463–474
30 Oxtoby, E. *et al.* (1991) Nucleotide and derived amino acid sequence of the cyanogenic β-glycoside (linamarase) from white clover (*Trifolium repens* L.), *Plant Mol. Biol.* 17, 209–219
31 Curtis, W. (1835) *Flora Londinensis IV* (Bohn, H., ed.), London

Anne Osbourn is at the Sainsbury Laboratory, The John Innes Centre, Colney Lane, Norwich, UK NR4 7UH.

Fungal hydrophobins: proteins that function at an interface

Joseph G.H. Wessels

Hydrophobins are small, moderately hydrophobic proteins secreted by fungi and containing eight cysteine residues in a conserved pattern. Most hydrophobins have been discovered as putative products of genes abundantly expressed in development, pathogenesis and symbiosis. Those that have been purified exhibit interfacial self-assembly into amphipathic protein films that can be extremely insoluble. These protein films arise at the surface of emergent structures, such as aerial hyphae, fruit bodies and air-borne spores and they line air channels in tissues, conferring hydrophobicity to these surfaces. Hydrophobins may also be responsible for adherence of hyphae to each other and to hydrophobic surfaces of other organisms, as in pathogenic interactions.

The intimate relationships between plants and fungi is best appreciated if it is realized that these organisms belong to different kingdoms that have evolved separately but in complete interdependence. Although fungi may superficially resemble plants, they appear evolutionarily closer to animals than plants[1]. That fungi depend on plant photosynthesis for nutrition is obvious, but that land plants could not have evolved without fungi is less generally appreciated.

First of all, fungi are the principal degraders of dead plant remains, particularly of the lignocellulosic cell wall, and thus return to the atmosphere an estimated $25–50 \times 10^9$ tons of carbon annually fixed by plants. Of course, this is because they secrete large quantities of cellulases, ligninases and other enzymes, but equally important is their mode of growth by means of apically extending hyphae[2]. Under turgor, while secreting the depolymerizing enzymes, such hyphae can penetrate solid substrata such as wood and digest them from within[3]. Many fungi also penetrate living plants, but only in a few cases does this lead to death of the plant. In most cases a symbiotic relationship is set up.

It is relatively recently that plant biologists have come to realize the enormous importance, at least in natural habitats, of the mutualistic symbioses between fungi and plant roots, known as mycorrhizas. These associations occur with nearly all plant species and without them there would be no rain forests nor heathlands, to mention just two well-known biotopes[4]. While obtaining photosynthates from the plant, the fungus aids the plant with recruitment of water and minerals from poor soils and even recycles organic carbon

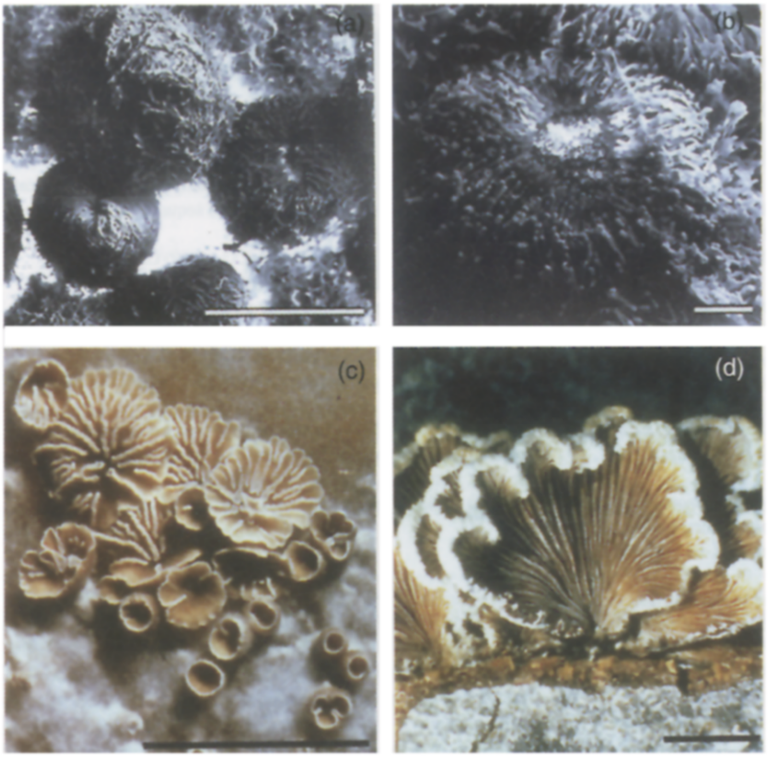


Fig. 1. Fruit-body development in *Schizophyllum commune*. (a) Cryo-scanning electron micrographs of a frozen fully-hydrated early stage of fruit-body formation, showing aggregation and upward growth of parallel hyphae that grow at their tips. (b) As (a) but at a somewhat more advanced state of development, showing the first signs of hymenium development at the bottom of the apical pit. (c) Macrograph of fruit bodies at the cup stage, grown in the laboratory on minimal agar medium. Note formation of the split gills. (d) Expanded fruit bodies from the wild. Bar in (a) represents 1 mm, in (b) 100 µm, and in (c) and (d) 10 mm.

and nitrogen back into the plant. Symbiotic fungi that infect aerial plant parts cannot give these returns and thus are biotrophic phytopathogens that have mastered the art of keeping the plant alive. Finally, a large number of fungi capture algae and cyanobacteria and extract the carbon and nitrogen fixed by these organisms with solar energy. This common symbiosis leads to the unique associations known as lichens, which occupy a vast land area particularly in tundra and high mountains where nothing else can grow[5].

Except for these lichens that live in and from the air, fungi thus always grow by tunnelling their way through moist substrata, dead or alive. They can grow over or through non-nutritious substrata by recycling their own cellular material or transporting water and nutrients from a food base, often by forming parallel assemblages of hyphae growing at their tips. Instances are known in which colonies cover several hectares underground[6]. But for reproduction by asexual or meiotic spores the hyphae have to come into the open and form aerial structures. Among these are asexual spores named conidia that are born on terminally branched aerial stalks as in *Aspergillus* and *Penicillium* spp., or fruit bodies such as mushrooms and brackets in which genetically different nuclei fuse and immediately undergo meiosis to form recombinant spores. It is important to emphasize that in all these developmental processes any comparison with plant development is superficial. For instance, fruit bodies such as those of *Schizophyllum commune* (Fig. 1) do not develop from meristems but from the interactions of hyphae that grow individually at their tips. How they know what direction to take, what neighbours to interact with, and what final differentiation to undergo, is a complete mystery. However, it may not be too far-fetched to assume that proteins at the surfaces of hyphae play important roles in these developmental processes as well as in interactions of hyphae with their environment.

Hydrophobins

Of all homobasidiomycetes ('higher fungi'), *Schizophyllum commune* is the easiest for producing fruiting stages in the laboratory; fully sporulating fruit bodies, as shown in Fig. 1c, are present about a week after inoculation. Therefore, among homobasidiomycetes, this organism is genetically the best known[7] and the most extensively studied at the molecular level[8].

Among the genes most abundantly expressed during emergent growth of aerial hyphae and fruit bodies (Fig. 1) is a family of genes putatively encoding small, moderately hydrophobic, cysteine-rich proteins with signal sequences for secretion[9]. Although high mRNA concentrations predicted abundant proteins, these proteins remained elusive until it was found that they occurred in SDS-insoluble complexes that could be dissociated only with agents such as concentrated formic acid (FA) or trifluoroacetic acid (TFA). These proteins, dubbed hydrophobins, were found in walls of aerial hyphae (SC3 hydrophobin) and

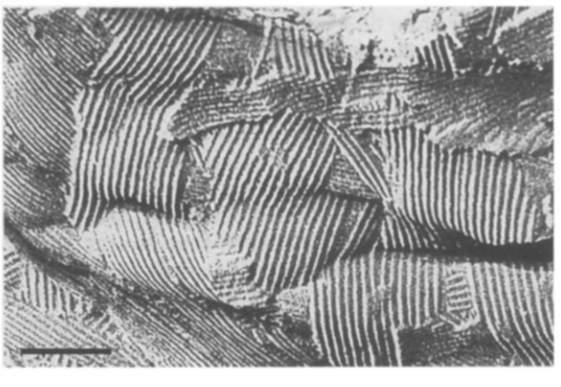


Fig. 2. The hydrophobic rodlet layer on conidiospores of *Aspergillus nidulans*, the product of the *rodA* gene, as seen after freeze-fracturing. Bar represents 100 nm. *Courtesy of Dr H.A.B. Wösten.*

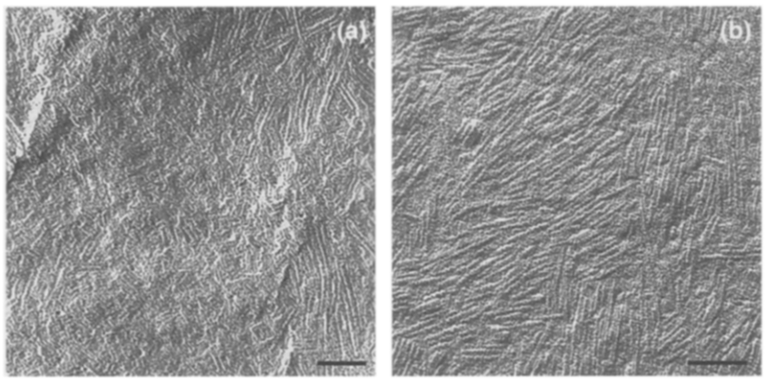


Fig. 3. Morphological appearance of assembled SC3 hydrophobin. (a) Freeze-fracturing and shadowing show the typical rodlets of assembled SC3 at the surface of aerial hyphae. (b) Similar rodlets are observed after drying down solutions of pure SC3 and shadowing the preparation. Bar represents 100 nm. *Courtesy of Dr H.A.B. Wösten.*

in walls of hyphae that constitute fruit bodies (SC4 hydrophobin), while they were also found in various states of aggregation in the culture medium but not in the cell walls of assimilative hyphae bathing in the culture medium[10].

The finding that a developmentally controlled hydrophobin gene of *Aspergillus nidulans* upon disruption caused disappearance of the hydrophobic rodlet layer on conidiospores of this organism[11] (Fig. 2) suggested an essential role of hydrophobins in formation of such layers, universally present on hydrophobic surfaces of fungi. Accordingly it was found that the SC3 hydrophobin coats the outer surface of aerial hyphae of *Schizophyllum commune* with an insoluble protein film, 10 nm thick, which provides for the non-wettable outer surface with the typical pattern of fascicles of parallel rodlets (Fig. 3)[12].

Interfacial self-assembly

After dissociation of SC3 hydrophobin complexes with TFA and removal of the TFA by evaporation, the monomers show remarkable properties, not known for other proteins (Box 1). The SC3 monomers assemble around air bubbles, coating these with SDS-insoluble amphipathic films about 10 nm thick[13]. At the side facing the air these films showed a pattern of parallel rodlets, 10 nm thick, in fascicles, very similar to those seen on the surface of aerial hyphae. Simply drying down a solution of SC3 in air produces the same pattern (Fig. 3). Moreover, this surface has about the same hydrophobicity as the surface of aerial hyphae. The hydrophobins assemble into an insoluble amphipathic film not only at the water–air interface but also at interfaces between water and any hydrophobic material[14]. In this way, oil droplets are stabilized in water and hydrophobic plastics become wettable. Other proteins also have the property of coating hydrophobic surfaces and making them easily wettable. However, the hydrophobin film is not only insoluble; its attachment to the hydrophobic surface is also very strong and not broken, for instance, by hot detergent. Compared to other proteins SC3 is also very surfactive, the lowering of the surface tension mainly resulting from a conformational change during assembly of the monomers into an amphipathic film[15].

Self-assembly of a protein reminiscent of that of the SC3 hydrophobin has earlier been described for cerato-ulmin[16], a protein abundantly secreted by *Ophiostoma ulmi*, the causative agent of Dutch elm disease. Sequencing of the protein[16] and its gene[17] showed cerato-ulmin to be a hydrophobin. However, contrary to the films produced by SC3, the assembled films of cerato-ulmin are unstable and soluble in 60% ethanol and SDS. Because of its deviating hydropathy pattern and solubility of assemblages in SDS, it was called a class II hydrophobin, SC3 belonging to class I hydrophobins[18]. Also cryparin, an abundant protein secreted by *Cryphonectria parasitica*, the causative agent of chestnut blight, is a hydrophobin with properties similar to those of cerato-ulmin[19]. Until now, the properties of none of the other (putative) hydrophobins, known from gene sequences, have been reported but presumably those known to be responsible for rodlet layers on spores[11,20–23] are class I hydrophobins because these layers are all resistant to hot-SDS treatments.

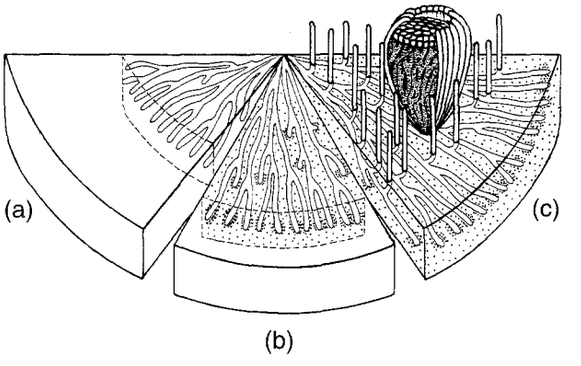


Fig. 4. Schematic representation of the roles of hydrophobins in development of the *Schizophyllum commune* secondary mycelium producing aerial hyphae and fruit bodies (a similar scheme can be drawn for the monokaryon which only produces the SC3 hydrophobin and aerial hyphae). (a) In juvenile cultures the hydrophobin genes are silent and a substantial amount of submerged mycelium can be produced. (b) The hydrophobin genes are then switched on and the hydrophobins are secreted as monomers by growing hyphal apices into the medium (stipples) whereas the walls of these hyphae are virtually devoid of hydrophobins (walls of substrate hyphae drawn as thin lines). The mycelium is now mature and ready to produce aerial structures. (c) While continuing apical secretion, emergent hyphae are now coated with an insoluble hydrophobin film due to interfacial self-assembly of the hydrophobins (hyphal walls drawn as thick lines). The SC3 hydrophobin coats aerial hyphae and hyphae at the surface of fruit bodies (here represented by a fruit body in which the pileus has not yet expanded). The SC4 hydrophobin coats air-exposed surfaces within the fruit bodies (shown shaded within the cut-away part of the fruit body). *From Ref. 34, with permission.*

Biological significance of hydrophobins

Formation of aerial hyphae

Of the four hydrophobin genes identified in *Schizophyllum commune*, *SC3*, *SC1*, *SC4* and *SC6*, only *SC3* is appreciably expressed in the primary mycelium that normally forms aerial hyphae but no fruit bodies. A role for the *SC3* gene in formation of aerial hyphae was suggested by the presence of the SC3 assemblage at the surface of these hyphae (Fig. 3) and by a strict correlation between activity of the gene and the appearance of aerial hyphae[8,18,24]. Indeed, a targeted mutation in the *SC3* gene[14] suppresses formation of aerial hyphae and aerial hyphae that still form have hydrophilic surfaces.

How can activity of the *SC3* gene lead to formation of hydrophobic hyphae that emerge from the substrate into the air? As shown in the cartoon of Fig. 4, hydrophobins are not formed in juvenile mycelia engaged in assimilation. This may be important because a certain amount of assimilative mycelium has to be formed to sustain the growth of aerial structures that themselves cannot take up nutrients. Then the hydrophobin genes switch on and hydrophobins are secreted into the medium – in the monokaryon the SC3 hydrophobin only (this hydrophobin was shown to be secreted at growing hyphal tips[12] where it can easily be translocated over the wall to the hyphal surface by the flow of still semi-fluid wall polymers[18]). In combination with what is known about the *in vitro* properties of this hydrophobin and its location *in vivo* it is plausible to assume that, in tips that breach the surface, secreted SC3 cannot diffuse away and assembles at the wall–air interface into the hydrophobic rodlet layer that determines these hyphae for further growth into the air[12,13]. At the moment it is unknown whether hyphal tips breach the liquid–air surface just by chance or if other factors are involved.

Fruit-body formation

Most homobasidiomycetes make fruit bodies only on a secondary mycelium originating from the mating of primary mycelia carrying differences at one or two mating-type genes. This ensures that nuclei participating in meiosis will be genetically different and the resulting spores recombinants.

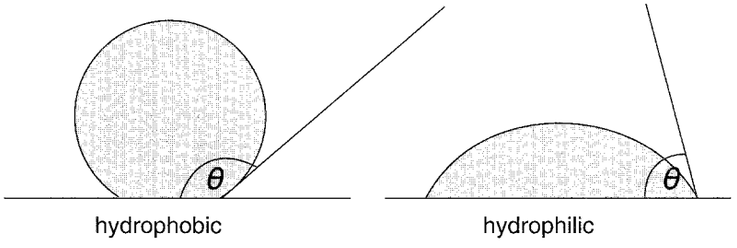
In *Schizophyllum commune* (Fig. 1) the activities of hydrophobin genes are

Box 1. Interfacial assembly of hydrophobins

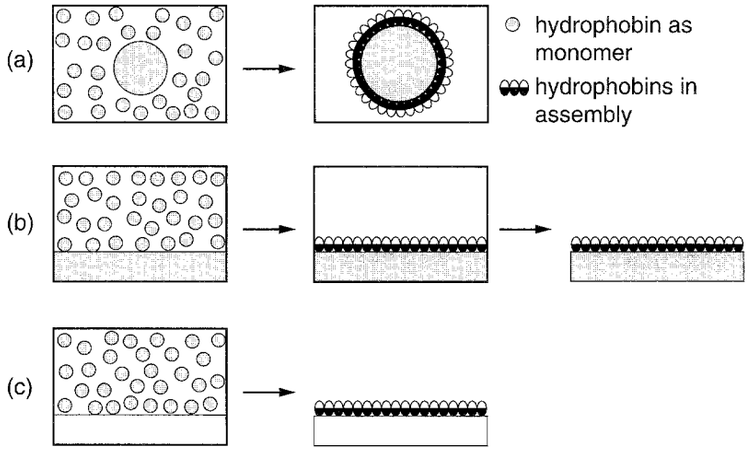
Hydrophobins (about 20 hydrophobin genes of different fungi have been sequenced) are small secreted proteins (100 ± 25 amino acids) that share a typical hydropathy pattern and have 8 cysteine residues spaced in a specific pattern,

$$X_{2-38}\text{-C-}X_{5-9}\text{-C-C-}X_{11-39}\text{-C-}X_{8-23}\text{-C-}X_{5-9}\text{-C-C-}X_{6-18}\text{-C-}X_{2-13}$$

in which X signifies any amino acid, with a high proportion of non-polar amino acids. The spacing of cysteine residues, the positions of the disulphide bridges as determined for cerato-ulmin[16] and the recurrent hydropathy patterns around the cysteine residues[18] suggest two-domain proteins. Although only explicitly shown for the SC3 hydrophobin, the evidence suggests that they all exhibit the property of self-assembly at hydrophilic–hydrophobic interfaces (interfacial self-assembly) into amphipathic films which may be very insoluble. For instance, the protein films formed by SC3 are insoluble in most aqueous and organic solvents and in 2% SDS at 100°C. The film can, however, be dissociated into monomers by a brief treatment with formic acid or trifluoroacetic acid and after removal of the acids the monomers can re-assemble.



Because of interfacial self-assembly into amphipathic protein films, hydrophobins can change the wettability of surfaces. One method to measure wettability is by estimating the contact angle (θ) that a water drop makes with the surface. A large θ indicates a hydrophobic, a small θ a hydrophilic surface.



(a) Air bubbles or oil droplets in an aqueous solution of SC3 hydrophobin become coated with an amphipathic film that stabilizes them in water.

(b) A sheet of hydrophobic plastic such as teflon (θ 110 deg) immersed in SC3 hydrophobin becomes coated with a strongly adhering protein film that makes the surface completely wettable (θ 48 deg), even after SDS extraction (θ 62 deg).

(c) SC3 hydrophobin monomers dried down on a hydrophilic surface make the surface hydrophobic (θ 110 deg).

under mating-type gene control; only in the secondary mycelium are the *SC1*, *SC4* and *SC6* hydrophobin genes highly expressed and they are continuously active in the developing fruit bodies[8,9,24,25]. The *SC3* hydrophobin gene is active only in cells that form aerial hyphae and the hydrophobic hyphae that cover the fruit bodies (Figs 1 and 4). These hyphae have assembled SC3 hydrophobin on their surfaces and seem to have reverted to a monokaryotic type of gene expression[24,25]. The hyphae making up the major fruit-body tissue do not express *SC3* but rather express the other hydrophobin genes.

Unfortunately, gene disruption experiments to test the functionality of these dikaryon-expressed genes have not yet succeeded because in this system a phenotype would require a knock-out of the hydrophobin gene in both constituent genomes. However, the SC4 hydrophobin has been isolated and antibodies have shown its location in the fruit bodies. In these fruit bodies, individual hyphae form a compact tissue by being packed in an extracellular mucilaginous matrix, traversed by air channels and cavities that probably serve gas exchange. The SC4 hydrophobin assembles at the interface between mucilage and the gas phase[25], thus providing the air spaces with a hydrophobic lining, showing rodlets, that probably prevents them from becoming filled with water during frequent cycles of wetting and drying as occur in nature.

Formation of conidia

It is now established that the hydrophobic rodlet layer on conidia of *Aspergillus nidulans*[11] (Fig. 2), *A. fumigatus*[22] and *Neurospora crassa*[20,21] disappear after disruption of identified hydrophobin genes. Although the encoded hydrophobins have not yet been shown to self-assemble into such layers, the fact that the isolated SC3 hydrophobin does form a similar layer *in vitro* (Fig. 3) indicates that this is a distinct possibility. In *A. nidulans* the hydrophobin gene, *rodA*, is active in the phialides that bud off conidia; the hydrophobin is probably transported into the developing conidia and there secreted, followed by self-assembly into a rodlet layer at the spore surface. Recently another hydrophobin gene, *dewA*, has been found and its product detected on the surface of the conidia[26]. This hydrophobin also contributes to hydrophobicity of the spores, but rodlets were not seen in a *rodA*$^-$*dewA*$^+$ mutant. These two hydrophobin genes have been shown to be targets of regulation by a cascade of regulatory genes involved in conidiogenesis. For instance, the promoter of *rodA* was shown to be activated by binding the product of the *brlA* gene and, when activated under an inducible promoter, *brlA* switched on conidiogenesis and *rodA* transcription in hyphae growing in liquid[27]. Significantly, on the conidia produced when submerged no rodlet layer is formed[26]; the hydrophobin probably diffuses into the medium because of the absence of an interface with air at the conidial surface.

From the results of the gene disruption experiments[11,20–22] it is obvious that the hydrophobic rodlet layer on fungal spores is an adaptation for dispersal of these propagules by wind and thus of great ecological significance. Possibly, this layer also serves the attachment of spores of pathogenic fungi to hydrophobic surfaces of plants and animals[28]. This possibility has been investigated in the opportunistic human pathogen *Aspergillus fumigatus*. Although disruption of a hydrophobin gene made the conidia easily wettable these spores were as pathogenic in mice as wild-type spores[22]. Apparently, once the spores are inhaled, wet conidia also lead to disease development.

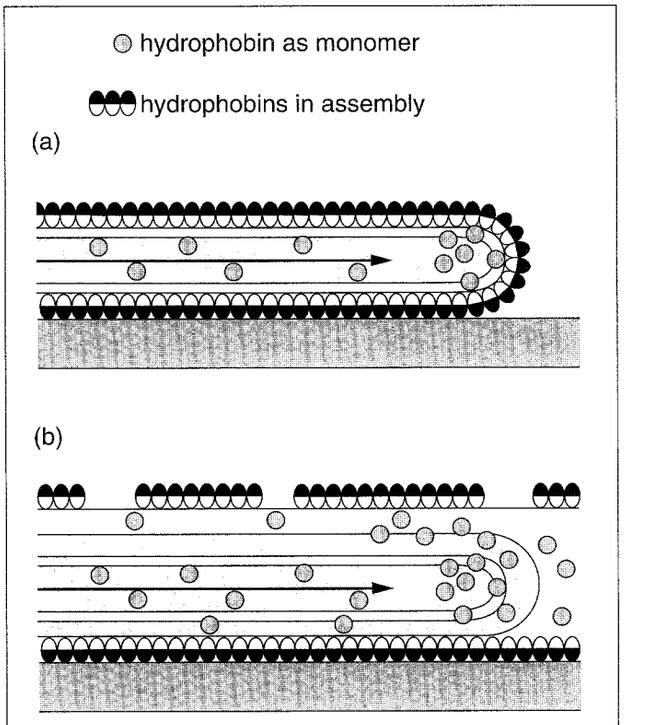


Fig. 5. Models for the attachment of hyphae to hydrophobic surfaces. In (a) the hypha is growing in air over a hydrophobic substrate and secreted hydrophobin monomers assemble directly on the hyphal surface exposed to air and between the wall and the hydrophobic substratum, firmly attaching the hypha. This was observed for hyphae of *Schizophyllum commune* forced to grow over dry teflon[14]. In (b) it is assumed that free hydrophobin monomers are secreted into the liquid or into mucilage surrounding the hyphae and that the hydrophobin, by assembling on the hydrophobic surface, creates a hydrophilic surface to which the hypha can be attached by means of the mucilage serving as a hydrophilic adhesive that may specifically bind to the primed surface.

Pathogenesis

To penetrate and infect plants and animals, fungi must tightly adhere to the host. A dramatic example is the rice blast fungus *Magnaporthe grisea*. A germ tube adheres to the highly hydrophobic leaf of rice, then forms an appressorium in which a turgor pressure of up to 8 MPa develops (the highest value measured in any biological system), after which the appressorium develops a hypha that by sheer mechanical force penetrates an epidermal plant cell[29]. Evidence that hydrophobins are involved in this and other cases of strong adherence to host surfaces comes from two lines. First, studying genes expressed during *in vitro* appressorium formation of *Metarhizium anisopliae*, an insect pathogen, it was found that one highly expressed gene (*ssgA*) encodes a hydrophobin[30]. Also, a gene (*MPG1*) of *Magnaporthe grisea*, highly expressed during early stages of infection of rice, proved to be a hydrophobin, while disruption of this gene caused a dramatic decrease in the

ability of germ tubes to form appressoria and to infect rice leaves[31]. However, a direct role of the hydrophobins in attachment to host surfaces was not established. Second, observing the ability of isolated SC3 hydrophobin to coat hydrophobic materials and make them easily wettable (Box 1) prompted an experiment in which the nonpathogenic *Schizophyllum commune* was forced to grow over a hydrophobic teflon surface. Indeed, it tightly adhered to this surface by means of SC3 assembling at the interface between the hydrophilic cell wall and the hydrophobic plastic, while adhesion was greatly diminished by a targeted mutation in the *SC3* gene[14]. It was therefore suggested (Fig. 5a) that a similar mechanism may be involved in attachment of germ tubes or appressoria of pathogenic fungi to their hosts[18]. However, under humid conditions (Fig. 5b) it is more probable that hydrophobins first wet the hydrophobic host surface and that hyphae or appressoria then adhere to this primed surface with a hydrophilic mucilaginous matrix commonly seen to be secreted during adherence[28].

Cerato-ulmin, a class II hydrophobin, has long been considered to be the phytotoxin of *Ophiostoma ulmi*, being responsible for clogging xylem vessels and thus causing wilting of elm trees[16]. This would occur by assemblage of the hydrophobin around gas bubbles regularly arising during water transport under negative pressure. With the cloning of the gene (*CU*)[17] has come the possibility to disrupt it and to test this proposition. Unexpectedly, aggressive strains with a mutation in the gene and not producing cerato-ulmin do not lose their pathogenicity but have little aerial growth (P.A. Horgen, pers. commun.). If a hydrophobin is involved in Dutch elm disease, it may be one that is only produced during growth of the fungus in the tree.

Symbiosis

Although both *Magnaporthe grisea* and *Ophiostoma ulmi* kill their hosts, most phytopathogenic fungi have a symbiotic relationship with their host, albeit of the parasitic kind; the fungus takes too much and gives too little. In the mutually beneficial mycorrhizal relationship many of the infection structures are similar to those in parasitic symbiosis but here both partners profit and the plant may even totally depend on the fungus for growth in certain biotopes. Because of the similarities it would not be surprising to find hydrophobins involved in these symbiotic association. Indeed, some genes (*HydPt-1* and *HydPt-2*) of *Pisolithus tinctorus* that are highly expressed during formation of ectomycorrhiza in *Eucalyptus globulus* roots encode hydrophobins[32]. The precise role of these hydrophobins is unknown and it would seem to require a mycorrhizal fungus more amenable to molecular-genetic manipulations to investigate such a role.

Equally difficult may be a study of the role of hydrophobins, if any, in the lichen symbiosis. A possible role for hydrophobins in this symbiosis is suggested by the general occurrence of rodlet layers that line the surfaces of air spaces within the thallus and surround the mucilage that binds the fungal hyphae and algae tightly together[33]. On the one hand, this thin layer may be responsible for apoplastic transport between fungus and alga[5]. On the other hand, it could prevent the thallus from becoming soaked by water infiltrating the air spaces during wetting after the regular dry periods to which these emergent fungi are submitted in nature.

Conclusion

Fragmentary evidence suggests that hydrophobins, probably among the most abundant proteins produced by fungi, play key roles in development and in the interactions of fungi with the environment and other organisms, particularly plants. A fungal species may contain several different hydrophobin genes, each tailored for a specific function. Hydrophobins seem to become functional only when reaching a hydrophilic–hydrophobic interface where they assemble into amphipathic films, often very insoluble. The challenges for the future will be, on the one hand, to understand the molecular mechanism of the remarkable property of hydrophobins to self-assemble into amphipathic films at interfaces. On the other hand, further studies on their biological roles may reveal a central role for these proteins in the evolution of fungi and hence the evolution of land plants. Finally, we envisage the use of these magic proteins in technologies involving, for instance, changing surface wettability, mediating attachment of materials to surfaces, and dispersing materials in liquids. They appear non-toxic because, unknowingly, we have been ingesting them for a long time when eating mushrooms and fungus-fermented foods.

References

1 Baldauf, S.L. and Palmer, J.D. (1993) Animals and fungi are each other's closest relatives: Congruent evidence from multiple proteins, *Proc. Natl Acad. Sci. USA* 90, 11558–11562
2 Wessels, J.G.H. (1986) Cell wall synthesis in apical hyphal growth, *Int. Rev. Cytol.* 104, 37–79
3 Wessels, J.G.H. (1993) Wall growth, protein excretion and morphogenesis in fungi, *New Phytol.* 123, 397–413
4 Read, D.J. (1991) Mycorrhizas in ecosystems – Nature's response to the 'law of the minimum', in *Frontiers in Mycology* (Hawksworth, D.L., ed.), pp. 101–130, CAB International
5 Honneger, R. (1993) Developmental biology of lichens, *New Phytol.* 125, 659–677
6 Smith, M.L., Bruhn, J.N. and Anderson, J.B. (1992) The fungus *Armillaria bulbosa* is among the largest and oldest living organisms, *Nature* 356, 428–431
7 Raper, C.A. (1988) *Schizophyllum commune*, a model for genetic studies of the basidiomycetes, in *Genetics of Plant Pathogenic Fungi* (Sidhu, G.S., ed.), pp. 511–522, Academic Press
8 Wessels, J.G.H. (1992) Gene expression during fruiting in *Schizophyllum commune*, *Mycol. Res.* 96, 609–620
9 Schuren, F.H.J. and Wessels, J.G.H. (1990) Two genes specifically expressed in fruiting dikaryons of *Schizophyllum commune*: homologies with a gene not regulated by mating type genes, *Gene* 90, 199–205
10 Wessels, J.G.H., de Vries, O.M.H., Ásgeirsdóttir, S.A. and Schuren, F.H.J. (1991) Hydrophobin genes involved in formation of aerial hyphae and fruit bodies in Schizophyllum, *Plant Cell* 3, 793–799
11 Stringer, M.A. *et al.* (1991) *Rodletless*, a new *Aspergillus* developmental mutant induced by directed gene inactivation, *Genes Dev.* 5, 1161–1171
12 Wösten, H.A.B. *et al.* (1994) The fungal hydrophobin Sc3p self-assembles at the surface of aerial hyphae as a protein membrane constituting the hydrophobic rodlet layer, *Eur. J. Cell Biol.* 63, 122–129
13 Wösten, H.A.B., de Vries, O.M.H. and Wessels, J.G.H. (1993) Interfacial self-assembly of a fungal hydrophobin into a hydrophobic rodlet layer, *Plant Cell* 5, 1567–1574
14 Wösten, H.A.B., Schuren F.H.J. and Wessels, J.G.H. (1994) Interfacial self-assembly of a hydrophobin into an amphipathic membrane mediates fungal attachment to hydrophobic surfaces, *EMBO J.* 13, 5848–5854
15 van der Vegt, W. *et al.* A comparison of the surface activity of the fungal hydrophobin SC3p with those of other proteins, *Biophys. Chem.* (in press)

16 Yaguchi, M. *et al.* (1993) Amino acid sequence and spectroscopic studies of Dutch elm disease toxin, cerato-ulmin, in *Dutch Elm Disease Research, Cellular and Molecular Approaches* (Sticklen, M.B. and Sherald, J.L., eds), pp. 152–170, Springer Verlag
17 Bowden, C.G. *et al.* (1993) Isolation and characterization of the cerato-ulmin toxin gene of the Dutch elm disease pathogen, *Ophiostoma ulmi*, *Curr. Genet.* 25, 323–329
18 Wessels, J.G.H. (1994) Developmental regulation of fungal cell wall formation, *Annu. Rev. Phytopathol.* 32, 413–437
19 Zhang, L. *et al.* (1994) Virus-associated down-regulation of the gene encoding cryparin, an abundant cell-surface protein from the chestnut blight fungus *Cryphonectria parasitica*, *Gene* 139, 59–64
20 Bell-Pedersen, D., Dunlap, J.C. and Loros, J.J. (1992) The *Neurospora* circadian clock-controlled gene, *ccg*-2, is allelic to *eas* and encodes a fungal hydrophobin required for formation of the conidial rodlet layer, *Genes Dev.* 6, 2382–2394
21 Lauter, F-R., Russo, V.E.A. and Yanovsky, C. (1992) Developmental and light regulation of *eas*, the structural gene for the rodlet protein of *Neurospora*, *Genes Dev.* 6, 2373–2381
22 Thau, N. *et al.* (1994) *rodletless* mutants of *Aspergillus fumigatus*, *Inf. Immun.* 62, 4380–4388
23 Parta, M. *et al.* (1994) HYP1, a hydrophobin gene from *Aspergillus fumigatus* complements the *rodletless* phenotype in *Aspergillus nidulans*, *Inf. Immun.* 62, 4389–4395
24 Ásgeirsdóttir, S.A., van Wetter, M.A. and Wessels, J.G.H. (1995) Differential expression of genes under control of the mating-type genes in the secondary mycelium of *Schizophyllym commune Microbiology* 141, 1281–1288
25 Wessels, J.G.H. *et al.* (1995) Genetic regulation of emergent growth in *Schizophyllum commune*, *Can. J. Bot.* 73 (Suppl. 1), S273–S281
26 Stringer, M.A. and Timberlake, W.E. (1995) *dewA* encodes a fungal hydrophobin component of the *Aspergillus* spore wall, *Mol. Microbiol.* 16, 33–44
27 Timberlake, W.E. (1993) Translational triggering and feedback fixation in the control of fungal development, *Plant Cell* 5, 1453–1460
28 Cole, G.T. and Hoch, H.C., eds (1991) *The Fungal Spore and Disease Initiation in Plants and Animals*, Plenum Press
29 Talbot, N.J. (1995) Having a blast: exploring the pathogenicity of *Magnaporthe grisea*, *Trends Microbiol.* 3, 9–16
30 St Leger, R.J., Staples, R.C. and Roberts, D.W. (1992) Cloning and regulatory analysis of starvation stress gene, *ssgA*, encoding a hydrophobin-like protein from the entomopathogenic fungus, *Metarhizium anisopliae*, *Gene* 120, 119–124
31 Talbot, N.J., Ebbole, D.J. and Hamer, J.E. (1993) Identification and characterization of *MPG1*, a gene involved in pathogenicity from the rice blast fungus *Magnaporthe grisea*, *Plant Cell* 5, 1575–1590
32 Martin, F. *et al.* Fungal gene expression during ectomycorrhiza formation, *Can. J. Bot.* (in press)
33 Honneger, R. (1991) Functional aspects of the lichen symbiosis, *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 42, 553–578
34 Wessels, J.G.H. (1994) Development of fruit bodies in homobasidiomycetes, in *The Mycota* (Esser, K. and Lemke, P.A., eds), Vol. I, *Growth, Differentiation and Sexuality* (Wessels, J.G.H. and Meinhardt, F., eds), pp. 351–366, Springer-Verlag

Joseph Wessels is at the Dept of Plant Biology, University of Groningen, Kerklaan 30, 9751 NN haren, The Netherlands (j.g.h.wessels@biol.rug.nl).

Transport proteins in the plasma membrane and the secretory system

Diane C. Bassham and Natasha V. Raikhel

The organelles of the plant secretory system and the plasma membrane each contain a specific complement of resident integral membrane proteins, which provide each organelle with some of its unique characteristics. Over the past few years, genes encoding some of these proteins have been isolated and this has allowed the function and localization of the encoded proteins to be determined. A number of the genes encode proteins involved in transport processes, both in the trafficking of proteins between membranes and the transport of solutes across membranes. However, little is known about the majority of membrane proteins within the endomembrane system. A more complete understanding of the processes occurring within this system awaits the identification and analysis of many more of its components and the interactions between them. This will also allow important questions to be addressed regarding the mechanisms by which membrane proteins are correctly localized and assembled in the secretory pathway.

The plant endomembrane system, or secretory pathway, consists of a series of organelles that include the endoplasmic reticulum (ER), Golgi apparatus and vacuole, as well as transport vesicles connecting these compartments and the plasma membrane (Fig. 1). Transport vesicles carry proteins, lipids and polysaccharides between the organelles, and to the cell's exterior, in a series of budding and fusion events. Each organelle of the secretory pathway contains a specific complement of soluble and membrane proteins, which determine some of the unique properties of the organelle: sorting signals are required for the targeting and retention of proteins in most of these compartments.

In recent years, a number of proteins have been identified that reside in membranes of the plant secretory system