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Review

Hydrophobins, from molecular structure to multiple functions in fungal development

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Mycelial fungi secrete small, cysteine-rich, proteins, called hydrophobins, that self-assemble at hydrophilic-hydrophobic interfaces into amphipathic membranes, highly insoluble in case of Class I hydrophobins. By self-assembly at the culture medium-air interface they greatly lower the surface tension enabling emergent structures to grow into the air. By self-assembly at the interface between the hydrophilic cell wall and the air or any other hydrophobic environment, these emergent structures are coated with a hydrophobin membrane. These properties allow hydrophobins to fulfil a broad spectrum of functions in fungal development. They are involved in formation of aerial (reproductive) structures, in aerial dispersion of spores, and they line air channels within fruiting bodies with a hydrophobic coating, probably serving gas exchange. Hydrophobins also mediate hyphal attachment to hydrophobic surfaces such as those of plants. Moreover, they appear involved in complex interhyphal interactions, and in interactions with algae in lichens. Their resistance towards chemical and enzymatic treatments suggests that assembled hydrophobins also protect fungal emergent structures against adverse environmental conditions.

Key Words——aerial growth; fruit body formation; fungal-host interaction; hydrophobin; hyphal attachment.

Mycelial fungi effectively penetrate and colonize moist (semi)solid substrates by means of branching hyphae that grow at their apices, while secreting lytic enzymes at the same site (Wessels, 1993a). At some stage of development, fungal hyphae may encounter a hydrophobic environment. For instance, hyphae can leave the moist substrate and grow into the air (aerial hyphae), where they may form reproductive structures like conidiophores and fruiting bodies. Hyphae may also traverse meter distances of non-nutrious substrate with rhizomorphs or cords to find a new food base, while many pathogenic fungi attach to and grow on the hydrophobic surface of their host prior to penetration. Growth and differentiation of multicellular entities in filamentous fungi are accomplished by the activities of individual hyphae and not by meristems as in plants. Each hypha senses, and responds to, its (a)biotic environment and to other hyphae by means of molecules located in the plasma membrane or at the surface of the hyphal wall. Molecules at the wall surface provide for electrostatic and hydrophobic interactions known to be important for interfacial interactions with other cells, liquids, solids, or the air (Doyle and Rosenberg, 1990). It has been shown that the biophysical properties of the hyphal surface change during development, and these changes correlate with changes in atomic composition (Hazen, 1990; Gerin et al., 1993; Wösten et al., 1993, 1994b). Yet, little is known about the molecular constitution of the fungal surface in relation to the environment or the developmental stage. With the discovery of hydrophobins (Schuren and Wessels, 1990), proteins were identified that are active at the fungal surface in interaction with the environment. Although the term "hydrophobin" was used earlier to denote any hydrophobic material on microbial surfaces (Rosenberg and Kjelleberg, 1986), the term hydrophobin was coined by Wessels (Wessels et al., 1991a, b) to name these surface proteins.

The most characteristic feature of hydrophobins is that they self-assemble at hydrophilic/hydrophobic interfaces (Wösten et al., 1993, 1994c, 1995). By self-assembly at the interface between the hydrophilic cell wall and a hydrophobic environment (air, oil, or a hydrophobic solid), emergent structures are covered with an amphipathic membrane (Wösten et al., 1994a, c). The hydrophilic side of this membrane faces the cell wall, while the hydrophobic side is exposed to the hydrophobic phase. The transition from a hydrophilic to a hydrophobic cell surface allows formation of aerial hyphae (Bowden et al., 1996; van Wetter et al., 1996; Temple et al., 1997), facile dispersion of spores by wind (Beever and Dempsey, 1978; Stringer et al., 1991; Bell-Pedersen et al., 1992; Lauter et al., 1992), formation of hydrophobic cavities for gas exchange in compact tissues such as

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fruiting bodies (Wessels et al., 1995a; Lugones et al., 1996), hyphal attachment (Wösten et al., 1994c), and signalling of surface hydrophobicity (Talbot et al., 1993, 1996; Beckerman and Ebbole, 1996). Hydrophobins also appear to function in the establishment of complex interhyphal interactions as in fruiting bodies (Wessels, 1993b; Wessels et al., 1995), and in interactions of hyphae with plants (Talbot et al., 1993; Martin et al., 1995a; Tagu et al., 1996), or with algae and cyanobacteria (lichens) (Honegger, 1993).

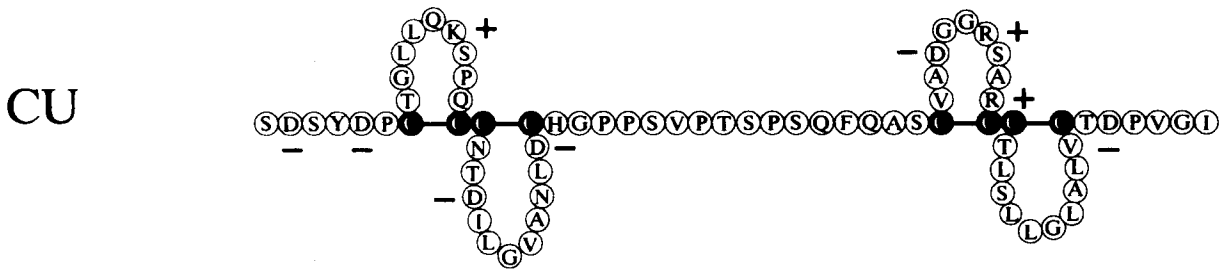
In this overview we will elaborate on the molecular and biophysical properties of hydrophobins and how these properties relate to their central role in fungal growth and development.

Molecular and biophysical characterization of hydrophobins

Although hydrophobins are diverse in amino acid sequence (Wessels, 1997), they all encode small (±100 aa) moderately hydrophobic secreted proteins with typical hydropathy patterns (Wessels, 1994) and eight cysteine residues in conserved spacing (Fig. 1). Based on differences in hydropathy patterns and biophysical properties Wessels (1994) proposed to distinguish between class I and class II hydrophobins. Most of the hydrophobins (Fig. 2) are only known as gene sequences. Only a few of the proteins have been isolated and studied, namely, SC3 and SC4 of *Schizophyllum commune*, ABH1 and ABH3 of *Agaricus bisporus*, EAS of *Neurospora crassa*, cerato-ulmin (CU) of *Ophiostoma ulmi* and *O. novo-ulmi*, and cryparin (CRP) of *Cryphonectria parasitica*.

Interfacial self-assembly of hydrophobins Hydrophobins self-assemble at hydrophilic/hydrophobic interfaces (e.g., between water and air, water and oil, or water and a hydrophobic solid like Teflon) into an amphipathic membrane as shown for the class I hydrophobins SC3 (Wösten et al., 1993, 1994c, 1995), ABH1, ABH3, and SC4 (Lugones et al., 1996; L. G. Lugones and J. G. H. Wessels, unpublished), and the class II hydrophobins CU (Richards and Takai, 1973; Takai and Richards, 1978; Russo et al., 1982; Richards, 1993) and CRP (Carpenter et al., 1992). The membranes formed by class I hydrophobins are highly insoluble (even resisting 2% SDS at 100°C), and can only be dissociated using agents like formic acid (FA) or trifluoroacetic acid (TFA) (Wessels et al., 1991a, b; de Vries et al., 1993). In contrast, assemblages formed by class II hydrophobins are less stable. Those of CU and CRP readily dissociated in 60% ethanol and in 2% SDS (Russo et al., 1982; Carpenter et al., 1992), while assembled CU also dissociated by applying pressure or by cooling (Russo et al., 1982). After dissociation of the assembled hydrophobin with TFA or FA, these solvents can be removed by evaporation, the solubilized hydrophobins dissolved in water, and the process of self-assembly repeated (Wösten et al., 1993; Lugones et al., 1996), showing that these proteins are extremely stable.

During the process of self-assembly the conformation of the monomers is apparently changed in such a way that hydrophilic and hydrophobic groups orient themselves at opposite sides of the membrane. The hydrophilic side of the SC3 membrane has a water contact angle (θ) of 36±3, while its hydrophobic side exhibits a water contact angle of 110±10 (Wösten et al.,



Class I X_{26-85}-C-X_{5-8}-C-C-X_{17-39}-C-X_{8-23}-C-X_{5-6}-C-C-X_{6-18}-C-X_{2-13}

Class II X_{17-67}-C-X_{9-10}-C-C-X_{11}-C-X_{16}-C-X_{6-9}-C-C-X_{10}-C-X_{3-7}

Fig 1. Cysteine linkages of CU (Yaguchi et al., 1993) and the length of the sequences spacing the cysteine residues in class I and class II hydrophobins.
Cys 1 of CU interacts with Cys 2 or 3, and Cys 2 or 3 is linked to Cys 4. Similarly, Cys 5 interacts with Cys 6 or 7, while Cys 6 or 7 is linked to Cys 8. Lengths of the intervening sequences between the cysteines of class II hydrophobins are less variable than those of class I hydrophobins. The amino acid sequence in between cysteine 3 and 4 in class I hydrophobins is relatively long compared to the eleven amino acids found in class II hydrophobins. The analysis was based on the structure of the hydrophobins listed in Fig. 2.

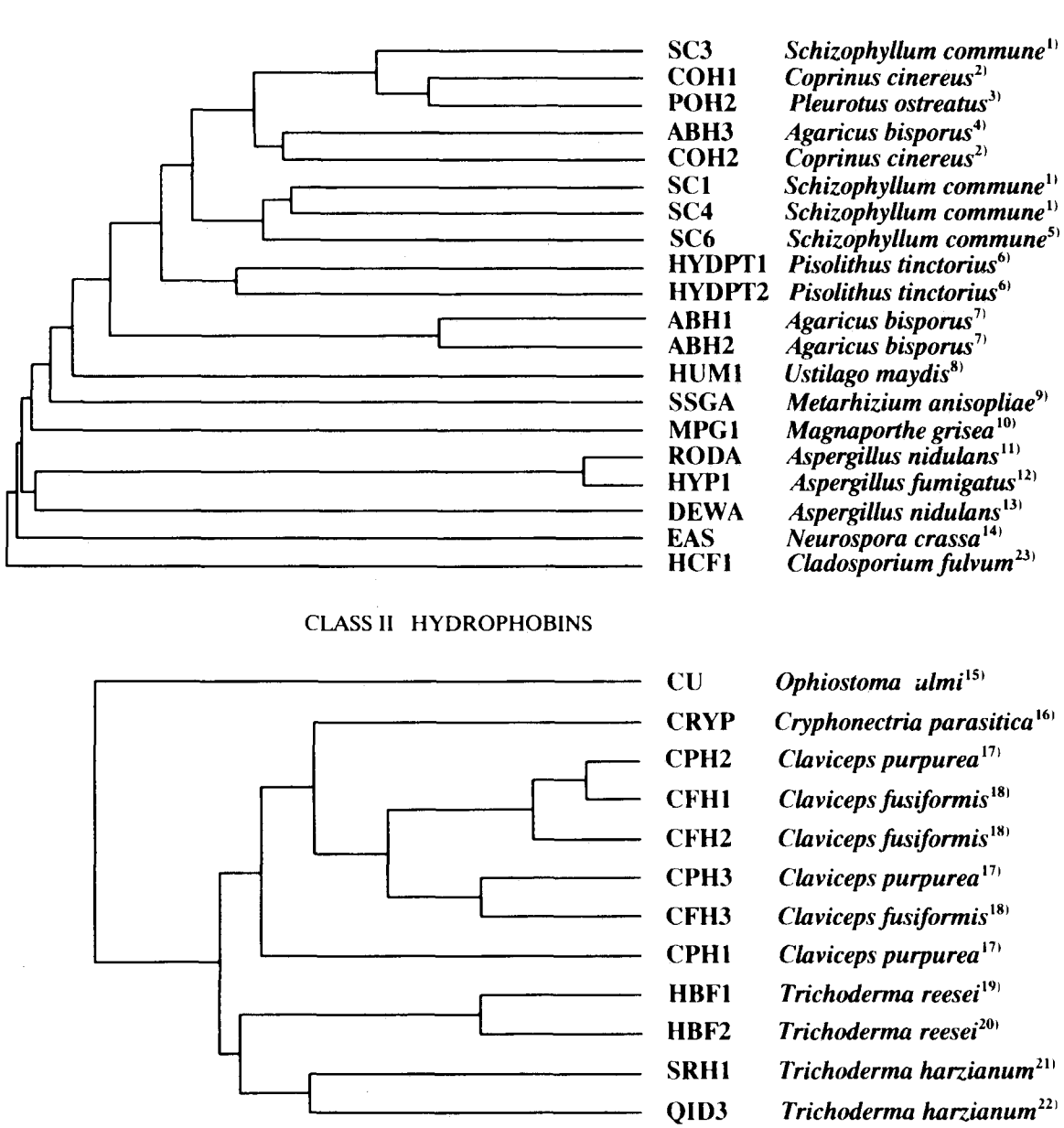


Fig 2. Dendrogram of similarities between aligned class I and class II hydrophobins obtained by the CLUSTAL programme of the PC/GENE programs package, version 6.60 (Higgins and Sharp, 1988).
In class I hydrophobins the assembled form is insoluble in SDS and in 60% ethanol, whereas assemblages of members of class II hydrophobins are soluble in these reagents. Numbers in superscript indicate references where sequence information was published or the source of unpublished data. Amino acid sequences at the *N*-terminal end preceding the first cysteine residue were omitted from the comparison since these include the signal sequence for secretion and in only in a number of cases (SC3, SC4, CoH1, ABH1, RodA, Eas, CU, and CRP) is the *N*-terminus of the mature protein known. [1)]Schuren and Wessels (1990); Wessels et al. (1991a). [2)] Ásgeirsdóttir et al. (1997). [3)] Ásgeirsdóttir, S.A. and Wessels, J. G. H. (unpublished data). [4)] Lugones, L. G. and Wessels, J. G. H. (unpublished data). [5)] Wessels et al. (1995). [6)] Tagu et al. (1996). [7)] Lugones et al. (1996), de Groot et al. (1996). [8)] Bohlmann (1996). [9)] St. Leger et al. (1992). [10)] Talbot et al. (1993). [11)] Stringer et al. (1991); Rhodes, J. and Timberlake, W.E., cited in Stringer and Timberlake (1995). [12)] Parta et al. (1994); Thau et al. (1994). [13)] Stringer and Timberlake (1995). [14)] Bell-Pedersen et al. (1992); Lauter et al. (1992); Templeton et al. (1995). [15)] Yaguchi et al. (1993); Stringer and Timberlake (1993); Bowden et al. (1994); [16)] Carpenter et al. (1992); Zhang et al. (1994). [17)] The three hydrophobins 1, 2, 3, in that sequence, are probably part of one protein in which the hydrophobin moieties are separated by NG-rich spacers (Arntz, C. and Tudzynski, P., unpublished data). [18)] The three hydrophobins 1, 2, 3, in that sequence, are part of one protein in which the hydrophobin moieties are separated by NG-rich spacers (Arntz and Tudzynski, 1997; de Vries, O. M. H., Moore, S., Wessels, J. G. H. and Tudzynski, P., unpublished data). [19)] Nakari-Setälä et al., (1996). [20)] Nakari-Setälä et al. (1995). [21)] Munoz, G., Nakari-Setälä, T., Agosin, E. and Penttilä, M. (unpublished data). [22)] Lora et al. (1994). [23)] Spanu (1997).

1993) (which is similar to that of Teflon). Chemical analysis and mass spectroscopy revealed that SC3 is exceptional in that it is a glycoprotein containing 17–22 mannose residues (de Vries, O. M. H., de Vocht, M. L. and Wessels, J. G. H., unpublished data). Most of the mannose residues, if not all, are present at the hydrophilic side of the SC3 membrane, and probably contribute to the high hydrophilicity of this side (Wösten et al., 1994b; de Vocht, M. L., Wessels, J. G. H. and Robillard, G. T., unpublished data). Mass spectra of the non-glycosylated SC4, ABH3 and CRP were in good agreement with the masses predicted from the primary amino acid sequence (Lugones, L. G., de Vocht, M. L., Robillard, G. T. and Wessels, J. G. H., unpublished data), showing that hydrophilicity and hydrophobicity at the two sides of these membranes is determined solely by amino acid residues and not by conjugated sugars and lipids.

By self-assembly, hydrophobins can change the nature of a surface. By letting a solution of hydrophobin evaporate on filter paper, the paper was made hydrophobic: water droplets stayed at the surface for hours instead of being sucked immediately into the cellulose fibres (Lugones et al., 1996). Similarly, glass can be made hydrophobic by drying down a hydrophobin solution (Wösten et al., 1993). Conversely, hydrophobic solids or oil droplets can be made hydrophilic by submerging or suspending these materials into a solution of hydrophobin (Wösten et al., 1994c, 1995; Lugones et al., 1996). The most dramatic change in hydrophobicity was observed after assembly of CRP on a Teflon surface. Water contact angles changed from 109 deg (bare Teflon) to 22 deg after assembly of the hydrophobin. The interaction between CRP and Teflon resisted washes with water, but SDS or 60% ethanol readily removed the protein layer from the hydrophobic surface (Wösten, H. A. B. and Wessels, J. G. H., unpublished data). In contrast, the class I hydrophobins (SC3, SC4, ABH3) assembled on Teflon were only removed by TFA or FA, showing that these proteins strongly interact with hydrophobic surfaces (Wösten et al., 1994c, 1995; Lugones, L. G. and Wessels, J. G. H., unpublished data). One milligram of SC3 hydrophobin was shown to be sufficient to coat 1 m^2 of Teflon surface with a monolayer about 10 nm thick (Wösten et al., 1994c), indicating that only small amounts of hydrophobin suffice to fulfil their role in nature (see below).

Surface activity of hydrophobins Hydrophobins belong to the most surface-active molecules (Table 1). With a maximal lowering of the water surface tension to 24 mJ m^{-2} at 50 μg ml^{-1}, SC3 is the most powerful biosurfactant known (van der Mei, H. C., Wösten, H. A. B. and Wessels, J. G. H., unpublished data). A slightly smaller lowering of surface tension (32 mJ m^{-2}) was found at 100 μg ml^{-1} SC3 (van der Vegt et al., 1996), similar to that of other hydrophobins at this concentration (Wösten, H. A. B., Lugones, L. G. and Wessels, J. G. H., unpublished data), but much higher than that of a synthetic surfactant like SDS, which lowers the surface tension to 37 mJ m^{-2} at 5 mg ml^{-1}. Surface activity of proteins is generally low (see van der Vegt et al., 1996), but the activity of hydrophobins is at least similar to that of traditional biosurfactants encompassing glycolipids, lipopeptides/lipoproteins, phospholipids, neutral lipids, substituted fatty acids, and lipopolysaccharides (for references see Jenny et al., 1991). In contrast to these surfactants, surface activity of hydrophobins is not dependent on a lipid molecule but is apparently caused solely by the amino acid sequences. Furthermore, surface activity depends on a conformational change of the molecules during assembly rather than on a diffusion-limited adsorption to the interface (van der Vegt et al., 1996), which is in good agreement with the properties of hydrophobins at hydrophilic/hydrophobic interfaces.

(Ultra) structure of monomeric hydrophobin and hydrophobin under assembly The hydrophilic side of assembled SC3 appears smooth in the electron microscope (Wösten et al., 1994c). In contrast, the hydrophobic side of SC3 and some other class I hydrophobins assembled in vitro and in vivo is characterized by a mosaic of parallel rodlets (Wösten et al., 1993, 1994c; Lugones et al., 1996; L. G. Lugones and J. G. H. Wessels, unpub-

Table 1. Minimal surface tension (γ_{lv}) of the most powerful biosurfactants compared to those of some synthetic surfactants.
Adapted from Georgiou et al. (1992) and van der Vegt et al. (1996). Note that water has a surface tension of 72 mJ m^{-2}.

Surfactant	Class	Organism	γ_{lv} mJ m^{-2}
SC3	Protein	*Schizophyllum commune*	24
Rubiwettins	Glycolipid	*Serratia rubidaea*	25.5
Pentasaccharide lipid		*Nocardia corynebacteroides*	26
Lipopeptides	Lipopeptide	*Bacillus licheniformis*	27
Surfactin		*Bacillus subtilis*	27
Fatty acids/Neutral lipids	Fatty acid/Neutral lipid	*Nocardia erythropolis*	32
Oleylaminefluoride	Synthetic surfactant		29
Dihydroaminefluoride			35
Sodiumdodecylsulphate			37

lished data). Such rodlets are also seen in assembled SC3 by atomic force microscopy (de Vocht, M. L., van der Vegte, E. W., Wessels, J. G. H. and Robillard, G. T., unpublished data and Fig. 3). Although the proteins were not isolated, outer spore layers made up by class I hydrophobins, as shown by gene disruption experiments, exhibit similar rodlets of about 10 nm thickness (Stringer et al., 1991; Bell-Pedersen et al., 1992; Lauter et al., 1992; Talbot et al., 1996), although those attributed to MPG1 (Fig. 2) were 5 to 7 nm thick instead of 10 nm (Talbot et al., 1996). Within class I hydrophobins, DewA seems to be an exception because no rodlets were seen on spores apparently containing this protein but no RodA (Stringer and Timberlake, 1995). Also, no rodlet pattern was seen at the hydrophobic side of assembled CRP, a class II hydrophobin (Wösten, H. A. B., Wessels, J. G. H. and Engelhardt, H., unpublished data). Whether this has any functional consequences is not yet known. Fourier Transform Analysis of assembled SC3 and SC4 negatively stained with uranyl acetate indicates that the membranes of these class I hydrophobins are relatively open structures with pores allowing for passage of small molecules like gasses and sugars (Wösten, H. A. B., Wessels, J. G. H. and Engelhardt, H., unpublished data), which could have considerable biological significance (see below).

Hardly anything is known about the 2D and 3D structure of hydrophobins and the changes that occur upon self-assembly. CU (Yaguchi et al., 1993) and SC3 (de Vocht, M. L., Wessels, J. G. H. and Robillard, G. T., unpublished data) have a high content in β-sheets, typical of stable proteins. The cysteine residues of SC3 (de Vries et al., 1993) and CU (Yaguchi et al., 1993) form intramolecular disulphide bridges, which probably also contribute to the stability of these proteins. The cysteine linkages in CU were determined (Yaguchi et al., 1993) (Fig. 1). Assuming that disulphide bridges in class I hydrophobins are identical to those of CU, both hydrophobin classes seem to contain two domains. Cysteine 1 to 4 is contained in the first domain, while the second domain encompasses cysteine 5 to 8 (Fig. 1). The presence of two domains is also indicated by the hydropathy pattern (Wessels, 1996) and by the similarity of the most prevalent amino acids surrounding the fourth and eighth cysteine residue of most class I hydrophobins. These cysteine residues are both preceded by L, V/I, X, Pho and X (X indicates any amino acid except for W, Pho any hydrophobic amino acid) and are followed by S/T, P, I, X, V/I residues.

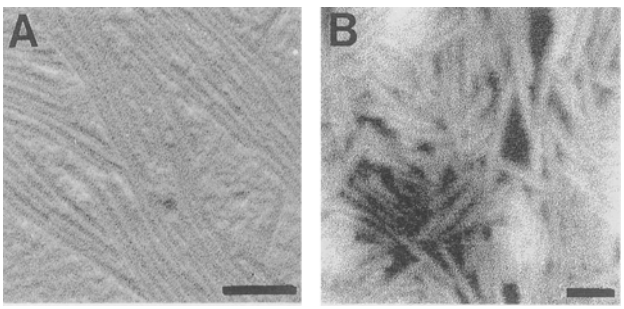


Fig 3. The hydrophobic side of assembled SC3 is characterized by a mosaic of parallel rodlets as shown by surface shadowing (**A**) and Atomic Force Microscopy (courtesy of de Vocht, M. L. and van der Vegte, E. W.) (**B**). Bar represents 100 nm.

Biological functions

Hydrophobin genes have been isolated from ascomycetes, deuteromycetes and basidiomycetes (Fig. 2), while hydrophobin-like proteins were shown to occur in zygomycetes as well (de Vries et al., 1993). To date, class II hydrophobins have not been identified in basidiomycetes, while no hydrophobin genes have been detected in yeasts. Inspection of the completed genome sequence of *Saccharomyces cerevisae* did not reveal hydrophobin sequences (Bussey, H., personal communication), possibly indicating that hydrophobins are adaptations related to the filamentous mode of growth. The dimorphic fungi *O. ulmi* and *Ustilago maydis* do, however, contain hydrophobin genes. The hydrophobin *Hum1* of *U. maydis* is only expressed at the filamentous stage (Bohlmann, 1996), while *Cu* is expressed in both the yeast-like and the filamentous stage (Temple et al., 1997).

Class I hydrophobins in aerial growth

Aerial hyphae SC3 is the best-studied class I hydrophobin. Its role in formation of aerial hyphae and in attachment of hyphae to hydrophobic surfaces (see below) probably exemplifies an important role of hydrophobins in fungal emergent growth and therefore will be discussed in some detail.

In young cultures the *SC3* gene is silent. It becomes activated only after a few days of submerged growth, its mRNA accumulates to as high as 1% of the total mRNA mass (Mulder and Wessels, 1986). Submerged growing hyphae secrete the protein into the medium at their tips (Wösten et al., 1994a) and SC3 remains monomeric unless confronted with the medium/air interface. At this interface SC3 self-assembles into an amphipathic membrane, which is accompanied by a tremendous drop in surface tension (from 72 to as low as 24 mJ m^{-2}) (Wösten, H. A. B., van Wetter, M.-A. and Wessels, J. G. H., unpublished data). This drop in surface tension seems to be crucial for escape of hyphae from the aqueous environment. In a strain in which the *SC3* gene was disrupted, surface tension of the culture medium decreased to only 45 mJ m^{-2} and hardly any aerial hyphae were formed under conditions of poor aeration. By supplementing to the culture medium with SC3, reduction of surface tension and formation of aerial hyphae were restored to wild-type levels. SC3 secreted by aerial hyphae cannot diffuse into the medium but will be confronted with the interface between the hydrophilic cell wall and the hydrophobic air, inducing self-assembly of the protein into a single amphipathic membrane

(Wösten et al., 1994a). The hydrophilic side of the SC3 membrane faces the cell wall, while its hydrophobic side, characterized by a rodlet pattern, is exposed (Wösten et al., 1993) and confers hydrophobicity to these hyphae. Aerial hyphae formed by the mutant strain after supplementing the culture medium with SC3 were hydrophilic (Wösten, H. A. B., van Wetter, M.-A. and Wessels, J. G. H., unpublished data), confirming that SC3 secreted by aerial hyphae is coating the surface and is not recruited from the culture medium. The inability of SC3 in the medium to coat aerial hyphae is explained by the fact that the growing hyphal apex becomes isolated from the medium soon after it grows into the air.

An impermeable membrane formed at the hyphal surface and at the medium/air interface would reduce gas exchange of aerial hyphae. The noted porosity of the SC3 membrane would permit free exchange of gases, which might explain why fungi use proteins instead of lipids to escape the hydrophilic environment and to grow into the air.

The isolation of the growing apices of aerial hyphae and fruiting bodies from the medium implies that water and nutrients have to be transported from the submerged mycelium to support growth of aerial emergent structures (see Wessels, 1997). As a consequence, a feeding mycelium has to be established before aerial growth can start. As mentioned, the *SC3* gene is silent during the first days of submerged growth (Mulder and Wessels, 1986). As a result, surface tension at the water/air interface remains high, preventing premature aerial growth and allowing formation of a sizeable amount of feeding mycelium. It is not clear which signal is responsible for the activation of the *SC3* gene, but it could be induced by a mechanism of quorum sensing as observed in bacteria (for a review see Swift et al., 1996). The *thn* mutation in *S. commune* might interfere with such a mechanism, because strains carrying this mutation fail to express any of the hydrophobin genes (Wessels et al., 1991b). Like SC3, other hydrophobins have been shown to be highly surface active and could induce aerial growth by breaking the surface tension.

Conidiospores Similar rodlet layers to those observed on aerial hyphae of *S. commune* have generally been observed at surfaces of fungal aerial structures (such as conidiospores, conidiophores, and fruiting bodies) (see Wessels, 1996, 1997). These layers are probably all formed by a mechanism as described for the SC3 membrane at surfaces of aerial hyphae of *S. commune* (Wösten et al., 1994a). Inactivation of the hydrophobin genes *RodA* of *Aspergillus nidulans* (Stringer et al., 1991) and *A. fumigatus* (Thau et al., 1994), *Eas* of *N. crassa* (Bell-Pedersen et al., 1992; Lauter et al., 1992), and *MPG1* of *Magnaporthe grisea* (Talbot et al., 1996) resulted in conidiospores that lacked the hydrophobic rodlet layer, while this layer was also absent at surfaces of conidiospores of *A. nidulans* formed in submerged cultures (Stringer et al., 1991), even though *RodA* mRNA accumulated to high levels by induced expression of the *brlA* gene, a regulator of *RodA* (Adams et al., 1988).

Fruiting bodies Fruiting bodies develop from individual aerial hyphae that form a tissue consisting of hyphae embedded in a hydrophilic mucilage and traversed by an elaborate system of air channels, which probably serve for gas exchange. The hydrophobins SC4 and ABH3 were shown to line air channels within the plectenchyma of *S. commune* and *A. bisporus*, respectively (Wessels et al., 1995; Lugones et al., 1996; Lugones, L. G. and Wessels, J. G. H., unpublished data), their proposed porosity probably allowing gas exchange. In the absence of hydrophobins, these air channel walls would be hydrophilic and therefore would easily collapse during repeated cycles of wetting and drying as occur in nature. These hydrophobins thus seem to have a function analogous to that of surfactants in alveoli of our lungs, preventing collapse of these structures. ABH1 also covers the outer surface of the fruiting body of *A. bisporus* (de Groot et al., 1996; Lugones et al., 1996), but in *S. commune* the outer covering of the fruiting body consists of hyphae that have SC3 on their surfaces (Ásgeirsdóttir et al., 1995).

Lichens Lichens are fungi, mostly ascomycetes, associated with algae and/or cyanobacteria. About 20% of all fungi are lichenized and occupy as the sole "vegetation" about 8% of the land area (Honegger, 1991). The fungal hyphae make simple contacts with the alga, the whole being ensheathed by a matrix which is covered with a rodlet layer at sites in contact with the air (Honegger, 1982, 1991). The hydrophobic rodlet layer, presumedly consisting of a class I hydrophobin, would allow apoplastic transport of water and solutes within the symbiotic system by shielding the apoplast of mycobiont and phycobiont from the air spaces. In addition, this would permit optimal gas exchange and prevent the air channels from becoming soaked with water during cycles of wetting and drying to which lichens are regularly subjected (Honegger, 1991, 1993; Wessels, 1996, 1997), a function that is analogous to that proposed for hydrophobins in fruiting bodies.

Class II hydrophobins in aerial growth

Class II hydrophobins seem to have a role in aerial growth similar to that of class I hydrophobins. CU was localized in the culture medium of *O. ulmi* (Richards and Takai, 1973; Takai, 1974; Takai and Richards, 1978) and was also shown to occur in the synnematal head fluid (Takai et al., 1980) and on surfaces of all structures formed by aggressive isolates of *O. ulmi* (vegetative hyphae, synnemata, synnematal spores, perithecia and ostiolar hairs) (Svircev et al., 1988).

Formation of aerial hyphae in a strain of *O. novo-ulmi* in which the *cu* gene was deleted was strongly reduced (Bowden et al., 1996; Temple et al., 1997), while aerial hyphae were abundantly formed in a non-aggressive strain of *O. ulmi*, normally exhibiting a waxy flattened morphology, by introducing multiple copies of the *cu* gene of *O. novo-ulmi* (Temple et al., 1997).

A role for CRP in formation of aerial structures is indi-

cated by the presence of this protein at surfaces of aerial hyphae, pycnidia (asexual spore-containing bodies) and fruiting bodies (Carpenter et al., 1992). In contrast, no cryparin was detected in mycelial fans, structures that are important in the penetration of the host, the chestnut tree (Hebard et al., 1984). Like SC3, CRP has a strong capacity to lower the surface tension of the culture medium (Wösten, H. A. B. and Wessels, J. G. H., unpublished data) and could thus plausibly participate in helping hyphae to escape the hydrophilic medium.

Whether the lower stability of the assembled class II hydrophobin membrane has any effect on its role in aerial growth is not known. It could be that an assembled membrane is stabilized by interaction of its hydrophilic side with the cell wall. Such an interaction might be mediated by a lectin-like activity, suggested by the haemagglutination of blood cells by CRP (Carpenter et al., 1992).

Class I and class II hydrophobins in attachment

SC3 not only assembles at the cell wall/air interface but also at the interface between the hydrophilic cell wall and a hydrophobic solid. When hyphae of *S. commune* were grown over a Teflon surface, a thin layer of SC3 was localized at the interface between the hydrophobic solid and the cell wall, which were shown to be firmly glued together (Wösten et al., 1994c). Adhesion of hyphae of a strain in which the *SC3* gene was inactivated was strongly reduced, showing that SC3 is involved in glueing together these basically incompatible surfaces, probably employing the amphipathic nature of the SC3 membrane, each side of which strongly interacts with one of the two surfaces. As shown in vitro, the interaction between the hydrophobic side of the SC3 membrane and a hydrophobic solid resisted heating at 100°C in 2% SDS (Wösten et al., 1994c, 1995). The affinity of the hydrophilic side of the membrane to the cell wall might be mediated by a lectin-like activity, suggested by the ability of SC3 to agglutinate rat blood cells (Wösten, H. A. B. and Wessels, J. G. H., unpublished data). The property of SC3 to mediate hyphal attachment to hydrophobic surfaces was suggested to aid in degradation of hydrophobic substrates such as lignin (Wösten et al., 1994c), ensuring close contact of the lignin with the fungal wall, while porosity of the SC3 membrane would allow transfer of electrons and uptake of breakdown products.

Hydrophobins in attachment in pathogenic relationships

Many pathogenic fungi attach to the hydrophobic surface of their host prior to penetration (Hazen, 1990; Cole and Hoch, 1991), and a role for hydrophobins in this process was suggested (Wessels, 1994). Infectious propagules are often conidiospores covered with a hydrophobic rodlet layer. This layer would not only facilitate spore dispersal by wind but would also allow attachment to insect vectors and to the host surface. Significantly, an isolated rodlet preparation from conidia and intact conidia of *Beauveria bassiana* bound equally well to insect cuticles (Boucias et al., 1988). Class II hydrophobins may have a similar role in attachment. A correlation was found between production of CU in strains of *O. ulmi* and *O. novo-ulmi* and attachment of yeast-like cells, the infectious propagules, to surfaces of the bark beetle vector *Scolytus multistriatus* (Temple et al., 1997).

Once the infectious propagule is attached to the host surface it must colonize the host. Conidiospores may germinate and assume hyphal growth followed by the formation of an appressorium. To penetrate the host, this structure has to attach firmly to the host surface. The hydrophobin *MPG1* of the rice pathogen *M. grisea* (Talbot et al., 1993) was isolated as a gene highly expressed in plants during formation of appressoria and was proposed to be involved in formation and attachment of appressoria since these processes were greatly reduced in a strain in which the *MPG1* gene was disrupted (Talbot et al., 1993, 1996). This might be established by self-assembly of MPG1 at the fungal/host interface, similar to SC3 attaching hyphae of *S. commune* to Teflon. However, many pathogenic fungi only germinate in a humid environment, and a hydrophilic mucilage was implicated in attachment (Hamer et al., 1988). Nevertheless, this does not exclude the involvement of hydrophobin in this process (Wessels, 1994). Any hydrophobin secreted with the mucilage could self-assemble at the host surface, making it hydrophilic. The change in surface hydrophilicity might then allow attachment of the hydrophilic mucilage to the hydrophobic host surface. This hypothesis is supported by the finding that formation of appressoria on a hydrophobic surface by a *MPG1* mutant strain was restored by coinoculation with a wild-type strain in an aqueous environment (Beckerman and Ebbole, 1996). The hydrophobin gene *ssgA* was expressed during in vitro appressoria formation of the insect pathogen *Metarhizium anisopliae* (St. Leger et al., 1992). The corresponding hydrophobin may likewise aid in attachment of the appressorium to the insect cuticle.

Attachment of a fungal cell to a hydrophobic surface appears to generate signals for induction of development. Such a role was proposed for the hydrophobin MPG1 of *M. grisea* (Beckerman and Ebbole, 1996; Talbot et al., 1996). In a strain in which the *MPG1* gene was inactivated, attachment of conidia and germlings to a hydrophobic surface was affected (Talbot et al., 1996), and this was proposed to cause the strong reduction in appressorium formation and pathogenicity (Talbot et al., 1993). Addition of cAMP to germinating spores bypassed the need of *M. grisea* for an inductive substrate (Lee and Dean, 1993), while it restored appressorium formation in the *MPG1* disruptant strain (Beckerman and Ebbole, 1996; Talbot et al., 1996), although these appressoria were more easily removed with hot SDS than wild-type appressoria (Talbot et al., 1996). These results suggest that strong attachment of *M. grisea* by MPG1 generates a signal for induction of appressoria formation, probably transduced via cAMP. The resulting deformation of the hypha might stretch the plasma membrane, activating mechano-sensitive channels (Wessels, 1994), which have been shown to be present at least in *S.*

cerevisae (Gustin et al., 1988), *Saprolegnia ferax* (Garrill et al., 1993), and the phytopathogen *Uromyces appendiculatus* (Zhou et al., 1991). In the latter fungus a correlation was found between the strength of adhesion of sporelings, the number of appressoria formed, and the hydrophobicity of surface bearing the sporelings (Terhune and Hoch, 1993).

A. fumigatus is a major cause of several respiratory diseases in immuno-compromised patients. It was suggested that adhesion of conidia to host proteins or cells is a primary event during establishment of infection (Annaix et al., 1992). The conidial surface is characterized by the presence of a rodlet layer formed by the hydrophobin RODA (HYP1) (Thau et al., 1994; Parta et al., 1994), suggesting a role for this hydrophobin in conidial attachment. However, conidia of a strain in which the *rodA* gene was disrupted adhered equally well to pneumocytes, fibrinogen and laminin compared to wild-type conidia, while adhesion to collagen and BSA was reduced. From these experiments it was concluded that RODA is not the major surface protein responsible for the adherence of conidia to host cells and proteins but serves in dispersion of the conidia (Thau et al., 1994). In addition, spores of the strain in which the *rodA* gene was disrupted were as pathogenic as spores of a wild-type strain in mice infection experiments (Thau et al., 1994).

Hydrophobins in attachment in mutually beneficial symbioses Fungi in mutually beneficial symbioses also have to attach to the surface of their host. In the lichen symbiosis between peltigerean mycobionts and their *Coccomyxa* phycobionts, cell walls of both partners appear to adhere tightly. The surface of the fungus in contact with the phycobiont consisted of a hydrophobic rodlet layer (Honegger, 1982, 1991), indicating a role of hydrophobins in attachment. A similar role for hydrophobins has been suggested in the ectomycorrhizal interaction. In contact with the plant root, the fungus differentiates a highly-branched, fan shaped mycelium (Jacobs et al., 1989), which aggregates around the plant root to form the multicellular ectomycorrhizal mantle. Coenocytic hyphae then penetrate between the root cells to establish an exchange interface, called the Hartig net (Kottke and Oberwinkler, 1987). Two class I hydrophobin genes (*HydPt-1* and *HydPt-2*) of *Pisolithus tinctorius* were isolated as genes highly expressed during formation of aerial hyphae and in early stages of ectomycorrhiza formation with roots of *Eucalyptus globulus* (Tagu et al., 1996). The differential expression of these hydrophobin genes indicates that they are not only involved in formation of aerial hyphae but could also be involved in attachment of hyphae to the plant roots (Martin et al., 1995a; Tagu et al., 1996). The latter process is not yet understood. Fibrillar adhesive material released by the fungal partner together with amorphous mucilage-like material secreted by the host root have also been suggested to play a role in the attachment process (for review see Martin et al., 1995b). Alternatively, the hydrophobins of *Pisolithus* may be involved in the formation of the ectomycorrhizal mantle by aggregating hyphae or may create the hydrophobic surface of this structure (Martin et al., 1995a; Tagu et al., 1996).

Do hydrophobins protect fungal structures? Assembled hydrophobins are extremely resistant towards chemical and enzymatic treatment. For instance, assembled SC3 resisted treatments with (aqueous solutions of) organic solvents or chaotropic agents and detergents in the presence or absence of 100 mM DTT (de Vries et al., 1993). In addition, SC3 appears to be insensitive to a number of proteases (de Vries, O. M. H. and Wessels, J. G. H., unpublished data). Similarly, rodlet layers on conidia of *Trichophyton mentagrophytes* (Hashimoto et al., 1976), *Nomuraea rileyi*, *B. bassiana*, *M. anisopliae* (Boucias and Pendland, 1991), and *A. niger* (Cole et al., 1979) resisted treatments with organic solvents, various detergents, proteases, cell wall lytic enzymes, mild acid and urea. Only heating at 100°C in 1 M NaOH completely degraded these rodlet layers. The resistance against enzymatic and chemical treatments indicates that hydrophobins could have a role in protection of emergent structures against adverse environmental conditions. It was proposed that the ABH1 membrane covering fruiting bodies of *A. bisporus* (de Groot et al., 1996; Lugones et al., 1996) protects against bacterial infection and other environmental influences (de Groot et al., 1996).

The tiny conidia of *Coccidioides immitis*, if inhaled, can initiate a human respiratory disease known as valley fever (coccidioidomycosis). These conidia have an outer wall layer decorated with rodlet fascicles (Cole et al., 1983). Removal of this hydrophobic layer by sonication prompted phagocytosis of the propagule by human polymorphonuclear neutrophils (PMNs) (Drutz and Huppert, 1983), while sheared conidia or a soluble cell wall fraction, but not intact conidia, induced an intense lymph node proliferation (Cole et al., 1987). When the isolated outer wall layer was added to the soluble wall fraction, cell proliferation was effectively blocked. These results suggest that the rodlet layer shields multiple antigenic molecules in the cell wall and represents a defence of the pathogen against attack by the host cellular immune system (Cole and Kirkland, 1991). Whether this property is more widespread is not yet clear. RODA (HYP1) of *A. fumigatus* forms the outer layer of conidiospores of this pathogenic fungus. It was suggested that this layer protects the infectious propagule from the hydrolytic activity of the phagocytic cells. However, conidia of a strain in which the *rodA* gene was disrupted were as pathogenic as wild-type conidia (Thau et al., 1994). In the pathogenicity assay, mice were challenged with at least 10^5 conidia. Pathogenicity factors may only be identified at lower dosages, because at higher dosage an alternative infection route could be operative. Such a dosage effect was shown for the involvement of the cutinase gene in pathogenicity of *Fusarium solani* for pea (Rogers et al., 1994).

Like animals, plants have developed mechanisms to detect and defend themselves against pathogenic fungi. Hydrophobins may mask β-glucan and chitin (Templeton

et al., 1994), main components of the fungal cell wall, derivatives of which exhibit a high elicitor activity (Sharp et al., 1984a, b; Roby et al., 1987). Hydrophobins could prevent release of these elicitors and thus permit the fungus to colonize the plant unnoticed.

CU was shown to be involved in protection against desiccation. Over-expression of CU in *O. ulmi* resulted in decreased susceptibility to desiccation, while susceptibility was increased in strains of *O. novo-ulmi* in which the *cu* gene was disrupted (Temple et al., 1997). This suggests that the assemblage of CU forms an impermeable coating, which might contrast with that formed by class I hydrophobins (see above).

Hydrophobins as toxins and elicitors? Of the hydrophobins isolated from pathogenic fungi, only CU of *O. novo-ulmi* and *O. ulmi* has been proposed to be a toxin (Takai, 1974). Injection of purified CU into the host white elm caused wilting, reduction in transpiration, increase in leaf respiration and electrolyte loss (Takai, 1974). At the electron microscopy level, symptoms of plants injected with CU or infected with *O. novo-ulmi* were indistinguishable (Takai and Hiratsuka, 1984). These symptoms were suggested to derive from plugging of xylem vesicles by CU-coated air bubbles (Ipsen and Abul-Hajj, 1982; Russo et al., 1982) and to increased host plasma membrane permeability (Stevenson et al., 1979). The toxicity of CU appeared to be selective and was highest with hosts of the pathogen (Richards, 1993). However, it was recently shown unambiguously that CU, despite its property to function as a wilt-toxin in vitro, is dispensable for pathogenicity. When *cu* was disrupted pathogenicity was unaffected (Bowden et al., 1996; Temple et al., 1997), while overexpression of *cu* in the relatively non-aggressive *O. ulmi* did not increase pathogenicity (Temple et al., 1997). Moreover, two naturally occurring isolates of *O. novo-ulmi* which exhibited a flat morphology were shown to be deficient in CU production but were still pathogenic (Brasier et al., 1995). These conflicting results about the role of CU as a toxin might be explained by the methods used to assay for wilt toxins: results obtained in these assays may not necessarily reflect the in vivo function (for a critical review see Van Alfen, 1989). Alternatively, CU might be one of several pathogenicity factors, each of which is dispensable, but which together give full pathogenicity (Bowden et al., 1996). Results could also be explained by the expression of another hydrophobin specifically produced in plants giving the same symptoms as CU (Wessels, 1997).

Isolated peptides eliciting plant defence show some resemblance to hydrophobins in being rich in cysteine residues. For instance, NIP1 of *Rhynchosporium secalis*, a pathogen of barley, contains 10 cysteine residues, 8 of which spaced in a way similar to those of hydrophobins (Rohe et al., 1995). However, the hydropathy pattern of this elicitor deviates significantly from those of both classes of hydrophobins. Nevertheless, such peptide elicitors might have originated from these proteins (Templeton et al., 1994).

Are hydrophobins tailored to fulfil specific functions? Four hydrophobin genes have been identified in *S. commune*, three in *A. bisporus* and three in *Pleurotus ostreatus* (Fig. 2). Isolation of these genes by cross hybridization is hampered by their low homology at the nucleotide level (see Wessels, 1997), and therefore the number of hydrophobin genes that are contained in the genome of these and other fungi might even be higher. We can only speculate whether these diverse hydrophobins are tailored to fulfil specific functions during fungal development. SC3 (van Wetter et al., 1996) and CU (Bowden et al., 1996; Temple et al., 1997) have been shown to be involved in formation of aerial hyphae, while correlative evidence indicated that COH1, POH3, ABH3 (Ásgeirsdóttir et al., 1997; Ásgeirsdóttir, S. A., Lugones, L. G., de Vries, O. M. H., and Wessels, J. G. H., unpublished data), HydPt1 and HydPt2 (Tagu et al., 1996) are also instrumental in formation of these structures. The class I hydrophobins SC3, COH1, POH1 and ABH3 are more related to each other than SC3 of *S. commune* and ABH3 *A. bisporus* are related to other hydrophobin genes in the same organisms (*SC1*, *SC4*, *SC6*, and *ABH1* and *ABH2*, respectively) which are involved in formation of fruiting bodies (Fig. 2). This suggests that functional similarity is reflected in the structural homology of hydrophobins. On the other hand, SC3 of *S. commune*, MPG1 of *M. grisea* and CU of *O. novo-ulmi* and *O. ulmi* are apparently multifunctional proteins. SC3 is involved both in formation of aerial hyphae (van Wetter et al., 1996) and in attachment of hyphae to hydrophobic surfaces (Wösten et al., 1994c), MPG1 is active in initial stages of pathogenicity and in coating conidiospores with a hydrophobic rodlet layer (Talbot et al., 1993, 1996), while CU is involved in formation of aerial hyphae and in attachment of yeast-like cells to hydrophobic surfaces (Temple et al., 1997). Structure-function analysis of hydrophobins should establish which requirements a hydrophobin should have to fulfil specific functions.

Literature cited

Adams, T. H., Boylan, M. T. and Timberlake, W. E. 1988. *brlA* is necessary and sufficient to direct conidiophore development in *Aspergillus nidulans*. Cell **54**: 353–362.

Annaix, V., Bouchara, J. P., Larcher, G., Chabasse, D. and Tronchin, G. 1992. Specific binding of human fibrinogen fragment D to *Aspergillus fumigatus* conidia. Infect. Immun. **60**: 1747–1755.

Arntz, C. and Tudzynski, P. 1997. Identification of genes induced in alkaloid-producing cultures of *Claviceps* sp. Curr. Genet. (In press.)

Ásgeirsdóttir, S. A., Halsall, J. R. and Casselton, L. A. 1997. Expression of two closely linked hydrophobin genes of *Coprinus cinereus* is monokaryon specific and down-regulated by the *oid*-1 mutation. Fungal Genet. Biol. (In press.)

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 *Schizophyllum commune*. Microbiology **141**: 1281–1288.

Beckerman, J. L. and Ebbole, D. J. 1996. MPG1, a gene encoding a fungal hydrophobin of *Magnaporthe grisea*, is in-

volved in surface recognition. Mol. Plant-Microbe Interact. **9**: 450–456.

Beever, R. E. and Dempsey, G. P. 1978. Function of rodlets on the surface of fungal spores. Nature **272**: 608–610.

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.

Bohlmann, R. 1996. Isolierung und Charakterisierung von filamentspezifisch exprimierten Genen aus *Ustilago maydis*. PhD thesis, University of Munich.

Boucias, D. G. and Pendland, J. C. 1991. Attachment of mycopathogens to cuticle. The initial event of mycoses in arthropod hosts. In: The fungal spore and disease initiation in plants and animals, (ed. by Cole, G. T. and Koch, H. C.), pp 101–127. Plenum Press, New York.

Boucias, D. G., Pendland, J. C. and Latgé, J. P. 1988. Nonspecific factors involved in the attachment of entomopathogenic deuteromycetes to host insect cuticle. Appl. Environ. Microbiol. **54**: 1795–1805.

Bowden, C. G., Hintz, W. E., Jeng, R., Hubbes, M. and Horgen, P. A. 1994. Isolation and characterization of the cerato-ulmin toxin gene of the Dutch elm disease pathogen, *Ophiostoma ulmi*. Curr. Genet. **25**: 323–329.

Bowden, C. G., Smalley, E., Guries, R. P., Hubbes, M., Temple, B. and Horgen, P. A. 1996. Lack of association between cerato-ulmin production and virulence in *Ophiostoma ulmi*. Mol. Plant-Microbe Interact. **9**: 556–564.

Brasier, C. M., Kirk, S. A. and Tegli, S. 1995. Naturally occurring non cerato-ulmin producing mutants of *Ophiostoma novo-ulmi* are pathogenic but lack aerial mycelium. Mycol. Res. **99**: 436-440.

Carpenter, C. E., Mueller, R. J., Kazmierczak, P., Zhang, L., Villalon, D. K. and Van Alfen, N. K. 1992. Effect of a virus on accumulation of a tissue-specific cell surface protein of the fungus *Cryphonectria (Endothia) parasitica*. Mol. Plant-Microbe Interact. **4**: 55–61.

Cole, G. T. and Hoch, H. C. 1991. The fungal spore and disease initiation in plants and animals. Plenum Press, New York.

Cole, G. T. and Kirkland, T. N. 1991. Conidia of *Coccidioides immitis*. In: The fungal spore and disease initiation in plants and animals, (ed. by Cole, G. T. and Hoch, H. C.), pp. 403–443. Plenum Press, New York.

Cole, G. T., Kirkland, T. N. and Sun, S. H. 1987. An immunoreactive, water soluble conidial wall fraction of *Coccidioides immitis*. Inf. Immun. **55**: 657–667.

Cole, G. T., Pope, L. M., Huppert, M., Sun, S. H. and Starr, P. 1983. Ultrastructure and composition of conidial wall fractions of *Coccidioides immitis*. Exp. Mycol. **7**: 297–318.

Cole, G. T., Sekiya, T., Kasai, R., Yokoyama, T. and Nozawa, Y. 1979. Surface ultrastructure and chemical composition of the cell walls of conidial fungi. Exp. Mycol. **3**: 132–156.

de Groot, P. W. J., Schaap, P. J., Sonnenberg, A.S.M., Visser, J. and Van Griensven, L. J. L. D. 1996. The *Agaricus bisporus hypA* gene encodes a hydrophobin and specifically accumulates in peel tissue of mushroom caps during fruit body development. J. Mol. Biol. **257**: 1008–1018.

de Vries, O. M. H., Fekkes, M. P., Wösten, H. A. B. and Wessels, J. G. H. 1993. Insoluble hydrophobin complexes in the walls of *Schizophyllum commune* and other filamentous fungi. Arch. Microbiol. **159**: 330–335.

Doyle, R. J. and Rosenberg, M. 1990. Microbial cell surface hydrophobicity. Amer. Soc. of Microbiol., Washington, D. C.

Drutz, D. J. and Huppert, M. 1983. Coccidioidomycosis: Factors affecting the host-parasite interaction. J. Infect. Diseases **147**: 372–390.

Garrill, A., Jackson, S. L., Lew, R. R. and Heath, I. B. 1993. Ion channel activity and tip growth: tip localized stretch-activated channels generate an essential Ca^{2+} gradient in the oomycete *Saprolegnia ferax*. Eur. J. Cell Biol. **60**: 358–365.

Georgiou, G., Lin, S.-C. and Sharma, M. M. 1992. Surface-active compounds from microorganisms. Bio/Technol. **10**: 60–65.

Gerin, P. A., Dufrene, Y., Bellon-Fontaine, M.-N., Asther, M. and Rouxhet, P. G. 1993. Surface properties of the conidiospores of *Phanerochaete chrysosporium* and their relevance to pellet formation. J. Bacteriol. **175**: 5135–5144.

Gustin, M. C., Zhou, X.-L., Matinac, B. and Kung, C. 1988. A mechanosensitive ion channel in the yeast plasma membrane. Science **242**: 762–765.

Hamer, J. E., Howard, R. J., Chumleym, F. G. and Valent, B. 1988. A mechanism for surface attachment in spores of a plant pathogenic fungus. Science **239**: 288–290.

Hashimoto, T., Wu-Yuan, C. D. and Blumenthal, H. J. 1976. Isolation and characterization of the rodlet layer of *Trichophyton mentagrophytes* microconidial wall. J. Bacteriol. **127**: 1543–1549.

Hazen, K. C. 1990. Cell surface hydrophobicity of medically important fungi, especially *Candida* species. In: Microbial cell surface hydrophobicity, (ed. by Doyle, R. J. and Rosenberg, M.), pp. 249–295. Amer. Soc. Microbiol., Washington, D. C.

Hebard, F. V., Griffin, G. J. and Elkins, J. R. 1984. Developmental histopathology of cankers incited by hypovirulent and virulent isolates of *Endothia parasitica* on susceptible and resistant chestnut trees. Phytopathology **74**: 140–149.

Higgins, D. G. and Sharp, P. M. 1988. Clustal: a package for performing multiple sequence alignments on a microcomputer. Gene **73**: 237–244.

Honegger, R. 1982. Cytological aspects of the triple symbiosis in *Peltigera aphthosa*. J. Hattori Bot. Lab. **52**: 379–391.

Honegger, R. 1991. Functional aspects of the lichen symbiosis. Ann. Rev. Plant Physiol. Plant Mol. Biol. **42**, 553–578.

Honegger, R. 1993. Developmental biology of lichens. New Phytol. **125**: 659–677.

Ipsen, J. D. and Abul-Hajj, Y. J. 1982. Fluorescent antibody technique as a means of localizing *Ceratocystis ulmi* toxins in elm. Can. J. Bot. **60**: 724–729.

Jacobs, P. F., Peterson, R. L. and Massicotte, H. B. 1989. Altered fungal morphogenesis during early stages of ectomycorrhiza formation in *Eucalyptus pilularis*. Scanning Microscopy **3**: 249–255.

Jenny, K., Käppeli, O. and Fiechter, A. 1991. Biosurfactants from *Bacillus licheniformis*: structural analysis and characterization. Appl. Microbiol. Biotechnol. **36**: 5–13.

Kottke, I. and Oberwinkler, F. 1987. The cellular structure of the Hartig net: coenocytic and transfer cell-like organization. Nordic J. Bot. **7**: 85–95.

Lauter, F.-R., Russo, V. E. A. and Yanofsky, C. 1992. Developmental and light regulation of *eas*, the structural gene for the rodlet protein of *Neurospora*. Genes Dev. **6**: 2373–2381.

Lee, Y.-H. and Dean, R. A. 1993. cAMP regulates infection structure formation in the plant pathogenic fungus *Magnaporthe grisea*. Plant Cell **5**: 693–700.

Lora, J. M., de la Cruz, J., Benitez, T., Llobell, A. and Pintor-Toro, J. A. 1994. A putative catabolite-repressed cell wall

protein from the mycoparasitic fungus *Trichoderma harzianum*. Mol. Gen. Genet. **242**: 461–466.

Lugones, L. G., Bosscher, J. S., Scholtmeijer, K., de Vries, O. M. H. and Wessels, J. G. H. 1996. An abundant hydrophobin (ABH1) forms hydrophobic rodlet layers in *Agaricus bisporus* fruiting bodies. Microbiology **142**: 1321–1329.

Martin, F., Laurent, P., de Cavalho, D., Burgess, T., Murphy, P., Nehls, U. and Tagu, D. 1995a. Fungal gene expression during ectomycorrhiza formation. Can. J. Bot. **73**: S541–S547.

Martin, F., Burgess, T., Carnero Diaz, M. E., de Carvalho, D., Laurent, P., Murphy, P., Nehls, U. and Tagu, D. 1995b. Ectomycorrhiza morphogenesis: insights from studies of developmentally regulated genes and proteins. In: Biotechnology of ectomycorrhizae: molecular approaches, (ed. by Stocchi, V.). Plenum Press, New York.

Mulder, G. H. and Wessels, J. G. H. 1986. Molecular cloning of RNAs differentially expressed in monokaryons and dikaryons of *Schizophyllum commune* in relation to fruiting. Exp. Mycol. **10**: 214–227.

Nakari-Setälä, T. (1995). Highly expressed *Trichoderma reesei* genes. Thesis. VTT Publications, Espoo, Finland.

Nakari-Setälä, T., Aro, N., Kalkkinen, N., Alatalo, E. and Penttilä, M. 1996. Genetic and biochemical characterization of the *Trichoderma reesei* hydrophobin HFB1. Eur. J. Biochem. **235**: 248-255.

Parta, M., Chang, Y., Rulong, S., Pinto-DaSilva, P. and Kwon-Chung, K. J. 1994. *HYP1, a* hydrophobin gene from *Aspergillus fumigatus* complements the *rodletless* phenotype in *Aspergillus nidulans*. Inf. Immun. **62**: 4389–4395.

Richards, W. C. 1993. Cerato-ulmin: a unique wilt toxin of instrumental significance in the development of Dutch elm disease. In: Dutch elm disease research: cellular and molecular approaches, (ed. by Sticklen, M. B. and Sherald, J. L.), pp. 89–151. Springer Verlag, New York.

Richards, W. C. and Takai, S. 1973. Novel technique for isolating microstructures present in shake cultures of the fungus *Ceratocystis ulmi*. Appl. Microbiol. **26**: 443–444.

Roby, D., Gadelle, A. and Toppan, A. 1987. Chitin oligosaccharides as elicitors of chitinase activity in melon plants. Biochem. Biophys. Res. Commun. **143**: 885–892.

Rogers, L. M., Flaishman, M. A. and Kolattukudy, P. E. 1994. Cutinase gene disruption in *Fusarium solani* f sp *pisi* decreases its virulence on pea. Plant Cell **6**: 935–945.

Rohe, M., Gierlich, A., Hermann, H., Hahn, M., Schmidt, B., Rosahl, S. and Knogge, W. 1995. The race-specific elicitor, NIP1, from the barley pathogen, *Rhynchosporium secalis*, determines avirulence on host plants of the Rrs1 resistance genotype. EMBO J. **14**: 4168–4177.

Rosenberg, M. and Kjelleberg, S. 1986. Hydrophobic interactions: role in bacterial adhesion. Adv. Microb. Ecol. **9**: 353–393.

Russo, P. S., Blum, F. D., Ipsen, J. D., Miller, W. G. and Abul-Hajj, Y. J. 1982. The surface activity of the phytotoxin cerato-ulmin. Can. J. Bot. **60**: 1414–1422.

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.

Sharp, J. A., McNeil, M. and Albersheim, P. 1984a. The primary structure of one elicitor active and seven elicitor-inactive hexa (β-D-glucopyranosyl)-D-glucitols isolated from the mycelial walls of *Phytophtora megasperma* f. sp. *glycinea*. J. Biol. Chem. **25**: 11321–11326.

Sharp, J. A., Valent, B. and Albersheim, P. 1984b. Purification and partial characterization of a β-glucan fragment that elicits phytoalexin accumulation in soybean. J. Biol. Chem. **25**: 11312–11320.

Spanu, P. 1997. HCf-1, a hydrophobin from the potato pathogen *Cladosporium fulvum*. Gene (In press.)

Stevenson, K. J., Slater, J. A. and Takai, S. 1979. Cerato-ulmin, a wilting toxin of Dutch elm disease fungus. Phytochemistry **18**: 235–238.

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.

Stringer, M. A., Dean, R. A., Sewall, T. C. and Timberlake, W. E. 1991. Rodletless, a new *Aspergillus* developmental mutant induced by directed gene inactivation. Genes Dev. **5**: 1161–1171.

Stringer, M. A. and Timberlake, W. E. 1993. Cerato-ulmin, a toxin involved in Dutch elm disease, is a fungal hydrophobin. Plant Cell **5**: 145–146.

Stringer, M. A. and Timberlake, W. E. 1995. *dewA* encodes a fungal hydrophobin component of the *Aspergillus* spore wall. Mol. Microbiol. **16**: 33–44.

Svircev, A. M., Jeng, R. S. and Hubbes, M. 1988. Detection of cerato-ulmin on aggressive isolates of *Ophiostoma ulmi* by immunocytochemistry and scanning electron microscopy. Phytopathology **78**: 322–327.

Swift, S., Throup, J. P., Williams, P., Salmon, G. P. C. and Stewart, G. S. A. B. 1996. Quorum sensing: a population density component in the determination of bacterial phenotype. TIBS **21**: 214–219.

Tagu, D., Nasse, B. and Martin, F. 1996. Cloning and characterization of hydrophobin-encoding cDNAs from the ectomycorrhizal basidiomycete *Pisolithus tinctorius*. Gene **168**: 93–97.

Takai, S. 1974. Pathogenicity and cerato-ulmin production in *Ceratocystis ulmi*. Nature **252**: 124–126.

Takai, S. and Hiratsuka, Y. 1984. Scanning electron microscope observations of internal symptoms of white elm following *Ceratocystis ulmi* infection and cerato-ulmin treatment. Can. J. Bot. **62**: 1365–1371.

Takai, S., Hiratsuka, Y., Krywienczyk, J., Richards, W. C. and Davies, Y. P. 1980. Evidence for the presence of the toxin cerato-ulmin in the synnema head fluid of *Ceratocystis ulmi*. Can. J. Bot. **58**: 669–675.

Takai, S. and Richards, W. C. 1978. Cerato-ulmin, a wilting toxin of *Ceratocystis ulmi*: isolation and some properties of cerato-ulmin from the culture of *C. ulmi*. Phytopathol. Z. **91**: 129–146.

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.

Talbot, N. J., Kershaw, M. J., Wakley, G. E., de Vries, O. M. H., Wessels, J. G. H. and Hamer, J. E. 1996. *MPG1* encodes a fungal hydrophobin involved in surface interactions during infection-related development by the rice blast fungus *Magnaporthe grisea*. Plant Cell **8**: 985–999.

Temple, B., Horgen, P. A., Bernier, L. and Hintz, W. E. 1997. Cerato-ulmin, a hydrophobin secreted by the causal agents of Dutch elm disease, is a parasitic fitness factor. Fungal Genet. Biol. (In press.)

Templeton, M. D., Greenwood, D. R. and Beever, R. E. 1995. Solubilization of *Neurospora crassa* rodlet proteins and identification of the predominant protein as the proteolytically processed *eas* (*ccg-2*) gene product. Exp. Mycol. **19**:

166–169.

Templeton, M. D., Rikkerink, E. H. A. and Beever, R. E. 1994. Small, cysteine-rich proteins and recognition in fungal-plant interactions. Mol. Plant-Microbe Interact. **7**: 320–325.

Terhune, B. T. and Hoch, H. C. 1993. Substrate hydrophobicity and adhesion of *Uromyces* urediospores and germlings. Exp. Mycol. **17**: 241–252.

Thau, N., Monod, M., Crestani, B., Rolland, C., Tronchin, G., Latgé, J.-P. and Paris, S. 1994. Rodletless mutants of *Aspergillus fumigatus*. Inf. Immun. **62**: 4380–4388.

Van Alfen, N. K. 1989. Reassessment of plant wilt toxins. Ann. Rev. Phytopathol. **27**: 533–550.

van der Vegt, W., van der Mei, H. C., Wösten, H. A. B., Wessels, J. G. H. and Busscher, H. J. 1996. A comparison of the surface activity of the fungal hydrophobin SC3p with those of other proteins. Biophys. Chem. **51**: 253–260.

van Wetter, M.-A., Schuren, F. H. J., Schuurs, T. A. and Wessels, J. G. H. 1996. Targeted mutation of the *SC3* hydrophobin gene of *Schizophyllum commune* affects formation of aerial hyphae. FEMS Microbiol. Lett. **140**: 265–269.

Wessels, J. G. H. 1993a. Wall growth, protein excretion, and morphogenesis in fungi. Tansley Review no. 45. New Phytol. **123**: 397–413.

Wessels, J. G. H. 1993b. Fruiting in the higher fungi. Adv. Microb. Physiol. **34**: 147–202.

Wessels, J. G. H. 1994. Developmental regulation of fungal cell wall formation. Ann. Rev. Phytopathol. **32**: 413–437.

Wessels, J. G. H. 1996. Fungal hydrophobins: proteins that function at the interface. Trends Plant Sc. **1**: 9–15.

Wessels, J. G. H. 1997. Hydrophobins: proteins that change the nature of a fungal surface. Adv. Microb. Physiol. **38**: 1–45.

Wessels, J. G. H., Ásgeirsdóttir, S. A., Birkenkamp, K. U., de Vries, O. M. H., Lugones, L.G., Schuren, F. H. J., Schuurs, T. A., van Wetter, M. A. and Wösten, H. A. B. 1995. Genetic regulation of emergent growth in *Schizophyllum commune*. Can. J. Bot. **73**: S273–S281.

Wessels, J. G. H., de Vries, O. M. H., Ásgeirsdóttir, S. A. and Schuren, F. H. J. 1991a. Hydrophobin genes involved in formation of aerial hyphae and fruit bodies in *Schizophyllum commune*. Plant Cell **3**: 793–799.

Wessels, J. G. H., de Vries, O. M. H., Ásgeirsdóttir, S. A. and Springer, J. 1991b. The *thn* mutation of *Schizophyllum commune*, which suppresses formation of aerial hyphae, affects expression of the *Sc3* hydrophobin gene. J. Gen. Microbiol. **137**: 2439–2445.

Wösten, H. A. B., Ásgeirsdóttir, S. A., Krook, J. H., Drenth, J. H. H. and Wessels, J. G. H. 1994a. The Sc3p hydrophobin self-assembles at the surface of aerial hyphae as a protein membrane constituting the hydrophobic rodlet layer. Eur. J. Cell Biol. **63**: 122–129.

Wösten, H. A. B., de Vries, O. M. H., van der Mei, H. C., Busscher, H. J. and Wessels, J. G. H. 1994b. Atomic composition of the hydrophobic and hydrophilic sides of self-assembled SC3P hydrophobin. J. Bacteriol. **176**: 7085–7086.

Wösten, H. A. B., de Vries, O. M. H. and Wessels, J. G. H. 1993. Interfacial self-assembly of a fungal hydrophobin into a rodlet layer. Plant Cell **5**: 1567–1574.

Wösten, H. A. B., Ruardy, T. G., van der Mei, H. C., Busscher, H. J. and Wessels, J. G. H. 1995. Interfacial self-assembly of a *Schizophyllum commune* hydrophobin into an insoluble amphipathic membrane depends on surface hydrophobicity. Coll. Surf. B: Biointerfaces **5**: 189–195.

Wösten, H. A. B., Schuren, F. H. J. and Wessels, J. G. H. 1994c. Interfacial self-assembly of a hydrophobin into an amphipathic membrane mediates fungal attachment to hydrophobic surfaces. EMBO J. **13**: 5848–5854.

Yaguchi, M., Pusztai-Carey, M., Roy, C., Surewicz, W. K., Carey, P. R., Stevenson, K. J., Richards, W. C. and Takai, S. 1993. Amino acid sequence and spectroscopic studies of Dutch elm disease toxin, cerato-ulmin. In: Dutch elm disease research: cellular and molecular approaches, (ed. by Sticklen, M. B. and Sherald, J. L.), pp. 152–170. Springer Verlag, New York.

Zhang, L., Villalon, D., Sun, Y., Kazmierczak, P. and Van Alfen, N. K. 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.

Zhou, X. L., Stumpf, M. A., Hoch, H. C. and Kung, C. 1991. A mechanosensitive channel in whole cells and in membrane patches of the fungus *Uromyces*. Science **253**: 1415–1417.