

3 Adhesion and Adhesives of Fungi and Oomycetes

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3.1 Introduction

During the past decade there has been an increased recognition of the importance of adhesion of fungi to host surfaces—both plant and animal—before penetration (Mendgen et al. 1996; Epstein and Nicholson 1997; Hardham 2001; Osheroov and May 2001; Tucker and Talbot 2001). Observational studies with microscopy indicate that many fungi adhere tenaciously onto inert surfaces such as polystyrene in addition to host substrata. This is perhaps not surprising since, for example, the aerial surfaces of plants are hydrophobic and relatively inert, and aquatic fungi can adhere to rocks. Microscopy of fungi that are in the process of adhering also indicates that fungal-substratum adhesion is mediated by a glue, i.e., a secreted macromolecule that extends from the fungus onto the adjacent surface and binds to it in a relatively non-specific manner. Here, we will primarily focus on fungal cell-substratum adhesion that is mediated by a glue.

We will use the term ‘adhesin’ to indicate a molecule that mediates a comparatively specific attachment between a ligand on a fungus and a receptor on its host’s substratum. However, whether an adhesin is somewhat non-specifically “sticky” or has a conformational “good fit” for a particular substratum is not always clear, particularly with the literature on adhesion of animal pathogenic fungi to the extracellular matrices of mammalian cells. The cells of the human commensal and pathogen *Candida albicans*, for example, adhere to inert substrata such as polystyrene (Masuoka et al. 1999). Consequently, while we will focus on examples of glue-mediated adhesion of fungi to substrata, we will also mention some of the best-characterized examples of cell-cell adhesion in which at least one of the cells is a fungus, and the adhesin may have glue-like properties.

3.2 Prevalence and Importance of Adhesion in Fungi and Oomycetes

Cell-substratum adhesion is common in all taxonomic classes of microscopic fungi (Nicholson and Epstein 1991; Jones 1994). Cell-substratum adhesion

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also is common in the oomycetes, which include such economically important plant pathogens such as *Phytophthora* spp., *Pythium* spp., and *Plasmopara* spp. (Hardham 2001). Although oomycetes are stramenopiles, i.e., are phylogenetically distinct from fungi, and are most closely allied to diatoms, brown algae, and some parasitic protozoans (Gunderson et al. 1987), we will include them here because they traditionally have been classified as fungi. More importantly, oomycetes and fungi share environments in which glues are secreted and function.

3.2.1 Adhesion as Part of Many Stages of Morphogenesis in Many Fungi

In a single organism, multiple stages of development can be adherent and can involve differing mechanisms of adhesion. With wind-disseminated plant pathogens such as *Botrytis cinerea* (Doss et al. 1993) and *Erysiphe graminis* (see Sect. 3.2.3.2), hydrophobic interactions between a hydrophobic spore and the leaf cuticle can secure the initial contact. With water-disseminated pathogens such as *Colletotrichum graminicola*, initial adhesion of spores requires the release of a glue (Nicholson and Epstein 1991; Epstein and Nicholson 1997). Since most fungal spores require free water for germination, later stages of adhesion for both air- and water-disseminated spores occur in an aqueous environment. Regardless, less perturbable adhesion is temporally and spatially associated with de novo material on the cell surface (Hamer et al. 1988; Kwon and Epstein 1993; Braun and Howard 1994a). It is important to note that the fungal interface with its environment is biochemically complex and multifunctional, i.e., glues are only one component of surface-associated compounds and adhesion is only one function of the extracellular matrix (Nicholson and Epstein 1991). In addition, non-glue components in the extracellular matrix can alter the surface and increase the strength of attachment; *Uromyces viciae-fabae* urediospores release cutinases and esterases that degrade the plant cuticle and enhance spore adhesion (Deising et al. 1992).

The strength of attachment varies with the fungus and the cell type. For example, *Nectria haematococca* mating population I conidia and germlings adhere to polystyrene and to plant surfaces, but they do not adhere as tenaciously as spores or germlings of *Colletotrichum graminicola* or germlings of the rust fungus *Uromyces appendiculatus*. Unfortunately, relatively few papers quantify the strength of adhesion. Comparisons between studies would be facilitated by utilization of flow cells in which shear force can be determined (Li and Palecek 2003). Mechanical penetration from appressoria requires firm adhesion. Using a microscopic method, Bechinger et al. (1999) estimated that *Colletotrichum graminicola* appressoria had a turgor pressure of 5.4 MPa and exerted an approximate force of 17 μ N. Consequently, the glue that affixes the appressorium to the plant surface must be able to withstand this force.

3.2.2 Functions of Adhesion

Even if we limit our discussion to fungal-substratum adhesion that occurs on the plant host surface before penetration, adhesion serves multiple functions (Table 3.1). Most obviously, adhesion keeps a fungus from being blown or rinsed from a potentially suitable environment. Whether contact is required can depend upon the environmental conditions; many species of fungal spores germinate readily regardless of contact in a nutritive liquid medium, but in an oligotrophic environment, efficient germination requires contact with a substratum (Chaky et al. 2001). In *Phyllosticta ampellicida*, adhesion of pycnidiospores is required for germination (Kuo and Hoch 1996). In fungi such as *Erysiphe graminis*, spore adhesion may facilitate “preparation of the infection court”, i.e., alteration of the host surface so that it favors fungal development (see Sect. 3.2.3.1). That is, a tightly adherent spore or germling may be able to effectively maintain a higher concentration of its lytic enzymes at the interface between its wall and the substratum surface. *Nectria haematococca* mutants with conidia and germlings

Table 3.1. Functions of fungal glues on the surface of the plant host

Benefits of adhesion for the fungus	Cell types	Examples of organisms ^a	References with experimental evidence
Prevents displacement by water and/or wind	Conidia, germlings, hyphae, appressoria	All	Reviewed in Epstein and Nicholson (1997)
Limits germination to potential host tissue (Required for contact-stimulated germination)	Conidia	Pa	Kuo and Hoch (1996)
Increases the surface area of contact with its host	Germlings, hyphae	Cg	Apoga et al. (2004)
Facilitates chemical interaction between pathogen and host	Conidia, germlings	Nh	Jones and Epstein (1990)
Required for thigmotropism	Germlings	Ua	Epstein et al. (1987)
Required for thigmodifferentiation	Appressoria, Hyphopodia	Cg Eg Ua	Chaky et al. (2001) Yamaoka and Takeuchi (1999) Epstein et al. (1985)
Required for host penetration	Appressoria,	Cg	Bechinger et al. (1999)
via mechanical pressure	hyphopodia, cysts	Mg	Howard et al. (1991)

^a This list provides examples and is a not comprehensive survey. All, applies to all fungi in this list; Cg, *Colletotrichum graminicola*; Eg, *Erysiphe graminis*; Mg, *Magnaporthe grisea*; Nh, *Nectria haematococca* mating population I (anamorph *Fusarium solani* f. sp. *cucurbitate* race I); Pa, *Phyllosticta ampellicida*; Ua, *Uromyces appendiculatus*

with reduced adhesiveness were less virulent than the wild type when deposited on the cucurbit fruit surface, but were equally virulent when deposited within the fruit tissue (Jones and Epstein 1990). Adhesion of germlings to a substratum maximizes fungal reception of surface signals and absorption of nutrients from the host substratum because adherent germlings and hyphae are flattened on the bottom; in contrast, germlings and hyphae that have not developed attached to a substratum are round in cross-section (Epstein et al. 1987).

Some fungi have contact-induced responses, and these generally require attachment. Rust fungi such as *Uromyces appendiculatus* display a type of thigmotropism, i.e., contact-dependent growth in which physical cues from leaf surface topography orient hyphal growth towards stomata (Hoch et al. 1987). Many fungi, including *U. appendiculatus* and *Colletotrichum* spp., display thigmodifferentiation, i.e., contact-dependent differentiation. Here, adhesion is required to induce formation of appressoria (Apoga et al. 2004); indeed the researchers demonstrated that to induce appressorial formation, *C. graminicola* germlings require 4 μm of continuous contact with a hydrophobic substratum. *U. appendiculatus* germlings that were rendered non-adherent with proteases continued to grow but were unable to establish pathogenicity because they were unable to grow thigmotropically or undergo thigmodifferentiation (Epstein et al. 1985, 1987). Finally, since fungi mechanically penetrate the plant surface from appressoria (Howard et al. 1991), we can deduce that firm adhesion is required for appressorial function. Indeed attachment is so integral to appressorial function that appressoria are often defined as adherent.

3.2.3 Selected Examples

Recent sequencing of whole fungal genomes makes comparisons of adherent and non-adherent fungi possible. The ascomycetous yeasts *Saccharomyces cerevisiae* and *Candida albicans* are comparatively non-adherent and adherent, respectively. The ascomycetes *Magnaporthe grisea* and *Botrytis cinerea* have adherent conidia, germlings and appressoria (Hamer et al. 1988; Doss et al. 1993, 1995; Braun and Howard 1994b; Xiao et al. 1994; Spotts and Holz 1996) but *Neurospora crassa* is non-adherent. The basidiomycete *Ustilago madydis* has hyphae that grow attached to the leaf and then produces appressoria (Snetselaar and McCann 2001).

Fungi that are adherent and that seem likely to be sequenced include *Colletotrichum graminicola* and *Erysiphe graminis*. *C. graminicola* is amenable to a range of molecular strategies (Epstein et al. 1998).

3.2.3.1 *Colletotrichum graminicola* (Teleomorph, *Glomerella graminicola*), Causal Agent of Anthracnose on Corn

The maize anthracnose pathogen, *C. graminicola*, produces conidia in acervuli. A complex mixture of high molecular weight glycoproteins surrounds the conidia. The glycoproteins have several roles. They act as an anti

desiccant allowing the conidia to survive for an extended time during periods of drought and severe changes in temperature (Nicholson and Moraes 1980; Bergstrom and Nicholson 1999). In addition, the mucilaginous matrix contains mycosporine-alanine, a self-inhibitor of germination (Leite and Nicholson 1992). The mucilage also contains a cutinase and non-specific esterase that assist in the process of adhesion and recognition of the infection court (Pascholati et al. 1993). Ungerminated conidia of *C. graminicola* adhere to artificial hydrophobic surfaces, yet the same conidia are unable to bind to hydrophilic surfaces (Mercure et al. 1994). Materials released by the conidia, including the adhesive material, were easily observed by scanning electron microscopy (Mercure et al. 1995). The matrix complex was isolated, partially characterized and contains a mixture of high molecular weight glycoproteins (Sugui et al. 1998).

3.2.3.2 *Erysiphe graminis* (Teleomorph, *Blumeria graminis*) Causal Agent of Powdery Mildew of Barley

The powdery mildew pathogen of barley *E. graminis* produces conidia that release a liquid exudate upon contact with the hydrophobic surface of the barley leaf (Kunoh et al. 1988). To ensure that contact is successful, the conidial exudate contains a cutinase that degrades the host cuticle at the contact site (Nicholson et al. 1988; Pascholati et al. 1992). The substrata and the geometry of the interface between the conidium and the substratum affect the release of extracellular material by *E. graminis* conidia (Carver et al. 1999; Wright et al. 2002). On hydrophobic surfaces, a pad of extracellular material is released within 1 min after contact with the substratum. This phenomenon did not occur on a hydrophilic surface, suggesting that the hydrophobicity of the surface is critical to recognition of the host by the pathogen.

3.3 Challenges in Identifying Adhesives in Fungi

As indicated above, great progress has been made in documenting the spatial and temporal expression of cell-substratum attachment and in the development of adhesiveness. However, identification of the components of glues can present both biochemical and genetic challenges (Vreeland and Epstein 1996).

3.3.1 Genetic 'Knockout' and 'Knockin' Strategies

Adhesion-reduced and non-adhesive mutants provide a superb tool to investigate the role of adhesion. For example, adhesion-reduced mutants of the plant pathogenic fungus *Nectria haematococca* were less virulent than the wild-type when deposited on the intact surface of cucurbit fruits, but were equally virulent when deposited into wounded fruits; the results indicated

that adhesion is a virulence factor in the “natural” environment, and suggested that adhesion prevents displacement by water and assists in efficient localization of secreted enzymes on the host surface (Jones and Epstein 1990). Mutants can be used to formulate hypotheses about the adhesive compound; in the biocontrol yeast *Rhodosporidium toruloides*, the mannose-binding lectin Concanavalin A (Con A) eliminated adhesion in the wild-type and bound to the region of bud development in the wild-type where the yeast adhered, but did not bind to the same region in the mutant (Buck and Andrews 1999b).

Site-directed mutagenesis provides the most powerful technique to demonstrate that a specific gene is involved in the adhesive process. With the medically important fungi, there are multiple examples in which a putative adhesin gene has been knocked out, and there is a resultant loss of adhesiveness and virulence (Gale et al. 1998; Brandhorst et al. 1999; Loza et al. 2004; Zhao et al. 2004); adhesion and virulence was restored after the mutant strain had the disrupted gene restored. Amongst the plant pathogenic fungi, researchers have focused more on genes that reduce pathogenicity; in a few cases, these genes also affect fungal attachment. For example, disruption of the *Colletotrichum lagenarium* mitogen-activated protein (MAP) kinase *CMK1* does not affect mycelial growth, but interrupts production of conidia, conidial adhesion, conidial germination, and appressorial formation (Takano et al. 2000). Disruption of *Magnaporthe grisea* *acr1* (Lau and Hamer 1998) affects conidiogenesis so that an *acr1*-mutant has conidiophores that produce conidia in a head-to-tail chain rather than sympodially. That is, a new conidium is produced from the tip of an older conidium rather than from a conidiophore. In the wild type, conidial adhesion is mediated by mucilage released from the spore tip. The *acr1*-conidia fail to produce the spore tip mucilage, are non-adherent, and are inefficient in forming appressoria. While mutants in regulatory genes are extremely informative about pathways (e.g., Liu and Kolattukudy 1999), they are not as useful for adhesion studies as knockouts of genes directly involved in glue production. However, if multiple compounds can serve as building blocks of a glue, single gene knock-outs may only result in, at best, quantitatively discernible differences from the wild-type.

Researchers have used ‘knockin’ strategies to identify individual genes from *C. albicans* that transform the generally non-adhesive *Saccharomyces cerevisiae* into an organism that adheres to polystyrene (Barki et al. 1993; Fu et al. 1998; Li and Palecek 2003). These researchers screened libraries rather than specific genes, which resulted in identification of potentially new adhesins. However, successful utilization of this strategy to identify a glue depends upon there being a single or perhaps tandem genes that are expressed in a sticky organism that are not expressed in the non-adherent organism. Using a strategy similar to that described in Barki et al. (1993), the Epstein laboratory was not successful in identifying *Colletotrichum graminiicola* genes that rendered *S. cerevisiae* transformants adhesive.

3.3.2 Biochemical Strategies

From a biochemical perspective, there are several reasons why identification of even a single fungal adhesive has remained a challenge. Particularly compared to *S. cerevisiae* and *C. albicans*, the cell surfaces of filamentous fungi are complex and poorly characterized. As discussed in greater detail below (Sect. 3.4.2), fungi probably produce glycoprotein-(or proteoglycan-) based glues. Perhaps not surprisingly, the external layer of fungal cell walls is largely composed of heavily glycosylated proteins (de Nobel et al. 2001). Indeed, all proteins in the wall may be glycosylated because glycosylation is part of the pathway of secreted proteins. Cell surface labeling of proteins by iodination and biotinylation indicate that there are many (glyco)proteins on the cell surfaces of fungi (Epstein et al. 1987; Apoga et al. 2001; de Nobel et al. 2001; de Groot et al. 2004; Weig et al. 2004). Adding to the complexity of identifying one or few proteins out of many, the macromolecules on the surface are highly cross-linked. For example, in *S. cerevisiae*, the glycosylphosphatidylinositol-dependent (GPI) cell wall proteins are bound to the plasma membrane and to β 1,6-glucan; the β 1,6-glucan is bound to chitin and to β 1,3-glucan, which also is bound to chitin (de Nobel et al. 2001). Non-GPI cell wall proteins are also cross-linked to the wall. For example, in the animal pathogen *Blastomyces dermatitidis*, the proteinaceous adhesin is secreted through the wall, localizes on the cell surface and then reassociates with cell wall chitin (Brandhorst and Klein 2000).

Several properties of glues also make identification a challenge. Although assays, such as retention of microspheres, can be useful in identifying potential adhesives, there are limitations in that glues are transiently sticky (Vreeland and Epstein 1996). That is, a glue must be sufficiently non-sticky to be secreted to the wall surface, sticky during glue formation, and then typically, non-sticky after “curing” or “hardening.” The ultimate loss of stickiness is accompanied by dramatic changes in solubility; fungal glues are extremely insoluble. Thus, there are always concerns that compounds that are not involved in adhesion are co-purified when the adhesive is sticky, and polymerized with the glue after hardening.

3.4 Fungal and Oomycete Glues

3.4.1 Features

Conidia of most fungal species must be alive in order for adhesion to occur (Slawewski et al. 2002). As indicated previously, although there may be some initial passive attachment of fungal spores to a surface, stronger adhesion seems mediated by secreted material on the cell surface (Epstein and Nicholson 1997; Apoga and Jansson 2000). Microscopic observations, including

transmission electron microscopy with freeze-substituted specimens (Caesar-TonThat and Epstein 1991) are consistent with the interpretation that adhesives are liquid upon release and spread over the surface. The spreading of the adhesive obviously occurs underwater in aquatic fungi (Jones 1994), but also occurs underwater for most terrestrial fungi, which require “free water” to germinate. Thus, the glue must either displace or completely mix with the water molecules that are already on the surface. Microscopic observations are also consistent with the interpretation that the cell surface changes over time as the adhesive material is polymerized into a stronger, crosslinked-compound (Caesar-TonThat and Epstein 1991; Apoga and Jansson 2000). Mature adhesives often appear to have a fibrous component (Watanabe et al. 2000).

As indicated above, many fungi adhere well to inert surfaces including teflon (Hamer et al. 1988). Overall, for the purpose of attachment, the predominant feature of a leaf surface appears to be its hydrophobicity. Many fungi adhere more tenaciously to hydrophobic than to hydrophilic surfaces (Epstein and Nicholson 1997). Terhune and Hoch (1993) found that the extent of adhesion of germlings of *Uromyces appendiculatus* to inert surfaces correlated closely with substratum hydrophobicity. However, other features on the leaf surface may also affect adhesion. The rust fungus *Uromyces viciaefabae*, for example, secretes cutinases and esterases which change the potential binding sites on the surface, and enhance adhesion (Deising et al. 1992). Buck and Andrews (1999b) utilized attachment mutants to conclude that localized positive charges, and not hydrophobic interactions, mediate attachment of the yeast *Rhodospiridium toruloides*. Adhesion to roots is not as well characterized as to leaves (Recorbet and Alabouvette 1997), and roots have potential carbohydrate receptors to which an adhesin could bind.

3.4.2 Composition of Glues

During the last two decades we have learned much about why and when fungi adhere. We still know relatively little about how fungi adhere. Most researchers have deduced the composition of the glues by testing for the loss of adhesiveness with enzymes, lectins, antibodies, and inhibitors of specific metabolic pathways. Such indirect evidence indicates that fungal adhesives are glycoproteins (Chaubal et al. 1991; Jones 1994; Kuo and Hoch 1996; Pain et al. 1996; Bircher and Hohl 1997; Epstein and Nicholson 1997; Sugui et al. 1998; Hughes et al. 1999; Apoga et al. 2001). The carbohydrate moieties on glycoproteins seem particularly important in adhesion. In many fungi, including *Bipolaris sorokiniana*, *Colletotrichum graminicola*, *Magnaporthe grisea*, *Nectria haematococca*, and *Phyllosticta ampellicida*, substratum adhesion is at least partially blocked by the lectin Con A (Hamer et al. 1988; Shaw and Hoch 1999; Apoga et al. 2001). Experimental evidence, including block-

age of adhesion with the snowdrop lectin, indicates that mannose residues are involved in the adhesive mechanism (Kwon and Epstein 1997a).

There have been a few reports that fungal adhesives are carbohydrates (Pringle 1981), but whether the adhesives are actually glycoproteins or proteoglycans is unknown. The carbohydrate residues on the *Magnaporthe grisea* conidial spore tip mucilage may be modified carbohydrates. Buck and Andrews (1999a) reported that a mannose-containing compound, and possibly a mannoprotein, was involved in attaching the yeast *Rhodospiridium toruloidesto* to polystyrene and barley leaves. Determination of whether lipids, for example, are part of the appressorial adhesive (Ebata et al. 1998; Ohtake et al. 1999) requires additional experimental evidence.

3.4.3 Secretion and Crosslinking, with a Focus on Transglutaminases

The precursors of fungal glues must be secreted in a water-soluble form so that the material migrates through the wall to the cell surface. Either in the wall or on the cell surface, the glue must polymerize into a high molecular mass. Theoretically, a variety of oxidases could polymerize the glue; a catechol oxidase polymerizes the glue in mussels and a haloperoxidase may polymerize algal glues (Vreeland et al. 1998). Transglutaminases (EC 2.3.2-13) may be the polymerizing agents of fungal glues. Transglutaminases form a calcium-dependent interpeptidic cross-link between a glutamic acid and a lysine residue. Certainly transglutaminases serve functions in fungal cell walls that are distinct from adhesives; transglutaminases crosslink proteins in ascomycete walls (Ruiz-Herrera et al. 1995; Iranzo et al. 2002) which helps to reduce the pore size of the fungal wall. In addition, transglutaminases in the oomycete *Phytophthora sojae* serve as elicitors of plant host defense (Brunner et al. 2002), which perhaps is an indication that transglutaminases are present on the cell surface. Fabritius and Judelson (2003) identified a family of transglutaminases (M81 M81C, M81D and M81E) in the plant pathogen *P. infestans*. Thus, transglutaminases are present, apparently on the surface, of fungal and oomycete walls. Adhesion of oomycete zoospores and some fungal spores requires Ca^{++} (Shaw and Hoch 2000, 2001), as do transglutaminases. For example, adhesion of encysting zoospores of *P. cinnamomi* was enhanced with increasing Ca^{++} concentration from 2 to 20 mmol/l; chelation of Ca^{++} with EGTA eliminated adhesion (Gubler et al. 1989). Interestingly, with the *Candida albicans* adhesin Hwp1 the host transglutaminase covalently links the pathogen to the host epithelial cell (Staab et al. 1999).

3.4.4 Cell-surface Macromolecules with Apparent Adhesive Properties

Potential fungal glues are summarized in Table 3.2 and several are discussed below.

Table 3.2. Fungal macromolecules that potentially function as fungal-substratum glues

Organism	Glue component	Comments	Reference
<i>Candida albicans</i>	Eap1	GPI-CWP ^a that is involved in adhesion to polystyrene and to epithelial cells	Li and Palecek (2003)
<i>Colletotrichum lindemuthianum</i>	110-kDa glycoprotein on the conidial wall	Adhesion disrupted by monoclonal antibody UB20, which binds to several glycoproteins including the 110 kDa	Hughes et al. (1999)
<i>Nectria haematococca</i> mating population I	90-kDa mannoprotein	Spatially and temporally associated with adhesion of macroconidia; specifically associated with conditions in which adhesion is induced	Kwon and Epstein (1997a,b)
<i>Rhodosporidium toruloides</i>	Mannose-containing compound	Involved in adhesion to polystyrene and barley leaves	Buck and Andrews (1999a,b)
<i>Phytophthora cinnamomi</i>	220-kDa protein with thrombospondin type 1 repeats	Thrombospondin type 1 repeats are found in adherent cells in animals and in apicomplexan parasites	Robold and Hardham (2005)
<i>Saccharomyces cerevisiae</i>	Flo11p	GPI-CWP that is involved in adhesion to substrata (including polystyrene), cell-cell adhesion, and filamentous growth	Reynolds and Fink (2001); Braus et al. (2003)

^a GPI-CWP, glycosylphosphatidylinositol-dependent cell wall proteins

3.4.4.1 *PcVsv1*, a Protein on Encysting Zoospores of *Phytophthora cinnamomi*

The oomycete *Phytophthora cinnamomi* causes serious diseases on a wide range of host plants. As with many oomycetes, *P. cinnamomi* are disseminated by motile zoospores. The zoospores contain an adhesive in secretory vesicles (Hardham and Gubler 1990). About 2 min after contact with a solid surface, the material in the vesicles is secreted onto the ventral surface of the zoospores and the spores become sticky. The adhesive process is part of the process of transformation of zoospores into adherent cysts. Within 20–30 min the encysted spores penetrate the underlying plant tissue. Adhesion is essential because a zoospore that is developing into a cyst must not be dislodged from a potential infection site, and because a mature cyst must be sufficiently glued to the host surface so that the fungus can penetrate the host tissue.

Hardham and Gubler (1990) identified a monoclonal antibody that specifically bound material in the secretory vesicles and on the developing adhesive

surface, i.e., the antigen was spatially and temporally consistent with being a component of the glue. Robold and Hardham (2005) screened a *P. cinnamomi* cDNA library with the antibody, and then selected and cloned a gene that encodes for PcVsv1 (*P. cinnamomi* ventral surface vesicle) protein with an approximate mass of 220 kDa. Not surprisingly for an adhesive material, PcVsv1 contains a repetitive motif—47 copies of a ca. 50 amino acid length segment that has homology to the thrombospondin type1 repeat. Thrombospondin motifs are found in proteins involved in attachment in animals and in apicomplexan parasites, including *Plasmodium*, *Cryptosporidium* and *Eimeria* (Tomley and Soldati 2001; Deng et al. 2002; Tucker 2004). In animals, the thrombospondin motifs occur in proteins in the extracellular matrix, i.e., potentially in the location in which adhesion occurs.

More experimental evidence is required before PcVsv1 can be declared the glue, or a component of the glue. Experimental strategies could include determination of the following: loss of adhesiveness with silencing of *Pcvsv1*; demonstration that PcVsv1 behaves as a glue in vitro; and interference with the development of adhesiveness with compounds that specifically bind PcVsv1, e.g., the antibody that binds to PcVsv1. To date, evidence that is consistent with PcVsv1 being involved in the adhesive process are 1) that the antibodies against PcVsv1 bind to multiple adherent oomycetes, i.e., *Phytophthora* spp., *Pythium* spp., *Plasmopara* spp., and *Albugo* sp. and 2) that Southern blotting and/or BLAST searches demonstrate the presence of PcVsv1 homologues in *Phytophthora* spp. (Robold and Hardham 2004, 2005). Even if thrombospondin is a glue component in oomycetes, it is probably not involved in adhesion of true fungi; based on a survey of sequences, Robold and Hardham (2005) found no evidence of thrombospondin motifs in either true fungi, or in plants or green algae.

Other proteins of *Phytophthora* spp. may be involved in some aspects of adhesion, although probably not as components of the glue per se. Göernhardt et al. (2000) identified the car90 (for cyst germination specific acidic repeat) protein on the surface of *Phytophthora infestans* germlings; the protein is transiently expressed during cyst germination and appressorium formation, i.e., during an adherent portion of the *P. infestans* lifecycle. The car90 protein contains 120 repeats of the consensus sequence TTYAPTEE. Because the repetitive sequence has homology with human mucins, the authors suggested that the car90 protein “may serve as a mucous cover protecting the germling from desiccation, physical damage, and adverse effects of the plant defense response.” Because the protein is potentially viscous and sticky, the authors further speculated that car90 “may assist in adhesion to the leaf surface.” Gaulin et al. (2002) identified the CBEL (for cellulose-binding elicitor lectin) glycoprotein on the surface of hyphae of *Phytophthora parasitica* var. *nicotianae*. However, while transgenic strains lacking CBEL did not adhere to and develop on cellulose in vitro, they appeared to have normal adhesion and development on the plant surface and in planta.

3.4.4.2 90-kDa Mannoprotein on Macroconidia of *Nectria haematococca*

We selected *N. haematococca* as a model biochemical system because, unlike *C. graminicola* and *U. appendiculatus*, the *N. haematococca* glue components seem somewhat detergent-soluble. When exposed to some but not all carbon sources, macroconidia of *Nectria haematococca* mating population I (anamorph, *Fusarium solani* f. sp. *curcurbitae* race I) become adhesive within 5 min and adhere at the spore apices to substrata and to other spores (Jones and Epstein 1989). At the same time, the macroconidia produce mucilage at the spore tips which binds Con A and the snowdrop lectin (SDL), and produce a 90-kDa glycoprotein which binds Con A and SDL; Con A and SDL inhibit macroconidial adhesion (Kwon and Epstein 1993, 1997a). When exposed to media that induce germination but not adhesiveness, neither the spore tip mucilage nor the 90-kDa glycoprotein are produced (Kwon and Epstein 1993). Adhesion of both ungerminated macroconidia and germlings was inhibited by a polyclonal IgG prepared against the 90-kDa glycoprotein (Kwon and Epstein 1997a). Anti-adhesive activity of the IgG was reduced by incubation of the antibodies with mannan; the mannan alone has no effect on adhesion. The anti-90-kDa IgG did not bind to the deglycosylated glycoprotein. Thus, we conclude that the 90-kDa glycoprotein and specifically its mannose residues are specifically involved in adhesion of *N. haematococca* macroconidia.

Visualization of the macroconidial tip mucilage and the detection of the 90-kDa glycoprotein is transient over time, consistent with compounds which become increasingly polymerized. Although *N. haematococca* macroconidia in zucchini extract are adherent for a 3-h incubation period, the macroconidial tip mucilage and the 90-kDa glycoprotein are primarily detectable only for the first hour. Macroconidia incubated in an adhesion-inducing medium with transglutaminase inhibitors (either 1 mM iodoacetamide or 10 mmol/l cystamine) adhere significantly less, have less of the macroconidial tip mucilage that is spatially associated with adhesion, and have less recoverable 90-kDa glycoprotein that is temporally associated with adhesion; the inhibitors do not affect the presence of other extractable compounds visualized on the protein blot. In addition, the fact that two reducing agents do not affect adhesion suggests that the iodoacetamide and cystamine are interfering with the adhesion process, and are not inhibiting adhesion by chemical reduction.

Thus the data are consistent with the following model. Within minutes of incubation in an adhesion-inducing medium, at the macroconidial apices, *N. haematococca* spores secrete a sticky lower-molecular-weight and more-water-soluble precursor of a 90-kDa glycoprotein (Kwon and Epstein 1993). At the spore apex, the glycoprotein is partially polymerized by a transglutaminase into a somewhat sticky 90-kDa form (Kwon and Epstein 1997a). After 1–2 h, the 90-kDa glycoprotein is extracellularly modified so that it is no longer sticky. After 2 h, adhesion is no longer localized at the spore apex; the macroconidia adhere along the entire lower spore surface, and later along the

germ tube substratum interface. Mutant analysis suggests that compounds other than the 90-kDa glycoprotein are involved in this later stage of adhesion (Epstein et al. 1994). However, inhibition of both spore and germling adhesion by anti-90-kDa IgGs suggest that related compounds may be involved in spore and germling adhesion (Kwon and Epstein 1997a).

The 90-kDa compound is hydrophobic, contains mannose, has N-linked carbohydrates, has sites for O-linked carbohydrates (18% serine and threonine), and has an amino acid composition with ca. 38% hydrophobic and 62% hydrophilic residues (Kwon and Epstein 1997b). Genetic data and an in vitro system are required to demonstrate that the 90-kDa glycoprotein is a fungal glue per se.

3.4.4.3 *The Mannoprotein SC3, a Schizophyllum commune Hydrophobin*

Aerial plant surfaces are hydrophobic and many fungi and oomycetes adhere more tenaciously to hydrophobic surfaces such as polystyrene than to hydrophilic surfaces such as glass (Sela-Buurlage et al. 1991; Terhune and Hoch 1993; Kuo and Hoch 1996; Wright et al. 2002). Fungal hydrophobins are small (approximately 100 amino acids), secreted, moderately hydrophobic proteins with eight conserved cysteine residues and a common hydrophobic profile, but highly variable amino acid sequences (Kershaw and Talbot 1998). Despite the variability in sequence, complementation experiments in *Magnaporthe grisea* with heterologous hydrophobin genes indicate that hydrophobins from different species encode for functionally related proteins (Kershaw et al. 1998).

Hydrophobins are interesting candidates as components of adhesives because they are structural proteins on the fungal surface that self-assemble into insoluble aggregates (Wösten et al. 1994). Moreover, the self-assembly occurs at the interface between a hydrophobic and a hydrophilic surface, which includes the environment in which most foliar plant pathogens have to adhere, i.e., a leaf surface covered with water. At least some hydrophobins, including Sc3 are glycosylated with mannose residues (de Vocht et al. 1998), as are many fungal glues. However, while the conidial rodlet hydrophobins, e.g., *Magnaporthe grisea* MPG1 (Kershaw et al. 1998) may allow for an initial hydrophobic interaction between a wind-blown spore and a leaf, it would be a comparatively weak attachment. Indeed, a hydrophobin mutant of *M. grisea* *Mpg1* adhered as well as the wild-type, indicating that the MPG1 hydrophobin is not involved in adhesion (Talbot et al. 1996).

Although currently there is no compelling evidence that a hydrophobin is a component of a strong glue, two *Schizophyllum commune* hydrophobins are involved in attachment. SC4 coats the surface of hyphae within the fruiting body (Schuren and Wessels 1990). As such, it serves in cell-cell adhesion. SC3 is involved in aerial hyphal formation. In comparison to the wild type, *sc3* mutants are reduced in adhesiveness to hydrophobic substrates (Wösten et al. 1994).

Hydrophobins have the interesting property of forming amphipathic membranes (Scholtmeijer et al. 2002). That is, hydrophobins form a membrane with a hydrophobic and a hydrophilic side; the hydrophobic side binds to hydrophobic surfaces and the hydrophilic side binds to hydrophilic surfaces. In so doing, a hydrophobin changes the polarity of its substratum. In the context of fungal-plant interactions, *Colletotrichum graminicola*, *Erysiphe graminis* and *Uromyces viciae-fabae* spores, for example, secrete material that increases the hydrophilicity of the surface (Nicholson et al. 1988; Deising et al. 1992; Pascholati et al. 1993). Here, we speculate that some of these changes could be mediated by hydrophobins.

3.4.4.4 Selected Glycosylphosphatidylinositol-dependent (GPI) Cell Wall Proteins

GPI cell wall proteins (GPI-CWP) are the most abundant proteins in fungal walls (de Groot et al. 2005). They have a GPI anchor to the plasma membrane at the C terminus and a signal peptide at the N terminus (de Nobel et al. 2001). GPI-CWP often have a repeated motif and a binding site that links the protein to the β 1,6-glucan part of the wall. Here we will discuss GPI-CWP that are clearly adhesins, but that may have more non-specific glue-like properties.

The human pathogen *Candida albicans* adheres to polystyrene and to medical devices, in addition to human epithelial cells; *C. albicans* is more adherent than *Saccharomyces cerevisiae*. Li and Palecek (2003) expressed a library of *C. albicans* in a strain of *S. cerevisiae flo8Δ* and screened for adhesive cells on polystyrene. They isolated a gene *EAP1* (enhanced adherence to polystyrene) which encodes for a GPI-CWP. Using expression in both *S. cerevisiae* and in a *C. albicans* mutant, they demonstrated that Eap1 affects adhesion on both polystyrene and on epithelial cells. In the diploid *C. albicans* SC5314, in one allele, the predicted protein product has 70 PATEST repeats in one allele (comprising 37% of the protein) and 13 in the other (NCBI accession numbers EAK95619 and EAK95520). Following the PATEST repeat, there are nine or ten repeats of TPAAPGTPVESQP. Eap1 has homology to *C. albicans* Hwp1p and to *S. cerevisiae* Flo11p, which are discussed below. The *C. albicans eap1* complements *flo 11* mutations in *S. cerevisiae*.

In low-glucose, *Saccharomyces cerevisiae* adheres to plastic and yeast cells agglutinate (Reynolds and Fink 2001). Genetic manipulation indicates that adhesion and agglutination requires the cell surface glycoprotein Flo11p. Flo11p increases cell surface hydrophobicity and is expressed when *S. cerevisiae* grows as filaments, pseudohyphae, and invasively through agar but is not expressed in yeast cells (Braus et al. 2003). Based on the deduced amino acid sequence, Flo11p (also named MUC1 and YIR019C) contains 1367 amino acids of which 50% are either serine or threonine and 10% are proline. Starting at amino acid 333, there are 38 repeats of SSTTESS. This residue,

which comprises 19% of Flo11p is contained within 22 copies of S/T SSTTESS S/V (A)P V/A PTP and 16 copies of S/T SSTTESSAPV.

3.5 Fungal Adhesins

Attachment of fungi to host cells is a critical part of fungal pathogenesis in animals (Sundstrom 2002). Examples of fungal adhesins that bind to animal cells are shown in Table 3.3. Similar to the fungal glues, most adhesins are glycoproteins, and at least several are mannoproteins (Fukazawa and Kagaya 1997), with mannan involvement in adhesion (Kanbe and Cutler 1998). Hazen and colleagues (Masuoka et al. 1999) have argued that cell surface hydrophobicity is an important factor in adhesion of *Candida albicans* to host epithelial cells and to plastic, that adhesion is a virulence factor, and that hydrophobic glycoproteins on the surface of the cells contribute, if not determine, cell surface hydrophobicity.

Table 3.3. Examples of animal pathogenic fungi that produce adhesins, and their receptors

Fungus	Fungal adhesin	Apparent host binding site	Reference
<i>Blastomyces dermatitidis</i>	BAD1 (=WI-1) protein	Binds yeast to CD11b/CD18 and CD14 receptors on human macrophages	Newman et al. (1995); Brandhorst et al. (1999); Brandhorst and Klein (2000)
<i>Candida albicans</i>	Members of the ALS (agglutinin-like sequence) gene family (GPI-CWP ^a): Als3, Als1, Als4, Als5 (=Ala1)	Different Als (in their N-terminal regions) may have different specificities, e.g., for laminin, collagen, fibronectin, endothelial and/or epithelial cells.	Fu et al. (1998); Hoyer (2001); de Groot et al. (2004); Loza et al. (2004); Zhao et al. (2004)
<i>Candida albicans</i>	Eap1, a GPI-CWP	Epithelial cells	Li and Palecek (2003)
<i>Candida albicans</i>	Int1, a RGD-protein	Epithelial cells	Gale et al. (1998)
<i>Candida albicans</i>	Mannan core and oligomannosyl side chains of the acid-stable portion of phosphomannoprotein	Marginal zone macrophages	Kanbe and Cutler (1998)
<i>Candida glabrata</i>	Epa1, a GPI-CWP	Epithelial cells	Cormack et al. (1999)

^aGPI-CWP, glycosylphosphatidylinositol-dependent cell wall proteins

Several of the fungal adhesins, including Hwp1, Ala1p/Als5p, Als1p from *Candida albicans* and Epa1p from *Candida glabrata* are GPI-cell wall proteins. The arginine-glycine-aspartic (RGD) tripeptide sequence appears to be a critical site in both some fungal adhesins and plant and animal host receptors (Hostetter 2000; Senchou et al. 2004). Corrêa et al. (1996) concluded that thigmosensing of appressorium-inductive surfaces by the rust fungus *Uromyces appendiculatus* involved an RGD sequence, probably on a transmembrane, integrin-like glycoprotein.

3.6 Conclusions

Although many fungi produce glues, we have yet to characterize one definitively. Fungal glues are typically formed in an aqueous environment, and as such they may provide models for commercial glues, including for biomedical applications. The commercially available Tisseel® (Baxter Corporation) is used during surgery as a sealant. Just before use, the Tisseel® ingredients, fibrinogen and thrombin, are mixed; in mammals, thrombin activates fibrinogen which forms fibrin clots. The example is particularly relevant because many animal pathogenic fungi adhere to host fibrinogen and a putative adhesive from the oomycete *Phytophthora cinnamomi* is a protein with thrombospondin type 1 repeats (Robold and Hardham 2005).

Adhesion is a common feature of pathogenic fungi, regardless of whether they are pathogens in plants or animals. In addition, adhesion can be disrupted experimentally using enzymes that degrade the glue or compounds that block adhesion without directly killing the cell (Epstein et al. 1987). Consequently, anti-adhesives provide an appealing disease control strategy because they do not require uptake into the fungus and they do require disruption of a eukaryotic pathway. At least some of the antifungal activity of surfactants and detergents may be because they render plant surfaces less hydrophobic. Stanley et al. (2002) demonstrated that the phenolic zosteric acid (*p*-(sulfo-oxy) cinnamic acid) inhibits adhesion and infection by conidia of *Magnaporthe grisea* and *Colletotrichum lindemuthianum*; concentrations that are effective as an anti-adhesive are not toxic to either the plant hosts or fungi.

Genetic technologies should facilitate identification of fungal glues (Robold and Hardham 2005). The adherent *Magnaporthe grisea* (Hamer et al. 1988; Howard et al. 1991) has been sequenced and is amenable to targeted gene disruption. Within the next five years, the sequences of several other filamentous fungi that are and are not adherent should be available. Similarly, cDNA microarrays from an adherent fungus at adherent and non-adherent stages of development should allow the selection of putative adhesives for targeted gene disruption. In addition, “in silico” proteomics (Weig et al. 2004; de Groot et al. 2005) will facilitate identification of putative adhesives.

Because fungal glues are generally glycoproteins or conceivably proteoglycans, we predict that selection of secreted proteins with repetitive motifs should allow an initial selection for further demonstration of function by either targeted gene disruption or gene silencing.

References

- Apoga D, Jansson H-B (2000) Visualization and characterization of the extracellular matrix of *Bipolaris sorokiniana*. *Mycol Res* 104:564–575
- Apoga D, Jansson H-B, Tunlid A (2001) Adhesion of conidia and germlings of the plant pathogenic fungus *Bipolaris sorokiniana* to solid surfaces. *Mycol Res* 105:1251–1260
- Apoga D, Barnard J, Craighead HG, Hoch HC (2004) Quantification of substratum contact required for initiation of *Colletotrichum graminicola* appressoria. *Fung Genet Biol* 41:1–12
- Barki M, Koltin Y, Yanko M, Tamarkin A, Rosenberg M (1993) Isolation of a *Candida albicans* DNA sequence conferring adhesion and aggregation on *Saccharomyces cerevisiae*. *J Bacteriol* 175:5683–5689
- Bechinger C, Giebel K-F, Schnell M, Leiderer P, Deising HB, Bastmeyer M (1999) Optical measurements of invasive forces exerted by appressoria of a plant pathogenic fungus. *Science* 285:1896–1899
- Bergstrom GC, Nicholson RL (1999) The biology of corn anthracnose: knowledge to exploit for improved management. *Plant Dis* 83:596–608
- Bircher U, Hohl HR (1997) Surface glycoproteins associated with appressorium formation and adhesion in *Phytophthora palmivora*. *Mycol Res* 101:769–775
- Brandhorst T, Klein B (2000) Cell wall biogenesis of *Blastomyces dermatitidis*: evidence for a novel mechanism of cell surface localization of a virulence-associated adhesin via extracellular release and reassociation with cell wall chitin. *J Biol Chem* 275:7925–7934
- Brandhorst T, Wüthrich M, Warner T, Klein B (1999) Targeted gene disruption reveals an adhesin indispensable for pathogenicity of *Blastomyces dermatitidis*. *J Exp Med* 189:1207–1216
- Braun EJ, Howard RJ (1994a) Adhesion of *Cochliobolus heterostrophus* conidia and germlings to leaves and artificial surfaces. *Exp Mycol* 18:211–220
- Braun EJ, Howard RJ (1994b) Adhesion of fungal spores and germlings to host surfaces. *Protoplasma* 181:202–212
- Braus GH, Grundmann O, Brueckner S, Moesch H-U (2003) Amino acid starvation and Gcn4p regulate adhesive growth and *FLO11* gene expression in *Saccharomyces cerevisiae*. *Mol Biol Cell* 14:4272–4284
- Brunner F, Rosahl S, Lee J, Rudd JJ, Geiler C, Kauppinen S, Rasmussen G, Scheel D, Nuernberger T (2002) Pep-13, a plant defense-inducing pathogen-associated pattern from *Phytophthora* transglutaminases. *EMBO J* 21:6681–6688
- Buck JW, Andrews JH (1999a) Attachment of the yeast *Rhodosporidium toruloides* is mediated by adhesives localized at sites of bud cell development. *Appl Environ Microbiol* 65:465–471
- Buck JW, Andrews JH (1999b) Localized, positive charge mediates adhesion of *Rhodosporidium toruloides* to barley leaves and polystyrene. *Appl Environ Microbiol* 65:2179–2183
- Caesar-TonThat TC, Epstein L (1991) Adhesion-reduced mutants and the wild type *Nectria haematococca*: an ultrastructural comparison of the macroconidial walls. *Exp Mycol* 15:193–205
- Carver TLW, Kunoh H, Thomas BJ, Nicholson RL (1999) Release and visualization of the extracellular matrix of conidia of *Blumeria graminis*. *Mycol Res* 103:547–560
- Chaky J, Anderson K, Moss M, Vaillancourt L (2001) Surface hydrophobicity and surface rigidity induce spore germination in *Colletotrichum graminicola*. *Phytopathology* 91:558–564

- Chaubal R, Wilmot VA, Wynn WK (1991) Visualization, adhesiveness, and cytochemistry of the extracellular matrix produced by urediniospore germ tubes of *Puccinia sorghi*. *Can J Bot* 69:2044–2054
- Cormack BP, Ghori N, Falkow S (1999) An adhesin of the yeast pathogen *Candida glabrata* mediating adherence to human epithelial cells. *Science* 285:578–582
- Corrêa A Jr, Staples RC, Hoch HC (1996) Inhibition of thigmostimulated cell differentiation with RGD-peptides in *Uromyces* germlings. *Protoplasma* 194:91–102
- De Groot PWJ, de Boer AD, Cunningham J, Dekker HL, de Jong L, Hellingwerf KJ, de Koster C, Klis FM (2004) Proteomic analysis of *Candida albicans* cell walls reveals covalently bound carbohydrate-active enzymes and adhesins. *Eukaryotic Cell* 3:955–965
- De Groot PW, Ram AF, Klis FM (2005) Features and functions of covalently linked proteins in fungal cell walls. *Fungal Genet Biol* 42:657–675
- Deising H, Nicholson RL, Haug M, Howard RJ, Mengden K (1992) Adhesion pad formation and the involvement of cutinase and esterases in the attachment of uredospores to the host cuticle. *Plant Cell* 4:1101–1111
- De Nobel H, Sietsma JH, van den Ende H, Klis FM (2001) Molecular organization and construction of the fungal cell wall. In: Howard RJ, Gow NAR (eds) *The Mycota*, vol VIII. *Biology of the fungal cell*. Springer, Berlin Heidelberg New York, pp 181–200
- Deng MQ, Templeton TJ, London NR, Bauer C, Schroeder AA, Abrahamsen MS (2002) *Cryptosporidium parvum* genes containing thrombospondin type 1 domains. *Infect Immun* 70:6987–6995
- De Vocht ML, Scholtmeijer K, van der Vegte EW, deVries OMH, Sonveaux N, Wösten HAB, Ruyschaert J-M, Hadziioannou G, Wessels JGH, Robillard GT (1998) Structural characterization of the hydrophobin SC3, as a monomer and after self-assembly at hydrophobic/hydrophilic interfaces. *Biophys J* 74:2059–2068
- Doss RP, Potter SW, Chastagner GA, Christian JK (1993) Adhesion of non-germinated *Botrytis cinerea* conidia to several substrata. *Appl Environ Biol* 59:1786–1791
- Doss RP, Potter SW, Soeldner AH, Christian JK, Fukunaga LE (1995) Adhesion of germlings of *Botrytis cinerea*. *Appl Environ Microbiol* 61:260–265
- Ebata Y, Yamamoto H, Uchiyama T (1998) Chemical composition of the glue from appressoria of *Magnaporthe grisea*. *Biosci Biotech Biochem* 62:672–674
- Epstein L, Nicholson RL (1997) Adhesion of spores and hyphae to plant surfaces In: Carroll G, Tudzynski P (eds) *The Mycota*, vol V. *Plant relationships*, part A. Springer, Berlin Heidelberg New York, pp 11–25
- Epstein L, Laccetti L, Staples RC, Hoch HC, Hoose WA (1985) Extracellular proteins associated with induction of differentiation in bean rust uredospore germlings. *Phytopathology* 75:1073–1076
- Epstein L, Laccetti LB, Staples RC, Hoch HC (1987) Cell-substratum adhesive protein involved in surface contact responses of the bean rust fungus. *Physiol Mol Plant Pathol* 30:373–388
- Epstein L, Kwon YH, Almond DE, Schached LM, Jones MJ (1994) Genetic and biochemical characterization of *Nectria haematococca* strains with adhesive and adhesion-reduced macroconidia. *Appl Environ Microbiol* 60:524–530
- Epstein L, Lusnak K, Kaur S (1998) Transformation-mediated developmental mutants of *Glomerella graminicola*. *Fungal Genet Biol* 23:189–203
- Fabritius A-L, Judelson HS (2003) A mating-induced protein of *Phytophthora infestans* is a member of a family of elicitors with divergent structures and stage-specific patterns of expression. *Mol Plant Microbe Interact* 16:926–935
- Fu Y, Rieg G, Fonzi WA, Belanger PH, Edwards JE Jr, Filler SG (1998) Expression of the *Candida albicans* gene *ALS1* in *Saccharomyces cerevisiae* induces adherence to endothelial and epithelial cells. *Infect Immun* 66:1783–1786
- Fukazawa Y, Kagaya K (1997) Molecular bases of adhesion of *Candida albicans*. *J Med Vet Mycol* 35:87–99
- Gale CA, Bendel CM, McClellan M, Hauser M, Becker JM, Berman J, Hostetter MK (1998) Linkage of adhesion, filamentous growth, and virulence in *Candida albicans* to a single gene, *INT1*. *Science* 279:1355–1358

- Gaulin E, Jauneau A, Villalba F, Rickauer M, Esquerre-Tugaye MT, Bottin A (2002) The CBEL glycoprotein of *Phytophthora parasitica* var. *nicotianae* is involved in cell wall deposition and adhesion to cellulosic substrates. *J Cell Sci* 115:4565–4575
- Göernhardt B, Rouhara I, Schmelzer E (2000) Cyst germination proteins of the potato pathogen *Phytophthora infestans* share homology with human mucins. *Mol Plant Microbe Interact* 13:32–42
- Gubler F, Hardham AR, Duniec J (1989) Characterising adhesiveness of *Phytophthora cinnamomi* zoospores during encystment. *Protoplasma* 149:24–30
- Gunderson JH, Elwood H, Ingold A, Kindle K, Sogin ML (1987) Phylogenetic relationships between chlorophytes, chrysophytes, and oomycetes. *Proc Natl Acad Sci USA* 84:5823–5827
- Hamer JE, Howard RJ, Chumley FG, Valent B (1988) A mechanism for surface attachment in spores of a plant pathogenic fungus. *Science* 239:288–290
- Hardham AR (2001) Cell biology of fungal infection of plants. In: Howard RJ, Gow NAR (eds) *The Mycota: biology of the fungal cell*, vol 7. Springer, Berlin Heidelberg New York, pp 91–123
- Hardham A, Gubler F (1990) Polarity of attachment of zoospores of a root pathogen and pre-alignment of the emerging germ tube. *Cell Biol Int Rep* 14:947–956
- Hoch HC, Staples RC, Whitehead B, Comeau J, Wolf ED (1987) Signaling for growth orientation and cell differentiation by surface topography in Uromyces. *Science* 235:1659–1662
- Hostetter MK (2000) RGD-mediated adhesion in fungal pathogens of humans, plants and insects. *Curr Opin Microbiol* 3:344–348
- Howard RJ, Ferrari MA, Roach DH, Money NP (1991) Penetration of hard substrates by a fungus employing enormous turgor pressures. *Proc Natl Acad Sci USA* 88:11281–11284
- Hoyer LL (2001) The ALS gene family of *Candida albicans*. *Trends Microbiol* 9:176–180
- Hughes HB, Carzaniga R, Rawlings SL, Green JR, O'Connell RJ (1999) Spore surface glycoproteins of *Colletotrichum lindemuthianum* are recognized by a monoclonal antibody which inhibits adhesion to polystyrene. *Microbiology* 145:1927–1936
- Iranzo M, Aguado C, Pallotti C, Canizares JV, Mormeneo S (2002) Transglutaminase activity is involved in *Saccharomyces cerevisiae* wall construction. *Microbiology* 148:1329–1334
- Jones EBG (1994) Fungal adhesion. *Mycol Res* 98:961–981
- Jones MJ, Epstein L (1989) Adhesion of *Nectria haematococca* macroconidia. *Physiol Mol Plant Pathol* 35:453–461
- Jones MJ, Epstein L (1990) Adhesion of macroconidia to the plant surface and virulence of *Nectria haematococca*. *Appl Environ Microbiol* 56:3772–3778
- Kanbe T, Cutler JE (1998) Minimum chemical requirements for adhesin activity of the acid-stable part of *Candida albicans* cell wall phosphomannoprotein complex. *Infect Immun* 66:5812–5818
- Kershaw MJ, Talbot NJ (1998) Hydrophobins and repellents: proteins with fundamental roles in fungal morphogenesis. *Fung Genet Biol* 23:18–33
- Kershaw MJ, Wakley GE, Talbot NJ (1998) Complementation of the *Mpg1* mutant phenotype in *Magnaporthe grisea* reveals functional relationships between fungal hydrophobins. *EMBO J* 17:3838–3849
- Kunoh H, Yamaoka N, Yoshioka H, Nicholson RL (1988) Preparation of the infection court by *Erysiphe graminis*: I. Contact mediated changes in morphology of the conidium surface. *Exp Mycol* 12:325–335
- Kuo K-C, Hoch HC (1996) Germination of *Phyllosticta ampellicida* pycnidiospores: prerequisite of adhesion to the substratum and the relationship of substratum wettability. *Fung Genet Biol* 20:18–29
- Kwon YH, Epstein L (1993) A 90-kDa glycoprotein associated with adhesion of *Nectria haematococca* macroconidia to substrata. *Mol Plant Microbe Interact* 6:481–487
- Kwon YH, Epstein L (1997a) Involvement of the 90 kDa glycoprotein in adhesion of *Nectria haematococca* macroconidia. *Physiol Mol Plant Pathol* 51:287–303
- Kwon YH, Epstein L (1997b) Isolation and composition of the 90 kDa glycoprotein associated with adhesion of *Nectria haematococca* macroconidia. *Physiol Mol Plant Pathol* 51:63–74

- Lau GW, Hamer JE (1998) *Acropetal*: a genetic locus required for conidiophore architecture and pathogenicity in the rice blast fungus. *Fungal Genet Biol* 24:228–239
- Leite B, Nicholson RL (1992) Mycosporine-alanine: a self-inhibitor of germination from the conidial mucilage of *Colletotrichum graminicola*. *Exp Mycol* 16:76–86
- Li F, Palecek S (2003) *EAP1*, a *Candida albicans* gene involved in binding human epithelial cells. *Eukaryotic Cell* 2:1266–1273
- Liu ZM, Kolattukudy PE (1999) Early expression of the calmodulin gene, which precedes appressorium formation in *Magnaporthe grisea*, is inhibited by self-inhibitors and requires surface attachment. *J Bacteriol* 181:3571–3577
- Loza L, Fu Y, Ibrahim AS, Sheppard DC (2004) Functional analysis of the *Candida albicans* *ALSI* gene product. *Yeast* 21:473–482
- Masuoka J, Wu G, Glee PM, Hazen KC (1999) Inhibition of *Candida albicans* attachment to extracellular matrix by antibodies which recognize hydrophobic cell wall proteins. *FEMS Immunol Med Microbiol* 24:421–429
- Mendgen K, Hahn M, Deising H (1996) Morphogenesis and mechanisms of penetration by plant pathogenic fungi. *Annu Rev Phytopathol* 34:367–386
- Mercure EW, Leite B, Nicholson RL (1994) Adhesion of ungerminated conidia of *Colletotrichum graminicola* to artificial hydrophobic surfaces. *Physiol Mol Plant Pathol* 45:421–440
- Mercure EW, Kunoh H, Nicholson RL (1995) Visualization of materials released from adhered, ungerminated conidia of *Colletotrichum graminicola*. *Physiol Mol Plant Pathol* 46:121–135
- Newman SL, Chaturvedi S, Klein BS (1995) The WI-1 antigen on *Blastomyces dermatitidis* yeasts mediates binding to human macrophage CD18 and CD14 receptors. *J Immunol* 154:753–761
- Nicholson RL, Epstein L (1991) Adhesion of fungi to the plant surface: prerequisite for pathogenesis. In: Cole GT, Hoch HC (eds) *The Fungal spore and disease initiation in plants and animals*. Plenum Press, New York, pp 3–23
- Nicholson RL, Moraes WBC (1980) Survival of *Colletotrichum graminicola*: importance of the spore matrix. *Phytopathology* 70:255–261
- Nicholson RL, Yoshioka H, Yamaoka N, Kunoh H (1988) Preparation of the infection court by *Erysiphe graminis*. II. Release of esterase enzyme from conidia in response to a contact stimulus. *Exp Mycol* 12:336–349
- Ohtake M, Yamamoto H, Uchiyama T (1999) Influences of metabolic inhibitors and hydrolytic enzymes on the adhesion of appressoria of *Pyricularia oryzae* to wax-coated cover-glasses. *Biosci Biotech Biochem* 63:978–982
- Osherov N, May GS (2001) The molecular mechanisms of conidial germination. *FEMS Microbiol Lett* 199:153–160
- Pain NA, Green JR, Jones GL, O'Connell RJ (1996) Composition and organisation of extracellular matrices around germ tubes and appressoria of *Colletotrichum lindemuthianum*. *Protoplasma* 190:119–130
- Pascholati S, Yoshioka H, Kunoh H, Nicholson RL (1992) Preparation of the infection court by *Erysiphe graminis* f. sp. *hordei*: cutinase is a component of the conidial exudate. *Physiol Mol Plant Pathol* 41:53–59
- Pascholati SF, Deising H, Leite B, Anderson D, Nicholson RL (1993) Cutinase and non-specific esterase activities in the conidial mucilage of *Colletotrichum graminicola*. *Physiol Mol Plant Pathol* 42:37–51
- Pringle RB (1981) Nonspecific adhesion of *Bipolaris sorokiniana* sporelings. *Can J Plant Pathol* 3:9–11
- Recorbet G, Alabouvette C (1997) Adhesion of *Fusarium oxysporum* conidia to tomato roots. *Lett Appl Microbiol* 25:375–379
- Reynolds TB, Fink GR (2001) Bakers' yeast, a model for fungal biofilm formation. *Science* 291:878–881
- Robold AV, Hardham AR (2004) Production of monoclonal antibodies against peripheral vesicle proteins in zoospores of *Phytophthora nicotianae*. *Protoplasma* 223:121–132
- Robold AV, Hardham AR (2005) During attachment *Phytophthora* spores secrete proteins containing thrombospondin type 1 repeats. *Curr Genet* 47:307–315

- Ruiz-Herrera J, Iranzo M, Elorza MV, Sentandreu R, Mormeno S (1995) Involvement of transglutaminase in the formation of covalent cross-links in the cell wall of *Candida albicans*. *Arch Microbiol* 164:186–193
- Scholtmeijer K, Janssen MI, Gerssen B, de Vocht ML, van Leeuwen BM, van Kooten TG, Wösten HAB, Wessels JGH (2002) Surface modifications created by using engineered hydrophobins. *Appl Environ Microbiol* 68:1367–1373
- Schuren FHJ, Wessels JGH (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
- Sela-Buurlage MB, Epstein L, Rodriguez RJ (1991) Adhesion of ungerminated *Colletotricum musae* conidia. *Physiol Mol Plant Pathol* 39:345–352
- Senchou V, Weide R, Carrasco A, Bouyssou H, Pont-Lezica R, Govers F, Canut H (2004) High affinity recognition of a *Phytophthora* protein by *Arabidopsis* via an RGD motif. *Cell Mol Life Sci* 61:502–509
- Shaw BD, Hoch HC (1999) The pycnidiospore of *Phyllosticta ampellicida*: surface properties involved in substratum attachment and germination. *Mycol Res* 103:915–924
- Shaw BD, Hoch HC (2000) Ca²⁺ regulation of *Phyllosticta ampellicida* pycnidiospore germination and appressorium formation. *Fung Genet Biol* 31:43–53
- Shaw BD, Hoch HC (2001) Ions as regulators of growth and development. In: Howard RJ, Gow NAR (eds) *The Mycota*, vol VIII. *Biology of the fungal cell*. Springer, Berlin Heidelberg New York, pp 73–89
- Slawecki RA, Ryan EP, Young DH (2002) Novel fungitoxicity assays for inhibition of germination-associated adhesion of *Botrytis cinerea* and *Puccinia recondita* spores. *Appl Environ Microbiol* 68:597–601
- Snetselaar KM, McCann MP (2001) From bud to appressorium: morphology of the *Ustilago maydis* transition from saprobic to parasitic growth. *Phytopathology* 91:S165 (abstract)
- Spotts RA, Holz G (1996) Adhesion and removal of conidia of *Botrytis cinerea* and *Penicillium expansum* from grape and plum fruit surfaces. *Plant Dis* 80:691–699
- Staab JF, Bradway SD, Fidel PL, Sundstrom P (1999) Adhesive and mammalian transglutaminase substrate properties of *Candida albicans* Hwp1. *Science* 283:1535–1538
- Stanley MS, Callow ME, Perry R, Alberte RS, Smith R, Callow JA (2002) Inhibition of fungal spore adhesion by zosteric acid as the basis for a novel, nontoxic crop protection technology. *Phytopathology* 92:378–383
- Sugui JA, Leite B, Nicholson RL (1998) Partial characterization of the extracellular matrix released onto hydrophobic surfaces by conidia and conidial germlings of *Colletotricum graminiicola*. *Physiol Mol Plant Pathol* 52:411–425
- Sundstrom P (2002) Adhesion in *Candida* spp. *Cell Microbiol* 4:461–469
- Takano Y, Kikuchi T, Kubo Y, Hamer JE, Mise K, Furusawa I (2000) The *Colletotrichum lagenarium* MAP kinase gene *CMK1* regulates diverse aspects of fungal pathogenesis. *Mol Plant Microbe Interact* 13:374–383
- Talbot NJ, Kershaw M, Wakley GE, De Vries OMH, Wessels JGH, Hamer JE (1996) *MPG1* encodes a fungal hydrophobin involved in surface interactions during infection-related development of the rice blast fungus *Magnaporthe grisea*. *Plant Cell* 8:985–999
- Terhune BT, Hoch HC (1993) Substrate hydrophobicity and adhesion of *Uromyces* urediospores and germlings. *Exp Mycol* 17:241–252
- Tomley FM, Soldati DS (2001) Mix and match modules: structure and function of microneme proteins in apicomplexan parasites. *Trends Parasitol* 17:81–88
- Tucker RP (2004) Molecules in focus. The thrombospondin type 1 repeat superfamily. *Int J Biochem Cell Biol* 36:969–974
- Tucker SL, Talbot NJ (2001) Surface attachment and pre-penetration stage development by plant pathogenic fungi. *Annu Rev Phytopathol* 39:385–417
- Vreeland V, Epstein L (1996) Analysis of plant-substratum adhesives. In: Jackson JF, Linskens H-F (eds) *Modern methods of plant analysis*, vol 17. *Plant cell wall analysis*. Springer, Berlin Heidelberg New York, pp 95–116

- Vreeland V, Waite JH, Epstein L (1998) Polyphenols and oxidases in substratum adhesion by marine algae and mussels. *J Phycol* 34:1–8
- Watanabe K, Parbery DG, Kobayashi T, Doi Y (2000) Conidial adhesion and germination of *Pestalotiopsis neglecta*. *Mycol Res* 104:962–968
- Weig M, Jansch L, Gross U, de Koster CG, Klis FM, de Groot, PWJ (2004) Systematic identification *in silico* of covalently bound cell wall proteins and analysis of protein-polysaccharide linkages of the human pathogen *Candida glabrata*. *Microbiology* 150:3129–3144
- Wösten HAB, Schuren FHJ, Wessels JGH (1994) Interfacial self-assembly of a hydrophobin into an amphipathic membrane mediates fungal attachment to hydrophobic surfaces. *EMBO J* 13:5848–5854
- Wright AJ, Thomas BJ, Kunoh H, Nicholson RL, Carver TLW (2002) Influences of substrata and interface geometry on the release of extracellular material by *Blumeria graminis* conidia. *Physiol Mol Plant Pathol* 61:163–178
- Xiao J-Z, Ohshima A, Kamakura T, Ishiyama T, Yamaguchi I (1994) Extracellular glycoprotein(s) associated with cellular differentiation in *Magnaporthe grisea*. *Mol Plant Microbe Interact* 7:639–644
- Yamaoka N, Takeuchi Y (1999) Morphogenesis of the powdery mildew fungus in water (4) The significance of conidium adhesion to the substratum for normal appressorium development in water. *Physiol Mol Plant Pathol* 54:145–154
- Zhao X, Oh S-H, Cheng G, Green CB, Nuessen JA, Yeater K, Leng RP, Brown AJP, Hoyer LL (2004) ALS3 and ALS8 represent a single locus that encodes a *Candida albicans* adhesin; functional comparisons between Als3p and Als1p. *Microbiology* 150:2415–2428