

Chapter 2

Adhesion and Adhesives of Fungi and Oomycetes

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Abstract Many fungi adhere strongly to an outer surface of a host or a substratum before penetration; the ability to adhere is a virulence factor for plant and animal pathogens. Because substrata such as the plant cuticle are largely inert hydrophobic surfaces, plant pathogenic fungi often adhere to plastics. Strong adhesion is generally mediated by a secreted glue. Fungal glues are typically formed in an aqueous environment, and as such they may provide models for commercial glues, including for biomedical applications. The best-characterized fungal glue is a galactosaminogalactan polymer that serves as an adhesive to plastic, fibronectin, and epithelial cells in the mammalian pathogen *Aspergillus fumigatus*. However, most fungal glues are apparently mannoprotein(s). Despite our advances in fungal genomics, most fungal glues are uncharacterized, in contrast to the well-characterized fungal pathogen-mammalian host adhesins produced by *Candida albicans*; these adhesins, which bind to specific host receptors, are primarily large, glycosylphosphatidylinositol (GPI)-anchored cell wall mannoproteins that are posttranslationally covalently cross-linked to the cell wall β 1,6-glucans. Adhesins typically have tandem repeats; adhesins such as the *C. albicans* ALS family bind to both specific cell types and to plastic. We postulate that the mannoprotein glues from plant pathogenic fungi are secreted in a more water-soluble form, are transiently sticky, and are cross-linked extracellularly. Anti-adhesive strategies may enable new plant disease control methods that are environmentally compatible because anti-adhesive strategies do not necessarily require any uptake of compounds into the fungus or disruption of a eukaryotic metabolic pathway.

Ralph Nicholson was deceased at the time of publication.

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2.1 Introduction

The adhesion of fungi to host surfaces—both plant and animal—before penetration has been well documented (Mendgen et al. 1996; Epstein and Nicholson 1997, 2006; 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. Adhesion onto hydrophobic surfaces is 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 nonspecific manner. Here, we will primarily focus on fungal cell-substratum adhesion that is mediated by a glue. Some researchers differentiate between an initial and reversible attachment and a time-dependent and at least relatively irreversible adhesion. We will focus on adhesion.

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. The term adhesin is sometimes also used to indicate a molecule involved in cell-to-cell adhesion within a fungal biofilm, in which the ligand and the receptor are on cells of the same species; biofilms of the medically important *Candida* spp. are typically attached to either inert substrata such as implants or onto host cells. Whether an adhesin is somewhat nonspecifically “sticky” or has a conformational “good fit” for a particular substratum is generally unclear; *Candida albicans* cells, for example, adhere to inert substrata such as polystyrene (Masuoka et al. 1999) and to a variety of cell types. 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. Flocculation as a cell-cell adhesion-mediated phenomenon in *Saccharomyces cerevisiae* has been reviewed relatively extensively and will only be mentioned briefly here (Brückner and Mösch 2012; Rossouw et al. 2015). Adhesins in biofilms produced by animal pathogens also will only be briefly mentioned (Ramage et al. 2009).

2.2 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 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 in similar ways.

2.2.1 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. 2.2.3.2), hydrophobic interactions between a hydrophobic spore and the leaf cuticle can secure the initial contact. With water-disseminated pathogens such as *Colletotrichum graminicola* (Nicholson and Epstein 1991; Epstein and Nicholson 1997) and *Venturia inaequalis* (Schumacher et al. 2008), initial adhesion of conidia 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 typically 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* urediniospores release cutinases and esterases that degrade the plant cuticle and enhance spore adhesion (Deising et al. 1992).

The strength of adhesion varies with the fungus and the cell type. For example, *Nectria haematococca* mating population I (synonym, *Fusarium solani* species complex 10) 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 strong 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. Using an indirect measure of turgor via osmotically induced collapse, Howard et al. (1991) estimated that *Magnaporthe oryzae* appressoria generated turgor pressures as high as

8.0 MPa. Consequently, the glue that affixes the appressorium to the plant surface must be able to withstand a very strong force.

2.2.2 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 2.1). Most obviously, adhesion keeps a fungus from being blown or rinsed off 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

Table 2.1 Examples of documented 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 thigmomodifferentiation	Appressoria, hyphopodia	Cg	Chaky et al. (2001)
		Eg	Yamaoka and Takeuchi (1999)
		Ua	Epstein et al. (1985)
Required for host penetration via mechanical pressure	Appressoria, hyphopodia, cysts	Cg	Bechinger et al. (1999)
		Mo	Howard et al. (1991)

^aThis list provides examples and is a not comprehensive survey. All applies to all fungi in this list; Cg, *Colletotrichum graminicola*; Eg, *Erysiphe graminis*; Mo, *Magnaporthe oryzae*; Nh, *Nectria haematococca* mating population I (synonyms *Fusarium solani* species complex 10, *Fusarium solani* f. sp. *cucurbitae* race I); Pa, *Phyllosticta ampellicida*; Ua, *Uromyces appendiculatus*

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. 2.2.3.1). 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 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 firm 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 toward 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 nonadherent with proteases continued to grow but were unable to establish pathogenicity because they were unable to grow thigmotropically or to 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.

Although most fungi are either saprophytic or associated with plants in either a parasitic or a mutualistic association, there are carnivorous fungi. The nematophagous fungi are the best studied of the carnivorous fungi; many of the nematophagous fungi produce adhesive hyphae or protuberances that are critical for nematode capture (Li et al. 2015; Nordbring-Hertz et al. 2006). More than 90 % of the carnivorous fungi are in a single monophyletic clade (the Orbiliomycetes) in the Ascomycota (Yang et al. 2012); additional nematophagous fungi are in the Zygomycotina and in the genus *Nematoctonus* in the Basidiomycota. Adhesive structures within the Orbiliomycetes include adhesive nets in *Arthrobotrys* spp., either adhesive columns or sessile adhesive knobs in *Gamsylella* spp., and stalked adhesive knobs in *Dactylellina* spp. (Yang et al. 2012).

Arbuscular mycorrhizal fungi in the Glomeromycota provide a critical ecosystem service by aggregating soil (Rillig 2004). Soil aggregation is apparently mediated by a highly expressed glycoprotein(s) which was named glomalalin, or later, a glomalalin-related soil protein (Gillespie et al. 2011). However, the glomalalin-related soil protein(s) co-extracts with nonproteinaceous compounds and has never

been identified. Nonetheless, the soil “super glue” apparently originates on fungal walls (Driver et al. 2005).

2.2.3 Selected Examples of Adhesiveness as a Part of a Developmental Sequence

Currently available sequences of whole fungal genomes make comparisons of the genes in adherent versus nonadherent fungi possible. The ascomycetous yeasts *Saccharomyces cerevisiae* and *Candida albicans* are comparatively nonadherent and adherent, respectively, for yeasts, but are far less adherent than many filamentous ascomycetes. The ascomycetes *Magnaporthe oryzae*, *Colletotrichum graminicola*, 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. 1994a; Spotts and Holz 1996), but *Neurospora crassa* is nonadherent. Among the model basidiomycetes, *Ustilago maydis* has hyphae that grow attached to the leaf and then produces appressoria (Snetselaar and McCann 2001). The following examples illustrate that adhesion is just one component of development on a plant surface.

2.2.3.1 *Colletotrichum 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 antidesiccant that allows 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 nonspecific 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 plastic, but the same conidia bind less to hydrophilic surfaces such as glass (Mercure et al. 1994). Materials released by the conidia, including the adhesive material, are easily observed by scanning electron microscopy (Mercure et al. 1995) and contain a mixture of high molecular weight glycoproteins (Sugui et al. 1998). Most if not all *Colletotrichum* spp. produce adherent conidia, germ tubes, and appressoria (Sela-Buurlage et al. 1991). Glycoproteins in the ECM are considered responsible for host adhesion in *Colletotrichum* spp. (Pain et al. 1996).

2.2.3.2 *Blumeria graminis* f. sp. *hordei* and f. sp. *tritici*, Causal Agent of Powdery Mildew of Barley and Wheat, Respectively

The powdery mildew pathogen of barley *B. graminis* produces conidia that release a liquid exudate on contact with the hydrophobic surface of the barley leaf (Kunoh et al. 1988). 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 *B. 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 for the initial host-pathogen interaction. The development of *B. graminis* f. sp. *tritici* is similar. A lipase, Lip1, is constitutively produced on the surface of the tips of mature conidia and, after surface contact, is induced on conidial germlings and secondary hyphae (Feng et al. 2009). Pretreatment of the wheat leaf with lipases reduced conidial adhesion, suggesting that the undigested cuticle is important for at least the initial attachment, which has been postulated to occur via hydrophobic interactions between the hydrophobic conidia, which are coated with long-chain fatty acids, and the hydrophobic leaf cuticle, which is also coated with long-chain fatty acids (Samuels et al. 2008). Feng et al. (2009) suggest that the Lip1 transesterification activity could result in the formation of covalent ester bonds between the fungus and the host cuticle. If so, this would result in an extremely strong attachment in the absence of a glue. However, such a covalent attachment would be limited to the plant cuticle and other lipase-sensitive surfaces and would not account for adhesion onto either inert substrata or root surfaces.

2.2.3.3 *Magnaporthe oryzae*, Causal Agent of Rice Blast

In ungerminated *M. oryzae* conidia, after the conidia are hydrated, a “spore tip mucilage” is released from the periplasmic space at the spore apex; the mucilage attaches the spore to a hydrophobic surface (Hamer et al. 1988). The spore tip mucilage contains protein, an α -1,2 mannose, and a lipid (Howard and Valent 1996). The germ tubes are also adherent and function in sensing features of the surface; appressoria are typically induced at the juncture of two epidermal cells. Xiao et al. (1994b) inferred that hardness was a key feature of an appressorial-inducing surface. On an inductive surface, germ tubes produce an (adherent) appressorium. An adherent appressorium can withstand turgor pressures as high as 8.0 MPa (Howard et al. 1991; de Jong et al. 1997; Ebbole 2007), and consequently, the glue that attaches the appressorial wall to the substratum must withstand this force too. Antibody and enzyme treatments suggest that the appressorial glue is a glycoprotein that can be digested by gelatinase B, a collagenase (Inoue et al. 2007, 2011).

2.3 Challenges in Identifying Adhesives in Fungi

As indicated above, progress has been made in documenting the spatial and temporal expression of the development of adhesiveness and of cell-substratum adhesion. However, identification of the components of glues presents both biochemical and genetic challenges (Vreeland and Epstein 1996). Despite the recent advances in gene characterization of filamentous fungi, the most common fungal cell-inert substratum glue has still not been clearly identified.

2.3.1 Genetic “Knockout,” “Knockin,” and Overexpression Strategies

Adhesion-reduced and nonadhesive mutants provide a superb tool to investigate the role of adhesion. Mutants have been used to formulate hypotheses about the adhesive compound(s); in the biocontrol yeast *Rhodospiridium 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 many examples in which a putative adhesin gene has been knocked out, and there is a resultant loss of adhesiveness and virulence (e.g., Gale et al. 1998; Brandhorst et al. 1999; Loza et al. 2004; Zhao et al. 2004; de Groot et al. 2013); adhesion and virulence were restored after the mutant strain had the disrupted gene restored. Fungi produce both adhesives and anti-adhesives; *Candida albicans* with a deleted *Ywp1* had increased adherence to several surfaces and had enhanced biofilm formation (Granger et al. 2005; Granger 2012). Similarly, transmission electron microscopy of freeze-substituted conidia of *Fusarium solani* strains with reduced adhesiveness showed an increased amount of extracellular material (Caesar-TonThat and Epstein 1991).

Among the plant pathogenic fungi, researchers have focused more on genes that reduce pathogenicity than on adherence per se; in a few cases, these genes also affect fungal adhesion. However, the mutations have apparently been in genes that function in pathways upstream of adhesion and the mutants are pleiotropically affected. 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). Somewhat similarly, disruption of a highly conserved transmembrane mucin (VdMsb) in the MAP kinase pathway in *Verticillium dahliae* resulted in a strain with reduced virulence, microsclerotial and conidial production, conidial adhesion, and invasive growth (Tian et al. 2014). The

Aspergillus nidulans signaling mucin MsbA regulates hyphal attachment onto low nutrient substrata in addition to starvation responses and cellulase secretion (Brown et al. 2014). Disruption of *Magnaporthe oryzae* *acr1* (Lau and Hamer 1998) affects conidiogenesis so that an Δ *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 Δ *acr1* conidia fail to produce the spore tip mucilage, are nonadherent, and are inefficient in forming appressoria.

Multiple authors have identified transcription factors that appear to activate genes involved in adhesion. Lin et al. (2015) demonstrated that the *Aspergillus fumigatus* transcription factor SomA, which is a homologue of *Saccharomyces cerevisiae* Flo8, is required for adhesion to host cells and for biofilm formation. Breth et al. (2013) demonstrated that mutants in the *M. oryzae* transcription factors *tra1*, *TDG2*, and to a lesser extent *TDG6* had reduced conidial adhesion in addition to other phenotypes. Nonetheless, *tra1*⁻ mutants appeared to form less spore tip mucilage, as determined by binding with the lectin concanavalin A-FITC. In *Colletotrichum lindemuthianum*, a mutant in the *Ste12p* transcription factor was reduced in its ability to adhere to artificial surfaces; an extracellular protein *Clsp1* was absent from the mutant strain (Hoi et al. 2007). However, 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. Nonetheless, if multiple compounds can serve as building blocks of a glue, single-gene knockouts 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 nonadhesive *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. Tran et al. (2014) transformed a non-flocculating *Saccharomyces cerevisiae* Δ *flo8* strain with a cDNA library from the filamentous plant pathogen *Verticillium longisporum*, selected for adhesion onto agar and flocculation, and then identified 22 genes that conferred adhesiveness in *S. cerevisiae*. The majority of the identified genes are not glue components, but two potential GPI cell wall anchored candidates were identified: *Fas1* and *Wsc1*. Successful utilization of this type of 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 nonadherent organism. Using a strategy similar to that described in Barki et al. (1993), the Epstein laboratory was not successful in identifying *Colletotrichum graminicola* genes that rendered *S. cerevisiae* transformants adhesive.

2.3.2 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 while advances have been made, they are still comparatively poorly characterized. As discussed in greater detail below (Sect. 2.4.2), many 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 indicates 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). 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). Similarly, the ALS proteins on *Candida albicans*, which adhere to both plastics and to host cells, are bound to the cell wall β 1,6-glucan (de Groot et al. 2013). Moreover, *C. albicans* produces a family of eight ALS proteins that bind to plastic (Table 2.3). However, despite attachment by multiple proteins, single-deletion mutants still have discernible adhesion-reduced phenotypes.

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 nonsticky to be secreted to the wall surface, sticky during glue formation, and then typically, nonsticky 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.

2.4 Fungal and Oomycete Glues

2.4.1 Features

Conidia of many but not all fungal species must be alive in order for adhesion to occur (Slawecki 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, cross-linked compound (Caesar-TonThat and Epstein 1991; Apoga and Jansson 2000). Mature adhesives often appear to have a fibrous component (Watanabe et al. 2000; Nordbring-Hertz et al. 2006).

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 viciae-fabae*, 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 *Rhodospodium 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.

2.4.2 Postulated Composition of Glues

During the last three decades, we have learned much about why and when fungi adhere. We still know relatively little about how fungi adhere. Many 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 most fungal adhesives are glycoproteins

(Chaubal et al. 1991; Jones 1994; Kuo and Hoch 1996; Pain et al. 1996; O'Connell et al. 1996; Bircher and Hohl 1997; Epstein and Nicholson 1997; Sugui et al. 1998; Hughes et al. 1999; Apoga et al. 2001; Rawlings et al. 2007). The carbohydrate moieties on glycoproteins seem particularly important in adhesion. In many fungi, including *Bipolaris sorokiniana*, *Colletotrichum graminicola*, *Magnaporthe oryzae*, *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 blockage of adhesion with the snowdrop lectin, indicates that mannose residues are involved in the adhesive mechanism (Kwon and Epstein 1997a). Importantly, Kahmann's and Corcelles' groups demonstrated that the yeast cells of the corn smut *Ustilago maydis* with a deletion in an intracellular o-mannosyltransferase *pmt4* had reduced adhesion onto starch agar compared to the wild type (Fernández-Álvarez et al. 2012). This supports the hypothesis that the *U. maydis* glue is a mannoprotein. In addition, the appressoria of the $\Delta pmt4$ strain were defective in penetration (Fernández-Álvarez et al. 2009), which could be due to defective appressorial adhesion, although this was not measured directly. Disruption of three *pmt* orthologs in the entomopathogen *Beauveria bassiana* was pleiotropically affected; adhesion of the defective strains was not examined (Wang et al. 2014).

Lectins, which are carbohydrate-binding proteins or glycoproteins, are candidates for cell-cell binding, such as on fungal-root interactions and on adhesive nets of nematophagous fungi (Li et al. 2015). Li et al. (2015) reviewed the recent literature on the role of lectins in the adhesiveness of traps of nematophagous fungi; based on a combination of lectin knockout and spatial/temporal expression of lectin genes, they suggest that lectins may not be involved in nematode trapping.

In the mammalian pathogen *Aspergillus fumigatus*, a galactosaminogalactan polymer serves as an adhesive to plastic, fibronectin, and epithelial cells (Gravelat et al. 2013); the polymer contains α -1,4-linked galactose and α -1,4-linked *N*-acetylgalactosamine. In addition to imparting adhesion, the polymer suppresses the host inflammatory response by preventing binding of the host dectin-1 to the fungal wall β -glucans (Gravelat et al. 2013). There have been a few other reports that fungal adhesives are carbohydrates (Pringle 1981), but in most cases, whether the adhesives are actually glycoproteins or proteoglycans was not determined. Buck and Andrews (1999a) reported that a mannose-containing compound, and possibly a mannoprotein, was involved in attaching the yeast *Rhodospiridium toruloides* to polystyrene and barley leaves. Howard et al. (1993) concluded that the adhesive *Magnaporthe oryzae* spore (conidial) tip mucilage is composed of a mixture of a polypeptide(s) with terminal mannose disaccharides and a lipid component. Determination of whether lipids are part of the *M. oryzae* appressorial adhesive (Ebata et al. 1998; Ohtake et al. 1999) requires additional experimental evidence.

2.4.3 *Secretion and Cross-Linking, 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 may cross-link proteins in ascomycete walls (Ruiz-Herrera et al. 1995; Iranzo et al. 2002) which would help 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 presumably 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).

2.4.4 *Cell Surface Macromolecules with Apparent Adhesive Properties*

Potential fungal glues are summarized in Table 2.2 and several are discussed below.

2.4.4.1 *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

Table 2.2 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>Metarhizium anisopliae</i>	MAD1	GPI-CWP that is involved in conidial adhesion onto locust wings	Wang and St. Leger (2007)
<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>Rhodospidium 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/Muc1	GPI-CWP that is involved in adhesion to substrata (including polystyrene), cell-cell adhesion (flocculation), and filamentous growth	Reynolds and Fink (2001); Braus et al. (2003); Brückner and Mösch (2012)

^aGPI-CWP, glycosylphosphatidylinositol-dependent cell wall proteins

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 (2004) 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. However, 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.

2.4.4.2 90-kDa Mannoprotein on Macroconidia of *Nectria haematococca* (Anamorph *Fusarium solani* f. sp. *cucurbitae*)

The Epstein laboratory 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 *N. haematococca* mating population I (anamorph, *Fusarium solani* f. sp. *cucurbitae* 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 is 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 are 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 mM 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 suggests 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*.

Prados-Rosales et al. (2009) characterized the cell wall proteome from hyphae in adhesion-inducing conditions of the related fungus, *Fusarium oxysporum*. They compared cell wall proteins of the wild type with a nonadherent and nonpathogenic mutant with a deletion in a regulatory kinase, *fmk1*. Eighteen differentially expressed proteins were identified, but none to date have been identified as involved in adhesion.

2.4.4.3 Hydrophobins: The Mannoprotein SC3, a *Schizophyllum commune* Hydrophobin; the Class I Hydrophobin BcHpb1 of *Botrytis cinerea*; and the Hcf-6 Hydrophobin of *Cladosporium fulvum*

As indicated above, 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 hydrophathy profile, but highly variable amino acid sequences (Kershaw and Talbot 1998). Despite the variability in sequence, complementation experiments in *Magnaporthe oryzae* 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 at least initial attachment 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. 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). Films of class I hydrophobins form amyloid-like fibrils (Kwan et al. 2006), which are consistent with many microscopic observations of adhesion.

However, while the conidial rodlet hydrophobins, e.g., *Magnaporthe oryzae* class I hydrophobin Mpg1 (Kershaw et al. 1998) may allow for an initial hydrophobic interaction between a wind-blown spore and a leaf, it is a comparatively weak attachment. Indeed, a hydrophobin mutant of *M. oryzae* Mpg1 adhered as well as the wild type, indicating that the MPG1 hydrophobin is not involved in adhesion (Talbot et al. 1996). In contrast, based on gene knockdown and knockout experiments, Inoue et al. (2016) concluded that Mpg1 expression was correlated with adhesion, germling differentiation, and pathogenicity. Based on a knockout mutant in the class II hydrophobin mhp1, Inoue et al. (2016) concluded that mhp1 was not involved in adhesion, pathogenicity, or spore viability, although it affected hydrophobicity and appressorium differentiation on the artificial surface.

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). We note that *S. commune* Sc3 is glycosylated with mannose residues (de Vocht et al. 1998), as many fungal glues appear to be. Similarly, deletion of the *Botrytis cinerea* class I hydrophobin BcHpb1 resulted in conidia that had reduced adhesion to a model hydrophobic surface, dimethylsilicated-coated glass (Izumitsu et al. 2010).

Cladosporium fulvum has six hydrophobins (Lacroix et al. 2008). Using epitope tagging, Lacroix et al. (2008) demonstrated that Hcf-6 was produced during infection, is secreted, and coats surfaces under and around growing hyphae. Although a deletion mutant indicates that Hcf-6 **inhibits** adhesion of conidia and hyphae to glass slides, Lacroix et al. (2008) suggest that it is involved in attachment of hyphae to solid surfaces. Teertstra et al. (2006) demonstrated that the two hydrophobins in *Ustilago maydis* had no effect on hyphal adhesion to Teflon but that deletion of the repellent gene *rep1* (which is processed into 11 secreted peptides) increased hyphal wettability and decreased adhesion onto Teflon. While *rep1-1* is not a hydrophobin, Teertstra et al. (2009) demonstrated that it formed amyloid-like filaments on the hyphal surface and concluded that it functioned as other hydrophobins.

2.4.4.4 MAD1 and MAD2 (Metarhizium Adhesion-Like Proteins) in the Entomopathic Fungus *Metarhizium anisopliae*

Two putative adhesins were identified in an entomopathic fungus *Metarhizium anisopliae* (Wang and St. Leger 2007), which adheres to insect cuticles, a hydrophobic surface, and to plant root surfaces (Hu and St. Leger 2002), which are less hydrophobic. *M. anisopliae* MAD1 is present on the surface of conidia but interestingly plays a role in cytoskeletal organization and cell division. MAD1 knockouts had conidia with reduced adhesion onto locust wings and reduced virulence, but no reduction of adhesion onto onion epidermal peels, a more hydrophilic surface. Expression of *Mad1* in *Saccharomyces cerevisiae* cells transformed the wild type from nonadherent to adherent on insect cuticles but did not affect adhesion to onion peels. Conidia of MAD2 knockouts in *M. anisopliae* had wild-type adhesion on locust wings and wild-type virulence but reduced adhesion to onion peels. Expression of *Mad2* in *Saccharomyces cerevisiae* cells transformed the wild type from nonadherent to adherent on onion peels but did not affect adhesion on locust wings. Liang et al. (2015) disrupted the *A. oligospora* homologue AoMAD1, which was upregulated during trap formation of the nematophagous fungus. While the Δ AoMAD1 has an altered surface and wall structure, AoMAD1 does not appear to be the adhesive protein per se (Liang et al. 2015) because the Δ AoMAD1 strain formed more adhesive traps than the wild-type strain. Consequently, the authors suggested that AoMAD1 may have a negative regulatory role in the fungus in switching from a saprophytic to a pathogenic lifestyle.

2.4.4.5 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. 2004). 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 nonspecific 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 the nonadherent *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 and biofilm formation (Li et al. 2007). 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 require 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.

The ALS (agglutinin-like sequences) family of GPI-CWP is enriched in the *Candida* clade but absent from the *Saccharomyces* clade (Klis et al. 2010). As indicated in Table 2.3, different ALS may have different specificities to different cell types; mutational analysis indicates that Als1, Als2, Als3, Als4, Als5, Als7 and Als9 also adhere to plastic (Table 2.3). The ALS have repeat regions, and longer repeat regions are associated with greater adherence and shorter repeat regions are associated with less adherence (Verstrepen and Klis 2006); this may be because the longer protein extends further outside of the wall. Interestingly, within the threonine, isoleucine, and valine-rich “T” regions of the ALS proteins (de Groot

Table 2.3 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> ^a	Members of the ALS (agglutinin-like sequence) gene family (GPI-CWP ^b): Als3, Als1, Als4, Als5 (=Ala1), Als2, Als6, Als7, Als9	Different ALS (in their N-terminal regions) have different specificities, e. g., for laminin, collagen, fibronectin, endothelial and/or epithelial cells. The indicated ALS also adhere to plastic	Fu et al. (1998); Hoyer (2001); de Groot et al. (2004); Loza et al. (2004); Zhao et al. (2004); Hoyer et al. (2008); Aoki et al. (2012); Alsteens et al. (2013); de Groot et al. (2013)
<i>Candida albicans</i>	Eap1 (enhanced adherence to polystyrene), a GPI-CWP	Epithelial cells; also polystyrene	Li and Palecek (2003); Li et al. (2007); Modrzewska and Kurnatowski (2015)
<i>Candida albicans</i>	Hwp1, a mannosylated, GPI-CWP	Epithelial cells	Tsuchimori et al. (2000); Staab et al. 2004
<i>Candida albicans</i>	Iff4	Epithelial cells; also plastic	Kempf et al. 2007; Fu et al. (2008)
<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 phosphor-mannoprotein	Marginal zone macrophages	Kanbe and Cutler (1998)
<i>Candida glabrata</i>	Epa1, a GPI-CWP	Epithelial cells	Cormack et al. (1999)
<i>Cryptococcus neoformans</i>	Cfl1, cell flocculation 1 (CNA07720)	Plastic biofilm	Wang et al. (2012)
<i>Metarhizium anisopliae</i>	MAD2, a GPI-CWP	Onion epidermal peels, presumably a model surface for plant roots	Wang and St. Leger (2007)

^aSee de Groot et al. (2013) for more extensive documentation on adhesins on *Candida albicans* and other human pathogens

^bGPI-CWP, glycosylphosphatidylinositol-dependent cell wall proteins

et al. 2013), Als1, Als3, and Als5, have sequences that form amyloid adhesion domains (Alsteens et al. 2010; de Groot et al. 2013). Fungal adhesins are discussed more below.

2.5 Fungal Adhesins

Attachment of fungal cells both within biofilms and to their host cells is a critical part of fungal morphogenesis and pathogenesis in animals (Sundstrom 2002; Wang et al. 2013). Adhesion of *Candida albicans* and other *Candida* spp. within biofilms, onto implanted medical devices and other model substrata, and onto host cells has been studied the most because invasive candidiasis is a common cause of nosocomial infections, and mortality, particularly with antibiotic-resistant isolates, has been correlated with a strain's ability to form biofilms and to adhere (Uppuluri et al. 2009). Examples of fungal adhesins that bind to animal cells are shown in Table 2.3; most are relatively well characterized (de Groot et al. 2013). Clf1 is a hyphal-specific adhesin from *Cryptococcus neoformans* involved in cell-cell adhesion in colonies and biofilms and in cell-substratum adhesion onto plastic (Wang et al. 2012); cells aggregated when CFL1 was overexpressed, but virulence was reduced.

Similar to the fungal glues, most adhesins are glycoproteins, and at least several are mannoproteins (Fukazawa and Kagaya 1997), with the mannan residues involved in adhesion (Kanbe and Cutler 1998). Timpel et al. (1998, 2000) demonstrated that strains of *Candida albicans* with either a disrupted mannosyltransferase Pmt1p or a Pmt6p adhered less to host cells and had reduced virulence; pmt1 mutants produced an Als1p adhesin with a lower molecular mass than the wild type. The results are consistent with a role of mannoproteins in cell-cell adhesion. Using *C. albicans* strains with deletions in the mannosyltransferases mnt1 and/or mnt2, Dutton et al. (2014) demonstrated that mannosylation is important for interkingdom (bacterial/*C. albicans*) biofilm formation.

Hazen and colleagues (Masuoka et al. 1999) have argued that cell surface hydrophobicity is an important factor in adhesion of *C. 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. Based on 184 clinical isolates of *Candida* spp., Silva-Dias et al. (2015) provide evidence that cell surface hydrophobicity is a reasonably good predictor of biofilm formation, but not of adhesion. *C. tropicalis* and *C. parapsilosis* were the most adherent; *C. parapsilosis*, *C. krusei*, and *C. guilliermondii* were moderately adherent; and *C. albicans* and *C. glabrata* were the least adherent.

As indicated above, 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 some fungal adhesins and in some 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.

2.6 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 of 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 may provide an appealing disease control strategy because they do not necessarily require uptake into the fungus or disruption of a eukaryotic metabolic 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 oryzae* and *Colletotrichum lindemuthianum*; concentrations that are effective as an anti-adhesive are not toxic to either the plant hosts or fungi.

Although it is perhaps surprising that more progress has not been made in the elucidation of fungal “superglues” in the past decade, we now have extremely powerful technologies with which to proceed—full genome assemblies in a variety of fungi which range from strongly adherent to weakly or essentially nonadherent, new tools in gene deletion and silencing using methods such as CRISPR/Cas (Schuster et al. 2016), and new technologies for imaging and analyzing adherent molecules (Alsteens et al. 2010; Beaussart et al. 2012). In combination, these tools should enable the fungal adhesion field to leap forward.

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