Quantifying the Importance of Galactofuranose in Aspergillus nidulans Hyphal Wall Surface Organization by Atomic Force Microscopy

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The fungal wall mediates cell-environment interactions. Galactofuranose (Galf), the five-member ring form of galactose, has a relatively low abundance in Aspergillus walls yet is important for fungal growth and fitness. Aspergillus nidulans strains deleted for Galf biosynthesis enzymes UgeA (UDP-glucose-4-epimerase) and UgmA (UDP-galactopyranose mutase) lacked immunolocalizable Galf, had growth and sporulation defects, and had abnormal wall architecture. We used atomic force microscopy and force spectroscopy to image and quantify cell wall viscoelasticity and surface adhesion of ugeAΔ and ugmAΔ strains. We compared the results for ugeAΔ and ugmAΔ strains with the results for a wild-type strain (AAE1) and the ugeB deletion strain, which has wild-type growth and sporulation. Our results suggest that UgeA and UgmA are important for cell wall surface subunit organization and wall viscoelasticity. The ugeAΔ and ugmAΔ strains had significantly larger surface subunits and lower cell wall viscoelastic moduli than those of AAE1 or ugeBΔ hyphae. Double deletion strains (ugeAΔ ugeBΔ and ugeAΔ ugmAΔ) had more-disorganized surface subunits than single deletion strains. Changes in wall surface structure correlated with changes in its viscoelastic modulus for both fixed and living hyphae. Wild-type walls had the largest viscoelastic modulus, while the walls of the double deletion strains had the smallest. The ugmAΔ strain and particularly the ugeAΔ ugmAΔ double deletion strain were more adhesive to hydrophilic surfaces than the wild type, consistent with changes in wall viscoelasticity and surface organization. We propose that Galf is necessary for full maturation of A. nidulans walls during hyphal extension.

The fungal wall supports and shields the hyphal cytoplasm and mediates interactions between the cell and its environment. Fungal walls are typically about 30% of the weight (dry weight) of the cell (7, 10), and a similar portion of the fungal genome is thought to contribute to cell wall biosynthesis and/or maintenance (11, 17). Fungal walls are composed of a variety of carbohydrate polymers (7, 11, 15); however, deleting many wall biosynthesis genes appears to be compensated by genetic redundancy and/or by induction of the cell wall integrity pathway (6, 7, 23).

Aspergillus wall is reinforced by chitin fibrils and has a matrix containing alpha- and beta-glucans, other sugars, including galactomannans, and proteins. Galactofuranose (Galf) is the five-member ring form of galactose that is found in the cell walls of Aspergillus spp. (6, 7, 23), other fungi (reviewed in reference 15), and certain other microbes (3). Deletion of UDP-galactopyranose mutase in several Aspergillus species has shown that Galf, despite its reported low abundance, is important for wild-type fungal growth, cell morphogenesis, hyphal adhesion, wall architecture, and spore development (6, 8, 9, 14, 16, 25) and may mediate pathogenesis (1, 21–23).

The A. nidulans gene products UgeA (UDP-glucose-4-epimerase) (8), and UgmA (UDP-galactopyranose mutase) (9) catalyze sequential steps in Galf biosynthesis (Fig. 1). The ugeAΔ and ugmAΔ deletion strains have similarly compact colonies, aberrant hyphal growth, and reduced sporulation. The hyphal walls of these strains differ from those of the wild type and from each other as visualized using transmission electron microscopy (TEM) (8).

Atomic force microscopy (AFM) imaging uses a fine-tipped probe mounted on a flexible cantilever to raster scan the surface of an object generating a topographic map. An approach-retract cycle of the AFM probe, called force spectroscopy (FS), can be used to calculate the viscoelastic modulus of the whole organism or its cell surface and surface adhesion. Previously we used AFM to show that A. nidulans cell walls of growing hyphal tips differ from those of mature regions (18) and to document changes associated with spore swelling and germination and the nonpolarized hyphal growth of temperature-sensitive mutants (19). Here, we compare the hyphal walls of the wild type and a suite of Galf biosynthesis gene deletion strains using TEM, AFM, and FS to gain a better understanding of the role played by Galf in Aspergillus nidulans cell wall organization.

MATERIALS AND METHODS

Strains and culture conditions. Aspergillus nidulans strains were grown as described in references 8 and 9. Deletion strain construction followed procedures described in references 8 and 9 using nkaAΔ strains, plasmids, and primers listed in Table SA in the supplemental material. The AN2951 (ugeB) gene was deleted.
all autoclaved for 20 min at 121°C, cooled to room temperature, and then stored sensitive. The vitamin solution was stored in a dark bottle, since riboflavin is light sensitive. The vitamin solution contains 100 mg each of biotin, pyridoxin, thiamine, riboflavin, zinc, manganese, copper, and molybdenum. The solution also contains 2.2 g ZnSO$_4$·7H$_2$O, 1.1 g H$_2$BO$_3$, 0.5 g MnCl$_2$·4H$_2$O, 0.5 g FeSO$_4$·7H$_2$O, 0.17 g CoCl$_2$·6H$_2$O, 0.16 g CuSO$_4$·5H$_2$O, 0.15 g Na$_2$MoO$_4$·2H$_2$O, and 5 g Na$_2$EDTA.

For TEM, wild-type and gene deletion strains were cultured in either liquid or solid double deletion strain AAE10. Construction of strain AAE10 (ugeAΔ ugeB Δ double deletion strain) was described in reference 8. All \textit{Aspergillus nidulans} strains were cultured in either liquid or solid medium. After 16 h of growth, the dialysis tubing was transferred to a glass coverslip. Sterile Whatman no. 4 filter paper placed beneath the dialysis tubing was used to deliver liquid growth medium by capillary action, ~20 µl at a time. Other control and experimental procedures were essentially identical to those described for liquid cultures.

AFM. Samples for imaging fixed hyphae were prepared for atomic force microscopy (AFM) as previously described (18, 19). Briefly, conidia were germinated in liquid growth medium between two glass coverslips for 16 h. The top coverslip was carefully removed, and the hyphae were fixed with 3.7% formaldehyde in 50 mM phosphate buffer (pH 7.0) containing 0.2% Triton X-100, followed by rinsing with distilled water and air drying. For live-cell AFM imaging, hyphae were grown on dialysis tubing (Spectrapor; 12 to 14 kDa) overlaying agar medium. After 16 h of growth, the dialysis tubing was transferred to a glass coverslip. Sterile Whatman no. 4 filter paper placed beneath the dialysis tubing was used to deliver liquid growth medium by capillary action,~20 µl at a time. An Explorer AP microscope (Veeco, Plainview, NY) with a dry scanner (model 5460-00; Veeco) was used for contact mode imaging and force spectroscopy (FS). The cantilever spring constant, $k_c$, was determined prior to each force measurement using resonance frequencies according to reference 5. Tip-sample interaction was tracked by cantilever deflection as a function of Z piezo elongation during probe approach and retraction. For soft materials, meaningful FS comparisons often depend on the velocity of the surface approach (4). In this case, the approach velocity did not significantly affect the hyphal spring constant (data not shown), but this parameter was kept constant (100 nms ($^{-1}$)) to facilitate comparison of viscoelastic moduli between samples. Repeated measurements of individual sites on mature walls gave consistent values, and images obtained before and after FS were unchanged (data not shown), indicating that the walls were not damaged during data collection. Values were averaged from force curves collected in triplicate at 10 separate points on the surfaces of mature hyphal walls ($>$40 µm) for each strain using typical three different samples.

Force approach curves measure the unit force (in nanonewtons) required to indent a surface a given distance (in nanometers), so the slopes corresponding to the b-c segment of the approach cycle (see Fig. 5A) was used to examine the relative cell wall elasticity. FS data were plotted as deflection (in nanoamperes) versus distance (in nanometers), converted to force (in nanonewtons) versus distance (in nanometers) curves using the piezo sensor response, and the slope of the line b-c (m) in nanonewtons per nanometer used to determine the spring constant $k_w$ of the cell wall according to the following equation:

$$ k_w = m_k = \frac{m}{m_m - m_s} $$ (1)

where $m_s$ is the sample slope and $m_m$ is the slope for a hard surface (mica). The value of $k_w$ was used for the subsequent determination of Young’s modulus according to the following equation (28):

$$ E = \frac{0.80 (k_w/h R_b) \nu}{\nu + 1.5 \nu} $$ (2)

where $E$ is the cell wall viscoelastic (Young’s) modulus, $R_b$ is the hyphal radius measured by either TEM or AFM, and $\nu$ is the thickness of the cell wall measured by TEM. Surface adhesion values were measured from the last segment of the retraction cycle (see Fig. 5A, segment e-f). If there is a chemical attraction between the sample and the Si$_3$N$_4$ AFM probe, which is hydrophilic, segment e-f will be a measure of its intensity in nanonewtons.

\textbf{Data processing and analysis.} AFM images were processed using horizontal leveling, with the maximal height adjusted for optimum contrast (SPMLab ver-
RESULTS

Building on our previous experience using atomic force microscopy (AFM) to study *Aspergillus nidulans* hyphae (18, 19), we compared a suite of *A. nidulans* strains deleted for galactofuranose (Galf) biosynthesis enzymes UgeA and UgmA, the near-isogenic wild-type strain AAE1, a strain deleted for an epimerase (UgeB) that did not affect hyphal morphogenesis, and double deletion strains (ugeAΔ ugeBΔ and ugeAΔ ugmAΔ). Double deletion strains were used to further explore the function of individual gene products. Even enzymes that mediate a known biochemical function may have collateral defects in a deletion strain based on protein-protein interactions, for example if the protein is part of a scaffold for a multi-enzyme.

Characterization of *Aspergillus nidulans* ugeB. *Aspergillus nidulans* ANID2951.4 (which we named UgeB) shares 38% amino acid sequence identity with UgeA (8) and had been annotated as a UDP-glucose/galactose-4-epimerase (www.broadinstitute.org/annotation/genome/aspergillus_group/). The ugeB genomic sequence has a single exon that encodes a 428-amino-acid peptide. The *ugeB* cDNA could not be amplified (three attempts), unlike *ugmA* (7) and *ugeA* (8). We deleted *ugeB* (see Fig. SA in the supplemental material) as described in reference 9 and confirmed the deletion using PCR (see Table SA and Fig. SA in the supplemental material). A *ugeAΔ ugeBΔ* double deletion strain was generated and confirmed (see Fig. SB in the supplemental material) as described in reference 8. The *ugmAΔ ugeAΔ* single deletion strain was described in reference 9, and the *ugeAΔ ugmAΔ* single deletion strain and *ugeAΔ ugmAΔ* double deletion strain were described in reference 8. UgeB was expressed *in vitro* using the genomic sequence, which does not contain introns, purified, and shown to convert UDP-galactose into a product that is not UDP-glucose by the procedure described in reference 8 (data not shown). This unknown product awaits conclusive identification.

The *ugeB* sequence was put under the control of the *alcA* promoter and tagged with red fluorescent protein (A. M. El-Ganiny and S. G. W. Kaminskyj, unpublished data) and then overexpressed by culturing on CM containing 100 mM threonine (CMT). Under these conditions, *palcA-ugeB-rfp* was expressed, albeit weakly, in conidia and to a lesser extent in mature hyphae (see Fig. SC in the supplemental material), but it was not detectable in hyphal tips (data not shown).

Morphology of *Aspergillus nidulans* strains deleted for Galf biosynthesis genes. Confocal microscopy images showing the hyphal morphology of the suite of *Galf* biosynthesis gene deletion strains examined in this study (strain AAE1, *ugeAΔ* and *ugeBΔ* single deletion strains, *ugeAΔ ugeBΔ* double deletion strain, *ugmAΔ* single deletion strain, and *ugeAΔ ugmAΔ* double deletion strain) are shown in Fig. 2. Strain morphometry is described in Table 1. Unlike the previously described *ugeAΔ* and *ugmAΔ* deletion strains, which had wide and highly branched hyphae and reduced sporulation (8, 9), the *ugeBΔ* strain had wild-type hyphal morphology and growth rate and abundant sporulation (Table 1 and Fig. 2; also data not shown). The *ugeAΔ ugeBΔ* double deletion strain was viable when grown on medium containing galactose as the carbon source, but it did not form colonies on medium containing galactose as the sole carbon source (due to *ugeAΔ*), and its hyphae were wide and branched like those of *ugeAΔ* (Table 1 and Fig. 2).

Transmission electron micrographs of hyphal cross sections showed that the walls of *ugeAΔ* and *ugmAΔ* single deletion strains were 2-fold and 4-fold thicker, respectively, than those of strain AAE1 (Table 1 and Fig. 3) (8, 9). Given the general correlation between hyphal morphology and wall thickness (e.g., see references 8, 9, 11, 12, 20, and 24), we expected that the hyphal wall thickness of the *ugeBΔ* strain might be similar

![Figure 2](image-url)
TABLE 1. Morphological characteristics, maximum dimension of surface subunits, and cell wall viscoelastic moduli of wild-type and Gal/
biosynthesis enzyme gene deletion strains

| Strain                  | Hyphal width* (μm) (mean ± SE) | Wall thickness* (nm) (mean ± SE) | Subunit maximum dimension (nm) (mean ± SD) | Viscoelastic moduli (MPa) (mean ± SD)* of: | Adhesion (nN) (mean ± SD) |
|-------------------------|---------------------------------|----------------------------------|------------------------------------------|-------------------------------------------|--------------------------|
| Wild-type (AAE1)        | 2.4 ± 0.0                       | 54 ± 2                           | 35 ± 5                                   | Fixed hyphal wall                         | 211 ± 15                 |
| ugeAΔ                   | 3.6 ± 0.1                       | 104 ± 10                         | 63 ± 10                                  | Live hyphal wall                          | 82.3 ± 12.9              |
| ugeBΔ                   | 2.5 ± 0.0                       | 95 ± 11                           | 39 ± 8                                   |                                          | 5.7 ± 1.6                |
| ugeAΔ ugeBΔ             | 3.5 ± 0.1                       | 217 ± 62                         | ND                                      |                                          | ND                       |
| ugmAΔ                   | 3.1 ± 0.1                       | 204 ± 10                         | 108 ± 35                                 |                                          | ND                       |
| ugeAΔ ugmAΔ             | 3.2 ± 0.4                       | 162 ± 8                          | 97 ± 23                                  |                                          | 8.1 ± 0.3                |

* There was no significant difference in the hyphal width for fixed or live hyphae measured by either confocal microscopy or atomic force microscopy (AFM).

b Measured from TEM hyphal cross sections. See Materials and Methods.

c Standard deviations (SDs) were calculated from the standard errors propagated through equations 1 and 2. See Materials and Methods.

d ND, not determined. See Results.

to that of strain AAE1. Instead, the ugeBΔ strain hyphal walls were almost 2-fold thicker than the hyphal walls of strain AAE1 (Table 1 and Fig. 3). Also unexpectedly, the hyphal walls of the ugeAΔ ugeBΔ double deletion strain were about twice as thick as those of either single deletion strain and even thicker than those of the ugeAΔ ugmAΔ double deletion strain (Table 1 and Fig. 3). TEM images of the ugeAΔ (see Fig. Cb in Appendix A in reference 8) and ugeBΔ (data not shown) strains grown in liquid shaking cultures accumulated debris, not observed for the same strains grown on dialysis tubing (Fig. 3) or for the ugeAΔ ugeBΔ double deletion strain (data not shown).

Atomic force microscopy imaging of wild-type and Gal/ gene deletion strains. We used AFM imaging to acquire high-spatial-resolution information about the hyphal wall surfaces of two wild-type strains and four Gal biosynthesis gene deletion strains. AFM imaging provides quantitative depth resolution, thus facilitating surface subunit measurements (18, 19). Our previous work demonstrated that for the wild-type strain, A28, the walls of hyphal tips and tips of lateral branches had matured by 3 μm behind the apex, at which point their surfaces resembled unbranched regions 20 μm and 40 μm behind the tip, respectively (18). To ensure that the wall surfaces were mature for all strains, we chose analysis sites that were at least 40 μm behind the hyphal tips, expecting that wall maturation might be slower in the Gal biosynthesis gene deletion strains. AFM data can be collected from living or fixed cells (e.g., reference 18). We present images of fixed hyphae for comparing wall surfaces among the suite of Gal biosynthesis gene deletion strains, since hyphal wall subunit size and distribution were similar to those of living cells but were more clearly defined (18).

Contact mode AFM imaging simultaneously collects topography and lateral force information. The latter represents a convolution of topography and tip-sample interactions for rough samples, thus producing relief images with more clearly defined edge features (18). High-resolution images of the hyphae from fixed wild-type strain (AAE1) and Gal biosynthesis gene deletion strain (Fig. 4) show distinct differences in their surface subunit size (Table 1) and packing. The hyphae of strain AAE1 had small rounded subunits with a consistent size and even packing. In contrast, both the ugeAΔ and ugmAΔ single deletion strains had substantially larger hyphae and more variable-sized hyphal wall surface subunits than strain AAE1 and also had less organized subunit packing. The hyphal wall of the ugeBΔ strain most closely resembled that of strain AAE1, but with slightly elongated subunits. The hyphal surface of the ugeAΔ ugeBΔ double deletion strain was notable in that its...
surface appeared fibrillar in the topographic images; hence, maximum subunit sizes were not measured. Thus, both UgeA and UgeB appear to be important for wild-type hyphal wall surface formation. The hyphal wall surface subunits of the $ugeA^{+/H9004}$ $ugmA^{+/H9004}$ double deletion strain were similar in size to those of the $ugmA^{+/H9004}$ single deletion strain. Taken together, each member of the suite of Galf deletion strains produced a distinctive wall phenotype.

**Cell wall viscoelasticity and adhesion of wild-type and Galf biosynthesis gene deletion strains.** Viscoelasticity is the property describing materials such as hyphal walls, which exhibit both viscous (fluid-like) and elastic mechanical properties. Cell wall spring constants measured by FS (Fig. 5A, segment b-c) were used to calculate the viscoelastic modulus for both fixed and live hyphae of strain AAE1 and the suite of Galf gene deletion strains (Table 1). Cell wall viscoelastic moduli for the single deletion strains, $ugeA^{+/H9004}$ and $ugeB^{+/H9004}$ single deletion strains, were significantly lower than that of strain AAE1 (Table 1). Viscoelastic moduli of the $ugmA^{+/H9004}$ single deletion strain and the double deletion strains ($ugeA^{+/H9004}$ $ugeB^{+/H9004}$ and $ugeA^{+/H9004}$ $ugmA^{+/H9004}$) were at least an order of magnitude smaller than strain AAE1 (Table 1).

**FIG. 4.** Atomic force microscopy images of wild-type and Galf biosynthesis gene deletion strains used in this study. The gray scale for lateral force images is $-2$ nA, and for topography images, it ranges from 50 to 70 nm. All images were photographed at the same magnification. Bar = 200 nm.
Notably, viscoelastic moduli of fixed hyphal walls were typically 3-fold higher than that of live ones (Table 1). Hyphal wall viscoelasticity for the suite of Galf deletion strains exhibited the same trend for fixed and live hyphae.

The Si$_3$N$_4$ AFM probes used in this study have hydrophilic surfaces. We used the e-f segment of the FS curve (Fig. 5A) to quantify adhesion between A. nidulans wall surfaces and the AFM tip during the retraction phase. The wall surface adhesion of strain AAE1 to Si$_3$N$_4$ is shown in Table 1. The hyphae of the ugmAΔ single deletion strain and ugeAΔ ugmAΔ double deletion strain had significantly ($P < 0.05$) stronger adhesion to the hydrophilic tip than wild-type hyphae.

**DISCUSSION**

Our most notable finding is that perturbing A. nidulans cell wall maturation by deleting genes in the Galf biosynthesis pathway has profound effects on wall surface subunit size and packing that are directly associated with cell wall viscoelasticity. This is despite the fact that none of these genes is essential for growth in culture. Previously, we used AFM and FS to show for the first time that growing hyphal tips of a wild-type A. nidulans strain, A28 (18), had wall surface characteristics that were consistent with long-accepted models of wall deposition and maturation (2, 26) that had yet to be quantitatively tested.

El-Ganiny et al. (8, 9) had shown using molecular biology, fluorescence microscopy, and TEM that the hyphal morphogenetic defects of the ugeAΔ and ugmAΔ deletion strains appeared to be correlated with a lack of immunolocalizable wall Galf and to aberrant hyphal wall architecture. Now, we have used high-spatial-resolution AFM imaging and FS to directly quantify wall defects in this suite of Galf biosynthesis gene deletion strains and to compare them to wild-type strains. 

**Galf is required for wild-type Aspergillus nidulans hyphal wall formation.** This AFM study is the first to make quantitative measurements of cell wall surface subunit features, wall viscoelasticity, and adhesive properties of A. nidulans strains that had been deleted for enzymes having roles in Galf biosynthesis. Strong but circumstantial data in the study by El-Ganiny et al. (9) showed that A. nidulans strains lacking UgmA had defective hyphal morphogenesis, colony growth, and spore development deficits that correlated with lack of immunodetectable wall Galf. Using TEM cross sections, El-Ganiny et al. (9) showed that hyphal walls of the ugmAΔ strain were more than four times the thickness of the hyphal walls of strain AAE1 and had poorly consolidated surfaces, suggesting that Galf may play a role in wall organization.

Previously, we showed using AFM imaging that growing tips of wild-type A. nidulans had ellipsoidal surface subunits that were larger and more variable in size than the round subunits found 3 μm or further back (Fig. 3 in reference 18). Consistent with the decrease in subunit size and improved organization as a function of maturation, we showed an increase in surface hydrophobicity (Fig. 6D in reference 18), which was attributed to decreased exposure of sugar hydroxyl groups. El-Ganiny’s study (9) suggested that the walls of the ugmAΔ strain were weaker than those of the wild type, since this phenotype was partially alleviated by growth on 1 M sucrose.

Our present study shows that both the ugeAΔ and ugmAΔ single deletion strains had substantially larger surface subunits than strain AAE1. In contrast, the ugeBΔ single deletion strain, which had wild-type colony phenotype and growth rate, had subunit sizes very similar to those of strain AAE1. Thus, it appears that A. nidulans surface subunit size is inversely correlated with cell wall maturation in strain A28 (18) and a suite of deletion mutants in the Galf biosynthetic pathway. Our data also show that wall surface organization correlates with wall viscoelastic moduli and that these data are mirrored by the thickness and surface layer characteristics visualized in hyphal cross sections using TEM.

Viscoelastic moduli of fixed and living cell walls had a strong positive correlation, demonstrating the value of comparing fixed strains, thus reducing data collection time. The viscoelastic moduli of fixed hyphal walls were consistently larger, revealing the relationship between chemical cross-linking of the cell wall and its viscoelasticity. The data offer insight into the enzymatic cross-linking of hyphal wall components as an integral step in wall maturation, where cross-linking likely contributes to wall integrity by increasing viscoelasticity.

Strains A4 (28), A28 (18), and AAE1 (current work) are
morphological wild-type strains. The cell wall viscoelastic modulus of the fixed AE1 strain was lower than that determined for fixed, rehydrated A4 by Zhao and coworkers (28). Although both studies used the same method to determine cantilever spring constants (5), it is only an estimate and can account for the difference in viscoelastic moduli. The viscoelastic modulus of live AE1 cell walls (Table 1) was lower than that reported previously for A28 (115 ± 31 MPa; 18). However, since the latter study compared viscoelastic moduli in different regions along single hyphae, cantilevers were not calibrated.

We used Zhao’s model (18), which assumes the indentation of a contiguous layer (cell wall) surrounding a large cylinder (hyphae; Fig. 5B), to calculate cell wall viscoelastic moduli. A plot of the dimensionless unit $Eh/k_0$ versus $(R/h)^1.5$ (data not shown) suggests that this model fits strain AE1 and ugeAΔ and ugeBΔ single deletion strains better than it does the poorly ordered walls of the ugmAΔ single deletion strain and the ugeAΔ ugeBΔ and ugeAΔ ugmAΔ double deletion strains. Differences can at least in part be attributed to the composition and organization of cell wall components (Fig. 5B), whereas the viscoelastic modulus data for the ugmAΔ single deletion strain and ugeAΔ ugeBΔ and ugeAΔ ugmAΔ double deletion strains suggest that the AFM tip may penetrate the loosely packed cell wall surface. The Si$_3$N$_4$ AFM tips used in this study have tips that are about 10 nm wide (18). The subunits of the ugmAΔ and ugeAΔ ugmAΔ strains are 10-fold larger (Table 1), so the AFM tip could possibly pierce an individual subunit. The surface of the ugeAΔ ugeBΔ strain has a fibrillar appearance (Fig. 5C), so its interaction with the AFM tip could be unlike the other deletion strains we studied.

Galf appears to mediate *Aspergillus nidulans* hyphal wall surface and hyphal adhesion. Lamarre et al. (14) suggested that hyphal wall Galf plays a role in *A. fumigatus* hyphal wall surface properties, which they showed qualitatively by the accumulation of material on $Afugm1Δ$ hyphal walls using scanning electron microscopy (SEM) and by hyphal adhesion to substrates, including glass and plastic coverslips, latex beads, and epithelial respiratory cells.

We quantified the adhesion between the hydrophilic Si$_3$N$_4$ AFM tip and walls of living hyphae of strain AE1, the ugmAΔ single deletion strain, and the ugeAΔ ugmAΔ double deletion strain. The increasing hyphal wall surface disorder in ugmAΔ and ugeAΔ ugmAΔ deletion strains correlates with effects on hyphal wall viscoelasticity and adhesion. The loose packing of hyphal wall surfaces observed by AFM imaging of Galf mutants would expose polar groups normally masked during wall maturation, increasing hydrophilic character of the wall surface and resulting in greater adhesion. Consistent with the results of Lamarre et al. (14), the ugmAΔ single deletion strain and the ugeAΔ ugmAΔ double deletion strain tended to adhere to microscope coverslips compared to strain AE1 and the ugeBΔ single deletion strain (data not shown). The adhesion values between the Si$_3$N$_4$ tip and the walls of ugmAΔ and ugeAΔ ugmAΔ deletion strains were comparable to those previously reported for A28 hyphae at growing tips, where the wall is newly deposited and not yet mature (~9 nN [18]). Thus, surface subunit size, wall viscoelasticity, and wall adhesion to hydrophilic surfaces show consistent trends for wild-type and Galf gene deletion strains.

Schmalhorst et al. (23) provided data to suggest that the *A. fumigatus* gflAΔ (homologous to *A. nidulans* ugmA [9]) strain had attenuated virulence in a murine model for systemic aspergillosis. However, scanning electron microscopy of cross-fractured gflA Δ hyphal walls were half the thickness of wild-type *A. fumigatus* walls, the opposite trend to our study that deserves further attention.

By combining gene deletion characterizations with TEM, AFM, and FS, we have shown that Galf is important for *A. nidulans* hyphal wall maturation. However, despite the strong correlation between our results and those of Lamarre et al. (14) we are not yet able to determine the likely location(s) of Galf-containing molecules in *A. nidulans* walls. Indeed, Latgé’s 2010 model (16) discusses these molecules without indicating many details relating to their deployment in the three-dimensional wall architecture.

We have shown that perturbing wild-type Galf cell wall deposition has substantial effects on the surface ultrastructure and viscoelasticity of the *Aspergillus nidulans* cell wall. Galf appears to have crucial and multiple roles in *Aspergillus* hyphal cell wall maturation and integrity. Studies addressing potential roles of mannose in *A. nidulans* wall structure and adhesive properties and potential roles of Galf in pathogenicity are under way.

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REFERENCES

1. Bar-Peled, M., C. L. Griffith, J. J. Ory, and T. L. Doering. 2004. Biosynthesis of UDP-GlcA, a key metabolite for capsular polysaccharide synthesis in the pathogenic fungus *Cryptococcus neoformans*. Biochim. Biophys. Acta 1681:131–136. doi: 10.1016/S0006-3495(04)00316-0.
2. Bartnicki-Garcia, S., C. E. Bracker, G. Gierz, R. Lopez-Franco, and H. Lu. 2000. Mapping the growth of fungal hyphae: orthogonal cell wall expansion during tip growth and the role of turgor. *Biophys. J.* 79:2382–2390. doi: 10.1016/S0006-3495(00)76483-6.
3. Beverley, S. M., et al. 2004. Native cryogenic UDP-galactopyranosyl mutase (GFL gene) in microbial and metazoal pathogens. *Eukaryot. Cell* 1147–1154. doi: 10.1128/EC.4.6.1147-1154.2005.
4. Cappella, B., and G. Dietler. 1999. Force-distance curves by atomic force microscopy. *Surf. Sci. Rep.* 34:5–104.
5. Cleveland, J. P., and S. Mann. 1993. A nondestructive method for determining the spring constant of cantilevers for scanning probe microscopy. *Rev. Sci. Instrum.* 64:403–405.
6. Danveld, R. A. 2008. A novel screening method for cell wall mutants in *Aspergillus niger* identifies UDP-galactopyranosyl mutase as an important protein in fungal cell wall biosynthesis. *Genetics* 178:783–811.
7. de Groot, P. W., et al. 2009. Comprehensive genomic analysis of cell wall genes in *Aspergillus nidulans*. *Fungal Genet. Biol.* 46(Suppl. 1):S72–S81.
8. El-Gamawy, A. M., I. Sheoran, D. A. R. Sanders, and S. G. W. Kaminskyj. 2010. *Aspergillus nidulans* ugmAΔ: UDP-glucose-4-epimerase UgeA has multiple roles in wall architecture, hyphal morphogenesis, and asexual development. *Fungal Genet. Biol.* 47:629–635. doi: 10.1016/j.fgb.2010.03.002.
9. El-Gamawy, A. M., D. A. R. Sanders, and S. G. W. Kaminskyj. 2008. *Aspergillus nidulans* UgdA GALACTOPYRANOXYLose mutase, encoded by ugm4 plays key roles in colony growth, hyphal morphogenesis, and conidiation. *Fungal Genet. Biol.* 45:1533–1542. doi: 10.1016/j.fgb.2008.09.008.
10. Gastebois, A. 2009. *Aspergillus fumigatus*: cell wall polysaccharides, their biosynthesis and organization. *Future Microbiol.* 4:583–595.
11. Harris, S. D. 2009. Morphology and development in *Aspergillus nidulans*: a complex puzzle. *Fungal Genet. Biol.* 46(4 Suppl):1582–892.
12. Kamps, J. G., S. G. W., and J. E. Hamer. 1998. Hyp loci control cell pattern formation in the vegetative mycelium of *Aspergillus nidulans*. *Genetics* 148:669–680.
13. Kaminskyj, S. G. W. 2001. Fundamentals of growth, storage, genetics and microscopy of Aspergillus nidulans. Fungal Genet. Newsl. 48:25–31.
14. Lamarre, C., et al. 2009. Galactofuranose attenuates cellular adhesion of Aspergillus fumigatus. Cell. Microbiol. 11:1612–1623.
15. Latgé, J.-P. 2009. Galactofuranose containing molecules in Aspergillus fumigatus. Med. Mycol. 47(Suppl. 1):S104–S109. doi:10.1080/13693780802258832.
16. Latgé, J.-P. 2010. Tasting the fungal cell wall. Cell. Microbiol. 12:863–872. doi:10.1111/j.1462-5822.2010.01474.x.
17. Lesage, G., and H. Bussey. 2006. Cell wall assembly in Saccharomyces cerevisiae. Microbiol. Mol. Biol. Rev. 70:317–343.
18. Ma, H., L. A. Snook, S. G. W. Kaminskyj, and T. E. S. Dahms. 2005. Surface ultrastructure and elasticity in growing tips and mature regions of Aspergillus hyphae describe wall maturation. Microbiology 151:3679–3688. doi:10.1099/mic.0.28328-0.
19. Ma, H., L. A. Snook, C. Tian, S. G. W. Kaminskyj, and T. E. S. Dahms. 2006. Fungal surface remodelling visualized by atomic force microscopy. Mycol. Res. 110:879–886. doi:10.1016/j.mycres.2006.06.030.
20. Momanly, M., P. J. Westfall, and G. Abramowsky. 1999. Aspergillus nidulans swo mutants show defects in polarity establishment, polarity maintenance and hyphal morphogenesis. Genetics 151:557–567.
21. Moyrand, F., I. Lafontaine, T. Fontaine, and G. Janbon. 2008. UGE1 and UGE2 regulation of the UDP-glucose/UDP-galactose equilibrium in Cryptococcus neoformans. Eukaryot. Cell 7:2069–2077.
22. Perfect, J. R. 2005. Nuances of new anti-Aspergillus antifungals. Med. Mycol. 43(Suppl. 1):S271–S276.
23. Schmalhorst, P. S., et al. 2008. Contribution of galactofuranose to the virulence of the opportunistic pathogen Aspergillus fumigatus. Eukaryot. Cell 7:1268–1277. doi:10.1128/EC.00109-08.
24. Shi, X., Y. Sha, and S. G. W. Kaminskyj. 2004. Aspergillus nidulans hypA regulates morpohogenesis through the secretion pathway. Fungal Genet. Biol. 41:75–88.
25. Wallis, G. L., F. W. Hemming, and J. F. Peberdy. 2001. Beta-galactofuranoside glycoconjugates on conidia and conidiophores of Aspergillus niger. FEMS Microbiol. Lett. 201:21–27.
26. Wessels, J. G. 1999. Fungi in their own right. Fungal Genet. Biol. 27:134–145. doi:10.1006/fgbi.1999.1125.
27. Xu, S., and M. F. Arnsdorf. 1994. Calibration of the scanning (atomic) force microscope with gold particles. J. Microsc. 173:199–210.
28. Zhao, L., et al. 2005. Elastic properties of the cell wall of Aspergillus nidulans studied with atomic force microscopy. Biotechnol. Prog. 21:292–299.