Characterization of Aspergillus nidulans Mutants Deficient in Cell Wall Chitin or Glucan

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Received 10 September 1991/Accepted 29 October 1991

By screening for the osmotically remediable phenotype, mutations in two genes (orlA and orlB) affecting the cell wall chitin content of Aspergillus nidulans were identified. Strains carrying temperature-sensitive alleles of these genes produce conidia which swell excessively and lyse when germinated at restrictive temperatures. Growth under these conditions is remedied by osmotic stabilizers and by N-acetylglucosamine (GlcNAc). Remediation by GlcNAc suggests that the mutations affect early steps in the synthesis of chitin. Temperature and medium shift experiments indicate that the phenotype is the result of decreased synthesis rather than increased chitin degradation and that osmotic stabilizers act to stabilize a defective wall rather than to stabilize the gene product. Two genes, orlC and orlD, which affect cell wall β-1,3-glucan content were also identified. Walls from strains carrying mutations in these genes exhibit normal amounts of α-1,3-glucan and chitin but reduced amounts of β-1,3-glucan. As for the chitin-deficient mutants, orlC and orlD mutants spontaneously lyse on conventional media but are remedied by osmotic stabilizers. These results indicate that both chitin and β-1,3-glucan are likely to contribute to the structural rigidity of the cell wall.

The cell wall plays an important role in the growth and development of the fungi. In addition to its function as the primary osmotic barrier of the cell, the temporal and spatial regulation of wall polymer synthesis is critical to the morphogenesis of the cell types characteristic of many fungi. The architecture and synthesis of the cell walls of fungi are poorly understood. For a majority of the fungi, including most of those of medical and economic importance, chitin and/or β-1,3-glucans are believed to be the most important structural polysaccharides (3). Many studies of wall synthesis have concerned the enzymology of chitin synthase and, to a lesser extent, β-1,3-glucan synthase.

In the yeasts or yeastlike fungi such as Saccharomyces cerevisiae, Candida albicans, and Schizosaccharomyces pombe, chitin is a relatively small fraction of the cell wall. In S. cerevisiae, chitin is found predominantly in the primary septa between cells (9, 10, 26). Three related genes (chs1, chs2, and cal1) involved in chitin synthesis from S. cerevisiae (7, 29, 32) and one from C. albicans (1) have been sequenced. The finding that the chs1 (chitin synthase 1) gene from S. cerevisiae, which codes for the major chitin synthase activity, could be disrupted without affecting chitin synthesis was unexpected (7). In addition, at least under some conditions, both the chs1 and chs2 genes can be disrupted without affecting the majority of chitin synthesis (5). Recently, a gene (cal1) with regions of homology to chitin synthases 1 and 2 and required for chitin synthase 3 activity was identified (32). Chitin synthase 3 was shown to be responsible for chitin synthesis in the portion of the wall not associated with the septum and to be responsible for the chitin ring formation which precedes budding (26). Chitin synthase 2 is apparently responsible for septal chitin synthesis (26, 30), and chitin synthase 1 is believed to be involved in repair synthesis (11). Mutants of S. cerevisiae have been obtained which possess less than 10% of the normal chitin content (6, 24), indicating that yeast cells are relatively insensitive to perturbations in chitin content.

In contrast to yeasts, chitin forms a larger fraction of the wall in most filamentous fungi and is found in substantial amounts in both lateral walls and in septa. Several years ago, Cohen et al. (15) and Katz and Rosenberger (18) isolated a conditional mutation in the tsE gene of the filamentous ascomycete Aspergillus nidulans. Strains carrying the mutant tsE6 allele possess less than 10% of the normal chitin levels at restrictive temperature. At restrictive temperature in conventional media, spores (conidia) from the mutant swell excessively and lyse, indicating the importance of chitin to the rigidity of the wall. The phenotype could be remedied by high osmotic pressure or the presence of glucosamine or N-acetylglucosamine (GlcNAc) in the medium. Presumably, the mutation resides in a gene affecting an early step in the biosynthesis of amino sugars. A mutation in a second gene, gcnA, was shown to possess a similar phenotype (2).

By screening for the osmotically remediable phenotype, we isolated mutations in two additional genes (orlA and orlB) involved in chitin synthesis by A. nidulans. We also identified mutations in two genes (orlC and orlD) which affect the alkali-insoluble β-1,3-glucan content. As for the chitin-deficient mutants, strains carrying mutations in the orlC and orlD genes lyse in conventional media and are osmotically remediable, indicating that the β-1,3-glucan is likely to contribute to the structural rigidity of the wall. Here, we report some characteristics of strains carrying those mutations.

MATERIALS AND METHODS

Strains and Growth Conditions. Strains of A. nidulans with an FGSC prefix were obtained from the Fungal Genetics Stock Center (University of Kansas, Kansas City). Strains with a PB or CF prefix were constructed in our laboratory by conventional techniques (14). The genotypes of the strains used are PB 001 (yA2 pabaAl); PB 005 (yA2 pabaAl orlA1);
Cultures were grown from conidia on a complex medium (YG) containing 0.5% yeast extract and 1% glucose. Osmotically stabilized media contained 1.0 M sorbitol or 0.6 M KCl. Solid media contained 1.5% agar. Conventional minimal medium and supplements were prepared by the method of Clutterbuck (14). In minimal medium, some of the mutants described tend to exhibit a more pronounced phenotype when the normal salt concentration is reduced. Consequently, in some experiments a low-salt minimal (LSM) medium was used which contained (per liter) 2.5 g of carbon source, 1.0 g of NaNO₃, 0.5 g of MgSO₄ · 7H₂O, and 0.75 g of KH₂PO₄. Trace metals were added at the usual concentrations (14).

Isolation of osmotically remediable mutants. Conidia of strain PB 001 were treated with N-methyl-N,N-nitro-N-nitrosoquinuclidine to about 10% survival by the method of Martinek and Clutterbuck (21). The spores were plated at a density of approximately 200 survivors per plate on YG medium containing 0.01% Triton X-100 and were incubated at 32°C for 2 to 3 days. Conidiating colonies were then replica plated onto YG medium and YG medium supplemented with 6% KCl or 6% NaCl. The replicas were incubated for 16 h at 42°C. Colonies requiring the increased salt content and showing microscopic evidence of lysis on the unsupplemented plates were retained for further examination.

In some experiments, mutagen-treated spores were initially plated on YG medium containing 0.01% Triton X-100, 100 µg of GlcNAc per ml, and 6% KCl; plates were incubated at 32°C. Colonies arising after 2 to 3 days were replica plated onto YG medium plus 100 µg of GlcNAc per ml and YG medium supplemented with 100 µg of GlcNAc per ml and 6% KCl. Replicas were incubated at 32°C for 16 h. Individual colonies requiring the increased salt content and showing microscopic evidence for lysis on the unsupplemented plates were retained for analysis.

Preparation and analysis of cell walls. The cells from a 24-h-old culture grown in YG medium were harvested by filtration on Miracloth (475855; Calbiochem). The mycelial pad was suspended in a convenient amount of 250 mM sodium phosphate buffer (pH 6.8). The suspension was blended for 30 s in a minicup of a Waring blender. The resulting suspension was passed through a French pressure cell twice at 20,000 lb/in². The homogenate was centrifuged at 1,400 × g max for 5 min at 4°C. The pellet was retained and washed with 40 ml of phosphate buffer and recentrifuged ten times. Finally, the pellet was washed five times with distilled water and lyophilized.

For the analysis of total GlcNAc content, 10-mg samples of walls were digested for 16 h in 1.0 ml of 50 mM sodium citrate-NaOH buffer (pH 5.8) containing 2.0 mg of Novozyme 234 (492004; Calbiochem) per ml, 10 µl of β-glucuronidase (G 0763; Sigma) per ml, and 0.02% sodium azide. Following digestion, the suspension was centrifuged for 1 min in a microcentrifuge, and aliquots of the supernatant were assayed for amino sugars by the method of Ghuysen et al. (17). Preliminary experiments had shown that the recovery of amino sugars by this method was almost twice that from any of a variety of methods employing acid hydrolysis.

For the analysis of total wall glucan, 10-mg samples of walls were suspended in 1.0 ml of concentrated formic acid and heated at 100°C with occasional mixing until a homogenous suspension was obtained (about 20 min). Aliquots of the suspension were transferred to test tubes and dried in a vacuum desiccator, and the amount of neutral carbohydrate was estimated with phenol-sulfuric acid (16).

For the analysis of distinct glucan fractions, 10-mg samples of walls were suspended in 1.0 ml of distilled water and then heated at 100°C for 30 min. The suspension was centrifuged for 1 min in a microcentrifuge, and the supernatant was removed and assayed with phenol-sulfuric acid. The pellet was dried and suspended in 1.0 ml of 1.0 M KOH and heated for 30 min at 70°C. The suspension was centrifuged, and the supernatant was removed and assayed with phenol-sulfuric acid. The KOH-insoluble pellet was dried and suspended in 1.0 ml of concentrated formic acid or water and heated at 100°C until a uniform suspension was obtained, and aliquots were assayed with phenol-sulfuric acid. In experiments in which the alkali-insoluble fraction was digested with β-1,3-glucanase, the fraction was suspended in 50 mM sodium citrate-NaOH buffer (pH 5.0) containing 100 mM of laminarinase (L 5144; Sigma) per ml and 0.02% sodium azide. Digestion was done at 37°C for 16 h.

Microscopy. Cells were grown on the surface of YG plates at the indicated temperatures. Cells were viewed and photographed directly on the plates after being covered with a coverglass. A Zeiss standard microscope equipped with an MC 100 camera attachment and a Planapo 63 oil immersion 1.4 N.A. objective was used. Bright-field illumination was utilized.

### RESULTS

**Mutant isolation and wall composition.** We screened approximately 40,000 colonies for the temperature-sensitive osmotically remediable lysis (orl) phenotype. Forty isolates showing cell lysis usually combined with hyphal and spore ballooning at the restrictive temperature were chosen for cell wall carbohydrate analysis. Walls from the strains were purified from cells grown at 32°C and at 42°C in the presence of 1.0 M sorbitol. In agreement with previous reports (8, 15, 18, 23, 35), acid hydrolysis of walls and then thin-layer chromatography showed that the predominant carbohydrates in the wall preparations were glucose and glucosamine, with minor amounts of galactose and mannose (data not shown). Thus, the phenol-sulfuric acid assay which reacts with all three hexoses provides a slight overestimate of total glucan content. Table 1 shows the carbohydrate composition from selected strains. Wild-type strains show about 45 to 50% glucan (by weight) and about 40% GlcNAc.

<table>
<thead>
<tr>
<th>Strain</th>
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<th>Permissive*</th>
<th>Restrictive*</th>
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<tr>
<td></td>
<td></td>
<td>Glucose</td>
<td>GlcNAc</td>
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<tr>
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<td>43.8</td>
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<td>orlAI</td>
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<td>37.1</td>
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<td>orlBI</td>
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<td>ts6E</td>
<td>48.5</td>
<td>36.0</td>
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<tr>
<td>CF 002</td>
<td>orlCl</td>
<td>—b</td>
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<tr>
<td>CF 020</td>
<td>orlDI</td>
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* For strains PB 001, PB 043, PB 060, and FGSC 725, permissive temperature was 32°C and restrictive temperature was 42°C. orlCl and orlDI are not temperature-sensitive alleles, and strains CF 002 and CF 020 were grown at 32°C.

b —, not determined.
irrespective of growth temperature. The recoveries of the sugars are considerably higher than those previously reported since acid hydrolysis was not used, thereby eliminating the concomitant degradation. About 60% of the mutants we isolated showed no significant deviations in glucan and chitin content from wild-type values at restrictive temperatures (data not shown), and this class was not further analyzed. About 40% of the isolates fell into a class which shows almost normal glucan and GlcNAc levels at permissive temperature but contain reduced GlcNAc content at the restrictive temperature (Table 1, strains PB 043 and PB 060). Presumably, these represent chitin deficiencies. Note that the chitin-deficient mutants show an increase in glucan content due to the fact that the data are expressed as grams of glucose equivalents per gram (dry weight) of walls.

In an attempt to isolate chitin-deficient mutants which could not be remedied by exogenous GlcNAc (see below), we modified the screening procedure by including GlcNAc in the plating medium and in the replica plating media. Surprisingly, none of the osmotically remediable isolates we obtained show any obvious deficiencies in chitin content. Two nonconditional isolates, however, show decreased glucan content (Table 1, strains CF 002 and CF 020). The decreased glucan content also results in an apparent increase in chitin content of the walls.

The walls of aspergilli have both α- and β-linked glucans. The α-glucans are soluble in hot alkali, while the β-glucans are insoluble in hot alkali and are believed to be covalently linked to chitin and thus contribute to the structural rigidity of the wall (27, 28, 31, 33). The walls of the glucan-deficient mutants were fractionated and assayed for neutral carbohydrates. The results are shown in Table 2. For both of the mutant strains, the hot-alkali-insoluble glucan content is about one-half that of the wild type. Digestion of the wild-type alkali-insoluble fraction with β-1,3-glucanase solubilized 80% of the phenol-sulfuric acid-reactive material, indicating that it is predominantly β-1,3-glucan. Digestion of the same fraction from strain CF 002 liberated 78% of the remaining phenol-sulfuric acid-reactive material, indicating that it is also predominantly β-1,3-glucan. For strain CF 020, glucanase digestion resulted in the solubilization of only 40% of the phenol-sulfuric acid-reactive material, indicating that this assay considerably underestimates the β-1,3-glucan deficiency in this strain. The nature of the glucanase-resistant material is unknown.

The alkali-soluble glucan (presumably α-glucan) content from the mutants (Table 2) is higher than that of the wild type. This elevation is likely to be due to the fact that the data are expressed on the basis of grams of glucose equivalents per gram (dry weight) and the alkali-soluble content is decreased. Thus, it is likely that the mutations do not affect alkali-soluble glucan (α-glucan) synthesis.

Complementation analysis and mapping. Thirteen temperature-sensitive chitin-deficient isolates formed a single complementation group based on analysis in heterokaryons, and the gene represented was designated orlA. Two isolates formed a second complementation group designated orlB. Strains carrying orlA or orlB mutant alleles complemented the tsE6 and gcnA95 mutations, and no isolates representing mutations in these previously described genes were obtained in our screening.

The orlA1, orlB1, and tsE6 mutations were mapped to chromosomes by constructing diploids between an appropriate mutant strain and a mitotic mapping strain. Haploid segregants were obtained by growth of the diploids in the presence of 0.40 μg of benomyl per ml. Linkage of the mutation to be mapped relative to markers from the mapping strain was determined (data not shown). orlA was mapped to chromosome VI, orlB was mapped to chromosome I, and tsE was mapped to chromosome V. All three of the mutations were recessive in heterokaryons and diploids. orlC1 and orlD1 were not mapped, but each of the alleles behaved as a single gene in crosses with wild-type strains (data not shown).

Preliminary mapping of orlA on chromosome VI indicated linkage to nicC and methB. PB 043 was crossed with PB 091, and random spore analysis was performed on 332 ascospores from a single cleistothecium. The orlA gene mapped 7.8 centimorgans from nicC and 4.2 centimorgans from methB (Table 3). The tsE gene was mapped in a cross between FGSC 725 and FGSC 491. Analysis of 343 ascospores from a single cleistothecium (Table 3) showed tsE to map between the pA gene (20.4 centimorgans) and the lsbB gene (39.4 centimorgans). Analysis of another 248 isolates from another cleistothecium from the same cross gave essentially the same results.

Following crosses of the primary glucan-deficient isolates into nutritionally complementing genetic backgrounds, heterokaryons derived from strains carrying each mutant allele were isolated on minimal medium containing KCl as an osmotic stabilizer. Complementation of the orl alleles was then tested by transferring mycelia from the heterokaryons to minimal medium lacking KCl and comparing the growth of the heterokaryons with that of the parental strains. Complementation was not detected. Thus, the isolates define two additional genes, orlC and orlD.

Remediation of orlA1 and orlB1 by osmotic stabilizers and GlcNAc. At restrictive temperatures, strains carrying either the orlA1 or orlB1 mutant allele can be remedied in the presence of 1.0 M concentrations of sorbitol, sucrose, KCl,
or NaCl (Fig. 1). At restrictive temperature in the absence of osmotic stabilizers, strains carrying either mutant allele can be remedied by the presence of 100 μg of GlcNAC per ml (Fig. 1). Glucosamine at the same concentration was capable of remediation of growth to a lesser degree than GlcNAC (data not shown). Figure 1 shows that GlcNAC in the presence of glucose does not completely restore the wild-type radial growth rate. In addition, although vegetative hyphal growth is partially remedied by GlcNAC, conidiation is blocked in the orlAI and orlBI mutants. In Fig. 1, the light color of the wild-type colony is the result of profuse spore (conidium) formation on the surface of the mycelium. In contrast, the dark color of the mutant colonies in the presence of GlcNAC results from a lack of spore formation. Similar results are obtained in the presence of 0.25% GlcNAC as the sole carbon source. GlcNAC is also unable to completely restore the normal growth rate and sporulation of tsE6 and gcnA95 at restrictive temperatures (data not shown).

Morphological properties of the mutants. Germination of wild-type conidia in YG medium is accompanied by a swelling of the spores to about twice their original diameter and then emergence of one or two hyphal germ tubes about 6 h after inoculation (Fig. 2A and B). At permissive temperatures, strains carrying the orlAI or orlBI allele appear morphologically normal (data not shown). Microscopic observation of the edges of colonies on solid media, however, showed that a fraction of hyphal tips had lysed (data not shown). In comparison, wild-type colonies seldom show tip lysis under normal conditions. This observation suggests that the mutant gene products have only partial activity at permissive conditions. At 42°C, the conidia of both the orlAI and orlBI strains swell excessively. Most of the swollen spores do not give rise to germ tubes but eventually lyse (Fig. 2C and D). A fraction of the spores do form germ tubes but are misshapen, and these cells also lyse (Fig. 2E). At 42°C in the present of 1.0 M sorbitol, the mutant spores swell to sizes larger than those of wild-type germinals but form hyphae with normal morphology (Fig. 2F and G). The normal hyphal morphology indicates that the polarity of deposition of other polymers is unaffected in the chitin-deficient mutants. The excessive swelling of the mutant spores suggests that the osmotic pressure within the germinating spore is greater than that of hyphae.

When cells from an orlAI mutant germinated at 42°C in the presence of osmotic stabilizers are shifted to media without the stabilizer, lysis occurs immediately irrespective of the incubation temperature (Fig. 3A). We interpret this as evidence that the wall synthesized in the presence of the stabilizer at restrictive temperature is inherently defective and that the stabilizer does not stabilize the mutant gene product to any substantial degree. This is in agreement with the finding of the reduced GlcNAC content of walls generated under these conditions. Mutants carrying the orlBI allele also act in the same manner (Fig. 3B).

When either orlAI or orlBI mutants are grown at 32°C in the absence of stabilizers and are then shifted to 42°C, lysis is growth dependent; the newly synthesized subapical portions of the hyphae become grossly swollen, and eventually lysis occurs in these zones (Fig. 3C through E). The hyphal apices of these cells often appear normal. These result are consistent with a defect in synthesis rather than increased degradation of chitin synthesized prior to the temperature shift. Subapical swelling and lysis of hyphae by osmotic shock or agents which inhibit wall synthesis has been repeatedly reported in the literature and has been reviewed by Wessels (33).

Figure 4 shows the morphological characteristics of glucan-deficient strains carrying the orlC1 or orlD1 alleles. As for the chitin-deficient mutants, growth of either strain in the absence of stabilizer also leads to spores which swell excessively and give rise to considerable lysis (Fig. 4A and B). Conidia from strains carrying the orlD1 allele swell enormously and often give rise to distorted hyphae which frequently lyse. In the presence of 1.0 M KCl or sorbitol, lysis of orlC1 and orlD1 strains is suppressed (Fig. 4C and D). Strains carrying the orlC1 allele do not form morphologically normal hyphae even in the presence of stabilizers. The hyphae are slow growing and highly vacuolated and exhibit excessive branching (Fig. 4C). Numerous regions of balloononed hyphae are also evident. Strains carrying this mutation form slow-growing compact colonies on solid media supplemented with osmotic stabilizers.

**DISCUSSION**

By screening for osmotically remediable mutants, we identified mutations in two genes involved in chitin synthesis and two genes affecting β-glucan synthesis. This method and modifications of it may be a relatively general way to identify genes participating in a positive manner to wall integrity in *A. nidulans*. These could include genes involved in the enzymatic steps for the synthesis of wall polymers as well as steps involved in the regulation and localization of wall synthesis. It is interesting that many of the osmotically remediable mutants we isolated had no apparent deficiencies in chitin or glucan content. This class might include mutations in genes involved in the synthesis of polymers other than chitin or glucans or in other factors important to wall integrity such as the cross-linking of wall polymers. Chitin and β-1,3-glucan are believed to be cross-linked to form the rigid alkali-insoluble material characteristic of fungal walls (27, 28, 31, 33).

**FIG. 1.** Remediation of orlAI and orlBI strains by osmotic stabilizers or GlcNAC. For all plates, the left colony is PB 001 (wild type), the center colony is PB 005 (orlAI), and the right colony is PB 060 (orlBI). Upper left plate, growth of the strains at 42°C on LSM medium. Upper right plate, growth of the strains at 42°C on LSM medium supplemented with 1.0 M sorbitol. Lower left plate, growth of the strains at 42°C on LSM medium supplemented with 100 μg of GlcNAC per ml. Lower right plate, growth of the strains at 42°C on LSM medium containing 0.25% GlcNAC as the sole carbon source.
Mutations in all four genes (tSE, gcNA, orlA, and orlB) thus far identified as affecting chitin synthesis are remediable by GlcNAc. The first two pathway-specific steps leading to chitin synthesis are the amination and isomerization of fructose-6-phosphate to form glucosamine-6-phosphate and the acetylation of glucosamine-6-phosphate to form N-acetylglicosamine-6-phosphate. Presumably, exogenous GlcNAc enters the pathway at the level of either glucosamine-6-phosphate or N-acetylglicosamine-6-phosphate and can thus remedy mutations affecting the first two enzymatic steps of the pathway. Consistent with this interpretation, strains carrying the tSE6 or orlAI allele exhibit defective fructose-6-phosphate amidotransferase activity (4). It is not clear why the screening procedure has only identified genes acting early in the pathway. In light of the finding of multiple chitin synthases in S. cerevisiae, one possible reason is redundancy of other genes involved in the pathway. We have shown that A. nidulans has at least two chitin synthases which can be distinguished on the basis of metal requirement and pH optimum (unpublished data). GlcNAc supplements to glucose minimal medium or GlcNAc as the sole carbon source is unable to completely restore the wild-type growth rate of orlA and orlB1 mutants. Presumably, the reduced growth rate is a reflection that GlcNAc transport or catabolism is rate limiting. More interesting is the inability of exogenous GlcNAc to support normal sporulation. A. nidulans conidia are produced on specialized aerial hyphae called conidiophores. Perhaps GlcNAc is not efficiently translocated from the surface vegetative hyphae to the aerial conidiophores and thus must be derived by localized synthesis.

It is evident that relatively small alterations in chitin synthesis adversely affect the growth and osmotic stability of A. nidulans cells. Strains carrying either the orlAI or orlB1 allele possess about 40 to 50% of the normal chitin content at restrictive temperature but still show osmotically remediable lysis. This is in contrast to the situation in S. cerevisiae in which strains with a 90% deficiency in chitin content are capable of normal growth (6, 24). This difference in sensitivity to perturbations in synthesis may be a reflection of the higher chitin content in the filamentous fungi and thus the greater importance of chitin to structural wall integrity in these organisms. Interestingly, the fission yeasts such as Schizosaccharomyces pombe have even less chitin than S. cerevisiae (22). In the yeasts, the β-linked glucans are likely to contribute to a greater degree to wall integrity. These differences in the relative importance of particular wall polymers could have implications in the design and utility of antifungal drugs acting at the level of wall polymer synthesis.

The available evidence indicates that α-glucans do not play a significant role in the rigidity of the fungal wall. Genes affecting α-glucan content in A. nidulans have been identified. Strains carrying the melBP2 allele produce hyphae which are deficient in α-1,3-linked glucan, do not secrete melanin, and fail to form cleistothecia (20, 23, 34). The fundamental defect is believed to be in glucan synthesis (23). These strains are not osmotically sensitive. The wetA gene encodes a regulatory protein necessary for the expression of conidium-specific genes including the wA gene (19). wA mutants produce white conidia which lack α-1,3-gluca (12). At restrictive temperatures, wetAB6 mutants lack two layers of the conidial wall formed late in conidial development (25).

FIG. 2. Morphological properties of chitin-deficient mutants. In all photographs, the size bar represents 20 μm. (A) Ungerminated conidia (spores) from strain PB 001 (wild type). (B) Conidia from strain PB 001 germinated at 32°C for 10 h. (C) Conidia from strain PB 005 (orlAI) incubated at 42°C for 18 h on YG medium. Note the high proportion of ungerminated conidia (UC), excessively swollen conidia (SC), and lysed swollen conidia (LSC). (D) Conidia from strain PB 060 (orlB1) incubated at 42°C for 18 h on YG medium. Note the excessively swollen conidia (SC) and lysed swollen conidia (LSC). (E) Conidia from strain PB 005 (orlAI) incubated at 42°C for 18 h on YG medium. Note the lysis of two hyphae at tips (TL). (F) Conidia from strain PB 005 (orlAI) incubated at 42°C for 12 h on YG medium supplemented with 1.0 M sorbitol. (G) Conidia from strain PB 060 (orlB1) grown at 42°C for 12 h on YG medium supplemented with 1.0 M sorbitol.
FIG. 3. Morphological properties of chitin-deficient strains in temperature and medium shift experiments. In all photographs, the size bar represents 20 μm. (A) Cells of strain PB 005 (orlA1) grown from conidia at 42°C for 12 h on YG medium supplemented with 1.0 M sorbitol were shifted to YG medium at 32°C. Note the lysis of the swollen conidia (LSC) and the hyphal apex (TL). (B) Cells of strain PB 060 (orlB1) grown from conidia at 42°C for 12 h on YG medium supplemented with 1.0 M sorbitol were shifted to YG medium at 32°C. Note the lysis of the swollen conidia (LSC) and the hyphal apex (TL). (C) Cells of strain PB 005 (orlA1) grown from conidia at 32°C for 10 h on YG medium were shifted to 42°C for 4 h. Note the swelling of the subapical region of the cells (SAS) and the lysis of a cell at the hyphal apex (TL). (D) Cells of strain PB 060 (orlB1) grown from conidia at 32°C for 10 h on YG medium were shifted to 42°C for 4 h. Note the swelling of the subapical region of most cells (SAS). (E) Edge of a PB 005 colony which had been grown from conidia at 32°C on YG medium for 15 h and whose cells were then shifted to 42°C for 6 h. Note the lysis of the majority of the hyphae in the subapical region.

The outermost layers of conidia are a protein-rich rodlet layer and the α-1,3-glucan layer (12, 13). Conidia from wetA6 strains autolize and are osmotically remediable (19). However, it seems unlikely that the lysis is due to the α-1,3-glucan deficiency since wA3 mutants lack the glucan but do not show spore autolysis. Presumably, other events affected by the wetA6 gene product are responsible for the autolysis. The hyphae of wA3 mutants have not been examined for glucan content but show no osmotic defects.

We identified mutations in two genes affecting the β-1,3-glucan content of cell walls. Strains carrying the mutant alleles lyse in conventional media, indicating that this poly-

FIG. 4. Morphological properties of glucan-deficient strains. In all photographs, the size bar represents 20 μm. (A) Conidia from strain CF 002 (orlC1) were incubated for 16 h at 32°C on YG medium. Note the excessive swelling of the conidia (SC) and the lysis of swollen conidia (LSC). (B) Conidia from strain CF 020 (orlD1) incubated for 16 h at 32°C on YG medium. Note the excessive swelling of the conidia (SC). (C) Conidia from strain CF 002 (orlC1) were incubated for 16 h on YG medium containing 1.0 M sorbitol. (D) Conidia from strain CF020 (orlD1) were incubated for 16 h on YG medium containing 1.0 M sorbitol.
mer(s) is likely to contribute to the structural rigidity of the wall. Thus, at least in *A. nidulans*, and probably in other fungi with the chitin-glucan wall type (3), both chitin and glucan are essential to normal wall function. The synthesis of both the α- and β-linked glucans utilizes uridine diphosphate glucose as a substrate. Since the α-glucan content of the walls of the mutants is apparently unaffected in *orlCl* strains, rather, the genes appear to encode products which directly or indirectly affect only the β-glucan synthesis or deposition in the wall.

**ACKNOWLEDGMENTS**

P.T.B. expresses his thanks for the interest, encouragement, and hospitality shown by William E. Timberlake, Department of Genetics, University of Georgia, in whose laboratory many of the experiments described were performed while P.T.B. was on sabbatical.

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