CELL WALL CHEMISTRY, MORPHOGENESIS, AND TAXONOMY OF FUNGI[1]

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Contents

The prominent role of the cell wall in the structure and behavior of fungi needs little elaboration; with few exceptions, the wall, more than any other cellular part, defines a fungus and distinguishes it from other living creatures. Only during the brief amoeboid or flagellate stages of a minority

[1] The survey of the literature pertaining to this review was concluded in January 1968.

of fungi do we find the fungal protoplast deprived of its characteristic housing. For most fungi, the wall is a permanent and highly versatile home —continuously expanded during growth, extensively remodelled during development. By manipulating cell wall construction, a fungus may assume a variety of characteristic morphologies to suit a wide variety of functions: vegetative growth, substrate colonization, reproduction, dispersal, survival, host penetration, animal predation, etc. In simplified terms, morphological development of fungi may be reduced to a question of cell wall morphogenesis.

This review deals primarily with recent chemical studies on fungal walls and how they relate to taxonomy (phylogeny) and to morphogenesis (ontogeny). For an introduction to the general features of fungal cell walls, the reader is referred to Aronson's chapter (1). The literature dealing specifically with yeast cell walls was the subject of a recent review by Phaff (2).

GENERAL STRUCTURE

Chemically, the fungal cell wall is 80 to 90 per cent polysaccharides with most of the remainder consisting of protein and lipid. Wide departures from these values are rare; e.g., the cell wall of the yeast *Saccharomycopsis guttulata* which contains 40 per cent protein (3). Sometimes substantial amounts of pigments (melanin), polyphosphate, inorganic ions, etc., may also be present. There is partial evidence for the presence of nucleic acids in fungal walls (4–7), but to date no decisive proof of their presence has been provided. Physically, the fungal cell wall is a fabric of interwoven microfibrils embedded in or cemented by amorphous matrix substances. Chitin and cellulose are well known as the microfibrillar or skeletal components of the wall of the vast majority of fungi; whereas, in most true yeasts, the skeletal part is seemingly composed of noncellulosic glucans. Proteins and various polysaccharides (glucans, mannans, galactans, heteropolysaccharides) are probably the cementing substances which bind together the different structural components of the wall into macromolecular complexes.

Protein.—Aronson's (1) cautious reservation on the validity of regarding the protein found in cell wall preparations of filamentous fungi as a true structural component does not seem justified any longer. Although part of the wall protein may be enzymic, and perhaps another portion could be a cytoplasmic contaminant, some of the protein is so firmly bound to the rest of the wall that drastic extractions usually fail to remove it completely [e.g. (4, 7, 8)]. The latter protein is probably an integral part of the structure of the cell wall. There is also growing evidence for glycoprotein complexes in the cell walls of filamentous fungi (9–11), adding to the earlier findings of glycoprotein complexes in yeast walls [see reviews by Nickerson (12, 13)].

Lipid.—Dyke's (14) investigations on *Nadsonia elongata* demonstrated clearly that the lipid found in the cell wall of this yeast was a bona fide component and not a cytoplasmic contaminant; contrary to cytoplasmic lip-

ids, cell wall lipids lack palmitoleic acid and are mainly composed of saturated fatty acids. The role of lipid in fungal cell walls has not been elucidated. Hurst (15) suggested that lipid contributes to the stiffness of the cell wall of *Saccharomyces cerevisiae*. Another plausible function would be to confer hydrophobic properties to certain cell structures such as sporangiophores and spores. Some of the lipid is firmly bound to the cell wall [e.g. (4, 7, 8, 16, 17)] and may have a structural role (13).

Polysaccharide.—Polysaccharides of fungal cell walls are built from a variety of sugars. At least 11 monosaccharides have been reported as occurring in fungal cell walls, but only three, D-glucose, N-acetylglucosamine, and D-mannose, are consistently found in most fungi. Their relative proportions, however, vary enormously from traces in certain organisms to principal components in others. The following monosaccharides are less frequently found and with a more or less characteristic distribution among certain groups of fungi: D-galactose and D-galactosamine (Ascomycetes), L-fucose (Mucorales and Basidiomycetes), D-glucosamine (Mucorales), xylose (Basidiomycetes), and D-glucuronic acid (see below). Occasionally, the presence of small quantities of rhamnose (17–19), ribose (4, 7, 17, 20), and arabinose (21, 22) has been reported. The role of these minor sugars is uncertain.

As recently as 1965, Aronson (1) commented on the lack of conclusive evidence for uronic acids in fungal walls; in the intervening time their presence has been clearly demonstrated (23–25). Gancedo et al. (23) detected as much as 2 per cent glucuronic acid in *Dactylium dendroides* and smaller amounts in *Alternaria, Fusarium, Penicillium,* and *Aspergillus*. There is also paper chromatographic evidence for small amounts of uronic acid in *Rhizoctonia* (40) and *Neurospora* (10). From the cell walls of *Pullularia pullulans,* Brown & Lindberg isolated a heteropolymer of mannose, galactose, glucose, and glucuronic acid (25). Perhaps the most striking example of the presence of uronic acids in fungal walls is that of *Mucor rouxii* in which polymers of D-glucuronic acid constitute as much as 25 per cent of the sporangiophore wall (24). There appear to be two kinds of polyuronide in these walls. One is an alkali-soluble heteropolymer ("mucoran") containing about 50 per cent D-glucuronic acid plus some fucose, mannose, and galactose. The other is a highly insoluble, acid-resistant polyuronide, probably a homopolymer of D-glucuronic acid (24). Skucas (26) found that the papillae of *Allomyces* sporangia were made of pectic acid material. This is perhaps the only available example of D-galacturonic acid associated with fungal walls.

Analytical techniques.—Powder X-ray diffraction continues to be the most reliable single technique for identifying cell wall polymers such as chitin and cellulose. Its usefulness may be extended to other less frequently investigated polymers, like noncellulosic glucans and chitosan (27). Infrared spectroscopy may be used as an auxiliary technique for distinguishing chitinous cell walls from nonchitinous ones (28).

CELL WALL CHEMISTRY AND TAXONOMY

Perhaps one of the most mutually satisfying by-products of fungal cell wall research, to taxonomists and biochemists alike, is the close correlation that can be established between chemical composition of the cell wall and major taxonomic groupings elaborated on morphological criteria. This correlation first became apparent last century when van Wisselingh (29) showed that cell walls of fungi could be either chitinous or cellulosic; later, von Wettstein (30) used this criterion to support the division of aquatic Phycomycetes into two major taxa. Although some fungi within these groups were incorrectly assigned owing to dubious cytochemical tests employed for identifying chitin and cellulose, the more recent X-ray work of Frey, Aronson, Fuller and co-workers [see review by Aronson (1)] reaffirmed the validity of equating the presence of chitin or cellulose with taxonomic position within the Phycomycetes. Confirmation of the simultaneous presence of cellulose and chitin in *Rhizidiomyces* led Fuller & Barshad (31) to the conclusion that a third wall category exists which more closely parallels the currently accepted tripartite division of the aquatic Phycomycetes, made on the basis of flagellation (Oomycetes, Chytridiomycetes, and Hyphochytridiomycetes).

With the advent of mechanical methods to prepare essentially pure cell walls, and of paper chromatography to characterize their monomeric components, there has been a recent surge of interest in cell wall chemistry of fungi. Although the data are exceedingly limited in the number of related organisms examined, diversity of groups selected, and analytical refinement, it has become increasingly clear that the *entire* spectrum of fungi may be subdivided into various categories according to the chemical nature of their walls, and that these categories closely parallel conventional taxonomic boundaries. The classification of fungal cell walls proposed in Table I is based on dual combinations of those polysaccharides which appear to be the principal components of vegetative walls. These include, in addition to chitin and cellulose, chitosan, mannans, a glycogen-like polymer, and ubiquitous noncellulosic glucans (probably β1, 3- and β1, 6-bonded) for which the admittedly ambiguous term "glucan" will be used pending further characterization. Galactose and galactosamine polymers are found in the borderline group VIII.

For the establishment of the eight wall categories, the presence of small amounts of other classifying polysaccharides was disregarded (for instance, traces of chitin are present in the yeasts of category VI). These eight categories probably represent a minimum of dual combinations. A detailed characterization of glucans (or other components) might, for example, provide a basis for subdividing the chitin-glucan category, the largest of all. Likewise, examination of neglected taxa may reveal new categories. The correlation between wall chemistry and taxonomy may be effectively extended to

the genus level, although in such close proximity the differences are apt to be minor and chiefly, if not entirely, quantitative. Thus, by measuring sugar ratios in alkali-soluble fractions from the walls of various Basidiomycetes, O'Brien & Ralph (33) showed clear differences between related genera (e.g., *Fomes* versus *Fomitopsis; Inonotus* versus *Polyporus*). Serological differences which are sometimes used to distinguish species could be reflections of minor differences in wall composition (34, 35).

Some broad generalizations result from examination of Table I. The

TABLE I

CELL WALL TAXONOMY OF FUNGI

Chemical Category	Taxonomic Group[a,b]	Distinctive Features
I. Cellulose-Glycogen[f]	Acrasiales	pseudoplasmodia
II. Cellulose-Glucan[g]	Oomycetes	biflagellate zoospores
III. Cellulose-Chitin	Hyphochytridiomycetes	anteriorly uniflagellate zoospores
IV. Chitosan-Chitin	Zygomycetes	zygospores
V. Chitin-Glucan[g]	Chytridiomycetes	posteriorly uniflagellate zoospores
	Ascomycetes[c]	septate hyphae, ascospores
	Basidiomycetes[d]	septate hyphae, basidiospores
	Deuteromycetes[e]	septate hyphae
VI. Mannan-Glucan[g]	Saccharomycetaceae	yeast cells, ascospores
	Cryptococcaceae	yeast cells
VII. Mannan-Chitin	Sporobolomycetaceae	yeasts (carotenoid pigment) ballistospores
	Rhodotorulaceae	yeasts (carotenoid pigment)
VIII. Polygalactosamine-Galactan	Trichomycetes	heterogenous group, arthropod parasites

[a] After Alexopoulos (32).

[b] Not all orders or families within each group have been examined for wall composition. Further segregation is possible.

[c] Except Saccharomycetaceae.

[d] Except Sporobolomycetaceae.

[e] Except Cryptococcaceae (and Rhodotorulaceae).

[f] Incompletely characterized.

[g] Incompletely characterized; probably β1,3- and β1,6-linked.

vast majority of fungi, including all forms with typical septate mycelium, have a chitin-glucan cell wall (category V). A departure from the mycelial to the yeast habit is accompanied by a substantial increase in the mannan

content of the wall (categories VI and VII), a relationship disclosed some time ago by Garzuly-Janke (36). Cellulose is characteristic of many but not all lower fungi. Significantly, the greatest departures in wall composition from the chitin-glucan axis are found in the Acrasiales and Trichomycetes, groups whose inclusion among the authentic fungi has long been argued.

Cell wall chemistry also serves to distinguish fungi from other living creatures. The lack of close kinship between Actinomycetes (37–39) and fungi may be deduced from their entirely different wall composition as well as other criteria. Likewise, although fungal walls exhibit some similarity to algal walls (41, 114), distinctive traits can be recognized. For instance, Parker et al. (42) found that two phylogenetically related groups, Vaucheriaceous algae and Saprolegniaceous fungi, differ greatly in wall features, such as content and crystallinity of cellulose, etc.

It seems certain that a greater availability of chemical data on fungal walls would be of help in dissipating taxonomic ambiguities. Similarly, the correlation between wall chemistry and taxonomy could serve as a guide to the biochemist in the exploration of cell wall features throughout the fungi, and to re-evaluate analytical results inconsistent with taxonomic position. No drastic differences in major structural components are apt to occur among fungi which are closely related by morphological criteria. For instance, a seemingly simple change like the inversion of the glycosidic linkage in a glucose polymer, from $\beta 1, 4$ to $\alpha 1, 4$, would cause such vast changes in the physical properties of the polymer that it is doubtful that the shape and integrity of the wall could be retained.

The constancy of wall composition is by no means absolute, and a certain latitude in quantitative, and sometimes qualitative, composition is seen. Frequently, these alterations are in response to changes in environmental conditions, bringing about concomitant changes in cellular shape; hence, the close interdependence of cell wall chemistry, environment, and morphology observed in fungi [e.g. (4, 43)].

Salient features and selected examples of each category are briefly discussed in the following sections.

Group I. Cellulose-Glycogen

By X-ray diffraction a poorly crystalline cellulose was found in the sorophores of *Dictyostelium* and *Acytostelium* (44–46) and the microcyst walls of *Polysphondylium* (47). Cellulose and a glycogen-like polysaccharide were found in spore walls of *Dictyostelium discoideum* prepared by alkali extraction (48). The extent to which cytoplasmic glycogen may have contributed to this fraction is uncertain. Microcyst walls of *Polysphondylium pallidum* prepared by sonic treatment contain approximately equal amounts of two glucans: cellulose and an alkali-soluble glucan assumed to be glycogen (47). These walls also contain high levels of protein and lipid.

Group II. Cellulose-Glucan

Recent papers described the structure of hyphal walls of Oomycetes of the orders Saprolegniales and Peronosporales with two phytopathogenic genera, *Phytophthora* and *Pythium*, receiving the most attention. Except for a questionable claim (49), there is agreement on the similarity of wall composition among these fungi. Traditionally, the Oomycetes have been regarded as the cellulosic fungi; surprisingly, cellulose was found not to be the major component of the hyphal walls of any of the examined genera: *Phytophthora* (7, 17, 50), *Pythium* (17, 51), *Achlya* (42, 50), *Saprolegnia* (17, 42), *Atkinsiella* (50), *Brevilegnia*, or *Dictyuchus* (42). Instead, the principal wall component was an alkali-insoluble and cuprammonium-insoluble glucan(s) containing β1, 3, and β1, 6 linkages (7, 50–52). Cellulose (type I) was apparently present in a poorly crystalline state (7, 42, 51). Significantly, removal of cellulose by cuprammonium extraction did not visibly affect the overall morphology of hyphal walls of *Phytophthora* (7). Possibly, the noncellulosic glucan plays a skeletal role in the architecture of these walls. Although glucose is the principal monomer, small amounts of mannose are usually detected in oomycete cell walls (7, 17, 20, 51). The relatively high estimates of mannose for some Saprolegniales may be due to incomplete removal of cytoplasmic components by the extractive procedure used in preparing the wall material (42).

The cell wall glucan of *Pythium butleri* was claimed to be a β1,2-linked polymer analogous to the crown-gall polysaccharide of *Agrobacterium tumefaciens*, mainly on the basis of partial similarity in X-ray reflections (49). However, Novaes-Ledieu et al. (17) and Aronson and collaborators (50, 51) showed convincingly that the hyphal walls of *Pythium butleri* and *P. debaryanum* contain mainly β1,4-, β1,3-, and β1,6-linked glucans as the walls of other Oomycetes do. Although the absence of a small proportion of β1,2 linkages cannot be ruled out, more definite proof is needed to substantiate their presence in the wall glucans of these fungi.

The absence of any appreciable amount of chitin in these fungi was confirmed (7, 51). Only a small amount of hexosamine (usually less than 1 per cent) can be detected in oomycete cell walls, but its polymeric state is unknown. Investigations on yeast cell walls suggested that hexosamine may be important in the formation of glycoprotein complexes (53, 54). A distinguishing feature of this wall category is the presence of hydroxyproline in the cell wall protein (7, 17, 20). Failure to detect this and other amino acids in *P. butleri* (49) may have resulted from incomplete release and destruction during alkaline hydrolysis. Hydroxyproline is not found in fungi with chitinous walls (20); it is a characteristic amino acid of cellulosic walls of either fungi, algae (55), or higher plants (56). Recent evidence indicates that hydroxyproline provides an important (glycosidic) link between polysaccharides and protein (57).

Group III. Cellulose-Chitin

Only the Hyphochytridiomycetes are presently considered as members of this category. The presence of both cellulose and chitin was convincingly demonstrated in *Rhizidiomyces* sp. (31, 58), the only representative critically studied so far. In the absence of quantitative data on wall composition, the validity of their inclusion in this category rests chiefly on the fact the Hyphochytridiomycetes constitute an intermediate group of Phycomycetes distinct from the cellulosic Oomycetes and the chitinous Chytridiomycetes.

The simultaneous presence of cellulose and chitin in the cell walls of a variety of fungi has long been claimed. Most reports, however, failed to provide confirmatory X-ray evidence [for examples refer to Frey (59); Aronson (1)]. At present, *Ceratocystis ulmi* is the only higher fungus in which both chitin and cellulose have been demonstrated by X-ray diffraction (60). In all other mycelial Ascomycetes and Basidiomycetes which have been critically examined, chitin but not cellulose was consistently detected. The anomaly posed by the case of *C. ulmi* may be reconciled in two ways. *Ceratocystis* and related genera of the Ophiostomaceae may be a special group of Ascomycetes legitimately belonging in this category of fungal walls. Alternatively, in the absence of quantitative information, it is permissible to suggest that the cellulose found on the walls of *C. ulmi* may represent a minor portion of the cell wall, the main component being noncellulosic glucan as in the cell walls of other Ascomycetes.

Group IV. Chitosan-Chitin

Several representatives of the Zygomycetes have been examined. By X-ray diffractometry, chitin was detected in the Mucorales [*Mucor, Rhizopus* (59); *Phycomyces* (61)] and Entomophthorales [*Basidiobolus* and *Entomophthora* (59)]. No representatives of the third order (Zoopagales) have been examined. The highly distinguishing properties of this cell wall category are derived from limited but consistent evidence regarding several members of the Mucoraceae: quantitative analyses of *Mucor rouxii* (4, 9, 24, 62), qualitative study of *Zygorhynchus vuilleminii* (20), and partial polymer characterization of *Phycomyces blakesleeanus* (27). Chitosan, the group characteristic polymer, is a poorly or nonacetylated polyglucosamine found by Kreger (27) in the cell walls of the mycelium and sporangiophores of *Phycomyces*. It also occurs in the mycelial, yeast, and sporangiophore walls of *Mucor* (4, 62). This polycation is probably neutralized by the concurrent presence of large amounts of phosphate (inorganic polyphosphate?) and polyuronides in the cell walls of *Mucor* (4, 24).

Fucose, mannose, galactose (4, 20), and substantial quantities of D-glucuronic acid (24) are present in this category of cell walls. These sugars may form part of heteropolyuronides such as the one isolated from *M. rouxii* cell walls (24). A conspicuous feature of the group is the absence of

glucose polymers from vegetative walls (*Mucor, Zygorhyunchus*). Possibly chitosan fulfills the structural role played by glucan in other fungi. The absence of glucose would constitute an absolute point of divergency from all other fungi (save for the disputable Trichomycetes) were it not for the fact that glucan abruptly appears as the main component of the spore wall of *Mucor rouxii* (9). The spore wall also differs from vegetative walls in other chemical features (Table II).

TABLE II

CHEMICAL DIFFERENTIATION OF THE CELL WALL IN THE LIFE CYCLE OF *MUCOR ROUXII* (4, 9, 24, 62)[a]

Wall Component	Yeasts	Hyphae	Sporangiophores	Spores
Chitin	8.4	9.4	18.0	2.1[b]
Chitosan	27.9	32.7	20.6	9.5[b]
Mannose	8.9	1.6	0.9	4.8
Fucose	3.2	3.8	2.1	0.0
Galactose	1.1	1.6	0.8	0.0
Glucuronic Acid	12.2	11.8	25.0	1.9
Glucose	0.0	0.0	0.1	42.6
Protein	10.3	6.3	9.2	16.1
Lipid	5.7	7.8	4.8	9.8
Phosphate	22.1	23.3	0.8	2.6
Melanin	0.0	0.0	0.0	10.3

[a] Values are per cent dry wt of the cell wall.

[b] Not confirmed by X ray. Value of spore chitin represents N-acetylated glucosamine; chitosan is nonacetylated glucosamine.

Apart from morphogenetic considerations (9), one could also conjecture that the shift in wall chemistry, from a chitosan-chitin type in vegetative cells to a glucan-polyglucosamine (chitin?) type in spores, represents a transition from group IV to group V; hence, we may be witnessing an ontogenetic recapitulation of a phylogenetic relationship.

GROUP V. CHITIN-GLUCAN

This is by far the most numerous category, harboring all mycelial forms of the Ascomycetes, Basidiomycetes, and Deuteromycetes. Also included is a segment of lower fungi, the Chytridiomycetes. This inclusion is tentative pending characterization of the wall glucan(s). Presently, the very limited information available on the glucose polymers of *Allomyces* walls (6, 63) does not rule out the possibility that they may be similar, at least in part, to the wall glucans of higher fungi. Confirmation of a close similarity in glucan

structure would strengthen those phylogenetic schemes which propose that higher fungi evolved from ancestral Chytridiomycetes [see Klein & Cronquist (148) for a recent review].

The chitin content oscillates widely from 3 to 5 per cent in *Schizophyllum commune* (64) to about 60 per cent in *Allomyces macrogynus* (6) or *Sclerotium rolfsii* (65). The balance consists largely of noncellulosic glucans which have been only superficially characterized, if at all. At least two different kinds of glucans may be present; these are usually separated by their solubility in alkali. Although seldom attempted, the noncellulosic glucans may be identified by X-ray diffraction [Kreger (27)]. Thus, the alkali-soluble fraction of cell walls of *Penicillium, Endomyces, Agaricus* (27), and *Schizophyllum* (64) exhibited sharp reflections corresponding to those of a glucan first discovered in *Schizosaccharomyces* (27). The nature of this soluble glucan, abbreviated S-glucan (64) is not well known; seemingly it contains mainly β1,6 linkages [Wessels (64)]. The alkali-insoluble fraction may be characterized as "yeast" glucan upon conversion to its hydroglucan by acid treatment, as has been done for the cell walls of various Endomycetales (27), *Schizophyllum commune* (64), and *Verticillium alboatrum* (66). It is not known if both glucans occur throughout the entire spectrum of fungi with chitin-glucan cell walls. There is already one exception, *Agaricus,* which apparently lacks "yeast" glucan (27).

The linkage most commonly reported in these noncellulosic glucans is β1,3. In addition to the β1,6 mentioned above, α-1,3 and α-1,4 have also been detected. Repeated claims for the presence of cellulose (β1,4 glucan) in walls of higher fungi have long been made. Some recent ones include *Fusarium* (67), *Aspergillus* (68), *Schizophyllum* (69), *Venturia* (70), and *Ceratocystis* (60). Except for the last one (see Group III) no conclusive X-ray evidence was submitted to substantiate these claims. In fact, X-ray analyses of the cell walls of *Fusarium* (59), *Aspergillus* (8), *Schizophyllum* (64), and a variety of other representatives of this wall category, have failed to reveal the presence of cellulose.

The major classes of fungi included in this category differ from one another with regard to the presence of minor sugars. Barring exceptions which could have been the result of incomplete hydrolysis or incorrect identification, the following rules seem applicable. Ascomycetes have galactose and galactosamine in their walls, while lacking xylose and fucose. The reverse is true in the Basidiomycetes. Mannose occurs in both classes (20, 33). In the Chytridiomycetes (*Allomyces*) none of these sugars was detected (6, 63).

Quantitative analyses of wall composition have been published for *Aspergillus* (5, 8, 21, 65), *Neurospora* (10, 43, 71), *Pithomyces* (72, 73), *Penicillium* (18, 74), *Sclerotium* (65, 75), *Rhizoctonia* (40, 76), *Fusarium* (77), *Venturia* (70), *Schizophyllum* (64), *Histoplasma* (78), *Trichophyton*

(79). *Aspergillus* and *Neurospora* will be discussed in some detail.

There is general agreement on the presence of N-acetylglucosamine, glucose, mannose, and galactose in *Aspergillus* (5, 8, 20, 21, 65) but galactosamine has not been always reported even though *Aspergillus* spp. were found to have the highest content of mycelial galactosaminoglycan from among a variety of fungi examined (80). The insoluble glucan of *Aspergillus* was proven long ago not to be cellulose (81). Enzymic digestion of cell walls of various species of *Aspergillus* indicated a preponderance of β1,3-linked glucose residues (5, 65, 77, 83). Johnston (21, 82) separated three glucan fractions from the mycelial walls of *A. niger*. Two glucans were alkali-soluble; one of them was further soluble in hot water and was identified as nigeran (alternating α1,3 and α1,4 bonds). The main component of this fraction was a glucan composed chiefly of α1,3 bonds and a small proportion of α1,4. The alkali-insoluble glucan was not characterized, but it probably contains the β1,3 linkages found by others in *Aspergillus* cell walls. According to Ruiz-Herrera (8), mannose and galactose are associated with the alkali-insoluble glucan. He found no evidence for nigeran in an *Aspergillus* sp. Nigeran was found to be only a minor component of the walls of a strain of *A. niger* examined by Johnston (21) and, even though its presence in the wall has been confirmed (84), its role in wall structure would seem doubtful; nigeran is apparently a dispensable component of *A. niger* (85).

About two thirds of the hyphal wall of *Neurospora crassa* has been accounted for as chitin and glucan (10, 43, 71, 86). The protein content was calculated from total N values to be as high as 14 per cent; this figure is probably an overestimate, since hexosamine-N is usually incompletely accounted for. The presence of β1,3 and β1,6 linkages in *Neurospora* cell wall glucan(s) has been reported (10, 71). Small amounts of galactosamine were detected in walls of *N. crassa* (10, 87) and *N. sitophila* (20). Mahadevan & Tatum (10) extracted the walls of a variety of strains and mutants of *N. crassa* into four fractions. Galactosamine was found in the hydrolyzate of an alkali-soluble fraction which also contained glucuronic acid, glucose, and amino acids. These monomers seemingly form part of a glycoprotein complex important to hyphal morphology (see below); the glycoprotein appears under the electron microscope as coarse microfibrils located in the outer portions of the wall (86). By comparing the digestive action of β1,3 glucanase and chitinase, tested alone and in combination, against the hyphal walls of *N. crassa,* it was concluded that chitin is probably surrounded or protected by glucan molecules (71, 86). Lytic studies of this sort may be helpful in determining structural differences among members of the chitin-glucan group. For instance, the combination β1,3 glucanase + chitinase dissolved only 40 per cent of the wall of *Neurospora crassa;* whereas, the same treatment completely disintegrated the walls of *Fusarium solani* [Potgieter & Alex-

ander (71)]. The exact structural reason for this discrepancy is not known; Manocha & Colvin (11) suggested that wall protein may have been responsible for the retention of morphology of *N. crassa* after enzymic attack.

Group VI. Mannan-Glucan

In this category are included the yeast forms of the Ascomycetes and Deuteromycetes. A description of the corresponding literature on yeast wall structure will not be attempted, the reader is advised to consult Phaff (2) and Nickerson (12, 13). Typical members of this category (*Saccharomyces, Candida, Hanseniaspora, Kloeckera,* etc.) have mannan(s) and glucan(s) as their principal wall components. Chitin is present only in very small amounts. Mannan and part of the glucan are alkali-soluble. Glycoprotein complexes of these polysaccharides have been isolated (12, 13). The alkali-insoluble glucan, the so-called "yeast" glucan, is usually considered the skeletal component of the wall, but its native fibrillar state is uncertain (88). Also, its fine chemical structure has yet to be clearly elucidated; the most recent report regards it as a branched polymer with β1,6-linked main chains and β1,3-linked branches (89). A predominant mannan-glucan composition cannot be established for all organisms belonging to the Hemiascomycetes. However, the discrepancies correlate to a large extent with the degree of morphological dissimilarity between the divergent organism and the typical budding yeasts. Thus, compared to budding yeasts, the mycelial Endomycetales (*Endomycopsis, Endomyces, Eremascus*) have a reduced "yeast" glucan content, a high chitin, and a very low mannan content (27); therefore, they are much closer to and probably belong with the typical mycelial fungi of group V. Yeasts with unusual characters also depart in chemical composition, e.g., the "triangular" yeast *Trigonopsis variabilis* (90) contains little or no alkali-insoluble "yeast" glucan. Several species of *Schizosaccharomyces,* the fission yeasts, contain "yeast" glucan but seem to lack chitin and manan (27). Instead, they have in their walls an alkali-soluble glucan (S-glucan, see group V) which is present in the Endomycetales and in typical higher filamentous fungi (27). The virtual absence of mannan in *Schizosaccharomyces* has been confirmed (20). In other yeasts, e.g., *Nadsonia elongata,* conflicting claims of presence or absence of mannose polymers have been made (14, 91).

Group VII. Mannan-Chitin

The pink yeasts of the genera *Rhodotorula* and *Sporobolomyces* fall in this category. Their cell walls differ from those of yeasts of group VI in that they contain only a small quantity of glucose polymers (20), seemingly lack "yeast" glucan, and their chitin content is high (27). Their mannan may also be different since it does not form an insoluble copper complex, which probably explains previous negative findings (27, 36) [see also comments on mannan detection (13)]. Actually, mannose occurred abundantly in

wall hydrolyzates of *Sporobolomyces* and *Rhodotorula* [Crook & Johnston (20)]. Furthermore, these yeasts are the only ones having galactose, fucose, and γ-aminobutyric acid in their walls (20). The presence of fucose is significant since it is also characteristic of other Basidiomycetes wherein these yeasts are classified. Interestingly, Tsuchiya et al. (35) reclassified *Rhodotorula* with *Sporobolomyces* in the same subfamily (Sporobolomycetoideae) on the basis of serological (wall antigens?) similarities (34, 35) as well as other known common properties. This relationship might also have been deduced from examination of their unique wall composition mentioned above.

Group VIII. Polygalactosamine-Galactan

The properties of this category are typified by those of *Amoebidium parasiticum* (92), the only Trichomycete so far examined. Since this is a heterogenous group, the cell wall composition may not be uniform throughout the Trichomycetes. In marked contrast to the walls of other fungi, the thallus walls of *A. parasiticum* are entirely soluble in alkali. The insoluble polymers of glucose and (acetyl) glucosamine, characteristic of other fungi, are absent in *A. parasiticum*. Instead, Trotter & Whistler (92) found chromatographic evidence for polymers of galactosamine, the predominant component, and galactose. Smaller amounts of xylose were also present; glucose was absent.

CELL WALL STRUCTURE AND MORPHOGENESIS

Nickerson's (93) pioneering approach to the elucidation of biochemical bases of morphogenesis in fungi via a better understanding of cell wall properties and behavior has continued to receive increasing attention. The evidence, though circumstantial, does indicate that morphological differentiation is correlated with changes directly affecting cell wall metabolism, thus strengthening the main premise of equating morphological differentiation with cell wall differentiation (94). Elucidating the nature of the wall bricks and cementing material is of course part of the story only. The cell wall-building enzymes and their chemical as well as physical relationship to the interior of the cell, constitute at the moment a formidable and barely understood complex. The following selected examples illustrate some of the chemical, enzymological, or cytological tactics that have been used in a strategic attack on the mystery of cell wall morphogenesis.

Chemical Differentiation of the Cell Wall

Vegetative development.—Neurospora crassa may be induced, phenotypically or genotypically, to change its normal long, straight, sparsely branched hyphae into short, undulating, highly branched ones (the so-called colonial growth). Tatum and co-workers (10, 43, 86) have shown that this morphological change is accompanied by a marked increase in the ratio of

hexosamine/glucose in the wall polymers. A glycoprotein seemingly made of glucose, glucuronic acid, and galactosamine was the wall component most critically affected during colonial growth of a variety of strains of *N. crassa* [Mahadevan & Tatum (10)]. Closely related is the observation that an "osmotic" mutant of *N. crassa,* with its less rigid and irregularly shaped walls, contained in its walls much more galactosamine and less glucosamine than the wild type (95). This suggests that a proper balance of hexosamine polymers may be essential for maintaining the rigidity and regularity of hyphal walls of this fungus. Interestingly, selective removal of various wall components from isolated normal hyphae of *N. crassa* (86) did not destroy the integrity of the wall—an indication that the critical time for attaining proper balance of wall polymers is during cell wall assembly. Colonial growth has been traced to single-gene mutations affecting, in one case, the structure of glucose-6-phosphate dehydrogenase with a resulting *in vivo* increase in the level of glucose-6-phosphate; in another colonial mutant there was a severe deficiency in phosphoglucomutase leading to the accumulation of glucose-1-phosphate [Brody & Tatum (96, 97)]. Conceivably, these superficially simple metabolic changes bring about, in an as yet unknown manner, an alteration of the ratios and(or) fine structure of wall polymers, ultimately resulting in "colonial" morphology.

The cell wall mannan content of various dimorphic fungi has been observed to vary according to morphology (mycelial versus yeastlike), albeit in conflicting directions. For instance, yeast walls of *Mucor rouxii* have 5 to 6 times as much mannose as the hyphal walls of aerobically grown mycelium. Since the architectural role of this carbohydrate is not known, phylogenetic reasoning was invoked to support a casual relationship between the increase in wall mannan and yeast morphogenesis (4, 98). In partial agreement, slightly more mannose was detected in yeast walls than in filamentous walls of *Pullularia pullulans* [Brown & Nickerson (19)]. On the other hand, Domer et al. (78) found that the yeast form of *Histoplasma capsulatum* had nearly one fifth the mannose content of the mycelial form. Likewise, 2.5 times less mannan was detected (Cu complex) in ellipsoidal cell walls compared to the walls of highly elongated cells of *Hansenula schneggii* [Sundhagul & Hedrick (99)]. By growing *Saccharomyces cerevisiae* under NH_4^+ limitation (100), cell shape may be varied from spheroid to cylindroid, yet the mannan content of the cell wall is not necessarily altered [McMurrough & Rose (101)]. From the foregoing, it is obvious that measurements of overall mannose content do not support a universal role of mannan in dimorphism. Yet, the possibility of mannose polymers playing a causal role in yeast morphogenesis cannot be ruled out entirely. The conflicting mannose content/morphology relationships could arise from the fact that the measurements did not discriminate between different types of mannans, i.e., glucomannans (16), phosphomannans (53, 102), heteropolyuronides (24, 25), each one possibly having a different architectural influence on the cell wall.

Germination and sporogenesis.—Chemical comparisons of vegetative cell walls versus spore walls may be useful in understanding the biochemical basis of sporulation and spore germination, but few studies have been made in this regard. Partial characterizations of hyphal walls and spore walls of *Aspergillus oryzae* (5), *Aspergillus phoenicis* (65), *Pithomyces chartarum* (72, 73, 103), *Fusarium culmorum* (67), hyphal walls of *Allomyces macrogynus* (6), and sporangial walls of *A. neo-moniliformis* (63), revealed differences that were largely quantitative. Among the qualitative changes recorded were the deposition of melanin (5, 63, 65) and the absence of a phospho-glycoprotein (73). It is quite possible that minor structural changes of morphogenetic significance may not be detectable by gross chemical analysis. The conclusion for the time being would be that asexual sporogenesis and spore germination in the above fungi (group V) are accomplished with retention of the basic chitin-glucan structure of the cell wall. In *Mucor rouxii* the situation is entirely different. A comparison of cell wall composition in four stages of development (Table II) revealed only quantitative differences between hyphae, yeast cells, and sporangiophores (4, 24, 62), but the spore wall manifested a drastically different qualitative composition (9). A glucan appears *de novo* during sporogenesis as the principal polymer of *M. rouxii* spore walls; various hyphal wall components are either diminished or absent. Upon germination, spore wall composition reverses to the chitosan-chitin type. These shifts in chemical composition indicate a recurring reorganization of cell wall metabolism in each turn of the asexual life cycle of *Mucor*. Accompanying the chemical shift, there is a structural discontinuity between the spore walls and vegetative walls (104). A phylogenetic interpretation for the shift was suggested above (group IV). It has also been proposed that the cell wall shift may be a critical part of a morphogenetic mechanism by which the fungus diverts its resources from the synthesis of vegetative walls to spore walls (9).

Cell Wall Splitting Enzymes

Endogenous enzymes capable of splitting cell wall polymers or their complexes seemingly play an indispensable role in a multitude of morphogenetic processes. Furthermore, it is conceivable that some of these degradative enzymes occur at the sites of cell wall formation and operate in harmony with cell wall synthetases.

Protein disulfide reductase.—Nickerson & Falcone (93) interpreted the dimorphism of *Candida albicans* as a case of different budding ability. The ellipsoidal, actively budding cell of *C. albicans* is believed to have an unimpaired capacity to plasticize localized areas of the cell wall where bud extrusion takes place. The softening of the wall was attributed to the specific action of a protein disulfide reductase capable of splitting S-S cross-links in the glycoproteins of the wall fabric. Accordingly, the levels of protein disulfide reductase found in a filamentous mutant of *C. albicans*, displaying little or no budding capacity, were much lower than those in the normal budding

form. Although the cytology and rapidity of budding are in controversy [compare (93, 105, 106, 109)], the correlation between protein disulfide reductase and budding is supported by the recent observation of Brown & Hough (107) who found that elongated cells of *Saccharomyces cerevisiae* contained considerably lower levels of the enzyme than did typical ellipsoidal cells. Moor's (108) freeze-etching electron microscopy of *S. cerevisiae* suggests a centrifugal progression of elements of the endoplasmic reticulum toward the budding sites; it was postulated that these vesicles, rather than mitochondria, were the carriers of the protein disulfide reductase.

Glucanases.—Enzymes active against cell wall glucans have been reported to participate in morphogenetic phenomena; for instance, in the fusion of conjugating cells of *Hansenula wingei* (110, 111), in the hormone-induced development of antheridial branchings in *Achlya* (112), and in the formation of pilei from dikaryons of *Schizophyllum commune* (64, 113). In the latter, Wessels observed that pileus morphogenesis was correlated with substantial increases in the levels of a specific, repressible glucanase which hydrolyzes the alkali-insoluble glucan of mycelial walls. The degraded mycelial polysaccharide was subsequently utilized for pileus development. Inhibition of pileus morphogenesis by exogenous glucose was interpreted as being a result of catabolite repression of the glucanase in question (113).

Ultrastructure of Cell Wall Development

The following are only two aspects of the rapidly expanding field of fine structure of developing cell walls of fungi. For a discussion of other selected areas, consult Hawker (115) and Bracker (116).

Spore germination.—Although relatively few fungi have been examined, it appears that there are three basically different mechanisms of vegetative wall formation during spore germination, each type being characteristic of certain groups of fungi.

Type I. The vegetative wall is derived directly as an extension of the spore wall, or one of its innermost layers. This mode of genesis is perhaps the most common and appears throughout the higher fungi; e.g., the conidia of *Botrytis* (117), *Aspergillus* (118, 119), uredospores of *Melampsora* (120), conidia and ascospores of *Neurospora* (121, 122). There is probably no fundamental change in the composition of the cell wall during this type of germination [e.g., in *Aspergillus* (5)].

Type II. *De novo* formation of a cell wall on a naked protoplast. This occurs during the encystment of the zoospores of aquatic Phycomycetes (123–125).

Type III. *De novo* formation of a vegetative wall under the spore wall. This kind, first observed by Hawker & Abbott in *Rhizopus* (126), is also found in other Mucorales: *Cunninghamella* (127), *Gilbertella* (128), *Mucor* (104). This unique mode of germination may be exclusive of fungi with

vegetative walls of the chitosan-chitin type (category IV) and may be a direct consequence of the changeover in cell wall composition that occurs during germination. Because of the distinctly different appearance of spore wall versus vegetative wall in the Mucorales, the formation of a new cell wall during germination could be ascertained with confidence. In other fungi, the demarcation between the older and newer parts of the wall was not as clear, and claims for the formation of an entirely new wall during germination may have been premature [e.g., in the conidia of *Fusarium culmorum* (129)].

Hyphal morphogenesis.—Fungal hyphae grow by the commonly known but poorly understood process of apical growth. Essentially, apical growth is a mechanism by which the fungus restricts cell wall synthesis to the hyphal apex. Some recent studies, seeking intracellular structures associated with and possibly responsible for apical growth, have shown an increased degree of vesicular differentiation in the apical cytoplasm. Bracker and collaborators (116) found an accumulation of vesicles, similar to those discharged by dictyosomes, in the hyphal apices of *Pythium ultimum*. Marchant et al. (130) proposed that vesicles from the endoplasmic reticulum were responsible for "primary" cell wall synthesis. They considered that lomasomes, structures previously suspected of having a role in cell wall synthesis (131, 132), were responsible for "secondary" wall synthesis. In germinating spores of *Mucor rouxii,* a seemingly unique organelle was found in association with the apical wall of germ tubes (104). It was postulated that this extracytoplasmic apical corpuscle could be responsible for both germ tube emergence and its continued apical growth.

Cell Wall Construction

Polymer synthesis.—Our knowledge of cell wall biosynthesis in fungi is restricted to data concerning a few individual polysaccharides. Chitin is synthesized from the same precursor by all fungi so far examined. The initial observation of uridine-diphospho-N-acetyl-glucosamine serving as the glucosyl donor for chitin synthesis in *Neurospora crassa* (133) has been extended to *Venturia inaequalis* (134), *Allomyces macrogynus* (134, 135), *Blastocladiella emersonii* (136), *Mucor rouxii* (137), and *Schizophyllum commune* (138). Uridine-diphospho-glucose (UDPG) was reported to be the natural precursor for the synthesis of noncellulosic glucan(s) of *Phytophthora cinnamomi;* both β1, 3 and β1, 6 bonds were formed (139). UDPG is also the glycosyl donor for the synthesis of a glycogen-like polymer of *Dictyostelium discoideum* spore coats (48). The mode of cellulose biosynthesis in fungi is not clear. Although cellulose is present in both *Phytophthora* and *Dictyostelium,* there was little or no cellulose synthesized in the cell-free preparations containing UDPG as precursor (48, 139). Guanosine-diphospho-glucose, the other known cellulose precursor (140) was not

effective either (139). Guanosine-diphospho-mannose serves as glycosyl donor for the synthesis of mannan in *Saccharomyces carlsbergensis* (141) and phosphomannan in *Hansenula holstii* (142). Cell-free extracts of *M. rouxii* catalyzed the synthesis of wall polyuronides from uridine-diphospho-glucuronic acid (143).

Polymerization sites.—Solutions to some of the most important problems of fungal morphogenesis probably depend on the availability of answers to the following questions: Where are cell wall structural polymers synthesized? Are they polymerized in some intracellular site (plasmalemma, dictyosomes, lomasomes, etc.) and somehow transported in an orderly way to their final destination in the cell wall? Alternatively, is it more reasonable to expect cell wall synthesis (polymerization) to take place *in situ* (94)? Data obtained from cell-free studies are too fragmentary and contradictory to permit definite conclusions about the subcellular site of cell wall polysaccharide synthesis. The only generalization that can be safely made is that the polymerizing enzymes are not soluble but particulate. The actual cellular organelles responsible for cell wall polysaccharide synthesis have not been conclusively characterized. Significantly, in those instances wherein the cell wall fraction was assayed, most of the enzymic activity was detected in this fraction (48, 139). These results tend to support the concept of *in situ* synthesis, at least for some cell wall polymers; however, additional evidence is needed to refute the possibility that the observed association between synthesizing enzyme and cell wall resulted from incomplete removal of cytoplasm.

Assembly patterns.—The techniques of autoradiography and fluorescent-antibody labeling have been employed to elucidate the patterns of cell wall assembly in budding yeasts, but they have led to conflicting conclusions. Autoradiographic studies of incorporation of glucose-^{3}H into the cell wall of *Pichia farinosa* and *Saccharomyces cerevisiae* led Johnson & Gibson (144, 145) to conclude that wall extension was primarily by tip growth (distal end of the bud). However, Chung et al. (146) observed that the buds of *S. cerevisiae* labeled with fluorescent antibody incorporated new wall material mainly into an annular region at the base of the bud. These two conflicting observations may be reconciled by the possibility that each technique followed the formation of entirely different wall components, each with a special distribution and function in the cell.

Autoradiographic evidence was collected to support the hypothesis (98) that the dimorphism of *M. rouxii* results from the operation of two different mechanisms of cell wall assembly (localized versus disperse). Accordingly, hyphae showed the expected strongly localized apical pattern of cell wall synthesis, whereas budding yeastlike cells of the same fungus exhibited a more diffuse pattern, with a tendency for increased wall synthesis toward the base of the bud. The increased synthesis at the base might be related to bud abscission rather than bud growth (147).

CONCLUSION

In the previous pages I have attempted to summarize and emphasize the role of the cell wall in two major aspects of the biology of fungi, systematics and morphogenesis. Despite our present level of ignorance, some close correlations can be established between cell wall properties and the properties of the whole fungus. This constitutes a reassuring indication of the potential value that cell wall studies may have in the elucidation of biochemical bases of ontogenetic and phylogenetic development of the fungi.

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