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REVIEW PAPERS

The Fungal Cell Wall: Modern Concepts of Its Composition and Biological Function

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Abstract—This review deals with the cell wall (CW), a poorly known surface structure of the cell of mycelial fungi. Data are presented concerning (i) isolation techniques and purity control methods securing the absence of the cytoplasm content in the CW fraction and (ii) the chemical composition of the CW. The structural (backbone) and intrastructural components of the CW, such as aminopolysaccharides, α- and β-glucans, proteins, lipids, uronic acids, hydrophobins, sporopollenin, and melanins, are discussed in detail. Special attention is given to chitin and its novel function in terms of protecting the cells from stress as well as to the differences of this fungal aminopolysaccharide from the chitin of algae and *Arthropoda*. The apical growth of hyphae and the involvement of special microvesicles in morphogenesis of a fungal cell are discussed. Data on the enzymes involved in CW synthesis and lysis are presented. In conclusion, the functional role of the fungal CW is discussed in juxtaposition to the surface structures of higher eukaryotes.

Keywords: mycelial fungi, cell wall, isolation techniques, chemical composition, apical growth, physiological functions, morphogenesis.

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One of the key issues of present-day biology deals with the question of how morphologically identical organisms are formed during developmental processes and what biochemical mechanisms and cell structures are involved in these processes that have been carried out on this planet for billions of years. Other important issues are concerned with the influence of stress on morphogenesis and the biopolymers that control a cell's shape. The results of recent studies reveal that the growing hypha of mycelial fungi is a unique model system for investigation of cell morphogenesis and for the mechanism responsible for the constancy of cell morphology during ontogeny. A very important contribution to the studies conducted on this phenomenon was made by obtaining data on the composition and biological function of the cell wall (CW) of mycelial fungi.

The plant CW was discovered in 1665, while the fungal CW was found only in the early 18th century. For a long time, this cell surface structure was virtually ignored by researchers. The CW was assumed to perform only a "backbone" function, like a house wall that carries the bearing load. The attitude to the CW drastically changed in the beginning of the 20th century. Intense research on the CW was started, but the main subjects were plants and bacteria. However, the rapid development of mushroom cultivation and biotechnological industry using mycelial fungi as producers of biologically active compounds intensified the progress of scientific research on fungal CWs. Data on this surface structure obtained before the 1980s were summed up in the world's first book on the fungal CW [1]. In the years to follow, the bulk of the research work was done at the turn of the 21st century. Fundamental studies were conducted concerning the systematics and chemical composition of the CW, apical growth of the hyphae, chitin metabolism, antifungal preparations, hydrophobins, covalently bound proteins, and enzymes involved in CW formation, hypha branching, and CW lysis [2–14]. These data have not been generalized yet. However, taken together, they demonstrate that this surface cell structure plays a multifunctional role and performs essential functions, such as protection from deleterious factors, regulation of morphogenesis, participation in fungal reproduction, determination of the antigenic and adhesive characteristics, control over dimorphism and formation of the dormant fungal cells, reception of an external signal, and its transfer to the membrane and intracellular messengers. Therefore, discussion of the data concerning the chemical composition and the structure of the CW, the biological function of its main biopolymers, intercellular interactions in fungal hyphae, their apical growth, and the main enzymes implicated in CW formation and degradation is of considerable interest.

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CHEMICAL COMPOSITION OF THE FUNGAL CELL WALL AND THE BIOLOGICAL FUNCTION OF ITS MAJOR COMPONENTS

Research on the chemical composition of the fungal CW begins with purification of the CW fraction from the cytoplasmic admixture. The initial procedure (disintegrating fungal cells and removing the cell content) is carried out at a low temperature (about 4–5°C) to prevent the destructive activity of the degradation enzymes. Prior to destruction, the cells are frozen at liquid nitrogen temperature. Cell disintegration is performed by homogenization or by the "solid pressure" method. Good results are obtained using an ultrasonic disintegrator. The cell content is removed with cold water; this procedure is repeated four or five times. More complete removal of the cytoplasmic content is achieved by washing the sample with NaCl, 8 M urea, 1 M ammonium, or 0.5 M acetic acid. Recently, methods of isolating fungal CWs at different stages of their ontogeny have been developed. There are significant differences between the techniques used to isolate CWs from mycelium and the dormant cells [15]. If the goal is, for example, to determine the polysaccharide composition of the CW, lipids are extracted with organic solvents, e.g., chloroform and methanol at a 2 : 1 ratio. In some cases, good results are obtained by additional treatment with diethyl ether. This solvent efficiently dries CWs, and in some cases this treatment can be used instead of lyophilization. The goal of the next stage is to assess the purity of the CW fraction obtained. For this purpose, light and electron microscopy and specific staining techniques are employed. The most widely used test is based on the interaction between I^{3-} and chitosan. Pure isolated CWs acquire a pink or violet color upon treatment with Lugol's solution, while intact CWs are colored red [16]. The DAPI (4,6-diamidino-2-phenylindol) test for the presence of nuclei is also used. It should be emphasized that isolating the pure CW fraction is a very important procedure the outcome of which determines the results obtained subsequently by analyzing its chemical composition. Work [17] is of much interest in this respect. It was revealed that the mycelium of representatives of mucoraceous fungi contains glucan or, more precisely, a chitosan–glucan complex, even though all previous studies indicated that it was lacking in the mycelium of *Mucorales* [1].

Currently, fungal CW components are subdivided into (i) structural components such as chitin, β(1–3)–β(1–6)-glucans, and β(1–4)-glucan (cellulose) and (ii) intrastructural components (termed the matrix), including mannoproteins, galacto–mannoproteins, xylo–mannoproteins, glucurono–mannoproteins, and α(1–3)-glucan [18]. The least information is available concerning the linear glucose polymers α-glucans. In *Schizosaccharomyces pombe*, such a glucan contains about 260 glucose residues. This polymer consists of two linear chains that are bound to one another and contain about 120 (1–3)-α-D-glucose residues and (1–4)-α-D-glucose residues at the ends of the polymer molecule. Presumably, this glucan is required for morphogenesis [19]. A water-insoluble glucan was isolated from the CW of *Penicillium roqueforti* mycelium. Upon desiccation, this glucan lost the ability to dissolve in alkali; however, it was soluble in 10% lithium chloride solution in dimethyl sulfoxide. Studies with glucan using the methylation method yielded two tri-O-methyl derivatives of glucose containing 1⟶ 3 and 1⟶ 4 bonds between monosaccharides, respectively, at a ratio of about 5 : 2, as well as trace amounts of a tetra-O-methyl derivative containing terminal nonreducing monosaccharide residues. This suggests a linear structure of the molecules that contain only 1⟶ 3 and 1⟶ 4 bonds between glucose residues. The data were confirmed by analyzing the ^{13}C-NMR spectrum of the polysaccharide, which also testified to an α-configuration of all glucose residues [20]. These polysaccharides can be used as biologically active biopolymers, especially after a chemical modification (sulfation or carboxymethylation) that renders them water-soluble. They are of considerable interest in terms of their biological function in the CW of fungi and plants.

Water-insoluble (1⟶ 3)-α-D-glucans were earlier isolated from several species of higher and lower fungi, including *Penicillium chrysogenum* [21]. Related α-D-glucans with (1⟶ 3)- and (1⟶ 4)-bonds at various ratios were detected in higher plants (*Aconitum kuznezoffii* Reichb. [22]).

Cellulose is a structural component characteristic of oomycete fungi that are classified into pseudofungi in terms of present-day taxonomy [23]. The monosaccharide components of the CW include glucose, mannose, and xylose. The predominant sugar is glucose, accounting for up to 68% of the monosaccharides involved. Fungal CWs also contain amino acids, lipids (up to 3%), and N-acetyl-D-glucosamine. Glucans such as 1,3-glucan form a stable complex with chitin termed the chitin–glucan complex (CGC), which forms the skeleton of a fungal cell. This complex is present in all fungi except zygomycetes, and this is a reliable systematic criterion. On the surface of the CW of some fungi, a mucous polysaccharide material was detected that was composed of β-1,3-glucans. Every third glucose units in their main chain is attached by β-1,6-bonds. On the outer surface of the hyphal CW, S-glucan consisting exclusively of 1,3-bonded glucose residues, special proteins termed hydrophobins, and the phenolic pigments melanins occur.

The bulk polysaccharide of the fungal CW is chitin (poly-N-acetyl-D-glucosamine) [24]. This biopolymer is widespread in nature. It is similar to cellulose in some physicochemical properties. Chitin does not occur in a pure state in nature. Like other biopolymers, it is bound by ionic or covalent bonds with other compounds. As we mentioned above, in fungi chitin is bound to glucans. Chitin is bound to melanin in the insect cuticle and to proteins (arthropodin and sclero-

tin) in the crustacean carapace. The chitin content in the crab carapace is 30 to 50%, which is higher than in the fungal CW [1]. Vertebrates lack chitin but contain N-acetylglucosamine that forms part of the mucopolysaccharides (glycoaminoglycans) of the connective tissue (such as hyaluronic acids, chondroitin sulfate, and heparin), blood group substances, and other glycoproteins [25]. The backbone component of most bacterial CWs is murein. It is composed of polysaccharide chains including alternate N-acetyl-D-glucosamine and N-acetylmuramic acid residues cross-linked with peptides. Interestingly, a polymer termed pseudomurein with the properties similar to those of murein was revealed in some methanogenic archaea. However, the main difference is that N-acetylmuramic acid is replaced by N-acetyltalosaminuronic acid in pseudomurein [26, 27].

Chitin microfibrils form the primary backbone of a cell. In our studies, it was shown for the first time that the chitin of mycelial fungi differs from that of other taxa in its physicochemical properties. For example, the chitin of all tested fungi contains less nitrogen than that of *Arthropoda* [28].

Chitin from fungi and other natural sources differ also in their crystallinity degree. The chitin of *Arthropoda* (of the crab *Cancer magister* and krill) and hydroids exist in the most crystalline form [29]. A comparison of fungal chitins reveals that the chitin of *Agaricus bisporus* fruiting bodies has a more crystalline form whereas the chitin of mucoraceous fungi exhibits the least orderly structure and a large number of amorphous areas [28].

Fungal chitins differ in their deacetylation degree. The chitin of *Arthropoda* has the highest number of acetyl groups, and the CGC of ascomycete fungi is less acetylated due to the presence of glucans. The chitin of *Cunninghamella japonica* and *Absidia coerulea* is characterized by a comparatively high acetyl group content among mucoraceous fungi. The chitin of insects, e.g., the cockroach *Blatella orientalis*, is acetylated to a greater extent, although the acetylation degree of crab chitin is still higher [28, 30].

Differences in the physical properties of natural chitins isolated from various organisms are also revealed by studying their IR spectra. The characteristic bands of chitin in the IR spectrum are located in the 3265, 3105, 1655–1620 (amide I) and 1550 cm^{-1} (amide II) ranges [28]. The 1100–1000 cm^{-1} range and the ratio between the peaks of amide I and amide II demonstrate the most prominent differences between chitins [31, 32].

The above data are of much interest in two regards. First, there is a relationship between the crystallinity degree and the size of the organism from which the biopolymer is obtained. Among the investigated fungi, *A. bisporus* basidiomes, a macroform, contain chitin with the highest crystallinity degree. The same pattern, an increase in the structural order of chitin with an increase in the organism size, applies to crustacean chitin, e.g., if krill and crab chitin is compared. Moreover, the crystallinity degree of chitin is probably related to the CW composition. The highest degree is revealed in higher fungi, more specifically, in those that contain the chitin–glucan complex [28]. The fungi containing chitin–chitosan in the cell wall are characterized by a lesser crystallinity degree. Apart from the support function, chitin may coordinate electrochemical processes and is involved in polyanion transport in these fungi [30]. Differences in the physicochemical properties of chitins obtained from natural sources also include different water sorption capacity and thermal stability.

Chitin performs a number of important functions in a fungal cell. In particular, it is involved in protecting it from stress [33]. The efficiency of cell conservation under unfavorable conditions varies depending on CW composition, including its chitin content. The transition of fungi to the idiophase, e.g., during starvation, results in the formation of dormant cells. This is accompanied by a drastic increase in chitin content. For instance, the level of this polymer in *Aspergillus niger* increases 2.5-fold on average [34, 35]. Intensified synthesis of chitin, which is appropriately dubbed a cell's "shield," occurs only under special conditions: against the background of changes in the composition of membrane and neutral lipids and in the microviscosity of the lipid bilayer. The membrane is enriched in phosphatidylinositol (almost threefold), and the sterol ester level of neutral lipids is increased [33]. These data suggest that the increase in chitin amount is controlled by alterations in the lipid bilayer composition involving the overproduction of a number of phospholipids and the sterol "mechanism."

The ratio between chitin and glucan, structural components of CWs, changes not only during the ontogeny of mycelial fungi. It also varies depending on the composition of the cultivation medium. In *A. niger*, the highest CGC amount is formed on media with sucrose and ammonium nitrogen upon considerable acidification of these media (down to pH 2.0). The highest chitin–glucan ratio and a glucan amount of 0.86 g/l occurred in media with a high nitrogen content [34, 35]. Currently, a number of methods of determining chitin and CGC in fungi are available. The most widely used are the methods given in [29, 36].

The question of whether proteins occur in the fungal CW used to be a controversial issue because the purity of isolated CWs was doubted. According to the generally accepted model of the CW structure, glucans are located on the CW surface. The glycoprotein reticulum is situated below the glucans, followed by a protein layer with chitin microfibrils embedded in it. The CW contains proteins involved in iron transfer and those promoting survival under stress. It was established that the protein composition of the fungal CW varies depending on the environmental conditions and the development stage. These proteins are covalently bound to polysaccharides [11]. Specific proteins

termed amyloid proteins are located on the surface of fungal cells. Amyloids are protein aggregates characterized by filamentous morphology and a high content of β-layers with β-strands that represent cross-β-structures. It has been recently suggested that the presence of such proteins in the cells is associated with the development of over 20 diseases in humans. One of these proteins, glucan transferase, was isolated from the yeast cell surface. Its physicochemical properties and physiological function were investigated, and its capacity for fibril formation in vivo and in vitro was demonstrated. A hypothesis was suggested concerning the possible mechanisms of interactions between the microbial amyloid and the proteins of the cells of the macroorganism [37]. Apart from transferase Bg112p, thioflavine-binding proteins that induce specific fluorescence and possess amyloid properties have been recently revealed in yeast [38]. It should be reemphasized that the isolation of proteins from a fungal CW suggests the possibility of their contamination by intracellular proteins. Until rather recently, both the enzymes and the structural proteins isolated from CWs were assumed to be merely cytoplasmic content admixtures. Therefore, a biotin-based method was developed that labels only the surface proteins of the CW [39]. In some studies, the method used for protein extraction from CWs provided a basis for their classification. For this purpose, EDTA, SDS, alkali, sodium chloride, and urea were used [40]. Recently, numerous proteins have been isolated from fungal CWs. It was revealed that they play an important role in the formation and operation of CWs [37].

Apart from the proteins discussed above, hydrophobic proteins referred to as hydrophobins were detected on the hyphal surface. These cysteine-rich proteins were originally revealed in *Schizophyllum commune*. They only occur in aerial hyphae; submerged mycelium lacks them [41]. Hydrophobins are small secretory proteins containing seven cysteine residues. Apart from aerial mycelium, these proteins are present in spores and fruiting bodies. Their biological function is to coordinate the adherence of hyphae to solid substrata. The hyphae are arranged perpendicularly to the substratum surface. Of paramount importance are hydrophobins for parasitic fungi during the first stage of parasite–host coexistence, i.e., attachment to the host organism [7]. Hydrophobins represent a new class of proteins. Their isolation involves methods that are not typically used for other proteins. Hydrophobins can only be extracted by trichloroacetic and formic acids. Hydrophobins form a film (~10 μm thick), covering the hyphal CW surface as a mosaic of parallel rods [42]. Polypeptide compounds that are functionally analogous to hydrophobins occur in actinomycetes. Their most widespread protein is streptofactin, an amphipathic peptide involved in the formation of aerial hyphae in *Streptomyces coelicolor*. Hydrophobins located between the CW and the aerial environment influence the morphogenetic processes including the formation of spores, basidiomes, and structures resulting from fungal infection of plant tissues. Hydrophobicity may be also due to another protein type termed repellents. They were detected in pathogenic fungi, e.g., *Ustilago maydis*. Hydrophobin formation is subject to control by two genes, *Le hyd-1* and *Le hyd-2*, revealed in *Lentinus edodes*. Recently, the nucleotide sequence of these genes was determined [44]. It has also been established that hydrophobins are necessary for the formation of chains of microconidia in *Fusarium verticillioides* [43].

The fungal CW surface may be covered with melanin, a black phenolic pigment. It is assumed that this pigment performs protective functions due to the presence of unpaired electrons in its molecule. In addition, melanin has a significant antioxidant activity. The main function of the black pigment is to protect a cell from UV radiation that can change the ion permeability of its membranes, resulting in cell swelling and rupturing. Melanin is an excellent filter that cuts off over 90% of UV radiation [45].

An additional component of the fungal CW is sporopollenin, a product of oxidative polymerization of β-carotene [46, 47]. Isolation of sporopollenin from natural sources involves consecutive degradation, dissolution, and hydrolysis of the compounds of the CW. Sporopollenin is even more resistant to environmental factors than chitin. It is resistant to alkali and strong (sulfuric, hydrochloric, hydrofluoric, and phosphoric) acids. It can be degraded with very strong oxidizing agents, e.g., ozone followed by the addition of H_2O_2, chromic acid, and nitric acid [48].

Sporopollenin is located in the outer layer of the zygospores of carotene-synthesizing fungi. Chitin and chitosan are also arranged in this layer. Cytochemical studies revealed that sporopollenin performs a structural function and maintains the integrity of the biopolymers of this CW layer. Being a highly unsaturated compound, sporopollenin probably also performs antioxidant functions, since reactive oxygen species may be generated on the surface of cells (e.g., pollen grains) [49].

Uronic acids are present in the fungal CW. For instance, the CW of mucoraceous fungi contains a unique heteropolysaccharide, mucoran, consisting mainly of uronic acid, fucose, galactose, and mannose [50]. Two types of polyuronide fractions were detected and partially characterized in *Mucor rouxii*: (i) mucoran, a readily hydrolyzable heteropolysaccharide and (ii) a homopolymer of D-glucuronic acid that is resistant to formic acid and insoluble in a number of solvents. Apart from these polysaccharides, a high molecular mass heteropolysaccharide with weak acidic properties that contains only glucuronic acid and D-mannose was detected in mucoraceous fungi that produce yeastlike forms.

The presence of uronic acids in fungal CWs is of considerable interest in several respects: (i) it demonstrates the similarity of fungi and plants because some

plant xylans contain glucuronic acids as supplementary components; (ii) there is evidence that uronic acids impart plasticity to CWs, providing for their extensibility; and (iii) the fact that the content of uronic acids varies depending on the fungal ontogeny stage is of much interest in biological terms. These monosaccharides are abundant in the sporangiophores of some fungi, while almost absent in the spore CW. Interestingly, the composition of the heteropolymer mucoran varies depending on the morphology of the fungi involved. In mucoraceous fungi, the transition from mycelium to the spherical form (dimorphism) is accompanied by alterations in the monosaccharide composition of mucoran: the galactose and fucose contents change. These changes are currently regarded as biochemical criteria of fungal morphogenesis.

The lipids of fungal CWs were investigated to a lesser extent than the other components of this surface structure. In chemical terms, they are analogous to the lipids of whole cells. Most attention is given to compounds occurring in spore CWs along with lipids and termed sporidismolides (depsipeptides). These compounds are extracted from spore CWs by benzene together with the lipids. Although sporidimolides were already discovered at the end of the 20th century, their exact function and the reason for their close association with lipids have not been elucidated yet. Presumably, they inhibit bacterial growth, creating more favorable conditions for spore conservation and germination [51].

Thus, the chemical composition of the fungal CW provides reliable protection for the cells, particularly if they are in a state of dormancy and exposed to such deleterious factors as UV, γ-, and other kinds of radiation; chemical reagents; lytic enzymes; etc. It is likely that this protective function is primarily performed by chitin [52] that is bound to glucan or mucoran (this compound functions in the fungi of the *Mucoraceae* family). An increase in chitin and CGC content, e.g., upon depleting the nutrient substrate and the onset of starvation, as well as the function of structural proteins can be regarded as a peculiar response of an organism to deleterious environmental factors. The response is aimed at protecting the cell from the deleterious factor by means of a stable biopolymer complex. Of special interest in this context is the suggestion that temperature can be the factor whose effect activates chitin's protective function. It was established that the chitin content in the CW of the mucoraceous fungus *C. japonica* drastically increases at a high temperature. Conversely, upon lowering the temperature, less chitin is synthesized than in the control system [52]. The content of water-soluble proteins is increased in the CW in this situation.

Nevertheless, chitin does not perform this function in all fungi. In some ascomycete yeast (*Hansenula polymorpha*), resistance to environmental functions is provided by special CW proteins, with these proteins playing a more important role in forming the CW structure than polysaccharides [53].

In addition to the above functions, the CWs of fungi, like those of plants, possess both cation- and anion-exchange properties. Studies were conducted with the mucoraceous mycelial fungus *C. japonica* using a novel method revealing the fungal CW as an integral structure without its chemical destruction. Potentiometric nonaqueous titration and elemental analysis were used. It was shown for the first time the CWs of fungi, like those of plants, contain amino groups with a pK_a of 3.5–4.0 and cation-exchange groups with a pK_a of 8.5–9.0. This enables a fungal CW to be a natural cation or anion exchanger, depending on the environmental conditions [54–56].

CW STRUCTURE AND INTERCELLULAR INTERACTIONS IN THE FUNGAL HYPHAE

The CW structure, including the number of its layers, displays a clear dependence on mycelium age. In fungi, the primary and secondary CW are to be distinguished. The primary CW is formed at the hyphal apex and becomes an internal layer of a mature hypha in the process of its growth. Using fluorescent wheat germ agglutinin and Cacofluor staining made it possible to establish that the primary CW chitin is more sensitive to these compounds than the mature CW chitin. It was also revealed that chitin is the first CW layer to be synthesized [57]. The primary CW contains chitin and a protein layer arranged below it, is very thin (130 nm), and stains weakly with periodic acid and silver thiocarbazide–proteinate. As the distance from the apex increases, the CW becomes appreciably thicker (up to 280 nm) and additional layers are visualized by microscopy. Hence, the formation of the fungal CW includes two stages. Initially, the primary chitin CW is generated. Subsequently, the secondary CW is synthesized; it contains glucans and other components located over the primary CW.

The apex of the hypha, i.e., its most actively growing part, contains a very large number of mitochondria. They perform two functions: they contain calcium and are involved in calcium transfer from the cytoplasm. Inhibiting the synthesis of DNA or mitochondrial protein for a short time does not affect the apical hyphal growth and the mitochondria's shape and distribution in the hypha [58]. One more difference between the apical and the lower part of hypha is due to the existence of cooperation among its cells in terms of energetics. At the very apex, the electric current is weaker than at a distance of 30 μm from it. The current disappears at a distance of 300–400 μm from the apex [59]. It was also revealed that the formation of a side branch of new mycelium is preceded by the arrival of a still unknown signal at this point. This results in local actin accumulation, a restructuring of the adjacent cytoplasm, and the activation of chitin wall-degrading enzymes. Interestingly, the minimum

mycelium growth unit that may be separated and grow independently is a fragment at least 350 μm in size. The above data testify to the existence of intercellular interactions and cooperation with respect to energetics in the hypha. Hence, the cells of a growing hypha can exchange energy, which promotes the mycelium's active growth. The secondary CW of mucoraceous fungi contains the fascinating heteropolysaccharide mucoran, which includes D-mannose, L-fucose, D-galactose, and D-glucose. Uronic acids also form part of this biopolymer [60].

PHENOMENON OF APICAL CELL WALL GROWTH

It is the apical growth of fungal hyphae that is responsible for the fact that only fungi can grow at a rate of 100 μm per minute and accumulate an extraordinarily large biomass. This process requires a high activity of special vesicles (actin filaments and microtubules) and the mechanisms of secondary substrate transfer.

At the hyphal apex, the new CW is continuously synthesized and substrate-degrading enzymes are released. All these growth-related processes in the CW are coordinated by vesicle aggregation centers, special formations that also control the rate of vesicle transfer, thereby determining the morphology of the growing hypha. The lower the rate of vesicle transfer, the more oval the growing cell; hyphae are formed only at a very high vesicle transfer rate [60, 61].

The growth of a fungal hypha is comparable to the propulsion of living substance along a tunnel formed by the hypha itself, which sustains a pressure of 4–18 atmospheres. The tunnel is made up of chitin [59]. It was established that the highest chitin synthetase activity occurs at the apex of the growing hypha and at points where branching hyphae and the septum are formed. For *Aspergillus nidulans*, it was established that the RhoA enzyme (belonging to the GTPase family) is involved in the polarized growth of hyphae, their branching, and CW synthesis. This enzyme is hypersensitive to CW-degrading agents such as calcofluor and caspofungin acetate. It increases the N-acetylglucosamine level in the fungal CW [62].

According to present-day concepts, the fungal CW is, therefore, a system of microfibrils embedded in an amorphous matrix. Such fibrils of backbone CW components may be composed of glucan, chitin, chitosan, or cellulose, depending on the species of the fungi involved. The rest of the CW components, such as proteins, polysaccharides, lipids, and pigments, are regarded as cementing substances that form ionic or covalent bonds with the microfibrillar part of the CW. Owing to such dissimilar components, the CW possesses discordant properties: the structure can be both strong and fragile, permeable for some substances and impermeable for others.

The two CW moieties differ with respect to their formation/biosynthesis. The data obtained with fungal protoplasts reveal that the matrix and the fibrils are not formed synchronously. The biosynthesis of the backbone part of the CW is catalyzed by constitutive polysaccharide synthetases that are uniformly distributed in the plasmalemma. However, the protein-bound polysaccharide matrix is synthesized from its precursors by enzymes located both in the rough and smooth endoplasmic reticulum. The polymerized products are transferred by vesicles to the site where the new CW is formed.

It was established that microfibril synthesis is sufficiently independent of protein synthesis. This was demonstrated in experiments with the translation inhibitor cycloheximide. In contrast, matrix microfibrils are formed with the involvement of enzymes synthesized de novo. Nevertheless, protein synthesis inhibition results in changes in CW morphology, including the apical part of the hypha (Feofilova, unpublished). Presumably, the fact that backbone polysaccharides are independent of de novo protein synthesis is essential for fungi. This is the mechanism that enables fungi to undergo certain morphogenesis stages under unfavorable conditions, e.g., under nitrogen limitation.

THE MAIN ENZYMES INVOLVED IN CELL WALL BIOSYNTHESIS AND LYSIS

Two enzymes are involved in the formation of backbone polysaccharides. One of them carries out the final stage of aminopolysaccharide synthesis, and the other is responsible for aminopolysaccharide hydrolysis. The first enzyme is termed chitin synthase or chitin synthetase (uridine-diphosphate-2-acetamido-2-deoxy-D-glucose: chitin 4-β-acetamidodeoxyglucosyltransferase, EC 2.4.1.16). The chitin-synthesizing factor (EC. 3.4.22.9) is proteinase B or protease B. Chitin synthase is usually in the zymogenic form. It is activated by the protease in a process that also requires lipids. A prerequisite for chitin synthesis is sterol, the neutral membrane lipid that is responsible for the relevant fluidity of the lipid bilayer. A unique glycolipid, acetylglucosaminoglycosyldiacylglycerol, is directly involved in chitin formation. In all likelihood, this lipid does not perform a metabolic function. It plays the role of the primer during chitin synthesis. Chitin synthetase exists in three different states in a hypha: (i) as a zymogen in the vesicles termed chitosomes, (ii) as a zymogen in the plasma membrane, and (iii) as an active enzyme in the plasmalemma. Chitosomes are characterized by a unique composition of the lipid membrane that contains almost 80% of sterols. Although chitosomes were discovered a long time ago [51], their lipid composition, including the extraordinarily high sterol level, still is of much interest presently. Plausibly, domain structures, e.g., lipid rafts and caveoles containing glycosphingolipids and sterols, are

implicated in regulating the chitin synthesize activity. Importantly, a relatively short time ago the main emphasis was still placed on phospholipid research. However, increasing attention has been recently given to membrane sterols and their complexes with glycolipids (ceramides) that provide for sufficiently quick changes in the microviscosity of a lipid membrane [63–66].

The other enzymes are chitinases (poly[1,4-β-(2-acetamido-2-deoxy-D-glucoside]glycan hydrolases, EC 3.2.1.14) that catalyze the degradation (lysis) of CW chitin molecules. This process is a prerequisite for the onset of hypha branching. Chitinase-involving lysis of a part of the hyphal CW proceeds, and the formation of a side hypha starts. Chitinases were detected during spore swelling and germination. These enzymes are induced upon mechanical damage in some zygomycetes, e.g., *Choanophora cucurbitarum*. Chitinases predominantly function as endoenzymes: they cleave chitooligosaccharides 2–6 N-acetylglucosamine residues long. Chitinases are produced by a large number of bacteria, and chitinase-involving chitin lysis is widespread in nature.

According to the data available in the literature, hundreds of millions of tons of chitin are produced in nature [67, 68]. Soil microorganisms exert their effects on fungal and insect chitin. Apart from the exoenzymes chitinases (glycoside hydrolases), they form chitobiases that degrade chitin to N-acetyl-D-glucosamine [69]. Chitinases are subdivided into two families, 18 and 19, that differ in their amino acid sequence, the molecular mechanism of catalytic reactions, and, presumably, the evolutionary background. Glycoside hydrolases (GH) of family 18 are found in such organisms as bacteria, fungi, viruses, animals, and higher plants, whereas the GH of family 19 are less common and chiefly occur in higher plants and some bacteria [70].

Mycelial prokaryotes (actinomycetes) are the most active chitinase producers in the soil [71]. Chitin carbon is consumed by microorganisms three times more actively than the carbon of soil organic matter [72]. Among other bacteria, the species *Serratia marcescens* exhibits high chitinase activity [73]. This organism synthesizes one intracellular and four extracellular proteins that are encoded by different genes and possess different physicochemical properties. These chitinases are referred to as ChiA (58.8 kDa), ChiB (55.4 kDa), ChiC (51.7 kDa), and CBP 21 (21.8 kDa). They are excreted into the environment, adhere to chitin particles, and depolymerize them. Protein CBP 21 lacks hydrolase activity but binds to chitin in such a way as to increase its susceptibility to hydrolysis involving the other chitinases. The main compound resulting from chitin hydrolysis is the N-acetyl-D-glucosamine dimer that is thereupon cleaved into monomers by the enzyme N-acetyl-D-glucosaminidase (EC 3.2.30) [74]. Presumably, the chitinases involved in the hydrolytic processes associated with hypha branching are insignificantly different from those described above.

The hydrolytic enzymes of microorganisms also include chitosanases (EC 3.2.1.132). They are glycosyl hydrolases that catalyze chitosan degradation in fungal CWs. Chitosan is a mixture of polysaccharides containing β-1,4-bound D-glucosamine and N-acetyl-D-glucosamine residues. Microbial chitosanases mainly hydrolyze the glucosamine–glucosamine bonds in the chitosan molecule. The capacity for chitosan degradation depends on its deacetylation degree. It was demonstrated that chitosanases are more active if the substrate is chitosan with a deacetylation degree of 70% [75]. Chitinases and chitobiases actively participate in the sexual reproduction of fungi, for example, during the interaction of heterothallic *Mucorales* strains resulting in zygote formation. The enzymes lyse the CWs of the (+) and (–) strain hyphae during this process [76].

In conclusion, we emphasize the undoubtedly fascinating comparison between the function of the integument in fungi and mammals that appears to enable us to highlight the functional role of the CW in lower eukaryotes. The backbone part of the hypha consisting of aminopolysaccharides and glucans is located on the surface of the fungal cell. In vertebrates, the skeleton, in contrast, occupies the internal part of the organism and performs other functional roles in addition to the support function. As for fungi, their CWs are responsible for the protective function apart from the role of the "skeleton." The protective function is performed by the fungal CW very efficiently, clearly surpassing human skin in this respect. The key role is played by chitin and chitosan. According to recent data, chitin is more chemically resistant to reagents than collagen. In combination with melanin, chitin protects the cell from UV light, other kinds of radiation, and heavy metals. It possesses pronounced anticancer and antibacterial activities. These properties are enhanced if chitin forms a complex with glucans. In this respect, the mammalian integument, the skin, is less efficient than the backbone polysaccharides of the fungal CW.

The outer fungal "skeleton" is essential for morphogenesis. The dimorphism phenomenon is widespread among fungi. This means that the mycelium form is replaced by a yeastlike form under unfavorable conditions. This adaptive response helps the fungi to survive under microaerobic conditions and at an elevated partial pressure of CO_2. The role of chitin is essential for this morphological change, as emphasized above. The CW also promotes the formation of dormant cells (spores), surviving in a state of anabiosis. Under these conditions, the CW composition undergoes drastic changes (compared to the vegetative mycelium) and becomes a peculiar "armor" consisting of aminopolysaccharides, glucan, sporopollenin, and melanin that are highly resistant to environmental factors. In contrast, animals are unable to radically

change their body shape and integument composition. As a rule, they cannot carry out any horizontal gene exchange [77, 78] and do not survive in an anabiotic state. Hence, the CW is a sufficiently progressive adaptation, at least in terms of species preservation. Importantly, the CW is the first cell component to alert the cell to stress. Special sensory compounds composed of a protein and a chromophore (a flavin or carotenoid) are located on the CW surface. They convey the stress signal causing the fungal cell to undergo radical metabolic changes and to make the transition from homeostasis to enantiostasis. Finally, it should be reemphasized that the presence of the CW enables the fungi to intensify their growth process and, accordingly, to colonize nutrient substrates to an extent which is unattainable for animals.

All the above ideas were nicely expressed in the words by the mycologist J. Gooday. As early as in 1973 [79], he emphasized that the cell wall directly participates in the formation of structures that we recognize as hyphae, spores, and fruiting bodies, i.e., it *represents a material expression of fungi*.

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