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Fungal Cell Walls: Their Structure, Biosynthesis and Biotechnological Aspects

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Summary

The article briefly reviews the present knowledge on various aspects of fungal cell wall structure, function and biosynthesis of its individual components. A special attention is devoted to biotechnological potential of cell walls and cell-wall linked phenomena.

Introduction

For a long time, the fungal cell wall has been considered as a more or less rigid shell surrounding the metabolically active protoplast and protecting it against the osmotic and mechanical stresses coming from the environment. It has been also noted that the cell wall plays an indispensable role in maintaining the cellular morphology and in cytokinesis.
The knowledge accumulated in the last two decades has shed new light on the fungal cell wall and its functions. It has been recognized that the cell wall is by no means an inert structure but that it is in fact a very active, "living" cellular organelle, fulfilling many vital functions in the life of the cells:

a) The cell wall is the site of somatic antigens located at the cell surface and playing role in such phenomena as mating, agglutination, interactions with the cells of other organisms and the interactions with the environment.
b) The cell walls are the site of various enzymes, both of those involved in the metabolism of the wall itself (e.g. synthases, hydrolases) or enzymes necessary for breakdown of certain complex nutrients before their entering the cell (e.g. glycanases, phosphatases, lipases, proteases, etc.).
c) The cell walls may serve as a transient station for the enzymes secreted by the cell and subsequently released into the medium.
d) Finally, the cell wall components may, in some situations, serve as a metabolic reserve of nutrients, such as carbohydrates, aminoacids, phosphate, cations, etc.

Since all these functions of the walls appear to be vital in some instances, it is obvious that they should be taken into consideration when solving various biotechnological problems connected with growth of the cells and their interactions with the environment.

Cell Wall Structure

Polysaccharides and protein-polysaccharide complexes are main components of the fungal walls, accompanied by minor quantities of compounds such as lipids, melanins and polyphosphates.

BARTNICKI-GARCIA [1] has first pointed out that there exists a close correlation between the taxonomic classification of fungi and the overall chemical composition of their cell walls.

In most cases, the first approach towards chemical characterization of the cell walls is their solubilization in solutions of alkali [2]. During this treatment, the amorphous matrix wall components are solubilized leaving behind crystalline cell wall polymers. Tab. 1 shows that individual taxonomic groups of fungi differ markedly in the chemical nature of their alkali-soluble and alkali-insoluble wall components [3].

Tab. 1. Alkali-soluble and alkali-insoluble polymers in fungal cell walls [8].

Taxonomic group	Cell wall polymers	
	Alkali-soluble	Alkali-insoluble
Basidiomycotina	Xylo-manno-protein α-1,3-D-glucan	β-1,3/β-1,6-D-glucan Chitin
Ascomycotina	(Galacto)-manno-protein α-1,3-D-glucan	β-1,3/β-1,6-D-glucan Chitin
Zygomycotina	Glucurono-manno-protein Polyphosphate	Polyglucuronic acid Chitosan Chitin
Mastigomycotina		
Chytridiomycetes	Glucan[a]	Glucan[a] Chitin
Hypochytridiomycetes	Not determined	Cellulose Chitin
Oomycetes	β-1,3/β-1,6-D-glucan	β-1,3/β-1,6-D-glucan Cellulose

[a] incompletely characterized (presumably β-1,3/β-1,4-D-glucan)

The solubility properties of cell wall polymers reflect not only their chemical nature, but also their functions in the wall. Thus, the alkali-insoluble cell wall polymers constitute the wall skeleton, giving to it the required mechanical strenght and flexibility, whereas the alkali-soluble ones play the role of cementing substances, linking together individual components of the wall skeleton and, in addition, they fulfil diverse biological functions.

Generally, there is a great similarity in the architecture of cell walls among fungi. The skeletal, microfibrillar wall components form the innermost wall layer embedded in an amorphous wall matrix which extends throughout the wall. The outer surface of the walls is usually smooth or slightly reticular, composed mainly of amorphous material.

The cross-linking between the individual wall polymers which adds to the mechanical strength of the walls can be of covalent and/or of noncovalent nature. For example, the existence of covalent links between glucan and chitin has been proved in the walls of *Aspergillus niger* [4]. In *Schizophyllum commune*, the link between β-glucan and chitin is accomplished by peptide bridges rich in lysine [5].

Covalent mannan-glucan complexes have been described in the cell walls of the yeast *Pichia polymorpha* [6]. Disulphide bridges between the protein moieties accomplish the cross-linking of mannoprotein molecules in the cell walls of yeasts [7]. Besides that, noncovalent interactions, such as hydrogen bonding and VAN DER WAALS forces may also contribute to adherence of individual cell wall components [8].
The schematic illustration of the structure of the cell wall of the yeast *Saccharomyces cerevisiae* depicted in the Fig. 1 is an integration of numerous results from chemical, biochemical and ultrastructure studies [9]. The periplasmic space located between the plasma membrane and the wall contains soluble mannoprotein components, some of them of enzymic nature. i.e. invertase, acid phosphatase, etc., which acumulate here before their incorporation into the wall or release into the medium. The innermost wall ayer formed by crystalline β-glucan is linked covalently to mannoprotein which extends through the wall up to the wall surface.

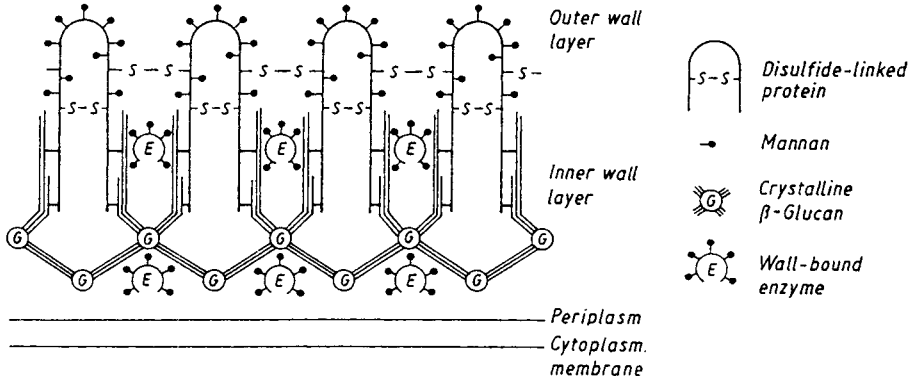

Fig. 1. Schematic illustration of the cell wall structure of the yeast *Saccharomyces cerevisiae*

The disulfide bridges are cross-linking the protein moieties of mannan and function as a molecular sieve preventing the escape of wall-located enzymes into the medium. For this reason, the treatment of the intact yeast cells with protease or thiol reagents is essential for liberation of periplasmic enzymes or for penetration of exogeneous enzymes used for preparation of protoplasts to the inner wall-layer polysaccharides [10]. An interesting feature of the yeast cell wall architecture is that the outer surface polymers (i.e. mannoproteins) are oriented radially whereas the β-glucan chains in the inner wall layer are parallel to the cytoplasmic membrane [11].

Biosynthesis of the Cell Walls

Owing to structural complexity of fungal cell walls, it is impossible to describe their biosynthesis as an unidirectional process in terms of simple biochemical reactions.
The methods employed to study the biosynthesis of fungal cell walls range from chemical and biochemical to physical and cytological ones. Naturally, the more complicated system is used in these studies, the more difficult is the straightforward interpretation of results.
For this reason, here is mainly concentrated on the biochemical mechanisms involved in the biosynthesis of individual cell wall components and their regulation.

Chitin

Chitin is the most caracteristic component of fungal cell walls. Its contents in the walls ranges from nil, as for example in the yeast *Schizosaccharomyces pombe*, to well over 40% of the dry cell wall weight in some filamentous fungi [12].

Autoradiographic investigations have shown that chitin is synthesized at plasma membrane/cell wall interface (e.g. [13, 14]). In filamentous fungi, the chitin is formed at the tips of the hyphae and at the sites of the forming septa [15, 16], whereas in the yeast *S. cerevisiae* the chitin is made only at the site of septum, and after separation of mother and daughter cells, it remains as the constituent of the bud scar on the wall of the mother cell [17]. The enzyme chitin synthase is localized in the plasma membrane, its active site facing the cell interior [18]. A prevalent part of chitin synthase is found in an inactive, zymogen state and "in vitro" it can be activated by limited proteolysis [19, 20]. It is not yet clear whether limited proteolysis is physiological mechanism to activate chitin synthase "in vivo". The originally isolated vacuolar protease believed to play the role of an "activating factor" of chitin synthase [21], has been shown to be identical with proteinase B [22], and to be dispensable for normal growth and formation of chitin in yeast [23]. A part of chitin synthase can be found in microvesicular, 40 to 70 nanometers in diameter particles termed "chitosomes" which are present in cytoplasm and considered to serve as conveyors of chitin synthase from its site of synthesis in the endoplasmic reticulum to its destination at the cell surface [24, 25].

Recent findings indicate that, at least in the yeast *S. cerevisiae*, there are in fact two chitin synthases. Chitin synthase 1 (chs 1) which accounts for about 95% of total chs activity is not essential for the formation of septa and mutants lacking it grow and divide normally [26]. The second enzyme, chs 2, shares certain properties with chs 1, such as activation by proteases and location at the plasma membrane, however, it differs in cation dependence and pH optimum [27]. Disruption of chs 2 gene is lethal to the cells and the chs 1 cannot substitute for chs 2 [28].

The location of chitin synthase on the inner face of the plasma membrane poses the question of how the chitin chains traverse the plasma membrane into the cell exterior. There is a fairly good evidence given by CABIB et al. [29] that the polymerization of N-acetyl-D-glucosamine residues from UDP-N-acetyl-D-glucosamine (UDP-GlcNAc) is a vectorial process during which the nascent chitin chains are extruded through the plasma membrane into the extracytoplasmic space.

Inhibition of Chitin Synthesis. The presence of chitin in the cell walls of most fungi, distinguishes them clearly from the cell surfaces of vertebrates and plants and makes chitin a highly perspective target for specific antifungal agents. In analogy with penicillin, which disturbs selectively the biosynthesis of bacterial wall peptidoglycan, the perspective fungicides should interfere specifically with the biosynthesis of chitin without having any side effects on biochemical reactions proceeding in the cells of the host.

One group of such compounds discovered to date are nucleoside antibiotics polyoxins produced by *Streptomyces cacaoi* [30, 31] and to a limited extent used in the field as fungicides for protection of plant crops [32]. The inhibitory properties of polyoxins upon the chitin synthase are based on their structural similarities with the natural substrate of chitin synthase, UDP-N-acetyl-D-glucosamine (Fig. 2). In vitro, the polyoxins are powerful competitive inhibitors of fungal chitin synthases with K_i's being in the micromolar range. Application of polyoxins to growing fungi leads to dissolution of mycelia at their growth zones [30, 33, 34].

However, not all fungi are equally sensitive towards polyoxin and resistance is developed in some strains [30]. The mechanism of this resistance is not clear, but there are indications that some peptides may prevent the effect of these antibiotics, probably by interfering with transport of the antibiotics into the intact cells [34, 35].

Other nucleoside-peptide antibiotics, structurally related to Polyoxins are Neopolyoxins and Nikkomycins [36]. They also are very effective in inhibiting chitin synthase "in vitro", but "in vivo" their effect varies [37, 38].
Besides these specific inhibitors of antibiotic nature, there is a great number of compounds described to act more or less selectively as inhibitors of chitin synthase. Among them are chlorinated hydrocarbons and cyclodienes, nucleoside and base analogs and other compounds (for review see ref. [39]).

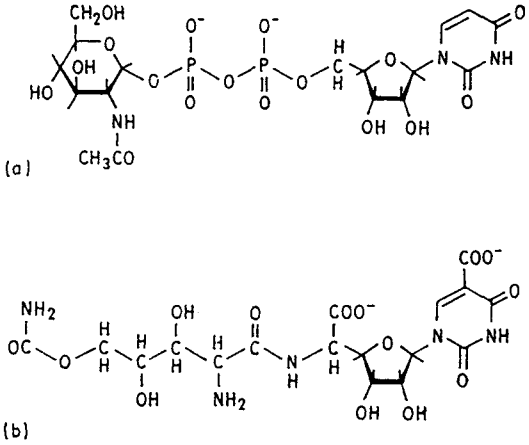

Fig. 2. Chemical structures of a) UDP-N-acetyl-D-glucosamine and b) Polyoxin D

A special group of inhibitors of chitin synthesis are various brighteners and dyes such as Calcofluor white, Primulin and Congo red. The principle by which these compounds inhibit synthesis of chitin is somewhat different from the previously described competitive inhibition of chitin synthase: "in vivo" they do not act on the synthase itself but rather, they interact through hydrogen bonding with nascent chains of polysaccharide and in this way prevent their crystallization and regular assembly [40, 41]. When assayed "in vitro", Calcofluor white and Congo red act as noncompetitive inhibitors of chitin synthase [42, 43].
Another approach how to inhibit selectively fungal growth via interactions with chitin is the dissolution of wall chitin with chitinases. There is accumulating evidence that this may be one of the principal defense mechanisms by which plants protect themselves from chitin-containing parasites [44–46]. It is interesting that plant chitinases are much more powerful inhibitors or fungal growth than their microbial counterparts. As little as 1 μg/ml of plant chitinases are capable to inhibit effectively spreading of fungal colonies on agar plates [45].

Chitosan

In some fungi e.g. *Zygomycetes*, chitosan, a linear polymer of β-1,4-linked D-glucosamine represents the principal cell wall component. Although studies concerning the chitosan biosynthesis are scarce, there is fairly good evidence that this polysaccharide is in fact made by deacetylation of nascent chitin. The enzymes chitin synthase and chitin deacetylase operate in tandem and neither enzyme alone is capable to catalyze the formation of chitosan [47, 48].

Glucan

Fungal β-glucan (Fig. 3) is made bypolymerization of glucosyl units from UDP-glucose (UDP-Glc). The enzyme glucan synthase, similarly as chitin synthase, is located at the plasma membrane, its active site facing the cell interior [49]. There are indications, that glucan molecule is elaborated while covalently attached to the enzyme or some other proteinaceous primer [50, 51].
Although fungi contain mostly glucan containing mixed β-1,3/β-1,6-glycosidic links [52], the particulate glucan synthase preparations from fungi are capable to synthesize "in vitro" almost exclusively only a linear β-1,3-glucan. For this reason, some authors suppose that the β-1,6-glycosidic links are elaborated as a postsynthetic modification of β-1,3-glucan by a branching enzyme located outside the plasma membrane [53, 54].

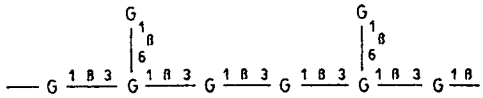

Fig. 3. Structure of the fungal cell-wall β-glucan. G, glucopyranosyl unit

The activity of particulate β-1,3-glucan synthase is influenced by a number of compunds. The most powerful stimulator of the enzyme from different sources is GTP whereby the concentration of the nucleotide sufficient for half maximal activation lies in micromolar range [55, 56]. The binding of GTP to the enzyme is mediated by an easily-dissociable regulatory protein. The soluble regulatory component interacts with GTP and on combination with the membrane fraction that has catalytic function brings about the activation of glucan synthase [57]. Among other activators of fungal glucan synthase are β-linked glucose disaccharides [50], glycerol, bovine serum albumin, EDTA [58], phosphatase- and protease inhibitors [59].
From practical point of view, interesting are compounds that inhibit selectively β-1,3-glucan synthesis in fungi. Among these are sorbose, δ-gluconolactone, papulacandin B and echinocandin B [60], aculeacin A [61] and neopeptins [62]. The unspecificity of action of these compounds on glucan synthase is reflected in a mixed- or uncompetitive types of inhibition that can be observed in kinetic assays with particulate β-1,3-glucan synthase [60].
Similarly as in the case of chitin, the dyes Congo red and Calcofluor white are capable to disorganize the "de novo" formed glucan microfibrils and cause distortion of fungal growth and morphology [40, 41]. Due to interference with the normal organization of the cell wall, the rate of chitin and β-glucan synthesis in regenerating protoplasts and growing cells is increased in the presence of these dyes, however, when tested "in vitro" with particulate polysaccharide synthases, the dyes are inhibitory [41—43].

Glycoproteins

This is a rather broad group of cell wall components, represented by proteins glycosylated to various degree with carbohydrates of different chain length and composition. Structural complexity and variability of glycoproteins enables them to fulfil in cells diverse vital functions which are derived from their enzymatic, antigenic and physico-chemical properties.
So far, the best studied fungal wall glycoprotein component are mannoproteins. Their general structure depicted in Fig. 4 stems mainly from studies made by BALLOU and his coworkers [63]. There are two types of linkages between carbohydrate and protein moie-

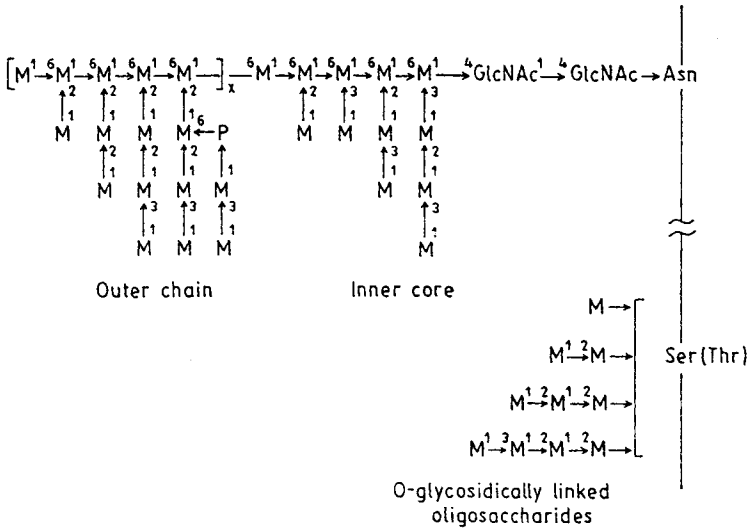

Fig. 4. Structure of mannan from the cell walls of the yeast *Saccharomyces cerevisiae*. M — mannose; GlcNAc — N-acetyl-D-glucosamine; Asp, Ser, Thr — aminoacids asparagine, serine and threonine [63]. All links are α, except 1.4 — bonds

ties in yeast mannoproteins. The polymannose chain, structurally resembling the carbohydrate moiety of the so-called "high-mannose" glycoproteins of higher organisms, is attached N-glycosidically via N,N'-diacetylchitobiose bridge to asparagine residues in the protein part of the molecule. The second carbohydrate moiety is represented by short mannooligosaccharides attached 0-glycosidically to serine and/or threonine residues in the protein. Some fungal glycoproteins contain both types of glycosidic linkages, whereas some are exclusively N-glycosylated or 0-glycosylated.

Reaction pathways leading to biosynthesis of individual carbohydrate moieties in yeast mannan are different and they can proceed independently [64]. Both involve in the first steps participation of glycosylated lipid intermediates of the type of dolichyl phosphate-mannose (Dol-P-Man, Fig. 5).

The mannosyl donor for the biosynthesis of yeast mannoproteins is guanosine-5'-diphosphate mannose (GDP-Man). During biosynthesis of 0-glycosidically linked mannooligosaccharides, the first mannosyl unit is added to serine or threonine from the lipid

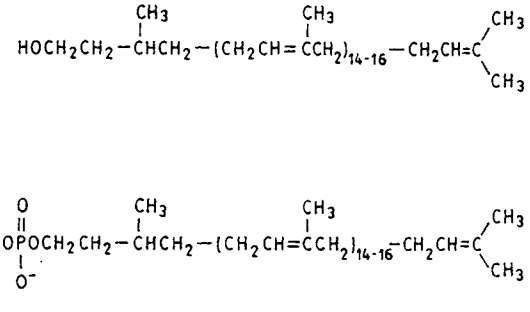

Fig. 5. Structures of a) dolichol and b) dolichyl-phosphate mannose

intermediate Dol-P-Man. The further additions of mannosyl units to growing oligomannosyl chains take place by mannosyl transfer directly from GDP-Man [65, 66].
The formation of N-glycosidically linked polymannose chain follows a rather complicated pathway, very similar to the reaction sequence involved in the biosynthesis of animal glycoproteins [67]. As depicted in the Fig. 6, the whole process can be divided into five stages which are separated both spatially and temporally.

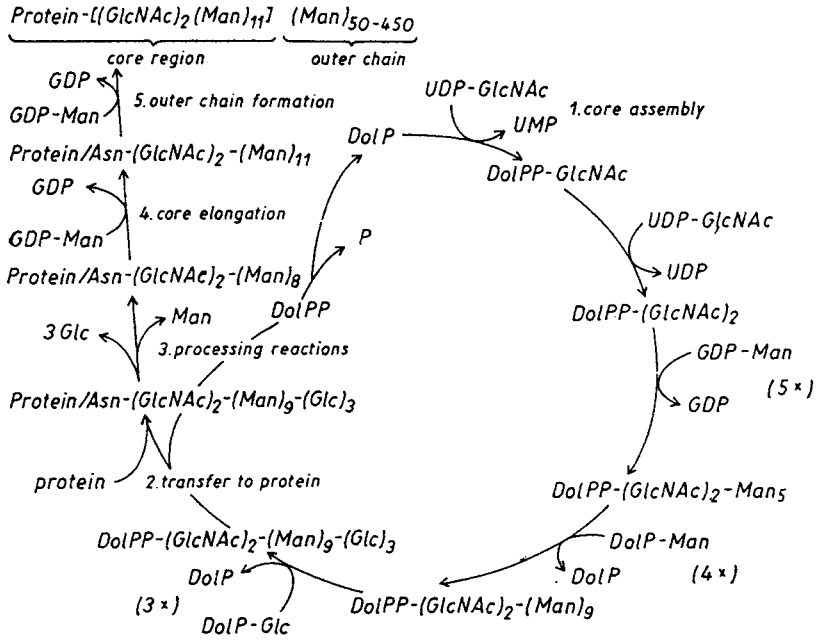

Fig. 6. Scheme illustrating the sequence of reactions leading to biosynthesis of polymannose part of mannan molecule [68]

In the first stage, the core consisting of two N-acetylglucosamine- and nine mannosyl units is assembled on the lipid under the formation of Dol-PP-$GlcNAc_2Man_9$. During this process, the first five mannosyl units are added to the growing oligosaccharide directly from GDP-Man, while the remaining four mannosyls stem from Dol-P-Man [68].
The reason why it is so is not completely clear. In an analogous mammalian system, the first five mannoses are added to the Dol-PP-$GlcNAc_2$ at the cytoplasmic side, whereas the next four mannosyls are transferred to lipid-oligosaccharide at the luminal side of the endoplasmic reticulum [69]. After successive addition of three glucosyl units to growing core, the oligosaccharide $GlcNAc_2Man_9Glc_3$ is transferred "en block" by a specific oligosaccharyl-transferase to the peptide.
Core oligosaccharides are added to protein in the endoplasmic reticulum (ER), where the three glucosyls and one mannosyl unit are removed by action of specific glycosidases [70, 71]. The "trimmed" glycoprotein is then transported from ER to GOLGI apparatus, where the addition of outer-chain mannosyl units takes place [72]. ER to GOLGI transport is energy-dependent and requires the presence of ATP [73, 74].
The finished mannoproteins are packed into GOLGI-derived vesicles and transported towards plasmalemma where they fuse with it and discharge their contents into the cell exterior or incorporate it into the growing plasma membrane.

The elucidation of the glycosylation and secretory pathways in yeast has been greatly facilitated by the use of secretory mutants (*sec* mutants) and mutants defective in individual steps of glycosylation and trimming of the inner core oligosaccharide (*alg* and *gls* mutants). Thus, the *sec* mutants have temperature-sensitive defects in protein transport between ER, GOLGI and cell exterior [73]. Accordingly, at the nonpermissive temperature, they accumulate secretory glycoproteins in the endoplasmic reticulum, GOLGI bodies or in secretory vesicles (Fig. 7). Using these mutants, it has been shown that reactions requiring dolichyl-phosphate intermediates, i.e. the formation of the inner core oligosaccharide and single mannosylation of serine and/or threonine residues, take place in the ER, whereas the formation of polymannose outer chain and elongation of 0-glycosidi-

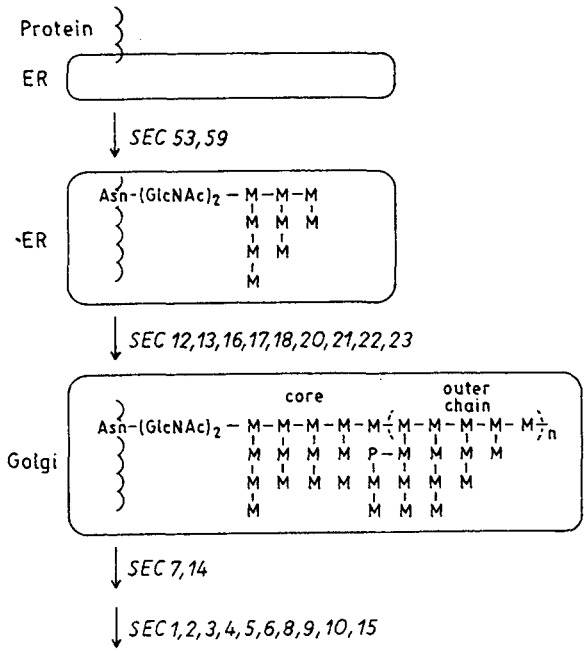

Fig. 7. Compartmentalized assembly of yeast mannan molecule. The numbers indicate individual *sec* mutants blocked at different stages of the secretory pathway at the nonpermissive temperature [100]

Tab. 2. Characterization of mutants defective in glycosylation and processing of the core oligosaccharide. Compiled from Refs. [77—79]

Mutation	Structure of the accumulating lipid-linked oligosaccharide	Defective enzyme
alg 1	$GlcNAc_2$	β-1,4-mannosyl transferase
alg 2	$Man_{1-2}GlcNAc_2$	α-mannosyl transferase
alg 3	$Man_5GlcNAc_2$	α-1,6-mannosyl transferase
alg 4	$Man_{1-3}GlcNAc_2$	oligosaccharyl transferase
alg 5	$Man_9GlcNAc_2$	Dol-P-Glc synthase
alg 6	$Man_9GlcNAc_2$	α-1,3-glucosyl transferase I
alg 8	$Glc_1Man_8GlcNAc_2$	α-1,3-glucosyl transferase II
gls 1	$Glc_3Man_9GlcNAc_2$	α-glucosidase I

cally bound mannooligosaccharides take place in GOLGI cisternae and in the GOLGI-derived secretory vesicles [75, 76].
The *alg* mutants are at the nonpermissive temperature blocked at various steps of the biosynthesis of lipid-linked oligosaccharide [77, 78], whereas the *gls* mutants [79] are defective in specific glycosidases involved in the core oligosaccharide processing (Tab. 2).

Cell Wall Assembly

Similarly as the complex man-made structures, the cell wall has to be assembled from its building stones by a process that is taking place outside the plasma membrane. The individual wall polymers are either secreted from cytoplasm (e.g. glycoproteins) or they are synthesized at the surface of plasma membrane (e.g. skeletal polysaccharides). There is a wide possibility of interactions between these polymers, such as crystallization, cocrystallization, covalent crosslinking and noncovalent bonds.
I was already noted that, in fungal hyphae, the wall synthetic activity is concentrated at the hyphal tip. Chitin and glucan are formed here from their respective precursors by the enzymes localized in the plasma membrane. Also the highest frequency of fusion of secretory vesicles with the plasma membrane occurs at the tip (for reviews see refs. [8, 80]).
The conversion of relatively plastic nascent wall, formed at the hyphal tip, into the stiff structure characteristic for lateral walls is a gradual process and its velocity apparently determines the diameter of the hypha. It can be envisaged that when the process of wall maturation is fast, the diameter of the growing hypha would be smaller than in the case when the cross-linking of the wall components proceeds at a slower rate.
WESSELS et al. [15] have observed that a hot-water soluble β-1,3-glucan formed at the hyphal tips of *Schizophyllum commune* is the precursor of the alkali-insoluble β-1,3-glucan bound in chitin-glucan complex found in the subapical regions. The insolubilization of β-1,3-glucan is thought to be a consequence of the linkage formation between β-glucan and chitin. Dissolution of chitin component with chitinase has led also to liberation of linked β-1,3-glucan [81]. In the process of wall maturation, also the amount of β-1,6-glucan increases with the distance from the tip, probably due to branching of the linear β-1,3-linked precursor [78].
Another difference between the growing tip and subapical regions is different degree of crystallinity of chitin and glucan. While no crystalline polysaccharides are observed at the hyphal apex, microfibrils of chitin and β-1,3-glucan appear in subapical regions in *Schizophyllum commune* [82].
In contrast to cylindrical hyphal cells, the spherical fungal cells grow by incoporation of new cell wall material uniformly over the entire wall surface [83–85]. Here, it can be envisaged that the cross-linking of wall components proceeds simultaneously with their deposition into the wall.
These facts indicate that fungal morphology is to a great degree determined by the orientation of cell wall growth. How this is regulated, is still one of the basic questions of fungal cytology.
Since polysaccharide synthases, as well as other membrane- and cell wall glycoproteins are transported to their destinations by means of secretory vesicles, it is highly probable that the polarization of hyphal growth is determined by the direction of vesicle streaming. Several mechanisms have been envisaged to play the role in directing the vesicle flow: a) electrophoresis, driven by an electrical potential formed between the hyphal apex and subapical regions of the hyphae [e.g. 86]. b) movement of secretory vesicles could be guided by microtubuli and/or microfilaments constituting the fungal cytoskeleton. Such proposals stem from the observations that compounds which interfere

with the proper function of cytoskeleton cause disorganization of hyphal tip growth [87, 88]. The indispensable function of filamentous ring constricting the plasma membrane at the site of septum initiation has been observed in yeast [89, 90].

Biotechnological Aspects

After this brief introduction into the problematics of cell wall formation in fungi, back to biotechnology and tring to answer the question: how this knowledge could be useful in solving various biotechnological problems?

Beginning with the cell wall structure: it has been generally accepted that the knowledge about the chemical composition and structure of individual wall components may be useful not only in taxonomy but also in such applied fields as immunology, medicine, food- or pharmaceutical industry. For example, defined cell wall components can be used as antigens for preparation of vaccines or immunodiagnostic reagents [91], or as nonspecific immunostimulants [92]. Cell wall components may also be used as additives or thickeners for various purposes or they may serve as starting materials for preparation of special sugars and other compounds. Furthermore, the knowledge on the cell wall structure is necessary for prediction of methods for covalent immobilization of fungal cells on various supports [93] or for their precipitation with natural or artificial flocculants [94].

The knowledge about the chemical nature of fungal cell walls may be useful in devising enzyme cocktails used for preparation of fungal protoplasts or for pretreatment of fungal cells before their mechanical rupture. Enzymatic dissolution of fungal cell walls may be also used as a gentle method for extraction of intracellular components, such as recombinant proteins, or it may be applied to increase digestibility of fungal cells used as single-cell protein additives in food or in animal feeds [95]. Undoubtedly, the use of microbial mutants with weakened cell walls (e.g. [96]) could be advantageous in these instances.

Fungi are often used as producers of extracellular enzymes. As shown above, these are manufactured in the endoplasmic reticulum and transported via the secretary pathway to the hyphal tips and discharged into the cell exterior. It can be inferred that the ability of fungal cells to overproduce extracellular enzymes would increase with the increased proliferation of endoplasmaic reticulum and with increasing the number of secretory hyphal tips, i.e. with increased braching of the hyphae. Indeed, it has been observed that the hypersecretory mutant of *Trichoderma reesei* Rut-C 30 producing cellulase, contains increased content of ER elements in its cytoplasm [97].

The number of branchings of mycelia can be influenced by mutation, but also by the growth conditions. We have observed that the frequency of branching of mycelia of *Aspergillus niger* grown in a fermenter has increased with the increased impeller speed (Fig. 8). As a consequence of higher branching, also the chitin content of the walls has increased from 8 to 14% [98].

It is also well known that the cellular morphology can greatly influence rheological properties of microbial suspensions and, consequently, the oxygen transfer rate and the uptake of the nutrients.

Another important field for exploiting the knowledge about the structure and biosynthesis of fungal cell walls is the search for efficient protection against fungi as pathogens of humans, animals and plants. Here, the fungal cell wall components may be used as specific targets for antifungal agents. In order to assess the usefulness of such compounds, preliminary testing is made in cell-free systems containing fungal wall-polysaccharide synthases [39, 99].

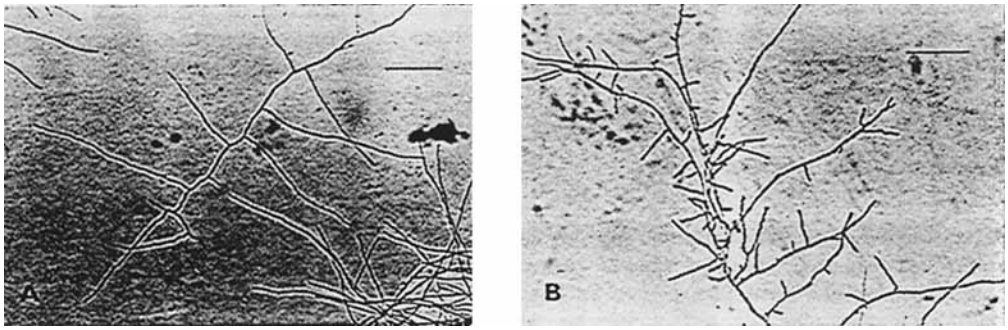

Fig. 8. Effect of impeller speed on the morphology of the mycelium of *Aspergillus niger* grown in a fermenter. A, 300 r.p.m.; B, 1200 r.p.m. [98]. The bar represents 100 μm. (Photo courtesy Dr. E. UJCOVÁ)

In future many new applications of the knowledge on fungal cell walls will be found. How we shall be able to exploit the biotechnological potential of fungal walls will largely depend on our ability to integrate all the available knowledge stemming from the basic research upon the wall structure, function and metabolism.

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