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*review*

# Cell Wall of the Yeast *Saccharomyces cerevisiae*

## Stanična stijenka kvasca *Saccharomyces cerevisiae*

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***Summary***

*The investigation of yeast cell walls attracts attention of scientists in practically all biological areas, from biotechnology to molecular biology. Among a wide range of yeast species and genera studied,* ***Saccharomyces cerevisiae*** *is certainly the best explored microorganism and a substantial amount of data is reported on the structure, biosynthesis, morphology and different changes in the cell wall during the growth, budding, mating and sporulation of cells. The aim of this review is to present a short survey of information available on this cellular structure, with a special emphasis on the individual components of the* ***S. cerevisiae*** *cell wall, glucane, mannan, chitin and proteins.*

***Sažetak***

*Istraživanja stanične stijenke kvasca privlače pozornost znanstvenika u gotovo svim biološkim područjima, od biotehnologije do molekularne biologije. Među mnogim izučavanim vrstama i rodovima kvasca,* ***Saccharomyces cerevisiae*** *je svakako najbolje proučeni mikroorganizam, te je objavljen značajan broj podataka o strukturi, biosintezi, morfologiji, kao i o različitim promjenama u staničnoj stijenci tijekom rasta, pupanja, parenja i sporulacije stanica. Cilj ovog prikaza je dati kratki pregled današnjih spoznaja o ovoj staničnoj strukturi, s posebnim naglaskom na pojedinačne komponente stanične stijenke kvasca* ***S. cerevisiae****, glukan, manan, hitin i proteine.*

### Introduction

The growing scientific interest in yeasts in the last several decades is mainly due to a variety of applications of this microorganism which makes it a subject of extensive studies of scientists in practically all biological sciences. The biochemical simplicity and, on the other hand, the commercial applicability of the yeast *Saccharomyces cerevisiae* brought about the general acceptance of this microorganism as a commonly used model of an eucariotic cell, a sort of »eucariotic *E. coli*«. This survey will focus on the peripheral structure of the *S. cerevisiae* cell, the cell wall. This structure is discussed in terms of a differentiated cellular compartment which, besides providing the necessary mechanical stability of the cell, serves for highly sophisticated interactions of the cell with other cells or surrounding ambiental factors. In the first part, an overview of physical and chemical properties of the wall is given and the most important changes of wall characteristics during the cell cycle are mentioned. The second part deals with individual cell wall components, their structure and properties, their biosynthesis and possible role these molecules might have in the wall of *S. cerevisiae*.

### Physico-chemical properties of the cell wall

The characteristic morphology of yeasts as budding cells of clearly defined shape results from properties of its cell wall. Therefore, the outermost cellular structure has been a subject of extensive studies in many laboratories in the world. Among different yeast genera which attracted the attention due to their importance in biotechnology, food technology, medicine, genetics, biochemistry, molecular biology and other fields, *Saccharomyces* species are probably the most extensively investigated. The information coming from laboratories of different scientific orientations clearly point out that the cell wall of *Saccharomyces cerevisiae* is not only the shell providing the required mechanical protection of the cell, but rather a complex living organelle with diverse and sophisticated functions which constantly change with the life of the cell.

The thickness of the wall and, presumably, its porosity is subjected to changes during the life cycle and is also to some extent strain-specific. Hagedorn *(1)* reported the thickness ranging from about 150 to about 400 nm. Structurally, *S. cerevisiae* cell wall consists of a carbohydrate network in which a number of proteins are anchored (Fig.1.).

The carbohydrate moiety of the wall is composed of three oligosaccharide polymers, glucane and mannan, each contributing with about 48-50 % of wall carbohydrate *(2,3)*, and chitin appearing in a much smaller amount of 2-3 % *(4)*. The protein part of the cell wall consists of at least 30 different proteins imbedded in the carbohydrate structure *(5,6)*. The content of different wall components

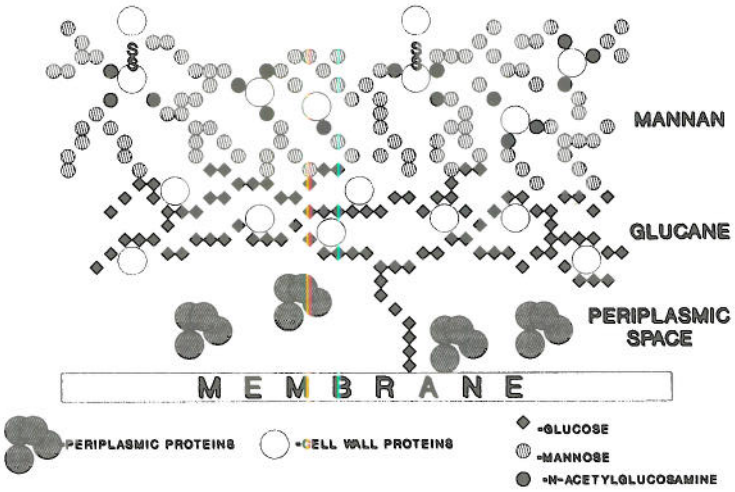


Fig. 1. Schematic presentation of the *Saccharomyces cerevisiae* cell wall
Slika 1. Shematski prikaz stanične stijenke kvasca *Saccharomyces cerevisiae*

is, at least to some extent, a function of growth conditions. The amount of glucane in the wall seems to be higher at a high glucose concentration in the medium *(7)*, while the mannan content is increased if cells grow on mannose as the carbon source *(8)*.

The ultrastructure of the wall and the distribution of different components has been the subject of several often contradictory reports. Early works of Bowden and Hodgson *(9)* and Bacon et al. *(10)* indicated that no layering in yeast cell wall exists. The authors even raised doubt that glucane fibers exist at all, although this idea was later recanted *(11)*. Electron micrographs of the *S. cerevisiae* cell wall, however, reveal the layered structure with usually three distinct layers. The periphery and the inner layer appear electron-dense, while the middle zone is electron-transparent. It is probable that all layers contain both glucane and mannan, but that their distribution is uneven, glucane being more concentrated in the electron-transparent middle part and mannan in electron-dense layers. The differentiated structure of *S. cerevisiae* cell wall was also proposed by Linnemans et al. *(12)* who found the accumulation of acid phosphatase, an extracellular glycoenzyme, in two layers corresponding to electron-dense zones of the wall. These results are, however, in contradiction with the reported periplasmic location of this enzyme *(13,14)*. Zlotnik et al. *(15)* also favored the model of the wall being an asymmetrical structure with glucane forming the inner layer and mannoproteins constituting the outer surface of the cell wall. It is the surface mannoprotein envelope which seems to establish the limited permeability of the wall *(15)*.

Porosity of the cell wall is one of the most intriguing properties of the yeast cell. Different investigators have been using different approaches to establish the size of pores through which molecules cross the wall, and different results have been obtained. Gerhardt and Judge *(16)* measured the size of polyethylene glycols able to penetrate the cell wall and concluded that the size limit corresponded to the molecular weight of 4 500. The reinvestigation of this problem *(17)* brought about the even smaller value of 700. These results, however, could not explain how some externally supplied proteins were able to transverse the wall and reach the cell membrane *(18,19)*. More recent reports indeed prefer the existence of much larger pores enabling the molecules of the protein size to cross the cell wall. The work performed on the periplasmic protein invertase showed that this protein in its dimeric form, having the molecular weight of about 240 000, is to a large extent secreted into the growth medium, while the native octameric form with the size of about 1 000 000 is retained within the periplasmic space *(20,21)*. It is tempting to conclude that octamerization of periplasmic proteins like invertase *(20)* and acid phosphatase *(22)* is actually necessary to prevent their leakage through the cell wall. Consistent with this are results of DeNobel et al. *(23)* who tried to calibrate the cell wall using fluorescently labeled dextranes. They found that dextranes with the maximal molecular weight of about 400 000 could reach the cell membrane and were transported into the cell by endocytosis. Dextranes of this size would correspond to globular proteins of the molecular weight of approximately 700 000.

Another crucial property of the cell wall is its growth. Two principal questions could be addressed considering the wall synthesis and expansion: where does it occur and how? First results obtained by Chung et al. *(24)* proposed the basal growth of the bud, based on the fluorescent antibody labeling. However, data reported by Johnson and Gibson *(25)*, who approached the problem by light-microscopic quantitative autoradiography, indicated the incorporation of $^{3}$H-glucose into glucane primarily at the tip of the bud. Similar conclusions have been reached by Tkacz and Lampen *(26)* who showed, using fluorescein-labeled concanavalin-A, that newly synthesized mannan is inserted at the tip of the bud. Further corroboration of such results came from two autoradiographic studies, one using tritiated glucose *(27)* and the other tritiated mannose *(28)*, clearly showing that both the deposition of newly synthesized glucane and mannan occur at the tip of the bud. Considering the technology of the cell wall

biosynthesis, the first proposal of Johnson *(29)* seems to be the generally accepted one. According to his study the existing glucane chains are first nicked by the action of endoglucanases, followed by the insertion of glucose or a sort of oligoglucane, expanding in this way the carbohydrate network. Although only hypothetical at the time, endoglucanase activity has really been found in the wall (see later in the text). The extension of the cell wall in the early phase of the bud emergence and development can proceed in two ways. In some yeast genera, like *Rhodotorula (30)*, *Candida (31)*, or *Sporobolomyces (32)*, the outer layer of the wall breaks, while the inner part extends into the bud cell wall which is in that case thinner than the wall of the parental cell. In other examples, among which also in *Saccharomyces cerevisiae*, the bud wall seems to be a direct extension of the cell wall of the mother cell having also approximately the same thickness and ultrastructure like the parental cell wall *(33)*. The formation of the bud is in *S. cerevisiae* always initiated by the formation of the chitin ring *(34)*. The subsequent centripetal growth of the chitin ring generates the primary septum at division. It may be interesting to mention that chitinous rings at the neck of the bud have only been reported for some yeast genera including *Saccharomyces* and *Candida*, while other have septa made of glucane *(35)*. Generally, it can be concluded that budding yeast septa markedly differ among genera and species in their composition, outlook and the type of pores if they have any. *S. cerevisiae* septa are not perforated. Upon completion of the septation process the scission begins with the erosion at the periphery of the neck *(36)*.

The study of chitin scars which remain on the cell wall after scission reveals the budding patterns of yeast cells. In a haploid *S. cerevisiae* cell first buds appear proximal to the birth scar, forming a rosette *(37,38)*. Diploids bud in a typical distal pattern. One of the first attempts to explain how the cell chooses the next budding site, by assuming that the cell buds at the point of the greatest curvature of the wall *(39)*, could reflect the situation in a diploid cell but cannot be true for haploids. Therefore, this important problem remains up to now unsolved. Another interesting question addressed was how many times a cell can bud during its lifetime. Muller et al. *(40)* managed to remove 67 consecutive buds by microdissection from the same cell. This is, at the same time, the highest number of scars reported for a single yeast cell. It should be mentioned that the work of Johnson and Lu *(41)* clearly showed that the number of scars is not determined by the surface area of the wall. Considering the diameter of scars, it seems to be more or less constant throughout the life of the cell, birth scars being always somewhat larger than bud scars *(42)*.

## Individual components of the *Saccharomyces cerevisiae* cell wall

Generally, the cell wall of *Saccharomyces cerevisiae* constitutes 15-25 % of the cell dry weight. Most of the wall (80-90 %) is composed of carbohydrates, mainly, as already mentioned, of glucane and mannan, chitin being present as only 2-3 % of the total carbohydrate. The noncarbohydrate part of the cell wall consists of proteins, but an on-going discussion about the presence of lipids in the wall should also be mentioned. Up till now, however, no definite data about the content and nature of wall lipids are available, mostly due to experimental difficulties in clear separation of walls from traces of membrane lipids. Yeast genera other than *Saccharomyces* seem to have quite different compositions of their cell walls *(43,44)*, although only for *S. cerevisiae* a detailed study of wall components has been performed.

### *Glucane*

When an exhaustive alkali treatment of *S. cerevisiae* cells with a subsequent acid extraction is performed, the remaining material is composed exclusively of glucose, thus representing cell wall glucane. The fact that purified glucane still retains the shape of the yeast cell points out this wall constituent as the major structural component of the cell wall, thus performing its main function in the mechanical and osmotic stabilization of the cell. Indeed, the lysis of the glucane component of the cell wall by glucanases results in the complete depletion of the wall and the lysis of the cell due to its higher osmotic pressure compared to the surrounding *(45,46)*. More recent data indicate another function glucane might have in anchoring cell wall proteins, thus indirectly enabling biochemical processes to take place in the wall *(47,48)*.

The first reported alkali-insoluble, acid-insoluble glucane *(49,50)* was for a number of years considered the only glucane present in the yeast cell wall. Later reports, however, described two other glucane types, an acid-soluble and an alkali-soluble form. Early purification procedures involved the hot alkali extraction to remove mannan, followed by the acetic acid extraction of glycogen. Glucane was the insoluble material that remained. Chemical analysis of alkali-insoluble, acid insoluble glucane obtained by this procedure revealed the predominance of $\beta$-(1→3)-linkages *(49)* with a small proportion of about 3 % of branching through $\beta$-(1→6)-linked glucose residues *(51)*. This type of glucane was shown to be resistant to the action of $\beta$-(1→6)-glucanases *(52)* as expected, but also to the digestion with some $\beta$-(1→3)-glucanases, indicating a specific conformation of $\beta$-(1→3)-glucane inaccessible to some hydrolases *(53)*.

The second type of glucane has first been reported by Bacon et al. *(54)*, and then studied in details by Manners et al. *(55)*. It represents the part of glucane material coextracted with glycogen by acetic acid after the alkali treatment of cell walls. The chemical analysis of this material revealed small molecules with a high $\beta$-(1→6)- linkage proportion and considerable branching. The predominance of $\beta$-(1→6)-linkages was confirmed by susceptibility of this type of glucane to the action of $\beta$-(1→6)-glucanases *(52)*. Several reports suggested the presence of two different subtypes of $\beta$-(1→6)-glucane. Hutchins and Bussey *(56)* reported that a part of this material remained acid-insoluble but could be isolated after the enzymatic digestion of $\beta$-(1→3)-glucane. On the basis of different capacities for binding of the killer toxin, a protein which interacts specifically with $\beta$-(1→6)-glucane, the authors concluded that the two glucane preparations structurally differed. Tkacz *(57)* has obtained similar results in an attempt to fractionate $\beta$(1→6)-glucane obtained by the enzymatic diges-

tion of β-(1→3)-glucane, by gel filtration. Two fractions were obtained, one eluting with mannoproteins, and the other as a disperse population of molecules of smaller size, corresponding to the molecular weight reported by Manners et al. *(55)*.

The work of Fleet and Manners *(58,59)* explained the structure of the third type of glucane. It is the glucane material obtained by the alkali extraction of walls after which it was separated from mannan by precipitation at neutral pH. This glucane has mainly β-(1→3)-linkages, low extent of branching, and resembled in structure and chemical composition the alkali-insoluble β-(1→3)-glucane. In spite of the extensive purification, the material always contained a small amount of mannan, suggesting possible covalent linkages between different polysaccharide species.

The summary of data reported on the structure of *S. cerevisiae* glucane clearly shows that it is a wall component with a much finer and more sophisticated structure than previously believed, probably covalenty linked with other polysaccharides and proteins in the wall.

The biosynthesis of glucane is still to a large extent unexplained. It takes place at the plasma membrane where a glucane synthase has been found and partially characterized *(60-62)*. The enzyme was able to catalyze the incorporation of glucose from UDP-glucose into a linear β-(1→3)-polymer. No evidence for other enzymes required for the synthesis of different types of glucane, particularly β-(1→6)-linkages have been reported.

### *Mannan*

Besides glucane, mannan represents the most abundant *Saccharomyces cerevisiae* cell wall carbohydrate. Studies of this polysaccharide structure revealed that mannan chains are always found covalently linked to proteins so that this material could more appropriately be described by the term mannoproteins. »Mannan« will therefore here be used to describe the carbohydrate part of cell wall mannoproteins. The structure of mannan has been studied in details during the last two decades and has been elucidated mainly due to the excellent work of Ballou and his coworkers *(63-67)*. As the information on the structure of mannan accumulated, the similarity between the carbohydrate material isolated from the cell wall and oligomannose chains of glycoproteins became obvious. Indeed, it is in the meantime well understood that the biosynthesis of both structures occurs through the same glycosylation pathway and that mannan is in fact nothing but a defined composition of glycoproteins, some of which are heavily glycosylated. Besides a structural role, these glycoproteins could have enzymatic, receptor, or other biochemical functions (see later in the text). Although not determining the cell form, as its removal does not bring about the destruction of the overall shape of the cell, mannan is essential for the cell survival since mutants with severely decreased mannan content have distorted shapes and lyse *(67-69)*. Mutants completely lacking mannan have never been isolated.

Mannan is usually isolated from whole cells or cell walls by alkali extraction *(50)*, or by autoclaving cells or walls in citrate buffer (pH = 7) *(70)*. Solubilized material is then precipitated by ethanol, which gives a product still containing some glucane *(71)*, or by Fehling's solution *(71,72)*, or Cetavlon *(72,73)*. All methods described, however, result in heterogeneous mannan which can further be fractionated into several subfractions by ion-exchange chromatography *(71-74)*, or gel filtration *(75,76)*. The heterogeneity of mannan obtained from whole cells is understandable, since the procedure allows the coisolation of most cell mannoproteins including intracellular and periplasmic ones. It should however be mentioned that most of the procedures described do not lead to the complete removal of mannan from cell walls *(58,77)*.

Due to the substantial destruction of wall material occurring during chemical extractions, methods have been developed for the enzymatic release of mannan from cell walls or whole yeast cells. Shibata et al. *(78)* described the procedure involving the treatment of cells with the β-glucanase preparation, Zymolyase-60000. Mannoproteins obtained by this method were about three times larger than those obtained by the chemical extraction. Interestingly, even after extensive purification, these mannoproteins still contained some glucose, indicating the existence of covalent linkages between mannan and glucane in the wall. Zymolyase extraction soon became a very popular method and was further used in studies of Pastor et al. *(79)*,Valentin et al. *(5)*, Zlotnik et al. *(15)*, Elorza et al. *(80)*, Herrero et al. *(81)* and others. Nevertheless, Zymolyase, like the chemical extraction, does not seem to remove all mannoproteins from the cell wall and this could only be achieved by the combined treatment with Zymolyase and SDS *(5)*.

The structure of *S. cerevisiae* mannan has been elucidated through the extensive work of Ballou and his coworkers. Lee and Ballou *(82)* used acetolysis for the selective cleavage of α-(1→6)-linkages, thus liberating side chains from the α-(1→6)-backbone of the mannan structure (see Fig. 2.). Side chains were then separated by gel filtration into mannose polymers ranging from mannobiose to mannotetraose *(71)*. Further experiments revealed that some of the side chains were phosphorylated *(83)*. Characterization of phosphorylated side chains and identification of the position of the phosphate group has been done revealing that the phosphate is associated with the mannan side chain by a diesther bond, as the mild acid hydrolysis releases mannose or mannobiose *(84-86)*. The α-(1→6)-linked mannose forming the backbone moiety of mannan chains was further proved by Jones and Ballou *(87,88)*, using an exomannanase from *Arthrobacter* sp., an enzyme which preferentially cleaves mannan side chains, leaving the unsubstituted linear α-(1→6)-mannose chain. The final structure resulting from the line of experiments mentioned above is shown in Fig. 2.

Clarification of the structure of *S. cerevisiae* mannan came also from another series of experiments, using the genetic approach and nicely matching with the results of the chemical analysis. Namely, mutants with truncated mannan chains were isolated. Mutants were particularly useful because they provided information not only about the structure, but also about the biosynthesis of mannan. In Table 1 are listed *mnn* mutants, as they were named, with corresponding phenotypes. Interestingly, all of these mutants grow with the unchanged generation time and do not differ morphologically from their parental strain,

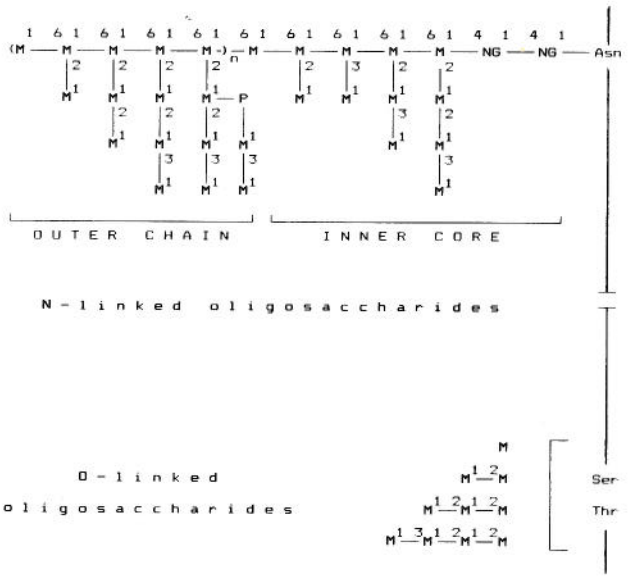


Fig. 2. *Saccharomyces cerevisiae* mannan structure
Slika 2. Struktura manana kvasca *Saccharomyces cerevisiae*

in spite of the marked difference in the mannan structure of some mutants, raising an obvious question of the importance of such a sophisticated carbohydrate moiety.

As already pointed out, mannan is actually composed of a number of highly mannosylated proteins. Therefore, much attention has been paid to the type of connection existing between the protein and carbohydrate chains. Sentandreu and Northcote *(89)* first observed that mannoproteins underwent $\beta$-elimination reaction when treated with diluted alkali, indicating the O-glycosidic linkage through serine or threonine. However, only a small amount of mannose could be removed in that way. Nakajima and Ballou *(72)* confirmed the existence of mannobiose, mannotriose and mannotetraose O-glycosidically linked to serine and threonine residues, but they also subjected mannan remaining after $\beta$-elimination to strong alkali treatment to remove protein. In the resulting material they were able to detect N-acetylglucosaminitol which could only arise from the reducing end of the polysaccharide chain, strongly indicating the involvement of N--acetylglucosamine in the protein-carbohydrate attachment. Besides, the same authors *(90)* subjected mannan from the *mnn*2 mutant lacking carbohydrate side chains (Table 1) to the digestion with an endo-$\alpha$-(1→6)-mannanase and an endo-N-acetylglucosaminidase. The latter enzyme cleaved the chitobiosyl unit at the attachment site of carbohydrates to asparagin. The mannanase digested the $\alpha$-(1→6)-backbone leaving a branched structure of 14--16 mannoses called »inner core« (Fig. 2.).

The biosynthesis of mannan has been elucidated to a great deal through the work in many laboratories dealing with protein glycosylation and secretion. Briefly, proteins are synthesized by ribosomes attached to the endoplasmic reticulum and translocated into the lumen where both O- and N-glycosylation start. »Inner core« is transferred *en bloc* from a lipid carrier dolichol phosphate to asparagin, after which the core-glycosylated proteins are transported to Golgi where some of the core units are elongated by the sequential addition of up to 250 mannose molecules forming the so called »outer chain« (Fig. 2). O-glycosylation starts also in the endoplasmic reticulum where the first mannose is transferred from dolichol-P-mannose to either serine or threonine, and continued in Golgi by the elongation of chains to not more than pentasaccharides, using GDP-mannose as the monosaccharide donor. Protein glycosylation in yeast, as well as in higher organisms has been extensively reviewed *(91-93)* and is in the meantime included in most textbooks. It is, therefore not discussed here in details.

## *Chitin*

The presence of chitin in cell walls of most yeast genera, *Schizosaccharomyces* apparently being an exception, has been reported although the amount of this polymer has been found to be much lower than in other fungi *(39,43)*. As the methodology improved, chitin has been detected in cell walls by X-ray diffraction patterns *(94)*, infrared spectra *(4)*, the presence of glucosamine in wall hydrolysates *(95)*, digestion with chitinase *(96)* and by specific staining with primulin *(97)*, Calcofluor *(98,99)* and gold-labeled wheat germ agglutinin *(100)*. The most intriguing questions in connection with the role of chitin in the wall were indicated already in the work of Bacon et al. *(4)* who showed that *S. cerevisiae* chitin is located primarily in bud scars remaining on the mother cell after detachment of newly formed daughter cells. It seems, however, that about 10 % of wall chitin is located outside of budding regions *(99,101)* and that even unbudded cells contain a small amount of chitin *(102)*. The physiological significance of chitin not associated with budding zones in unknown. The fact that most chitin is localized in bud scars forming characteristic rings, indicated that this polymer could have a role in cell division *(103-105)*. It has been proposed that the bud scar remains from the primary septum formed between the mother and the daughter cell. The accumulation of chitin in the form of the ring indicated a possible structural role in the fortification of the bud neck as the particularly sensitive region during the bud formation. However, Cabib and Bowers *(98)* showed that the development of the neck region remained unchanged if cells were grown in the presence of polyoxin D, an antibiotic which inhibits the chitin synthesis. Such results indicated that the significance of chitin in cell division is not connected with the deposition in the neck of the bud, but rather in the formation of the septum. Indeed, polyoxin D treated cells do not deposit septal plug and lyse at the bud junction *(98)*. Therefore, it seems clear that the strictly localized deposition of chitin is essential for the yeast cell division, although it is not clear why the cell prefers chitin to widely distributed glucane for this purpose. Covalent linkage between chitin and glucane in bud scars has been suggested *(106,107)*, but chemical evidence, reported for *Candida albicans* where glucosyl-N-acetyl-glucosamine has been found after the enzymatic degradation of glucane *(108)*, still lack for *S. cerevisiae*.

Chitin biosynthesis is located on the plasma membrane and performed by two enzymes, chitin synthase 1 and 2 *(109,110)*. Both enzymes have been found to catalyze

the incorporation of N-acetylglucosamine into a $\beta$-(1→4)-linked polymer *in vitro*. The function of these enzymes in the cell is somewhat unclear. Chitin synthase 2 seems to be the main chitin producing enzyme and its elimination causes the total block in the chitin biosynthesis. The mutation of the chitin synthase 1 gene, however, did not affect the production of chitin and its role in the biosynthesis of this wall component is still not clarified *(111,112)*.

### *Proteins*

While the structure of carbohydrate components of the *S. cerevisiae* cell wall has been the subject of thorough studies, little is known about proteins embedded in the polysaccharide moiety of the wall. The presence of at least 30 different proteins in the cell wall has been reported *(5, 6,79,81,113)*, but the function of only few of them has been elucidated. Four genes coding for cell wall proteins have been cloned and sequenced *(47,114-116)*. Recently, one of them has been characterized as a $\beta$-glucanase. The protein, isolated by heating purified cell walls was shown to bind firmly to glucane and chitin *in vitro*. It is a glycoprotein with one N-glycosidically linked carbohydrate chain consisting only of the »inner core«. The disruption of BGL2 gene, coding for this protein, did not result in any phenotype *(47)*, but the overproduction of the protein significantly increased the generation time of yeast cells (Mrša et al., unpublished). The physiological role of this protein is still unknown although a function in the limited hydrolysis of wall glucane might be assumed.

Three other cell wall proteins whose genes have been cloned are associated with the mating process and their biosynthesis is regulated by mating pheromones, a- and $\alpha$- factor. Two proteins, a- and $\alpha$-agglutinin were shown to interact one with another causing agglutination of cells of opposite mating types *(116)*. a-agglutinin has been purified from mercaptoethanol extracts of *S. cerevisiae* walls as an O-glycosylated glycoprotein with a molecular weight of 22 000. *(117,118)*. Cloning of the corresponding AGA2 gene revealed the molecular weight of the protein part of only 7 500 *(116)*. This protein seems to be anchored in the cell wall through a disulphide bridge with another protein called »core protein«, the AGA1 gene product. The mutation of the AGA1 gene causes the release of a-agglutinin into the growth medium *(115)*. $\alpha$-agglutinin has been isolated by the Zymolyase digestion of cell walls and was shown to be highly N-glycosylated *(114)*. The molecular weight of the entire molecule was estimated to 200 000-300 000 while the size of the protein moiety was 68 000.

Another cell wall protein has been purified and partially characterized by Frevert and Ballou *(119)*, although the corresponding gene has not been cloned. The protein was shown to be highly glycosylated and could only be detected in *mnn9* mutant cells, which are not able to synthesize carbohydrate »outer chains«, thus producing glycoproteins with appreciably smaller carbohydrate content (Table 1). Authors proposed the structural role of this protein, but only due to the absence of any other tentative function.

It should be mentioned that a small amount (approximately 10 %) of two proteins found normally in the growth medium, chitinase and exo-$\beta$-(1→3)-glucanase (EXG1 gene product, 120), remains localized in the cell wall *(121,* Mrša et al., unpublished). Kuranda and Robbins *(121)* proposed a role of the cell wall associated chitinase in cell separation, while the physiological significance of the exoglucanase in the wall is unclear.

Table 1. *mnn* mutants
Tablica 1. *mnn* mutanti

| Mutation<br>Mutacija | Phenotype<br>Fenotip |
|---|---|
| *mnn1* | lack of terminal $\alpha$-(1→3)-linked mannoses from side chains<br>nedostatak krajnjih $\alpha$-(1→3)-vezanih manoza u pobočnim lancima |
| *mnn2* | lack of complete side chains in »outer chain«<br>nedostatak cijelih pobočnih lanaca u »vanjskom lancu« |
| *mnn3* | lesser number of side chains<br>manji broj pobočnih lanaca |
| *mnn4* | lack of phosphorylated side chains<br>nedostatak fosforiliranih pobočnih lanaca |
| *mnn5* | side chains of »outer chain« consist of only one mannose<br>pobočni lanci u »vanjskom lancu« sadržavaju samo jednu manozu |
| *mnn6* | lack of mannose-6-phosphate in »outer chains«<br>nedostatak manoza-6-fosfata u »vanjskom lancu« |
| *mnn7* | truncated »outer chains«<br>nepotpun »vanjski lanac« |
| *mnn8* | truncated »outer chains«<br>nepotpun »vanjski lanac« |
| *mnn9* | lack of complete »outer chains«<br>nedostatak cijelog »vanjskog lanca« |
| *mnn10* | truncated »outer chains«<br>nepotpun »vanjski lanac« |

Molecular mechanisms by which cell wall mannoproteins reach their final cellular location after being secreted through the cell membrane are still unknown. *In vitro* experiments showed that yeast glucane possesses the high affinity for binding proteins at acidic pH, thus suggesting nonspecific interactions allowing the first contact between extracellular proteins and the cell wall *(48)*. Proteins could further be entrapped by newly formed carbohydrate chains and perhaps cross-linked by disulphide bonds as proposed by Valentin et al. *(5)*. Further work is required for the clarification of functions of other cell wall proteins, as well as for understanding of biochemical processes in the wall during the life cycle of the cell.

## Concluding remarks

Substantial amount of information on the structure, biosynthesis and function of the *S. cerevisiae* cell wall which has accumulated over the years still does not provide a complete and satisfactory picture about this cell structure. However, the present state of knowledge enables the understanding of most important functions of different cell wall components, thus pointing out possibilities for biochemical or genetic modifications of these functions for scientific or biotechnological reasons. Modifications of wall components could lead to altered physico-

-chemical properties of the yeast cell, having impact on the use of this microorganism for production purposes. On the other hand, variations in the expression of cell wall proteins could alter particular wall functions. This may have particular significance in the application of yeast cells for the production of heterologous proteins using systems for the secretion of synthesized protein. Further work should improve our understanding of the *S. cerevisiae* cell wall, thus providing tools for biochemical and genetic manipulations of this important microorganism.

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