

## Structure of the *Saccharomyces cerevisiae* Cell Wall

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Cell wall of the yeast *S. cerevisiae* is composed of the structural polysaccharides glucan, mannan and chitin, and of proteins. Possible interactions between the wall components have not yet been studied in detail. Molecular organization of the cell wall constituent molecules is also, to a great extent, unclear. To elucidate the structure of the cellular outermost compartment, intermolecular interactions responsible for the constitution of the cell wall were studied. Results revealed protein-glucan, protein-chitin, glucan-chitin, and glucan-glucan interactions. Mannan chains reacted neither with proteins nor with other polysaccharides. Intact cell walls were found to bind proteins approaching the wall from the inside but not those reaching the cell wall from the outer side, suggesting a marked asymmetry of the wall having an outer mannan layer, and an inner glucan network. Mannoproteins seem to be attached to glucan through their protein moieties.

### INTRODUCTION

The cell wall of the yeast *Saccharomyces cerevisiae* has attracted considerable attention in the last several years since it became clear that the yeast outermost cellular compartment has a much more sophisticated structure and diverse functions than previously supposed. Besides providing a mechanical protection of the cell, a number of protein-mediated processes have been described, important for the regular cell cycle events, like budding, mating or sporulation.<sup>1-3</sup> This turned the attention from the studies of the structure of carbohydrate components of the wall to yeast extracellular proteins which are now in the centre of interest in many laboratories.

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The *Saccharomyces cerevisiae* cell wall is composed of about 80–85% carbohydrates, the rest consisting of proteins interspersed throughout the carbohydrate network.<sup>4,5</sup> Three polysaccharides comprise the carbohydrate moiety of the wall, glucan and mannan account for about half of the total carbohydrate content, with chitin forming only about 2–3% of the wall and being localized mainly in bud scars remaining after the detachment of the daughter from the mother cell in the budding process.<sup>6</sup> The structure of carbohydrate constituents of the *S. cerevisiae* cell wall has been studied in detail and, although new emerging data still accumulate completing the general picture, the main structural features of glucan, mannan and chitin have been described.<sup>7,8</sup> It should be mentioned that the term »mannan« actually describes oligomannose chains of cell wall glycoproteins, which can be extracted from wall preparations by alkaline extraction.

Little is known, however, about the molecular organization of different constituents in the cell wall, and practically nothing about the cellular mechanisms for achieving this organization, and about the mechanism(s) for the incorporation of proteins in the cell wall. The work described in this paper was, therefore, performed with the aim of establishing possible interconnections between the *S. cerevisiae* cell wall constituents, and elucidating the organization of these molecules in the wall.

## INTERMOLECULAR INTERACTIONS BETWEEN PURIFIED YEAST CELL WALL COMPONENTS

### *Protein-glucan interactions*

To establish possible intermolecular interactions between different yeast cell wall constituents, an *in vitro* binding assay was developed and optimized (Figure 1.). The assay was designed so that the binding of any water-soluble component of the wall to an insoluble one could be detected. In this way, interactions of yeast extracellular proteins with glucan, the main structural constituent of the wall have been studied.<sup>9</sup> It has been found that glucan binds most cell wall proteins, but also most yeast intracellular proteins which, under physiological conditions, never come in contact with glucan, as well as proteins from other sources. The binding occurs in a rather nonspecific way by the formation of a number of hydrogen bonds at acidic pH (Figure 2.). Interactions were found to be strictly pH-dependent, and when the pH was above 7.0, no binding could be recorded. Three exceptions were found among *S. cerevisiae* extracellular proteins studied. Periplasmic proteins, invertase and acid phosphatase, were found not to interact with glucan, as it could be expected for proteins which, due to their cellular location, come in contact with glucan chains at the inner surface of the cell wall upon

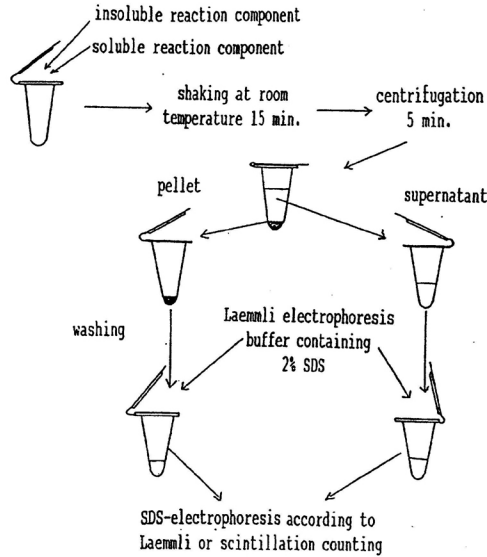


Figure 1. »Binding test« for the determination of intermolecular interactions between different yeast cell wall components.

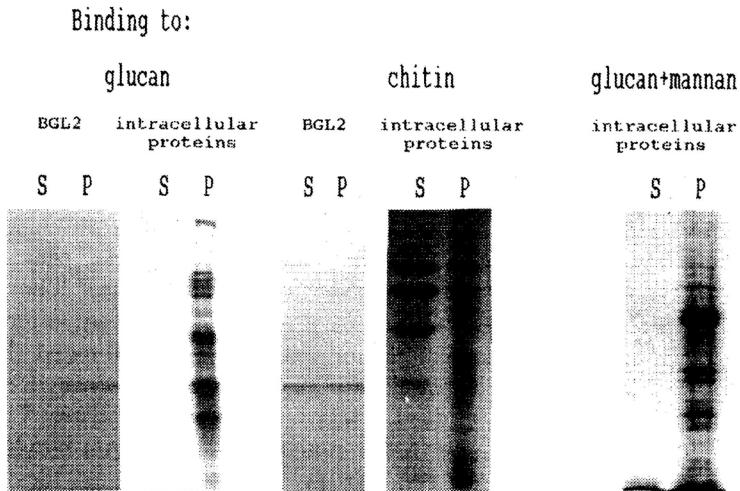


Figure 2. Binding of proteins to different cell wall polysaccharides. Cell wall  $\beta$ -glucanase (Bgl2p), and a mixture of yeast intracellular proteins were bound to glucan, chitin, or to glucan in the presence of mannan, respectively, as described in the text. The bound (P), as well as the unbound (S) proteins were analyzed either by immunoblot (Bgl2p), or by SDS-electrophoresis (intracellular proteins).

their secretion through the membrane, but still remain soluble periplasmic proteins, readily released if cell walls are broken mechanically. Although both periplasmic proteins are heavily glycosylated, it is not the mannan moiety that prevents them from binding to glucan, since it has been shown that the intracellularly located, nonglycosylated invertase possesses the same property.<sup>9</sup>

Another protein that was found to react with yeast glucan with a much lower affinity than most other proteins was the *exo*- $\beta$ -1,3-glucanase (EXG1 gene product), a protein which was reported to be secreted into the growth medium.<sup>10</sup> Thorough investigation of the localization of this protein revealed that about 10% of the enzyme was cell wall-associated while 90% was indeed found in the medium. When the binding of EXG1 glucanase to glucan was attempted, it reacted with the polysaccharide, but the measurement of the amount of glucanase bound (assayed both for activity and proteins), compared to the amount of bound yeast intracellular proteins (assayed for proteins), revealed a 3–4 times lower capacity of glucan for this enzyme than for most other proteins. This result was a strong indication that protein-glucan interactions could be responsible for the localization of yeast extracellular proteins in the cell wall.

#### *Protein-chitin interactions*

The described binding test was further used to study the interactions between yeast proteins and chitin.<sup>9</sup> Although forming only 2–3% of the *S. cerevisiae* cell wall, chitin plays a very important role in the process of yeast budding since it accumulates at the bud neck region providing mechanical stability for the buds. When the bud reaches the size of the mother cell, the primary septum dividing the two cells is also made of chitin.<sup>11</sup> Finally, small amounts of chitin deposited in lateral walls were reported to play a crucial role in the covalent cross-linking of  $\beta$ -1,3-glucan subunits, resulting in a highly rigid, insoluble and resistant carbohydrate network.<sup>12</sup> Therefore, interactions between yeast proteins and chitin might have a physiological significance in budding and the cell wall formation. Results showed that chitin binds proteins similarly to glucan (Figure 2.), but with a somewhat smaller capacity and the optimal pH shifted towards more acidic values. The nature of interactions was again shown to be nonspecific hydrogen-bonding, since reagents interfering with other types of protein interactions, like high salt concentrations, EDTA,  $\beta$ -mercaptoethanol, nonionic detergents, or group specific reagents, had little or no effect on protein-chitin interactions. Urea, however, affected the binding (not shown). Similarly to what has already been described for protein-glucan interactions, chitin also interacted with proteins from the yeast cell extract, which normally do not come in contact with the cell wall, showing that the binding is not specific for wall proteins.



### *Protein-mannan interactions*

The third *S. cerevisiae* cell wall polysaccharide, mannan, consists of oligomannose chains of extracellular mannoproteins obtained by the alkaline extraction of yeast walls. Potential protein-mannan interactions could account for additional interconnections between protein moieties and carbohydrate chains of wall mannoproteins. In this case, however, the standard binding test could not be used since mannan is a water-soluble carbohydrate. Therefore, an indirect approach was used in the way that mannan was added to the protein-glucan, or protein-chitin reaction mixtures as a potential binding inhibitor. If mannan reacted with proteins, an inhibition of the binding of proteins to insoluble polysaccharides could have been expected. However, no inhibition in either reaction mixture was recorded (Figure 2.), indicating that mannan could not interact with proteins.

### *Interactions between different carbohydrate constituents*

To establish if mannan chains can react with glucan, or chitin, the binding test was employed and the amount of mannan remaining in the supernatant after the binding reaction was determined by the orcinol- $\text{H}_2\text{SO}_4$  method.<sup>13</sup> Again, no interactions were observed, since no decrease in the amount of mannan in the supernatant was recorded, suggesting that mannan represents an inert cell wall component with a low binding potential for proteins, as well as for wall carbohydrates. Hence, cell wall mannoproteins should be connected with other wall constituents, primarily with glucan, through their protein moieties.

Interactions between glucan and chitin chains, as well as between different glucan chains could be important in determining the density of the cell wall and, therefore, its porosity. As both glucan and chitin are insoluble polysaccharides, a soluble glucan analogue, laminarin, was used in the binding test. Laminarin differs from yeast glucan only in size, shorter chains making it water-soluble. The use of radioactively labelled  $^{14}\text{C}$ -laminarin enabled simple detection of the bound polysaccharide by radioactivity counting. Interactions of laminarin with both glucan and chitin were detected in a broad pH range between 3 and 9, but having optimal values between 4 and 5 (not shown). To establish whether laminarin and proteins use the same binding sites at the glucan molecule, a competition experiment was performed in which laminarin was added to the protein-glucan reaction mixture. Interestingly, addition of laminarin did not affect protein binding (not shown), indicating that laminarin chooses other binding sites than proteins, so both reactions can occur simultaneously producing complex, sophisticated structures in the cell wall. Intermolecular interactions between different *S. cerevisiae* cell wall constituents are summarized in Table I.

TABLE I

Intermolecular interactions between *S. cerevisiae* cell wall components

	Glucan	Chitin	Mannan	Proteins
Glucan	+	+	—	+
Chitin	+	?	?	+
Mannan	—	?	—	—

### INTERACTIONS BETWEEN PROTEINS AND INTACT YEAST CELL WALLS

Binding properties detected in the *in vitro* assay correspond to purified cell wall constituents. Therefore, experiments are required to check if the obtained results could be extrapolated to an *in vivo* situation. To establish if wall components possess the recorded properties in intact cell walls, the binding of proteins to *S. cerevisiae* cell walls was attempted. If proteins were added to intact yeast cells under the conditions required for protein-glucan or protein-chitin interactions (pH 3.8), practically no binding to cell walls could be observed (Figure 3.). Cell walls were then purified by disrupting the cells in a mechanical homogenizer, using glass beads which produce cracks in the wall through which the cell content leaks out leaving empty cell walls, still having the original shape of the cell. The binding to yeast cell walls purified in this way was slightly better, but the intensity of binding was still much below the one expected from the interactions of proteins with purified glucan (not shown). Such a result could be explained by assuming a layered structure of the cell wall, in which the outer layer is composed of mannan which could, according to our results, form an inert layer which does not bind proteins. The wall moiety localized innerly should, therefore, consist of glucan, the protein-binding component of the wall. To check if the inner surface of the wall could interact with proteins, cells were disrupted in a mechanical homogenizer under the binding conditions (pH 3.8), allowing released intracellular proteins to reach the inner surface of the wall. Cell debris was then washed at different pH. Results presented in Figure 3. show that the washing with a basic buffer (pH 8.0) released proteins from cell walls, in contrast to the washing with an acidic buffer (4.0), showing that intracellular proteins released during the cell disruption reacted with the inner surface of the cell wall, and could be released at basic pH. In this case, the binding reaction had the same properties as the binding of proteins to glucan.

Results presented in this paper indicate that the cell wall of the yeast *Saccharomyces cerevisiae* is a complex structure in which all constituents, except mannan, could interact with one another forming a compact sophis-

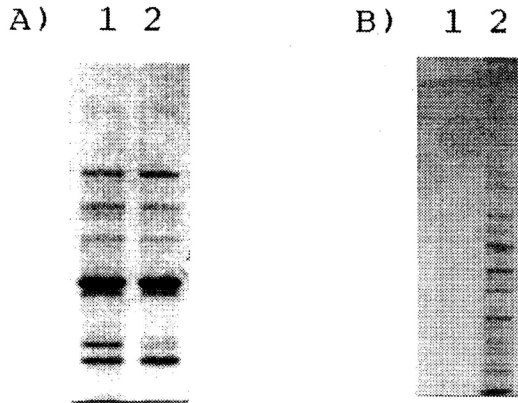


Figure 3. Binding of proteins to yeast cell walls. A) Binding of proteins added to cell walls externally. Lanes: 1. mixture of yeast intracellular proteins added to the binding reaction with intact yeast cells; 2. proteins remained in the supernatant after the binding. B) Binding of proteins reaching the cell wall from inside. Yeast cells were disrupted under the binding conditions (pH 4.0), and cell debris were washed at different pH. Lanes: 1. pH 4.0; 2. pH 8.0.

ticated and organized network. It should be mentioned that noncovalent interactions described, do not seem to be the only type of intermolecular coupling in the cell wall. The increasing amount of information about covalent bonds between different wall constituents<sup>12</sup> indicates that the attachment of newly formed glucan as well as secreted mannoproteins to the existing cell wall network through noncovalent interactions might represent the first step in the wall formation, followed by the covalent fixation of incorporated structures. Based on the results described, an asymmetric structure of the cell wall could be proposed with an outer inert layer incapable of binding proteins and an inner protein-binding network. According to the properties of different wall constituents tested, the outer layer should be formed of mannan chains, and the inner one of glucan. Mannoproteins could be linked to the glucan network through protein moieties of their molecules. A tentative model of the *S. cerevisiae* cell wall is presented in Figure 4. A layered composition of the cell wall was first proposed by Zlotnik *et al.*<sup>14</sup> based on the fact that cell walls could only be lysed by purified glucanases if they were first treated with a proteolytic enzyme, indicating that glucan is coated with mannoproteins in the wall. Besides, a layered composition of the wall could be seen in most electron microscopic studies of yeast cells.<sup>15-17</sup> Our results indicate that different layers of the wall have very different chemical properties, as well. Such organization could have a significant physiological importance for the yeast cell, allowing interactions of secreted proteins with the glucane layer of the wall as the first step of their inclusion in this cel-

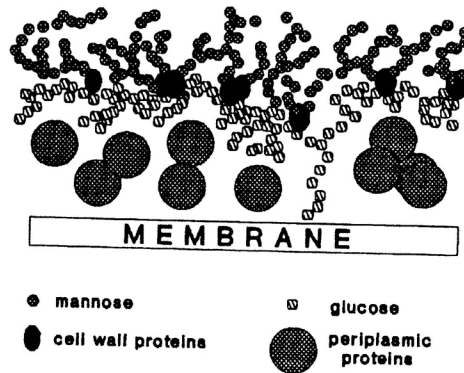


Figure 4. Tentative model of the *S. cerevisiae* cell wall.

lular structure. At the same time, it prevents the binding of potentially hazardous proteins from the environment to the cell surface. However, further work is required to support such molecular organization in the yeast cell wall, as well as to elucidate biochemical mechanisms by which this organization is accomplished.

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### SAŽETAK

#### Struktura stanične stijenke kvasca *Saccharomyces cerevisiae*

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Stanična stijenka kvasca *S. cerevisiae* izgrađena je iz strukturnih polisaharida glukana, manana i kitina, te od proteina. Moguće interakcije među komponentama stijenke nisu detaljno proučene. Također je nejasno postoji li određena molekulska organizacija molekula iz kojih je stijenka sastavljena. Kako bi se razjasnila struktura ovog vanjskog staničnog odjeljka, studirane su međumolekulske interakcije odgovorne za izgradnju stanične stijenke. Rezultati su pokazali da dolazi do protein-glukan, protein-kitin, glukan-kitin i glukan-glukan međudjelovanja. Lanci manana nisu reagirali niti s proteinima, niti s drugim polisaharidima. Nađeno je da čitave stanične stijenke vežu proteine koji do stijenke dolaze s unutrašnje strane, ali ne i proteine koji dospijevaju do stanične stijenke izvana, što ukazuje na izraženu asimetričnost stijenke čiji je vanjski sloj izgrađen od manana, a unutrašnji od glukana. Manoproteini bi mogli za glukan biti povezani preko proteinskih dijelova molekula.