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CELL WALL DYNAMICS IN YEAST

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The dynamics of cell wall biogenesis in yeast
The yeast *Saccharomyces cerevisiae* is the first fungus for which the structure of the cell wall is known at a molecular level. It is a dynamic and highly regulated structure. This is vividly illustrated when the cell wall is damaged and a salvage pathway becomes active, resulting in compensatory changes in the wall.

**Introduction**

Fungi devote a considerable amount of metabolic energy to building a cell wall, which accounts for 20-30 percent of the cell dry weight. Not only does the fungal cell wall have a skeletal function, it also plays a key role in morphogenesis and cell-cell recognition. We present a tentative molecular model of the cell wall of *Saccharomyces cerevisiae*. We further show that the composition and structure of the cell wall are strictly regulated and vary in response to a wide range of environmental conditions. We also touch upon the relation between the cell wall and morphogenesis. Finally, we present evidence for the existence of a salvage pathway, designated as the cell wall integrity pathway, allowing the cell to compensate for various forms of cell wall damage. Although the cell wall of *Saccharomyces cerevisiae* is certainly not representative of all fungi, we believe that our model has a strong predictive value for studying the cell wall of the Ascomycotina, and especially *Candida albicans*.

**A molecular model of the cell wall**

Based on recent work by various groups (Kapteyn et al. 1999b, Kollar et al. 1995, Kollar et al. 1997, Mrsa et al. 1997) (see for reviews Kapteyn et al. 1999a, Lipke and Ovalle 1998) we present a tentative model of the cell wall of yeast at a molecular level (Figure 1). The main features of this model are:

1. An internal skeletal framework formed by a three-dimensional network of β-1,3-glucan molecules which surrounds the entire cell and is largely responsible for the mechanical strength of the wall. Because mature β-1,3-glucan molecules are branched (Manners et al. 1973), they have multiple nonreducing ends. These may function as attachment sites for the other components of the cell wall (Kollar et al. 1995, Kollar et al. 1997).

2. The skeletal framework is strengthened by chitin chains (Kollar et al. 1995), which are mainly found close to the plasma membrane. However, some
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Figure 1. A molecular model of the cell wall of *Saccharomyces cerevisiae*. The internal skeletal layer consists of β-1,3-glucan molecules that form a three-dimensional network surrounding the entire cell. This network is kept together by local alignments between segments of β-1,3-glucan molecules allowing the formation of multiple hydrogen bridges. At the outside of the skeletal layer cell wall proteins are linked to the nonreducing ends of β-1,3-glucan molecules either directly (Pir-CWPs) or indirectly through an interconnecting β-1,6-glucan moiety (GPI-CWPs). Some GPI-CWPs such as Cwp1p may be linked both ways. After cytokinesis, the skeletal layer becomes strengthened by the coupling of chitin chains to nonreducing ends of β-1,3-glucan chains. This takes place mainly at the inside of the skeletal layer. β-1,6-Glucan is much more branched than β-1,3-glucan (Kapteyn et al. 1997, Manners et al. 1973b), probably explaining why the mature β-1,6-glucan molecule is water-soluble. Thus, β-1,6-glucan probably functions as a flexible tether for GPI-CWPs. Note that branched polysaccharides such as β-1,3-glucan and β-1,6-glucan in principle have a single reducing end and multiple nonreducing ends. For reasons of clarity, non-covalently bound proteins and proteins linked through disulfide bridges to other cell wall proteins have been omitted. CWP, cell wall protein. GPI, glycosyl phosphatidylinositol. Pir, protein with internal repeats. This model is based on data from (Kapteyn et al. 1999a, Kapteyn et al. 1999b, Kollar et al. 1995, Kollar et al. 1997, Lipke and Ovalle 1998, Manners et al. 1973a, Mrsa et al. 1997, Rees 1977).
chitin chains become linked to short side-chains of β-1,6-glucan (Kapteyn et al. 1997).

(3) Mature β-1,6-glucan molecules are mainly found at the outside of the skeletal framework and interconnect a particular class of cell wall proteins (GPI-CWPs) with the framework (Kollar et al. 1997).

(4) Two classes of covalently linked cell wall proteins (CWPs) are known, the already mentioned GPI-CWPs (Caro et al. 1997, Hamada et al. 1998, Hamada et al. 1998) and the Pir-CWPs (Kapteyn et al. 1999b, Mrsa, et al. 1997, Yun et al. 1997). They differ from each other in that Pir-CWPs seem to be directly linked to β-1,3-glucan molecules without an interconnecting β-1,6-glucan moiety (Kapteyn et al. 1999) and can be released from the cell wall by mild alkali (Mrsa et al. 1997). In contrast to the structural complex GPI-CWP→β-1,6-glucan→β-1,3-glucan, which has been extensively investigated (Kollar et al. 1997), the Pir-CWP→β-1,3-glucan complex is yet ill defined.

Glucan remodeling and cell wall assembly take place outside the plasma membrane. Likely candidates for glucan remodeling enzymes are Gaslp, a GPI-anchored plasma membrane protein (reviewed in Popolo and Vai 1999, see also Ram et al. 1998) and the proteins belonging to the Bgl2 family (Cappellaro et al. 1998). Interestingly, homologs of Gaslp have been found in various other (mycelial) fungi (Nakazawa et al. 1998, Popolo and Vai 1998, Popolo and Vai 1999).

The question arises in how far our model has predictive value for other fungi. The cell wall of C. albicans, recently reviewed in (Chaffin et al. 1998), is a good test case. First, there is strong evidence for the existence of a family of GPI-CWPs (Hoyer et al. 1998, Hoyer et al. 1998). Although it has not yet been shown that the proteins in this family are linked through an interconnecting β-1,6-glucan moiety to β-1,3-glucan, there is strong evidence for the presence of the structural complex GPI-CWP→β-1,6-glucan→β-1,3-glucan in C. albicans (Kapteyn et al. 1995). Second, the cell wall of C. albicans contains several proteins that like the Pir-CWPs in S. cerevisiae can be released by mild alkali (Mormeneo et al. 1994), suggesting that also in C. albicans a Pir-CWP→β-1,3-glucan-like complex may exist. Another interesting finding concerns a putative GPI-CWP at the cell surface of the mycelial fungus Penicillium marneffei (Cao et al. 1998). Taken together, these data strongly indicate that our model has predictive value for other Ascomycotina.
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Functions of cell wall proteins

Following a genomic approach, we were able to predict the existence of approximately 40 different GPI-CWPs in yeast (Caro et al. 1997), and this has been confirmed experimentally (Hamada et al. 1998). Although it has been shown that cell wall proteins, collectively, limit cell wall permeability (de Nobel et al. 1990), the function of most individual cell wall proteins remains a mystery. Some are clearly involved in cell-cell adhesion such as the flocculins Flo1p, Flo5p, Flo9p, and Flo10p (Teunissen and Steensma 1995) and the sexual agglutinins Aga1p, Aga2p, and Sag1p (Lipke and Kurjan 1992). Flo11p forms a special case (Lo and Dranginis 1996, Lo and Dranginis 1998, Robertson and Fink 1998). Discovered first as another flocculin, Flo11p appears to be required for invasive growth into agar, and possibly also pseudohyphal growth in response to nitrogen starvation (Lo and Dranginis 1998, Robertson and Fink 1998, Rupp et al. 1999). This indicates that Flo11p may play a role in determining cellular morphology. Fig2p, another GPI-CWP, also seems to affect cell morphology. When fig2Δ haploid cells mate, they form a narrow mating projection and fusion bridge, which interferes with nuclear fusion and migration (Erdman et al. 1998). Finally, Egt2p, which is also a GPI-CWP, seems to be required for cell separation (Kovacech et al. 1996).

Several cell wall protein-encoding genes show homology to the glucoamylase gene STA1, which is specific for the variant strain S. cerevisiae var. diastaticus. However, STA1 is probably the result of a recombination event between FLO11 and SGA1, the normal glucoamylase. As the homology is limited to the FLO11 domain, this probably excludes a glucoamylase-like function for the gene products involved.

Regulation of cell wall protein expression

The recent transcript profiling studies strongly suggest that expression of many cell wall proteins is cell cycle regulated (Caro et al. 1998, Cho et al. 1998, Spellman et al. 1998) and is affected by nutrient availability (Chu et al. 1998, DeRisi et al. 1997). It is further known that pheromones and various other environmental conditions affect the expression of CWP-encoding genes (Table 1; see also below).
Nutrient availability and environmental conditions

Batch-cultured cells growing on glucose rapidly consume the glucose by fermentation thereby producing ethanol. Subsequently, they switch to respiratory growth and use up the ethanol before entering stationary phase. The transcript levels of many CWP-encoding genes change when the cells switch to respiratory growth or enter stationary phase (DeRisi et al. 1997) (Table 1). For example, Sed1p, a GPI-CWP (Hamada et al. 1998), becomes a major cell wall protein in stationary phase cells (Shimoi et al. 1998). Fermentative growth can also be triggered by hypoxic conditions, and a similar set of cell wall proteins is then induced. However, other wall proteins are specifically induced under hypoxic conditions, indicating that they are regulated differently (Donzeau et al. 1996, Sertil et al. 1997) (Table 1).

When diploid yeast cells are starved for nitrogen, they switch to pseudohyphal growth. Interestingly, this is accompanied by increased expression of FLO11 (Lo and Dranginis 1998, Rupp et al. 1999) and presumably also by HSP150/PIR2 (Russo et al. 1993), raising the question if still more cell wall proteins are preferentially used by the cell during pseudohyphal growth. Without both suitable carbon and nitrogen sources, cells activate the sporulation program. This is accompanied by up- and downregulation of various CWP-encoding genes (Chu et al. 1998) (Table 1).

The cell cycle

Approximately 13% of the genes of S. cerevisiae are regulated in a cell cycle-dependent manner (Spellman et al. 1998). Intriguingly, more than half of all CWP-encoding genes (22/43) are cell cycle regulated, including PIR1, the most strictly cell cycle-regulated gene in yeast. Although during each phase of the cell cycle specific CWP-encoding genes seem to be expressed (Caro et al. 1998, Cho et al. 1998, Spellman et al. 1998), most of them are active in late M- and early G1-phase, around the time of cell separation and the subsequent period of isotropic growth by the daughter (Table 1). Finally, consistent with the extensive cell cycle-dependent expression of cell wall proteins, some cell wall proteins are indeed known to be localized to specific regions of the cell wall (Bony et al. 1998, Ram et al. 1998b).
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Table 1. Overview of CWP-encoding genes which vary in expression levels during the cell cycle and under various growth conditions.

<table>
<thead>
<tr>
<th>Signal</th>
<th>Regulated genes</th>
<th>References</th>
</tr>
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<tbody>
<tr>
<td>Cell cycle</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G1</td>
<td>PRY3, YGR189c, YNL300w</td>
<td>(Spellman et al. 1998, Cho et al. 1998)</td>
</tr>
<tr>
<td>G2</td>
<td>CIS3, CWP1, CWP2, TIR1</td>
<td>(Cho et al. 1998)</td>
</tr>
<tr>
<td>M</td>
<td>SED1, YIP1, YOR383c</td>
<td>(Caro et al. 1998)</td>
</tr>
<tr>
<td>M/G1</td>
<td>AGA1, AGA2, EGT2, HSP150, PIR1, PIR3, SAG1, TIP1, TIR5, UTR2, YER150c, YHR126c, YLR194c</td>
<td></td>
</tr>
<tr>
<td>Pheromone</td>
<td>AGA1, CWP1, FIG2, SAG1</td>
<td>(Erdman et al. 1998, Lipke and Kurjan 1992)</td>
</tr>
<tr>
<td>Nutrients carbon source</td>
<td>CIS3, CWP1, EGT2, FLO1, FLO5, FLO9, SED1, SUN4, TIP1, TIR1, TIR6, UTR2, YER150w, YOR383c</td>
<td>(DeRisi et al. 1997, Donzeau et al. 1996, Shimoi et al. 1998, Teunissen and Steensma 1995,a)</td>
</tr>
<tr>
<td>nitrogen</td>
<td>FLO11, HSP150</td>
<td>(Lo and Dranginis 1998, Russo et al. 1993)</td>
</tr>
<tr>
<td>Sporulation</td>
<td>AGA2, BAR1, CIS3, CWP1, CWP2, EGT2, FIG2, FLO11, HSP150, PIR1, PIR3, SED1, TIP1, TIR2, TIR5, UTR2, YDR134c, YER150w, YGR189c, YIB1, YLR110c, YLR194c, YOL155c, YOR214c, YOR382w, YOR383c</td>
<td>(Chu et al. 1998)</td>
</tr>
<tr>
<td>Stress weakened wall</td>
<td>CIS3, CWP1, HSP150, PIR3, SED1, SSR1</td>
<td>(Kapteyn et al. 1999a, Ram et al. 1998a,b)</td>
</tr>
<tr>
<td>temperature</td>
<td>HSP150, TIP1, TIR1, TIR2</td>
<td>(Kowalski et al. 1995, Russo et al. 1993)</td>
</tr>
<tr>
<td>aluminum</td>
<td>SED1, HSP150</td>
<td>(Ezaki et al. 1998)</td>
</tr>
</tbody>
</table>

b. J.C. Kapteyn, unpublished data
When haploid yeast cells sense the mating pheromone of the opposite mating type, they arrest in G1 and form a mating projection (Gustin et al. 1998). The density of sexual agglutinins in the wall of the mating projection dramatically increases (Lipke and Kurjan 1992). In addition, as discussed above, expression of FIG2 (Erdman et al. 1998), which encodes a putative GPI-CWP, is upregulated. Also more chitin is deposited in the wall of the mating projection (Lipke and Kurjan 1992). These observations clearly indicate that the wall of the mating projection differs from normal walls.

The cell wall integrity pathway

There is increasing evidence that weakening of the cell wall results in activation of a salvage pathway, leading to compensatory changes in the wall. We propose to call it the cell wall integrity pathway. The existence of such a pathway might explain why so many cell wall mutants show hypersensitivity to caffeine (Lussier et al. 1997). Caffeine activates protein kinase A, which represses many stress responses (Kronstad et al. 1998). Thus, mutant cells might be hypersensitive to caffeine because they depend on the cell wall integrity pathway for their survival.

A speculative scheme of the cell wall integrity pathway is presented in Figure 2. The sensing of cell wall weakening, possibly through membrane stretch, is thought to occur by the Wsc and Mid families of membrane proteins (Gustin et al. 1998). The signal is believed to be relayed to Rho1p through the exchange factor Rom2p (Bickle et al. 1998, Schmidt et al. 1997). Rho1p modulates the protein kinase C (PKC) pathway (Cabib et al. 1998) as well as the β-1,3-glucan synthase, resulting in increased synthesis of chitin and glucan (Dallies et al. 1998, Kapteyn et al. 1997, Ram et al. 1998a), altered cross-linking of glucan and proteins (Kapteyn et al. 1997, Kapteyn et al. 1999a), and to increased expression of Cwp1p and Pir-CWPs (Kapteyn et al. 1997, Kapteyn et al. 1999a, Ram, et al. 1998a). Other stress conditions which are also relayed through the PKC pathway, such as high temperatures and low osmolarity, might be sensed by the same or similar membrane sensors and lead to similar effects. This might be relevant for several cell wall proteins (HSP150/PIR2, TIP1 and TIR2), which are more strongly expressed at high temperatures (Kowalski et al. 1995, Russo et al. 1993).
Figure 2. Model for the cell wall integrity pathway. Cell wall stress is sensed at the cell surface by sensor membrane proteins for which the Wsc and Mid proteins are likely candidates. The signal from Slglp/Wsclp is relayed to the exchange factor Rom2p, which activates Rholp. Rholp can directly activate the glucan synthase complex as well as the protein kinase C pathway, and thus leads to an increased β-1,3-glucan synthesis and, through the Slt2p/Mpk1p MAP kinase cascade and an as yet unidentified transcription factor, to increased expression of cell wall biosynthetic enzymes and CWPs. Whether alterations in chitin synthesis, and possibly also in β-1,6-glucan synthesis, and the altered cross-linking of proteins to the cell wall matrix are achieved through the same signal transduction pathway remains to be clarified.
Chapter 5

Perspectives

Although many questions concerning cell wall biogenesis remain unanswered, our model of the cell wall allows to formulate them in molecular terms. Its predictive value concerning mycelial fungi is an important question that needs to be further addressed. It is also clear that the wall is highly dynamic and forms an integral part of cell metabolism, raising fascinating questions about the control mechanisms involved.

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