Cell wall dynamics in yeast

Gertien J Smits, Johan C Kapteyn, Herman van den Ende and Frans M Klis*

The yeast *Saccharomyces cerevisiae* is the first fungus for which the structure of the cell wall is known at the 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.

Addresses
BioCentrum Amsterdam, University of Amsterdam, Institute of Molecular Cell Biology, Developmental Biology of Fungi, Kruislaan 318, 1098 SM Amsterdam, The Netherlands
*e-mail: klis@bio.uva.nl

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Abbreviations

CWP	cell wall protein
GPI	glycosyl phosphatidylinositol
Pir	protein with internal repeats
PKC	protein kinase C

Introduction

Fungi devote a considerable amount of metabolic energy to building a cell wall, which accounts for 20–30% 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 *S. 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

On the basis of on recent work by various groups ([1–3,4••]; see [5•,6•] for reviews) we present a tentative model of the cell wall of yeast at a molecular level (Figure 1). The main features of this model are discussed below.

Firstly, an internal skeletal framework, formed by a three-dimensional network of β1,3-glucan molecules, surrounds the entire cell and is largely responsible for the mechanical strength of the wall. Because mature β1,3-glucan molecules are branched [7], they have multiple nonreducing ends. These may function as attachment sites for the other components of the cell wall [1,2].

Secondly, the skeletal framework is strengthened by chitin chains [1], which are mainly found close to the plasma membrane. Some chitin chains, however, become linked to short side-chains of β1,6-glucan [8].

Thirdly, mature β1,6-glucan molecules are mainly found at the outside of the skeletal framework and interconnect a particular class of cell wall proteins (CWPs), glycosyl phosphatidylinositol (GPI)-CWPs, with the framework [2].

Finally, two classes of covalently linked CWPs are known, the already mentioned GPI-CWPs [9,10•,11••] and the protein with internal repeats (Pir)-CWPs [3,4••,12]. They differ from each other in that Pir-CWPs seem to be directly linked to β1,3-glucan molecules without an interconnecting β1,6-glucan moiety [4••] and can be released from the cell wall by mild alkali [3]. In contrast to the structural complex GPI-CWP→β1,6-glucan→β1,3-glucan, which has been extensively investigated [2], the Pir-CWP→β1,3-glucan complex is as yet ill defined.

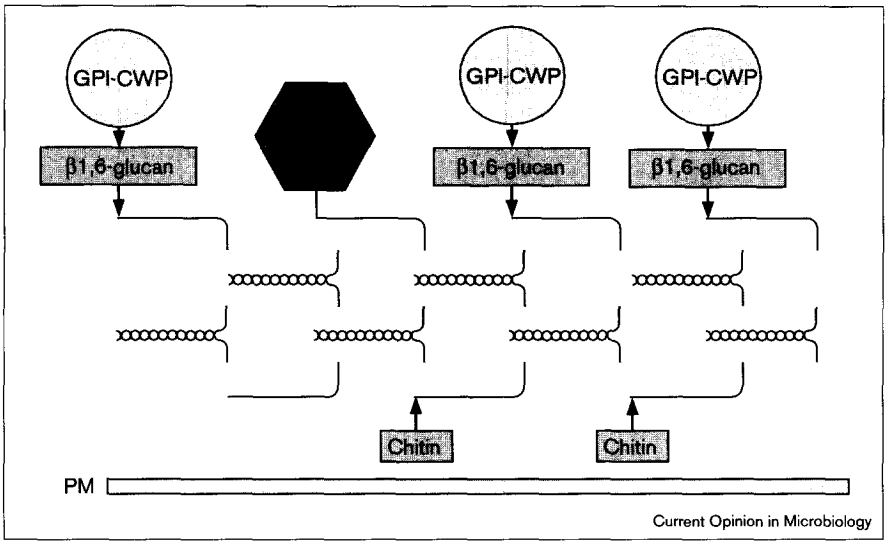
Glucan remodeling and cell wall assembly take place outside the plasma membrane. Likely candidates for glucan remodeling enzymes are Gas1, a GPI-anchored plasma membrane protein (reviewed in [13•]; see also [14••]), and the proteins belonging to the Bgl2 family of endo-beta-1,3-glucanases and/or transglucosylases [15]. Interestingly, homologs of Gas1 have been found in various other (mycelial) fungi [13•,16,17•].

The question arises in how far our model has predictive value for other fungi. The cell wall of *C. albicans*, recently reviewed in [18•], is a good test case. First, there is strong evidence for the existence of a family of GPI-CWPs in *C. albicans* [19••,20]. Although it has not yet been shown that the proteins in this family are linked through an interconnecting β1,6-glucan moiety to β1,3-glucan, there is strong evidence for the presence of the structural complex GPI-CWP→β1,6-glucan→β1,3-glucan [21]. Second, the cell wall of *C. albicans* contains several proteins that like the Pir-CWPs in *S. cerevisiae* can be released by mild alkali [22], suggesting that also in *C. albicans* a Pir-CWP→β1,3-glucan-like complex might exist. Another interesting finding concerns a putative GPI-CWP at the cell surface of the mycelial fungus *Penicillium marneffei* [23•]. Taken together, these data strongly indicate that our model has predictive value for other Ascomycotina.

Functions of cell wall proteins

Following a genomic approach, we were able to predict the existence of approximately 40 different GPI-CWPs in yeast [9], and this has been confirmed experimentally

Figure 1



A molecular model of the cell wall of *Saccharomyces cerevisiae*. The internal skeletal layer consists of β1,3-glucan molecules that form a three-dimensional network surrounding the entire cell. This network is kept together by local alignments between segments of β1,3-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 β1,3-glucan molecules either directly (Pir-CWPs) or indirectly through an interconnecting β1,6-glucan moiety (GPI-CWPs). Some GPI-CWPs, such as Cwp1, may be linked both ways. After cytokinesis, the skeletal layer becomes strengthened by the coupling of chitin chains to nonreducing ends of β1,3-glucan chains. This takes place mainly at the inside of the skeletal layer. β1,6-Glucan is much more branched than β1,3-glucan [7,8], probably explaining why the mature β1,6-glucan molecule is water-soluble. Thus, β1,6-glucan probably functions as a flexible tether for GPI-CWPs. Note that branched polysaccharides such as β1,3-glucan and β1,6-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. PM, plasma membrane. This model is based on data from [1–3,4••,5•,6•,52,53].

[10•]. Although it has been shown that CWPs, collectively, limit cell wall permeability [24], the function of most individual CWPs remains a mystery. Some are clearly involved in cell–cell adhesion such as the flocculins Flo1, Flo5, Flo9, and Flo10 [25] and the sexual agglutinins Aga1, Aga2, and Sag1 [26]. Flo11 forms a special case [27,28••,29]. Discovered first as another flocculin, Flo11 appears to be required for invasive growth into agar, and possibly also pseudohyphal growth in response to nitrogen starvation [28••,29,30•]. This indicates that Flo11 may play

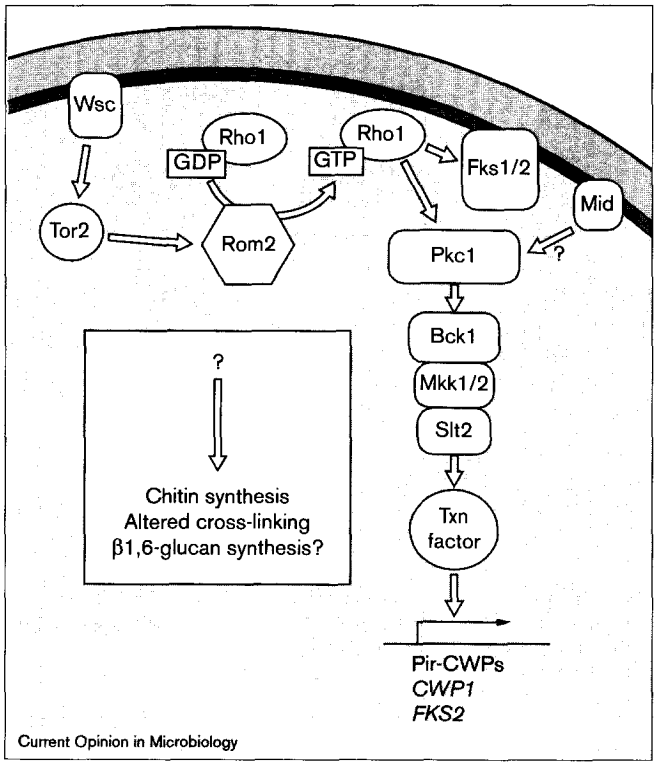
Table 1

Overview of CWP-encoding genes that vary in expression levels during the cell cycle and under various growth conditions.

Signal	Regulated genes	References
Cell cycle		
G1	*PRY3*, *YGR189c*, *YNL300w*	[32,33••,34,35]
G2	*CIS3*, *CWP1*, *CWP2*, *TIR1*	
M	*SED1*, *YIP1*, *YOR383c*	
M/G1	*AGA1*, *AGA2*, *EGT2*, *HSP150*, *PIR1*, *PIR3*, *SAG1*, *TIP1*, *TIR5*, *UTR2*, *YER150c*, *YHR126c*, *YLR194c*	
Pheromone	*AGA1*, *CWP1*, *FIG2*, *SAG1*	[26,31••]
Nutrients		
Carbon source	*CIS3*, *CWP1*, *EGT2*, *FLO1*, *FLO5*, *FLO9*, *SED1*, *SUN4*, *TIP1*, *TIR1*, *TIR6*, *UTR2*, *YER150w*, *YOR383c*	[25,36,38•,39]*
Nitrogen	*FLO11*, *HSP150*	[28••,41]
Sporulation	*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*	[37••]
Stress		
Weakened wall	*CIS3*, *CWP1*, *HSP150*, *PIR3*, *SED1*, *SSR1*	[4••,14••]†
Temperature	*HSP150*, *TIP1*, *TIR1*, *TIR2*	[41,51]
Hypoxia	*DAN1*, *TIP1*, *TIR1*	[39,40,54]
Aluminum	*SED1*, *HSP150*	[55]

*T Fujii, H Shimoi, I Fujishige, T Ohba, abstract 246, Yeast Genetics and Molecular Biology Meeting, College Park, Maryland, July 28 to August 2 1998. †JC Kapteyn, unpublished data.

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 Slg1/Wsc1 is relayed through the phosphatidylinositol-4 kinase Tor2 to the exchange factor Rom2, which activates Rho1. Rho1 can directly activate the glucan synthases Fks1 and Fks2, as well as the protein kinase C (PKC) pathway, and thus leads to increased β1,3-glucan synthesis and, through the Slt2/Mpk1 MAP kinase cascade and an as yet unidentified transcription factor (represented as Txn factor), to increased expression of cell wall biosynthetic enzymes and CWPs. Whether alterations in chitin synthesis, and possibly also in β1,6-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.

a role in determining cellular morphology. Another GPI-CWP, Fig2, 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 [31••]. Finally, Egt2, which is also a GPI-CWP, seems to be required for cell separation [32].

Several genes encoding CWPs show homology to the glucoamylase gene *STA1*, which is specific for the variant strain *S. cerevisiae* var. *diastaticus*. *STA1*, however, 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 CWPs is cell cycle regulated [33••,34,35] and is affected by nutrient availability [36,37••]. 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 [36] (Table 1). For example, Sed1, a GPI-CWP [10•], becomes a major CWP in stationary phase cells [38•]. Fermentative growth can also be triggered by hypoxic conditions, and a similar set of CWPs is then induced. Other wall proteins, however, are specifically induced under hypoxic conditions, indicating that they are regulated differently [39,40] (Table 1).

When diploid yeast cells are starved for nitrogen, they switch to pseudohyphal growth. Interestingly, this is accompanied by increased expression of *FLO11* [28••,30•] and presumably also of *HSP150/PIR2* [41], raising the question whether still more CWPs 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 down-regulation of various CWP-encoding genes [37••] (Table 1).

The cell cycle

Approximately 13% of the genes of *S. cerevisiae* are regulated in a cell-cycle-dependent manner [33••]. Intriguingly, more than half of all CWP-encoding genes (22 out of 43) are cell cycle regulated, including *PIR1*, the most strictly cell-cycle-regulated gene in yeast. Although specific CWP-encoding genes seem to be expressed during each phase of the cell cycle [33••,34,35], 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 cell (Table 1). Finally, consistent with the extensive cell-cycle-dependent expression of CWPs, some CWPs are indeed known to be localized to specific regions of the cell wall [42,43•].

When haploid yeast cells sense the mating pheromone of the opposite mating type, they arrest in G1 and form a mating projection [44••]. The density of sexual agglutinins in the wall of the mating projection dramatically increases [26]. In addition, as discussed above, expression of *FIG2* [31••], which encodes a putative GPI-CWP, is upregulated. Also more chitin is deposited in the wall of the mating projection [26]. 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 [45]. Caffeine activates protein kinase A, which represses many stress responses [46]. 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 [44••]. The signal is believed to be relayed to Rho1, a small GTP-binding protein, through Tor2, a phosphatidylinositol-4 kinase, and the exchange factor Rom2 [47,48••]. Rho1 modulates the protein kinase C (PKC) pathway [49•] as well as the β1,3-glucan synthase, resulting in increased synthesis of chitin and glucan [8,14••,50•], altered cross-linking of glucan and proteins [4••,8], and increased expression of Cwp1 and Pir-CWPs [4••,8,14••]. Other stress conditions that 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 CWPs (*HSP150/PIR2*, *TIP1* and *TIR2*), which are more strongly expressed at high temperatures [41,51].

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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