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Review

Cell wall construction in *Saccharomyces cerevisiae*

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Abstract

In this review, we discuss new insights in cell wall architecture and cell wall construction in the ascomycetous yeast *Saccharomyces cerevisiae*. Transcriptional profiling studies combined with biochemical work have provided ample evidence that the cell wall is a highly adaptable organelle. In particular, the protein population that is anchored to the stress-bearing polysaccharides of the cell wall, and forms the interface with the outside world, is highly diverse. This diversity is believed to play an important role in adaptation of the cell to environmental conditions, in growth mode and in survival. Cell wall construction is tightly controlled and strictly coordinated with progression of the cell cycle. This is reflected in the usage of specific cell wall proteins during consecutive phases of the cell cycle and in the recent discovery of a cell wall integrity checkpoint. When the cell is challenged with stress conditions that affect the cell wall, a specific transcriptional response is observed that includes the general stress response, the cell wall integrity pathway and the calcineurin pathway. This salvage mechanism includes increased expression of putative cell wall assemblases and some potential cross-linking cell wall proteins, and crucial changes in cell wall architecture. We discuss some more enzymes involved in cell wall construction and also potential inhibitors of these enzymes. Finally, we use both biochemical and genomic data to infer that the architectural principles used by *S. cerevisiae* to build its cell wall are also used by many other ascomycetous yeasts and also by some mycelial ascomycetous fungi. Copyright © 2006 John Wiley & Sons, Ltd.

Keywords: *Candida*; *Aspergillus*; glycosylphosphatidylinositol-modified proteins; cell wall proteins; cell wall integrity; cell cycle; anaerobic growth; stationary phase

Contents

Introduction

The yeast cell wall has four major functions:

1. Stabilization of internal osmotic conditions. The osmolality of the cytoplasm of *S. cerevisiae* and other fungi is usually higher than outside the cells. To limit the resulting water influx, which would perturb the internal reaction conditions and cause excessive swelling of the cell eventually leading to rupture of the plasma membrane, fungi construct a sturdy and elastic wall. Extension of the wall creates a counteracting pressure by the wall, which stops water influx.
2. Protection against physical stress. The cell wall is not only involved in maintaining osmotic homeostasis but also functions as a protective coat. The combination of considerable mechanical strength and high elasticity allows the wall to transmit and redistribute physical stresses, thus offering efficient protection against mechanical damage [86,106].

3. Maintenance of cell shape, which is a precondition for morphogenesis. Yeast cells can grow as oval cells, or in a more elongated form during nitrogen limitation or when growing pseudohyphally. The formation of a mating structure in response to pheromone from the opposite mating partner also illustrates this function of the cell wall. The spectacular forms of higher fungi are a striking example of how the cell wall may contribute to morphogenesis.
4. The cell wall as a scaffold for proteins. The stress-bearing polysaccharides of the cell wall of baker's yeast and other fungi function as a scaffold to an external layer of glycoproteins [115,154,169]. Collectively, these glycoproteins, and particularly their *N*-linked carbohydrate side-chains, limit the permeability of the cell wall for macromolecules, thus shielding the skeletal polysaccharides from attack by foreign proteins [40,169]. Conceivably, they may also limit the escape into the medium of soluble intermediates in cell wall construction. The limited permeability of the external protein layer may also allow the creation of a microenvironment in the inner region of the wall adjacent to the plasma membrane, particularly in stagnant cultures and in colonies. The high degree of glycosylation of the cell wall proteins and the presence of negatively charged phosphate groups in their carbohydrate side-chains probably also contribute to water retention.

In addition to these collective functions, specific functions of individual proteins are important. It has become clear that the external protein layer of the cell wall may at any time consist of at least 20 different glycoproteins and that the composition of this protein layer may vary depending on growth conditions [33,35,78,140,164]. This creates the opportunity for the cell to introduce a wide variety of new functions. Cell wall proteins allow the cells to flocculate, recognize mating partners, form a biofilm and grow pseudohyphally and invasively; they also help the cells to retain iron and facilitate sterol uptake and are required for growth under anaerobic conditions [1,5,20,84,85,120,127,160]. Cell wall proteins may also strongly affect hydrophobicity of the cells, which is important for adhering to polystyrene and other abiotic surfaces and for brewing alcoholic beverages [127,141,147]. As discussed below, some cell wall proteins seem to be specifically involved in cell wall repair. In addition, species-specific functions of cell wall proteins seem likely. For example, cell walls of the medically important fungus *Candida albicans* may contain Sod4p and Sod5p, GPI-modified superoxide dismutases that offer protection against oxidative stress [33,48,98].

Cell wall construction is tightly controlled. Polysaccharide composition, structure and thickness of the cell wall vary considerably, depending on environmental conditions [4]. Not surprisingly, cell wall formation is strictly coordinated with the cell cycle. For example, the majority of the cell wall protein-encoding genes are cell cycle-regulated [6,142]. Oxygen levels also strongly affect the protein composition of the wall. In this review, we will mainly focus on recent progress in our understanding of cell wall construction and on new insights coming from genomic approaches. For earlier work the reader is referred to reviews by Orlean [114], Lipke and Ovalle [95], Cabib *et al*. [18] and Klis *et al*. [83]. In particular, the recent and extensive review by Lesage and Bussey [92], which is largely complementary to this work, is strongly recommended.

Molecular structure of the cell wall

S. cerevisiae spends a considerable amount of metabolic energy in cell wall construction. Depending on growth conditions, its mass in terms of dry weight may account for about 10–25% of the total cell mass [4]. It consists of an inner layer of load-bearing polysaccharides, acting as a scaffold for a protective outer layer of mannoproteins that extend into the medium (Table 1, Figure 1) [83,95,114,164]. The major load-bearing polysaccharide is a moderately branched 1,3-β-glucan [43,97]. Due to the presence of side-chains, 1,3-β-glucan molecules can only locally associate through hydrogen bonds, resulting in the formation of a continuous, three-dimensional network. This network is highly elastic and is considerably extended under normal osmotic conditions. When cells are transferred to hypertonic solutions, they rapidly shrink and may lose up to 60% of their original volume, which corresponds to an estimated surface loss of about 40–50% [106]. Shrinkage is

Table 1. Macromolecules of the cell wall of *S. cerevisiae*

Macromolecule	% of wall mass[a]	Mean M_r (DP) (kDa)
Mannoproteins[b]	30–50	Highly variable
1,6-β-Glucan	5–10	24 (150)
1,3-β-Glucan	30–45	240 (1500)
Chitin	1.5–6	25 (120)

The cell wall components are presented in the order in which they are found in the cell wall from the outside to the inside. Cell wall stress may lead to dramatically increased chitin levels. DP, degree of polymerization.

[a] [4].

[b] The actual protein content is about 4–5%; the remaining mass is from protein-linked, mannose-containing carbohydrate side-chains.

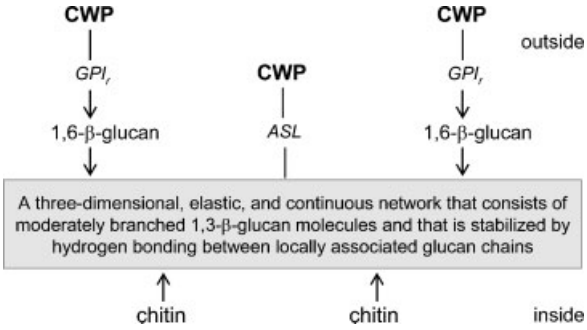

Figure 1. Molecular organization of the cell wall of *S. cerevisiae*. The GPI–CWPs form the majority of the covalently linked cell wall proteins. The ASL–CWPs include the Pir–CWPs (Pir1p, Hsp150p, Pir3p and Cis3p). GPI_r, lipidless remnant of a GPI-anchor. ASL, alkali-sensitive linkage. Adapted from Klis *et al.* [83]

A. CWP–GPI_r → 1,6-β-glucan → 1,3-β-glucan

B. CWP–*ASL* - 1,3-β-glucan

C. CWP–GPI_r → 1,6-β-glucan ← chitin

D. CWP[- *ASL* - 1,3-β-glucan]–GPI_r → 1,6-β-glucan → 1,3-β-glucan

E. CWP[–*ASL* – 1,3-β-glucan]- GPI_r

Figure 2. Known cell wall protein–polysaccharide complexes in *S. cerevisiae*. (A) This complex between a GPI–CWP and 1,6-β-glucan → 1, 3-β-glucan is normally the most abundant one. The arrows represent glycosidic linkages and point from the reducing end of a macromolecule to a non-reducing end of the acceptor polysaccharide. (B) The second most abundant category of CWP–polysaccharide complexes. The precise nature of the linkage between an ASL–CWP and 1,3-β-glucan is unknown. Pir–CWPs belong to this category. (C) This complex becomes more prominent in response to cell wall stress. (D and E) A small set of hybrid GPI–CWPs is directly connected to both 1,6-β-glucan (through a GPI remnant) and to 1,3-β-glucan (through an alkali-sensitive linkage; complex D) or to 1,3-β-glucan only (through an alkali-sensitive linkage; complex E). The glycosidic linkage between chitin and 1,3-β-glucan is omitted for clarity. CWP, cell wall protein; GPI_r, lipidless GPI remnant connecting the carboxyterminal end of a mature GPI-modified cell wall protein to the cell wall polysaccharide network. ASL, alkali-sensitive linkage

fully reversible, as seen when the cells are transferred to the original medium. This observation also explains the discrepancy between the permeability of isolated walls, which have an estimated exclusion limit of about 760 Da [133], and the cell wall permeability of intact cells, which is much higher and allows easy passage of medium-sized proteins [39,166]. Another consequence of the elasticity of the wall is that, after fixation, cells tend to shrink and as a result may become considerably smaller than living cells. This probably explains the invaginations that, after fixation, are frequently observed in the plasma membrane [105,149]. The elasticity of the cell wall reflects the structure of the individual 1,3-β-glucan molecules, which have a flexible and helical shape, like a wire spring that can exist in various states of extension [126]. When other yeasts, such as *Candida* spp., *Cryptococcus* spp., *Pichia* spp. and *Schizosaccharomyces* spp., are exposed to hypertonic solutions, they also rapidly shrink, indicating that the combination of mechanical strength and elasticity is a widely used architectural principle in the construction of yeast walls [106].

The non-reducing ends of the 1,3-β-glucan molecules may function as attachment sites for covalent attachment of other polysaccharides. At the external face of the 1,3-β-glucan network, highly branched (and thus water-soluble) 1,6-β-glucan chains are found, which in turn may be connected to a GPI-modified mannoprotein (Figures 1, 2) [50,71,88]. At the inside of the 1,3-β-glucan network in the lateral walls, chitin chains may become attached, but only after cytokinesis has taken place [87,138]. In other words, the lateral walls of the growing bud generally do not contain chitin, demonstrating that chitin is not essential for the mechanical strength of the lateral walls. Chitin may not only become glycosidically linked to non-reducing ends of 1,3-β-glucan but also of 1,6-β-glucan chains, particularly in response to cell wall

stress (Figure 2). Normally, however, most chitin is not found in the lateral walls, but in the chitin ring in the neck of the mother cell, in the primary septum and in bud scars (see below). Intriguingly, Fontaine *et al*. [47] have found a galactomannan in the wall of *Aspergillus fumigatus* that is directly linked to the β-glucan network. This galactomannan is similar in structure to the GPI-anchored, lipid-bound galactomannan recently identified in the same organism, raising the possibility that the lipid-bound form is a precursor of the cell wall-bound form and that a similar transfer mechanism is used as for GPI–CWPs [27]. Might that mean that, in *S. cerevisiae* and other ascomycetous yeasts, some mannan may be directly linked to the β-glucan network as well? There is some evidence pointing in this direction. A careful analysis of the alkali-soluble glucan in the cell wall of *S. cerevisiae* shows the presence of on average 15–45 mannose residues per glucan molecule of about 1500 residues, which remain behind despite extensive purification [44]. Our existing knowledge cannot explain such high numbers of mannose residues in alkali-extracted glucan molecules unless it is assumed that, before alkali extraction, each glucan molecule was attached to multiple GPI–CWPs, each with its own tetramannoside derived from the GPI remnant (note that alkali extraction cleaves the phosphodiester bridge in the GPI remnant, leaving the tetramannoside of the GPI remnant linked to 1,6-β-glucan). This seems unlikely in view of the data by Katohda *et al*. [75], as recalculated by Klis *et al*. [81], which indicate that the number of wall proteins is comparable to the number of 1,3-β-glucan molecules in the wall.

The majority of cell wall proteins (CWPs) are GPI-modified (GPI–CWPs) and are thus indirectly linked to the 1,3-β-glucan network (Figures 1, 2). To prove the presence of a putative GPI–CWP in the cell wall, and to determine how it is linked to the cell wall, the following evidence may be used: (a) immunofluorescence studies or immunogold labelling should show its presence in intact cells and SDS-extracted cell walls [19,125,130,131,136]; (b) deletion of the carboxyterminal 30–40 amino acids, which contains the GPI anchor addition signal, should result in secretion of the protein into the medium [139,161]; (c) extending a secretory reporter protein with the carboxyterminal 30–40 amino acids of the putative GPI–CWP should direct the reporter protein to the wall [58,156]; (d) the protein should be releasable from isolated walls by cleaving the phosphodiester bridge in the GPI remnant, using either HF-pyridine or a phosphodiesterase [33,71]; (e) the proteins should be released from isolated walls by either a 1,6-β-glucanase (resulting in a protein-bound 1,6-β-glucan epitope), a 1,3-β-glucanase (resulting in protein-bound 1,6- and 1,3-β-glucan epitopes) or, in some special cases, also by a chitinase (resulting in protein-bound 1,6-β-glucan and chitin epitopes) [71,72].

In addition to the GPI–CWPs, a smaller group of proteins are directly linked to the 1,3-β-glucan network through an unidentified linkage that is sensitive to mild alkali. These proteins are called ASL (alkali-sensitive linkage)–CWPs and include the family of Pir–CWPs (Pir, proteins with internal repeats; Figure 3) [35]. Whereas the GPI–CWPs are found in the outer layer of the wall, the Pir–CWPs seem to be uniformly distributed throughout the inner skeletal layer, which is consistent with their being directly connected to 1,3-β-glucan macromolecules [74]. In contrast to the other three Pir–CWPs, Cis1p has only a single repetitive sequence. Interestingly, Castillo *et al*. [24] have shown that this particular sequence is required for binding to 1,3-β-glucan. If these repeats, which all contain the

A. SP – functional region – spacer region – GPI anchor addition signal

B. Protein – C(=O) – **NH-CH_2-CH_2 – P_i – tetramannoside** → 1,6-β-glucan → 1,3-β-glucan

C. SP – pro-peptide – repeat region – conserved cysteine domain

Figure 3. Domain structure of covalently linked cell wall proteins in *S. cerevisiae*. (A) Predicted organization of GPI–CWPs, illustrating their modular nature. The functional region may be a catalytic domain or responsible for flocculation, sexual agglutination, or otherwise. (B) A mature GPI cell wall protein linked at its C-terminal end to 1,6-β-glucan through a lipidless remnant of the original GPI anchor (shown in bold), which consists of ethanolamine linked through a phosphodiester bridge to the third residue of a tetramannoside/pentamannoside [45,46,88,151]; residues 1 and 2 of the mannoside are also substituted with ethanolamine phosphate [8,55,65]; in turn, 1,6-β-glucan is glycosidically linked to 1,3-β-glucan or to chitin (not shown). Note that the functional domain of the protein extends into the environment. (C) Pir–CWPs. These represent an important group of the ASL–CWPs. The repeats all contain the signature sequence DGQJQ, in which J represents a hydrophobic amino acid. The pro-peptide terminates in a Kex2 cleavage site (–KR–). SP, N-terminal signal peptide

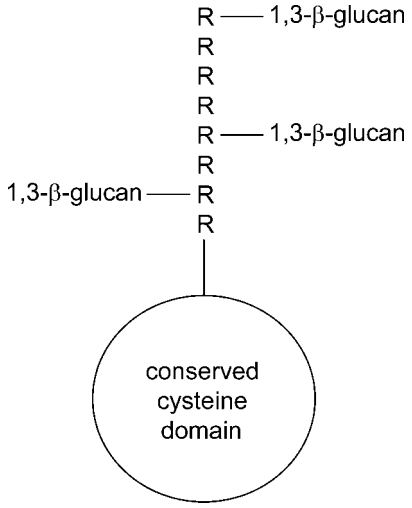

Figure 4. Do Pir–CWPs function as potential cross-linkers between 1,3-β-glucan chains? PIR, proteins with internal repeats. The internal repeats seem to be involved in interconnecting Pir–CWPs and 1,3-β-glucan [24], suggesting that Pir–CWPs contribute to the strength of the 1,3-β-glucan layer. Pir1p and Pir3p have each eight repeats containing the signature sequence DGQJQ; Hsp150p has 10 such repeats and Cis3p only one. Note that Pir1p, Hsp150p and Pir3p are primarily used during periods of isotropic growth and in response to cell wall damage. The function of the conserved cysteine domain is unknown. R, repeat

signature sequence DGQJQ (J is any hydrophobic amino acid), are indeed directly involved in binding 1,3-β-glucan, it seems conceivable that Pir–CWPs may interconnect two or even more 1,3-β-glucan molecules (Figure 4). This would considerably strengthen the wall. Interestingly, the Pir–CWPs Pir1p, Hsp150p/Pir2p, and Pir3p are highly expressed during the early G_1 phase of the cell cycle, when the cell grows isotropically and new cell wall components have to be inserted in and connected to the existing macromolecular network. It may therefore be advantageous to the cell to use Pir–CWPs during a period of isotropic growth. Consistent with their presumed 1,3-β-glucan cross-linking role in cell wall construction, the permeability of the wall is much lower during early G_1 phase than in any other phase of the cell cycle [38]. In addition, the same set of Pir–CWPs is strongly expressed in response to cell wall stress, consistent with a role for these Pir–CWPs in cell wall reinforcement [12,74], and their collective deletion results in hypersensitivity to cell wall-perturbing compounds such as Calcofluor white and Congo red [111]. *ScHSP150* has been successfully expressed in the mycelial fungus *Fusarium oxysporum*, which is a vascular wilt pathogen, resulting in efficient incorporation of Hsp150p into the cell wall. This is accompanied by higher resistance against a plant defence protein and by increased virulence of the fungus towards tobacco seedlings [112]. Possibly, the presumed 1,3-β-glucan cross-linking role of Pir–CWPs may limit the permeability of the wall and thus access of foreign enzymes. Interestingly, a subset of hybrid GPI–CWPs, viz. Cwp1p, Cwp2p, Tir1p, Tir2p, and the putative GPI–CWP Yhr126c, also contain the signature sequence DGQJQ found in Pir–CWP repeats, suggesting that these proteins might be linked through an alkali-sensitive linkage to the skeletal polysaccharides as well (Figure 2). This has been experimentally confirmed for Cwp1p [73].

Three of the four Pir–CWPs contain multiple internal repeats. Also some GPI–CWPs, such as the flocculins, Aga1p, Tir1p, Tir4p and Dan4p, contain multiple repeats [157]. Indeed, multiple repeats are predominantly found in cell surface proteins. In addition, the number of repeats often varies between strains and this is due to a comparatively high frequency of recombination events in the repeat domain. This variability affects cell surface properties, such as hydrophobicity and flocculation competence in baker's yeast and, conceivably, may contribute to virulence in pathogenic fungi as well [76,159].

The cell wall also contains proteins that are either retained in a noncovalent fashion, such as Bgl2p, or via disulphide bridges to other proteins, such as the agglutinin subunit Aga2p in *MAT***a** cells [19,80]. When intact cells are labelled with a membrane-impermeable biotinylation reagent that reacts with amino groups and subsequently extracted with the reducing agent dithiothreitol, a limited number of proteins are released, seven of which could be identified, viz. Bgl2p, Cis3p, Cts1p, Exg1p, Scw4p, Scw10p and Sun4p [21]. They are all secretory proteins with a predicted N-terminal signal peptide (SP/\/\/\; SP, signal peptide; /\/\/\ polypeptide chain) and are probably mannosylated because they bind to the lectin Concanavalin A, indicating that they follow the classical protein export pathway.

It has been claimed that many cytosolic proteins, such as glycolytic enzymes, may also be intrinsic components of the cell wall and that they arrive

at the cell surface through a non-classical protein export pathway [25,102]. However, when intact cells were treated with a membrane-impermeable biotinylation reagent to label amino groups, e.g. in lysine residues, and their cell walls were subsequently isolated and extracted with hot SDS under reducing conditions, only a limited number of proteins were identified, most of them corresponding to the set of biotinylated secretory proteins that could be directly extracted from intact cells (see previous paragraph) [21]. This excludes the presence of large numbers of cytosolic proteins at the cell surface and indicates that their presence in isolated walls may (often) be an artifact. For a further discussion of this topic, the reader is referred to a recent review [35].

Dynamics of the cell wall

It has been estimated that about 1200 genes show a cell wall-related phenotype when deleted, supporting the notion that cell wall construction is an integral part of cell physiology [36]. It is therefore not surprising that, as mentioned earlier, the cell wall may vary in composition and thickness, depending on the composition of the growth medium, growth temperature, external pH and oxygen levels [4]. This is sensitively reflected in the resistance of intact cells to cell wall-degrading enzymes [4]. Much less is known about possible corresponding changes on the molecular level in terms of the length and the degree of branching of the structural polysaccharides, and the degree of glycosylation and phosphorylation of the cell wall proteins. It is also not known whether the cell favours the use of specific CWP-polysaccharide complexes in specific phases of the cell cycle or in morphogenetic processes such as mating, pseudohyphal growth, biofilm formation, or when entering stationary phase or during sporulation. Interestingly, it has been shown that the usage of the CWP–$GPI_r \rightarrow$ 1, 6-β-glucan $\leftarrow$ chitin complex strongly increases in response to cell wall stress, raising the possibility that usage of specific CWP–polysaccharide complexes may take place in other situations as well [72,88]. It has further been shown that the walls of exponentially growing cells cultured in rich medium contain at least 20 different cell wall proteins [21,110,164]. As there are many more predicted cell wall proteins [23], this raises the question of whether the usage of specific cell wall proteins may depend on growth conditions. There is ample evidence for this, in particular from global transcript analyses [10,12,52,91,150,152], but also from studies focusing on particular genes [1,2] (see also the review by Smits *et al*. [142]). Here, we will discuss two striking examples, viz. exponentially growing cells vs. stationary phase cells, and aerobically vs. anaerobically grown cells. In the following two sections, we will discuss the changes occurring in the cell wall in response to cell wall stress and during the cell cycle.

The walls of cells that grow exponentially in rich medium differ strongly from post-diauxic shift cells that are on the verge of entering the stationary phase. Post-diauxic cells possess less permeable walls and are more resistant to glucanases [40]. Interestingly, the number of disulphide bridges in the walls of such cells increases about six-fold [40]. This is accompanied by a radical change in the protein profiles obtained by gel filtration of proteolytically released CWP fragments [140]. For example, Sed1p, which is a minor protein in exponentially growing cells, becomes the most abundant CWP in stationary phase cells. Global transcript analyses of cells in stationary phase are consistent with these results, showing that the *SED1* transcript level increases more than 10-fold [53]. As Sed1p has seven potential *N*-glycosylation sites and is heavily *N*-glycosylated, which limits cell wall permeability, and contains four cysteine residues, this may largely explain the observed phenotypes in stationary phase cells [40,140]. Indeed, deletion of *SED1* results in a strongly reduced resistance of intact cells to zymolyase [140]. Interestingly, *SED1* is also strongly upregulated during cell wall stress, heat stress, oxidative stress and hyperosmotic stress, suggesting that Sed1p has a general stress-protective function [12,53,57]. Other genes that are strongly upregulated in cells entering the stationary phase are the GPI–CWP-encoding gene *SPI1* and also *GSC2*, which codes for the alternative catalytic subunit of the 1,3-β-glucan synthase complex [53,121]. Sobering and co-workers [144] have identified a putative transcription factor, Rpi1p, that prepares the cells for entry into the stationary phase, resulting among other things in fortification of the cell wall. Consistent with this, overexpression of *RPI1* results in increased transcript levels of *SED1, GSC2* and *BGL2* (encoding a cell wall-associated transglucosidase).

Shimoi and co-workers later showed that the gel filtration profiles of proteolytically released CWP fragments of cells from shaken and non-shaken cultures also sharply differ and that Tir1p is strongly enriched in cell walls from non-shaken cells [78]. As stagnant cultures have to cope with low oxygen levels, it was proposed that these are responsible for the observed differences in the gel filtration profiles of CWPs. This work has been extended by several groups [1,2,26,150,152]. They have shown that under anaerobic conditions the transcript levels of the putative and confirmed GPI–CWP-encoding genes *DAN1, DAN4, MUC1/FLO11, TIR1, TIR2, TIR3* and *TIR4* are strongly upregulated. Importantly, when either *TIR1, TIR3* or *TIR4* is deleted, the cells are not able to grow under nitrogen, demonstrating that these proteins play a vital, but as yet not well understood, role under these growth conditions [1]. The appearance of a new group of GPI–CWP-encoding genes expressed under anaerobic conditions is accompanied by a strong reduction in the transcript levels of the GPI–CWP-encoding genes *CWP1* and *CWP2*, which are highly expressed under aerobic conditions [1]. Intriguingly, the transcript levels of the *PAU1, PAU3, PAU4, PAU5* and *PAU6* genes, which encode secretory proteins (SP/\/\/\) of unknown function and destination, are also upregulated under anaerobic conditions [122,150].

Reinforcement of the cell wall triggered by cell wall damage

Cell wall damage activates the so-called cell wall integrity (CWI) pathway (reviewed by Levin [93] and Heinisch [61]). This pathway consists of various plasma membrane proteins, such as Wsc1p and Mid2p, that act as sensors for the pathway during vegetative growth and pheromone-induced morphogenesis, respectively. Their signal results in activation of Rho1p and is then further transferred through the Pkc1p-controlled MAP kinase cascade to the transcription factors Rlm1p and SBF; the latter is a complex of Swi4p and Swi6p [69]. The majority of the Rlm1p-controlled genes are involved in cell wall biogenesis, such as: the glucan synthase-encoding genes *FKS1* and *GSC2; GFA1* and *CHS3*, which encode genes involved in chitin synthesis; the GPI–CWP-encoding genes *CCW14, CWP1, FIT2*, and *SED1*; the Pir–CWP-encoding genes *PIR1, HSP150, PIR3* and *CIS3*; the (trans)glycosidase-encoding genes *BGL2, CRH1*, and *DFG5*; the GPI–PMP-encoding genes *PST1* and *YPS3* (encoding an aspartyl protease); and also *SLT2/MPK1*, the gene that codes for the MAP kinase in the cell wall integrity pathway [68,90,93,153]. Activation of this pathway in response to cell wall stress results, among other things, in a massive deposition of chitin in the lateral walls of the mother cell and also of the growing bud. This is accompanied by increased formation of the CWP–polysaccharide complex GPI–CWP $\rightarrow$ 1, 6-β-glucan $\leftarrow$ chitin and increased resistance of intact cells to 1,3-β-glucanase, probably protecting the cells in their natural environment from cell wall-degrading enzymes [12,37,72,91]. As discussed above, the Pir–CWPs Pir1p, Hsp150p and Pir3p are also highly expressed during early G_1 phase and, conceivably, may interconnect two or more 1,3-β-glucan molecules. Their increased expression in response to cell wall damage would offer an ideal defence against 1,3-β-glucanases, which abound in plant tissues, both by limiting entry of foreign enzymes and by repairing the 1,3-β-glucan network. Interestingly, the hybrid GPI–CWP Cwp1p can be double-linked (complex D in Figure 1), i.e. directly linked to 1,3-β-glucan through an alkali-sensitive linkage and to 1,6-β-glucan $\rightarrow$ 1, 3-β-glucan through a GPI remnant [73], allowing it to interconnect two 1,3-β-glucan molecules as well. One may therefore speculate that the cell uses these particular CWPs to repair damage to the 1,3-β-glucan network. Finally, in the mycelial fungus *Aspergillus niger*, a homologue of Rlm1p has been described that is involved in the response to cell wall stress, indicating that at least in this fungus the function of Rlm1p has not diverged [31].

Until now, we have largely focused on individual genes. An alternative approach might be to monitor the expression behaviour of predefined gene groups [13], such as groups of genes with a common transcription factor-binding site in their promoter region (motif-based gene groups) or groups of genes that have been experimentally shown, using chromatin immune precipitation, to be bound by a specific transcription factor (transcription factor-based gene groups) [59]. A suitable method for this is T-profiler, a method that uses the *t*-test to score changes in the average expression levels of pre-defined gene groups [13].

The consensus binding site of Rlm1p has been well defined and is CTA(T/A)$_4$TAG [42,68]. When this motif is used in T-profiler analysis, the Rlm1 motif-based gene group is significantly activated: (a) immediately, but temporarily, after a shift of the cells to hypotonic conditions [53]; (b) in a constitutive fashion in mutants with a defective cell wall (*fks1, kni4, kre1, kre6, mnn9*) [62,91]; (c) in cells treated with cell-wall-perturbing compounds, such as Calcofluor white and Congo red, or with the cell-wall-degrading enzyme preparation zymolyase [12,37,52,91]; (d) as a delayed response in cells treated with α-pheromone and in cells treated with the plasma membrane-perturbing, polycationic compound chitosan [129,167]; and (e) as a delayed and transient response in heat-stressed cells [100]. Although the various conditions under which the CWI pathway becomes activated may vary widely, they all seem to lead to increased membrane tension. Transfer of cells to hypotonic conditions leads to rapid uptake of water, the pressure on the walls will rise and this will cause stretching of the walls and the plasma membrane. Similarly, cells with a weakened cell wall (either by genetic means or by growing the cells in the presence of compounds that cause cell wall weakening) tend to be swollen because their walls are less capable of withstanding turgor pressure [132]. This will result in constitutive stretching of the plasma membrane. In the presence of chitosan, the induction of the Rlm1-controlled gene group is delayed, indicating that this is an indirect response. Conceivably, perturbance of the plasma membrane affects the activity of plasma membrane-bound cell wall construction enzymes and thus indirectly weakens the cell wall. The transient activating effect of heat stress in the CWI pathway correlates with a transient accumulation of trehalose, which causes the osmolality of the cytosol to rise, resulting in water influx and increased pressure on the wall, and thus increased membrane tension. Furthermore, in a mutant that is unable to synthesize trehalose, activation of the CWI pathway at increased temperatures is strongly reduced. Consistent with this, increasing the osmotic strength of the medium also reduces the activation of the CWI pathway [100].

Interestingly, upregulation of the Rlm1p motif-based gene group is generally accompanied by upregulation of the Msn2p/Msn4p-based gene groups, which are involved in the general stress response, and the gene group controlled by Crz1p, a calcineurin-regulated transcription factor involved in calcium-mediated signalling (reviewed in Cyert [30]) [13,91]. Possibly, the increased plasma membrane tension resulting from cell wall weakening activates mechanosensitive channels in the membrane, resulting in a rapid increase in the cytosolic Ca^{2+} concentration [56].

Cell wall construction during the cell cycle

Cell wall construction is tightly coordinated with progress in the cell cycle. For example, chitin is laid down at three locations: (a) dispersed in the cell wall of the growing daughter cell after cytokinesis has taken place (G_1 cells); (b) as a ring at the presumptive bud site (late G_1 cells); and (c) in the primary septum (M/G_1 cells) [17]. Cells also switch between isotropic and apical growth during the cell cycle. Young daughter cells grow mainly isotropically, requiring the insertion of new cell wall macromolecules into the existing polymer network. During this phase of the cell cycle Pir–CWPs are highly expressed and, as discussed above, they seem to be particularly suited for coping with the difficulties related to isotropic growth. During this phase of the cell cycle, and not during any other phase of the cell cycle, chitin synthase III (CSIII)-produced chitin is laid down in the lateral walls as well. This is largely in the form of CWP–$GPI_r \rightarrow 1,6$-β-glucan $\leftarrow$ chitin complexes [17], presumably thereby stabilizing the wall of the future mother cell. After the cell has committed itself to cell division, a site is selected for the formation of a new bud. This site is delineated by a chitin ring, which is also formed by CSIII. This ring will determine the diameter of the neck between mother and bud. The chitin ring is anchored in the existing glucan network by attachment to 1,3-β-glucan chains [17]. The cell wall at the bud site is then weakened and a tiny bud emerges. Initially, the new bud grows apically. However, when the bud becomes larger, the contribution of isotropic growth increases and finally takes over from apical growth. Full independence of the bud from the mother cell begins with the formation of a primary septum, consisting of CHII-produced chitin growing out centripetally from the chitin ring. Interestingly, CHII-produced chitin is largely unbound to

other cell wall polymers, probably allowing crystallization, whereas the remainder is linked to 1,3-β-glucan to anchor it in place. The primary septum becomes covered on both sides with a secondary septum. Secretion of a chitinase by the daughter cell dissolves the primary septum and, in collaboration with other cell wall-degrading enzymes, allows the release of the daughter cell, leaving the mother cell with a prominent bud scar and the daughter cell with a much less conspicuous birth scar [16]. Cell separation may go amiss, probably explaining why the cell expresses, among other enzymes, a specific chitin synthase (*CHSI*) during this phase of the cell cycle. The daughter cell will grow further until, in turn, it has reached the critical size for the next round of cell division.

Amazingly, the cell does not only use specific chitin synthases during various phases of the cell cycle, but also seems to use specific cell wall proteins during the cell cycle. Microarray studies offer a fascinating glimpse of the interrelationships between cell wall synthesis and the cell cycle. In those cases in which the transcript data have been experimentally validated, they correspond well with the actual protein levels and the expected protein locations [7,130,131,143]. Using promoter swap experiments, Smits *et al*. [143] have shown that incorporation of the GPI–CWPs Cwp2p and Tip1p in the walls of medium-sized buds and mother cells, respectively, is indeed fully determined by the timing of transcription during the cell cycle. The GPI–CWP Cwp1p does not follow this rule. Although *CWP1* is transcribed at about the same time as *CWP2*, Cwp1p is incorporated into the cell wall at a much later stage and is found exclusively in the birth scar [22,143].

Our knowledge of the temporal and spatial control of cell wall construction during the cell cycle is, however, far from fully understood. Slt2p/Mpk1p phosphorylation is periodically stimulated during the cell cycle at the time when cells begin to bud, indicating a role for the CWI pathway in coordinating cell wall construction and the cell cycle [93,168]. Recently, a cell wall integrity checkpoint has been described, which operates when the size of the bud corresponds to approximately one-third the size of the mother cell [148]. As a result, cell wall defects, e.g. caused by temperature-sensitive alleles of *FKS1* and *DFG5*, cause the cells to arrest in the cell cycle before the separation of the spindle pole bodies and the formation of a spindle [77,148]. A similar cell cycle arrest (as cells with a small bud) has been observed in a *gas1/cwh52* and an *fks1/cwh53* mutant treated with the cell-wall-perturbing compound Calcofluor white, and in cells containing a conditional allele of *PKC1* [94,123]. Possibly, this checkpoint is also involved in the switch from apical to isotropic growth. Table 2 presents an overview of proteins involved in cell wall construction, for which the transcript levels of the corresponding genes have been shown to vary in a cell cycle-dependent fashion. It clearly illustrates the interdependency between cell wall construction and the cell cycle and indicates that cell wall-related genes are largely expressed in waves.

Enzymes involved in cell wall construction

The cell surface proteins that are actively involved in cell wall construction can be divided into synthases, remodelling enzymes, assembly enzymes and degrading enzymes, e.g. the ones that are active during isotropic growth and cytokinesis and during the mating process. For an extensive discussion of these enzymes, the reader is referred to recent reviews by Adams [3], Lesage and Bussey [92] and Yeong [163]. We shall briefly discuss each major cell wall component separately, with the main focus on remodelling and assembly enzymes (Table 3).

1,3-β-Glucan is synthesized as a linear polymer, which during or after extrusion through the plasma membrane is remodelled, resulting in branching and possibly further extension and coupling to existing 1,3-β-glucan molecules. The FKS gene family encodes the putative catalytic subunits (Fks1p, Gsc2p/Fks2p and Fks3p), using UDP-glucose as substrate. Recently, it has been demonstrated that the FKS protein of *Neurospora crassa* binds UDP-glucose, indicating that FKS proteins indeed represent the catalytic subunits of 1,3-β-glucan synthases [134]. How and when branchpoints in the molecule are introduced is still unknown. Nevertheless, this is a crucial step in cell wall construction, because otherwise 1,3-β-glucan molecules would fully crystallize, resulting in an abnormal wall. One of the better studied protein families involved in 1,3-β-glucan remodelling is the Gas family, consisting of five members (Gas1/2/3/4/5p) [108,109,118]. Gas1p, which is an abundant GPI-anchored plasma membrane

Table 2. Differential expression of cell wall-related genes during the cell cycle

t_{max} (min)	Gene	Protein function and other comments
0		Cells are released from cell cycle arrest at START in G_1
18	*CLN2*	Cyclin expressed late in G_1 that activates Cdc28p kinase to promote the G_1–S phase transition. Late G_1
28	*HTA1*	Core histone. S phase
	CRH1	GPI–CWP. Predicted transglycosidase, which at this phase of the cell cycle is located at the site of bud emergence and in the wall of tiny buds [130]. GH 16
	DFG5	GPI–PMP homologous to Dcw1p and presumably involved in processing plasma membrane-bound intermediates of GPI–CWPs [77,79]. GH 76
42	*CIS3*	Cis3p found in walls of small buds [107]. G_2 phase
	CWP1	GPI–CWP. Incorporation in the wall is delayed. It is targeted to the birth scar of the daughter cell [143]
	CWP2	Major GPI–CWP targeted to the walls of medium-sized buds [143]
	DCW1	GPI–PMP homologous to Dfg5p [77,79]. GH 76
	FKS1	1,3-β-glucan synthesis. Transcript levels peak at the same time as *GAS1* [63,123]
	GAS1	Abundant GPI–PMP with transglucosidase activity [118]. Some Gas1p is found in the wall [164]. GH 72
	GAS3	GPI–CWP. Putative transglucosidase. GH 72
	KRE6	Golgi membrane protein involved in the synthesis of 1,6-β-glucan. GH 16. Data from Igual *et al.* [63]
	MNN1	Golgi membrane protein. α-1,3-Mannosyltransferase that extends both *O*- and *N*-linked carbohydrate side-chains. Data from Igual *et al.* [63]
	VAN2	Golgi GDP-mannose transporter. Data from Igual *et al.* [63]
49	*CLB2*	B-type cyclin that activates Cdc28p to promote the transition from G_2 to M phase. It accumulates during G_2 and M phase. M phase
	GAS5	GPI–CWP. Putative transglucosidase. GH 72
56	*CHS2*	Formation of the primary septum. M/G_1
70	*CHS1*	Chitin synthesis during cytokinesis. Early G_1 phase
	DSE4/ENG1	Daughter cell-specific secreted protein with endo-1,3-β-glucanase activity that probably helps to degrade the cell wall surrounding the septum [7]. Deletant shows defective cell separation. GH 81
	EGT2	GPI–CWP found in the septal region [51]. Deletant suffers from defective cell separation
	HSP150	Abundant Pir–CWP and potential 1,3-β-glucan cross-linker
	PIR1	Abundant Pir–CWP and potential 1,3-β-glucan cross-linker; transcription requires Swi5p
	PIR3	Abundant Pir–CWP and potential 1,3-β-glucan cross-linker
	PST1	GPI–PMP homologous to Ecm33p
77	*CHS3*	Incorporation of chitin into the lateral walls during G_1
	CRH1	GPI–CWP. Predicted transglycosidase, which at this phase of the cell cycle is located in the neck of mother cells with medium-sized buds and in bud scars [130]. GPI–CWP. GH 16
	SCW11	Daughter cell-specific secretory protein homologous to Bg12p, Scw4p and Scw10p. Transcription requires Ace2p. GH 17
84	*CTS1*	Daughter cell-specific secreted protein that degrades primary septum; transcription requires Ace2p. GH 18
	DSE2	Daughter cell-specific secretory protein involved in cell separation transcription; transcription requires Ace2p.
	EXG1	Major exo-1,3-β-glucanase of the cell wall (SGD). GH 5
	TIP1	GPI–CWP in the wall of young daughter cells [143]

Most data are from Spellman *et al.* [145]. The cells were arrested in G_1 at START using α-pheromone and after full synchronization released from the arrest by transfer to fresh medium; the cells were cultured at 30 °C. For some genes data from other authors have been used and, when necessary, the data were normalized to the growth temperature used by Spellman *et al.* [145]. t_{max} corresponds to the time (min) at which the transcript levels reach their maximal value after release from cell cycle arrest. Note that the proteins presented here are exported through the secretory pathway with an estimated transit time of 5–10 min [113] and that the mature proteins therefore reach their maximal levels on average 5–10 min after t_{max}. *CLN2, HTA1* and *CLB2* have been included as cell cycle markers. GH numbers, glycosyl hydrolase family number; the classification into families is according to Coutinho and Henrissat [28]. GPI–CWP, GPI-modified cell wall protein. GPI–PMP, GPI-modified plasma membrane protein.

protein — although some Gas1p has also been found in the wall [164] — has transglucosidase activity. It can cleave internal 1,3-linkages in 1,3-β-glucan and transfer the newly generated reducing end to a non-reducing end of another 1,3-β-glucan molecule [108]. This would make it an attractive candidate for inserting new 1,3-β-glucan polymers into the existing 1,3-β-glucan network. Deletion of *GAS1* results in swollen cells with a severely weakened wall that are

Table 3. Postulated cell wall assembly enzymes

1,3-β-glucan—chitin assemblase
1,3-β-glucan—1,6-β-glucan assemblase
1,3-β-glucan—ASL-CWP assemblase
1,6-β-glucan—chitin assemblase
1,6-β-glucan—GPI–CWP assemblase

hypersensitive to the chitin synthesis inhibitor nikkomycin and other cell wall perturbants, and in constitutive activation of the CWI pathway [12,72,91,117,124]. Ecm33p is another abundant GPI-anchored plasma membrane protein. Similarly to Gas1p, some of it is also found in SDS-extracted cell walls [164]. The cell wall-related phenotypes of *ecm33*Δ cells are comparable to those of *gas1*Δ cells: the cells are swollen and spherical, they are hypersensitive to Calcofluor white, and the CWI pathway is constitutively activated — indicating that Ecm33p, and presumably also the related protein Pst1p, are directly involved in cell wall construction [36]. In contrast to *gas1*Δ cells, in which an external mannoprotein layer is still clearly observable, the mannoprotein layer of *ecm33*Δ cells is strongly reduced, suggesting that either the attachment of GPI–CWPs to 1,6-β-glucan or the attachment of 1,6-β-glucan to 1,3-β-glucan is affected [116,117].

Chitin is a linear polymer that may become attached through its reducing end to 1,3-β-glucan and 1,6-β-glucan. The three synthases CSI, CSII and CSIII, including their respective catalytic subunits Chs1p, Chs2p and Chs3p, are located in the plasma membrane and extrude the growing chain through the membrane. Chitin synthesis and its role in various key events of the cell cycle and during cell wall stress have been studied extensively (see also below). The assembly enzymes involved in coupling chitin to 1,3-β-glucan and 1,6-β-glucan are not known, but evidence is emerging that the Crh family (Crh1p, Crr1p and Utr2p) may be involved. The evidence for a role of the GPI protein Crh1p in interconnecting chitin to β-glucan is as follows:

1. Crh1p shows similarity to 1,3-/1,4-β-glucanases [28].
2. Mutagenesis of amino acids that are predicted to have a catalytic function results in loss of function of the protein [131].
3. Loss of function results in a decrease of alkali-insoluble glucan in the wall [130]. As the attachment of chitin to 1,3-β-glucan results in 1,3-β-glucan becoming less soluble in alkali [60,101], this observation is consistent with a role for Crh1p in attaching chitin to 1,3-β-glucan.
4. The temporal expression of *CRH1* during the cell cycle, which is characterized by two maxima, coincides with the temporary location of Crh1p at the presumptive bud site, when a chitin ring is synthesized and in a later phase of the cell cycle at the bud neck, when the primary septum is being synthesized and in the bud scar of the mother cell (Figure 5) [131,145].
5. When cells are confronted with cell wall stress, the chitin content of the walls is enhanced and also the CWP–GPI_r $\rightarrow$ 1, 6-β-glucan $\leftarrow$ chitin complex seems to be strongly increased [72]. This is accompanied by strong upregulation of *CRH1* [12,52,91]. It seems likely that Crr1p and Utr2p may have a similar function.

1,6-β-Glucan is a highly branched polymer. Synthesis seems to take place at the plasma membrane, but several ER and Golgi proteins are also in some unknown way involved in 1,6-β-glucan synthesis [104,137]. It is not known whether this polymer is synthesized as a linear molecule, and subsequently becomes branched, or is formed by repetitively attaching a preformed branched oligosaccharide to a growing chain. Recently, a method for *in vitro* synthesis of 1,6-β-glucan has been developed, which will help to identify the genes directly

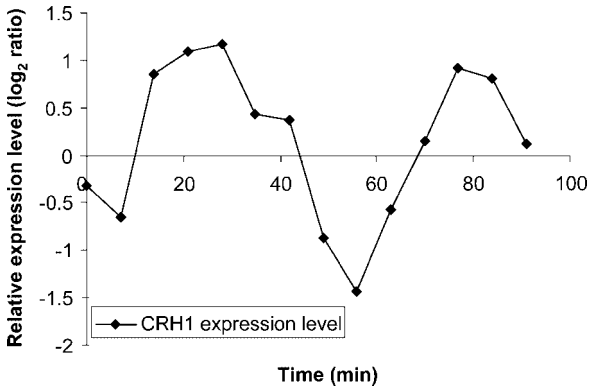

Figure 5. Transcript levels of *CRH1* show two optima during the cell cycle. The data are from Spellman *et al.* [145]. The expression ratios are from a pheromone-synchronized culture compared to an asynchronous culture of exponentially growing cells

involved in its synthesis [158]. The Kre9 family consists of two secretory proteins (SP/\/\/\), Kre9p and Knh1p, with a partially redundant function [14,41]. Deletion of both genes is lethal, indicating that they have an essential function in cell wall construction. Interestingly, the 1,6-β-glucan formed in the single *kre9*Δ mutant is considerably smaller than its wt equivalent and has a different structure [14]. This might mean that Kre9p and Knh1p might be directly involved in 1,6-β-glucan formation but, as both are secreted proteins, it seems unlikely that they are synthases requiring nucleotide sugars as substrate. Alternatively, they might be involved in coupling 1,6-β-glucan to 1,3-β-glucan.

GPI-proteins destined for the cell wall arrive at the cell surface with their GPI anchor intact, whereas mature GPI–CWPs possess a truncated, lipidless GPI anchor that has been cleaved between the first mannose residue and glucosamine [50,88]. Which enzymes are involved in releasing membrane-bound intermediates of GPI–CWPs from the plasma membrane is still uncertain, but the two-member protein family, consisting of the GPI–PMPs Dfg5p and Dcw1p, are likely candidates: the double deletant is non-viable, even in an osmotically stabilized growth medium, and, importantly, they show homology to bacterial endomannanases [77,79]. As homologues of these proteins are widespread in ascomycetous fungi, including mycelial fungi such as *Aspergillus fumigatus* (SGD), they represent an attractive target for antifungal compounds.

How ASL–CWPs are incorporated into the cell wall is at present unknown. At acidic pHs, Pir–CWPs and also Cwp1p are more efficiently incorporated into the cell wall, indicating that the enzyme involved in forming the alkali-sensitive linkage functions optimally at low pHs [73].

Although cell wall assembly enzymes (assemblases) have been, until now, only poorly characterized, it is possible to speculate about potential assemblase inhibitors. In principle, 1,3-β-glucan and 1,6-β-glucan oligosaccharides should inhibit several cell wall assemblases. Indeed, it has been shown that 1,6-β-glucan oligosaccharides affect the incorporation of CWPs and sensitize the cells to an antimicrobial peptide [11]. However, relatively high concentrations are needed to see an effect, presumably because of partial degradation of the added sugars. This argues for the use of non-degradable sugars and sugar mimics, such as iminosugars [54]. Aniline blue, a sulphonated aromatic dye, is known to bind to single helixes of 1,3-β-glucan [165]. Conceivably, it could act as an inhibitor of the cell wall assemblases, in which 1,3-β-glucan chains are involved. Similarly, two other sulphonated aromatic dyes, Calcofluor white and Congo red, which both preferentially bind to chitin in *S. cerevisiae* [64,119], might affect assemblases, which use chitin as a co-substrate.

Cell wall construction in other ascomycetous fungi

Does the molecular model of the cell wall of *S. cerevisiae* have predictive value for other ascomycetous fungi, including mycelial species? An important point to remember is that the cell wall in bakers' yeast does not contain α-glucan and that its chitin content is relatively low compared to that of many other species. On the other hand, this does not exclude the possibility that architectural principles identified in *S. cerevisiae* may also be used by fungi, which do contain α-glucan and high chitin levels in their walls. An interesting example is the dimorphic pathogenic fungus *Candida albicans*, which can grow in the yeast form and in the hyphal form and in the latter case even possesses an authentic Spitzenkörper [29]. All available evidence points to a similar molecular organization of the cell wall of both yeast and hyphal form of *C. albicans* compared to *S. cerevisiae* [70,82,96,128]. This seems also to be the case for *C. glabrata* [49,159]. In another dimorphic pathogenic fungus, *Exophiala dermatitidis*, cell wall proteins have been identified that are covalently linked to 1,6-β-glucan [103]; in addition, the walls of cryofixed cells of this fungus show a fibrillar outer layer extending into the medium [9]. Both observations are consistent with a cell wall organization similar to that found in *S. cerevisiae*. In the dimorphic yeast *Yarrowia lipolytica*, both a Pir–CWP and a GPI–CWP have been identified [66,67]. The wall of the dimorphic pathogen *Sporothrix schenkii* consists of an external fibrillar layer, which contains peptidorhamnomannan, and an internal skeletal layer. The internal layer contains 1,3-β-glucan,

1,6-β-glucan and, interestingly, also some 1,4-β-glucan; in addition, chitin is present [155]. Collectively, these data suggest that a cell wall protein–polysaccharide may be present in the cell wall of *S. schenckii* similar to that found in *C. albicans* and *S. cerevisiae*. Interestingly, in some filamentous fungi, such as *Fusarium oxysporum* and *Aspergillus niger*, cell wall proteins covalently linked to 1,6-β-glucan have also been identified [15,135]. Furthermore, HF-pyridine, which preferentially cleaves phosphodiester bridges, releases at least seven protein bands from isolated SDS-extracted walls of *A. niger*, suggesting that they are GPI–CWPs [32]. Indeed, the encoding gene of one of them has been cloned and the predicted amino acid sequence has all the hallmarks of a GPI–CWP. On the other hand, the alkali-insoluble fraction of the cell wall of *A. fumigatus* does not contain 1,6-β-glucan, in contrast to a similar fraction of *S. cerevisiae* walls, suggesting that nature might have more surprises in store [47]. For a more extensive discussion of cell wall organization in ascomycetous fungi, the reader is also referred to De Groot *et al*. [35].

Another approach to answering the question of how far cell wall construction in *S. cerevisiae* is representative of that in other fungi, is to look for homologues of known cell wall construction enzymes in the genomes of other fungi. For example, *FKS* and *CHS* synthase-encoding genes are widespread among ascomycetous and basidiomycetous fungi, as are homologues of *ScCRH1*, the gene that encodes the potential assembly enzyme between 1,3-β-glucan and chitin, (**http://www.yeastgenome.org/**, **SGD**). Homologues of the *ScGAS* and the *ScECM33* genes, and of *ScDFG5* and *ScDCW1*, are generally found in ascomycetous species [34,99,146], including mycelial species such as *A. fumigatus* (SGD). In addition, homologues of the Pir–CWP-encoding genes in *S. cerevisiae* are widely used by ascomycetous yeasts and may also be functional in mycelial ascomycetes [35]. On the other hand, homologues of *ScKRE9* are widespread among ascomycetous yeasts [96], but to a much lesser extent among mycelial species. Summarizing, the cell wall organization of *S. cerevisiae* has strong predictive power for ascomycetous yeasts and, to a lesser but still considerable extent, for ascomycetous mycelial species. In basidiomycetous fungi, however, the similarities are less, but certainly not negligible, and may depend on the taxonomic group to which they belong.

Concluding remarks

The cell wall biology of *S. cerevisiae* is a rapidly expanding field with a great potential for understanding cellular processes related to morphogenesis, for elucidating the regulatory networks that help to coordinate cellular processes with cell cycle progression, and for practical applications, such as the development of new antifungals [16,162]. Genetic engineering of cell wall surface properties by the introduction of new proteins is also attracting active interest [89]. Importantly, the findings offer valuable predictions for how the cell walls of other fungi are constructed, how their construction might be regulated and how their cell surface properties might be re-engineered [35].

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