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# Integrative studies put cell wall synthesis on the yeast functional map

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The fungal cell wall field, traditionally focused on polysaccharide composition and synthesis, retains a certain static architectural imagery of structural rigidity and integrity, with the wall offering protection from a harsh environment. This picture of the wall is increasingly changing to that of a bustling construction site, as research uncovers the organizational complexity of its assembly. With recent molecular and genomic studies on *Saccharomyces cerevisiae*, cell wall synthesis and biology appear increasingly to be dynamic and adaptable processes that are fully integrated with the underlying cytoskeletal and polarity machinery that drive cell cycle progression.

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## Abbreviations

**Chs3** chitin synthase III  
**MAPK** mitogen-associated protein kinase

## Introduction

The cell wall is an organelle common to yeasts and filamentous fungi localized at the interface between the microorganism and the environment. The fungal cell wall has been studied extensively in *Saccharomyces cerevisiae* [1], and in the human fungal pathogens *Candida albicans* [2] and *Aspergillus fumigatus* [3]. Cell-wall synthesis has long attracted attention as an antifungal drug target because of its fungal specificity and its essential function [4]. Indeed, echinocandins, the first novel human antifungal drug class approved in this generation, target the synthesis of the cell-wall polysaccharide  $\beta$ -1,3-glucan [4,5].

From the early *S. cerevisiae* genome project, fungal research has moved towards a genomic era in an attempt to go beyond reductionism, to a more ‘systems biology’

level of understanding [6,7]. As highlighted in a recent editorial in this journal, ‘in recent years it has become increasingly clear how many cellular processes that traditionally have been studied in isolation are tied together in large cellular regulatory networks that exhibit enormous signal-integrating power’ [8]. Here, we aim to show how this is increasingly the case for fungal cell-wall synthesis. During cell life, the cell wall is continuously subject to change, and so has to continuously balance its plasticity and tensile strength. To allow the cell to grow or to bud, the cell wall must increase its plasticity in coordination with the cell cycle program. Conversely, under stress conditions, a cell-wall remodeling response to increase strength must take place rapidly to ensure cell integrity.

In this review, we highlight recent findings on the spatial and temporal regulation of the cell-wall synthesis machinery and its integration into a more global network of cellular function.

## The fungal cell wall: an overview of constituents

Fungal cell wall research has focused on a molecular description of cell-wall macromolecules and their interactions, with reviews offering comprehensive descriptions of fungal cell-wall composition and organization [1–3]. In *S. cerevisiae*, the cell wall consists of a matrix of  $\beta$ -1,3-glucan,  $\beta$ -1,6-glucan, chitin and cell-wall proteins, surrounding the plasma membrane.  $\beta$ -1,3-glucan is the major constituent of the cell-wall inner layer, forming a microfibrillar backbone to which other components are cross-linked. The plasma membrane  $\beta$ -1,3-glucan synthase complex, although still not fully characterized, is known to consist of a putative catalytic subunit Fks1 (or the related Fks2) and a regulatory subunit Rho1 [1].  $\beta$ -1,6-glucan is a flexible minor component of the yeast cell-wall involved in linking cell-wall proteins to the  $\beta$ -1,3-glucan network, but understanding of  $\beta$ -1,6-glucan biosynthesis remains limited [9]. The cell-wall outer layer is involved in many interactions with the environment and is formed by cell-wall proteins, which are often highly mannosylated and often in families. The remaining cell-wall component is chitin, a polymer required during bud-site selection and septation. Normally, chitin is not abundant, but under stress conditions its level can be massively elevated [10].

## Cell wall synthesis: an integrated process Genome-wide approaches

Genome-scale studies in *S. cerevisiae* have provided information on genes that are involved in cell-wall synthesis;

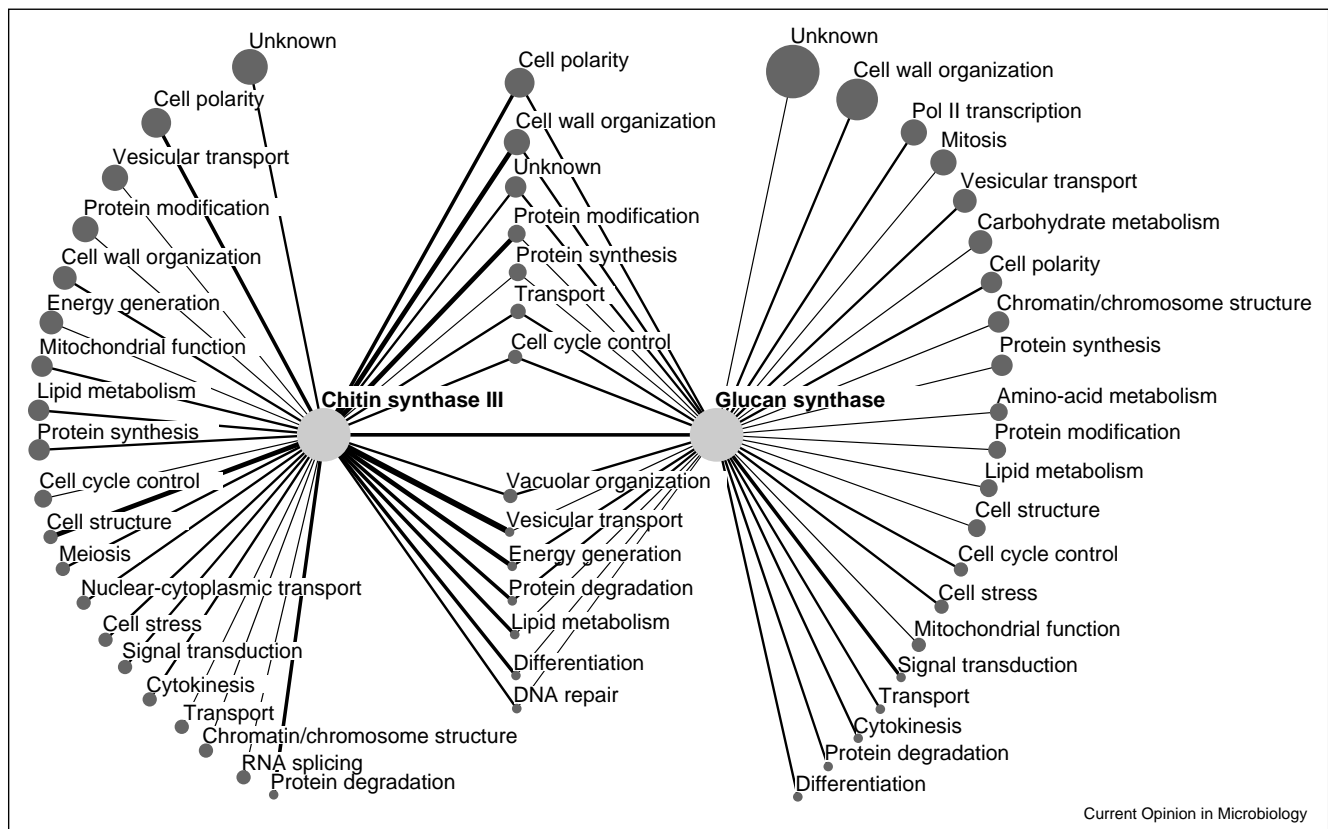
on their expression [11], their genetic interactions [12] and on the localization [13], abundance [14] and physical interactions (reviewed in [15]) of their products. More directly focused on cell-wall synthesis, a pioneering hierarchical screen identified 145 genes with cell wall phenotypes among 620 deletion mutants [16]. Screening of the genome-wide yeast mutant collection [17,18] has been performed for size homeostasis [19<sup>•</sup>,20<sup>•</sup>,21<sup>•</sup>], for budding pattern alteration [22], and for sensitivity to both caspofungin (an inhibitor of  $\beta$ -1,3-glucan synthase) [23<sup>••</sup>] and the K1 killer toxin (a lethal protein that binds to  $\beta$ -1,6-glucan) [24]. A complementary approach, focused on cell-wall synthesis, identified a genetic interaction network that is involved in  $\beta$ -1,3-glucan synthesis and regulation [23<sup>••</sup>].

These pathway-perturbing genomic and chemo-genomic studies help to unravel cell-wall synthesis and reveal its connections (Figure 1). A common theme is the multi-

licity of mechanisms that link cell growth and division, and in particular, linking cell-wall synthesis to the cell cycle. For example, transcription of the glucan synthase encoding gene *FKS1*, the chitin synthase encoding genes *CHS1* and *CHS2*, and the majority of cell-wall protein-encoding genes are regulated in coordination with the cell cycle [25,26]. These genomic studies reveal some broad-connecting trends between cell-wall synthesis, protein mannosylation, lipid and membrane biosynthesis, secretory vesicle trafficking and cell-surface signaling pathways (Figures 1 and 2). Functional links identified by genetic interactions underscore the importance of cell polarity, secretory pathways and endocytic processes for the synthesis of  $\beta$ 1,3-glucan [23<sup>••</sup>].

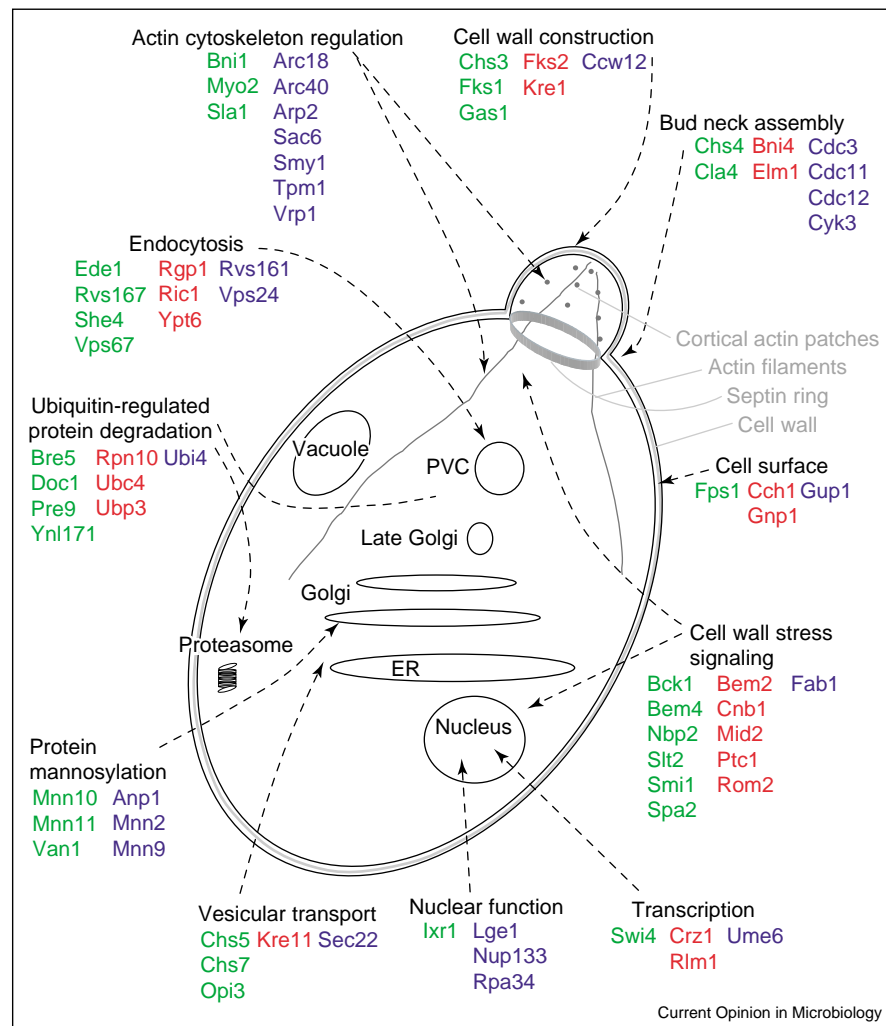
Large-scale studies also provide an increasingly comprehensive picture for a given phenotype and can suggest roles for genes of unknown function in cell-wall synthesis. This is exemplified by the identification of 268 genes

Figure 1



Integration of the chitin synthase III and glucan synthase genetic interaction networks. This graph illustrates the wide variety of processes (functional nodes are represented by dark grey circles) that buffer defects in chitin synthase III or glucan synthase pathways (light grey nodes). The connections between nodes represent synthetic lethal or synthetic sick interactions [12], with genes required for the function of the chitin synthase III (*BNI4*, *CHS3*, *CHS4*, *CHS5*, *CHS6* or *CHS7*, grouped in the chitin synthase III node) or with genes required for  $\beta$ -1,3-glucan assembly (*FKS1*, *FKS2*, *GAS1* or *SMI1*, grouped in the glucan synthase node). Genes with the same Gene Ontology annotation are grouped within functional nodes, whose circle size reflects the number of genes in the node. The line thickness is weighted according to the average number of interactions linking a functional node to the glucan synthase or the chitin synthase network. For clarity, functional nodes containing a single gene and connected by a unique interaction are not shown.

Figure 2



Overview of the interaction network in the cell context. The main genes found in the chitin synthase III network, the glucan network and in both networks (see Figure 1) are shown in blue, red and green, respectively. ER, endoplasmic reticulum; PVC, prevacuolar compartment. Figure modified from [22].

showing a K1 killer toxin phenotype, with only 15 of these previously known, and with 42 of these genes in uncharacterized open reading frames [24]. The unique set of genetic interactions for each gene also reveals specific functions and requirements; for example, the glucan synthesis regulator *SMI1* has connections with polarity establishment and chromosome segregation [23\*\*].

### The integrating role of Rho1

$\beta$ -1,3-glucan synthesis is dependent on the coupling of Fks1 with the regulatory subunit Rho1, a small GTPase that exists in an inactive GDP-bound form and an active GTP-bound form [27]. In addition to its function as a regulatory subunit of Fks1, Rho1 is a multi-effector protein, linking  $\beta$ -1,3-glucan synthesis to other cellular processes. Studies of *RHO1* alleles with specific func-

tional defects has allowed the characterization of Rho1 effectors [28–30]. One example of Rho1 functional plurality is given by its interaction with the formin Bni1, which contributes to the regulation of cell polarization through the formin-dependent assembly of actin cables [30,31\*\*,32–34]. Rho1 interaction with Bni1 is necessary for the maintenance of cell polarity, a process that is itself necessary for cell-cycle progression [30]. Another effector of Rho1 is Sec3, a member of the exocyst complex that defines the sites of active exocytosis [35\*\*]. Sec3 localization is Rho1-dependent, but actin-independent, allowing spatial control of vesicular secretion to sites of active cell-wall growth.

How is the regulatory subunit of  $\beta$ -1,3-glucan synthesis Rho1 regulated? A key activator of Rho1 is the GDP/GTP

exchange factor Rom2. In intracellular secretory vesicles, Rho1 is kept in its inactive GDP-bound form, owing to a lack of Rom2, which is independently transported to the plasma membrane, resulting in a restriction of glucan synthesis to the plasma membrane [36\*\*]. The activation of  $\beta$ -1,3-glucan synthase has been analyzed by genetic approaches that reveal many upstream regulators of Rho1 (*BAG7*, *BEM2*, *LRE1*, *MID2*, *MTL1*, *ROM2*, *SAC7*, *WSC1*, *WSC3* and *ZDS1*) that control distinct Rho1 effectors [37\*\*,38\*]. Thus, Rho1 functions by integrating multiple input signals and regulating its effectors in a manner that is dependent on its localization, its level of activation and effector availability.

### Spatial and temporal regulation

#### Spatial regulation of $\beta$ -1,3-glucan synthesis

The spatial distribution of the cell-wall synthesis machinery, including Fks1, is tightly regulated in coordination with the cell cycle. The generation of a new cell begins with the selection of a new bud site, the assembly of a septin ring and cell polarization along the mother-bud axis during the G1 phase of the cell cycle [39]. The initial localization of the cell wall machinery has the characteristics of an amplification loop, involving Rho1. By its participation in the formin-dependent polarization of the cell and in the activation of the exocyst complex, the recruitment and activation of Rho1 at the site of bud emergence allows the further recruitment of new Rho1 molecules and the necessary constituents for cell-wall synthesis. During apical growth, Fks1 is concentrated at the bud site and remains in the bud tip when the daughter cell has emerged [40\*\*]. Following this, Fks1 is isotropically dispersed over the daughter cell in coordination with the G2 phase of the cell cycle, and is re-concentrated at the neck between the mother and the daughter cells in coordination with mitosis [40\*\*]. After completion of mitosis, cytokinesis and septation can occur. These processes are characterized by a balance of cell wall degradation and synthesis to retain wall integrity and efficient separation of the two cells.

A key observation from the Ohya group [40\*\*] is that Fks1 localization correlates with cortical actin patch distribution, and in *las17* and *arc18* mutants that are impaired in actin patch movement, Fks1 movement is also impaired. This immobilization of Fks1 leads to cells that are prone to lysis as a result of an irregular cell wall, which is thick at the sites of Fks1 immobilization and thin elsewhere [40\*\*]. Actin patches are involved in endocytosis, although their exact functions remain unclear [41,42]. From this work, it seems that Fks1 internalization and recycling to the plasma membrane would allow proper distribution and movement of glucan synthase to sites of active growth without cell-wall synthesis down-regulation. However, although mutants in endocytic pathways show cell wall defects, they do not display the same cell wall defect phenotypes that are seen in mutants with

actin patch movement impairment [40\*\*] and as such, the link between cell wall synthesis and endocytosis needs more investigation [43].

### Reactivity to stress conditions

A remarkable feature of the cell wall is its adaptability in response to environmental stresses and to genetic perturbations. Cell integrity is ensured by variation in the relative proportion and the degree of cross-linking of wall polymers. The syntheses of the different cell-wall constituents are closely coupled and the reduction or absence of any single component can be buffered by increased synthesis of other polymers. The classical example is the increased amount of chitin in mutants with defects in  $\beta$ -1,3-glucan synthesis, such as *fks1*  $\Delta$ , with levels making up to 20% of the lateral cell wall by comparison to 1–2% in a wild-type cell [1]. A central stress regulatory system is the cell integrity pathway, involving membrane sensors (mainly the cell Wall integrity and Stress response Component (WSC) family and Mid2), mediation by Rho1 and activation of a kinase cascade through the protein kinase C, Pkc1, and the mitogen-activated protein kinase (MAPK) pathway Bck1–Mkk1/Mkk2–Slr2 (reviewed in [44]).

The first relatively rapid response is ensured by delocalization and hyper-activation of Rho1 and Pkc1, enabling the repair of local cell wall damage by direct activation of  $\beta$ -1,3-glucan and chitin synthases [45,46,47\*\*]. Activation and recruitment of Chs3, the principal chitin synthase, involves a process of regulated secretion [47\*\*]. In contrast to most cell wall genes, *CHS3* expression is not cell-cycle regulated, with Chs3 activation occurring through post-translational modifications and through regulation of its spatial distribution. A pool of Chs3 is stored in specialized intracellular vesicles, called chitosomes, that allow this synthase to be directly mobilized to the plasma membrane at sites of cell-wall damage by activated Rho1 and Pkc1 [47\*\*]. One hypothesis is that Pkc1-dependent phosphorylation of Chs3 regulates the transport of Chs3 to transient cell-wall damage sites, allowing a rapid cellular response that bypasses the need for MAPK activation [47\*\*].

### Adaptation to stress conditions

Cell wall integrity has been investigated by transcriptional profiling of cells subjected to environmental stresses that induce reversible cell-wall damage [48,49,50,51\*\*] and in mutants that are altered in cell-wall synthesis [51\*\*,52\*\*]. Adaptation to persistent cell-wall damage caused by chemical agents, drugs or genetic perturbations is mediated through the sequential activation of the cell-integrity MAPK cascade, resulting in an activation of transcriptional factors that trigger the expression of a large common gene set. The main transcriptional regulator of this cell integrity pathway appears to be Rlm1, which controls transcription of many of the genes

involved in cell wall remodeling. However, other signaling pathways and transcriptional activators also participate, including the *SWI4-SWI6* dependent cell cycle box Binding Factor (SBF) complex that acts at the G1-S transition, the calcineurin-dependent transcription factor Crz1 that regulates expression of *FKS2* (the alternative catalytic subunit of  $\beta$ -1,3-glucan synthase) and the *HOG1*-MAPK pathway [51<sup>••</sup>,52<sup>••</sup>]. In addition, activation of the cell-integrity MAPK pathway is mediated not only by Rho1 and Pkc1 but also appears to be dependent on input signals acting on intermediate components of the pathway, a phenomenon that itself depends upon the type of cell wall stress [53<sup>••</sup>].

## Conclusions

Following the successful introduction of the echinocandins, our growing understanding of fungal cell-wall synthesis can be applied to the development of much needed novel and specific antifungal drugs. As an essential part of the fungal cell-cycle program, cell-wall synthesis must be tightly regulated in time and space. Large-scale studies are poised to give a comprehensive picture of yeast biology and more traditional approaches are complementary in describing specific interactions. The integrating role of Rho1 is central to cell-wall synthesis, due to its direct action on  $\beta$ -1,3-glucan synthesis and its multiple interactions. Through the contribution of Rho1 to the larger regulatory network of small G-proteins, such as Cdc42, involved in cell polarity establishment [31<sup>••</sup>,32,54], we can look forward to a wider integration of cell-wall biosynthesis pathways with the yeast functional map.

## Update

The cell wall is even more dynamic and connected than expected. Pelling *et al.* [55] observed the pulsing nano-mechanical activity of the cell wall, using the cantilever of an atomic force microscope and the Ohya group [56] propose a novel checkpoint in the cell-cycle program, coupling cell-wall synthesis and mitosis mediated by the dyactin complex.

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