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MicroReview

The cell wall: a carbohydrate armour for the fungal cell

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Summary

The cell wall is composed of a polysaccharide-based three-dimensional network. Considered for a long time as an inert exoskeleton, the cell wall is now seen as a dynamic structure that is continuously changing as a result of the modification of culture conditions and environmental stresses. Although the cell wall composition varies among fungal species, chemogenomic comparative analysis have led to a better understanding of the genes and mechanisms involved in the construction of the common central core composed of branched β1,3 glucan-chitin. Because of its essential biological role, unique biochemistry and structural organization and the absence in mammalian cells of most of its constitutive components, the cell wall is an attractive target for the development of new antifungal agents. Genomic as well as drug studies have shown that the death of the fungus can result from inhibition of cell wall polysaccharide synthases. To date, only β1,3 glucan synthase inhibitors have been launched clinically and many more targets remain to be explored.

Introduction

The cell wall of fungi provides both protective and aggressive functions. It is protective, as it acts as an initial barrier that is in contact with hostile environments encountered by the fungus. If removed or weakened, the fungi die unless they are osmotically protected. It also provides an aggressive function, as it harbours many hydrolytic and toxic molecules, most of them being in transit in the cell wall and required for a fungus to invade its ecological niche. Furthermore, its rigid structure is useful as a force for the penetration of insoluble substrates that it colonizes or invades.

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In spite of its essential role, the cell wall of most fungi remains insufficiently studied and its biosynthesis incompletely understood, especially among filamentous fungi (Latgé and Calderone, 2005; Lesage and Bussey, 2006). Further difficulty in the analysis of the cell wall comes from the accumulation of data showing that the cell wall can no longer be considered as an inert exoskeleton, as its structure continuously changes over time. The role of the environment and signal transduction cascades regulating cell wall synthesis will not be discussed here; instead, the focus of this review will be the downstream events and enzymes responsible for the establishment of the specific three-dimensional (3D) polysaccharide network of the cell wall. Rather than summarizing all published literature on the cell wall, the aim of this review is to present new ideas and hypotheses on cell wall polysaccharide biosynthesis and remodelling that emerged from a comparative analysis of the cell wall of yeast and moulds, especially *Aspergillus fumigatus*.

Structure of the cell wall polysaccharides

Polysaccharides account for > 90% of the cell wall. The cell wall is an insoluble structure that must be solubilized to be precisely analysed. Hot alkali is the reference chemical treatment used to solubilize the cell wall polysaccharides that can be then analysed using recombinant glycosylhydrolases and liquid chromatography, nuclear magnetic resonance and mass spectrometry methodologies. For almost all fungi, the central core of the cell wall is a branched β1,3, 1,6 glucan that is linked to chitin via a β1,4 linkage. Interchain, β1,6 glucosidic linkages account for 3% and 4% of the total glucan linkages, respectively, in *Saccharomyces cerevisiae* and *A. fumigatus* (Fleet, 1991; Kollar *et al*., 1995; Fontaine *et al*., 2000; Perez and Ribas, 2004), the only two fungi whose cell wall structure has been investigated in detail. This structural core, which is differently decorated depending on the fungal species (Fig. 1), is generally thought to be fibrillar and embedded in an amorphous cement (usually removed by alkali treatment) (Fig. 2).

Our understanding of the overall organization of all these different polysaccharides in the cell wall remains quite vague, especially for the alkali-soluble fraction. For example, it is unknown to date how mannan is integrated

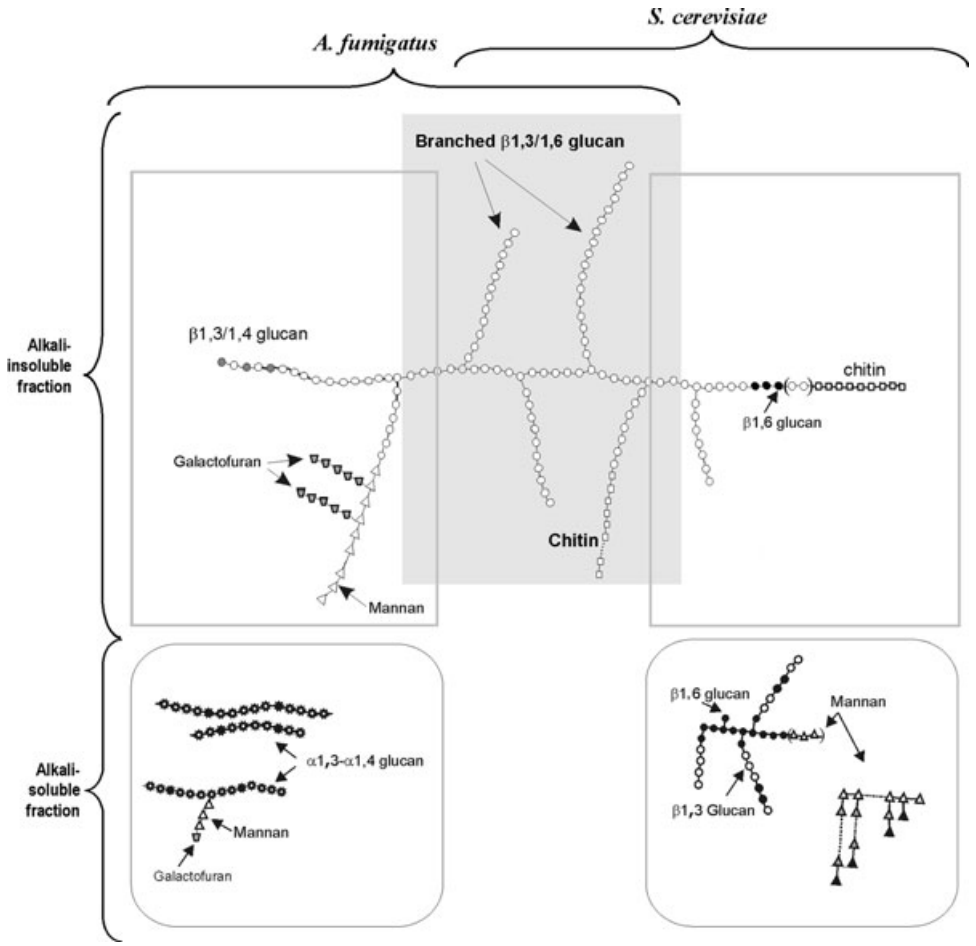

Fig. 1. Polysaccharides of the cell wall of the mould *Aspergillus fumigatus* and the yeast *Saccharomyces cerevisiae*. Components of the alkali-insoluble and alkali-soluble fractions are presented. α1,3 (☼), α1,4 glucan (✱); β1,3 (○) 1,4 (●) 1,6 (●) glucan; chitin (□); α1,2 (▲), 1,3 (▲),1,6 (△) mannan; β1,5 galactofuran (▼). The core that is common to the vast majority of fungi is in grey.

into the fungal cell wall. In yeast, simple calculations suggested that mannans cannot remain exclusively associated to proteins bound covalently to the cell wall. If we imagine that an yeast cell wall glycoprotein has four chains of mannan with 150 mannose residue per chain, mannan would account for 100 kDa per glycoprotein; if the average polypeptide moiety of the yeast proteins is 40 kDa, the cell wall should then contain > 10% polypeptides as mannans account for 40% of the yeast cell wall carbohydrates. The amount of protein detected is actually much lower and never exceed 2–3% of the cell wall (Klis *et al*., 2002; de Groot *et al*., 2007). Moreover, analysis of the sequences of many of the covalently bound cell wall proteins (such as the *TIR*, *TIP* or *DAN* families) shows that they do not have potential N-glycosylation sites. In addition, mannans are removed by boiling entire cells at neutral pH which suggests they are not covalently bound to other cell wall polysaccharides. In moulds, mannan structure is completely different: in *A. fumigatus*, for example, the mannan chains are shorter and seems to be bound covalently to the glucans without intermediary peptide moiety. A similar mystery holds true for α1,3 glucan, the major mould alkali-soluble polysaccharides.

Decorating molecules and even polysaccharides belonging to the structural core have constantly evolved since the appearance of fungi almost a billion years ago. There is some correlation between the appearance or the loss of a polysaccharide over time and fungal taxonomy, but this correlation is not absolute (Latgé and Calderone, 2005). Differences also have been noticed among fungal morphotypes in the same species, in agreement with a tight regulation of cell wall synthesis throughout the cell cycle. Many studies using fluorescent markers or radiolabelled precursors (Wessels, 1986; Humbel *et al*., 2001; Cortes *et al*., 2007) suggest that septa and apices have different structures to the lateral, older cell wall regions (Fig. 3). These differences have never been fully characterized chemically as septa and apices represent a small proportion of the total cell wall (e.g. in *A. fumigatus*, septa account for around 2% of the lateral cell wall) and cannot

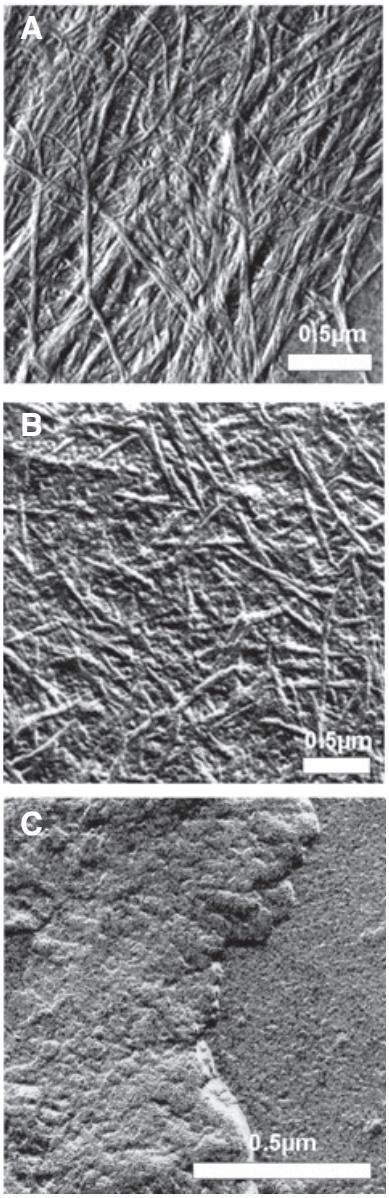

Fig. 2. Cell wall polysaccharides have different structures. See as an example carbon-platinum replicas of the amorphous and fibrillar materials recovered from the cell wall of the zygomycete *Conidiobolus obscurus*: (A) insoluble material recovered after cold 1N NaOH treatment; (B) insoluble material recovered after successive hot treatments in 1N NaOH and 0.5N CH3COOH; (C) alkali-soluble material recovered after cold 1N NaOH treatment.

be isolated separately from the mycelial bulk. To take into account this cellular heterogeneity, chemical data should be complemented by immunocytochemical analysis that will require the development of a complete library of monoclonal antibodies specific of the different fungal glycosidic linkages encountered among cell wall polysaccharides. With such library, the dynamics and sequential events in the construction of the fungal cell wall will be dissected cytochemically, as it is done in plant science (Persson *et al*., 2007). Moreover, the localization of the polysaccharides on the cell wall has some direct implication for the fungal cell life (Netea *et al*., 2006).

Polysaccharide biosynthesis

Synthesis of the skeleton chitin and β1,3 glucan polysaccharides of the cell wall

The chitin synthases that are responsible for the synthesis of linear chains of β1,4 *N*-acetylglucosamine from the substrate UDP-*N*-acetylglucosamine are a family of integral membrane proteins with molecular weight of 100–130 kDa (Roncero, 2002). Chitin biosynthesis is understood best in the model yeast *S. cerevisiae*. Three chitin synthases (*CHS1–3*) are responsible for the synthesis of the *S. cerevisiae* chitin (Cabib *et al*., 2001). The non-zymogenic Chs3p is responsible for the synthesis of the bulk chitin of the cell wall and for the increase in chitin synthesis as a response to cell wall stress. Chs2p is responsible for synthesis of septal chitin. Chs2p acts in the formation of the primary septum. Its function depends directly on the formation of the acto-myosin ring (Schmidt

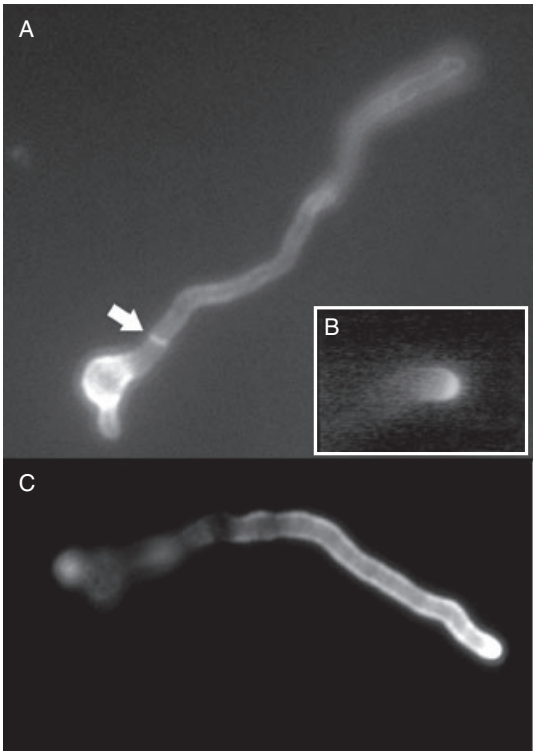

Fig. 3. Heterogeneity in the cellular localization of the different cell wall polysaccharides. Germ tubes of *Aspergillus fumigatus* stained with WGA-FITC for chitin (A) [note that the septum (arrow) and the conidium are intensively labelled whereas the germ tube is only poorly labelled.], with aniline blue for β1,3 glucans (B) [only accessible at the apex of germ tubes] and with an anti-galactofuran monoclonal antibody (C) binding to the entire germ tube surface but not to the conidium.

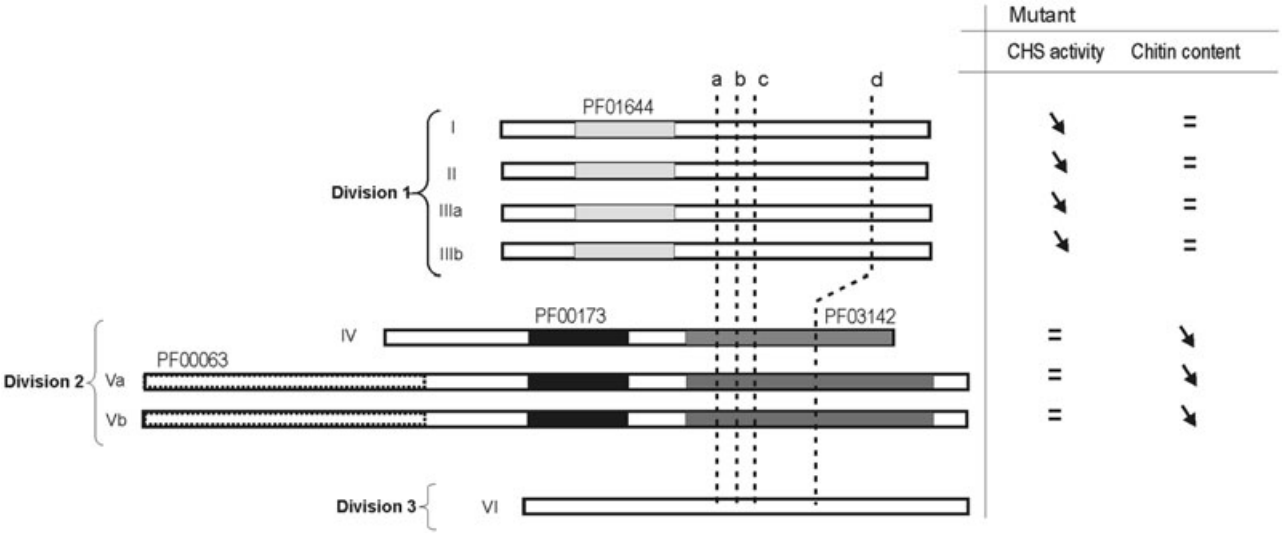

Fig. 4. Structure of the different chitin synthases of moulds and the contribution of the different members of each division to chitin synthase activity or chitin content of the cell wall deduced from the analysis of individual mutants (based on data from Mellado *et al*., 2003; Choquer *et al*., 2004; and the CADRE website). =: no difference with the wild type; and ↘: reduction of activity or chitin content of the cell wall of the mutant compared with wild type. The role of division 3 Chsp is unclear.

et al., 2002). Chs1p acts as a repair enzyme during cell separation. Chs1p and Chs2p activities *in vitro* were originally described as zymogenic as they require proteolytic activation, but direct evidence for this type of regulation *in vivo* is lacking. Among the CHSs, only the regulation of *CHS3 has* been investigated. The enzyme is transported in an inactive form to the plasma membrane, where it is activated by Chs4p (Trilla *et al*., 1997). The complex containing Chs3p/Chs4p is positioned at the myosin ring of the septum site through its interaction with the Bni4p/septin complex (Sanz *et al*., 2004). In *Aspergillus*, which lacks *BNI4* orthologues (Roncero, 2002), localization of CHS at the septum might be regulated differently. At least one gene in the CHS families of *Aspergillus* and other moulds has a consensus domain that is homologous to kinesin or myosin motor-like domains (Takeshita *et al*., 2005). This myosin motor-like domain of class V localizes near actin structures at the hyphal tips and septation sites. It binds to actin and this binding is necessary for chitin synthase activity.

The number of chitin synthase genes varies according to the fungal species, from one gene in the ancestral fungus *Encephalitozon cuniculi* to > 20 genes in *Rhizopus oryzae*. BLAST analysis of amino acid sequences identifies six families of fungal chitin synthases. Three are specific for filamentous fungi (class III, V and VI) (Choquer *et al*., 2004). A QRRRW motif present in the cytosolic nucleotide sugar transferase domain of all CHS genes belong to the catalytic domain because mutations affecting this domain result in a loss of chitin synthase activity (Cos *et al*., 1998). These six families can be grouped into three major divisions (Fig. 4). Division 1, containing families I, II and III, has a Pfam domain 01644 at the N-terminal region of the catalytic domain. Division 2 (families IV and V, including Chs3p of *S. cerevisiae*) has the same catalytic domain preceded by a cytochrome *b*5-like domain (Pfam 00173, which has replaced Pfam 01644) and a myosin head-like domain (Pfam 00063) in moulds. A pfam domain 03142 is conserved at the C-terminus of all Chsps of division 2. Division 3 enzymes that contains only the family VI (i.e. *CHS D* of *A. fumigatus*) have the conserved catalytic sequences but do not display any of the characteristic Pfams encountered in the other Chsps (Choquer *et al*., 2004; http://www.cadre-genomes.org.uk/Aspergillus_fumigatus/). Interestingly, despite the fact that fungi are ancestors of animals, insect and nematode CHSs have homologous sequences that are quite different from those of fungi (*E*-value of $2e^{-14}$ between the *Drosophila* gene 72965213 and its closest fungal orthologue from *E. cuniculi*, ECU 01g1390). Interestingly, the insect and nematode CHSs, like the unique CHS of the ancestral fungus *E. cuniculi*, belong to division 2, suggesting that this division might be closest to the ancestral chitin synthase.

Although relatedness and divergent domain structures could be correlated with function, expression and mutagenesis studies in fungi have not been very informative in this area. It remains to be seen whether there is a relationship between a specific fungal CHS and the structure of the chitin polymer at a cellular location as well as their association with chitin deacetylases that modify chitin in the less rigid deacetylated chitosan (Baker *et al*., 2007). The significance of each of these six *CHS* classes is indeed not understood, as mutations affecting members of a common family do not always

result in a similar phenotype. Two groups of mutants can be identified, however: the first group (from division 2) has reduced chitin content but normal chitin synthase activity *in vitro*. In contrast, mutants from the other divisions are affected in enzyme activity but have a normal cell wall chitin content (Fig. 4). The requirement for specific chitin synthase genes also varies. In *S. cerevisiae*, none of the *CHS* genes is essential, although the triple mutant is very sick, and its survival results from the acquisition of suppressors (Schmidt, 2004). In contrast, *Candida albicans* has four *CHS* genes, of which the family II gene *CHS1* is essential for cell viability (Munro *et al*., 2001). None of the *CHS* genes of *A. fumigatus* is essential (Mellado *et al*., 2003; C. Jimenez and C. Roncero, pers. comm.). These data suggest that the different fungal *CHS* perform distinct and specific functions in every fungus, even though they have homologous sequences. Overall regulation of chitin synthesis in fungi seems indeed quite complex as it is under the co-ordinated control of the PKC, HOG and Calcineurin pathways (Munro *et al*., 2007). A similar situation exits in insects, where functional analysis of the CHS genes revealed unique and complementary roles for each CHS. For example, one of the Chsp in *Tribolium* is specialized in the synthesis of the epidermal cuticle, whereas the other one is responsible for the midgut peritrophic matrix (Arakane *et al*., 2005). The amount of chitin produced per species is not directly correlated to the number of genes: although chitin is the most abundant component of insect cuticle, only one to two chitin synthases are encoded by these species (Merzendorfer, 2006).

β1,3 glucans are also synthesized by a plasma membrane-bound protein complex that uses UDP-glucose on its intracellular side as a substrate and extrudes linear β1,3 glucan chains through the membrane into the cell wall space (Douglas, 2001). This protein complex contains both catalytic and regulatory subunits. The putative catalytic subunit (Fksp) is encoded by large proteins (> 200 kDa) with as many as 16 transmembrane helices. A central hydrophilic domain of about 580 amino acids displays a remarkable degree of identity (> 80%) among all known FKS protein sequenced (Douglas, 2001). This region might be located on the cytoplasmic face of the plasma membrane and essential for function of the glucan synthase (Douglas, 2001). However, neither of two proposed consensus UDP-glucose binding sites (R/K)XGG or D,D,D35QXXRW is found in any of the Fks1p orthologues of the CAZy database (http://www.cazy.org/). In contrast, the product of the *CrdS* gene of *Agrobacterium* sp. ATCC 31749 that uses nucleotide sugars to produce also a linear β1,3 glucan contains a UDP glucose consensus binding site but the bacterial gene is not homologous to any eukaryotic *FKS* (Karnezis *et al*., 2003). The regulatory subunit of the β1,3 glucan synthase is the GTPase Rho1p that switches between a GDP-bound inactive state and a GTP-bound active state via conformational changes (Mazur and Baginsky, 1996; Qadota *et al*., 1996). After synthesis in the ER, Rho1p is geranylgeranylated, a modification required for Rho1p attachment to the membrane and transport. Geranylgeranylated Rho1p and Fks1p are transported to the plasma membrane as an inactive complex through the classical secretory pathway. Rho1p is activated on its arrival at the plasma membrane by Rom2p, the GDP/GTP exchange factor of Rho1p that is only localized at the plasma membrane. This activation and the movement of Fks1p on the plasma membrane are required for proper cell wall β1,3 glucan localization (Inoue *et al*., 1999; Abe *et al*., 2003). The role of Rho1-GTP in the regulation of β1,3 glucan synthesis has been shown in many fungi such as *Schizosaccharomyces pombe* and *Aspergillus* species (Lesage and Bussey, 2006). The role of GEFs might be interchangeable, however, as, in contrast to yeast, the unique orthologue of *ROM2* is not essential in *A. fumigatus* (Hu *et al*., 2007).

Like chitin synthases, the number of genes encoding β1,3 glucan synthases and the essentiality of each individual gene vary with the fungal species. Three *FKS* genes have been identified in *S. cerevisiae*, but none is essential, although FKS1 and FKS2 are synthetically lethal (Ishihara *et al*., 2007). In *S. pombe*, four genes *BGS1–4* sharing homology with β1,3 glucan synthase catalytic subunits have been identified, of which three (encoding Bgs1, 3 and 4p) are essential (Martin *et al*., 2003; Cortes *et al*., 2005; 2007). In contrast to yeasts, all filamentous ascomycetes sequenced to date have one *FKS* orthologue that is essential (Mouyna *et al*., 2004). β1,3 glucan synthase genes have also been found in plants, where they are responsible for callose synthesis. Although β1,3 glucan is a lot less abundant in plants than in fungi, the number of β1,3 glucan synthase genes is much higher in plants than in fungi. In the plants *Arabidopsis thaliana* or *Oryza sativa*, 12–18 callose synthase genes have been identified whereas the highest number of genes in fungi never exceeds 4. Plant callose is involved in several steps in pollen development (primary and secondary cell wall of the pollen, germination pore and tube, pollen tube plug) as well as wound repair after injury or disease (Enns *et al*., 2005). All steps in which callose participates should involve different callose synthase genes, which would explain the existence of multiple callose genes in plants. Despite the fact that plant and fungi belong to two different kingdoms, plant and fungal glucan synthases display high sequence similarities (*E*-value of $6e^{-80}$ between the *A. thaliana* gene At1g05570 and the *S. pombe* SPAC 19B12.03). However,

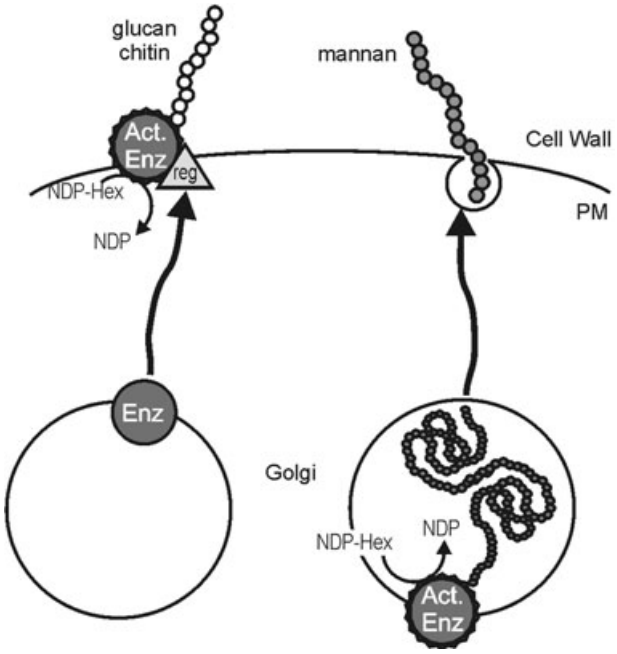

Fig. 5. Two different strategies used by fungi to bring polysaccharides to the cell wall. (i) Catalytic subunits of synthases are stored and transported (inactive) from the Golgi vesicles to the plasma membrane. They become activated *in situ* at the plasma membrane by regulatory subunits and use nucleotide sugars (NDP) as substrates. Examples are β1,3 glucan and chitin synthases. (ii) Polysaccharides are synthesized in the Golgi from NDP sugars and are brought to the plasma membrane as a complete polysaccharide that is directly secreted into the cell wall. An example is the mannosyltransferases.

PSI-BLAST comparisons align fungal and plant genes into two different clusters.

In spite of the lack of structural data for transmembrane glucan and chitin synthases, the biosynthesis of the two major fibrillar polysaccharides of the cell wall is basically understood. β1,3 glucan and chitin synthases are transported in an inactive form to the plasma membrane, where they are arranged as complexes and become activated after contact with resident activators (Fig. 5). This is a perfect cellular localization for these enzymes that use soluble intracellular nucleotide sugars and extrude-insoluble products into the cell wall space.

Synthesis of alkali-soluble polysaccharides

In contrast to chitin and β1,3 glucan, our knowledge of the synthesis of the other polysaccharides linked to or embedded in the chitin-glucan fibrillar core remains extremely limited. For genes that have been identified through genetic screens, the substrate of the enzymes and/or the biochemical activity of the encoded protein(s) are totally unknown. This is the case for the *KRE* and *AGS* genes involved in the synthesis of β1,6 and α1,3 glucan synthesis respectively (Beauvais *et al*., 2005; Lesage and Bussey, 2006). For other enzymes, even though the nucleotide sugar substrate is known, the synthase activity and acceptor have not been shown *in vitro*. This is the case for UDP-galactofuranose, the substrate for the synthesis of galactofuran in *A. fumigatus*, as mutations in the UDP-galactose mutase gene lead to the production of a mutant devoid of galactofuran (C. Lamarre and J.P. Latgé, unpublished data).

The subcellular site where these amorphous polysaccharide are synthesized is also a matter of debate. Localization of synthases at the plasma membrane should favour the extrusion of polymers like α1,3 glucans, with a degree of polymerization > 200. In contrast, the yeast mannoproteins or peptidomannan with *N*-mannan chains of around 150 mannose residues are synthesized in the Golgi compartment and then brought to the cell wall (Fig. 5). Although the organization of the mannans in the mould and yeast cell walls is different (namely long alkali-soluble chains in yeast versus short chains bound directly to glucan) in filamentous fungi, comparative genomic analysis suggests, however, that synthesis of mannan in moulds is via mannosyltransferases orthologous to the yeast genes such as *OCH1* or the mannosyltransferase complex *MNN9/VAN1/ANP1* (Nierman *et al*., 2005). The subcellular site of cell wall mannan synthesis in moulds and its further transfer to the glucan chain remains unknown.

Anchoring and cross-linking of cell wall polysaccharides

Two of the major questions in cell wall studies that remain unresolved are the holy grail of all ‘cellwallogists’. First, how are polysaccharides anchored to the plasma membrane? In *Mycobacterium*, the cell wall is attached to fatty acylated phosphatidylinositolmannans that are inserted in the plasma membrane (Chatterjee, 1997). Do glycolipids play a similar role in fungi, as lipo(galacto)mannans anchored to the plasma membrane through phosphatidyl anchors have been identified recently in yeasts and moulds (Trinel *et al*., 2002; Costachel *et al*., 2005)? Second, how are the neo-synthesized polysaccharides branched and cross-linked (Fig. 6)? A major breakthrough in the analysis of cell wall that might answer this question is the finding that a major glucanosyltransferase (Gelp) that elongates β1,3 glucan chains is anchored to the plasma membrane by a glycosylphosphatidyl inositol (GPI) moiety. Anchoring of proteins by a GPI anchor would allow the protein to face the cell wall space, a perfect cellular location to fulfil the glycosyltransferase function of these proteins (Fig. 7). Another argument reinforcing the putative involvement of this enzyme activity in the establishment of a branched β1,3 glucan comes from the fact that no orthologue of Gelp has been found in the plant kingdom where callose, the plant β1,3 glucan, is a linear polysaccharide

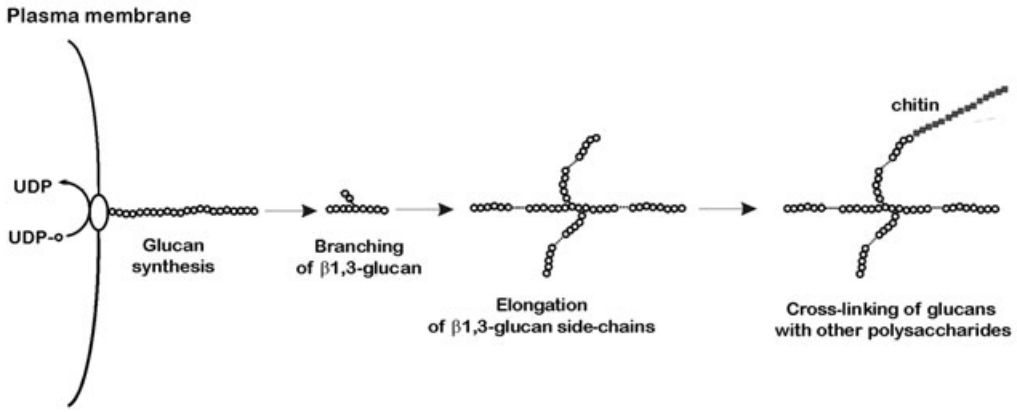

Fig. 6. Hypothetical successive steps that lead to the production of the alkali-insoluble core of the fungal cell wall.

without side-chains. As the composition of the alkali-insoluble core of the cell wall of most ascomycetes sequenced to date is similar, transferases playing an essential role in branching and cross-linking of glucan with chitin should be common to all these species. Comparative genomic and proteomic analyses have indeed shown that six families of membrane-bound GPI proteins are present in all ascomycetes sequenced to date: *SPS2*, *GAS/GEL*, *DFG*, *PLB*, *CRH*, *YPS* (Bernard *et al*., 2002). Four of the GPI-bound proteins (*SPS2*, *GAS/GEL*, *DFG*, *CRH*) are involved in cell wall construction as *SPS2 (ECM 33)*, *DFG1/DCW5*, *CRH1/CRH2* and *GEL/GAS* mutants have defects in cell wall associated to reduced growth phenotype (Mouyna *et al*., 2000; Rodriguez-Pena *et al*., 2000; Kitagaki *et al*., 2002; Tougan *et al*., 2002; Cabib *et al*., 2007). However, the *in vitro* glycosyltransferase activity of all of them except Gelp/Gasp remains to be determined.

A new vision for cell wall proteins

Most cell wall proteins are water- or detergent-soluble while in transit to the external milieu. A few of the cell wall proteins are covalently linked to polysaccharides. Two groups of proteins covalently bound to the cell wall have been identified in yeast. The first group of proteins (Ccwps) are removed by a mild alkali treatment (30 mM NaOH overnight at 4°C) and are attached to β1,3 glucans through a glutamine residue following a transglutaminase reaction (Ecker *et al*., 2006). The second family of proteins extracted by β1,3 glucanases or β1,6 glucanases is initially anchored to the plasma membrane by a GPI anchor. These GPI-CWPs are later transmannosylated by hydrolysing the oligomannosyl of the GPI anchor, which is then transferred to the glucan of the β1,3/β1,6 glucan core of the yeast cell wall through an unknown transglycosylation mechanism (Kapteyn *et al*., 1996; Kollar *et al*., 1997). The chemical proof of a covalent linkage between a GPI protein and the polysaccharide through the GPI remnant has been, however, only obtained for the GPI-anchored protein Tip1p (Fuji *et al*., 1999).

Studies in *S. cerevisiae*, mainly by the group of Klis and collaborators, suggest that the GPI proteins covalently linked to the polysaccharides play an essential structural role in cell wall organization. However, there are several arguments against an essential role of these proteins in the construction of the cell wall. (i) Proteins putatively covalently bound to the cell wall are only found in minute amounts (see above). (ii) Disruption of genes encoding the major GPI proteins isolated from the cell wall did not affect growth (van der Vaart *et al*., 1995). (iii) If these GPI-CWPs were essential in establishing the cell wall structure in yeast, they should also be found in other species. A careful comparative chemogenomic analysis of various yeast and moulds shows that this is not to be the case: orthologues of yeast GPI-CWPs have not been found in other ascomycetes such as *S. pombe* or

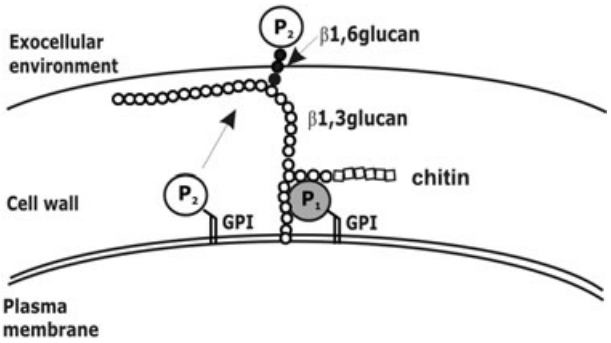

Fig. 7. Putative functions of GPI-anchored proteins during the construction of the fungal cell wall. (i) GPI-anchored proteins (P1) that remain attached at the plasma membrane remodel cell wall polysaccharides (examples are Gel/Gas or Crh proteins). (ii) GPI-anchored proteins (P2) become covalently bound to β1,3 glucans through β1,6 glucans. This is a way for protein P2 to remain at the cell surface to fulfil its biological function, for example, in cell-to-cell interactions. See Fig. 1 for the legend of polysaccharide linkages.

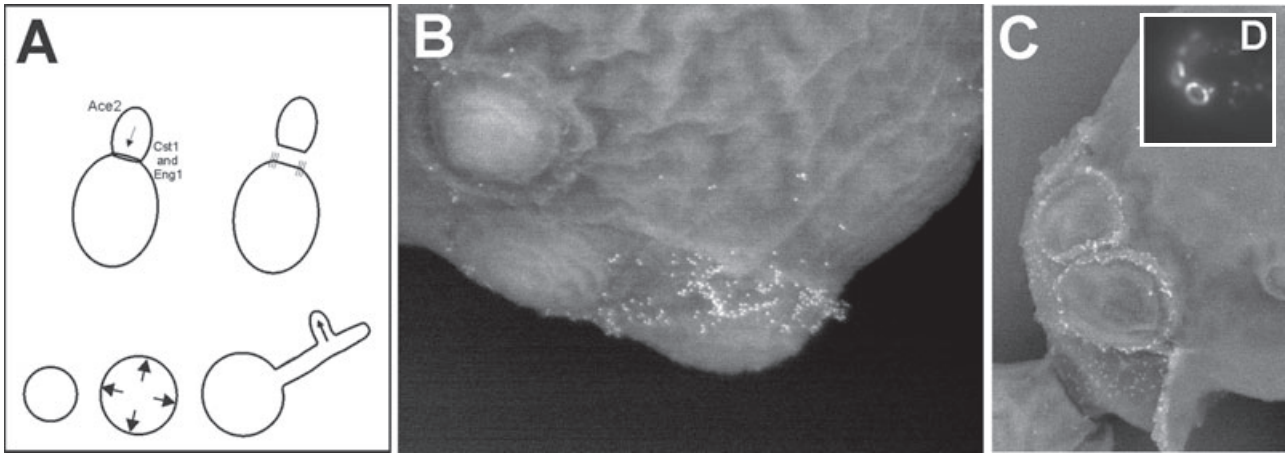

Fig. 8. Putative role of glycosylhydrolases during fungal morphogenesis.
A. In yeasts, the chitinase Cts1p and the endo β1,3 glucanase Eng1p, under the regulation of Ace2p in the daughter cell, lyse the septum and induce the separation of the mother and daughter cells. In moulds (lower part), glycosylhydrolases only plasticize the cell wall during conidial swelling, hyphal branching or hyphal anastomosis.
B–D. In addition to chitin, β1,3 glucans compose the bud scar ring of *Saccharomyces cerevisiae*. Labelling of β1,3 glucans was obtained with a Dectin1-Fc chimeric protein (provided by G. Brown) and an anti-human IgG coupled to 10 mm colloidal gold particles (B and C) or FITC (D). Note the inner layer of the yeast cell wall is labelled in (C).

A. fumigatus. (iv) Even after extensive boiling in a SDS-mercaptoethanol buffer, further incubation of the treated cell wall of *A. fumigatus* in the same buffer still releases soluble antigens after a few hours, indicating that soluble proteins can be tightly bound to cell wall through non-covalent linkages (Bernard *et al*., 2002). (v) Strikingly, some of the proteins with glycosyltransferase activity such as the Gasp, Ecm33p or Crhp are now claimed to be covalently linked to their product, the cell wall polysaccharides (Yin *et al*., 2005)! (vi) Soluble proteins such as the acid phosphatase PhoAp in *A. fumigatus* can be released by β1,3 glucanase treatment that loosens the polysaccharide net to which this protein is associated; these data show that the postulated 'release by β1,3 glucanase equals covalent linkage to the cell wall polysaccharide' is obviously not always true (Bernard *et al*., 2002). These comparative analyses suggest that proteins do not play any role in the establishment of the 3D polysaccharide cell wall network, even in yeast.

The majority of the polysaccharide-covalently bound proteins in yeast, such as Flop, Figp or Agap in *S. cerevisiae* or Alsp and Epap in *Candida*, are involved in cell–cell interactions such as flocculation, biofilm formation, mating, or adhesion to host cell surfaces (Verstrepen *et al*., 2005). Our current hypothesis on the role of covalently bound proteins is illustrated in Fig. 7. The covalent binding of proteins to polysaccharides is a way for the protein to remain at the surface of the cell wall, where it has to bind directly to its ligand to fulfil its biological function. Interestingly, many of the genes encoding these adhesins in yeast contained many intragenic tandem repeats with large (< 40 nt) repeats. Variation in intragenic repeat numbers results in gradual and reversible functional changes. This variability at the cell surface might permit evasion of the host immune response (Verstrepen *et al*., 2005) in a way similar to antigenic variation in parasites.

Lysis of structural polysaccharides is needed for fungal growth

Glycosylhydrolases are essential for the separation of mother and daughter yeast cells (Fig. 8). The hydrolases identified reflect the chemical nature of the septum. In *S. cerevisiae*, a chitinase (Cts1p) and an endo β1,3 glucanase (Eng1p) present in the daughter cells are required for cell separation due to the presence of chitin in the primary septum and of β-glucan in the secondary septum (Kuranda and Robbins, 1991; Baladron *et al*., 2002). Separation in yeast is under the control of Ace2p, a transcription factor present in the daughter cells (King and Butler, 1998). Other glucanase-associated enzymes such as Scw11p, Dse1p and Egt2p are involved in cell separation but their role is unknown. In *S. pombe*, separation of the two yeast daughter cells requires the endo β1,3 glucanase Eng1p, which degrades the primary septum that is composed of β1,3 glucan and Agn1p, an endo α1,3 glucanase that degrades the septum edging composed of α1,3 glucan (Martin-Cuadrado *et al*., 2003; Alonso-Nunez *et al*., 2005). The secondary septum, mainly composed of linear or branched β1,6 glucans, seems to remain untouched. In filamentous fungi, cells do not separate during colony extension and orthologues of *ACE2* are absent in moulds. However, there are two stages, conidial swelling and hyphal branching, that require cell wall softening. In *A. fumigatus*, endo α and

β1,3 glucanase and chitinase activities are present in resting conidia (J.-P. Debeaupuis and J.-P. Latgé, unpublished). They could be responsible for cell wall hydrolysis once water has penetrated into the conidium. The comparison of these three different fungal situations suggests that correct localization of the glycosylhydrolases is essential to fulfil their biological role. This has been shown in yeast, where removal of the 'carbohydrate-binding module' in the Cts1p of *S. cerevisiae* or Eng1p of *S. pombe* abolishes their function in cell separation (A.B. Martin-Cuadrado *et al.*, unpublished data).

Cell wall polysaccharides are essential drug targets

Three families of natural β1,3 glucan synthase inhibitors have been identified: (i) the glycolipid papulacandins, which consist of a modified disaccharide linked to two fatty-acyl chains, (ii) acidic terpenoids and (iii) cyclic hexapeptides with an *N*-linked fatty-acyl side-chain (Onishi *et al.*, 2000). Included in the later group are the non-competitive echinocandin inhibitors that are currently the only one of the three families used in clinical practice. Although these molecules are active in patients, mechanistic details of the non-competitive glucan inhibition by echinocandins still remain to be fully elucidated. Drugs inhibiting chitin synthesis, such as the peptide nucleoside antibiotics polyoxins and nikkomycins and their analogues, are substrate analogues of UDP-*N*-GlcNAc and are strong competitive inhibitors of chitin synthase *in vitro*. These compounds are poorly active *in vivo*, however. New strategies are presently being investigated to identify more efficient inhibitors (Behr, 2003; Yeager and Finney, 2004).

Although genetic data indicate that yeast glycosylhydrolase mutants are affected in growth, the search for antiglycosylhydrolases is in its infancy and focuses only antichitinase drugs. They are either substrate analogues, such as the pseudotrisaccharide allosamidin, which inhibit the oxazolinium reaction intermediate unique to chitinase, or new inhibitors that also bind to the chitinase active site. Only their role *in vitro* has been analysed (Schuttelkopf *et al.*, 2006). Inhibitors of glucanases have not been identified or proposed as antifungal drugs.

Combination therapy with drugs inhibiting the synthesis and hydrolysis of the same polysaccharide or the synthesis of the two main polysaccharides has been recently proposed (Stevens, 2000). Such combinatorial strategies might be very efficient, as compensatory reactions leading to increased synthesis of a cell wall component occur when the synthesis of another component is perturbed (Lagorce *et al.*, 2003). In yeast, an increase in chitin synthesis is a common compensatory reaction when the cell integrity pathway is affected. Other reported compensatory reactions might relate to the level of expression of different genes of the same family (Maubon *et al.*, 2006). These compensatory modifications might target unrelated cell wall components; for example, an increase in melanin can be induced by mutation of chitin synthase gene in *Wangiella dermatitidis* or α1,3 glucan synthase in *A. fumigatus* (Liu *et al.*, 2004; Maubon *et al.*, 2006); an increase in α1,3 glucan follows the disruption of chitin synthase genes in *A. fumigatus* (Mellado *et al.*, 2003). Such compensatory reactions explain why antifungal therapy with cell wall inhibitors is difficult, but also reflect the dynamics of the synthesis of cell wall component.

Another way to interfere with the polysaccharide synthesis is to use killer antibodies instead of chemical inhibitors. A remarkable example of the direct antimicrobial effects of antibodies is provided by the broad-spectrum antimicrobial activities of anti-idiotypic antibodies to a neutralizing monoclonal antibody to *Pichia anomala* killer toxin that binds to β1,3 glucan (Magliani *et al.*, 1997). These antibodies mediate antimicrobial activity by mimicking the internal image of the toxin in the toxin binding site and reproducing the antimicrobial effects of the killer toxin. Anti-β1,3 glucan antibodies showed a direct antifungal effect on the fungus *in vitro* and mediated protection against *Candida* and *Aspergillus* infection (Torosantucci *et al.*, 2005). Potentiation of the fungicidal activity of the antibody is possible by radiolabelling the antibody. Treatment of *Cryptococcus neoformans*-infected mice with a monoclonal antibody to the capsular polysaccharide, conjugated with either rhenium-188 or bismuth-213, was recently shown to prolong significantly the survival of lethally infected mice and to reduce organ fungal burden (Dadachova *et al.*, 2003).

Perspectives

In spite of recent progress, the fungal cell wall remains a poorly understood structure in terms of both composition and biosynthesis. A major issue is technical and is related to the harshness of treating cell walls to analyse its composition. Does boiling it in NaOH reflect the true structure? Is carboxymethylation a better approach to solubilize cell wall polysaccharides? How are polysaccharides that appear after chemical treatment fibrillar or amorphous under electron microscopy really organized *in vivo*? What are the linkages (non-covalent or alkali-labile covalent) and mechanisms governing the insertion of alkali-soluble material in the fibrillar core? Other issues are strictly biochemical. What transferases are responsible for establishing the polysaccharide 3D network of the cell wall? Are constitutive polysaccharides of the cell wall bound to specific membrane anchors? How can cell wall transferases work on insoluble substrates?

The structure of the cell wall has been at least partly chemically analysed. This remains insufficient, as it does not reflect the mechanical properties of the cell wall.

Atomic force microscopy has been introduced recently for such measurements (Zhao *et al*., 2005). This approach showed that the environment has a strong effect on cell wall elasticity; high osmotic conditions (0.6 M KCl) in the culture medium induce hyphae of *Aspergillus nidulans* to assemble a cell wall with a weaker molecular structure, resulting in lower elasticity and a reduction in the tensile strength of the apical cell wall. Associated with a better knowledge of the environmental sensors and the different signal transduction cascades activated under different environments, such biophysical approaches should lead to a better understanding of the dynamics of cell wall construction. A better analysis of the cell wall regeneration process might help this understanding and identify new pathways in cell wall synthesis. Several fungal orders include morphotypes without cell walls. A classical example is the motile zoospore of Chytridiomycota (one of the most ancient fungal phyllum), which is required for survival of the organism in an aquatic environment. Other cell wall-less stages are produced by some fungi to avoid recognition by its host. For example, *Pneumocystis carinii* forms cell wall-less commensal trophozoites that associate with pneumocytes (de Souza and Benchimol, 2005). This is also the case for the entomopathogenic zygomycetous *Entomophaga* or *Entomophthora* protoplasts that, in contrast to their β1,3 glucan-rich cell-walled stage, do not induce a defence prophenoloxidase cascade in arthropods. Wall shading results from a reduction or even a shut off of polysaccharide synthase activity (Beauvais and Latgé, 1989). The association between cell wall synthesis inhibition and water or glycerol pumps regulating the internal osmotic pressure of the cytoplasm, which must play an essential role for these zoospores or protoplasts to withstand the external milieu in the absence of cell wall, has never been investigated. The genomic exploitation of such dimorphism between cell wall-less morphotypes and cells covered by a cell wall within a species might lead to the discovery of new regulatory pathways of cell wall biosynthetic enzymes.

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References

Abe, M., Qadota, H., Hirata, A., and Ohya, Y. (2003) Lack of GTP-bound Rho1p in secretory vesicles of *Saccharomyces cerevisiae*. *J Cell Biol* **162:** 85–97.

Alonso-Nunez, M.L., An, H., Martin-Cuadrado, A.B., Mehta, S., Petit, C., Sipiczki, M., *et al.* (2005) Ace2p controls the expression of genes required for cell separation in *Schizosaccharomyces pombe*. *Mol Biol Cell* **16:** 2003–2017.

Arakane, Y., Muthukrishnan, S., Kramer, K.J., Specht, C.A., Tomoyasu, Y., Lorenzen, M.D., *et al.* (2005) The *Tribolium* chitin synthase genes *TcCHS1* and *TcCHS2* are specialized for synthesis of epidermal cuticle and midgut peritrophic matrix. *Insect Mol Biol* **14:** 453–463.

Baker, R.L.G., Specht, C.A., Donlin, M.J., and Lodge, J.K. (2007) Chitosan, the deacetylated form of chitin, is necessary for cell wall integrity in *Cryptococcus neoformans*. *Eukaryot Cell* **6:** 855–867.

Baladron, V., Ufano, S., Duenas, E., Martin-Cuadrado, A.B., del Rey, F., and Vazquez de Aldana, C.R. (2002) Eng1p, an endo-1,3-beta-glucanase localized at the daughter side of the septum, is involved in cell separation in *Saccharomyces cerevisiae*. *Eukaryot Cell* **1:** 774–786.

Beauvais, A., and Latgé, J.P. (1989) Chitin and beta(1-3) glucan synthases in the protoplastic Entomophthorales. *Arch Microbiol* **152:** 229–236.

Beauvais, A., Maubon, D., Park, S., Morelle, W., Tanguy, M., Huerre, M., *et al.* (2005) Two alpha(1-3) glucan synthases with different functions in *Aspergillus fumigatus*. *Appl Environ Microbiol* **71:** 1531–1538.

Behr, J.B. (2003) Chitin synthase as an antifungal target: recent advances. *Curr Med Chem* **2:** 173–189.

Bernard, M., Mouyna, I., Dubreucq, G., Debeaupuis, J.P., Fontaine, T., Vorgias, C., *et al.* (2002) Characterization of a cell-wall acid phosphatase (PhoAp) in *Aspergillus fumigatus*. *Microbiology* **148:** 2819–2829.

Cabib, E., Roh, D.H., Schmidt, M., Crotti, L.B., and Varma, A. (2001) The yeast cell wall and septum as paradigms of cell growth and morphogenesis. *J Cell Biol* **276:** 19679–19682.

Cabib, E., Blanco, N., Grau, C., Rodriguez-Pena, J.M., and Arroyo, J. (2007) Crh1p and Crh2p are required for the cross-linking of chitin to β(1-6) glucan in the *Saccharomyces cerevisiae* cell wall. *Mol Microbiol* **63:** 921–935.

Chatterjee, D. (1997) The mycobacterial cell wall: structure, biosynthesis and sites of drug action. *Curr Opin Chem Biol* **1:** 579–588.

Choquer, M., Boccara, M., Goncalves, I.R., Soulie, M.C., and Vidal-Cros, A. (2004) Survey of the *Botrytis cinerea* chitin synthase multigenic family through the analysis of six euascomycetes genomes. *Eur J Biochem* **271:** 2153–2164.

Cortes, J.C., Carnero, E., Ishiguro, J., Sanchez, Y., Duran, A., and Ribas, J.C. (2005) The novel fission yeast (1,3)beta-D-glucan synthase catalytic subunit Bgs4p is essential during both cytokinesis and polarized growth. *J Cell Sci* **118:** 157–174.

Cortes, J.C., Konomi, M., Martins, I.M., Munoz, J., Moreno, M.B., Osumi, M., *et al.* (2007) The (1,3)beta-D-glucan synthase subunit Bgs1p is responsible for the fission yeast primary septum formation. *Mol Microbiol* **65:** 201–217.

Cos, T., Ford, R.A., Trilla, J.A., Duran, A., Cabib, E., and Roncero, C. (1998) Molecular analysis of Chs3p participation in chitin synthase III activity. *Eur J Biochem* **256:** 419–426.

Costachel, C., Coddeville, B., Latgé, J.P., and Fontaine, T. (2005) Glycosylphosphatidylinositol-anchored fungal polysaccharide in *Aspergillus fumigatus*. *J Biol Chem* **280:** 39835–39842.

Dadachova, E., Nakouzi, A., Bryan, R.A., and Casadevall, A.

(2003) Ionizing radiation delivered by specific antibody is therapeutic against a fungal infection. *Proc Natl Acad Sci USA* **100:** 10942–10947.

Douglas, C.M. (2001) Fungal beta(1,3)-D-glucan synthesis. *Med Mycol* **39** (Suppl. 1)**:** 55–66.

Ecker, M., Deutzmann, R., Lehle, L., Mrsa, V., and Tanner, W. (2006) Pir proteins of *Saccharomyces cerevisiae* are attached to beta-1,3-glucan by a new protein-carbohydrate linkage. *J Biol Chem* **281:** 11523–11529.

Enns, L.C., Kanaoka, M.M., Torii, K.U., Comai, L., Okada, K., and Cleland, R.E. (2005) Two callose synthases, GSL1 and GSL5, play an essential and redundant role in plant and pollen development and in fertility. *Plant Mol Biol* **58:** 333–349.

Fleet, G.H. (1991) Cell walls. In *The Yeast*. Rose, A.H., and Harrisson, J.D. (eds). Vol. 4. New York: Academic press, pp. 199–277.

Fontaine, T., Simenel, C., Dubreucq, G., Adam, O., Delepierre, M., Lemoine, J., *et al.* (2000) Molecular organization of the alkali-insoluble fraction of *Aspergillus fumigatus* cell wall. *J Biol Chem* **275:** 27594–27607.

Fujii, T., Shimoi, H., and Iimura, Y. (1999) Structure of the glucan-binding sugar chain of Tip1p, a cell wall protein of *Saccharomyces cerevisiae*. *Biochim Biophys Acta* **1427:** 133–144.

de Groot, P.W., Yin, Q.Y., Weig, M., Sosinska, G.J., Klis, F.M., and de Koster, C.G. (2007) Mass spectrometric identification of covalently bound cell wall proteins from the fission yeast *Schizosaccharomyces pombe*. *Yeast* **24:** 267–278.

Hu, W., Sillaots, S., Lemieux, S., Davison, J., Breton, A., Linteau, A., *et al.* (2007) Essential gene identification and drug target prioritization in *Aspergillus fumigatus*. *PLoS Pathogens* **3:** e24.

Humbel, B.M., Konomi, M., Takagi, T., Kamasawa, N., Ishijima, S.A., and Osumi, M. (2001) In situ localization of beta-glucans in the cell wall of *Schizosaccharomyces pombe*. *Yeast* **18:** 433–444.

Inoue, S.B., Qadota, H., Arisawa, M., Watanabe, T., and Ohya, Y. (1999) Prenylation of Rho1p is required for activation of yeast 1, 3-beta-glucan synthase. *J Biol Chem* **274:** 38119–38124.

Ishihara, S., Hirata, A., Nogami, S., Beauvais, A., Latge, J.P., and Ohya, Y. (2007) Homologous subunits of 1,3-beta-glucan synthase are important for spore wall assembly in *Saccharomyces cerevisiae*. *Eukaryot Cell* **6:** 143–156.

Kapteyn, J.C., Montijn, R.C., Vink, E., de la Cruz, J., Llobell, A., Douwes, J.E., *et al.* (1996) Retention of *Saccharomyces cerevisiae* cell wall proteins through a phosphodiester-linked beta-1,3-/beta-1,6-glucan heteropolymer. *Glycobiology* **6:** 337–345.

Karnezis, T., Epa, V.C., Stone, B.A., and Stanisich, V.A. (2003) Topological characterization of an inner membrane (1→3)-beta-D-glucan (curdlan) synthase from *Agrobacterium* sp. strain ATCC31749. *Glycobiology* **13:** 693–706.

King, L., and Butler, G. (1998) Ace2p, a regulator of CTS1 (chitinase) expression, affects pseudohyphal production in *Saccharomyces cerevisiae*. *Curr Genet* **34:** 183–191.

Kitagaki, H., Wu, H., Shimoi, H., and Ito, K. (2002) Two homologous genes, DCW1 (YKL046c) and DFG5, are essential for cell growth and encode glycosylphosphatidylinositol (GPI)-anchored membrane proteins required for cell wall biogenesis in *Saccharomyces cerevisiae*. *Mol Microbiol* **46:** 1011–1022.

Klis, F.M., Mol, P., Hellingwerf, K., and Brul, S. (2002) Dynamics of cell wall structure in *Saccharomyces cerevisiae*. *FEMS Microbiol Rev* **26:** 239–56.

Kollar, R., Petrakova, E., Ashwell, G., Robbins, P.W., and Cabib, E. (1995) Architecture of the yeast cell wall. The linkage between chitin and beta(1-3)-glucan. *J Biol Chem* **270:** 1170–1178.

Kollar, R., Reinhold, B.B., Petrakova, E., Yeh, H.J.C., Ashwell, G., Drgonova, J., *et al.* (1997) Architecture of the yeast cell wall: β-1-6-glucan interconnects mannoprotein, β(1-3)-glucan, and chitin. *J Biol Chem* **272:** 17762–17788.

Kuranda, M.J., and Robbins, P.W. (1991) Chitinase is required for cell separation during growth of *Saccharomyces cerevisiae*. *J Biol Chem* **266:** 19758–19767.

Lagorce, A., Hauser, N.C., Labourdette, D., Rodriguez, C., Martin-Yken, H., Arroyo, J., *et al.* (2003) Genome-wide analysis of the response to cell wall mutations in the yeast *Saccharomyces cerevisiae*. *J Biol Chem* **278:** 20345–20357.

Latgé, J.P., and Calderone, R. (2005) The fungal cell wall. In *The Mycota I. Growth, Differentiation and Sexuality*. Kües U., and Fischer R. (eds). Berlin, Heidelberg: Springer-Verlag, pp. 73–104.

Lesage, G., and Bussey, H. (2006) Cell wall assembly in *Saccharomyces cerevisiae*. *Microbiol Mol Biol Rev* **70:** 317–343.

Liu, H., Kauffman, S., Becker, J.M., and Szaniszlo, P.J. (2004) *Wangiella* (*Exophiala*) *dermatitidis* WdChs5p, a class V chitin synthase, is essential for sustained cell growth at temperature of infection. *Eukaryot Cell* **3:** 40–51.

Magliani, W., Conti, S., de Bernardis, F., Gerloni, M., Bertolotti, D., Mozzoni, P., *et al.* (1997) Therapeutic potential of antiidiotypic single chain antibodies with yeast killer toxin activity. *Nat Biotechnol* **15:** 155–158.

Martin, V., Garcia, B., Carnero, E., Duran, A., and Sanchez, Y. (2003) Bgs3p, a putative 1,3-beta-glucan synthase subunit, is required for cell wall assembly in *Schizosaccharomyces pombe*. *Eukaryot Cell* **2:** 159–169.

Martin-Cuadrado, A.B., Duenas, E., Sipiczki, M., Vazquez de Aldana, C.R., and del Rey, F. (2003) The endo-beta-1,3-glucanase eng1p is required for dissolution of the primary septum during cell separation in *Schizosaccharomyces pombe*. *J Cell Sci* **116:** 1689–1698.

Maubon, D., Park, S., Tanguy, M., Huerre, M., Schmitt, C., Prévost, M.C., *et al.* (2006) *AGS3*, an α(1-3) glucan synthase gene family member of *Aspergillus fumigatus*, modulates mycelium growth in the lung of experimentally infected mice. *Fungal Genet Biol* **43:** 366–375.

Mazur, P., and Baginsky, W. (1996) *In vitro* activity of 1,3-beta-D-glucan synthase requires the GTP-binding protein Rho1. *J Biol Chem* **271:** 14604–14609.

Mellado, E., Dubreucq, G., Mol, P., Sarfati, J., Paris, S., Diaquin, M., *et al.* (2003) Cell wall biogenesis in a double chitin synthase mutant (*chsG–/chsE–*) of *Aspergillus fumigatus*. *Fungal Genet Biol* **38:** 98–109.

Merzendorfer, H. (2006) Insect chitin synthases: a review. *J Comp Physiol [B]* **176:** 1–15.

Mouyna, I., Fontaine, T., Vai, M., Monod, M., Fonzi, W.A., Diaquin, M., *et al.* (2000) GPI-anchored glucanosyltrans-

ferases play an active role in the biosynthesis of the fungal cell wall. *J Biol Chem* **275:** 14882–14889.

Mouyna, I., Henry, C., Doering, T.L., and Latge, J.P. (2004) Gene silencing with RNA interference in the human pathogenic fungus *Aspergillus fumigatus*. *FEMS Microbiol Lett* **237:** 317–324.

Munro, C.A., Winter, K., Buchan, A., Henry, K., Becker, J.M., Brown, A.J., *et al.* (2001) Chs1 of *Candida albicans* is an essential chitin synthase required for synthesis of the septum and for cell integrity. *Mol Microbiol* **39:** 1414–1426.

Munro, C.A., Selvaggini, S., de Bruijn, I., Walker, L., Lenardon, M.D., Gerssen, B., *et al.* (2007) The PKC, HOG and Ca^{2+} signalling pathways co-ordinately regulate chitin synthesis in *Candida albicans*. *Mol Microbiol* **63:** 1399–1413.

Netea, M.G., Gow, N.A., Munro, C.A., Bates, S., Collins, C., Ferwerda, G., *et al.* (2006) Immune sensing of *Candida albicans* requires cooperative recognition of mannans and glucans by lectin and Toll-like receptors. *J Clin Invest* **116:** 1642–1650.

Nierman, W.C., Pain, A., Anderson, M.J., Wortman, J.R., Kim, H.S., Arroyo, J., *et al.* (2005) Genomic sequence of the pathogenic and allergenic filamentous fungus *Aspergillus fumigatus*. *Nature* **438:** 1151–1156.

Onishi, J., Meinz, M., Thompson, J., Curotto, J., Dreikorn, S., Rosenbach, M., *et al.* (2000) Discovery of novel antifungal (1,3)-beta-D-glucan synthase inhibitors. *Antimicrob Agents Chemother* **44:** 368–377.

Perez, P., and Ribas, J.C. (2004) Cell wall analysis. *Methods* **33:** 245–251.

Persson, S., Caffall, K.H., Freshour, G., Hilley, M.T., Bauer, S., Poindexter, P., *et al.* (2007) The *Arabidopsis* irregular *xylem8* mutant is deficient in glucuronoxylan and homogalacturonan, which are essential for secondary cell wall integrity. *Plant Cell* **19:** 237–255.

Qadota, H., Python, C.P., Inoue, S.B., Arisawa, M., Anraku, Y., Zheng, Y., *et al.* (1996) Identification of yeast Rho1p GTPase as a regulatory subunit of 1,3-beta-glucan synthase. *Science* **272:** 279–281.

Rodriguez-Pena, J.M., Cid, V.J., Arroyo, J., and Nombela, C. (2000) A novel family of cell wall-related proteins regulated differently during the yeast life cycle. *Mol Cell Biol* **20:** 3245–3255.

Roncero, C. (2002) The genetic complexity of chitin synthesis in fungi. *Curr Genet* **41:** 367–378.

Sanz, M., Castrejon, F., Duran, A., and Roncero, C. (2004) *Saccharomyces cerevisiae* Bni4p directs the formation of the chitin ring and also participates in the correct assembly of the septum structure. *Microbiology* **150:** 3229–3241.

Schmidt, M. (2004) Survival and cytokinesis of *Saccharomyces cerevisiae* in the absence of chitin. *Microbiology* **150:** 3253–3260.

Schmidt, M., Bowers, B., Varma, A., Roh, D.H., and Cabib, E. (2002) In budding yeast, contraction of the actomyosin ring and formation of the primary septum at cytokinesis depend on each other. *J Cell Sci* **115:** 293–302.

Schuttelkopf, A.W., Andersen, O.A., Rao, F.V., Allwood, M., Lloyd, C., Eggleston, I.M., and van Aalten, D.M. (2006) Screening-based discovery and structural dissection of a novel family 18 chitinase inhibitor. *J Biol Chem* **281:** 27278–27285.

de Souza, W., and Benchimol, M. (2005) Basic biology of *Pneumocystis carinii*: a mini review. *Mem Inst Oswaldo Cruz* **100:** 903–908.

Stevens, D. (2000) Drug interaction studies of a glucan synthase inhibitor (IY 303366) and a chitin synthase inhibitor (Nikkomycin Z) for inhibition and killing of fungal pathogens. *Antimicrob Agents Chemother* **44:** 2547–2548.

Takeshita, N., Ohta, A., and Horiuchi, H. (2005) CsmA, a class V chitin synthase with a myosin motor-like domain, is localized through direct interaction with the actin cytoskeleton in *Aspergillus nidulans*. *Mol Biol Cell* **16:** 1961–1970.

Torosantucci, A., Bromuro, C., Chiani, P., De Bernardis, F., Berti, F., Galli, C., *et al.* (2005) A novel glyco-conjugate vaccine against fungal pathogens. *J Exp Med* **202:** 597–606.

Tougan, T., Chiba, Y., Kakihara, Y., Hirata, A., and Nojima, H. (2002) Meu10 is required for spore wall maturation in *Schizosaccharomyces pombe*. *Genes Cells* **7:** 217–231.

Trilla, J.A., Cos, T., Duran, A., and Roncero, C. (1997) Characterization of *CHS4 (CAL2)*, a gene of *Saccharomyces cerevisiae* involved in chitin biosynthesis and allelic to *SKT5* and *Csd4 Yeast* **13:** 795–807.

Trinel, P.A., Maes, E., Zanetta, J.P., Delplace, F., Coddeville, B., Jouault, T., *et al.* (2002) *Candida albicans* phospholipomannan, a new member of the fungal mannose inositol phosphoceramide family. *J Biol Chem* **277:** 37260–37271.

van der Vaart, J.M., Caro, L.H., Chapman, J.W., Klis, F.M., and Verrips, C.T. (1995) Identification of three mannoproteins in the cell wall of *Saccharomyces cerevisiae*. *J Bacteriol* **177:** 3104–3110.

Verstrepen, K.J., Jansen, A., Lewitter, F., and Fink, G.R. (2005) Intragenic tandem repeats generate functional variability. *Nat Genet* **37:** 986–990.

Wessels, J. (1986) Cell wall synthesis in apical hyphal growth. *Int Rev Cytol* **104:** 37–39.

Yeager, A.R., and Finney, N.S. (2004) The first direct evaluation of the two-active site mechanism for chitin synthase. *J Org Chem* **69:** 613–618.

Yin, Q.Y., de Groot, P.W., Dekker, H.L., de Jong, L., Klis, F.M., and de Koster, C.G. (2005) Comprehensive proteomic analysis of *Saccharomyces cerevisiae* cell walls: identification of proteins covalently attached via glycosylphosphatidylinositol remnants or mild alkali-sensitive linkages. *J Biol Chem* **280:** 20894–20901.

Zhao, L., Schaefer, D., Xu, H., Modi, S.J., LaCourse, W.R., and Marten, M.R. (2005) Elastic properties of the cell wall of *Aspergillus nidulans* studied with atomic force microscopy. *Biotechnol Prog* **21:** 292–299.

