Mini-Review

Correspondence
d.j.adams@leeds.ac.uk

Fungal cell wall chitinases and glucanases

David J. Adams

School of Biochemistry and Microbiology, University of Leeds, Leeds LS2 9JT, UK

The fungal cell wall is a complex structure composed of chitin, glucans and other polymers, and there is evidence of extensive cross-linking between these components. The wall structure is highly dynamic, changing constantly during cell division, growth and morphogenesis. Hydrolytic enzymes, closely associated with the cell wall, have been implicated in the maintenance of wall plasticity and may have roles during branching and cross-linking of polymers. Most fungal cell wall hydrolases identified to date have chitinase or glucanase activity and this short article reviews the apparent functions of these enzymes in unicellular and filamentous fungi, and the mechanisms that regulate enzyme activity in yeasts.

Introduction

The fungal kingdom is very diverse, with species growing as unicellular yeasts and/or branching hyphae that produce a remarkable array of spores and other reproductive structures. In each case, the shape and integrity of the fungus is dependent upon the mechanical strength of the cell wall, which performs a wide range of essential roles during the interaction of the fungus with its environment (Gooday, 1995). The fungal wall is a complex structure composed typically of chitin, 1,3-β- and 1,6-β-glucan, mannan and proteins, although wall composition frequently varies markedly between species of fungi. For diagrammatic representations of part or all of the cell wall of yeasts or filamentous fungi see recent reviews by Bernard & Latgé (2001), McFadden & Casadevall (2001), Smits *et al.* (2001) and Odds *et al.* (2003).

Enzymes of chitin and glucan biosynthesis manufacture long, linear chains of β1,4-linked *N*-acetylglucosamine and β1,3-linked glucose, respectively. However, the fungal cell wall contains abundant quantities of branched 1,3-β-, 1,6-β-glucan and there is evidence of extensive cross-linking between chitin, glucan and other wall components (Cabib *et al.*, 2001; Klis *et al.*, 2002). Furthermore, the wall is a highly dynamic structure subject to constant change, for example, during cell expansion and division in yeasts, and during spore germination, hyphal branching and septum formation in filamentous fungi. Cell wall polymer branching and cross-linking, and the maintenance of wall plasticity during morphogenesis, may depend upon the activities of a range of hydrolytic enzymes found intimately associated with the fungal cell wall. Most of the fungal cell wall hydrolases characterized to date have chitinase or glucanase activity and a number of these enzymes also exhibit transglycosylase activity. They may therefore contribute to breakage and re-forming of bonds within and between polymers, leading to re-modelling of the cell wall during growth and morphogenesis. Well-defined and putative glucanolytic and chitinolytic activities are discussed here if there is evidence of a close association of the enzyme with the cell wall. Alternatively, or additionally, a hydrolase is included if disruption of the gene encoding the enzyme leads to an altered phenotype that may be attributable to changes in cell wall structure. Mycoparasitic *Trichoderma* species secrete chitinases and glucanases that attack cell wall polymers in other fungi and have been exploited in the development of biocontrol strategies (Chet & Inbar, 1994). These enzymes are not considered here but see, for example, Kim *et al.* (2002) for an account of multiple chitinases and glucanases from *Trichoderma virens*.

Cell wall chitinases of yeasts

Work with the yeast *Saccharomyces cerevisiae* provided the first clear evidence of a role for a cell wall hydrolase in fungal growth. During budding in *S. cerevisiae* a division septum is laid down between the cells and within a ring of chitin deposited at the bud site. Degradation of this material leads to cell separation and involves an extensively glycosylated endochitinase with an apparent molecular mass of approximately 130 kDa. Disruption of the *CTS1* gene encoding this enzyme led to a defect in cell separation (Kuranda & Robbins, 1991), complementing results obtained by Sakuda *et al.* (1990) who demonstrated inhibition of yeast cell separation by the potent chitinase inhibitor demethylallosamidin. Parting of yeast cells during budding is therefore dependent upon hydrolysis of chitin joining mother and daughter. More recently, King & Butler (1998) demonstrated that disruption of *CTS1* can also promote pseudohyphal growth. The chitin-binding domain of Cts1p may have a role in localizing the enzyme to the cell wall (Kuranda & Robbins, 1991), where its activity appears to be counterbalanced by the 'repair' enzyme chitin synthase 1 (Cabib *et al.*, 2001). Most recently, a second apparent chitinase-encoding gene, *CTS2*, was identified in *S. cerevisiae*. It seems clear that the gene product has a role during sporulation, as

disruption of *CTS2* led to abnormal spore wall biosynthesis and failure to form mature asci (Giaver *et al.*, 2002).

McCreath *et al.* (1995, 1996) cloned three chitinase genes, *CHT1*, *CHT2* and *CHT3*, from the dimorphic pathogen of humans, *Candida albicans*. *CHT1* encodes a protein with a predicted molecular mass of 42 kDa which may correspond to a chitinase of 45 kDa purified from this organism by Mellor *et al.* (1994). *CHT2* and *CHT3* encode proteins with a predicted molecular mass of 61 kDa. The deduced amino acid sequences for Cht2p and Cht3p are 36 % similar to one another and 36–38 % similar to *S. cerevisiae* Cts1p. Levels of transcription of *CHT2* and *CHT3* were greater when *Ca. albicans* was grown in a yeast phase as compared to a mycelial phase and preliminary results from disruption of *CHT2* suggested a role for the gene product in cell separation, similar to the role noted for Cts1p of *S. cerevisiae* (Kuranda & Robbins, 1991; McCreath *et al.*, 1995). A C-terminal chitin-binding domain was not immediately apparent in Cht2p or Cht3p although analysis of deduced amino acid sequences using the DGPI facility at the SIB website (http://ca.expasy.org/) identified putative glycosyl-phosphatidylinositol (GPI)-anchor and cleavage sites at the C-terminus of CHT2. This chitinase may therefore be GPI-anchored to the cell membrane or wall of *Ca. albicans*. In this regard it is interesting to note that Iranzo *et al.* (2002) provided evidence for covalent linkage of Cht2p to the *Ca. albicans* yeast cell wall while Dickinson *et al.* (1991) demonstrated a close association of chitinase with membrane fractions from this organism.

Cell wall glucanases of yeasts

S. cerevisiae contains a wide range of endo- and exo-1,3-β-glucanases and it seems likely that approximately 15 genes in this yeast encode polypeptides with glucanase or related enzymic activities (Baladrón *et al.*, 2002). Some of these glucanases have roles during cell separation while others exhibit transglycosylase activity and may be involved in extending and rearranging 1,3-β-glucan chains, and cross-linking these polymers to other wall components.

ENG1

Deletion of *ENG1*, a gene encoding an *S. cerevisiae* endo-1,3-β-glucanase, led to clumping of cells, suggesting that Eng1p, like Cts1p, is involved in dissolution of the mother–daughter septum during cell separation (Baladrón *et al.*, 2002). *Ca. albicans* and the fission yeast *Schizosaccharomyces pombe* contain genes related to *S. cerevisiae ENG1* which encode products required for cell separation in these organisms. CaEng1p partially complemented the separation defect of a *S. cerevisiae eng1* mutant, suggesting a close functional relationship between the glucanases from these organisms (Martín-Cuadrado *et al.*, 2003; Vázquez de Aldana *et al.*, 2003). Failure of *eng1* mutant cells of *Schiz. pombe* to part was shown to be due mainly to an inability to degrade the 1,3-β-glucan-rich primary septum that separates sister cells (Martín-Cuadrado *et al.*, 2003).

SCW and SUN genes

Single knockouts of three *S. cerevisiae* genes, *SCW3*, *SCW4* and *SCW10*, that may encode soluble cell wall glucanases, had no significant effect on phenotype but disruption of the related gene, *SCW11*, caused marked inhibition of cell separation after division (Cappellaro *et al.*, 1998). The double knockout of *SCW3* and *SCW10* resulted in slower growth, an increase in the amount of protein released from cell walls by dithiothreitol and decreased mating efficiency. Interestingly, these effects were partly antagonized by disruption of the gene encoding the endoglucanase/transferase Bgl2p (Cappellaro *et al.*, 1998). *SCW3* is also known as *SUN4* and is the fourth member of the *SUN* gene family, which encodes a further group of yeast proteins, each with a different cellular function, that exhibit homology with a β-glucosidase. In a more recent study, Mouassite *et al.* (2000) demonstrated a role for the *SCW3/SUN4* gene product during cell separation in *S. cerevisiae*.

YHR143W/DSE2

This gene encodes a secreted protein, with sequence similarity to glucanases, that localizes to regions of the *S. cerevisiae* cell wall connecting mother and daughter cells (Colman-Lerner *et al.*, 2001; Doolin *et al.*, 2001). Deletion of the *YHR143W/DSE2* gene led to inhibition of cell separation after division and enhanced pseudohyphal growth (Doolin *et al.*, 2001).

GAS1

Gas1p is a glycoprotein that is GPI-anchored to the plasma membrane of *S. cerevisiae*. Disruption of the *GAS1* gene led to several morphogenetic defects and an apparent decrease in the degree of cross-linking of 1,3-β-glucan to other wall polymers (reviewed by Popolo & Vai, 1999). A proposed transglycosylase activity for Gas1p was confirmed by Mouyna *et al.* (2000a; see below). *S. cerevisiae* contains four genes (*GAS2–5*) that are homologues of *GAS1*, and related genes have been identified in several fungal pathogens of humans (Popolo & Vai, 1999). *GAS5*, like *GAS1*, is expressed during vegetative growth while *GAS2*, *-3* and *-4* exhibit differing expression patterns during meiosis and sporulation; diploid *gas2* and *gas4* mutants displayed defects in sporulation (Ragni *et al.*, 2003).

BGL2

Purified preparations of the *BGL2*-encoded endo-1,3-β-glucanase from *Ca. albicans* and *S. cerevisiae* were subjected to detailed kinetic analyses (Goldman *et al.*, 1995). Bgl2p catalyses a transferase reaction, with separate 1,3-β-glucan chains as donor and acceptor, in which the reducing terminus of the activated donor is connected to the non-reducing end of the acceptor in a β1,6 linkage. The product is therefore a linear but 'kinked' glucan chain. Bgl2p may have the apparently important role of introducing β1,6 linkages into the 1,3-β-glucan of the fungal cell wall.

However, in this regard, it should be noted that disruption of the *S. cerevisiae* *BGL2* gene had no effect on phenotype (Klebl & Tanner, 1989) although overproduction of Bgl2p caused a decrease in growth rate (Mrsa *et al.*, 1993).

EXG1, *EXG2* and *SSG1*

S. cerevisiae contains three related exoglucanases, ExgI, ExgII and Ssg1. The transcript of the *SSG1* gene was detected only in sporulating diploids and the appearance of mature asci during sporulation was delayed in diploid strains homozygous for *SSG1* disruption (reviewed by Larriba *et al.*, 1995). Disruption of both alleles of *EXG1* and/or *EXG2* had no significant effect on phenotype, and sporulation in a strain homozygous for disruption of all three exoglucanase genes was indistinguishable from sporulation in the strain homozygous for *SSG1* disruption. However, when Jiang *et al.* (1995) investigated the relationship between *EXG1* expression and sensitivity of *S. cerevisiae* to K1 killer toxin they found that disruption of *EXG1* increased sensitivity while overexpression of *EXG1* led to resistance to the toxin. Overexpression of *EXG1* also resulted in a modest reduction in cell wall β-1,6-glucan while disruption of the gene led to a small increase in this component. Jiang *et al.* (1995) propose that the Exg1p exo-β-glucanase may therefore have a functional role in cell wall glucan metabolism. An Exg1p-related exoglucanase is secreted by *Ca. albicans* but no role has been identified for this enzyme (Larriba *et al.*, 1995).

CRH1, *CRH2* and *CRR1*

Rodríguez-Peña *et al.* (2000) identified a family of three related gene products in *S. cerevisiae* with homology to bacterial β-glucanases and eukaryotic endotransglycosidases. Both Crh1 and Crh2 appear to be GPI-anchored to yeast cell wall components (Hamada *et al.*, 1998). The combined disruption of all three genes was not lethal. However, taken together, the results of gene knockout, gene expression and reporter gene localization studies suggest that these putative glycosidases may perform a common function in cell wall construction at different stages of the yeast life cycle (Rodríguez-Peña *et al.*, 2000).

Cell wall glucanases of filamentous fungi

Several glucanolytic activities have been detected in the cell walls of the respiratory pathogens *Aspergillus fumigatus*, *Coccidioides posadasii* and *Coccidioides immitis*. Some of these enzymes exhibit both glucanase and transglycosidase activities and, like the glucanases of yeasts, may have roles in cell wall remodelling during morphogenesis.

Fontaine *et al.* (1997) detected monomeric and dimeric exo-1,3-β-glucanases of molecular mass 82 kDa and 230 kDa, respectively, in cell wall autolysates of *A. fumigatus*. An endo-1,3-β-glucanase of molecular mass 74 kDa was also detected in *A. fumigatus* wall autolysates; disruption of the *ENGL1* gene encoding this enzyme did not lead to a

phenotype distinct from that of the parental strain (Mouyna *et al.*, 2002). The *A. fumigatus* cell wall also contains a number of glucanases which exhibit 1,3-β-glucanosyltransferase activity. These include a 49 kDa endoglucanase which cleaves 1,3-β-glucan then transfers the newly generated reducing end to the non-reducing end of another 1,3-β-glucan molecule. With the formation of a new β1,3 linkage the 1,3-β-glucan chain is elongated and the gene encoding this enzyme is described as *GEL1* for glucan elongating glucanosyltransferase (Mouyna *et al.*, 2000a). *GEL1* encodes a GPI-anchored protein homologous to the *GAS1* and *PHR* gene products of *S. cerevisiae* and *Ca. albicans*, respectively. *S. cerevisiae* Gas1p, and *Ca. albicans* Phr1p and Phr2p, were known to have roles during morphogenesis but no biochemical function had been identified for these proteins until Mouyna *et al.* (2000a) demonstrated that recombinant Gas1p, Phr1p and Phr2p each has 1,3-β-glucanosyltransferase activity similar to that of *A. fumigatus* Gel1p. Furthermore, expression of *A. fumigatus* *GEL1* cDNA in a Δ*gas1* strain of *S. cerevisiae* rescued the morphological defects of this mutant, leading Mouyna *et al.* (2000a) to propose that the transglycosidase, GPI-anchored to the plasma membrane, has an active role during cell wall biosynthesis.

A. fumigatus appears to contain a family of at least three closely related 1,3-β-glucanosyltransferases that includes Gel1p (Mouyna *et al.*, 2000b). Similarly, there is evidence for the presence of a family of several structurally and functionally related 1,3-β-glucanosyltransferases in *Co. posadasii* (Delgado *et al.*, 2003). The presence of multiple genes encoding these enzymes in *A. fumigatus* and *Co. posadasii* may explain the lack of alteration of the normal phenotype in mutants containing a disrupted 1,3-β-glucanosyltransferase gene (Mouyna *et al.*, 2000b; Delgado *et al.*, 2003). That is, the expression of a functionally related gene(s) can be expected to compensate for the loss of a *GEL* gene in each of these species. Interestingly in this regard, Bruneau *et al.* (2001) report a marked effect on phenotype for a double glucanosyltransferase mutant (Δ*GEL1*/Δ*GEL2*) of *A. fumigatus*.

Mouyna *et al.* (1998) identified a 1,3-β-glucanosyltransferase, distinct from members of the Gelp family, in the cell wall of *A. fumigatus*. This 37 kDa protein is a homologue of the Bgl2p β-glucanosyltransferase of *S. cerevisiae* and *Ca. albicans*, releasing a disaccharide unit from the reducing end of 1,3-β-glucan and transferring the newly generated reducing end to the non-reducing end of another 1,3-β-glucan molecule to form a new intrachain β1,6 linkage. The gene for this enzyme appears to be present as a single copy in the *A. fumigatus* genome. However, the results of gene disruption experiments were similar to those obtained following *S. cerevisiae* *BGL2* disruption: the phenotype of a null mutant did not differ from that of the parental strain, suggesting that the 37 kDa enzyme does not play a major role during cell wall morphogenesis in *A. fumigatus*.

The cell wall of *Co. immitis* contains a 120 kDa β-glucosidase with 1,3-β-glucanase activity. A considerable

body of evidence has accumulated suggesting that this enzyme has a morphogenetic role during the parasitic phase of growth of *Co. immitis* (Cole & Hung, 2001; Hung *et al.*, 2001). Disruption of the gene encoding the 120 kDa glucosidase led both to a reduction in the rate of development of the parasitic growth phase and to a reduction in the mycelial growth rate. Furthermore, the gene knockout appeared to cause a marked decrease in the virulence of this organism in a mouse model of infection (Cole & Hung, 2001).

Cell wall chitinases of filamentous fungi

Chitinases, like glucanases, are closely associated with the cell walls of *Aspergillus* and *Coccidioides* species (Hearn *et al.*, 1996; Reichard *et al.*, 2000). As genome sequencing programmes for *A. fumigatus*, *A. nidulans* and *Co. immitis* near completion (http://www.tigr.org; http://www-genome.wi.mit.edu) it is apparent that these species contain large numbers of chitinase-encoding genes. For example, interrogation of the *A. fumigatus* database, using the protein sequences of the ChiA1p and ChiB1p chitinases from this organism, reveals that *A. fumigatus* contains at least 11 conserved active-site domains for chitinases of glycosyl hydrolase family 18. These enzymes may be divided into 'fungal/bacterial' chitinases, similar to chitinases found in bacteria, and 'fungal/plant' chitinases, which are similar to chitinases from plants. Fungal/bacterial chitinases from *Aspergillus* species and *Co. immitis* have deduced molecular masses of approximately 46–48 kDa. Disruption of the gene encoding the ChiB1p chitinase of *A. fumigatus* or the Cts1p chitinase of *Co. immitis* had no effect on growth or morphogenesis in these organisms (Reichard *et al.*, 2000; Jaques *et al.*, 2003). It is possible that related enzymes in *A. fumigatus* and *Co. immitis* compensate for the loss of ChiB1p or Cts1p. Alternatively, these secreted enzymes may not have morphogenetic roles. Instead they may contribute to the digestion and utilization of exogenous chitin as a source of organic nutrients for energy and biosynthesis. The fungal/plant chitinases of *Aspergillus* species and *Co. immitis* (deduced molecular mass 83–97 kDa; Takaya *et al.*, 1998; Reichard *et al.*, 2000; Jaques *et al.*, 2003) are much larger than enzymes of the fungal/bacterial class. Furthermore, each fungal/plant chitinase contains a serine/threonine-rich domain and a putative GPI anchor and cleavage site. The domains of the *A. fumigatus* fungal/plant chitinase ChiA1p are compared with those of ChiB1p, a fungal/bacterial chitinase from this organism, in Fig. 1. Fungal/plant chitinases, GPI-anchored to the cell membrane or anchored to the cell wall by a GPI anchor remnant, may have roles during growth and morphogenesis. In support of a morphogenetic role for these enzymes, disruption of the gene encoding the *A. nidulans* fungal/plant chitinase, ChiA, led to a decrease in the frequency of spore germination and a lower hyphal growth rate (Takaya *et al.*, 1998). Fungal cell wall chitinases may also have roles during sporulation in filamentous fungi, as the specific chitinase inhibitors allosamidin or demethylallosamidin inhibited fragmentation of
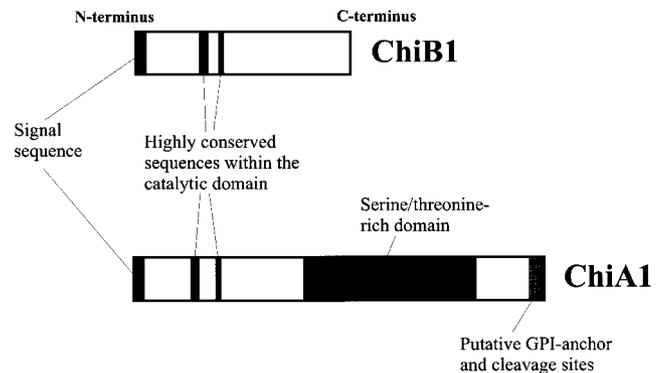
Fig. 1. Comparison of the domains of the ChiB1 fungal/bacterial chitinase (433 amino acid residues) and ChiA1 fungal/plant chitinase (825 amino acids) of *A. fumigatus*.

hyphae into arthroconidia (Yamanaka *et al.*, 1994; Sándor *et al.*, 1998).

Regulation of cell wall hydrolases
Regulation of gene expression in yeasts

Following budding in *S. cerevisiae*, the daughter cell displays a circular 'birth scar' in which chitin has been degraded while the mother retains a ring of chitin known as the 'bud scar' (Pringle, 1991). The basis for this asymmetry is the induction of daughter-cell-specific genes, including those encoding the Cts1p chitinase, the Eng1p glucanase and three further apparent glucanases (Colman-Lerner *et al.*, 2001; Doolin *et al.*, 2001; Baladrón *et al.*, 2002). The zinc finger transcription factor Ace2p regulates expression of a number of genes including *CTS1* (Dohrmann *et al.*, 1996; O'Conalláin *et al.*, 1999; Doolin *et al.*, 2001). Daughter-cell-specific expression of *CTS1* and other genes during cell separation is due to localization of Ace2p to the daughter nucleus and this, in turn, is dependent upon the Cbk1p kinase and its interacting protein Mob2 (Racki *et al.*, 2000; Bidlingmaier *et al.*, 2001; Colman-Lerner *et al.*, 2001; Weiss *et al.*, 2002).

Expression of the eng1 glucanase of *Schiz. pombe* is regulated by a zinc finger protein which shows significant similarity to *S. cerevisiae* Ace2p. Deletion of the gene encoding this regulatory protein led to a defect in cell separation that was more severe than that noted following *eng1*[+] gene disruption (Martín-Cuadrado *et al.*, 2003). Regulatory mechanisms in *Schiz. pombe* may therefore be similar to those found in *S. cerevisiae*, with an Ace2p-type regulator controlling expression of genes encoding hydrolases and other activities required for dissolution of the septum during cell division.

Other kinases besides Cbk1p have been implicated in the regulation of cell wall hydrolase activities in *S. cerevisiae*. A mutation in the gene encoding protein kinase C (Pkc1p)

of *S. cerevisiae* led to a fragile, 'hypo-osmolarity-sensitive' phenotype (Shimizu *et al.*, 1994). The amount of alkali-, acid-insoluble glucan in the cell wall of the mutant was reduced to about 30 % of wild-type, while the level of the Bgl2p glucanase in the wall was two- to threefold higher than in wild-type cells. Disruption of the *BGL2* gene in the *PKC1* mutant did not rescue the hypo-osmolarity-sensitive phenotype, suggesting that the *PKC1* kinase cascade regulates not only the expression of *BGL2* but also that of other genes involved in the biosynthesis and metabolism of the cell wall.

Finally, the results of Jiang *et al.* (1995) suggest that the Pbs2p kinase and the Ptc1p phosphatase play opposing regulatory roles in a MAP kinase signal transduction pathway that modulates the expression of the Exg1p glucanase and the expression of other proteins with roles in cell wall glucan assembly.

Regulation of gene expression in filamentous fungi

During recent years, Cbk1p and related serine/threonine protein kinases have emerged as important regulators of cell morphogenesis and proliferation in a range of eukaryotes. For example, the *COT1* gene of *Neurospora crassa* encodes a member of this group that is essential for growth (Yarden *et al.*, 1992; Bidlingmaier *et al.*, 2001). Conditional, temperature-sensitive mutants of *COT1* exhibit slow growth and excessive hyphal branching at the restrictive temperature. It is tempting to speculate that the phenotype of the *cot-1* mutant may be attributable, at least in part, to lack of regulation of cell wall lysins by Cot1p. However, no link has been established, as yet, between regulatory kinases/phosphatases and the expression of lytic enzymes with roles during growth and morphogenesis in filamentous fungi.

Conclusions

The work summarized in this review indicates that *S. cerevisiae*, *A. nidulans* and *A. fumigatus* contain multiple glucanase/glucanosyltransferase-encoding genes. The results of gene disruption studies suggest that several of these enzymes have roles during cell separation in unicellular organisms, and the development of cell wall architecture in yeasts and filamentous fungi. This battery of enzymes presumably helps to facilitate the complex pattern of lysis, branching and cross-linking that involves the glucan layers of the fungal cell wall. In contrast, *S. cerevisiae* contains only two chitinase-encoding genes, *CTS1* and *CTS2* (Kuranda & Robbins, 1991; Giaver *et al.*, 2002). Furthermore, the dimorphic, opportunistic pathogen *Ca. albicans* appears to contain only four or five chitinase genes (http://www-sequence.stanford.edu/); interrogation of the *Ca. albicans* database, using the protein sequences of *A. fumigatus* ChiA1p and ChiB1p, and *S. cerevisiae* Cts1p, identified only the chitinase-encoding sequences already deposited in the GenBank database and one other putative fungal/bacterial chitinase. This suggests that only a small number of these

hydrolases may be required for cell wall modification in organisms that exhibit budding yeast and/or filamentous growth patterns. Many of the multiple chitinase-encoding genes of *A. fumigatus* and other filamentous fungi may therefore encode secreted enzymes with nutritional roles rather than cell wall hydrolases with roles during growth and morphogenesis.

A number of distinct, non-catalytic regions have been identified in fungal cell wall hydrolases with apparent roles during growth and morphogenesis. These include serine/threonine-rich, GPI-anchoring and chitin-binding domains. However, these domains are not found in all of the apparent cell wall chitinases and glucanases characterized to date. For example, it appears that localization of the *S. cerevisiae* Gas1p and the *A. fumigatus* Gel1p endo-glucanase/glucanosyltransferase activities, to a specific site in the fungal cell envelope, is dependent upon the expression of the C-terminal GPI-anchor domain of these proteins (Mouyna *et al.*, 2000a). In contrast, the Cts1p chitinase of *S. cerevisiae* has no apparent GPI-anchor domain. Localization of this hydrolase to the division septum during cell separation appears to depend upon the expression of a chitin-binding domain (Kuranda & Robbins, 1991). Several apparent fungal cell wall hydrolases contain a serine/threonine-rich domain located towards the C-terminus of the enzyme. This is a common feature of many yeast cell wall proteins, and the serine and threonine residues within the domain are frequently heavily *O*-mannosylated. This pattern of glycosylation is thought to confer an extended, rod-like configuration on proteins and, in the case of cell wall hydrolases, may ensure that the active site of the enzyme is projected towards the surface of the cell or elsewhere within the wall structure (for references, see Popolo & Vai, 1999).

This short review has focused on hydrolases that cleave, and in some cases re-form, bonds in cell wall glucan or chitin. However, it should be stressed that during recent years, many novel linkages between cell wall components have been identified in yeasts and filamentous fungi (Cabib *et al.*, 2001; Klis *et al.*, 2002). Neither the enzymes that introduce these linkages into the cell wall nor, for the most part, the hydrolases that may cleave these unique bonds have been identified (it appears that chitinase can cleave a $\beta1,4$ linkage between chitin and glucan; Kollar *et al.*, 1995). Clearly this is an important area for future research with regard to both our understanding of fungal growth and morphogenesis, and the exploitation of these highly specific enzymes as additional, novel targets for antifungal drugs and agricultural fungicides. During the last decade, our understanding of the regulation of glucanase and chitinase activities in yeasts has increased markedly. It is equally important that our knowledge of the regulators of cell wall hydrolases in yeasts and filamentous fungi should improve, as underlying regulatory mechanisms may themselves be exploitable in the design of novel drugs. Thus, disruption of regulators may lead to inappropriate or

excessive expression of glucanase and/or chitinase activities in the wall, leading to cell lysis.

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