Independent regulation of chitin synthase and chitinase activity in *Candida albicans* and *Saccharomyces cerevisiae*

Serena Selvaggini,1 Carol A. Munro,1 Serge Paschoud,2 Dominique Sanglard2 and Neil A. R. Gow1

1School of Medical Sciences, University of Aberdeen, Institute of Medical Sciences, Foresterhill, Aberdeen AB25 2ZD, UK
2University Hospital Lausanne (CHUV), Institute of Microbiology, CH-1011 Lausanne, Switzerland

Chitin is an essential structural polysaccharide in fungi that is required for cell shape and morphogenesis. One model for wall synthesis at the growing cell surface suggests that the compliance that is necessary for turgor-driven expansion of the cell wall involves a delicate balance of wall synthesis and lysis. Accordingly, de novo chitin synthesis may involve coordinated regulation of members of the CHS chitin synthase and CHT chitinase gene families. To test this hypothesis, the chitin synthase and chitinase activities of cell-free extracts were measured, as well as the chitin content of cell walls isolated from isogenic mutant strains that contained single or multiple knock-outs in members of these two gene families, in both *Candida albicans* and *Saccharomyces cerevisiae*. However, deletion of chitinase genes did not markedly affect specific chitin synthase activity, and deletion of single CHS genes had little effect on in vitro specific chitinase activity in either fungus. Chitin synthesis and chitinase production was, however, regulated in *C. albicans* during yeast–hypha morphogenesis. In *C. albicans*, the total specific activities of both chitin synthase and chitinase were higher in the hyphal form, which was attributable mainly to the activities of Chs2 and Cht3, respectively. It appeared, therefore, that chitin synthesis and hydrolysis were not coupled, but that both were regulated during yeast–hypha morphogenesis in *C. albicans*.

**INTRODUCTION**

Chitin and (1,3)-β-D-glucan represent the main structural components of the fungal cell wall (Klis et al., 2001; Roncero, 2002; Munro & Gow, 2001). Chitin synthase therefore plays a fundamental role in the growth and morphogenesis of most fungal cells, and chitin synthesis is believed to be an essential process for most fungi (Cabib, 1987; Wessels, 1990; Shaw et al., 1991; Sietsma & Wessels, 1994; Gooday, 1995; Munro et al., 2001). Assembly and moulding of nascent chitin in the fungal cell wall has been proposed to involve an interplay between two enzymes: chitin synthase and chitinase. This investigation examines the hypothesis that chitin synthesis involves a coordinated interplay between chitin synthesis and chitin hydrolysis.

Several models have been proposed for the role of chitin synthesis and chitin lysis during hyphal growth. In the ‘unitary model’ of cell wall growth (Bartnicki-Garcia, 1973), chitinase is suggested to play an active role in cell wall biosynthesis at the growing tips of buds of yeast and the hyphae of filamentous fungi. Accordingly, wall growth represents a delicate balance between biosynthetic and hydrolytic processes. It has been proposed that hydrolytic enzymes such as chitinase cause sufficient cell wall lysis to maintain the wall in a plastic, compliant condition that allows insertion of new chitin fibrils as well as turgor-driven expansion of the cell surface. Zymogenic, membrane-associated forms of chitinase, which seem to be regulated in a way compatible with this role, have been described (Humphreys & Gooday, 1984a, b; Dickinson et al., 1991). However the chitin synthase and zymogenic chitinase activities of *Candida albicans* did not co-purify (Dickinson et al., 1991). Fungi have also been shown to possess complex chitinase families, suggestive of a range of roles in addition to the hydrolysis of chitin for nutrition (Rast et al., 1991; Gooday et al., 1992). The regulation of spore germination, budding, hyphal growth, hyphal branching and septum formation may all involve the direct participation of cell wall hydrolysis as well as synthesis, and thus these enzymic functions may be coordinately regulated (Gooday et al., 1992). Further evidence for the association

Abbreviations: CFW, Calcofluor White; FCS, fetal calf serum; 4-MU-[GlcNAc]₄, 4-methylumbelliferyl-β-D-N-tetraacylchitotetraoside.
of chitinase with chitin synthase was suggested by the parallel stimulation of the two activities during spore germination of *Mucor mucedo*, and exponential growth of yeast cells of *Mucor rouxii* (Rast et al., 1991) and *C. albicans* (Barrett-Bee & Hamilton, 1984).

An alternative steady-state model for fungal cell growth suggests that the plasticity of the hyphal apex and growing bud does not require the participation of hydrolytic enzymes such as chitinases (Wessels, 1984, 1986, 1990; Sietsma & Wessels, 1994), but instead is an inherent property of the process of chitin and glucan synthesis. Nascent polysaccharides have been suggested to be plastic. They are poorly cross-linked (Wessels et al., 1983) and are amorphous (Vermeulen & Wessels 1984), because the polysaccharide chains that are synthesized *de novo* have not yet hydrogen-bonded and crystallized into structural microfibrils (Wessels, 1986, 1990). Nascent chitin is more susceptible to chitinase digestion (Vermeulen & Wessels, 1986), which supports the view that chitinase could play a role in moulding and shaping the expanding cell wall. The most recent models of hyphal tip morphogenesis downplay the importance of the cell wall and emphasize instead the importance of the mechanism that deposits secretory vesicles from the vesicle supply centre to the cell surface (Bartnicki-Garcia, 2002).

Most or all fungi have multiple genes encoding chitin synthase families (Munro & Gow, 1995, 2001; Roncero, 2002). Most or all fungi have multiple genes encoding chitin synthase genes – *CHS1*, *CHS2*, *CHS3* and *CHS8* (Munro & Gow, 2001; Munro et al., 2003) – and at least four chitinases whose functions have yet to be fully explored. *CaCHT1* is not expressed under any known conditions *in vitro* (McCreath et al., 1995, 1996). *CHT2* and *CHT3* are expressed preferentially in the yeast form of the fungus (McCreath et al., 1995). *CHT4* has been identified recently in the *C. albicans* genome, but has yet to be characterized. *Saccharomyces cerevisiae* has only three chitin synthase enzymes (*CHS1*, *CHS2* and *CHS3*), and a single chitinase enzyme (*CTS1*) (Bulawa et al., 1986; Bulawa, 1992; Bulawa & Osmond, 1990; Cabib et al., 1982, 1989; Kuranda & Robbins, 1991; Shaw et al., 1991; Valdivieso et al., 1991).

In this study, we examine the hypothesis that chitin synthase and chitinase activity are coupled, by measuring the consequences of disruptions in specific chitin synthase and chitinase genes on both chitin synthesis and chitinase activity. We show that the total specific chitin synthase activity is not affected by mutations in chitinase genes, and reciprocally that the specific chitinase activity in cell extracts is not altered in any of the *chs* mutant backgrounds tested. The data do not provide support for the hypothesis that chitin synthesis and chitin hydrolysis are coordinately regulated.

### Methods

#### Strains and media.

*Candida albicans* strains used in this study are listed in Table 1. *C. albicans* cultures were maintained on solid YPD medium, consisting of 1% (w/v) yeast extract, 2% (w/v) peptone, 2% (w/v) glucose and 2% (w/v) peptone. Yeast cells of *C. albicans* and *Saccharomyces cerevisiae* were grown at 30 °C in YPD with shaking at 150 rpm. 

Table 1. *C. albicans* and *S. cerevisiae* strains used in this study

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>C. albicans</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CAF2-1*</td>
<td><em>ura3Δ::imm434/URA3</em></td>
<td>Fonzi &amp; Irwin (1993)</td>
</tr>
<tr>
<td>KW340</td>
<td><em>chs1Δ::hisGpSK-URA3-MRP1p-CHS1</em></td>
<td>Munro et al. (2001)</td>
</tr>
<tr>
<td>chs2Δ</td>
<td><em>chs2Δ::hisG::chs2Δ::hisG-URA3-hisG</em></td>
<td>Mio et al. (1996)</td>
</tr>
<tr>
<td>Myc03</td>
<td><em>chs3Δ::hisG::chs3Δ::hisG-URA3-hisG</em></td>
<td>Bulawa et al. (1995)</td>
</tr>
<tr>
<td>NGY126</td>
<td><em>chs8Δ::hisG::chs8Δ::hisG-URA3-hisG</em></td>
<td>Munro et al. (2003)</td>
</tr>
<tr>
<td>DSY1768</td>
<td><em>cht2Δ::hisG-URA3-hisG::cht2Δ::hisG</em></td>
<td>This work</td>
</tr>
<tr>
<td>SPY24</td>
<td><em>cht3Δ::hisG-URA3-hisG::cht3Δ::hisG</em></td>
<td>This work</td>
</tr>
<tr>
<td>DSY1741</td>
<td><em>cht2Δ::hisG-URA3-hisG::cht2Δ::hisG, cht3Δ::hisG::cht3Δ::hisG</em></td>
<td>This work</td>
</tr>
<tr>
<td><strong>S. cerevisiae</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BY4741</td>
<td><em>MATα, his3Δ1; leu2Δ0; met15Δ0; ura3Δ0</em></td>
<td>EUROSCARF†</td>
</tr>
<tr>
<td><em>chs1Δ</em></td>
<td><em>YNL192w::kanMX4</em> (derived from BY4741)</td>
<td>EUROSCARF</td>
</tr>
<tr>
<td><em>chs3Δ</em></td>
<td><em>YBR023c::kanMX4</em> (derived from BY4741)</td>
<td>EUROSCARF</td>
</tr>
<tr>
<td><em>cts1Δ</em></td>
<td><em>YLR286c::kanMX4</em> (derived from BY4741)</td>
<td>EUROSCARF</td>
</tr>
<tr>
<td>BY4743</td>
<td><em>MATα/MATα; his3Δ1/his3Δ1; leu2Δ0/leu2Δ0; met15Δ0/MET15; LYS2/lys2Δ0; ura3Δ0/ura3Δ0</em></td>
<td>EUROSCARF</td>
</tr>
<tr>
<td><em>chs2Δ</em></td>
<td><em>YBR038w::kanMX4/YBR038w::kanMX4</em> (derived from BY4743)</td>
<td>EUROSCARF</td>
</tr>
</tbody>
</table>

*All *C. albicans* strains are derived from CAF-2.

†European *Saccharomyces cerevisiae* Archive for Functional Analysis (euroscarf@em.uni-frankfurt.de).
200 r.p.m. Germ-tubes were induced using 20% (v/v) fetal calf serum (FCS) at 37 °C (Gov & Gooday, 1982). For the MRP1p: CHSI/ chs1Δ conditional mutant, cells were first grown under permissive conditions in maltose-containing medium, then washed and resuspended in glucose-containing SD medium, which repressed expression of CHSI1, as described by Munro et al. (2001).

Construction of chtΔ mutants. Published data obtained from McCreath et al. (1995) were used to clone the C. albicans CHT2 (U15800) and CHT3 genes (U15801). CHT2 and CHT3 were amplified from C. albicans genomic DNA with primers CHT2-XBA (5′-CCG-TCTAGAGGGCGATGCTTCAATACGATTTGCTTCAT-3′), CHT2-XHO (5′-CGCCGCTGAGGATGCTATCCTCTTTCTTATATATTTACCTTTCTCAGG-3′), CHT3-BAMHI (5′-CCGGCCATGCTGATCATCTAGTTTCTCTATGTTAATCTATT-3′) and CHT3-XHO (5′-CCCGTCGGATCTATACATATCCGTTATGTTAATCTATT-3′). The fragments obtained were cloned into compatible restriction sites of pBluescript KS+ to yield pDS594 and pDS595. Deletions were created for CHT2 between nt +587 (with respect to first ATG) and nt +1076, using primers CHT2-PST (5′-AAATACTCGAGCATCAACCAGAGTCATCAGGTATGACG-3′) and CHT2-BGL (5′-GAAAGATCTTCCTCTCTTCTTCTTCAATCTTCTT-3′), with pDS594 as a template. Deletions were created for CHT3 between nt +525 and +1294, using primers CHT3-PST (5′-AAATACTCGAGGAGGTATCCTATTTGTTATGTTAATCTATTGTTAATCTATT-3′) and CHT3-BGL (5′-GAAAGATCTTCCTCATCCTCTTTCTTCAATCTTCTTCTT-3′), with pDS593 as a template. The PCR products were digested with PstI and BglII and ligated to a PstI–BglII 3.7 kb fragment from pMB7, containing the hisG-URA3-hisG ‘Ura-blaster’ cassette (Fonzi & Irwin, 1993) to yield pDS597 and pDS598 for CHT2 and CHT3 disruptions, respectively. The disruption cassettes were liberated by PstI and BglII digestion, and the linear fragments used for sequential disruption of both alleles of the CHT2 and CHT3 genes after marker regeneration, as described by Fonzi & Irwin (1993). CHT2 and CHT3 disruption was carried out in strain CAF4-2 (Fonzi & Irwin, 1993). The double cht2Δ/cht2Δ cht3Δ/cht3Δ mutant was generated by disrupting CHT2 in the cht3Δ mutant background SPY24 (see Table 1), after marker regeneration.

Measurement of chitinase activity. The fluorogenic microtitre plate assay developed by McCreath & Gooday (1992) was used to measure endochitinase activity, employing the substrate 4-methylumbelliferyl-β-D-N-acetylglucosaminide (4-MU-[GlcnAc]). The substrate is sensitive to endochitinase activity and is relatively insensitive to action by N-acetylgalactosaminidases (Jackson et al., 1996). Chitinase activity was measured both in the extracellular supernatant and in washed whole cells. For hyphal cultures, bovine serum (FCS) at 37 °C, 20% (v/v) fetal calf serum (FCS) at 37 °C, and after a further 5 min incubation at 40 °C, the reaction was stopped by adding 120 μl 5M Na2CO3 was added, and the absorbance was measured. Chitin content was measured by assaying the glucosamine released by acid hydrolysis of chitin (Kapteyn et al., as described by Munro et al., 2001).

Measurement of chitinase activity. The fluorogenic microtitre plate assay developed by McCreath & Gooday (1992) was used to measure endochitinase activity, employing the substrate 4-methylumbelliferyl-β-D-N-acetylglucosaminide (4-MU-[GlcnAc]). The substrate is sensitive to endochitinase activity and is relatively insensitive to action by N-acetylgalactosaminidases (Jackson et al., 1996). Chitinase activity was measured both in the extracellular supernatant and in washed whole cells. For hyphal cultures, bovine serum (FCS) at 37 °C, 20% (v/v) fetal calf serum (FCS) at 37 °C, and after a further 5 min incubation at 40 °C, the reaction was stopped by adding 120 μl 5M Na2CO3 was added, and the absorbance was measured. Chitin content was measured by assaying the glucosamine released by acid hydrolysis of chitin (Kapteyn et al., as described by Munro et al., 2001).

RESULTS

Chitinase activities in chtΔ and chsΔ mutant backgrounds

The activity of endochitinase was measured in strains harbouring single or double chts (chitinase) and chsΔ (chitin synthase) mutations (Fig. 1a). Higher chitinase activity was found in hyphal cells than yeast cells for the conditions that were examined. The specific activity of cell-associated chitinase was higher than the supernatant activity for yeast cells, but the reverse was true for hyphae, where most activity was associated with the supernatant fraction. In the yeast form of C. albicans, the cht2Δ/cht2Δ mutant had a small but significant decrease in chitinase activity while the cht3Δ/cht3Δ mutant had less than 50% of the chitinase activity of the wild-type. Chitinase activity in the double cht2Δ/cht2Δ cht3Δ/cht3Δ mutant was further reduced, confirming that both CaCht2p and CaCht3p contribute to yeast chitinase activity.

The higher level of chitinase activity in the hyphal form of C. albicans was unaffected in the cht2Δ/cht2Δ mutant (Fig. 1b), but low activities were found in the cht3Δ/cht3Δ mutant. This suggests that CaCht3p accounted for most of the enhanced chitinase activity of the hyphal form and that CaCht2p was not expressed, or was poorly expressed, during hyphal growth. These results contrast with the transcription profiles of the CHT2 and CHT3 genes described
previously, which showed both genes to be transcribed preferentially during yeast cell growth (McCreath et al., 1995).

Chitinase activities were also examined in four C. albicans chitin synthase mutant strains: MRP1: CHS1/chs1Δ, chs2A/chs2Δ, chs3Δ/chs3Δ and chs8Δ/chs8Δ. The conditional MRP1-CHS1 strain was grown under repressing conditions in the absence of maltose and the presence of glucose. In the yeast form, all four chsΔ mutants had a slight but statistically significant decrease in cell-associated chitinase activity (Fig. 1a). Supernatant chitinase activity was only significantly lowered in chs1Δ and chs8Δ mutants grown in the yeast form. In the hyphal form, chs1Δ had significantly lower cell-associated chitinase activity, and significantly higher activity in the supernatant. Likewise, the chs1Δ, chs3Δ and chs8Δ hyphal forms had slightly but significantly higher supernatant chitinase activity (P<0.05). The chitinase activity in the other mutants remained unchanged (Fig. 1a).

Chitinase activity was also examined in three S. cerevisiae chitin synthase mutant strains (chs1Δ, chs2Δ, chs3Δ) and in the chitinase mutant cts1Δ. For the chitinase mutant, low residual chitinase activity was observed (Fig. 2). No difference in chitinase activity against 4-MU-[GlcNAc]4 was observed for any of the three chitin synthase mutants. Therefore, although the level of total chitinase activity was in general higher in C. albicans than in S. cerevisiae, mutations in any of the three chitin synthase genes of S. cerevisiae or four chitin synthase genes of C. albicans had little or no effect on the total chitinase activity expressed by either fungus.

**Chitin synthase activities in chtΔ and chsΔ mutant backgrounds**

Chitin synthase activity was measured in cell membrane fractions prepared from a parental strain and chsΔ and chtΔ mutant strains. The *in vitro* chitin synthase activity of both C. albicans and S. cerevisiae predominantly reflects the contributions of the class I ScChs1p and CaChs2p, CaChs8p enzymes. In C. albicans, this activity was not affected in any of the chitinase mutant strains during hyphal growth. However, the cht3Δ and cht2Δ cht3Δ mutants had
significantly higher Chs activity in yeast membranes after trypsin activation (Fig. 3). For the chitin synthase mutants the lowest activity was found, as expected, for the chs2Δ/chs2Δ mutant.

Chitin synthase activities in the membranes of the S. cerevisiae chitin synthase mutants, chs2Δ and chs3Δ, and in the cts1Δ mutant were not affected, and the activity for chs1Δ after trypsin digestion was reduced (Fig. 4). Therefore mutations in ScCTS1 and CaCHT2 chitinase genes did not alter the in vitro chitin synthase activity in either organism.

However, the Cacht3Δ and Cacht2Δ cht3Δ mutants did have significantly higher Chs activity in the yeast form.

**Cell wall chitin content in the chitinase mutants**

If chitinase enzymes act in delicate balance with chitin synthase to achieve the net biosynthesis of chitin, deletions in chitinase genes may alter the chitin content of the cell walls. The total chitin content of both the yeast and the hyphal forms of C. albicans is related mainly to the activity of Chs3, with a minor contribution from Chs2 (Munro et al., 1998, 2003). The chitin content of the yeast and hyphal forms of the C. albicans wild-type strain CAF-2 and cht2Δ/cht2Δ, cht3Δ/cht3Δ and cht2Δ/cht2Δ cht3Δ/cht3Δ chitinase null mutants was therefore determined (Fig. 5).

![Fig. 3](image-url) **Fig. 3.** Chitin synthase activity of chsΔ and chtΔ null mutant strains compared to wild-type strain CAF-2 of C. albicans yeast and hyphal cells. Chitin synthase enzymes are zymogenic and are activated in vitro by addition of trypsin. For this reason the activities were measured with and without trypsin pre-treatment. Error bars are SD, based on three means from three separate experiments. Units of chitin synthase activities are nmol substrate incorporated into chitin (mg protein min⁻¹). Results that were statistically different from control levels (t-test, P<0.05) are marked with an asterisk.

![Fig. 4](image-url) **Fig. 4.** Measurement of chitin synthase activity of chs1Δ, chs2Δ and chs3Δ and cts1Δ null mutant strains compared to their parental strains of S. cerevisiae (BY4741 for chs1Δ and chs3Δ and BY4743 for chs2Δ). The activities were measured with and without trypsin pre-treatment. Error bars are SD, based on three means from three separate experiments. Unfilled bars, non-trypsin-treated samples; solid bars, trypsin-treated. Units of chitin synthase activities are nmol substrate incorporated into chitin (mg protein) min⁻¹.

![Fig. 5](image-url) **Fig. 5.** Chitin content of the yeast and hyphal cells of C. albicans strain CAF-2 and the three chitinase null mutants cht2Δ, cht3Δ, cht2Δ cht3Δ. Assays were performed a minimum of three times, and each sample was measured in triplicate. Error bars are SD, based on three means from three separate experiments. The chitin content of the mutants was significantly different (P<0.01, but not P<0.05). Chitin contents of yeast and hyphal cells are shown as solid and clear bars respectively.
The walls of the yeast and hyphal cells of the cht3Δ/cht3Δ mutant strain showed a small but not statistically significant increase in chitin content, compared to the wild-type strain. In addition, the chitinase mutants showed some hypersensitivity to Calcofluor White, which can reflect an increase in the chitin content of a strain (Fig. 6). These results suggest that the Cht2p and Cht3p chitinases of C. albicans may act on cell wall chitin during normal growth.

**DISCUSSION**

The rationale for this work was based on the hypothesis that wall synthesis and hydrolysis are delicately balanced in the growing regions of fungal cells. Hence, the equilibrium between chitin synthesis and chitin hydrolysis may be regulated in order to maintain the overall plasticity of the expanding bud or hyphal apex. To test this hypothesis, we assessed whether chitinase activity was affected by mutations in chitin synthase genes and, vice versa, whether in vitro chitin synthase activity was influenced by gene deletions in specific chitinase genes. Experimental evidence has been provided for the existence of membrane-associated, zymogenic chitinase enzymes (Humphreys & Gooday, 1984a, b) that could act at the site of chitin content of a strain (Fig. 6). These results suggest that the Cht2p and Cht3p chitinases of C. albicans may act on cell wall chitin during normal growth.

Our investigations did reveal, however, that both chitin synthase and chitinase activity were regulated during yeast–hypha morphogenesis in C. albicans and that CaCht2p and CaCht3p were regulated differentially in hyphal and yeast forms. Both chitinases were active in the yeast form, and loss of function of either of the two chitinase genes resulted in significantly decreased total endochitinase activity. However, during hyphal development, chitinase activity was inferred to be due mainly to the CHT3 gene product. These results would not be predicted by earlier reports of the transcriptional analysis of these genes, where both CHT2 and CHT3 were shown to be expressed preferentially in the yeast form of C. albicans (McCreath et al., 1995). However, in the latter study, hyphae were induced using pH and temperature to regulate morphogenesis, while our experiments used serum to induce hypha formation. The residual chitinolytic activity of single and double cht mutant strains may be due to the CHT4 gene product, recently identified in the C. albicans genome (GenBank AAG35112 and C. Specht, personal communication). Expression of CHT1 has not been detected in either yeast or hyphae and is therefore unlikely to contribute to measured chitinase activity in these experiments (McCreath et al., 1995).

Chitinase activity was not greatly affected by deletion of chitin synthase genes in either growth form of C. albicans. During hyphal growth, most chitinase activity was measured in the supernatant, while in the yeast form, most chitinase activity fractionated with whole cells. These results are compatible with reports showing that CaCht2p is attached to the yeast cell wall (Iranzo et al., 2002) and that...
CaChT3p is a secreted protein (C. Specht, personal communication).

Similar findings were obtained for *S. cerevisiae* mutations in the chitin synthase genes had little effect on chitinase activity. In *C. albicans*, deletion of *CHT2* and *CHT3* led to a slight increase in the overall cell chitin content and slightly increased sensitivity to Calcofluor White. This suggests that Cht2p and Cht3p may act on the cell wall of *C. albicans* and influence its chitin content.

The results obtained for the chitin synthase activities of the chsΔ mutants in *C. albicans* and *S. cerevisiae* are consistent with previous findings. *CaCHS2* and *ScCHS1* encode the major *in vitro* chitin synthase activity for *C. albicans* and *S. cerevisiae*, respectively (Bulawa et al., 1986; Munro et al., 1998). In the yeast form, cht3Δ and cht2Δ chsΔ mutant strains of *C. albicans* showed significant changes in chitin synthase activity. However, chitin synthase activity in the hyphal form of these mutants was unaffected. In addition, in *S. cerevisiae*, no change in chitin synthase activity was observed in the cts1Δ mutant. Chitin synthase genes are known to be regulated by post-transcriptionally (Bulawa, 1992; Choi et al., 1994; Chuang & Schekman, 1996). Little transcriptional regulation of *CHS3* has been observed, although the gene product is activated by the Slt2-dependent salvage pathway that responds to cell wall damage (Popolo et al., 1997, 2001). The formal possibility remains that specific chitin synthase and chitinase enzymes may be coordinately regulated, but our *in vitro* enzyme assays were not sufficiently discriminating to evaluate how particular genes are regulated under different mutant backgrounds. It is possible that specific zymogenic forms are regulated through activation of proenzyme forms of the enzymes at specific cellular sites. To carry this study forward, we are now constructing reporter fusions to examine the transcriptional responses of *CHS* genes to specific genetic and environmental perturbations. However, taken at face value, the results presented here do not support the hypothesis that there is a strong interplay between total chitinase and chitin synthase activities in these fungi.

**ACKNOWLEDGEMENTS**

This work was initiated by Professor Graham Gooday, and is dedicated to his memory as a pioneer of research into chitin synthesis of fungi. We thank Charlie Specht for comments on this manuscript and Oxford GlycoSciences for permitting the use of chitinase mutants. We thank the EC for financial support for a studentship for Serena Selvaggini (EUROCELL WALL – QKL3-2000-01537) and the Wellcome Trust (063204 and 055015) and BBSRC (1/0014870) for support of our laboratory.

**REFERENCES**

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


