Survival and cytokinesis of *Saccharomyces cerevisiae* in the absence of chitin

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Most fungal cell walls are constructed with significant amounts of chitin, a linear polysaccharide that contributes mechanical resistance to the structure. In the yeast *Saccharomyces cerevisiae*, chitin is synthesized by three different isozymes, each of which has a separate cellular function.

In this yeast, the most important role of chitin is in cytokinesis, when a thin primary septum is synthesized by chitin synthase II to separate mother and daughter cells. If no primary septum can be formed, an irregular remedial septum is synthesized, a process that relies on chitin synthase III.

It was found that, with osmotic stabilization, *S. cerevisiae* tolerates a loss of all chitin synthase activities. Chitin-deficient mutants display a cytokinesis defect which leads to the formation of cell chains with incompletely separated cytoplasms. In these mutants septa are formed rarely.

The few septa found are bulky structures which contain inclusions of cytoplasm. Nuclear division proceeds under these conditions, demonstrating that there is no cell cycle arrest triggered by a failure to form a septum between mother and daughter cell. A genetic suppressor arises quickly in chitin-deficient mutants, giving rise to the synthesis of chitin-free remedial septa.

The suppressed chitin-free mutants grow well without osmotic stabilization and display hyper-resistance against the chitin-synthase inhibitor polyoxin D.

INTRODUCTION

The cell walls of fungi and plants consist of two components: a malleable, gel-like substance, and fibres of great tensile strength. In most fungal species, the fibrous component is chitin, a linear polymer of β-1,4-linked N-acetylglucosamine (GlcNAc) residues. Chitin is distributed over the entire cell wall, and in most fungi it appears to be necessary to prevent cell lysis (Ruiz-Herrera & San-Blas, 2003). The cell walls of the model yeast *Saccharomyces cerevisiae* contain comparatively low amounts of chitin, but nevertheless this substance has long been seen as essential to the survival of this organism (reviewed by Cabib et al., 2001). In *S. cerevisiae*, most of the chitin is concentrated at the bud neck, where it plays an important role in cytokinesis. Mutants lacking chitin at the cell periphery have been isolated and were shown to have only a minor growth defect (Shaw et al., 1991). In *S. cerevisiae*, the lack of chitin in the cell wall does not cause cell lysis, showing that cell wall integrity can be maintained even without reinforcement by chitin.

There are three chitin synthase activities in *S. cerevisiae*, two of which are involved in cytokinesis (Shaw et al., 1991). At the beginning of the cell cycle, chitin synthase III activity (CSIII) lays down a ring of chitin at the presumptive bud site through which the bud emerges. At the end of mitosis, chitin synthase II activity (CSII) forms a primary septum from chitin between mother and daughter cell. Finally, after mother and daughter cells have separated through the action of a chitinase, chitin synthase I activity (CSI) acts on the daughter cell wall to repair damage caused by excessive chitinolysis.

Our understanding of the importance of chitin synthesis in *S. cerevisiae* has evolved over time. Based on tetrad analysis of heterozygous deletion mutants, it was first concluded that CSII was essential (Silverman et al., 1988). It was later shown that the apparent inviability of a CSII-deficient spore was due to a germination defect and that CSII was not necessary for growth and cytokinesis (Bulawa & Osmond, 1990). A loss of CSII affects – but does not prevent – cytokinesis. Due to a lack of a primary septum, cells lacking the CSII catalytic moiety Chs2p fail to separate after cytokinesis and form cell clumps. These *chs2* mutants achieve separation of mother and daughter cytoplasms by constructing a remedial septum, which is a bulky structure of cell wall material deposited at the bud neck. The completion of the remedial septum requires CSIII (Cabib & Schmidt, 2003). It was shown that mutants defective in primary septum formation rely on CSIII for cytokinesis and survival. This correlation has been successfully exploited for the isolation of septation mutants by means of a synthetic lethality screen (Osmond et al., 1999; Schmidt et al., 2002).

This study was initiated after the surprising finding that

Abbreviations: CSI, CSII, CSIII, chitin synthase I, II, III; WT, wild-type.
in the presence of osmotic stabilizer a loss of both CSII and CSIII is tolerated. Based on this observation, a mutant lacking the catalytic moiety of all three known chitin synthase activities was constructed. After an initial period of fragility, the mutant acquires a suppressor and grows under normal culture conditions. The aim of this study was to show that growth and cytokinesis are possible in the absence of chitin.

**METHODS**

**Media and culture conditions.** Media and culture conditions were as described by Burke et al. (2000). Growth media and all solutions for strain YMS348 contained 1 M sorbitol unless stated otherwise. YMS348 was grown at 26°C; all other yeast strains were grown at 30°C. Growth of cultures was monitored by following the OD600 and by counting c.f.u. in a hematocytometer.

**Polyoxin D survival.** To assess the toxicity of polyoxin D, cells were grown overnight at 30°C in YPD medium to a titre of 1–2 × 10^8 cells ml^-1. Wet cell mass per ml was determined with an accuracy of 0.1 mg. The cultures were then diluted in fresh medium to 12 μg cells ml^-1, equalling 1 × 10^8 c.f.u. ml^-1 for YPH499 and 2 × 10^8 c.f.u. ml^-1 for YMS348. To 0.1 ml of cells, polyoxin D was added at the specified concentrations and the cultures were incubated overnight at 30°C with agitation. At the beginning and the end of the experiment, cells were counted in a haematocytometer, diluted appropriately and plated on solid YPD medium.

**Strain construction.** All strains were constructed using the procedures described by Crotti et al. (2001). Transformation of yeast strains was achieved with a lithium acetate protocol (Burke et al., 2000). To construct the chs1 chs2 chs3 triple deletion strain YMS348, ECY46 was sporulated and a chs1::HIS3 chs3::LEU2 sporule was isolated. The resulting strain ECY46-1-20D was transformed with a chs2::TRP1 fragment and the transformed cells were incubated on Trp- omission medium containing 1 M sorbitol at 26°C for 10 days. The chs2::TRP1 deletion was verified by PCR as described by Crotti et al. (2001). YMS348 cells with a suppressor (designated YMS348s) were isolated by streaking cells on Trp- omission medium without sorbitol and incubating at 30°C. Strains used in this study are listed in Table 1.

**Determination of chitin synthase activity.** A protocol of Choi & Cabib (1994) was used. First, membranes were isolated according to Orlean (1987) and resuspended in 50 mM sodium phosphate pH 7.5, 33% glycerol at a protein concentration of 4 mg ml^-1. The chitin synthase assay contained, in a total volume of 46 μl, 20 μl membrane suspension, 5 μl 0.5 M Tris/HCl pH 7.8, 5 μl 50 mM magnesium acetate, 5 μl 10 mM [14C]UDP-GlcNAc (5000 c.p.m. μl^-1) and 2 μl of a trypsin solution containing trypsin (Sigma) at concentrations from 0.125 to 1 mg ml^-1. Proteolysis was stopped after incubating for 15 min at 30°C by adding 2 μl of a soybean trypsin inhibitor solution at 1:5 × the concentration of the trypsin solution. Chitin synthesis was initiated by adding 2 μl 0.8 M GlcNAc. After incubating for 45 min at 30°C, chitin synthesis was stopped by adding 1 ml 10% trichloroacetic acid. Reaction mixtures were filtered through a Pall A/E glass fibre filter. Radioactivity was detected in a scintillation counter after adding 5 ml cytosine ES scintillation cocktail (ICN) to the washed and dried filters.

**Vital staining.** Staining of dead cells was achieved by washing cells once with water and 1 M sorbitol and incubating in 20 mg ml^-1 methylene blue in 50 mM KH₂PO₄ with or without 1 M sorbitol for 5 min at room temperature.

**Cell wall digestion and nuclear staining.** Analysis of cytokinesis by fixation and cell wall digestion was accomplished as previously described (Cabi & Schmidt, 2003). Cells were grown overnight in YPD medium to a titre of 1–2 × 10⁹ cells ml^-1. Then 5 ml of cells were fixed by addition of formaldehyde to a final concentration of 5%. After overnight incubation at 4°C, fixed cells were washed once with PBS and suspended in 1 ml citrate/phosphate buffer (40 mM Na₂HPO₄ and 20 mM citric acid mixed 39:61 to obtain pH 6.5, 0.8 M sorbitol, 1 mM EDTA, 50 mM β-mercaptoethanol). After this step, mechanical stress was carefully avoided. One hundred microlitres of Glusulase (Perkin-Elmer Life Sciences) was added to the fixed cells, and cell walls were digested at 30°C for 90 min with gentle shaking (60 r.p.m.). Spheroplast-like appearance of the digested cells indicated complete cell wall removal. Nuclei were stained by adding 1 μl of 0.1 mg ml^-1 Hoechst 33342 to the fixed, digested cells and incubating for 5 min at room temperature. Fluorescence was monitored with a standard DAPI filter set on a Zeiss Axioskop 2 fluorescence microscope.

**Electron microscopy.** Cells were grown in 100 ml YPD medium to a titre of 1–3 × 10⁹ cells ml^-1, spun down, washed once with water and resuspended in 1 ml 3% glutaraldehyde, 0.1 M cacodylate containing 5 mM CaCl₂ and 5 mM MgCl₂. Cells were dispersed and embedded in agarose, cooled and cut. Blocks were fixed in 4% KMnO₄ for 1 h at room temperature, washed thoroughly with water and incubated with 0.5% sodium metaperiodate for 15 min at room temperature. After washing with 50 mM potassium phosphate (pH 7.4), blocks were incubated in 50 mM ammonium phosphate (pH 7.4) for 5 min. After washing twice with water, blocks were placed overnight into 2% uranyl acetate (pH 4.5) at room temperature in the dark. Blocks were dehydrated through a graded series of ethanol solutions (50–100%, v/v, at 40°C) and left overnight in

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**Table 1.** Strains used in this study

<table>
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<tr>
<th>Strain</th>
<th>Genotype</th>
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<td>YPH499</td>
<td>MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-A1</td>
<td>Sikorski &amp; Hieter (1989)</td>
</tr>
<tr>
<td>YMS11</td>
<td>MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-A1 chs2::TRP1</td>
<td>Crotti et al. (2001)</td>
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<td>ECY46</td>
<td>MATa/MATα ura3-52/ura3-52 lys2-801/lys2-801 ade2-101/ade2-101 trp1-Δ63/trp1-Δ63 his3-Δ200/his3-Δ200 leu2-Δ1/leu2-Δ1 CHS1/chs1::HIS3 CHS2/chs2::TRP1 CHS3/chs3::Leu2</td>
<td>Crotti et al. (2001)</td>
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<td>This study</td>
</tr>
<tr>
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<td>MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-A1 chs1::HIS3 chs2::TRP1 chs3::LEU2</td>
<td>This study</td>
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<td>This study</td>
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fresh 100% ethanol at room temperature. They were then washed twice with 100% ethanol and twice with propylene oxide before being embedded in Spurr resin. From these blocks, sections were cut and post-stained with lead citrate for 2-5 min.

RESULTS

Deletion of chitin synthase genes causes a severe growth defect

The transformation of ECY46-1-20D with a chs2::TRP1 fragment yielded 1 chs2::TRP1 deletant per μg DNA. The deletion of the CHS1, CHS2 and CHS3 reading frames in the genome of the transformants was verified by PCR. One deletant, YMS348, was studied further. The triple deletant cells formed large clumps containing several hundred cells (Fig. 1a), grew with a generation time of about 7.5 h (YPD + 1 M sorbitol, 26 °C) and were not able to grow without 1 M sorbitol or at 30 °C. Repeated streaking of mutant cells on medium without sorbitol and incubating at 30 °C yielded several colonies of the triple mutant with a genetic suppressor. The suppressed strain, designated YMS348s, grew well on solid medium without sorbitol even at 30 °C and formed small clumps when cultured in liquid medium (Fig. 1b). The genotype of YMS348s with respect to chitin synthase genes was again verified by PCR. The generation time of YMS348s was about 5.5 h at 26 °C in YPD + 1 M sorbitol. The suppressed strain did not depend on osmotic stabilization in liquid medium and had a remarkably short generation time of about 3 h when grown at 30 °C in YPD without sorbitol. This is comparable to the growth of the chs2 mutant strain YMS11 under the same

![Fig. 1. Morphology and viability of chs1 chs2 chs3 triple mutants with and without suppressor. Cells were grown in YPD + 1 M sorbitol at 26 °C. Scale bars, 10 μm. (a) YMS348, differential interference contrast (DIC) image of the outer region of a cell clump; (b) YMS348s, DIC image of cell clump; (c) methylene blue staining of a normal-sized cell clump of YMS348; (d) methylene blue staining of normal-sized clumps of YMS348s. The staining reveals many dead cells in the centre of the aggregates. In (c) and (d) the size difference between clumps of YMS348 and YMS348s is evident.](http://mic.sgmjournals.org)
conditions (3.25 h). Vital staining with methylene blue showed that a considerable amount of the cells within the clumps were dead, regardless of the presence of suppressor: 37.9% of YMS348 and 36.5% of YMS348s (minimum 1000 cells counted, Fig. 1c, d).

**chs1 chs2 chs3 triple deletion mutants do not synthesize chitin**

The cell walls of YMS348 contain low amounts of Calcofluor-white-stainable material (data not shown). Because of the aggregation of the cells and the bright fluorescence emitted from dead cells in the centre of the clumps, the exact distribution of this fluorescence was difficult to determine. To exclude the possibility that this fluorescent material was chitin synthesized by a novel, fourth chitin synthase activity, chitin synthase activities of isolated membrane vesicles of YPH499 and YMS348s were compared. No chitin synthase activity ($-0.9\pm2.5\%$ of WT, $n=2$) could be detected in YMS348s, either with or without activation by trypsinization. The confusing staining of the cell walls of strain YMS348 and YMS348s with Calcofluor white hence must be due to binding of the dye to cell components other than chitin.

**Chitin-free mutants become hyper-resistant to polyoxin D**

The fungicidal effect of the chitin synthase inhibitor polyoxin D was examined in exponentially growing cultures (Fig. 2). In the WT, polyoxin D was fungistatic at 0.45 mg ml$^{-1}$ and resulted in 90% killing at 0.75 mg ml$^{-1}$. In these experiments, the chitin-free mutant YMS348s was not at all inhibited by polyoxin D. In a separate experiment, cells were incubated with polyoxin D at 2 mg ml$^{-1}$, which resulted in 99.5% killing of YPH499 cells but had no inhibitory effect on strain YMS348s. In order to determine whether the occasional survival of YPH499 cells at high doses of polyoxin D was due to chance or intrinsic resistance, three colonies of cells that had survived exposure to 2 mg polyoxin ml$^{-1}$ were incubated overnight with 1 mg polyoxin D ml$^{-1}$. All three strains previously exposed to polyoxin D were more resistant ($75.1\pm33.9\%$ killing) than the control strain that had not been exposed (99.7% killing).

**The suppressor mechanism affects septum formation**

Nuclear division and abscission in YMS348 and YMS348s was studied in order to learn about the ultrastructural defect of a chitin-free mutant and the nature of the suppressor mutation. By fixing the cells with formaldehyde and subsequently removing the cell wall, it could be determined whether cells which appeared by light microscopy to be connected indeed shared cytoplasm. It was found that YMS348 frequently failed to form septa, leading to the formation of chains containing up to eight cells (Fig. 3b, e). The formation of cell chains (as opposed to globular structures) also indicates that the mutant switches from the axial budding pattern of the wild-type to a polar budding pattern. The suppressor present in YMS348s leads to a significant shortening of the cell chains (Fig. 3c, e), showing that this strain has a better ability to form septa. Since a chs2 single mutant does not form cell chains at all, the formation of chains in YMS348s shows that even in the presence of the suppressor the lack of CSIII is a disadvantage for a chitin-deficient mutant (Fig. 3d, e).

**Nuclear division continues despite failure to construct a septum**

Nuclear division and migration was examined by fixing the cells, removing the walls and staining the remains with Hoechst 33342. It was found that almost all cells in chains of chitin-deficient mutants did contain at least one nucleus (Fig. 4), regardless of the presence of the suppressor. A quantitative analysis confirmed this finding (Table 2). It is evident that nuclear division is not affected by incomplete abscission. In over 9% of cells of YMS348, however, two separate nuclei could be observed (Fig. 4a, Table 2). This indicates an occasional failure to correctly distribute nuclei to daughter cells.

**Ultrastructure of chitin-free septa with and without suppressor**

The ultrastructure of the septal region of YMS348 and YMS348s was examined in order to characterize the default mechanism of septation in the absence of chitin (Fig. 5). It was found that abscission in both strains is achieved by thickening of the lateral cell walls of the bud neck. This leads to the formation of bulky structures which frequently contain cytoplasmic inclusions (lacunae). The presence of the suppressor does not affect the appearance of the default septa (Fig. 5c).

![Fig. 2. Survival in the presence of polyoxin D. The figure shows the means and standard deviations of cell growth relative to starting cell titre of two independent experiments. Cells were incubated for 18 h with polyoxin D. △, YPH499 (WT); ○, YMS348s.](image-url)
Fig. 3. Formation of cell chains due to incomplete abscission in YMS348. Cells were fixed and cell walls were enzymically removed. (a) YPH499 (WT), (b) YMS348, (c) YMS348s, (d) YMS11. YMS348 cells stay connected by thin stalks of cytoplasm (arrow), indicating the absence of a septum. (e) Statistical analysis of cell group size after cell wall removal. YMS348 forms chains with up to eight cells. It may well be possible that in culture the chains even extend beyond eight cells. The thin cytoplasmic stalks connecting the cells are fragile and may break during spheroplasting (Cabib & Schmidt, 2003). Chains of YMS348s are considerably shorter than chains of YMS348, which shows an improved ability to form septa.

Fig. 4. Composite figure of Hoechst 33324-stained cells from which the cell wall has been removed. (a) YMS348. Nuclei are properly replicated and divided. Binucleate cells can be observed occasionally (arrow), indicating a problem with nuclear segregation. (b) YMS348s. The suppressor enables the strain to form septa more efficiently. Binucleate cells are not observed. Scale bar, 10 μm.
**DISCUSSION**

A yeast strain in which the genes for all three known chitin synthase genes have been destroyed is viable and, after an initial period of fragility, acquires a suppressor mutation that enables it to grow under normal culture conditions. In contrast to the situation in other fungi, in *S. cerevisiae* chitin is not necessary for reinforcement of the peripheral cell wall. Mutants devoid of CSIII do not contain peripheral chitin but grow well even without osmotic stabilization (Shaw et al., 1991). The cause of the growth defects in the absence of chitin can be found in the last stage of cytokinesis, abscission, in which a primary septum is synthesized centripetally to separate mother and daughter cells. It has long been known that construction of the primary septum requires chitin synthesis (Bacon et al., 1966), whereas the mechanism that ensures cell survival when primary septum formation is abolished has been fully explained only recently (Cabib & Schmidt, 2003). A primary septum is normally formed through the close interaction between chitin synthase II and a contractile actomyosin ring at the bud neck (reviewed by Cabib, 2004). The contraction of the actomyosin ring pulls the plasma membrane inwards while CSII in the membrane extrudes chitin into the extracellular space, thus forming a thin chitin disk. If no primary septum can be formed, cells lay down cell wall material at the bud neck constriction, forming a crude structure which eventually separates mother from daughter cytoplasm (Shaw et al., 1991). This structure, designated the remedial septum, is rich in chitin which is synthesized by CSIII. Because of this function of CSIII, cells unable to synthesize a primary septum rely on CSIII for survival under normal culture conditions (Cabib & Schmidt, 2003). The construction of a viable chitin-synthase-deficient mutant has been attempted previously by segregation of a plasmid containing a

<table>
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<th>Strain</th>
<th>Cell clumps with more than one nucleus missing (%)</th>
<th>Binucleate cells (%)</th>
<th>Clumps analysed for nuclei</th>
<th>Mean clump size (cells per clump)</th>
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<td>YMS348s</td>
<td>0</td>
<td>0.3</td>
<td>262</td>
<td>2.02</td>
<td>738</td>
</tr>
</tbody>
</table>

**Table 2. Distribution of nuclei within cell clumps**

Cells were fixed, the cell walls removed and the nuclei stained with Hoechst 33324.

**Fig. 5.** Ultrastructure of the septa formed in (a) YPH499 (WT), (b) YMS348 and (c) YMS348s. The lack of a primary septum leads to the formation of bulky remedial septa at the bud neck, regardless of the presence or absence of the suppressor mutation. In the top panel of (c), a remedial septum under construction is shown. Note the layering of cell wall material at the bud, which indicates growth by thickening of the lateral cell wall. Scale bar, 1 μm.
repressible copy of CHS2 from a chs2 chs3 double mutant (Shaw et al., 1991). Although the attempt was unsuccessful, the authors did report a beneficial effect of osmotic stabilization on short-term survival of chitin-deficient mutants.

The apparent viability of a strain deleted in all of the known chitin synthase genes poses three questions. First, is there really no chitin synthesis in this strain? Second, how is cytokinesis achieved without the assistance of chitin? And third, how does the cytokinesis defect affect nuclear division and cell cycle progression?

The complete absence of chitin synthase activity in isolated membrane vesicles of YMS348s confirms that there are no more than three chitin syntheses encoded in the genome of S. cerevisiae. This could be expected, although this is the lowest number encountered so far in fungi (see overview by Roncero, 2002). Chitin syntheses can be identified based on genomic sequences because of highly conserved domains (Bowen et al., 1992). In the S. cerevisiae genome, only the three known chitin syntheses contain these domains, which made the existence of a fourth chitin synthase activity highly unlikely.

How is cytokinesis achieved in the absence of chitin? The data presented here indicate that the loss of chitin synthase activity causes a failure to synthesize a septum and leads to the formation of cell chains. Clearly, the synthesis of chitin does help with the timely completion of remedial septa. It has been suggested that the unique mechanism by which fungi divide -- contraction of an actomyosin ring coupled to synthesis of cell wall -- is necessary because a high cellular turgor pressure works against the advancing septa (Cabib, 2004). In the same way, reinforcement of remedial septa by chitin becomes necessary to overcome the turgor pressure (Cabib & Schmidt, 2003). A chitin-deficient mutant initially grows only in the presence of 1 M sorbitol. This hyperosmotic growth condition is known to lower the intracellular turgor pressure, which lowers the resistance that the advancing septa must overcome. However, hyperosmotic growth conditions trigger an adaptive response that aims at increasing the intracellular pressure back to the equilibrium (reviewed by Hohmann, 2002). Because of this physiological response, the actual cellular turgor pressure under hyperosmotic growth conditions is hard to predict.

An appealing interpretation of the present results is that it is not the mechanical qualities that make chitin essential for the construction of remedial septa but rather its abundance. The presence of the suppressor enables yeast cells to form remedial septa even in the absence of chitin, which suggests that the function of chitin in these structures can be taken over by other cell wall components. As can be seen in the electron micrographs, the remedial septa contain significant amounts of cell wall material. The timely completion of these structures from glucan and mannoproteins alone may exceed the cell’s biosynthetic abilities. This study shows that the suppressor changes the rate of synthesis rather than the ultrastructure of the remedial septa. The apparent rate of septum synthesis is a function of synthesis and degradation of cell wall material. Consequently, the suppressor either increases the deposition of septum material or decreases septum degradation by inhibiting enzymes such as Eng1p (Baladron et al., 2002). The molecular nature of the suppressor mechanism is still obscure and will be the subject of further studies. Unfortunately, classic genetic analysis is complicated by low mating efficiency, the germination defect of chs2 mutant spores and the rapid accumulation of suppressor in non-suppressed cultures.

How does a failure to close the septum affect nuclear division and cell cycle progression? Interestingly, completion of cell separation is not necessary for the initiation of a new cell cycle in S. cerevisiae. This is illustrated by the fact that a failure to assemble the septation apparatus or to contract the actomyosin ring at the bud neck gives rise to chains of incompletely separated cells, which nevertheless have properly divided nuclei (Bi et al., 1993; Vallen et al., 1995; Luca et al., 2001; Lim et al., 2003). If the cells were arresting the cell cycle at the M/G1 border because of the abscission defect, it would cause the accumulation of large budded cells with separated nuclei (Hartwell et al., 1973; Jaspersen et al., 1998). This could not be observed in chitin-deficient mutants. The analysis of nuclear division in YMS348 reveals that even with mother and daughter cells not separating their cytoplasm, the cells continue the mitotic cycles -- which then leads to the formation of multinucleolate cell chains.

Although a failure to form a septum in a timely manner does not arrest the cell cycle, it apparently does influence nuclear segregation. Analysis of nuclear distribution showed that binucleate cells can be found frequently in cell chains of YMS348. Binucleate cells are a consequence of incorrect positioning of the mitotic spindle. The proper positioning of the spindle is a process that depends on the interaction of cytoplasmic microtubules with the cell cortex (Cottingham & Hoyt, 1997; De Zwaan et al., 1997; Lee et al., 1999). In some way, growth in the absence of chitin disturbs this interaction. It goes beyond the scope of this study to satisfactorily explain the defect leading to nuclear missegregation. It should be noted, however, that the formation of bulky remedial septa is known to influence some cortical landmark proteins (Schmidt et al., 2002), which makes it likely that the cortical component of the microtubule interaction is impaired in the chitin-deficient strain.

Finally, what does the possibility of life without chitin mean for medical mycology? Despite strong similarities in some areas, S. cerevisiae is very different from pathogenic fungi in many other respects. Especially the physical properties of the cell wall seem to be different, since S. cerevisiae is able to grow without chitin reinforcement of the lateral walls whereas even its close relative, Candida albicans, is not (Munro et al., 2001). In pathogenic fungi, chitin synthesis is a prime target for antifungal drugs, and several drugs
with strong in vitro inhibitory potential are available. However, none of these drugs has been very effective in vivo (reviewed by Ruiz-Herrera & San-Blas, 2003). This has been attributed to a variety of factors, including limited uptake of the inhibitor into the fungal cell and different susceptibilities of chitin synthases to different inhibitors, with no inhibitor being equally effective on all isozymes. The present study shows that growth of yeast in the absence of chitin is not only possible, but also leads to a hyper-susceptibility of chitin synthases to different inhibitors. Although uptake of the inhibitor into the fungal cell and different locations of chitin is not only possible, but also leads to a hyper-susceptibility of chitin synthases to different inhibitors, with no inhibitor being equally effective on all isozymes.

in Saccharomyces cerevisiae, there might also be a similar resistance to chitin synthase inhibitors. Although a late mitotic regulatory network controlling cyclin destruction in Saccharomyces cerevisiae. Mol Biol Cell 9, 2803–2817.

Chitin synthase I and II are not required for chitin synthesis in budding yeast. Mol Biol Cell 21, 6972–6983.

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