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Chitin synthase 2 is essential for septum formation and cell division in *Saccharomyces cerevisiae*

(cell wall/yeast)

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ABSTRACT **Previous work led to the puzzling conclusion that chitin synthase 1, the major chitin synthase activity in *Saccharomyces cerevisiae*, is not required for synthesis of the chitinous primary septum. The mechanism of *in vivo* synthesis of chitin has now been clarified by cloning the structural gene for the newly found chitin synthase 2, a relatively minor activity in yeast. Disruption of the chitin synthase 2 gene results in the loss of well-defined septa and in growth arrest, establishing that the gene product is essential for both septum formation and cell division.**

Chitin is an important constituent of fungal cell walls. In *Saccharomyces*, it is the major, if not the only, component of the primary septum that forms between mother and daughter cells at cell division. This localization led us to study the mechanism of regulation of chitin synthesis in an effort to dissect, at the molecular level, the steps leading to septum morphogenesis and cell division (1, 2).

Recently, the structural gene for the major chitin synthase of *Saccharomyces cerevisiae* (chitin synthase 1 or Chs1) was cloned (3). Gene disruption experiments led to the surprising conclusion that Chs1 is not required for chitin synthesis and septum formation (3) and indicated that another enzyme must be responsible for chitin formation in vegetative cells. A candidate for this function, chitin synthase 2 (Chs2) was subsequently found in cells harboring a disrupted *CHS1* gene (4). A chitin synthase activity was detected in the same strain by Orlean (5). Chs2 shares certain properties with Chs1, including activation by proteases and localization to the plasma membrane; it differs in cation dependence and pH optimum (4). To ascertain whether Chs2 is required for chitin synthesis and cell division, we undertook to clone the corresponding gene. This report describes the successful cloning of the *CHS2* gene and the effects of its disruption.

MATERIALS AND METHODS

Strains and Media. *S. cerevisiae* SS504-6D (*MAT***a**, *chs1*::*URA3(3)*,*ura3-52*, *leu2-3,112*) and *Schizosaccharomyces pombe* ATCC 38399 (*h*$^-$,*leu1-32*) were used as recipients in transformations with plasmids carrying the *CHS2* gene. *S. cerevisiae* SS543 (*MAT***a**/*MATα*,*ura3-52*/*ura3-52*,*leu2-3,112*/*leu2-3,112*,His−/+, +/His−,*hom3*/*HOM3*,*TYR1*/*tyr1*) and JW17 (*MAT***a**/*MATα*,*chs1*::*URA3(3)*/*chs1*::*URA3*-(3),*leu2-3,112*/*leu2-3,112*,*ura3-52*/*ura3-52*) were recipients in gene disruption experiments. *S. cerevisiae* 7882-1B (ref. 6; *MAT***a**,*his4-912δ*,*arg11*) was a source of RNA for RNA blots. *Escherichia coli* strain DH5-α (Bethesda Research Laboratories, competent cells) was used for transformation and plasmid preparation. Media for *S. cerevisiae* were as described (7). *Sc. pombe* was grown in 2% glucose/0.67% Bacto yeast nitrogen base with amino acids or in YED (0.5% Bacto yeast extract/3% glucose). *E. coli* was grown in Luria broth supplemented with ampicillin (50 mg/liter).

Enzyme Preparations and Chitin Synthase Assay. Membrane preparations from protoplasts (Table 1) were obtained as described (4). Membranes were also obtained after disruption of intact cells with glass beads, essentially as reported by Orlean (5) but with the following modifications: to 0.5 g of cells (wet weight) in a 15-ml Corex tube was added 1.66 g of glass beads (Braun Melsungen, Burlingame, CA; diameter, 0.5 mm) and 1.5 ml of 50 mM Tris chloride (pH 7.5) containing 5 mM magnesium acetate. The tube was Vortex mixed at maximum speed for sixteen 15-sec intervals. In between Vortex mixings, the tube was cooled in ice water until the temperature reached 2°C. The maximum temperature attained was 6°C. The assay of chitin synthase activity has been described (4).

Yeast Transformations. *S. cerevisiae* was transformed with the lithium acetate procedure as described (8). *Sc. pombe* protoplasts were transformed as described by Beach and Nurse (9).

Isolation of a *CHS2* Clone. Strain SS504-6D was transformed to leucine independence by a library of yeast DNA in the yeast/*E. coli* shuttle vector YEp13 (ref. 10; kindly provided by Dennis Thiele, National Cancer Institute). The library had been constructed by inserting yeast DNA fragments partially digested with *Sau3*AI into the YEp13 *Bam*HI site. Approximately 18,000 colonies were screened for overproduction of chitin synthase activity by transferring the colonies to filter paper, followed by drying to permeabilize the cells (3). The assay mixture for Chs2 contained 30 mM Tris chloride (pH 8.0), 2.5 mM $Co(CH_3COO)_2$, 32 mM *N*-acetylglucosamine, 1 mM uridine diphospho-*N*-acetyl-D-[U-^{14}C]glucosamine (400,000 cpm/μmol), and trypsin (40 μg/ml). Incubation of filter-bound dried colonies was carried out in a humidified chamber at 30°C for 3 hr. Filters were washed (3) and exposed to Kodak XAR-2 film at room temperature for up to 1 week. Colonies that showed a much higher than average signal were reassayed for enzyme activity and plasmid stability. An isolate with the desired properties was chosen for analysis of its plasmid (pSS1).

Plasmid Constructions. Restriction endonucleases were from New England Biolabs and T4 DNA ligase was from Promega Biotec (Madison, WI). Calf intestinal phosphatase was from Boehringer Mannheim. The entire yeast DNA insert of pSS1 was subcloned into YEp351 (ref. 11; kindly provided by Alan Myers, Iowa State University) by cleaving pSS1 with *Hin*dIII and *Sph* I and inserting the resulting ≈6-kilobase (kb) DNA fragment into YEp351 cleaved with the same enzymes. The resulting plasmid, pSS2, was used to construct plasmids pSS2B, pSS2X, and pSS2S by cleavage with *Bgl* II, *Xba* I, or *Sal* I, respectively. The larger of the two

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Abbreviations: Chs1, chitin synthase 1; Chs2, chitin synthase 2; *CHS1* and *CHS2*, the corresponding structural genes.

fragments obtained in each case was isolated by agarose electrophoresis. Its ends were then ligated to yield a plasmid similar to the original one but lacking a single restriction fragment. Plasmid pSS2XP was plasmid pSS2X cleaved with *Pst* I and religated in a similar fashion.

Two plasmids were constructed for the gene disruption experiments. pSS2X and pSS2XP were cleaved with *Bgl* II and *Sal* I. Into each linearized plasmid was ligated the 2.3-kb *Bgl* II/*Sal* I fragment of YEp13 containing the *LEU2* gene. Each of these resulting plasmids was cleaved with both *Pst* I and *Xba* I and the restriction fragments containing *chs2::LEU2* were isolated by agarose gel electrophoresis and electroelution for yeast transformation.

Two plasmids were constructed for producing a radioactive probe for *CHS2* DNA or RNA. pGEM3Z (Promega Biotec) was cleaved with *Xba* I or *Pst* I and a 5.4-kb *Xba* I restriction fragment from pSS1 or a 3.5-kb *Pst* I fragment from pSS2, each containing a large portion of the original Chs2 DNA insert, was ligated into the appropriate site yielding, respectively, pGX and pGP.

DNA and RNA Preparations and Blots. Yeast DNA was prepared as described by Sherman *et al.* (ref. 7; 40-ml minipreparation). Blotting of plasmid or genomic DNA to nitrocellulose (Schleicher & Schuell) was achieved by standard capillary transfer (12). Poly(A)$^+$ RNA preparation, transfer to nitrocellulose membranes, and hybridization to radiolabeled DNA or RNA was performed as described (13). Radiolabeled RNA (with [^{32}P]CTP; Amersham) was transcribed from pGX or pGP plasmids by using SP6 or T7 polymerase, as described by the manufacturer (Promega Biotec) to produce single-stranded probes. Radiolabeled DNA (with [^{32}P]dCTP; Amersham) was produced by nick-translation of restriction fragments with an Amersham kit to produce probes 1 and 2 from plasmid pSS1.

Microscopy. Spores that had germinated overnight were first micromanipulated onto yeast extract/peptone/dextrose (YEPD) agar containing Calcofluor (50 μg/ml) and left at 30°C for 90 min. Cells were then micromanipulated two more times to a thin layer of Calcofluor-free YEPD agar on a microscope slide. A coverslip was placed directly on the agar and the slide was mounted on the microscope for normal phase or fluorescence examination with a Zeiss 1M microscope with a G365 UV filter, FT395 mirror, and LP420 barrier filter.

RESULTS

Cloning the *CHS2* Gene. Since no mutant in *CHS2* was available, the gene could not be cloned by complementation. Therefore, we decided to rely on overproduction of the enzyme conferred by the presence of *CHS2* on a high-copy plasmid. Accordingly, a strain containing a *leu2* mutation and a disrupted *CHS1* gene (*chs1::URA3*, SS504-6D) was transformed with a YEp13 shuttle plasmid (which contains a *LEU2* gene) carrying a yeast genomic library. Chitin synthase activity was measured directly on colonies of transformants. The method was similar to that used for *CHS1* (3), except that trypsin and Co^{2+} were added to the incubation mixture to optimize the activity of Chs2.

With the procedure described above, a transformant was isolated that overproduced chitin synthase activity by a factor of 50–80 as compared to the recipient cells (Table 1). Growth of the transformant in nonselective medium led to loss of leucine independence, indicating the presence of a plasmid (pSS1) that could be lost by mitotic segregation. DNA from the transformant strain was amplified in *E. coli*; plasmid was isolated and used to transform the original recipient. The new transformants again showed high chitin synthase activity (data not shown). The enzymatic activity resembled that of Chs2 in cation dependence—i.e., the activity was higher with Co^{2+} than with Mg^{2+} (Table 1), whereas Co^{2+} is without effect on Chs1 (4). Furthermore, the enzyme showed a neutral to alkaline pH optimum, in contrast to Chs1, which has maximal activity at pH 6–6.5 (4). Despite the high activity in the transformant, all the enzyme was membrane bound, as in the recipient strain.

Table 1. Chitin synthase activity in transformant containing plasmid pSS1

Strain	Chitin synthase activity, milliunits per mg of protein			
	With Mg^{2+}		With Co^{2+}	
	− trypsin	+ trypsin	− trypsin	+ trypsin
Recipient	0.013	0.093	0.085	0.29
Transformant	0.54	7.6	1.0	10

Membrane fractions were obtained from protoplasts and assayed as described. Mg^{2+} was 4 mM and Co^{2+} was 2.5 mM. One unit is defined as the amount of enzyme that catalyzes the incorporation of 1 μmol of *N*-acetylglucosamine per min into chitin at 30°C. The recipient strain is SS504-6D.

The activity of the enzyme was inhibited by polyoxin D, an inhibitor of Chs1 and Chs2. The reaction product was hydrolyzed by a chitinase from *Serratia* (14) as found for Chs2 from wild-type cells (results not shown). It may be observed in Table 1 that the activity of the synthase in the transformed strain is highly dependent on previous trypsin treatment, even more so than in the recipient strain. Thus, the chitin synthase of the transformed strain appears to be mainly in the zymogen form.

Restriction Map of the Insert and Effect of Deletions. The original DNA insert was excised and inserted in the vector YEp351 (Fig. 1, plasmid pSS2). The restriction map of the insert differs from that of *CHS1* (3). Genomic Southern blots showed hybridization to a single locus (results not shown). A radiolabeled probe from pSS2 detected pSS2 restriction fragments on a Southern blot but did not hybridize to a 10-fold higher amount of *CHS1* DNA on the same blot under conditions of high or low stringency. These findings are in agreement with the previous observation that *CHS1* hybridizes to a single chromosomal gene (3).

Deletions of the DNA insert were carried out in an attempt to define the minimal sequence required for Chs2 activity in

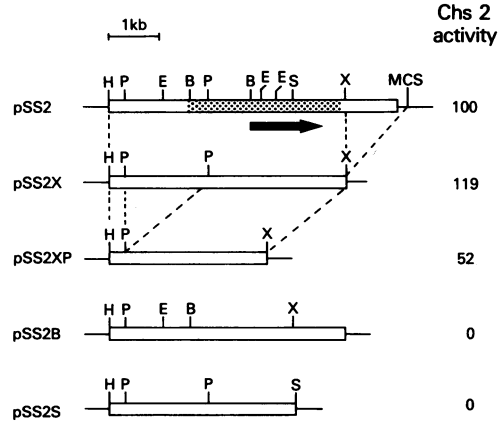

FIG. 1. Structure of different plasmids used in this study. Arrow points in the direction of transcription. Stippled area, approximate location of the 3.1-kb RNA transcript. B, *Bgl* II; E, *Eco*RI; H, *Hin*dIII; P, *Pst* I; S, *Sal* I; X, *Xba* I; MCS, multiple cloning site. Column at right shows the relative values of Chs2 specific activity found in cells harboring the different plasmids. The first three data were recalculated from those of Table 2 (activity with Co^{2+} after trypsin treatment). The activity determined in untransformed cells was subtracted in each case.

transformed yeast cells (see *Materials and Methods*). Deletion of the fragment between the *Xba* I site and the multiple cloning site did not alter enzymatic activity (Fig. 1, plasmid pSS2X). However, further deletion of the fragment between the two *Pst* I sites (Fig. 1, plasmid pSS2XP) led to a 50% loss in chitin synthase activity, suggesting that part of the promoter or part of the coding sequence had been eliminated. Deletion of the 1.1-kb *Bgl* II fragment of pSS2 (Fig. 1, plasmid pSS2B), or deletion of DNA from the *Sal* I site to the multiple cloning site (Fig. 1, plasmid pSS2S) was accompanied by total loss of enzymatic activity. On the basis of these findings, it is concluded that, among those tested, the minimal sequence that gives rise to at least partial enzymatic activity is that of pSS2XP.

mRNA Size and Direction of Transcription. RNA blot analysis (15) of yeast poly(A)$^+$ RNA was performed with the DNA and RNA probes described in Fig. 2*A*. Fig. 2*B* shows a typical result: probe 1 DNA hybridized to one major transcript of 3.1-kb and to an ≈1-kb transcript of lower abundance. Probe 2 DNA hybridized only to the 3.1-kb transcript. Single-stranded RNA probes (Fig. 2*A*) were used to determine the orientation of the transcripts: probe 5 detected only the 3.1-kb transcript, and probe 3 detected the 3.1-kb and an ≈1.2-kb transcript. Probe 4 did not detect any transcripts (data not shown). The 3.1-kb RNA is therefore transcribed from left to right (Fig. 2*A*) as is the smaller downstream RNA. The 1-kb RNA upstream of the 3.1-kb RNA is transcribed in the opposite direction. The 3.1-kb transcript is therefore the only RNA that corresponds to the minimal DNA sequence (Fig. 1, pSS2XP) responsible for the high Chs2 activity in transformants.

Expression of *CHS2* in *Sc. pombe*. To ascertain whether the cloned gene was the structural gene for Chs2, plasmids with different inserts were used to transform cells of *Sc. pombe*, an organism devoid of chitin or chitin synthase.

Transformation of an *Sc. pombe* leucine auxotroph with the YEp13 vector (16) resulted in complementation of the Leu$^-$ phenotype, but did not result in the presence of chitin synthase activity (Table 2). The pSS1 plasmid, however, conferred an activity of the same order of magnitude as that in wild-type *S. cerevisiae* (cf. Table 1). It is possible that in this case the plasmid was maintained at a low copy number (16) or had integrated into a chromosome. The enzymatic activity was ≈3 times higher with pSS2X and, surprisingly, was 65-fold higher with pSS2XP (activity with Mg^{2+} after trypsin treatment). The resulting level of enzymatic activity is similar to that obtained with the same plasmid in *S. cerevisiae* (Table 2).

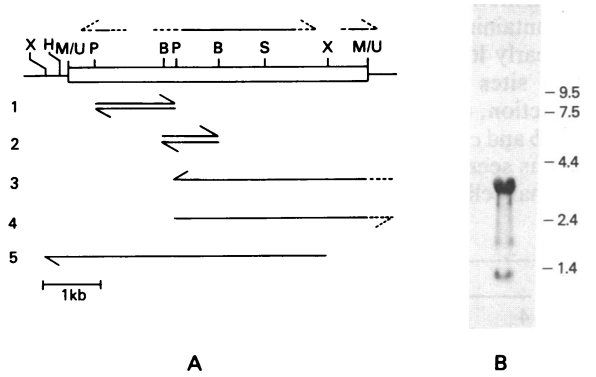

FIG. 2. Restriction map and transcript analysis of pSS1. (*A*) Positions of restriction enzyme sites are indicated by letters above the ≈6-kb insert (open box) and YEp13 vector (solid line). Probes homologous to parts of pSS1 are listed below the map. Probes: 1 and 2, double-stranded DNA; 3–5, single-stranded RNA synthesized from plasmids pGP (probes 3 and 4) and pGX (probe 5); arrowhead denotes 3′ end. The transcript directions (arrowhead 3′) and approximate positions of mRNA-encoding sequences are indicated above the map (solid and dotted arrows). M/U, *Bam*HI + *Sau3*AI hybrid site. Other abbreviations are as described in the legend of Fig. 1. (*B*) Poly(A)$^+$ RNA was prepared from strain 7882-1B, subjected to electrophoresis in a 1% agarose/formaldehyde gel, and transferred to nitrocellulose. Probe 3 was used to detect *CHS2* homologous RNA. Molecular sizes of standards are indicated in kb.

Table 2. Expression of Chs2 in *S. cerevisiae* and *Sc. pombe*

Plasmid	Organism	Chitin synthase activity, milliunits per mg of protein: With Mg^{2+}, No trypsin	With Mg^{2+}, After trypsin	With Co^{2+}, No trypsin	With Co^{2+}, After trypsin
YEp13	*Sc. pombe*	0.007	0.007	0	0
pSS1	*Sc. pombe*	0.008	0.04	0.017	0.21
pSS1	*S. cerevisiae*	0.47	3.7	0.87	10.4
pSS2	*S. cerevisiae*	0.57	5.7	1.0	11.8
pSS2X	*Sc. pombe*	0.009	0.12	0.045	0.6
pSS2X	*S. cerevisiae*	0.74	5.5	1.6	14
pSS2XP	*Sc. pombe*	0.13	2.6	0.52	4.2
pSS2XP	*S. cerevisiae*	0.36	1.8	0.59	6.1

Membrane fractions were isolated from logarithmic-phase cells disrupted with glass beads. Concentrations of Mg^{2+} and Co^{2+} were the same as for Table 1.

In every case, the activity of chitin synthase expressed in *Sc. pombe* was highly dependent on trypsin treatment—i.e., the enzyme behaved as a zymogen. The ratio of Co^{2+}-stimulated to Mg^{2+}-stimulated activity was even higher than in *S. cerevisiae*, except for pSS2XP (Table 2). The pH optimum was 8, both with Mg^{2+} and with Co^{2+} (results not shown).

Whereas the reasons for differential expression of the synthase with the several plasmids are unknown, there is no question that the transformed cells of *Sc. pombe* exhibit an activity with the properties of Chs2. This finding indicates that the cloned gene is the structural gene for the enzyme—i.e., *CHS2*.

Disruption of *CHS2* and Its Effect. To determine whether the *CHS2* gene was required for growth, two plasmids were constructed that contained an intact *LEU2* gene inserted in place of essential *CHS2* sequences. Linear fragments from these plasmids were then used to transform diploids that harbored a *leu2* mutation in both chromosomes. One of the strains used was wild type for *CHS1* (SS543), whereas the other was *chs1*::*URA3* in both chromosomes (JW17). In either case, successful transformation to leucine independence should give rise to an insertional deletion at the *CHS2* locus in one chromosome (see Fig. 3).

Disruption 1 was generated in plasmid pSS2X by inserting the *LEU2* gene between the leftmost *Bgl* II site and the *Sal* I site of the insert. In disruption 2 (derived from plasmid pSS2XP by first deleting its single *Bgl* II/*Sal* I restriction fragment), coding sequences were present on both sides of *LEU2* and the deleted fragment consisted of only internal *CHS2* coding sequences. In each disruption, only portions of the sequence that hybridizes to the 3.1-kb transcript were deleted. The deleted portions include part of the minimal sequence required for expression of *CHS2* (compare Figs. 1 and 3). The structures of the disrupted genes were confirmed by digestion of the chromosomal DNA with appropriate restriction endonucleases, followed by Southern blot analysis with a *CHS2* probe (Fig. 3).

Several transformants of this type were isolated and sporulated to assay viability of the haploid meiotic progeny. Four-spored tetrads from each were dissected. Analysis of these spores after germination (Table 3) showed no tetrads with three or four survivors. Most tetrads gave rise to two

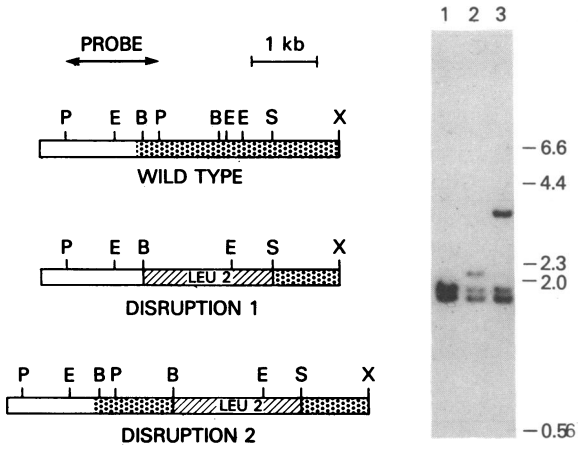

FIG. 3. Structure and Southern blot analysis of chromosomal DNA in wild-type and *chs2::leu2* strains. See *Materials and Methods* for plasmid construction and Table 3 for strain nomenclature. The abbreviations for restriction enzymes are the same as for Fig. 1. A DNA blot of *Eco*RI-digested DNA from strains SS543 (lane 1), SS543-Δ1 (lane 2), and SS543-Δ2 (lane 3) was probed with radiolabeled RNA homologous to probe 1 of Fig. 3. The molecular sizes (in kb) indicate the mobilities of λDNA *Hin*dIII cleavage fragments. Each insertion of the *LEU2*-containing *Bgl* II/*Sal* I fragment of YEp13 results in a deletion of a pair of *Eco*RI sites and reintroduces a single site of known distance from the remaining chromosomal *Bgl* II site (1382 base pairs of LEU2 DNA). The *Eco*RI fragment of wild-type cells that is altered by these insertions is the one at ≈1.75 kb. Since each disruption is lethal in a haploid, Southern blot analysis was performed on diploids containing one normal and one disrupted *CHS2* gene; therefore, the normal *Eco*RI fragments are present along with those corresponding to the disruption.

colonies, and some produced one or none. In every case, the colonies were shown by replica plating to consist of *leu2* cells—i.e., all spores that gave rise to colonies contained a normal *CHS2*. The results were not modified by inclusion of 1 M sorbitol in the germination medium—i.e., osmotic protection did not correct the phenotype. We conclude that *CHS2* is essential for cell growth. Because the same results were obtained irrespective of the presence of a functional *CHS1* gene in the cells (Table 3), it is clear that *CHS1* cannot substitute for *CHS2*.

Observation of those spores that did not give rise to colonies in two-colony tetrads showed that the spores had actually germinated and that the resulting cells had grown to a limited extent. These cells were larger than normal and displayed aberrant shapes. To investigate the presence of chitin, the cells were stained with Calcofluor White M2R New (see *Materials and Methods*), which gives rise to fluorescence when bound to chitin in intact cells (17, 18). Normal cells were fluorescent only in the septal region between mother and daughter cells (Fig. 4*a*). The misshapen cells containing the disrupted *CHS2* gene, however, showed less clearly localized staining (Fig. 4 *b* and *c*). Although at certain sites a fluorescent area coincided with a partial constriction, it was clear that a septum had not been formed (Fig. 4 *b* and *c*, arrows). In the aberrant microcolonies, no cell divisions seem to have been completed because, in contrast to normal cells, those harboring the *CHS2* disruption always

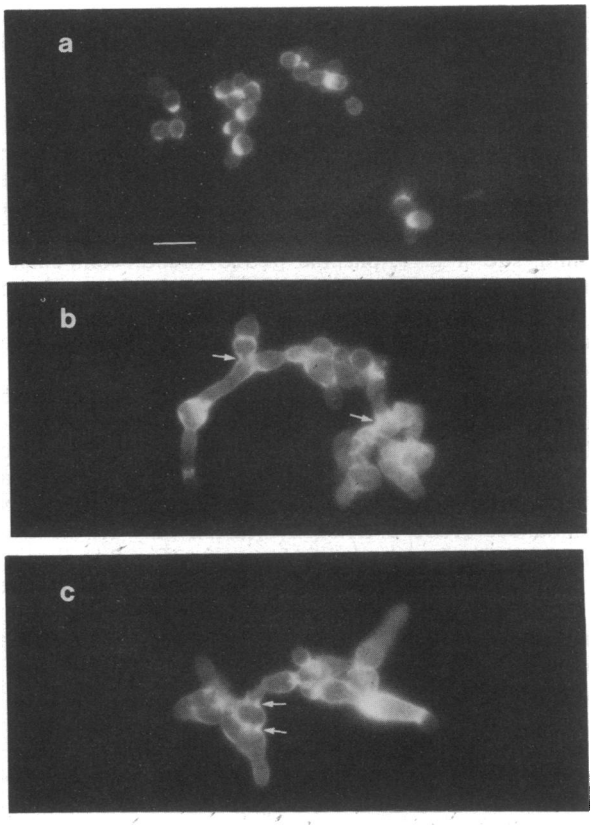

FIG. 4. Fluorescence micrographs of yeast cells stained for chitin with Calcofluor White. (*a*) Normal cells with bright chitin septa. (*b* and *c*) Cells harboring a disrupted *CHS2* gene that cannot form a septum. In all cases, cells were from spores of strain SS543-Δ1. Cells from spores of JW17-Δ1, containing a disrupted *CHS1* gene, yielded similar results. Cells were photographed on agar. Arrows, regions of constriction devoid of septa. (Bar = 10 μm.)

Table 3. Spore viability of recipient and *chs2::LEU2* strains

Strain	Relevant genotype		Colonies per tetrad				
			4	3	2	1	0
SS543	*CHS1*/*CHS1*	*CHS2*/*CHS2*	8	2	0	0	0
SS543-Δ1	*CHS1*/*CHS1*	*chs2::LEU2(1)*/*CHS2*	0	0	30	8	1
SS543-Δ2	*CHS1*/*CHS1*	*chs2::LEU2(2)*/*CHS2*	0	0	21	5	3
JW17	*chs1::URA3(3)*/*chs1::URA3(3)*	*CHS2*/*CHS2*	2	4	4	5	1
JW17-Δ1	*chs1::URA3(3)*/*chs1::URA3(3)*	*chs2::LEU2(1)*/*CHS2*	0	0	12	6	1
JW17-Δ2	*chs1::URA3(3)*/*chs1::URA3(3)*	*chs2::LEU2(2)*/*CHS2*	0	0	13	5	1

Δ1 and Δ2, disruption 1 and disruption 2, respectively.

held together as a unit when transferred with the micromanipulator needle.

DISCUSSION

The chitin synthase that is overproduced in strains that carry the plasmids described in this study has the characteristics of Chs2 with respect to cation stimulation and pH dependence: these criteria distinguish it clearly from Chs1. The enzyme expressed in *Sc. pombe* has similar properties except for a higher ratio of Co^{2+}-stimulated to Mg^{2+}-stimulated activity with plasmids pSS1 and pSS2X.

The overproduction of Chs2 activity in *S. cerevisiae* cells transformed with *CHS2*-carrying plasmids suggested that *CHS2* is the structural gene for the enzyme. This notion was confirmed by the presence of a similar activity in cells of the unrelated organism *Sc. pombe* only after transformation with the same plasmids. The zymogenic behavior of Chs2, when expressed in *Sc. pombe* from a single gene, shows that the enzyme, like Chs1 (3), is a genuine zymogen.

The lack of similarity between the *CHS1* and the *CHS2* genes, indicated both by restriction maps and by failure to hybridize in Southern blots, was unexpected, since the corresponding enzymes catalyze the same reaction. These results, however, do not preclude a partial homology at the DNA and/or at the protein level.

The results of gene disruption experiments establish unambiguously that *CHS2* is essential for growth. Apparently, in the absence of Chs2 the chitinous primary septum cannot be formed and cell division is interrupted. There is no block, however, in spore germination. The majority of spores carrying disrupted *CHS2* germinated and the resulting cells went through a short period of abnormal growth. The behavior of the cells, which held together as a unit during micromanipulation, suggests, however, that attempts at division were unsuccessful. In fact, constrictions were visible that clearly stopped short of closing the channel between cells (Fig. 4). Calcofluor, a fairly specific stain for chitin *in vivo*, gave rise to fluorescent areas, sometimes in coincidence with the constrictions, suggesting that some chitin was synthesized. Since the spores are formed in a diploid that contains one intact *CHS2* gene, the spores and the first germinated cell probably contain Chs2 in normal amounts. During subsequent growth, however, the enzyme cannot be replenished, so that its concentration in the cell becomes limiting. An alternative interpretation is that the Calcofluor staining at the constrictions is caused by another chitin synthetase (chitin synthetase 3?), which in a normal cell cycle might be responsible for the chitin rings formed in early budding (17, 18).

Finally, the tubular shapes of the aberrant cells are reminiscent of those observed in cytokinesis mutants—i.e., *cdc3, -10, -11,* and *-12* (19), which are also defective in septum formation.

We have shown here unequivocally that synthesis of a cell wall polysaccharide is specifically required for growth of a fungal cell. Aside from its general interest, this finding suggests that formation of cell wall polysaccharides is a valid target in the search for antifungal agents.

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