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cps1^{+}, a *Schizosaccharomyces pombe* Gene Homolog of *Saccharomyces cerevisiae FKS* Genes Whose Mutation Confers Hypersensitivity to Cyclosporin A and Papulacandin B

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The *Schizosaccharomyces pombe cps1-12* (for chlorpropham supersensitive) mutant strain was originally isolated as hypersensitive to the spindle poison isopropyl *N*-3-chlorophenyl carbamate (chlorpropham) (J. Ishiguro and Y. Uhara, Jpn. J. Genet. 67:97–109, 1992). We have found that the *cps1-12* mutation also confers (i) hypersensitivity to the immunosuppressant cyclosporin A (CsA), (ii) hypersensitivity to the drug papulacandin B, which specifically inhibits 1,3-β-D-glucan synthesis both in vivo and in vitro, and (iii) thermosensitive growth at 37°C. Under any of these restrictive treatments, cells swell up and finally lyse. With an osmotic stabilizer, cells do not lyse, but at 37°C they become multiseptated and multibranched. The *cps1-12* mutant, grown at a restrictive temperature, showed an increase in sensitivity to lysis by enzymatic cell wall degradation, in in vitro 1,3-β-D-glucan synthase activity (173% in the absence of GTP in the reaction), and in cell wall biosynthesis (130% of the wild-type amount). Addition of Ca^{2+} suppresses hypersensitivity to papulacandin B and septation and branching phenotypes. All of these data suggest a relationship between the *cps1*$^{+}$ gene and cell wall synthesis. A DNA fragment containing the *cps1*$^{+}$ gene was cloned, and sequence analysis indicated that it encodes a predicted membrane protein of 1,729 amino acids with 15 to 16 transmembrane domains. *S. pombe* cps1p has overall 55% sequence identity with Fks1p or Fks2p, proposed to be catalytic or associated subunits of *Saccharomyces cerevisiae* 1,3-β-D-glucan synthase. Thus, the *cps1*$^{+}$ product might be a catalytic or an associated copurifying subunit of the fission yeast 1,3-β-D-glucan synthase that plays an essential role in cell wall synthesis.

The immunosuppressants cyclosporin A (CsA) and FK506 form complexes with their binding proteins cyclophilin and FKBP-12, respectively, inhibiting T-cell activation by blocking the Ca^{2+}/calmodulin-dependent protein phosphatase 2B calcineurin. Since the receptor proteins are ubiquitous and highly conserved from yeast to human, the drugs may have a common mechanism of inhibition of the calcium signaling pathways (see reference 37 for a review). In the budding yeast *Saccharomyces cerevisiae*, these drugs prevent recovery from α-mating-factor arrest, although calcineurin is not essential for vegetative cell growth (20). An *fks1* (for FK506 sensitivity) mutant of *S. cerevisiae* that displays hypersensitivity to FK506 under vegetative growth conditions was isolated (44). Molecular characterization of the *FKS1* gene revealed that it encodes a 215-kDa integral membrane protein and that hypersensitivity of *fks1* mutant cells is due to loss of calcineurin function caused by the drugs CsA and FK506 (13, 17). *FKS1* was independently identified by several groups as a gene whose mutations are responsible for altered sensitivity to different cell wall synthesis inhibitors, namely, the echinocandin target gene *ETG1*, which confers resistance to the drug (12, 13), the calcofluor white hypersensitivity gene *CWH53* (49), *CND1* (for calcineurin dependent, since it is synthetically lethal with calcineurin mutants) (22), and *PBR1* (for papulacandin B resistance) (9). It was also identified as *GSC1* (for glucan synthase of *S. cerevisiae*) (29), obtained from partial purification and amino acid sequencing of proteins associated with 1,3-β-D-glucan synthase activity. *S. cerevisiae* has two closely related genes, *FKS1* and *FKS2*, whose products have 88% amino acid sequence identity to each other. The genes are proposed to code for two 1,3-β-D-glucan synthase isozymes. These proteins share an essential function for cell wall synthesis (simultaneous disruption of both genes is lethal). *FKS1* is predominantly expressed in a cell cycle-dependent manner under vegetative growth conditions, while transcription of *FKS2* depends on calcineurin activity, particularly during the mating process. Calcineurin is highly sensitive to CsA and FK506. Therefore, in the absence of *FKS1*, when *FKS2* becomes essential for cell wall synthesis, cells become sensitive to CsA and FK506 at concentrations that wild-type cells can resist, even under vegetative growth conditions (40).

Other genes from *S. cerevisiae* whose mutants and/or disruptants also display reduced levels of cell wall 1,3-β-D-glucan synthesis and/or 1,3-β-D-glucan synthase activity have been described: *KRE6* (for killer resistant) (52), *GNS1* (for 1,3-β-D-glucan synthase) (16), *HKR1* (for *Hansenula mrakii* killer toxin resistant) (33, 59), and *KNR4* (for killer nine resistance) (26). Mutations in *Schizosaccharomyces pombe* cell wall glucan genes *cwg1*$^{+}$ and *cwg2*$^{+}$ exhibiting similar phenotypes were also described (11, 51). However, none of these genes have been shown to form part of the 1,3-β-D-glucan synthase enzyme.

Fungal 1,3-β-D-glucan synthase consists of at least two components, a membrane-bound catalytic subunit and a detergent-soluble regulatory subunit (32). The latter is a GTP-binding protein proposed to play an important role for regulation of

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cell wall biosynthesis during the cell cycle (42). Rho1p GTPase is involved in both budding and fission yeast as the GTP-binding regulatory subunit of the enzyme (4, 14, 39, 48). In *S. pombe*, however, the catalytic subunit of the enzyme has not yet been identified.

In this report, we show that the *S. pombe cps1*$^{+}$ gene codes for a homolog of the *S. cerevisiae FKS1* and *FKS2* gene products. *FKS1* and *FKS2* are both proposed to encode a 1,3-β-D-glucan synthase catalytic subunit. We found that the *cps1-12* mutation confers hypersensitivity to the immunosuppressant CsA and to papulacandin B, a drug that specifically inhibits 1,3-β-D-glucan synthesis both in vivo and in vitro. *cps1-12* mutants displayed several phenotypes typical of cell wall synthesis defects, namely, increased sensitivity to enzymatic cell wall degradation and thermosensitivity with lysis that can be prevented with an osmotic stabilizer. Also, *cps1-12* mutants showed both altered 1,3-β-D-glucan synthase activity and altered cell wall components. We propose that *cps1*$^{+}$, like *S. cerevisiae FKS1* or *FKS2*, is a component of *S. pombe* 1,3-β-D-glucan synthase.

MATERIALS AND METHODS

Strains and culture conditions. *S. pombe* 972 (h^{-}, wild type), CP1-1 (h^{-} *cps1-12*), CP1-2 (h^{+} *cps1-12*), and CP911 (h^{-} *cps1-12 leu1-32*) have been described previously (31). Routine yeast growth medium (YE), selective medium (EMM) supplemented with the appropriate amino acids (1), and sporulation medium (SPA) (15) and bacterial LB medium (53) were as described elsewhere. The drugs isopropyl *N*-3-chlorophenyl carbamate (CIPC; Sigma), CsA (Sandoz), and papulacandin B (Ciba-Geigy) (kept in stock solution; 10 mg/ml in methanol at −20°C) were added to rich (YE) or selective (EMM) medium, after cultures were autoclaved and allowed to cool below 55°C, to the final concentrations specified in text and figures. General methods for yeast culture and genetic manipulation were carried out as described elsewhere (1, 43).

Escherichia coli HB101 [*supE44 hsdS20*($r_B^{-}m_B^{-}$) *recA13 ara-14 proA2 lacY1 galK2 rpsL20 xyl-5 mtl-1*] and MV1184 [*ara* Δ(*lac-proAB*) *rpsL thi* (φ80 *lacZ*ΔM15) Δ(*srl-recA*)306::Tn*10*(Tet^{r})] (53) were used for recovery from yeast and routine propagation of plasmids as described previously (53).

Plasmids and DNA techniques. Transformation of yeast cells was done by electroporation as described previously (30). Cloning and subcloning of a DNA fragment complementing the *cps1-12* mutant phenotype was done by using an *S. pombe* genomic DNA library inserted into *S. pombe-E. coli* shuttle vector pAL-KS (57), as described elsewhere (55). Strain CP911 was transformed with the genomic library by electroporation; transformants were grown on EMM plates and replica plated on YE–260 μM CIPC plates. Plasmids were recovered from a transformant capable of growing on the CIPC-containing medium as described elsewhere (43). This plasmid, able to complement the *cps1-12* mutation, was named pCP1 (8 kb). Three kinds of fragments, pCP1-1 (5.2 kb), pCP1-2 (4.5 kb), and pCP1-3 (3.5 kb), generated by *Sal*I or *Sac*I digestion, were subcloned into the pAL-KS vector and tested for the ability to complement the above-described mutant phenotype (see Fig. 5A).

DNA sequencing was done by making nested deletions of both strands from the 3.5-kb SalI-SalI and the 5.2-kb SacI-BamHI fragments cloned into pUC119 (53) (see Fig. 5A), by the modified method of Henikoff (24). Twenty-five deletion fragments were sequenced by the dideoxy-chain termination method (54), using an A.L.F. DNA Sequencer (Pharmacia). DNA sequence analysis was performed by using DNASIS software (Hitachi Software Engineering) and GenomeNet WWW databases. Other DNA manipulations were carried out essentially as described elsewhere (53).

Physical mapping and genomic Southern analysis. Physical mapping was performed by using an ordered cosmid clone bank (25) provided by Resource Center/Primary Database (RZPD; Berlin, Germany). DNAs prepared from cosmid clones 24C6c (ICRFc60C0624), 32H8c (ICRFc60H0832), and 29B5c (ICRFc60B0529) were digested with *Hin*dIII, *Kpn*I, *Pst*I, and *Bam*HI separately, and the fragments were resolved by 0.6% agarose gel electrophoresis. Yeast genomic DNA was prepared as described previously (43), digested with the same restriction enzymes, and used as a hybridization control. After alkali denaturation, DNA was blotted onto nylon membranes and fixed by UV irradiation. DNA labeling and detection were carried out by the nonradioactive digoxigenin–enzyme-linked immunosorbent assay method as recommended by the supplier (Boehringer Mannheim), using a 0.8-kb *Sal*I-*Xho*I fragment of the cloned DNA as a probe (see Fig. 5A).

Labeling and fractionation of cell wall polysaccharides. Exponentially growing cultures of *S. pombe* wild-type and mutant strains incubated at 28 or 37°C in YE or YE–1.2 M sorbitol medium, respectively, were diluted with the same medium, supplemented with D-[U-^{14}C]glucose (3 μCi/ml), and incubated overnight. Total glucose incorporation was monitored by measuring radioactivity in trichloroacetic acid-insoluble material. Cells were harvested at early logarithmic phase, supplemented with unlabeled cells as the carrier, washed twice with 1 mM EDTA, and broken with glass beads in a FastPrep FP120 apparatus (Savant; Bio 101, Inc.) (three times, 20-s pulse at a speed of 6). Cell walls were purified by repeated washing and differential centrifugation (once with 1 mM EDTA, twice with 5 M NaCl, and twice with 1 mM EDTA) at 1,000 × *g* for 5 min. Finally, purified cell walls were heated at 100°C for 5 min. Cell wall samples were extracted with 6% NaOH for 60 min at 80°C and neutralized with acetic acid. Precipitation of the galactomannan fraction from the neutralized supernatant was performed with Fehling reagent by adding unlabeled yeast mannan as the carrier, as described previously (2). Other samples of cell wall suspension were incubated with Zymolyase 100T (250 μg of enzyme and a cell wall volume equivalent to 150 μl of initial cell culture; Seikagaku Kogyo Co.) in 50 mM citrate-phosphate buffer (pH 5.6) for 36 h at 30°C. Samples without enzyme were included as a control. After incubation, samples were centrifuged and washed with the same buffer. One milliliter of 10% trichloroacetic acid was added to the pellets, and their radioactivity levels were measured as described elsewhere (58). These pellets were considered the 1,3-α-glucan fraction, and supernatants were the β-glucan-plus-galactomannan fraction. β-Glucan was also calculated as radioactivity remaining after subtraction of galactomannan and 1,3-α-glucan from total cell wall incorporation. All determinations were carried out in duplicate, and data for each strain were calculated from four independent cultures.

Enzyme preparation and 1,3-β-D-glucan synthase assay. Cell extracts were prepared and the 1,3-β-D-glucan synthase assay was performed essentially as described previously (58), with some modifications. Early-logarithmic-phase cells grown at 28 or 37°C in YE or YE–1.2 M sorbitol medium, respectively, were collected, washed once with 1 mM EDTA, and washed once with buffer A (50 mM Tris-HCl [pH 7.5], 1 mM EDTA, 1 mM β-mercaptoethanol). Cells were suspended in 100 μl of buffer A per g (wet weight) of cells and broken with glass beads in a FastPrep FP120 apparatus (Savant; Bio 101) (twice, 15-s pulse at a speed of 6). Broken material was collected, and cell debris was removed by low-speed centrifugation (5,000 × *g*, 5 min at 4°C). The supernatant was centrifuged at 48,000 × *g* for 30 min at 4°C, and the pellet was resuspended in buffer A containing 33% glycerol (at a concentration of 5 to 10 mg of protein per ml) and stored at −70°C. The standard 1,3-β-D-glucan synthase assay mixture contained 5 mM UDP-D-[^{14}C]glucose (4×10^{4} cpm/200 nmol), 150 μM GTP, 0.75% bovine serum albumin, 2.12 mM EDTA, 75 mM Tris-HCl (pH 8.0), 7.5% (vol/vol) glycerol, and enzyme (50 to 100 μg of protein) in a total volume of 40 μl. The reaction mixture was incubated for 30 min at 30°C unless specified otherwise and stopped by addition of 1 ml of 10% trichloroacetic acid. Protein was quantified by the Bradford dye-binding procedure (7), using the Bio-Rad protein assay dye reagent concentrate and bovine serum albumin as the standard. One unit of 1,3-β-D-glucan synthase was defined as the amount of enzyme that catalyzes incorporation of 1 μmol of glucose into glucan per min at 30°C. Specific activity was expressed as milliunits per milligram of protein. All reactions were carried out in duplicate, and data for each strain were calculated from four to six independent cultures.

Enzymatic lysis of cell suspensions. Early-logarithmic-phase cells grown at 28 or 37°C in YE or YE–1.2 M sorbitol medium, respectively, were collected, washed with 50 mM citrate-phosphate buffer (pH 5.6), suspended in a small volume of the same buffer, and adjusted to an optical density at 600 nm (OD_{600}) of 2.25. Cell suspensions were treated with no enzyme as control, with Zymolyase 100T (10 μg/ml), or with Novozyme 234 (30 μg/ml; Novo Industries) and incubated for 8 h at 30°C with shaking. The OD_{600} was monitored repeatedly, considering 100% at each time the absorbance of a control sample with no enzyme. Enzyme concentration and incubation time were adjusted to obtain approximately a final 50% absorbance decrease in the wild-type strain.

Nucleotide sequence accession number. The nucleotide sequence reported here was deposited in the DNA Data Bank of Japan (DDBJ) with accession no. D78352 (it can be accessed through EMBL, GenBank, and DDBJ databases).

RESULTS

Effects of CsA on *cps1-12* mutant cells. Originally, *cps1-12* was identified as a mutation that confers hypersensitivity to the spindle poison CIPC and to the inhibitor of calmodulin trifluoperazine (31). In the course of screening other cross-sensitivity tests, the *cps1-12* mutant was also found highly sensitive to CsA, a potent inhibitor of the Ca^{2+}/calmodulin-dependent protein phosphatase 2B calcineurin (Fig. 1). Wild-type cells were able to grow at 10 μg of CsA per ml. However, under such conditions a significant population of cells became elongated, often with two septa, with some cells displaying swelling pear-like shapes (Fig. 2B). Cytokinesis and cell morphology of wild-type cells thus appear to be affected by high concentrations of CsA, although cell growth is not noticeably inhibited (see also reference 60). A more severe defect in cell morphology was observed in *cps1-12* mutant cells. Even in the absence of the drug, *cps1-12* mutant cells resembled wild-type cells

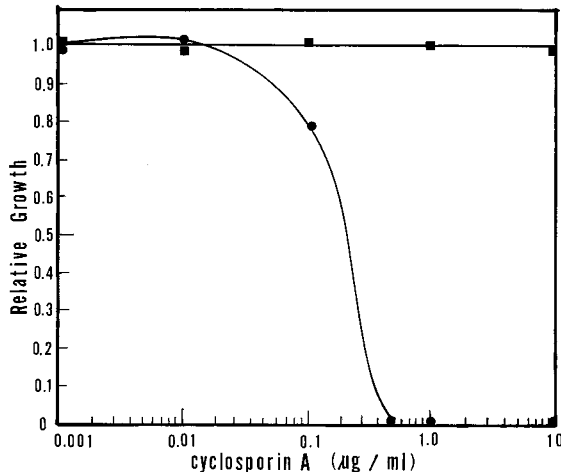


FIG. 1. Effects of different CsA concentrations on *S. pombe* cell growth. Cells from 972 (h^-, wild type; ■) and CP1-2 (h^+ *cps1-12*; ●) strains were spread (200 cells per plate) onto EMM plates supplemented with different concentrations of CsA as indicated and incubated at 30°C. Relative growth is considered the number of colonies grown on EMM plus CsA divided by the number of colonies grown on EMM. The mean values of five experiments are plotted.

grown in the presence of 10 μg of CsA per ml (Fig. 2C). Furthermore, growth of *cps1-12* mutant cells was completely inhibited at CsA concentrations higher than 0.5 μg/ml. At these concentrations, mutant cells swelled up and eventually lysed (Fig. 2D). When CsA-containing medium contained an osmotic stabilizer, mutant cell lysis was partially suppressed (data not shown). This finding suggests that CsA may promote a defect in cell wall formation that is enhanced in *cps1-12* mutants.

cps1-12 **mutant cells have other cell wall-related phenotypes.** The fragile phenotype that *cps1-12* mutant cells displayed in the presence of CsA suggested that they might have other mutant phenotypes related to cell wall integrity. Papulacandin B specifically interferes with 1,3-β-D-glucan synthesis both in vivo and in vitro in yeasts and fungi (5, 46, 58). *cps1-12* mutant cells, growing at 28°C in YE or EMM medium, were sensitive to papulacandin B at concentrations lower than MICs for the wild type (10 and 17.5 μg/ml in YE and EMM media, respectively). The degree of hypersensitivity varied with the medium (5 and 10 μg/ml in YE and EMM media, respectively) (data not shown) and was suppressed when cells were grown in the presence of 1.2 M sorbitol, indicating that this defect affects the cell wall structure. The hypersensitivity to CsA of the *cps1-12* mutant suggested a possible regulation of some mutant phenotypes by Ca^{2+}. When wild-type and *cps1-12* mutant strains were grown in YE–10 mM $CaCl_2$ medium in the presence of papulacandin B, the inhibitory concentration became the same in the two strains (10 to 12.5 μg/ml) (data not shown). Thus, the papulacandin B hypersensitivity of the *cps1-12* mutant depends on limiting concentrations of Ca^{2+} in the medium.

Mutant cells grown at 28°C showed a phenotype close to that of the wild type, with fewer abnormal cells than when cultured at 30°C (compare Fig. 2C and 3). In the presence of 1.2 M sorbitol as an osmotic stabilizer, mutant and wild-type cells were indistinguishable. When the *cps1-12* mutant was grown at 37°C, it showed a thermosensitive phenotype. Cells swelled up, displaying pear, lemon, and round shapes, became more refringent, and eventually lysed (after 15 to 20 h in liquid medium or at the second patch on solid medium). When 1.2 M sorbitol was added to the medium, cell lysis disappeared, the cells then showing a morphology resembling that of pseudohyphae or filamentous fungi. Cell size increased enormously, and cells displayed multiple septa and branches (Fig. 3). This finding suggests that the *cps1-12* mutation might affect different cellular processes, one related to cell wall structure and the other related to cell division and polarity. Addition of 10 mM $CaCl_2$ did not affect growth or cell shape in either the mutant or wild-type strain grown at 28°C. However, at 37°C, *cps1-12* mutant and wild-type cells displayed phenotypes of elongated, multiseptated, and often multibranched cells (slightly swelled compared to mutant cells in the presence of 1.2 M sorbitol) except that mutant cells stopped growing and eventually lysed (data not shown). Wild-type cells had a normal phenotype in the presence of 10 mM $CaCl_2$ plus 1.2 M sorbitol at 37°C. *cps1-12* mutant cells showed partial recovery under these conditions, becoming shorter and less septated and branched than when grown only in the presence of 1.2 M sorbitol (data not shown).

cps1-12 **mutant cells have increased sensitivity to lysis by enzymatic cell wall degradation.** Zymolyase 100T is an enzymatic complex containing mainly 1,3-β-glucanase and protease activities and lacking other activities such as 1,3-α-glucanase (35) whose substrate, 1,3-α-glucan, is present in *S. pombe* cell walls. Novozyme 234 is an enzymatic complex able to degrade all *S. pombe* cell wall polymers. Cell suspensions from wild-type or *cps1-12* mutant strains exponentially growing at 28 or 37°C were incubated at 30°C with limiting concentrations of Novozyme 234 (30 μg/ml) or Zymolyase 100T (10 μg/ml). The lysis rates of mutant and wild-type cell suspensions grown at 28 or 37°C were indistinguishable with Zymolyase 100T. In the presence of Novozyme 234, there was a faster lysis of mutant cells grown at 28°C, an effect that was more pronounced in mutant cells grown at 37°C (Fig. 4). Thus, at 3.20 h in the presence of Novozyme 234, the percentages of residual absorbance in wild-type and mutant cells grown at 28°C were 88 and 77, respectively, while in cells grown at 37°C they were 93 and 43, respectively. Therefore, the *cps1-12* mutant has a cell wall defect with a thermosensitive component, increasing noticeably the susceptibility to lysis in cultures grown at 37°C. The lysis rate in wild-type and mutant strains in the presence of Zymolyase 100T indicates that the galactomannan–β-glucan degradation rates are similar, suggesting that the amounts of the two polymers are similar in these strains.

The same results were obtained when cells were grown in the presence of 10 mM $CaCl_2$ except that both strains were about twice as resistant to lysis by either of these two enzymatic complexes than when grown with no added calcium (data not shown). This general increase in resistance to degradation from cultures grown with added 10 mM $CaCl_2$ was due to a modification in their cell wall structure and not to a calcium interference with the activity of hydrolytic enzymes (data not shown).

In vitro 1,3-β-D-glucan synthase activity of *cps1-12* mutant cells. The results presented above suggested that *cps1-12* mutant cells might display alterations in some cell wall components. The hypersensitivity of mutant cells to papulacandin B and the specific inhibition of 1,3-β-D-glucan synthase activity by such a drug implied that mutant cell wall defects might be promoted by an alteration in this activity. 1,3-β-D-Glucan synthase activity was determined from wild-type and *cps1-12* mutant strains grown at permissive (28°C in YE medium) and nonpermissive (37°C in YE–1.2 M sorbitol medium) temperatures. In all cases, both basal and GTP-activated activities were assayed. As shown in Table 1, there was no significant difference in specific activity, with or without GTP, from wild-type

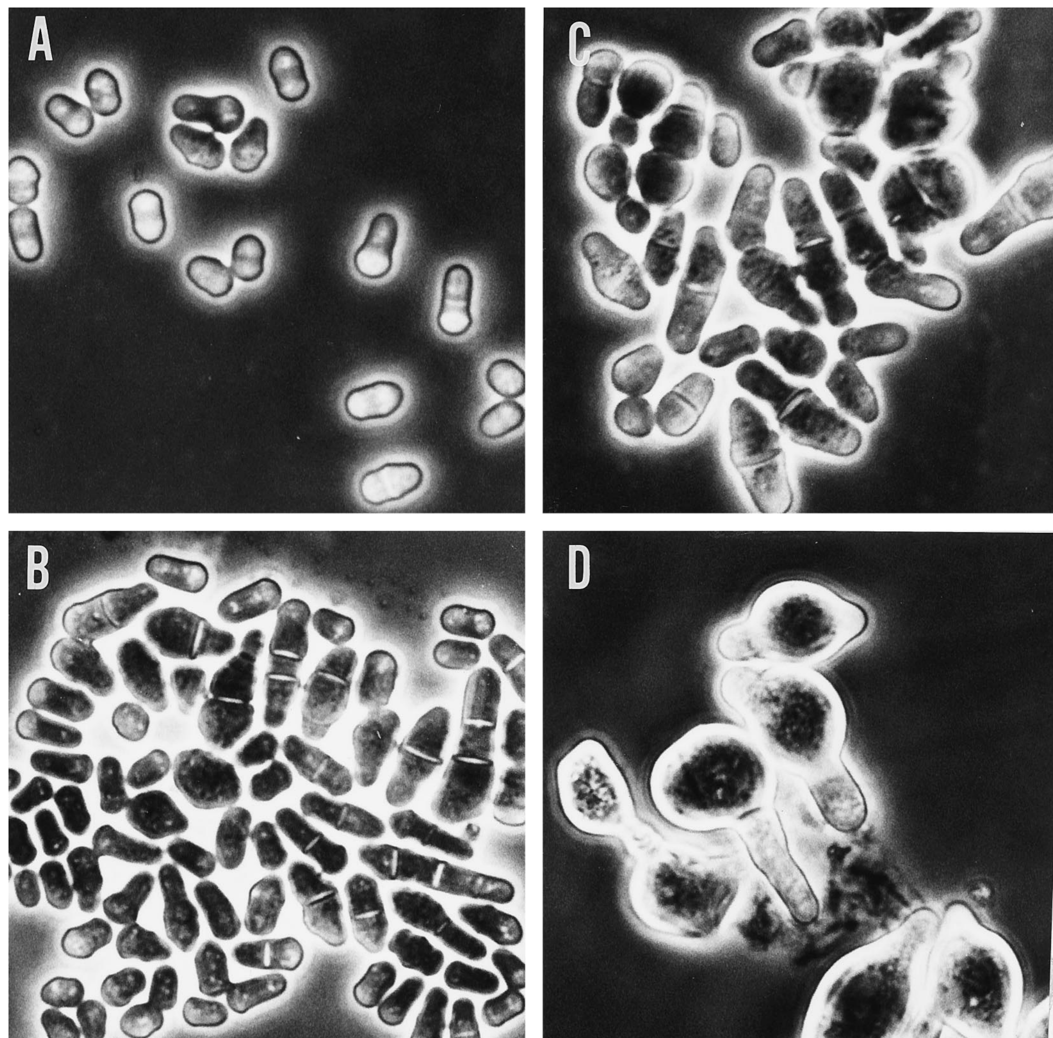


FIG. 2. Phase-contrast micrographs of *S. pombe* cells grown on EMM liquid medium in the presence of CsA. Precultured cells in late log or early stationary phase were diluted into fresh EMM medium with or without CsA and grown for 24 h at 30°C. Strain 972 (wild-type) cells were grown in the absence (A) or presence (B) of CsA (10 μg/ml), and CP1-2 mutant cells were grown in the absence (C) or presence (D) of CsA (10 μg/ml)

and mutant extracts grown at 28°C. Nonetheless, the activity from the *cps1-12* mutant was always slightly higher (109% with GTP and 119% without GTP compared to wild-type levels). Surprisingly, when cells were grown at 37°C, *cps1-12* mutant extracts showed a noticeable increased 1,3-β-D-glucan synthase activity compared to wild-type extracts. This increase was higher in the absence of GTP (173%) than in its presence (149%).

No differences between the two strains in susceptibility to the reaction temperature or in activity from cells grown with added $CaCl_2$ were detected (data not shown). Neither in vivo nor in vitro hypersensitivity of mutant 1,3-β-D-glucan synthase activity to papulacandin B was observed (data not shown). However, the increased activity in extracts from the mutant strain grown at 37°C implies a change in the properties of the enzyme that could be responsible for the mutant phenotypes.

Cell wall composition of *cps1-12* mutant cells. Although mutant 1,3-β-D-glucan synthase activity is increased at restrictive temperatures, previous data pointed to a cell wall weakness defect in *cps1-12* mutant cells. To determine the quantitative composition of *cps1-12* mutant cell wall polysaccharides, cell wall fractionation of ^{14}C-labeled mutant and wild-type cells grown at permissive (28°C in YE medium) and nonpermissive (37°C in YE–1.2 M sorbitol medium) temperatures was performed (Table 2). Measurement of total radioactivity in cell walls of cells grown at 28°C showed a slight increase in cell wall amount for the mutant strain (31.5 and 35.0% incorporation into wild-type and mutant cell walls, respectively). Levels of incorporation of radioactivity into β-glucan and galactomannan were similar in these strains, whereas α-glucan was increased in the *cps1-12* mutant (12.68% in the wild-type strain and 15.6% in the mutant strain). That increase could explain the total cell wall increase in the mutant strain. When grown at 37°C, the *cps1-12* mutant displayed an increase in total cell wall incorporation compared to the wild type (34.5 and 44.8% in wild type and mutant, respectively). Levels of incorporation of radioactivity into β-glucan and galactomannan were similar in these strains, whereas α-glucan was increased in the *cps1-12* mutant (12.68% in the wild-type strain and 15.6% in the mutant strain). That increase could explain the total cell wall increase in the mutant strain. When grown at 37°C, the *cps1-12* mutant displayed an increase in total cell wall incorporation compared to the wild type (34.5 and 44.8% in the wild type and mutant, respectively). When each cell wall polymer in the mu-

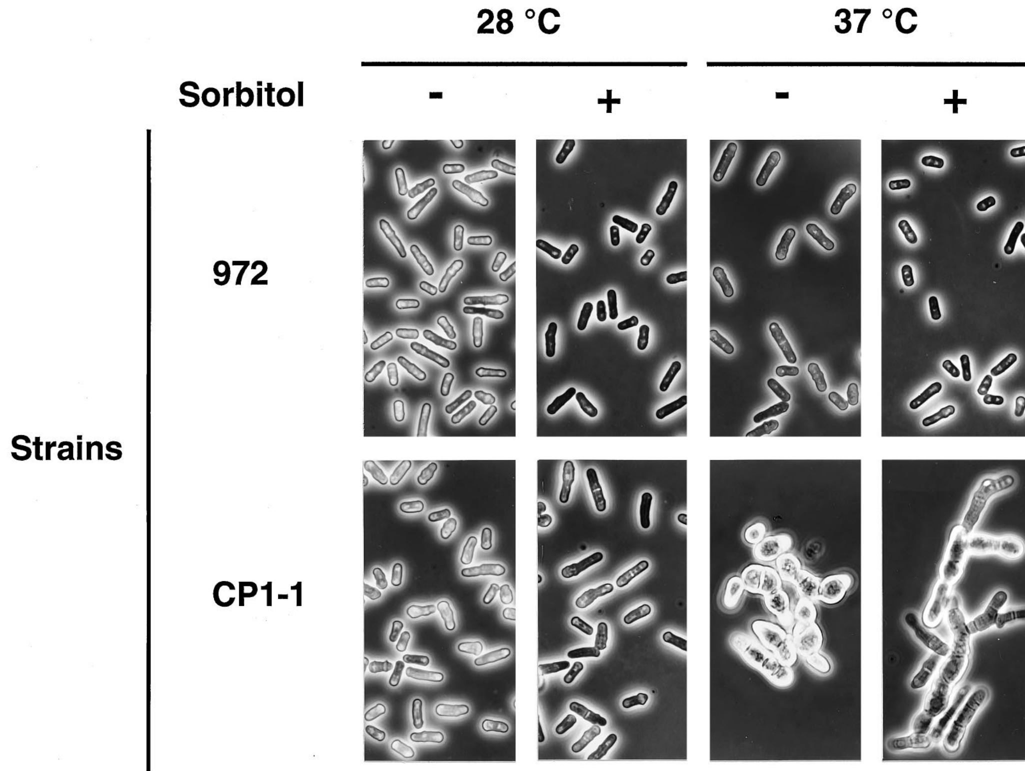


FIG. 3. Phase-contrast micrographs of *S. pombe* 972 and CP1-1 cells grown on YE solid medium at 28 or 37°C in the presence or absence of 1.2 M sorbitol.

tant strain was analyzed, we noticed that the percentage of every one in the cell was changed. The most remarkable finding is the α-glucan increase to 172% of the wild-type amount, which is responsible for the cell wall increase of the *cps1-12* mutant mentioned above. Cell wall weakness in mutant cells grown at the nonpermissive temperature could be due to an α-glucan excess that results in alteration of the percentages of other polymers in the cell wall. In fact, the level of β-glucan in mutant cells dropped to 85%, while α-glucan rose to 133%, of the wild-type amount.

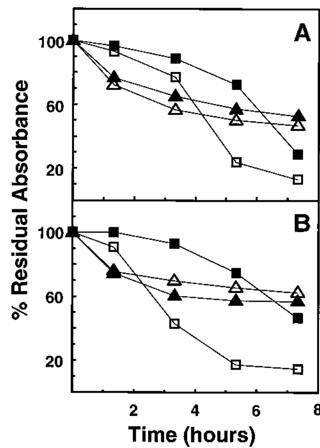


FIG. 4. Lysis of *S. pombe* cell suspensions from strains 972 (closed symbols) and CP1-1 (open symbols) grown either at 28°C in YE liquid medium (A) or at 37°C in YE–1.2 M sorbitol liquid medium (B). Cells were concentrated to an OD_{600} of 2.25 in 50 mM citrate-phosphate buffer (pH 5.6) and incubated at 30°C in the presence of Zymolyase 100T (10 μg/ml; triangles) or Novozyme 234 (30 μg/ml; squares).

Cloning of the *cps1*$^+$ gene. A strain bearing the *cps1-12* mutation is incapable of growing on medium containing 260 μM CIPC (31). To isolate the *cps1*$^+$ gene, strain CP911 was transformed with a fission yeast genomic plasmid library, constructed in the vector pAL-KS with partially *Hin*dIII-digested DNA fragments. Approximately 3.6×10^4 Leu$^+$ transformants were obtained and replica plated on YE–260 μM CIPC plates. A single transformant able to grow on CIPC plates was isolated. Plasmids were recovered, and all were identified as containing the same DNA fragment. Plasmid pCP1 contained an approximately 8.0-kb *Hin*dIII insert, as shown in Fig. 5A. To examine if pCP1 can also complement all the above-described mutant phenotypes, transformants were spread on plates containing CsA (10 μg/ml) or papulacandin B (10 μg/ml) and grown at 28°C. Another plate was incubated at 37°C to test for

TABLE 1. 1,3-β-D-Glucan synthase activities from *S. pombe* wild-type (972) and mutant (h$^-$ *cps1-12*) strains grown at 28 and 37°C

Growth temp (°C)	Strain genotype	Sp act (mU/mg of protein, mean ± SD)[a]	
		−GTP	+GTP[b]
28	972	3.57 ± 0.62 (100)	14.08 ± 4.31 (100)
	h$^-$ *cps1-12*	4.24 ± 0.99 (118.7)	15.40 ± 1.67 (109.3)
37	972	2.39 ± 1.57 (100)	7.26 ± 3.64 (100)
	h$^-$ *cps1-12*	4.14 ± 2.34 (173.2)	10.79 ± 4.94 (148.6)

[a] Calculated from six (at 28°C) or four (at 37°C) independent experiments. Values in parentheses are percentages of wild-type specific activity in the same conditions.

[b] Final concentration in the assay, 150 μM.

TABLE 2. Incorporation of radioactivity from [^{14}C]glucose into cell wall polysaccharides of *S. pombe* wild-type (972) and mutant (h$^-$ *cps1-12*) strains grown at 28 and 37°C

Growth temp (°C)	Strain genotype	% Incorporation of [^{14}C]glucose (mean ± SD)[a]			
		Galactomannan	α-Glucan	β-Glucan	Cell wall
28	972	2.09 ± 0.26 (6.56)	12.68 ± 0.27 (40.22)	16.73 ± 0.62 (53.22)	31.50 ± 0.66
	h$^-$ *cps1-12*	2.60 ± 0.06 (7.43)	15.60 ± 0.39 (44.46)	16.80 ± 0.74 (48.11)	35.00 ± 0.76
37	972	2.99 ± 0.25 (8.66)	12.77 ± 0.98 (37.00)	18.75 ± 0.90 (54.34)	34.50 ± 1.63
	h$^-$ *cps1-12*	2.09 ± 0.18 (4.66)	22.02 ± 0.56 (49.15)	20.69 ± 1.20 (46.19)	44.81 ± 1.62

[a] Determined as cpm incorporated per fraction × 100/total cpm incorporated. Values were calculated from four independent experiments. Values in parentheses are percentages of the value for the corresponding polysaccharide in the cell wall composition.

the thermosensitive phenotype. As shown in Fig. 5B and C, *cps1-12* mutant cells transformed with pCP1 were capable of growing on CIPC-, CsA-, or papulacandin B-containing plates at 28°C and on EMM plates at 37°C. pCP1 also suppressed the pseudohyphal phenotype of *cps1-12* mutant cells grown with 1.2 M sorbitol at 37°C (data not shown). To identify the functional region of the 8.0-kb insert, three fragments generated by *Sal*I or *Sac*I digestion were subcloned into pAL-KS and tested for the ability to complement *cps1-12* mutant phenotypes (Fig. 5A). Both pCP1-1 (5.2-kb *Sac*I-*Hin*dIII) and pCP1-2 (4.5-kb *Sal*I-*Hin*dIII) subclones partially complemented mutant phenotypes, while pCP1-3 (3.5-kb *Sal*I-*Hin*dIII) did not, indicating that rescuing activity spans the *Sal*I site and that a functionally more important region may extend 3′ to the *Sal*I site.

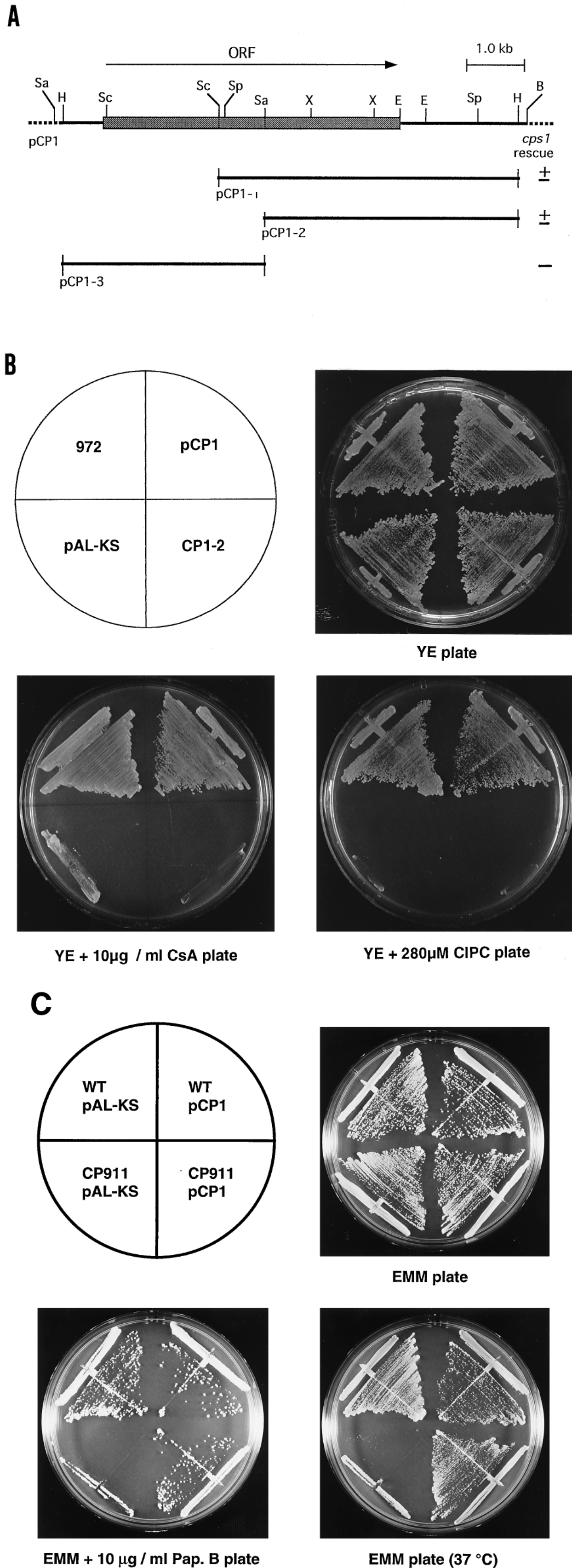
To determine whether the cloned gene is *cps1*$^+$ itself or is acting as a suppressor, physical and genetic mapping was carried out. The *cps1-12* mutant was previously mapped by tetrad analysis on the right arm of chromosome II, 11.3 centimorgans (cM) distal to *his5* and 31.9 cM proximal to *glu2* (31). Analysis of other *S. pombe* genetic markers distal to *his5* and proximal to *cdc10* (23, 25, 38) showed that *cdc14* (18) and *cps1* are very closely linked (analysis of 78 tetrads resulted in a *cdc14-cps1* segregation of 73 parental ditypes versus 5 tetratypes versus 0 nonparental ditypes, a genetic distance of 3.2 cM) (47). *cdc14*$^+$ is located in cosmid 24C6c (ICRFc60C0624, RZPD) of an *S. pombe* cosmid clone bank (25) and therefore *cps1*$^+$ as well. To determine whether the cloned gene is *cps1*$^+$, cosmid 24C6c (ICRFc60C0624, RZPD) and other cosmids from chromosome II (used as controls) were analyzed by Southern hybridization using a fragment of pCP1 as the probe. S. pombe chromosomal DNA was processed as the cosmid clones and used as a control to verify that the cosmid clones did not contain DNA rearrangements. Southern blot analysis showed that the 0.8-kb *Sal*I-*Xho*I fragment from pCP1 hybridized only with cosmid clone 24C6c (ICRFc60CO624, RZPD) and that the restriction pattern is the same in cosmid and genomic DNA (see Fig. 7A). This result indicates that the isolated gene is *cps1*$^+$. pCP1 was tested for suppression of cell wall-related phenotypes from such other *S. pombe* mutant genes as *pbr1-8*, which confers resistance to papulacandin B (9), and *cwg1-1*, which displays thermosensitivity due to a decrease in cell wall 1,3-β-D-glucan content (51), but in both cases it failed to complement their phenotypes.

Sequencing and characterization of *cps1*$^+$ gene. To sequence the isolated *cps1*$^+$-containing DNA fragment, nested deletions from two pUC119 clones containing 5.2-kb *Sac*I-*Bam*HI and 3.5-kb *Sal*I-*Sal*I fragments (Fig. 5A) were generated. By using a total of 25 independent deletion clones of both DNA strands, the entire nucleotide sequence of the 6.3-kb *Hin*dIII-*Eco*RI fragment was determined. The complete DNA sequence (6,283 bp) is shown in Fig. 6. It contains a single open reading frame (ORF) of 5,190 nucleotides, encoding a predicted protein of 1,729 amino acids (Fig. 6). In the noncoding sequence there are two TATAA elements for transcription initiation, at nucleotides -467 and -432 upstream of the ORF start codon, and two polyadenylation consensus sequences, AATAAA and AATATA, at positions 106 and 151 downstream of the ORF termination codon. A search in the SWISSPROT database by using BLASTP (3) revealed overall 55% sequence identity between *S. pombe* cps1p and *S. cerevisiae* Fks1p (13, 17) or Fks2p (40). A third *S. cerevisiae* ORF homologous to Fks1p and Fks2p (40), here called Fks3p, and an *Aspergillus nidulans* homologous protein, named FksAp (34), have been described. Lipman-Pearson protein alignment (45) shows sequence identity of 53.5, 54.5, 51.5, or 57.7% between cps1p and Fks1p, Fks2p, Fks3p, or FksAp, respectively. Hydropathy analysis of cps1p (56) predicts the gene product to be an integral membrane protein with 15 to 16 transmembrane helices that coincide with those of FksAp from *A. nidulans* and those of Fks1p and Fks2p from *S. cerevisiae*. The high degree of similarity between amino acids 801 to 1022 of cps1p and 939 to 1160 of Fks2p is noticeable (69% identity and 81% similarity [Fig. 7B]). An equivalent high degree of similarity can be also found between cps1p and Fks1p, Fks3p, or FksAp. These regions are presumed to localize to the cytoplasm and may function as a catalytic domain of the enzyme. cps1p contains a proposed UDP-glucose binding consensus sequence, RXTG (28), at amino acid 1410. These findings strongly suggest that cps1p, like its *S. cerevisiae* homologs, may be a catalytic subunit of the 1,3-β-D-glucan synthase. The *FKS1* gene contains two MCB (Mlu cycling box) and five SCB (Swi cycling box) elements in the promoter region (49), while the *cps1*$^+$ gene does not, suggesting that transcription of *cps1*$^+$ may not be regulated in a cell cycle-dependent manner at the G_1/S boundary. Also, the codon bias index of the *cps1*$^+$ gene is calculated as nearly 0.1 (6), suggesting that it may be expressed at an ordinary level.

To examine if other *cps1*$^+$-related sequences are present in *S. pombe* as they are in budding yeast (40), Southern blot analysis was carried out, using as the probe DNA encoding two highly conserved regions of cps1p (0.8-kb *Sal*I-*Xho*I and 1.0-kb *Xho*I-*Xho*I fragments from pCP1). However, no *cps1*$^+$ gene homologs were detected in the fission yeast genome, as shown in Fig. 7A. A more detailed study will be necessary to ascertain if *cps1*$^+$ is the only *FKS* homolog in *S. pombe*.

DISCUSSION

All processes that affect the cell wall structure throughout the fungal life cycle are responsible for changes in cell morphology. Obviously, this structure is essential for yeast and fungi survival, and therefore its synthesis and regulation constitute a morphogenetic model. To understand how cells control cell wall synthesis, one must know how genes regulate or act in this process (reviewed in references 8, 10, 28 and 36). The *S. pombe* cell division process resembles that of plant and



vertebrate cells, particularly from a cytological point of view. Hence, the fission yeast might provide an attractive model system to elucidate regulatory mechanisms of fungal cell wall synthesis and other morphogenetic events.

Previously, the *S. pombe cps1-12* mutation was shown to confer hypersensitivity to the spindle poison CIPC (31). A search for other possible effects of the *cps1-12* mutation showed that the mutant is highly sensitive to CsA, an inhibitor of the Ca^{2+}/calmodulin-dependent protein phosphatase 2B, calcineurin. *S. cerevisiae* strains with different *fks1* mutant alleles were described as sensitive to CsA (13, 17) or dependent on calcineurin (22), and the *FKS1* gene was found to encode a protein necessary for cell wall 1,3-β-D-glucan synthesis. The fact that CsA-mediated lysis of the *cps1-12* mutant can be suppressed to some extent with an osmotic stabilizer suggested that *cps1-12* may have some other cell wall-related phenotypes. It was found that *cps1-12* displays two lytic phenotypes, hypersensitivity to papulacandin B and thermosensitivity, both osmotically prevented, suggesting a defect in cell wall structure. In fact, several *fks1* mutant alleles from *S. cerevisiae* strains hypersensitive (*cwh53-1*) (50) or resistant (*pbr1-1*) (9) to papulacandin B have been described. Furthermore, lysis by enzymes that degrade cell wall polymers reflected a quantitative or qualitative defect in *cps1-12* cell wall synthesis.

The described *cps1-12* phenotypes and the remarkable homology of the *cps1*$^{+}$ gene to *S. cerevisiae FKS* genes suggested that the *cps1-12* mutant strain could be affected in the 1,3-β-D-glucan synthase activity. Previous results indicated that neither the *cwh53-1* nor the *pbr1-1* mutant allele of *FKS1* induced a 1,3-β-D-glucan synthase activity defect in the cell, but that the *fks1-1* allele and *fks1*Δ, *pbr1*Δ, and *gsc1*Δ partial disruptions of *FKS1* did (9, 13, 29, 40, 49). The *cps1-12* mutant displayed a 150 to 175% specific activity increase relative to the wild type at the restrictive temperature. This increase, although surprising, denotes a change in enzyme properties. That the mutant enzyme does not show increased sensitivity to either in vivo or in vitro papulacandin B treatment could be due to a protein modification in a region other than the binding site responsible for the papulacandin B effect.

The cell wall composition analysis showed that the amounts of galactomannan plus β-glucan per cell are similar in wild-type and mutant strains grown at both temperatures. The cell wall weakness in mutant cells grown at the nonpermissive temperature could be due to the α-glucan excess (Table 2) that, either accumulated in some areas or uniformly extended into the whole cell wall, interferes with the proper cell wall rigidity conferred by β-glucan.

A relationship between Ca^{2+} and genes affecting cell integrity is well known (10, 22, 27, 41, 60). The *S. cerevisiae FKS2*

FIG. 5. (A) Restriction map and subcloning of a DNA fragment that complements *cps1-12* mutant phenotypes from *S. pombe*. The grey box represents the predicted *cps1*$^{+}$ ORF, and the arrow shows its 5′-to-3′ direction. The ability of subclones to complement *cps1-12* mutant phenotypes is indicated as ± (partial rescue) and − (no rescue). Restriction sites: B, *Bam*HI; H, *Hin*dIII; E, *Eco*RI; Sa, *Sal*I; Sc, *Sac*I; Sp, *Spe*I; X, *Xho*I. (B) Complementation of CsA- and CIPC-hypersensitive *cps1-12* mutant phenotypes by plasmid pCP1. Strain CP911 (h^{-} *cps1-12 leu1-32*) was transformed with plasmid pCP1 or pAL-KS, and transformants were streaked out on YE plates containing either CsA (10 μg/ml) or CIPC (280 μM). Strains 972 (wild type) and CP1-2 (mutant) were included as controls. The plates were incubated at 28°C for 4 days. (C) Complementation of papulacandin B-hypersensitive and thermosensitive mutant phenotypes by plasmid pCP1. Wild-type (WT; h^{-} *leu1-32*) and CP911 (h^{-} *cps1-12 leu1-32*) cells were transformed with pCP1 or pAL-KS, and transformants were streaked out on EMM plates in the presence or absence of papulacandin (Pap.) B (10 μg/ml). The plates were incubated at 28 (for papulacandin B hypersensitivity assay) or 37°C (for thermosensitivity assay) for 4 days.

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-661 AAGCTTCTGCATACGACAATTTAATTGTTCAATCTAAAGTTTATGTGGAAAGCGATTTTATATTTTCTCCTTTTCTTTTAAAAAAACGTCCTTCCTTGAATCGCTTTTTTAAAAATTTGGTCAGTTTCCTAGG -529
-528 TGTGCATTTGTAAGTACTTCTTTTGGTGCTTGCTGTTTTATGGTATTATTATTGTTGTTTTTATAATTATTTCTTCTTTTTTTCGCCTCTTTTTTTATAATTTGCATACTGTATTTTTAGGTTCCGTTCAT -397
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   1 ATGGATCAGTATTGGCGTGAACAAGAGGGTCGTGGACTCTTTGAAGATGATGCCAATAGTTATGTCTCTGACGATGATACTATGAGCTCTCTGACCCGTATGATTTACGATAAAAACTCTTCTCGTGATGCA 132
   1  M  D  Q  Y  W  R  E  Q  E  G  R  G  L  F  E  D  D  A  N  S  Y  V  S  D  D  D  T  M  S  S  L  T  R  M  I  Y  D  K  N  S  S  R  D  A  44
 133 TTACATTCTGATTATGATAGCTCTTCATTTAATGTCGATTCCAGTTCAGTTGCTTATCCGGCTTGGAATCAAGCTGGTGAAGAGGCTCCTGTTACTATGGAAGGTGTTCAAGAGATCTTGTTGGACCTTACA 264
  45  L  H  S  D  Y  D  S  S  S  F  N  V  D  S  S  S  V  A  Y  P  A  W  N  Q  A  G  E  E  A  P  V  T  M  E  G  V  Q  E  I  L  L  D  L  T  88
 265 AATAAACTTGGTTTTCAAAAAGATAACATGCGTAACATTTTTGATTATGTTATGGTTCTTTTAGATTCTCGGGCATCTCGTATGTCTCCTTCCTCGGCACTGCTTACTATCCATGCCGATGTCATTGGTGGT 396
  89  N  K  L  G  F  Q  K  D  N  M  R  N  I  F  D  Y  V  M  V  L  L  D  S  R  A  S  R  M  S  P  S  S  A  L  L  T  I  H  A  D  V  I  G  G  132
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1981 TACCATCAAGTACCTTCTGAAAAGGCAGGCTATCATACTCTTAGAGCTCCCAACTTTTTCTACTCCCAACAGGTTAAGCATTATAAGCAAGATTTGTTTCCGGCTAATTCAGAAGCTGCTCGAAGAATCTCC 2112
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 925  E  R  H  L  Y  S  V  L  I  D  G  H  C  P  I  M  E  N  G  K  R  R  P  K  Y  R  I  R  L  S  G  N  P  I  L  G  D  G  K  S  D  N  Q  N  968
2905 ATGTCTATTCCTTACATTAGAGGTGAATATGTACAAATGATCGATGCCAACCAGGATAATTATTTGGAAGAATGTCTAAAGATTCGTAGCATTCTTGCTGAATTTGAGCAACTTACTCCTCCCTTACATAGC 3036
 969  M  S  I  P  Y  I  R  G  E  Y  V  Q  M  I  D  A  N  Q  D  N  Y  L  E  E  C  L  K  I  R  S  I  L  A  E  F  E  Q  L  T  P  P  L  H  S  1012
3037 CCTTACTCGGTAAATGCCAAAGCTGCTGACAATCATCCCGTAGCTATTCTCGGTGCTAGAGAATACATTTTTTCAGAAAATACAGGTATGCTTGGAGATGTAGCTGCTGGAAAGGAACAAACATTTGGTACT 3168
1013  P  Y  S  V  N  A  K  A  A  D  N  H  P  V  A  I  L  G  A  R  E  Y  I  F  S  E  N  T  G  M  L  G  D  V  A  A  G  K  E  Q  T  F  G  T  1056
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3433 GCGGAACAAATGCTTTCAAGAGAATATTTCAACCTTGGCACACAATTACCGTTTGATAGGTTTTTGTCATTTTTCTATGCCCACGCTGGATTTCACGTCAATAACATGGTCATCATGTTTTCTTTACAGTTA 3564
1145  A  E  Q  M  L  S  R  E  Y  F  N  L  G  T  Q  L  P  F  D  R  F  L  S  F  F  Y  A  H  A  G  F  H  V  N  N  M  V  I  M  F  S  L  Q  L  1188
3565 TTGATGCTTGTTATTATTAATTTGGGAGCAATGTACACTGTTGTTCCGGTTTGTAGATACCGCCAATTTGACTCCTTGACAGCTTCTTTGTACCCTGAAGGTTGTTATCAATTAAAACCAGTTCTCGAGTGG 3696
1189  L  M  L  V  I  I  N  L  G  A  M  Y  T  V  V  P  V  C  R  Y  R  Q  F  D  S  L  T  A  S  L  Y  P  E  G  C  Y  Q  L  K  P  Vx L  E  W  1232
3697 TTGAAGCGTTGTATTTTGTCTATTTTCATTGTCTTTGGTATTGCTTTTGTTCCACTTGCTGTTTGTGAATTAGGGGAACGCGGTGCCATCCGAATGGTTATTCGTTTAGCGAAACAAATATTCTCTCTATCT 3828
1233  L  K  R  C  I  L  S  I  F  I  V  F  G  I  A  F  V  P  L  A  V  C  E  L  G  E  R  G  A  I  R  M  V  I  R  L  A  K  Q  I  F  S  L  S  1276
3829 CCTATTTTTGAAATTTTCACATGCCAAATATATGCGCAGAGTTTAATTGCAAATCTTACGTTTGGTGGTGCACGCTATATTGGTACTAGCAGAGGTTTTGCTACTGTTCGTGTGCCGTTCTCACTATTGTAT 3960
1277  P  I  F  E  I  F  T  C  Q  I  Y  A  Q  S  L  I  A  N  L  T  F  G  G  A  R  Y  I  G  T  S  R  G  F  A  T  V  R  V  P  F  S  L  L  Y  1320
3961 TCTCGATTCTCTGGACCATCACTTTATTTTGGGTCACGACTTATGTACATGTTATTGTTTGGTTCAATTACTGCTTGGTTGCCGCATTATATTTACTTTTGGATTACGCTGACTGCTCTTTGCATTAGTCCA 4092
1321  S  R  F  S  G  P  S  L  Y  F  G  S  R  L  M  Y  M  L  L  F  G  S  I  T  A  W  L  P  H  Y  I  Y  F  W  I  T  L  T  A  L  C  I  S  P  1364
4093 TTCCTTTACAATCCCCATCAATTCGCCTGGACCGATTTCTTTGTTGATTATCGTGAGTTTATGCGCTGGCTATTCCGCGAAAATTCACGCAATCAAGCAAATTCATGGATTGGCAACTGTCAACTTTGCCGT 4224
1365  F  L  Y  N  P  H  Q  F  A  W  T  D  F  F  V  D  Y  R  E  F  M  R  W  L  F  R  E  N  S  R  N  Q  A  N  S  W  I  G  N  C  Q  L  C  R  1408
4225 ACCAGAGTAACTGGGTATAAACGTAAAATATATGGTAAGAAGGCAGATAAAATTGCTATGGATTCCCCGCGTGCGAGAATTACTACAATGTTTTATGGTGAAATATTAGGGCCTCTAGGAACACTATTTTTC 4356
1409  T  R  V  T  G  Y  K  R  K  I  Y  G  K  K  A  D  K  I  A  M  D  S  P  R  A  R  I  T  T  M  F  Y  G  E  I  L  G  P  L  G  T  L  F  F  1452
4357 ACGTGTATTCCATTTCTCTTTATCAATTCCCAACCTGGTAACGATGATGAGACCCAATCTACTAATGCTTTTATTCGCTTGATTATTATGTCAGTTGCTCCTTTGGTTTTAAGTGCAATTATTGCTTTTTTC 4488
1453  T  C  I  P  F  L  F  I  N  S  Q  P  G  N  D  D  E  T  Q  S  T  N  A  F  I  R  L  I  I  M  S  V  A  P  L  V  L  S  A  I  I  A  F  F  1496
4489 TTTTTCTGTTTAGGAATCATGTTACGTCCTATTCTCGGCGATAGATCTAAAACGTATGGTGTATATTTGGCTGGAGTGGCCCATTTCCTTTTTGTGTGTGTTGATGTTGTTGTCTTTGAAGTATTGGGCTAT 4620
1497  F  F  C  L  G  I  M  L  R  P  I  L  G  D  R  S  K  T  Y  G  V  Y  L  A  G  V  A  H  F  L  F  V  C  V  D  V  V  V  F  E  V  L  G  Y  1540
4621 TTGGAAGGTTGGTCATTTTCAAAAACTTTACTTGGATTTGTAGCAATCATTTCAATTCACAGGTTTGCTCATAAATTTTTTATCATATGTTTCCTGTCTCGAGAATTTCGTCATGATGGTGCCAATTTAGCT 4752
1541  L  E  G  W  S  F  S  K  T  L  L  G  F  V  A  I  I  S  I  H  R  F  A  H  K  F  F  I  I  C  F  L  S  R  E  F  R  H  D  G  A  N  L  A  1584
4753 TGGTGGTCTGGACGTTGGAATGGCCAAGGTTTTGGATATATGGTTTTAACGCAACCATGGAGAGAATTTGTGTGTAAAACTACAGAGTTGAATATGTTTGCTGGTGATTTCCTTTTAAGTCATCTTTTGTTG 4884
1585  W  W  S  G  R  W  N  G  Q  G  F  G  Y  M  V  L  T  Q  P  W  R  E  F  V  C  K  T  T  E  L  N  M  F  A  G  D  F  L  L  S  H  L  L  L  1628
4885 TTTTTGCAAGCGCCTGTTATTTTGATTCCTTATATCGACAAACTTCACTCGATCATATTATTCTGGCTTGTGCCCAGCAGACAAATTCGACCCCCAATTTATACCATAAGACAAAACAAACTTCGTCGTCAA 5016
1629  F  L  Q  A  P  V  I  L  I  P  Y  I  D  K  L  H  S  I  I  L  F  W  L  V  P  S  R  Q  I  R  P  P  I  Y  T  I  R  Q  N  K  L  R  R  Q  1672
5017 ATAGTTCTTCGCTATGCCACTTTGTACTTTTCATTGTTTATTGCCTTTTTTGTTCTTTTAATTTTGCCTTTTGTGTTTGGTAAATCAGCTGCTGGAACTTCAATGGATAAATTTAATCTGATTCAGCCAGCG 5148
1673  I  V  L  R  Y  A  T  L  Y  F  S  L  F  I  A  F  F  V  L  L  I  L  P  F  V  F  G  K  S  A  A  G  T  S  M  D  K  F  N  L  I  Q  P  A  1716
5149 ACAAAGATTGTTTATTCTTCAACAAAGAATTCTTCTGTCTAAAGCATTTTATTTTTTTTAAAGGATTTGTGTGATGTGGATGCTTATTTCTCATTAGAAATGCTCTCAATTAGGGTAAATAATAATTATTA 5280
1717  T  K  I  V  Y  S  S  T  K  N  S  S  V  *                                                                                              1730
5281 TATTTGCATTTTGTTAAATAAAAAATGACTTGAGTGTTGATTAAGTATCTAGTACTTGAGAATATATTATCTAAATAAGTAATGAAGAACGATTGATGGTTAGAGCAAACACAAACTTAATGTCTGTTACAT 5412
5413 GAGAAAAAATTATTGGTGCACCGTCTGCCCGTAATGTGATATTGATTATGTTATTACGTTCACATTTACAAATCGCTTGAAGAGATAAGGCTAACGGATTTTGAAACGTTTTGTTTAGCATCTTTAGTACGG 5544
5545 TGGTACTCATCTGAAAGTTTCTTCATAAGTTCCTTGTGAGTGATGTGTGGGTGTAGCTTTCGTAGTCTCTTAGAATTC                                                      5622
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FIG. 6. Nucleotide and predicted amino acid sequences of the *S. pombe* $cps1^{+}$ gene. See Results for details.

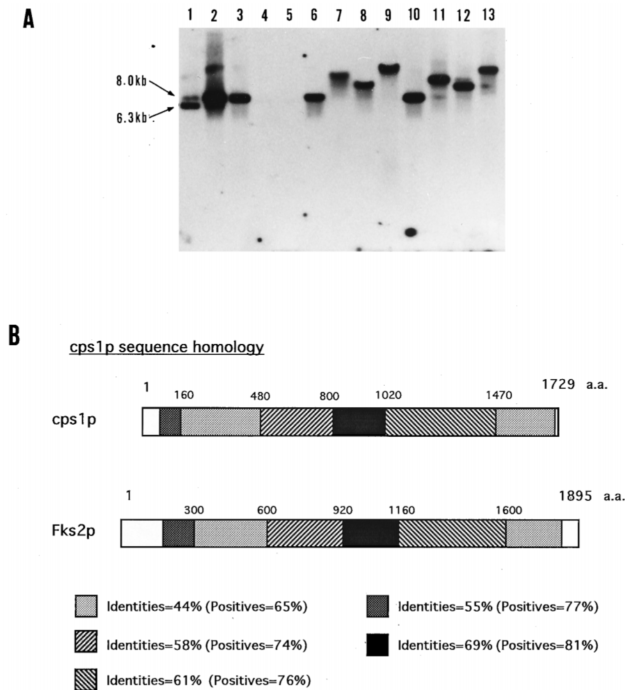

FIG. 7. (A) Southern blot analysis of the *S. pombe cps1*$^+$ gene. DNAs were digested with different restriction enzymes as indicated below, separated in a 0.6% agarose gel, and hybridized as indicated in Materials and Methods. Lane 1 and 2, pCP1; lane 3, cosmid clone ICRFc60C0624; lane 4, cosmid clone ICRFc60H0832; lane 5, cosmid clone ICRFc60B0529; lanes 6 to 9, strain 972 (h$^-$, wild type) genomic DNA; lanes 10 to 13, cosmid clone ICRFc60C0624. Cosmid clone nomenclature was according to RZPD. DNAs were digested with *Hin*dIII (lanes 1 to 6), *Kpn*I, *Pst*I, and *Bam*HI, (lanes 7 to 9, respectively) and *Hin*dIII, *Kpn*I, *Pst*I, and *Bam*HI, (lanes 10 to 13, respectively). An approximately 0.8-kb *Sal*I-*Xho*I fragment from the *cps1*$^+$ gene sequence was used as the probe. Labeled pCP1 was used as a control in lane 1. The sizes of plasmid (6.3 kb) and insert (8.0 kb) after *Hin*dIII digestion of pCP1 are indicated to the left. (B) Sequence homology between cps1p from *S. pombe* and Fks2p from *S. cerevisiae*. BLASTP (3) was used for the homology search. A similar sequence homology was obtained upon comparing cps1p from *S. pombe* with Fks1p or Fks3p from *S. cerevisiae* or FksAp from *A. nidulans*. a.a., amino acids.

gene was described as a component of another 1,3-β-D-glucan synthase activity, induced by addition of Ca^{2+} to the medium and responsible for the CsA sensitivity of *fks1* mutants (40). Some *cps1-12* mutant phenotypes seem to be dependent on calcium concentration in the medium. With increasing calcium, the hypersensitivity to papulacandin B is lost and cytokinesis and cell polarity defects are partially corrected. However, the facts that other mutant phenotypes, such as sensitivity to enzymatic lysis and thermosensitive lytic phenotypes, are not modified and that sorbitol protection from lysis induces mutant cells to show cytokinesis and cell polarity defects suggest that the *cps1-12* mutation may affect different cellular processes, one related to cell wall structure (lysis that can be prevented by an osmotic stabilizer) and the other related to cell division and polarity (when cells do not lyse multiseptation and branching appear).

The cps1p identity with all known Fks proteins and the coincidence of phenotypes between *cps1-12* and different *fks1* mutant alleles suggest that cps1p may be a catalytic or associated copurifying subunit of 1,3-β-D-glucan synthase as well. All are predicted integral membrane proteins with 15 to 16 transmembrane helices, and none contains the proposed UDP-glucose binding consensus (R/K)XGG found in glycogen synthase of several species (19, 21). Nevertheless, all contain another proposed homologous motif in their putative cytosolic face, RXTG (28) as RITG at 1532 on Fks1p and at 1560 on FksAp, RVTG at 1551 on Fks2p and at 1410 on cps1p, or RFTG at 1445 on Fks3p. Another modified motif for a proposed glucan synthase was suggested to be RVSG (52), but neither this nor another consensus sequence was found among the Fks proteins. RXTG is the most likely consensus sequence for the proposed 1,3-β-D-glucan synthase subunits, but it needs to be proven.

That some *cps1-12* mutant phenotypes are corrected with Ca^{2+} resembles the Fks2p effect in *fks1* mutants. On the other hand, *cps1*$^+$ does not contain in its promoter MCB and SCB elements, while *FKS1* does, and it contains the UDP-glucose binding consensus RVTG, like Fks2p. It is possible that *cps1*$^+$ shares some functions with *FKS1* and others with *FKS2*. *FKS3* has not been studied yet, and *cps1*$^+$ may also have some functions in common with it. The *S. pombe* regulatory mechanisms of glucan or cell wall synthesis may be different from those of *S. cerevisiae* in some aspects, including number or role of *FKS* homologous genes. Also, some *cps1-12* thermosensitive and Ca^{2+}-dependent phenotypes seem to be more related to cytokinesis and cell polarity than to those of *fks1* and *fks2* mutants. Calcineurin mutants display similar phenotypes (41, 60). Alternatively, the pleiotropic phenotypes described here might indicate that *cps1*$^+$ affects some membrane and cell wall functions rather than specifically 1,3-β-D-glucan synthesis. Further study, including analysis of the *cps1*$^+$ gene disruption, a complementation test between *cps1*$^+$ and *FKS1* or *FKS2*, and a fine search of possible *cps1*$^+$ homologs, will elucidate in more detail the functions of *cps1*$^+$.

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ADDENDUM IN PROOF

A recent report (T. Mio, M. Adachi-Shimizu, Y. Tachibana, H. Tabuchi, S. B. Inoue, T. Yabe, T. Yamada-Okabe, M. Arisawa, T. Watanabe, and H. Yamada-Okabe, J. Bacteriol. **179:**4096–4105, 1997) described the cloning of three *Candida albicans* genes, *GSC1*, *GSL1*, and *GSL2*, with predicted amino acid sequences having significant homologies to Fks1p, Fks2p, and FksAp (64 to 73% for Gsc1p and 47 to 55% for Gsl1p and Gsl2p).

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