Saccharomyces cerevisiae HOC1, a Suppressor of *pkc1*, Encodes a Putative Glycosyltransferase

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ABSTRACT

The *Saccharomyces cerevisiae* gene *PKC1* encodes a protein kinase C isozyme that regulates cell wall synthesis. Here we describe the characterization of *HOC1*, a gene identified by its ability to suppress the cell lysis phenotype of *pkc1-371* cells. The *HOC1* gene (Homologous to *OCH1*) is predicted to encode a type II integral membrane protein that strongly resembles Och1p, an α-1,6-mannosyltransferase. Immunofluorescence studies localized Hoc1p to the Golgi apparatus. While overexpression of *HOC1* rescued the *pkc1-371* temperature-sensitive cell lysis phenotype, disruption of *HOC1* lowered the restrictive temperature of the *pkc1-371* allele. Disruption of *HOC1* also resulted in hypersensitivity to Calcofluor White and hygromycin B, phenotypes characteristic of defects in cell wall integrity and protein glycosylation, respectively. The function of *HOC1* appears to be distinct from that of *OCH1*. Taken together, these results suggest that *HOC1* encodes a Golgi-localized putative mannosyltransferase required for the proper construction of the cell wall.

THE *Saccharomyces cerevisiae* protein kinase C isozyme encoded by *PKC1* is a key regulator of cell wall synthesis (KLIS 1994). Mutants in *pkc1* display abnormally thin and brittle cell walls (PARAVICINI *et al.* 1992; LEVIN *et al.* 1994). This defect in cell wall structure manifests itself as a cell-cycle-specific cell lysis phenotype (LEVIN and BARTLETT-HUEBUSCH 1992). That defects in the cell wall are the primary cause of lethality of *pkc1* mutants is shown by the fact that a deletion of *PKC1* can be rescued by the inclusion of osmotic stabilizers, such as sorbitol, in the growth medium (LEVIN and BARTLETT-HUEBUSCH 1992).

Pkc1p is a cytoplasmic enzyme that appears to regulate cell wall synthesis indirectly, via a signal transduction pathway. Pkc1p activity initiates signalling through a MAP kinase cascade consisting of the *BCK1, MKK1* or *MKK2,* and *MPK1* gene products (LEE and LEVIN 1992; IRIE *et al.* 1993; LEE *et al.* 1993). Overexpression of any of these downstream kinases can partially rescue the lysis phenotype of a *pkc1* mutant. Mutations of *bck1, mpk1,* or an *mkk1 mkk2* double mutant all result in cell wall defects producing a temperature-sensitive cell lysis phenotype (LEE and LEVIN 1992; IRIE *et al.* 1993; LEE *et al.* 1993).

In addition to regulation of this kinase cascade, Pkc1p may have a second function in cell wall synthesis (LEE and LEVIN 1992). The nature of this function has not yet been elucidated, but one potential regulatory target is the *KRE6* gene product. Kre6p is required for synthesis of β-1,6-glucan. Overexpression of *KRE6* will partially rescue the cell lysis defect of a *pkc1* mutant, but not of an *mpk1* mutant or an *mkk1, mkk2* double mutant (ROEMER *et al.* 1994). These observations suggest a defect in *pkc1* mutants, suppressible by *KRE6,* which is not present in strains mutant in the downstream kinases (ROEMER *et al.* 1994).

The cell wall itself is constructed of an outer mannan layer and an inner layer consisting largely of β-1,6- and β-1,3-glucans as well as some chitin. The β-1,3-glucan and chitin polymers are synthesized in the periplasm, while the β-1,6-glucans are assembled in the endoplasmic reticulum (ER) and Golgi (KLIS 1994). The mannan layer consists primarily of heavily glycosylated mannoproteins that have oligosaccharide chains attached via asparagine (*N*-linked) or serine and threonine (*O*-linked) linkages. These chains are assembled as the proteins transit through the secretory pathway (HERSCOVICS and ORLEAN 1993).

One critical protein in the extension of *N*-linked oligosaccharide chains is the *OCH1* gene product (NAKAYAMA *et al.* 1992). *OCH1* encodes an α-1,6-mannosyltransferase that resides in the Golgi. Och1p catalyzes the addition of an α-1,6-linked mannose to the core oligosaccharide that is attached to the protein in the ER (NAKANISHI-SHINDO *et al.* 1993). In the absence of Och1p, extension of *N*-linked oligosaccharide chains is blocked. In this report we describe the isolation and characterization *HOC1,* a high copy suppressor of a temperature-sensitive *pkc1* mutation. *HOC1* encodes a putative glycosyltransferase related to Och1p.

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TABLE 1

Strains used in this study

Strain	Genotype	Source
SEY6210	*MATα ura3-52 his3-Δ200 trp1Δ901 lys2-801 suc2-Δ9 leu2-3,112*	S. EMR
RSY255	*MATα ura3-52 leu2-3,112*	R. SCHEKMAN
371-4C-5C-4D	*MAT***a** *ura3 trp1 leu2 pkc1-371*	This study
VMY2	As RSY255 plus, *hoc1::LEU2*	This study
VMY2-2D	*MATα ura3 leu2 pkc1-371 hoc1::LEU2*	This study
VMY2-7D	*MAT***a** *ura3 leu2 pkc1-371 hoc1::LEU2*	This study

MATERIALS AND METHODS

Strains and media: *S. cerevisiae* strains used in this study are listed in Table 1. Yeast were routinely grown on YPAD (1% yeast extract, 2% bacto-peptone, 50 mg/l adenine, 2% glucose) or SD (0.67% Difco yeast nitrogen base, 2% glucose) containing appropriate nutritional supplements. Sensitivity to calcofluor white (CFW) (Sigma) was tested by streaking yeast onto YPAD plates supplemented with 1.5 mg/ml CFW (RAM *et al.* 1994). Hygromycin B sensitivity was tested on YPAD plates supplemented with 50 μg/ml hygromycin B (Boehringer) (BALLOU *et al.* 1991).

Plasmids: Plasmid p371B is a library clone of *PKC1* in the plasmid pUV1 (2μ *URA3*). Plasmid p371A is the original clone of *HOC1* in the library plasmid p366 (*CEN LEU2*). p371A *Hin*dIIIΔ was made by digestion of p371A with *Hin*dIII and religation of the backbone fragment. Similarly, p371A *Bam*HIΔ was constructed by digestion of p371A with *Bam*HI and religation of the backbone. Plasmid pRS425::HOC1 was made by cloning the 2.5-kb *Hin*dIII-*Bam*HI genomic fragment encoding the *HOC1* gene into *Hin*dIII-*Bam*HI-cut plasmid pRS425. Similarly, plasmid YEp352::HOC1 was constructed by cloning the same 2.5-kb *Hin*dIII-*Bam*HI genomic fragment into *Hin*dIII-*Bam*HI-cut plasmid YEp352. pCDC11 (from J. KONOPKA) carries the *CDC11* gene under control of the *ADH1* promoter.

Plasmid pVM3 was constructed as follows: first, convenient restriction sites were placed at the 5′ and 3′ ends of the *HOC1* coding sequence by PCR. Primer 1 (5′-CTCGGAATTCAGCAGATAGATATAT-3′) introduced an *Eco*RI site before the initiation codon and primer 2 (5′-GAGGATCCTCTAGATGCATTTCTGCTCCACCTTTG-3′) introduced *Nsi*I and *Bam*HI sites at the 3′ end of the coding sequence. The PCR fragment was digested with *Eco*RI and *Bam*HI and cloned into *Eco*RI-*Bam*HI-cut pBlueScript SK⁻, generating the plasmid pVM1. A *Hin*dIII-*Nsi*I fragment from pVM1 was then ligated into a plasmid containing the *c-myc* epitope, generating plasmid pVM2. This places the *c-myc* epitope at the 3′ end of the *HOC1* coding sequence, changing the carboxy-terminal amino acid sequence from PKEVQK to PKEVQKCMEQKLISEEDLNSAEAARL. pVM2 was cut with *Bam*HI, treated with Klenow to fill in the ends, and then digested with *Hin*dIII. The *Hin*dIII-blunt end fragment carrying *HOC1-myc* was subcloned into a *Hin*dIII-*Sma*I-cut deriviative of YIp56X (PELHAM 1988), an integrating plasmid that carries the *URA3* gene with a *Xho*I site inserted into it and the constitutive triose phosphate isomerase (*TPI*) promoter. This construct (pVM3) places the *HOC1-myc* sequence under control of the *TPI* promoter. Before transformation of yeast strains, pVM3 was cut at the unique *Xho*I site within the *URA3* gene to target the integration.

The disruption plasmid pVM4 was made as follows. Plasmid pVM1 was cut at the unique *Pst*I site in the *HOC1* sequence and blunt-ended by mung bean nuclease treament. The *LEU2* gene was isolated by *Bam*HI digestion of plasmid YDpL (BERBEN *et al.* 1991), blunt-ended by Klenow treament, and ligated to the linearized pVM1. This fusion interrupts the *HOC1* coding sequence after amino acid residue 45, separating the transmembrane domain from the region homologous to *OCH1*. The disruption fragment was liberated by *Bam*HI-*Hin*dIII digestion.

To construct pTiOCH-HA, the *OCH1* gene was first cloned in-frame to three tandem copies of the influenza hemagglutinin epitope (HA) tag. A fragment containing the *OCH1* ORF, lacking the stop codon and flanked by a 5′ *Hin*dIII and a 3′ *Nsi*I site was isolated by PCR. This *OCH1* fragment was cloned into *Hin*dIII-*Pst*I digested pSK-HA3, a Bluescript SK⁻ derivative (Stratagene). pSK-HA3 carries a 108-bp fragment containing sequences that encode three tandem copies of the HA epitope (YPYDVPDY) cloned between the *Pst*I and *Xba*I sites. Thus, the SKOCH1-HA construct contains an in-frame fusion of the three copies of the HA epitope to the carboxy terminus of *OCH1*. Finally, pTiOCH-HA was contructed by isolating the *Hin*dIII (blunted with Klenow)-*Xba*I fragment containing the triple HA-tagged *OCH1* gene and cloning it into YIp56X digested with *Eco*RI (blunted with Klenow) and *Xba*I. This plasmid carries the HA-tagged *OCH1* gene under control of the *TPI* promoter.

Cloning and sequencing the *HOC1* gene: Strain 371-5C-4C-1D was transformed by the lithium acetate method (ITO *et al.* 1983) with a genomic library in the *CEN ARS LEU2* vector p366 (from PHIL HIETER). Plates selective for transformants were placed at 23° overnight and then shifted to 37° to select for plasmids suppressing the temperature-sensitive phenotype. One clone of *HOC1* was obtained from ~5000 transformants.

Sequence alignments were performed using the Megalign program (DNASTAR). The sequence of the cosmid carrying *HOC1* was obtained as follows: total cosmid DNA was nebulized and treated with T4 DNA polymerase and Klenow, the blunt end fragments were then cloned into the *Eco*RV site of M13mp90, and sequencing was performed using an ABI373 sequencer and dye primers. The sequence of *HOC1* is available in GenBank, accession number Z49575.

Integration and disruption of the *HOC1* gene: One-step gene disruption using plasmid pVM4 and the haploid strain RSY255 was performed as described by ROTHSTEIN (1983) to give strain VMY2. Disruption of the genomic copy of *HOC1* was confirmed by PCR analysis of total yeast DNA using the primers used for PCR amplification of *HOC1*. That disruption of *HOC1* directly in a haploid had not resulted in the isolation of suppressor mutations was confirmed by back-cross of VMY2 to a wild-type strain (data not shown).

Detection of c-myc epitope-tagged Hoc1p and invertase in protein extracts: Protein extracts of yeast strains were prepared as described (POSTER and DEAN 1996). Protein samples were boiled for 5 min before fractionation on an 8% SDS-polyacrylamide gel and transferred to nitrocellulose. The Hoc1p protein was visualized by incubating nitrocellulose blots with 1/10 diluted mouse monoclonal antibody 9E10 culture supernatant (EVAN *et al.* 1985), followed by 1/3000

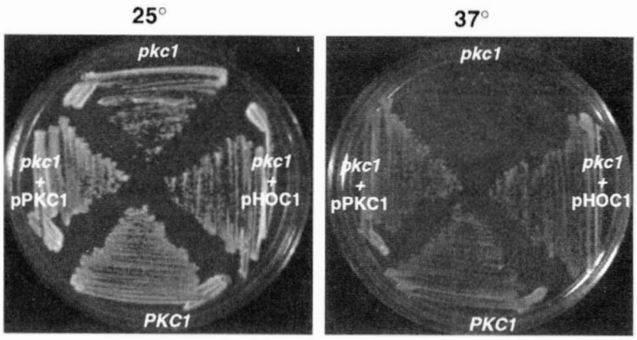


FIGURE 1.—*HOC1* rescues *pkc1-371*. Strain 371-5C-4C-1D (*pkc1-371*) transformed with a plasmid carrying *PKC1* (pPKC1 is p371B), untransformed, or transformed with a plasmid carrying *HOC1* (pHOC1 is p371A) was streaked out on YPAD plates and incubated at 25° or 37° as indicated. The *PKC1* strain is RSY255.

diluted goat-anti-mouse IgG horseradish peroxidase conjugate and chemiluminscent detection (Amersham).

Indirect immunofluorescence: Immunofluorescence was performed largely as described by REDDING *et al.* (1991). Epitope-tagged proteins were detected using 1/5 diluted 9E10 antibody culture supernatant. Pdi1p was detected using 1/90 diluted rabbit anti-Pdi1p polyclonal antibody (J. LUZ, SUNY Stony Brook). Diluted goat anti-mouse or goat anti-rabbit fluorescein-conjugated (FITC) secondary antibodies (1/200, Cappell-Organon Technika) were used to detect *c-myc* tagged proteins and Pdi1p, respectively. Nuclei were stained with the fluorescent stain 4′,6′-diamidino-2-phenylindoledihydrochloride (DAPI). Samples were visualized with a Bio-Rad MRC600 laser scanning confocal attachment and a Nikon Diaphot inverted microscope.

RESULTS

Isolation of *HOC1* as a dosage suppressor of a *pkc1-371* temperature-sensitive mutant: A screen for mutants with abnormal nuclear membrane morphology was conducted by staining a collection of temperature-sensitive strains (HARTWELL 1967) with the lipophilic dye $DiOC_6$ (KONING *et al.* 1993; A. M. NEIMAN and R. STERNGLANZ, unpublished data). Sequence of a complementing clone for one of the mutants obtained in this screen showed the rescuing activity to be the *PKC1* gene. Complementation tests of this mutant with previously identified alleles of *PKC1* demonstrated that the mutant is a new temperature-sensitive allele of *PKC1*, designated *pkc1-371* (data not shown).

In the process of cloning *PKC1*, a second library plasmid was found that did not contain the *PKC1* gene but that rescued the temperature-sensitive growth defect of a strain carrying the *pkc1-371* mutation (Figure 1). Subcloning of the genomic insert localized the suppressing activity to a 2.5-kb *Bam*HI-*Hin*dIII fragment (Figure 2). A partial sequence of the insert indicated that the genomic fragment came from the right arm of chromosome *X*. Analysis of the complete sequence of this region of chromosome *X* revealed that the *Bam*HI-*Hin*dIII fragment contains the 3′ end of the *CDC11* gene and a complete open reading frame that we designated *HOC1*. A clone containing the complete *CDC11* gene but not *HOC1* did not rescue the temperature-sensitivity of *pkc1-371* (Figure 2).

The lethality of *pkc1* mutations has previously been shown to be caused by cell lysis due to defects in the cell wall (LEVIN and BARTLETT-HUEBUSCH 1992; PARAVICINI *et al.* 1992). Two observations suggest that the temperature sensitivity of *pkc1-371* is likely to result from a similar defect. First, *pkc1-371* mutants display abnormally thin cell walls when observed in the electron microscope and second, the inclusion of 1 M sorbitol in the medium will allow growth of *pkc1-371* strains at high temperature (data not shown). The rescue of *pkc1-371* by *HOC1* therefore suggests a role for *HOC1* either directly in cell wall metabolism or in the signal transduction pathway downstream of *PKC1*.

***HOC1* encodes a protein similar to the Och1p glycosyltransferase:** The nucleotide and predicted amino acid sequence of *HOC1* is shown in Figure 3A. *HOC1* is predicted to encode a protein of 396 amino acids with a calculated molecular weight of 46.2 kDa. A search of the Genbank database using the Blastp algorithm (ALTSCHUL *et al.* 1990) revealed significant homology between Hoc1p and the α-1,6-mannosyltransferase encoded by the *S. cerevisiae OCH1* gene. Over the entire length of the protein, Hoc1p displays 33% identity and 58% similarity to Och1p (Figure 4A), with the strongest homology lying between residues 196 and 329 of Hoc1p (50% identity and 71% similarity). In addition, Hoc1p and Och1p share more limited homology with the products of the *SUR1* gene, the *S. cerevisiae* chromosome *II* open reading frame YBR161w and the *Schizosaccharomyces pombe* chromosome *I* open reading frame SPAC17G8.11 (Figure 4B). *SUR1* has been identified both by a mutation that suppresses the starvation-sensitive phenotype of an *rvs161* mutant (DESFARGES *et al.* 1993) and as a high copy suppressor of the Ca^{2+}-sensitive mutant *cls2* (TAKITA *et al.* 1995). The function of *SUR1*, however, is unclear. No function has been reported for YBR161w or for the *S. pombe* open reading frame.

Och1p is predicted to be a type II integral membrane protein with a short cytoplasmic amino-terminal domain and a lumenal carboxy-terminal catalytic domain (NAKAYAMA *et al.* 1992). This organization is characteristic of a variety of mammalian and yeast glycosyltransferases (PAULSON and COLLEY 1989). A hydropathy analysis predicts that Hoc1p possesses a single potential transmembrane spanning region near the amino-terminus of the protein (Figure 3B) (KYTE and DOOLITTLE 1982), suggesting that it too may be a type II membrane protein. The sequence homology and organizational similarity between Hoc1p (Homologous to *OCH1*) and Och1p suggests that *HOC1* might also encode a glycosyltransferase.

A synthetic interaction between *hoc1* and *pkc1-371*: To assess the function of *HOC1 in vivo*, a mutant allele was constructed by the insertion of the *LEU2* gene into the *HOC1* coding region. A strain carrying the

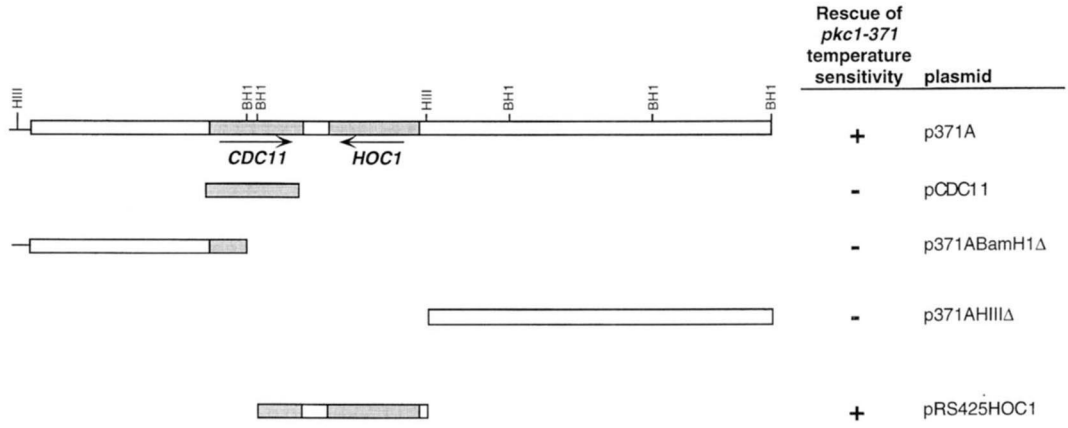


FIGURE 2.—Sucloning of *HOC1*. The top line shows the extent of the genomic insert in the original *HOC1* clone. ▭ indicate the position of the *CDC11* and *HOC1* genes and arrows indicate the direction of transcription. The vertical lines above the insert indicate the position of restriction sites. BHI, *Bam*HI; HIII, *Hin*dIII. The names of the plasmids used are given at right.

hoc1::LEU2 allele, VMY2, was constructed as described in MATERIALS AND METHODS. *hoc1* mutants are viable, morphologically normal, and display no obvious growth defects. Unlike *och1* mutants, which are temperature sensitive for growth, *hoc1* mutants grew well at both high (37°) and low (23°) temperatures. Thus, *HOC1* appears not to be required for growth under normal culture conditions.

Increased dosage of *HOC1* can rescue the temperature-sensitive phenotype of strains harboring *pkc1-371* (Figure 1). We therefore wished to examine if loss of *HOC1* might exacerbate the defect of a *pkc1-371* strain. A diploid heterozygous for both *hoc1::LEU2* and *pkc1-371* was constructed by mating strain VMY2 to 371-5C-4C-1D. Upon sporulation, this diploid gave rise to four viable progeny at 23° indicating that the *hoc1::LEU2 pkc1-371* double mutants are viable. However, when we tested growth at a range of temperatures between 23° and 37°, we found that the restrictive temperature of the *hoc1 pkc1-371* double mutant (identified as tempera-

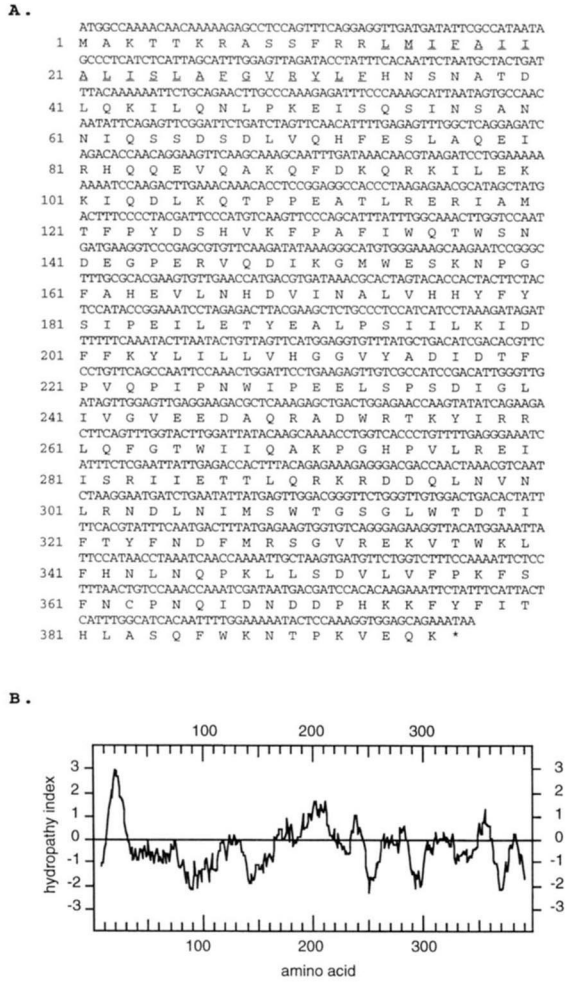


FIGURE 3.—Sequence of the *HOC1* gene. (A) Nucleotide and predicted amino acid sequence of *HOC1*. The potential transmembrane domain is underlined. (B) Kyte-Doolittle hydropathy plot of the Hoc1p amino acid sequence.

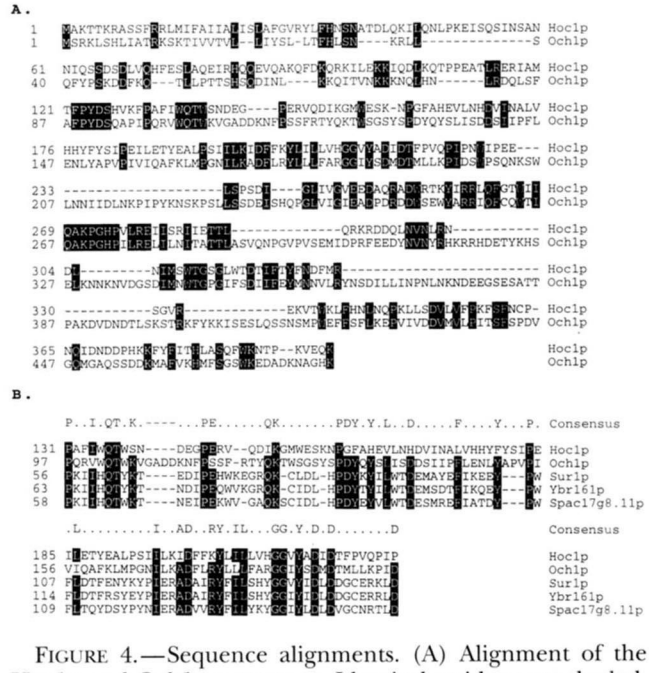


FIGURE 4.—Sequence alignments. (A) Alignment of the Hoc1p and Och1p sequences. Identical residues are shaded. (B) Alignment of the homology domain found in Hoc1p, Och1p, Sur1p, Ybr161p, and Spac17g8.11p. Consensus is four out of five identical.

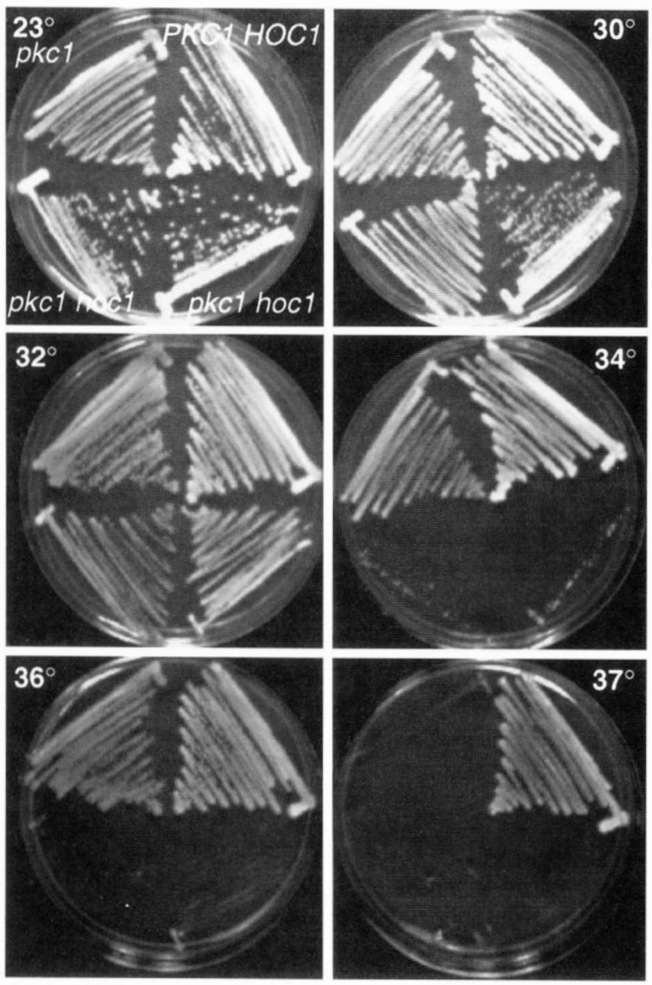


FIGURE 5.—*hoc1* exacerbates the *pkc1-371* defect. Strains 371-5C-4C-1D (*pkc1-371*, upper left), RSY255 (*PKC1 HOC1*, upper right), VMY3 (*pkc1-371*, *hoc1::LEU2*, lower right), and VMY4 (*pkc1-371*, *hoc1::LEU2*, lower left) were streaked out on YPAD plates and incubated at the indicated temperatures.

ture-sensitive, Leu$^+$ progeny) had decreased relative to the parental *pkc1-371* strain from 37° to 34° (Figure 5).

***hoc1* mutants are sensitive to Calcofluor White and hygromycin B:** The genetic interactions with *pkc1-371* suggest a role for *HOC1* in cell wall synthesis. To test this possibility, we asked whether a *hoc1* mutant strain was sensitive to Calcofluor White (CFW). CFW is a fluorescent dye that binds to chitin and disrupts cell wall assembly. Mutants defective in various aspects of cell wall synthesis have been isolated on the basis of sensitivity to CFW (RAM *et al.* 1994). As shown in Figure 6, inclusion of CFW in the medium completely inhibited the growth of a *hoc1* strain, though the isogenic wild-type strain was unaffected. Introduction of the wild-type *HOC1* gene on a plasmid rescued the sensitivity of the mutant. This result provides a second indirect piece of evidence that *HOC1* is important for proper synthesis of the cell wall.

Given the homology of Hoc1p to Och1p, it seemed likely that Hoc1p might function in cell wall synthesis as a glycosyltransferase. Mutants defective in most classes of *N*-linked glycosylation are sensitive to hygromycin B (BALLOU *et al.* 1991; DEAN 1995). We therefore examined the effect of this drug on a *hoc1* mutant strain. At a concentration of 50 μg/ml, hygromycin B completely inhibited the growth of a *hoc1* strain, though the isogenic wild-type strain was unaffected (Figure 6). As a wide variety of glycosylation-defective mutants are sensitive to this concentration of hygromycin B (DEAN 1995), this result does not indicate a specific function for Hoc1p in glycosylation. It does suggest, however, that *hoc1* mutants are defective in some aspect of protein glycosylation.

***hoc1* mutants display no detectable defect in protein glycosylation:** To explore more directly the role of *HOC1* in protein glycosylation, we examined a variety of glycosylated proteins in wild-type and *hoc1* mutant cells. Invertase, a secreted protein, is subject to extensive *N*-linked glycosylation during transit through the secretory pathway (REDDY *et al.* 1988). Immunoblot analysis of a *c-myc*-tagged version of invertase revealed no reproducible change in the mobility of invertase from *hoc1* cells (Figure 7, lane 2) relative to that from *HOC1* cells (Figure 7, lane 1). Using a similar approach we were unable to detect any alteration in the *N*-linked glycosylation of the vacuolar protease carboxypeptidase Y (data not shown). We have also examined *O*-glycosylation by studying the mobility of secreted chitinase, a protein exclusively decorated with *O*-linked sugars (KURANDA and ROBBINS 1991). Again, disruption of *HOC1* did not detectably alter the mobility of the protein (data not shown). To date, we have been able to find no gross changes in protein glycosylation in the *hoc1* mutant.

Overexpression of *HOC1* cannot rescue an *och1* mutant: One possible explanation for the modest phenotype of the *hoc1* mutant is redundancy between Hoc1p and the related Och1p. We attempted to assess this possibility by comparing the phenotype of an *och1 hoc1* double mutant strain to isogenic *och1* and *hoc1* single mutants. However, in the two strain backgrounds we examined (A364A and RSY255) the *och1* single mutant was either dead or extremely slow growing, making analysis of the double mutant problematic. Therefore, as an alternative way to assess redundancy of *OCH1* and *HOC1*, we tested the ability of overexpression of *HOC1* to rescue the growth defects in the original *och1* mutant strain (NAKAYAMA *et al.* 1992). Strains deleted for *OCH1* are blocked in extension of *N*-linked oligosaccharide chains beyond the core glycosylation added to the protein in the ER. *OCH1* is not essential in this background, but deletion strains are temperature-sensitive and sensitive to hygromycin B. Introduction of *HOC1* on a 2μ plasmid had no discernible effect on growth of the *och1* mutant at high temperature or on plates containing hygromycin B (data not shown).

As a further test for overlap in *HOC1* and *OCH1* function, we tried the reciprocal experiment of overexpressing *OCH1* in a *hoc1* mutant. The hygromycin B

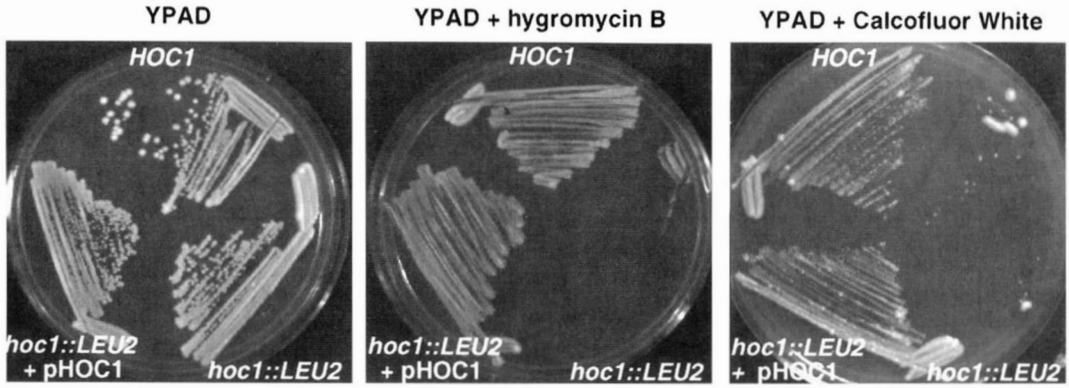


FIGURE 6.—*hoc1* mutants are sensitive to Calcofluor White and Hygromycin B. Isogenic strains RSY255 (*HOC1*), VMY2 (*hoc1::LEU2*), and VMY2 transformed with pYEp352::HOC1 were streaked out on YPAD plates containing no additive, 50 mg/ml hygromycin B, or 1 mg/ml Calcofluor White.

sensitivity of strain VMY2 (*hoc1::LEU2*) was not suppressed by introduction of a plasmid that expresses *OCH1* from the *TPI* promoter (Figure 8). Thus, overexpression of *OCH1* cannot suppress *hoc1* and vice versa. These results suggest that Hoc1p function is distinct from that of Och1p.

Hoc1p is localized in the Golgi: To localize the Hoc1 protein, we constructed a fusion of a sequence encoding the *c-myc* epitope (EVAN *et al.* 1985) to the 3′ end of the *HOC1* gene. This *HOC1-myc* fusion was expressed constitutively from the *TPI* promoter and could be detected by immunoblotting in total cell protein extracts (Figure 8E). Expression of this fusion protein in the *hoc1::LEU2* strain VMY2 rescued its hygromycin B-sensitive phenotype, indicating that the Hoc1-myc fusion protein was functional *in vivo* (data not shown).

We next performed indirect immunofluorescence experiments using the Hoc1-myc fusion protein. We also stained cells with antibodies to Pdi1p (LAMANTIA and LENNARZ 1993) and a *c-myc*-tagged Mnt1p (DEAN and POSTER 1996) as standards for ER and Golgi localization, respectively. Cells expressing *HOC1-myc* showed a punctate staining pattern (Figure 8B) with an average of three to seven bright dots per cell. These bright dots are not visible in the same strain without the *HOC1-myc* fusion (Figure 8A). The Hoc1-myc fusion protein staining pattern is distinct from the ER staining of Pdi1p (Figure 8D) and very similar to the pattern of the *c-myc*-tagged Mnt1p control (Figure 8C) and other Golgi-localized proteins (FRANZUSOFF *et al.* 1991; COOPER and BUSSEY 1992). Additionally, Hoc1p cofractionated with a different Golgi marker, Mnn1p (LUSSIER *et al.* 1995) in differential centrifugation experiments (Figure 10). Hoc1p fractionation was distinct from that of the ER marker Kar1p. We conclude that Hoc1p is probably localized to the Golgi apparatus in yeast.

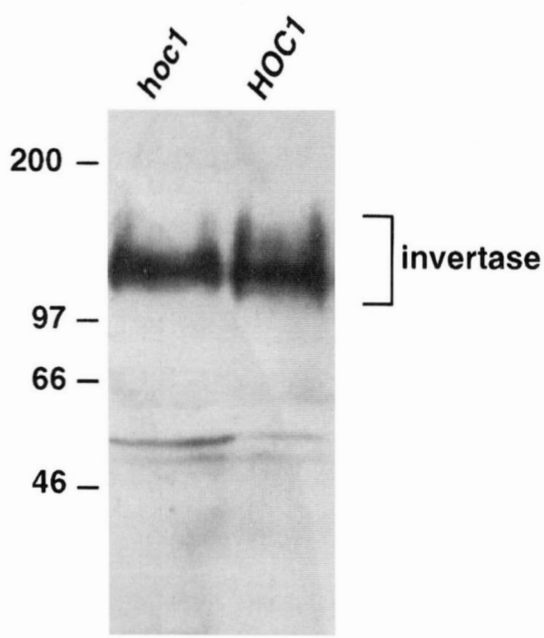


FIGURE 7.—Glycosylation of invertase in *hoc1* mutants. Protein extracts of isogenic strains RSY255 (*HOC1*) and VMY2 (*hoc1::LEU2*) carrying a plasmid expressing *c-myc*-tagged invertase were Western blotted and probed with anti-*myc* antibodies. Positions of molecular size standards are indicated on the right.

DISCUSSION

We report here the isolation of *HOC1* as a dosage suppressor of a temperature-sensititve *pkc1* mutant. The

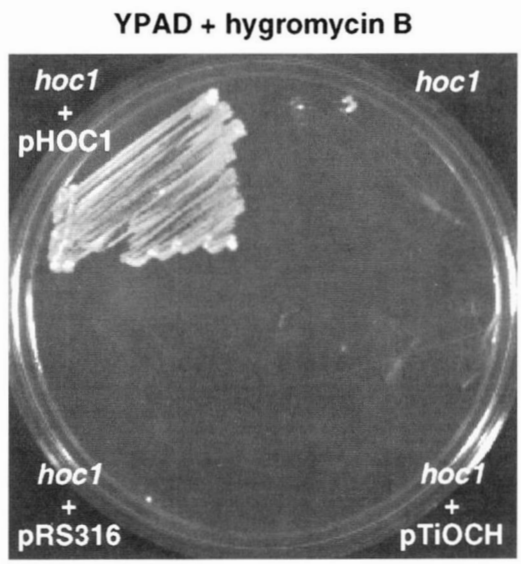
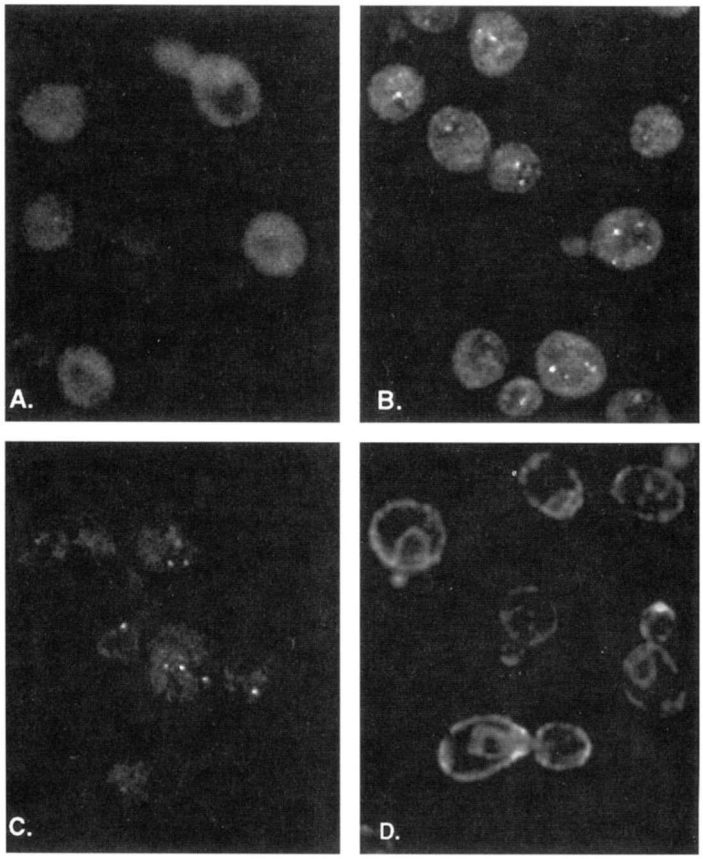


FIGURE 8.—Overexpression of *OCH1* does not suppress the hygromycin B sensitivity of a *hoc1* mutant. Strain VMY2 was transformed with pYEp352::HOC1 (pHOC1), pTiOCH1-HA (pTiOCH1), or pRS316 (vector) as indicated and streaked out on a YPAD plate containing 50 μg/ml hygromycin B.



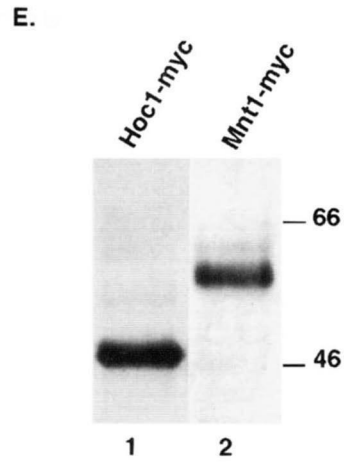


FIGURE 9.—Hoc1p localizes to a Golgi-like compartment. Strain SEY6210 carrying no plasmid (A and D), *c-myc*-tagged *HOC1* (B), or *c-myc*-tagged *MNT1* (C) was stained for indirect immunofluorescence with anti-myc antibodies (A—C) or anti-PDI antibodies (D). (E) Protein extracts of strain SEY6210 carrying a plasmid expressing either *c-myc*-tagged *HOC1* or *c-myc*-tagged *MNT1* were Western blotted and probed with anti-*myc* antibodies. Positions of molecular size standards are indicated on the right.

sequence of *HOC1* predicts a protein with significant similarity to the α-1,6-mannosyltransferase, Och1p. Both the homology to Och1p and the predicted topology of Hoc1p are suggestive that the Hoc1p might itself be a glycosyltransferase.

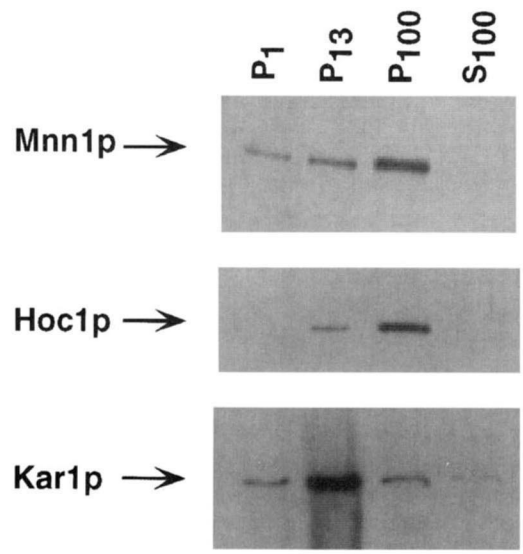


FIGURE 10.—Hoc1p cofractionates with the Golgi protein Mnn1p. Extracts of strain SEY6210 expressing HA-tagged Mnn1p (LUSSIER *et al.* 1995) and *c-myc*-tagged Hoc1p were subjected to differential centrifugation. Samples from the 1000 × *g* (P1) 13,000 × *g* (P13) and 100,000 × *g* (P100) pellets as well as the 100,000 × *g* supernatant (S100) were analyzed on a Western blot using anti-HA, anti-*c-myc*, or anti-HDEL (HARDWICK *et al.* 1990) antibodies. Kar1p is recognized by the anti-HDEL antibodies and serves as a marker for the ER.

Apart from the synthetic interaction with *pkc1-371*, the *hoc1* mutant displays no obvious cell growth phenotypes. It is, however, sensitive to the inclusion of CFW or hygromycin B in the growth medium (Figure 6). Mutants defective in cell wall synthesis are sensitive to CFW (RAM *et al.* 1994) while mutants defective in *N*-linked glycosylation are commonly sensitive to aminoglycosides such as hygromycin B (DEAN 1995).

Based on these observations, an economical hypothesis is that *HOC1* is a glycosyltransferase required for proper glycosylation of cell wall proteins. To test this possibility we examined two *N*-glycosylated (invertase and CPY) proteins and one *O*-glycosylated (chitinase) protein in the *hoc1* mutant. None of these proteins show any glycosylation defect in the *hoc1* mutant (Figure 7; data not shown). Although we cannot exclude the possibility that *HOC1* has a severe effect on glycosylation of a specific protein or set of proteins that we have not assayed, our current data suggest at best a minor role for Hoc1p in *N*-linked or *O*-linked glycosylation.

Hoc1p has a distinct role from Och1p: The *OCH1* and *HOC1* gene products are similar in primary sequence, and both Hoc1p and Och1p are localized to the Golgi. These results raise the possibilty that Hoc1p and Och1p have overlapping functions. Certainly there are numerous examples in yeast of genes with completely or partially redundant function (see *e.g.*, THOMAS 1993). To assess this possibility we overex-

pressed each gene in a strain mutant for the other. Neither overexpression of *HOC1* in an *och1* mutant nor overexpression of *OCH1* in a *hoc1* mutant (Figure 8) suppressed the mutant phenotypes. These results and the disparate phenotypes of *och1* and *hoc1* mutants suggest that Och1p and Hoc1p play distinct roles in the cell.

It is perhaps not surprising to find related glycosyltransferases with distinct functions. *S. cerevisae*, for example, contains a family of at least six putative glycosyltransferases related to the α-1,2-mannosyltransferase encoded by *KRE2/MNT1* (LUSSIER *et al.* 1993; A. M. NEIMAN, unpublished data). Mutation of *KRE2* results in a defect in *O*-linked glycosylation. Specifically, *O*-linked oligosaccharide chains are truncated after the second mannose residue. Mutation of one of these *KRE2*-related genes, *KTR2*, results in partial resistance to killer toxin, suggestive of a perturbation in cell wall structure (LUSSIER *et al.* 1993). However, reminiscent of our results with *HOC1*, *ktr2* mutants display no detectable alteration in protein glycosylation.

If *HOC1* has a distinct role from *OCH1* in protein glycosylation, why is the mutant phenotype so modest? A sequence search of *S. cerevisiae* protein sequences available in the database using known or suspected glycosyltransferases as probes (*e.g.*, Mnn1p, Mnn10p, Kre2p) reveals at least 20 putative glycosyltransferases falling into at least five distinct sequence families (A. M. NEIMAN, unpublished data). This large number of potential glycosyltransferases suggests that there may be extensive redundancy among enzymes involved in glycosylation. Alternatively, it may be that certain glycosyltransferases are specific for particular proteins. In either event, and the two possibilities are not mutually exclusive, it may be difficult to detect gross abnormalities of protein glycosylation in single mutants.

***HOC1* interactions with *PKC1*:** *HOC1* displays two types of genetic interactions with *pkc1-371*. First, overexpression of *HOC1*, even from a *CEN* plasmid, allows growth of *pkc1-371* strains at high temperature (Figure 1). Second, mutation of *HOC1* in a *pkc1-371* mutant background lowers the restrictive temperature from 37° to 34° (Figure 5). Thus, the phenotype of a *pkc1-371* mutant can be suppressed or enhanced depending on the dosage of *HOC1*.

An effect on *pkc1* mutants similar to that which we describe here for *HOC1* has previously been described for the *KRE6* gene (ROEMER *et al.* 1994). *KRE6* encodes a Golgi-localized protein required for the synthesis of β-1,6-glucan. Overexpression of *KRE6*, even from a CEN plasmid, can rescue the lysis defect of a *pkc1*Δ mutant, whereas *kre6 pkc1* mutants are dead even on osmotically stabilized media. In the same study, a variety of other *KRE* genes were tested for suppression and synthetic lethality with *pkc1*. While *pkc1* showed synthetic lethality with a number of *kre* mutants, only *KRE6* when overexpressed could rescue a *pkc1* mutant.

Though overexpression of *KRE6* rescues a *pkc1*Δ mutant, it does not rescue the lethality of mutations in kinases downstream of *PKC1*, such as *MPK1* (ROEMER *et al.* 1994). This observation suggests that overexpression of *KRE6* is suppressing a requirement for a function of *PKC1* that is distinct from regulation of the MAP kinase cascade. We have found that overexpression of *HOC1* will not rescue the temperature-sensitive growth phenotype of *bck1* or *mpk1* mutants (N. DEAN, unpublished data), further strengthening the parallel between *KRE6* and *HOC1*.

What is the function of HOC1p?: Though there is some reduction in the mannan content, the weakened cell wall in *pkc1* mutants appears to result primarily from defects in the inner, β-glucan layer of the cell wall (LEVIN and BARTLETT-HUEBUSCH 1992; PARAVICINI *et al.* 1992; ROEMER *et al.* 1994). This may be due in part to the upregulation of a β-glucanase encoded by *BGL2* in *pkc1* mutants (SHIMIZU *et al.* 1994). A primary defect in the β-glucan layer is also consistent with the genetic interactions seen between *pkc1* and the *kre* genes (ROEMER *et al.* 1994) that are involved in β-1,6-glucan synthesis.

By contrast, the homology of Hoc1p to Och1p suggests that Hoc1p is a mannosyltransferase. We expect, therefore, that *hoc1* should influence the structure of the mannan layer of the cell wall by altering *N*-linked or *O*-linked glycosylation. If so, then how might overexpression of *HOC1* suppress a cell lysis phenotype caused by defects in the β-glucan layer of the cell wall? We suggest two possibilities. First, Hoc1p might be required for the proper modification (and therefore function) of some protein involved in β-glucan synthesis. Consistent with this possibility, a number of the proteins required for β-glucan assembly, including Kre6p, are known to be glycoproteins (BROWN and BUSSEY 1993; ROEMER *et al.* 1994).

A second possibility is suggested by the description (MONTIJN *et al.* 1994) of a third class of oligosaccharide chains in some *S. cerevisiae* cell wall proteins. These side chains, which are distinct from *N*-linked and *O*-linked sugars, have a mixed composition containing both β-1,6-linked glucose residues and α-1,6-linked mannose residues. The proteins attached to these oligosaccharides can be released from the cell wall by digestion with the β-1,3-glucanase laminarase (MONTIJN *et al.* 1994) suggesting that they may reside in the β-glucan layer of the cell wall. We speculate that Hoc1p might be involved in the addition of α-1,6-mannose to this class of oligosaccharide. It has also been suggested (ROEMER *et al.* 1994) that Kre6p might be required for incorporation of β-1,6-glucose into these glycans. If both *HOC1* and *KRE6* were required for production of this type of side chain, this could explain the similar interactions that these genes display with *pkc1*.

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