Suppressors of a *Saccharomyces cerevisiae* pkc1 Mutation Identify Alleles of the Phosphatase Gene *PTC1* and of a Novel Gene Encoding a Putative Basic Leucine Zipper Protein

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**ABSTRACT**

The *PKC1* gene product, protein kinase C, regulates a mitogen-activated protein kinase (MAPK) cascade, which is implicated in cell wall metabolism. Previously, we identified the *pkc1-4* allele in a screen for mutants with increased rates of recombination, indicating that *PKC1* may also regulate DNA metabolism. The *pkc1-4* allele also conferred a temperature-sensitive (ts) growth defect. Extragenic suppressors were isolated that suppress both the ts and hyperrecombination phenotypes conferred by the *pkc1-4* mutation. Eight of these suppressors fell into two complementation groups, designated *KCS1* and *KCS2*. *KCS1* was cloned and found to encode a novel protein with homology to the basic leucine zipper family of transcription factors. *KCS2* is allelic with *PTC1*, a previously identified type 2C serine/threonine protein phosphatase. Although mutation of either *KCS1* or *PTC1* causes little apparent phenotype, the *kcs1Δ ptc1Δ* double mutant fails to grow at 30°C. Furthermore, the *ptc1* deletion mutation is synthetically lethal in combination with a mutation in *MKP1*, which encodes a MAPK homologue proposed to act in the *PKC1* pathway. Because *PTC1* was initially isolated as a component of the Hog1p MAPK pathway, it appears that these two MAPK cascades share a common regulatory feature.

**Protein** kinase C (PKC) plays a critical role in the regulation of growth and proliferation in all eukaryotic cells (reviewed in Nishizuka 1992). A PKC homologue in the yeast *S. cerevisiae* is encoded by the essential gene *PKC1* (Levin et al. 1990). Deletion of *PKC1* results in cell lysis (Levin and Bartlett-Heubusch 1992; Levin and Errede 1993). Two temperature-sensitive alleles of *PKC1* and one Ca2+-dependent allele were isolated by Levin and Bartlett-Heubusch (1992), and additional alleles were found to be allelic with *cyl15*, a temperature-sensitive cell lysis mutant (Paravicini et al. 1999), and *hpo2*, a hypo-osmosality-sensitive mutant (Shimizu et al. 1994). Several components of a Pkc1p-regulated kinase cascade have been identified in yeast. These were isolated based on their ability to suppress lysis in a strain deficient in either Pkc1p or in another component of the Pkc1p-governed cell wall metabolism pathway (Lee and Levin 1992; Lee et al. 1993a,b; Irie et al. 1993). Epistatic analysis suggests that Pkc1p activates Bck1p, which activates the mitogen-activated protein kinase kinase (MAPKK) homologues Mkk1p and Mkk2p, which in turn activate Mpklp, a MAPK homologue. Members of the MAPK and MAPKK families have also been implicated in PKC-mediated responses in mammalian cells (Kolch et al. 1993; Levin and Errede 1993).

A second MAPK cascade in yeast regulates the osmosensing pathway, which increases intracellular glycerol production in response to increases in extracellular osmolarity. *HOG1* and *PBS2* were identified as essential for viability on high-osmolarity medium and encode members of the MAPK and MAPKK gene families, respectively (Brewster et al. 1993). Phosphorylation of Hog1p in response to increased osmolarity is dependent on *PBS2* (Brewster et al. 1993). Activation of this MAPK cascade is likely to be mediated by the Sln1p/Ssk1p protein complex. Sln1p, on the basis of its homology to histidine kinase sensory systems in bacteria, is proposed to recognize the osmolarity of the medium and regulate the phosphorylation state of Ssk1p (Maeda et al. 1994). Deletion of *SLN1* or a dominant negative mutation in *SSK1* results in constitutive activation of the Hog1p pathway and inviability. Three phosphatases were identified as potential negative regulators of this pathway based on their ability to suppress this lethality in a dosage-dependent manner. These include the *PTP2*, *PTC1* and *PTC3* gene products (Maeda et al. 1994). *PTP2* encodes a protein tyrosine phosphatase, and *PTC1* and *PTC3* encode type 2C serine/threonine protein phosphatases. *PTC1* was previously identified by mutations that were synthetically lethal in combination with a *ptp1 ptp2* double mutation, which removes the two known protein tyrosine phosphatases (Maeda et al. 1998). *PTC1* was also identified as *TPD1*, based on its role in tRNA splicing (Robinson et al. 1994).

Previously we described a colony sectoring assay in
which the rate of sectoring is proportional to the rate of recombination at two ade2 alleles in direct repeat (Huang and Symington 1994). Using this assay, an allele of PKCl was isolated which promoted a marked increase in the rate of sectoring and conferred a temperature-sensitive (ts) growth defect (Huang and Symington 1994). We proposed that the regulation of cell wall metabolism and recombination were mediated through separate PkCl-controlled pathways, based on two observations. First, the addition of osmotically supportive agents to the medium, such as 1 M sorbitol or 100 mm CaCl2, suppressed the growth defect but did not affect the hyperrecombination phenotype conferred by the pckl-4 allele. Second, deletion of MPK1, which encodes a downstream component of the PkCl cell wall metabolism pathway, had no effect on the rate of recombination. To identify regulatory components of the PkCl-dependent pathways controlling growth and recombination, we isolated second site mutations that suppressed both the ts and hyperrecombination phenotype conferred by the pckl-4 allele or that suppressed either phenotype alone. Here we describe the mutations that suppressed both of these phenotypes.

MATERIALS AND METHODS

Strains: The yeast strains used in this study (Table 1) are derivatives of W303-1A or W303-1B (Thomas and Rothstein 1989). The kcs mutant strains YKH138 and YKH139 were derived from UV-induced mutagenesis of YKH29a. The kcs1 deletion strain was made by introducing the HIS3 gene between the EcoRV and HindIII sites of YCS1 (nucleotides 457 and 2731 of the coding sequence, respectively). This construct was introduced to the KCS1 locus by one-step gene replacement (Rothstein 1983). The kcs2/pcl1 deletion strains were made by introducing the LEU2 gene between the Smal and BamHI sites of KCS2/PCT1 (nucleotide 118 of the coding region and nucleotide 7 in the 3' untranslated region, respectively) (Maeda et al. 1993). A DNA fragment containing the kcs2/pcl1::LEU2 construct was introduced to the KCS2/PCT1 locus by one-step gene replacement (Rothstein 1983). For kcs1 and kcs2/pcl1 disruptions, transformants were subjected to Southern analysis to ensure integration at the correct locus (Southern 1975).

YKH40-3 was made by crossing DL523 to YKH12a. The resulting Leu+ segregants were backcrossed twice more to YKH12a or YKH12a to produce YKH40-3.

YKH110, 21, 146, 147, 149, 150, 151, 153, 161, 164, 163, 162 and 165 are Ade+ Ura- derivatives of YKH12a, 29a, 114, 141, 138, 139, 112, 142, 74, 113, 144, 140 and 145, respectively. These have lost the ade2 direct repeat recombination substrate.

Standard procedures were used for mating, sporulation and tetrad analysis (Guthrie and Fink 1991). Transformation of yeast was performed using the lithium acetate method (Ito et al. 1988).

Media and culture conditions: Media for yeast growth were prepared as described by Sherman et al. (1986). YEPD medium (1% yeast extract, 2% Bacto-peptone, 2% dextrose, and 2% agar for plates) was used for nonselective growth. 100 mm CaCl2 or 1 M sorbitol was added to YEPD medium as indicated. Nutritional requirements were determined on synthetic medium lacking one amino acid or nucleic acid base (2% dextrose, 0.67% yeast nitrogen base, 2% agar). Escherichia coli strains were grown at 37°C in LB medium or LB supplemented with 100 μg of ampicillin per ml.

Determination of recombination rates: To determine the rate of adenine prototroph formation, cells were plated at 30°C on YEPD plates. Seven colonies were picked from each plate, and dilutions were plated on medium lacking adenine to determine the number of Ade+ cells and on YEPD to determine the total number of cells in the colony. The median frequency was used to determine the rate of recombination per cell per generation (Lea and Coulson 1948). Each experiment was repeated three times; the median rate for each strain is given in Table 2.

Colonies hybridization: A yeast genomic library (Rose et al. 1987) was transformed into E. coli, and transformants were transferred to nitrocellulose filters. The colonies on the filters were lysed and probed for plasmids of interest using the protocol of Sambrook et al. (1989).

DNA sequence analysis: DNA sequencing was performed using the method of Sanger et al. (1977), using the Sequenase kit (United States Biochemical).

Northern analysis: Strains were grown at room temperature in YEPD medium to early log phase. The cultures were divided and a sample of each strain was concentrated by centrifugation and resuspended in YEPD containing 1 M glucose. The cultures were grown for 3 hr at 30°C prior to RNA extraction. RNA preparation and Northern analysis were performed as described (Sherman et al. 1986).

RESULTS

Isolation of mutants: To identify recombination mutans in yeast, we developed a colony sectoring assay that uses two heteroalleles of the ade2 gene (Stotz and Linden 1990) in direct repeat orientation at the ADE2 locus (Figure 1). The mutations in ade2 cause the initial strain to form red colonies (Reaume and Tatum 1949; Roman 1956). If recombination between the two alleles produces a wild-type ADE2 gene, a white sector will be formed within the red colony. Thus, the rate of sectoring indicates the rate of recombination within this strain, designated YKH12a (Huang and Symington 1994).

Using this assay, we previously identified an allele of PCK1 (pckl-4) that increased the rate of recombination in a temperature-dependent manner and conferred a temperature sensitive growth defect (Huang and Symington 1994). To identify components of the PkCl-dependent pathways controlling cell wall metabolism and recombination, we initiated a genetic screen for second site mutations that suppressed either the hyperrecombination or ts phenotype conferred by the pckl-4 allele. To isolate suppressors of the recombination phenotype, approximately 20,000 colonies of the pckl-4 strain YKH29a were plated on either YEPD or YEPD supplemented with 100 mm CaCl2, mutagenized by UV irradiation, and grown at 30°C. The addition of CaCl2 to the medium suppresses the temperature-dependent growth defect of pckl1 strains, but not the hyperrecombination phenotype. Colonies that displayed a decreased rate of sectoring were diluted into water and plated on medium lacking adenine to ensure that they were still capable of adenine prototroph formation, indicating that they retained the direct repeat construct. To identify suppressors of the ts defect, YKH29a was mutagen-
Suppressors of a pkcl Defect

**TABLE 1**

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<th>Yeast strains used in this study</th>
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* All strains are in the YKH12 background (ade2-2::ura3::ade2-a leu2-3, 112 his3-11, 15 can1-100 trp1-1) (HUANG and SYMINGTON 1994) except SJR13 and DL523, which are unrelated strains.

A total of 26 mutants falling into 20 complementation groups were isolated. Sixteen of these mutants, comprising 16 complementation groups, suppressed only the hyperrecombination phenotype and not the ts growth defect conferred by the pkcl-4 allele. An additional 10 mutants defined four complementation groups, KCS1-4, which suppressed both phenotypes. The KCS1 complementation group, containing five mutant alleles, and the KCS2 complementation group, containing three mutant alleles, showed the strongest suppression and were characterized further. When backcrossed to YKH29a, kcs1 and kcs2 mutations were determined to be recessive and to segregate as single gene traits. The kcs mutations were crossed to SJR13, a strain with wild-type ADE2 and PKCl alleles, and the resulting diploids were dissected. Because many segregants from these crosses displayed the ts and hyperrecombination phenotypes of YKH29a, the kcs mutations were determined not to be linked to either the ade2 direct repeat construct or the PKCl locus.
Cloning of the KCS1 gene: From the crosses to SJR13, which contains a wild-type TRP1 gene, it was determined that both the kcsI and kcs2 mutations were linked to the TRP1 locus. Therefore, a chromosome walking strategy was used to identify a plasmid containing the KCS1 gene. A cosmid clone containing approximately 40 kb of yeast DNA, including the TRP1 locus (American Type Culture Collection cosmid #70938) (Figure 2), was restriction mapped, and DNA fragments from different regions were isolated and used as probes to screen a yeast genomic library (ROE et al. 1987) transformed into E. coli. Fragments from yeast plasmids that extended beyond the ends of the cosmid clone were used to continue walking along the genome. A total of nine independent plasmids were obtained, which contained overlapping DNA inserts spanning 70 kb of chromosome IV (Figure 2). Each of these was tested for the ability to confer temperature sensitivity to the kcsI pkcI-4 and the kcs2 pkcI-4 strains YKH149 and YKH150, respectively. One plasmid, #23, complemented the phenotype of the kcsI pkcI-4 double mutant, yet none complemented the kcs2 pkcI-4 phenotype.

Subsequent subcloning and sequence analysis of plasmid #23 determined that the minimal complementing region contained a previously unidentified gene (Fig-
Suppressors of a pkcl Defect

Figure 3.—Nucleotide and predicted amino acid sequence of the KCS1 gene. Nucleotide 1 is the A of the initiating methionine. Boxes have been placed around leucine residues that could participate in the formation of two leucine zippers. The asterisk indicates the termination codon. Components of a possible transcription termination consensus sequence are underlined (Zaret and Sherman 1982).

This coding region was partially replaced with the HIS3 gene, and the resulting construct was used to replace the wild-type locus in the pkcl-4 strain YKH29a. Analysis of the KCS1 sequence predicts that it encodes a protein 1050 amino acids in length, with a predicted molecular weight of 119 kD. The sequence is

indicating that this coding region contains the KCS1 gene (data not shown).

Analysis of the KCS1 sequence predicts that it encodes a protein 1050 amino acids in length, with a predicted molecular weight of 119 kD. The sequence is
most closely related to the basic leucine zipper (bZIP) family of transcription factors (reviewed in BAXEVANIS and VINSON 1993; COHEN and PARRY 1994). KCS1 is unusual in that it contains two potential leucine zipper motifs, although the presence of two glycine residues in the last seven residues of the first putative leucine zipper region suggests that this segment may not participate in the formation of an alpha-helical structure. In addition to two putative leucine zipper elements, the KCS1 gene product contains a number of basic residues N-terminal to these elements. It should be noted that the consensus sequence NXX(A/X)(A/X)XXXR, which is conserved in most but not all bZIP proteins (BAXEVANIS and VINSON 1993), is only loosely matched in the predicted sequence of Kcs1p. Upstream of the first leucine zipper element, the sequence NKPCAL-DLK is found, and upstream of the second, the sequence most closely matching the consensus is QFAR-VLAR.

Cloning the KCS2 gene: None of the genomic clones described above and shown in Figure 2 conferred temperature sensitivity to the kcs2 pke1-4 strain. An alternative cloning strategy was enabled by the observation that kcs2 mutants were unable to grow on medium containing 100 mM CaCl$_2$ (Figure 4). This phenotype was specific for the addition of CaCl$_2$, rather than a general sensitivity to increased osmolarity, as the kcs2 mutants were able to grow on plates containing 1 M glucose, 0.5 M NaCl, or 1 M sorbitol (data not shown). To isolate the gene altered in the kcs2 mutant, a yeast genomic library (ROSE et al. 1987) in the vector YCp50 was introduced to YKH151, and transformants were selected on YEPD plates containing 100 mM CaCl$_2$. Colonies were subsequently tested for uracil prototrophy to test for the presence of plasmids. Three transformants were isolated and found to contain the same plasmid. This plasmid also conferred temperature sensitivity to the kcs2 pke1-4 strain YKH150. Subsequent subcloning and sequence analysis showed that the minimal complementing fragment contained the previously identified PTC1 gene (MAEDA et al. 1993). The PTC1 gene was deleted, and crosses using this deletion confirmed linkage to the TRPI locus, as previously reported (MAEDA et al. 1993; ROBINSON et al. 1994). Our analysis suggests that PTC1 is approximately 8-10 kb from the centromere on the left arm of chromosome IV. This estimate

![Figure 4](image_url) - Calcium sensitivity of ptc1 strains. From top: YKH10 (wild type), YKH21 (ptc1-4), YKH114 (ptc1Delta::LEU2 pke1-4), YKH162 (ptc1Delta::LEU2). Serial dilutions of each strain were grown on YEPD medium (A) or YEPD supplemented with 100 mM CaCl$_2$ (B) at 34°C.

![Figure 5](image_url) - kcs1 and ptc1 mutations reduce the rate of Ade+ sectoring in pke1-4 and pke1-2 strains. Recombination between the two ade2 alleles can produce a wild-type ADE2 gene, resulting in the formation of a white sector in the red colony. The rate of sectoring is therefore proportional to the rate of recombination in these strains. (A) YKH12a (wild type); (B) YKH29a (pke1-4); (C) YKH114 (kcs1Delta::HIS3 pke1-4); (D) YKH141 (ptc1Delta::LEU2 pke1-4); (E) YKH74 (pke1-2); (F) YKH144 (kcs1Delta::HIS3 pke1-2); (G) YKH145 (ptc1Delta::LEU2 pke1-2). All strains were grown on YEPD plates at 30°C.
Suppressors of a \( pckl \) Defect

YKH 162

\#I248432

YKH147

grown on YEPD plates with 1 M sorbitol (B, D and F). Serial dilutions of each strain were grown on YEPD plates with 1 M sorbitol (A, C and E) or YEPD plates without sorbitol (B, D and F) at 24° (A and B), 34° (C and D), or 37° (E and F).

is made by combining information from the genome sequencing project (a genomic clone with accession #Z48432 contains the \( PTCl \) gene), the chromosome maps compiled by L. Ruels and M. Olson (personal communication), and the YCp50 genomic clones generated in this study.

The \( ptcl \) mutation suppressed the ts and hyperrecombination phenotypes conferred by the \( pckl-4 \) allele, and the \( pckl-1 \) strain failed to complement \( kcs1 \) mutants. We shall henceforth refer to \( KCS2 \) as \( PTCl \).

Because the disruption alleles of \( KCS1 \) and \( PTCl \) had the same phenotypes as the original alleles, all subsequent experiments were performed using the disruption strains.

Rates of recombination at the \( ade2 \) locus: As shown in Figure 5, mutation of \( KCS1 \) or \( PTCl \) reduced the rate of sectoring of the \( pckl-4 \) and \( pckl-2 \) strains to approximately wild-type levels. The rate of recombination at the \( ADE2 \) locus was determined by measuring the frequency of adenine prototroph formation. The \( kcs1 \) and \( ptcl \) disruption mutations suppressed the rate of recombination in the \( pckl-1 \) background to near wild-type levels, but did not affect recombination in the wild-type background (Table 2). This suggests that the \( KCS1 \) and \( PTCl \) gene products are involved in the \( pckl-1 \) depen-
dent stimulation of recombination rather than the control of recombination in general.

Allele-specific suppression of the ts phenotype: As shown in Figure 6, a \( kcs1 \) or \( ptcl \) mutation suppresses the ts growth defect of the \( pckl-4 \) strain at 34°. Although \( ptcl \) \( pckl-4 \) and \( kcs1 \) \( pckl-4 \) mutants grow as patches and are identifiable when replica plated to a plate incubated at 37°, they fail to grow well as single colonies at this temperature (Figure 6). For the \( ptcl \) \( pckl-4 \) double mutant this was not unexpected, since a \( ptcl \) mutation alone results in a ts phenotype at 34° (Maeda et al. 1993; Robinson et al. 1994). Mutation of \( KCS1 \) or \( PTCl \) failed to suppress the inviability of the \( pckl \) deletion mutant on medium lacking an osmotic agent (Figure 7). In addition, the \( ptcl \) mutation only weakly suppressed the growth defect conferred by the \( pckl-2 \) allele at 32°, and neither the \( kcs1 \) nor \( ptcl \) mutation suppressed the growth defect of the \( pckl-2 \) strain at 34° (Figure 8). Because the activity of the \( pckl-1 \) and \( pckl-2 \)-encoded kinases are likely to decrease with increasing temperature, these results indicate that \( kcs1 \) and \( ptcl \) mutations are able to compensate for reduced Pkc1p kinase activity, yet cannot compensate for loss of the kinase.

To determine if a \( kcs1 \) or \( ptcl \) mutation could suppress the ts phenotype associated with mutation of another component of the Pkc1p cascade, \( kcs1 \) \( ptcl \) double mutants were crossed to an \( mpkl \) deletion strain. The resulting diploids were sporulated and tetrads were dissected. From the haploid progeny, it was determined that a \( kcs1 \) mutation failed to suppress the inviability of the \( mpkl-2 \) strain. The resulting diploids were sporulated and tetrads were dissected. From the haploid progeny, it was determined that a \( kcs1 \) \( ptcl \) double mutant was inviable when a \( ptcl \) \( mpkl \) haploid was crossed to an \( mpkl \) haploid, no Leu+ Trp+ segregants were recovered from 28 tetrads analyzed. However, 17 Leu+ Trp+ spores were obtained, indicating that the two loci are unlinked.

\( kcs1 :: HIS3 \) \( ptcl :: LEU2 \) cells display a severe growth defect: To determine the phenotype of the \( kcs1 \) \( ptcl \) double mutant, the \( PTCl \) gene was dis-

\textbf{Figure 6.}—\( kcs1 \) and \( ptcl \) mutations suppress the temperature sensitivity of the \( pckl-4 \) strain. From top: YKH10 (wild type), YKH21 (\( pckl-4 \)), YKH146 (\( kcs1 :: HIS3 \) \( pckl-4 \)), YKH147 (\( ptclA :: LEU2 \) \( pckl-4 \)), YKH164 (\( kcs1 :: HIS3 \)), and YKH162 (\( ptclA :: LEU2 \)). Serial dilutions of each strain were grown on YEPD plates with 1 M sorbitol (B, D and F). Serial dilutions of each strain were grown on YEPD plates with 1 M sorbitol (A, C and E) or YEPD plates without sorbitol (B, D and F) at 24° (A and B), 34° (C and D), or 37° (E and F).

\textbf{Figure 7.}—Mutation of \( ptcl \) or \( kcs1 \) fails to suppress the inviability caused by deletion of \( pckl \). From top: YKH10 (wild type), YKH1403 (\( pcklA :: LEU2 \)), YKH159 (\( kcs1 :: HIS3 \) \( pcklA :: LEU2 \)), YKH150 (\( ptclA :: LEU2 \) \( pcklA :: LEU2 \)), YKH164 (\( kcs1 :: HIS3 \)), and YKH162 (\( ptclA :: LEU2 \)). Serial dilutions of each strain were grown on a YEPD plate with 1 M sorbitol (A) or a YEPD plate without sorbitol (B) at 24°.
PTP2 transcript levels are reduced in mpkl and pkel-4 strains: It has previously been reported that a ptc1 mutation results in a synthetic growth defect in combination with ptp2, which encodes a protein tyrosine phosphatase (MAEDA et al. 1994). PTP2 would therefore be a possible target for regulation by the KCS1 gene product. Failure to induce expression of PTP2 in a kes1Δ strain would result in a kes1Δ ptc1 synthetic growth defect similar to that observed in a ptp2 ptc1 strain.

Northern analysis was performed to determine PTP2 transcript levels in wild type, kes1, ptc1, mpkl, and pkcl-4 strains. Because PTP2 has been implicated as a component of the Hog1p MAPK pathway, which regulates the response to osmotic stimulation, cells were grown in either YEPD or in YEPD supplemented with 1 M glucose. As shown in Figure 10, although the level of PTP2 mRNA was not reduced in a kes1 mutant strain, the level was markedly reduced in both the mpkl strain and in the pkcl-4 strain, suggesting that expression of PTP2 is dependent on components of the Pkc1p MAPK cascade. In addition, expression of PTP2 was increased slightly when cells were grown in the presence of 1 M glucose.

The role of the PTP2 gene product in the Pkc1p MAPK cascade: Because PTC1 and PTP2 were both identified as negative regulators of the Hog1p MAPK pathway, and our work suggested that the PTC1 gene product similarly regulates the Pkc1p MAPK cascade, we disrupted the PTP2 gene in the pkcl-4 background to determine if this would affect either the ts or hyperrecombination phenotype conferred by the pkcl-4 allele. It was determined that deletion of PTP2 affected neither of these phenotypes (data not shown).

DISCUSSION

The isolation of the pkel-4 allele in a screen for hyperrecombination mutants identified a novel role for Pkc1p, as the regulation of DNA metabolism was a previously unidentified role for protein kinase C in any system (HUANG and SYMINGTON 1994). We proposed that failure to phosphorylate one or more proteins involved in DNA metabolism results in the formation of recombinogenic DNA lesions and the observed hyperrecombination phenotype. The isolation of compensa-
Suppressors of a *pck1* Defect  

Cells were grown at 30° in either YEPD or YEPD with 1 M glucose. Ten µg of total RNA was loaded in each lane. Strains analyzed include YKH12a (wild type, lane 1), YKH113 (*kes1A::HIS3*, lane 2), YKH140 (*pct1A::LEU2*, lane 3), YKH41 (*mpk1A::TRP1*, lane 4), and YKH29a (*pck1-4*, lane 5). The blot was first probed with a fragment of the *YKH41* glucose. Ten pg of total RNA was loaded in each lane. Strains analyzed include suppress both the ts and hyperrecombination phenotype of *Ptc1p* kinase cascade. The ability of *Ptclp* to suppress the growth defect of *Ptc1p* null mutant. In addition, a *pct1* mutation fails to suppress the ts phenotype of a *pck1-2* strain at 34°, yet weakly suppresses the growth defect at 32° and suppresses the hyperrecombination defect conferred by the *pck1-2* allele at 30°. It is likely that the activity of the kinase encoded by the *pct1-2* allele decreases with increasing temperature, and the ability of a *pct1* mutation to compensate for the defects conferred by the *pct1-2* allele is similarly temperature dependent.

The *Kcs1* gene encodes a previously unidentified protein most closely related to the bZIP family of transcription factors. The target(s) of *Kcs1p* are not known at this time. One possible role for *Kcs1p* is to regulate the expression of a phosphatase gene. This is consistent with our observation that, as with *pct1* mutations, a *kes1* mutation suppresses the *pck1* growth defect in an allele-specific manner. Our data show that expression of the phosphatase gene *PTP2* is not altered in a *kes1* strain. A second possible target for regulation by *Kcs1p* is the *Ptc1* gene. This would be consistent with the ability of *kes1* and *pct1* mutations to suppress the phenotypes conferred by the different *pck1* alleles in a similar fashion. However, *Ptc1* expression could not be solely controlled by *Kcs1p* since a *kes1* mutation does not display many of the phenotypes displayed by the *pct1* mutant, such as sensitivity to CaCl$_2$. In addition, *Ptc1* could not be the sole target of *Kcs1p*, since the *kes1A::HIS3 ptc1A::LEU2* double mutant has a more severe phenotype than the *ptc1Δ::LEU2* single mutant. Northern analysis confirmed that *Kcs1p* is not required for the expression of *Ptc1* (not shown). It is interesting that *Ptc1* has been isolated in connection with two MAPK cascades. In this study, we report the isolation of three *pct1* alleles that suppress a *pck1* defect. Previously, *Ptc1* was found to act as a dosage-dependent suppressor of an *sln1* deletion mutant (Maeda et al. 1994). Deletion of *SLN1* results in lethality due to the constitutive activation of the Hog1p/Pbs2p MAPK cascade. It is proposed that *Ptc1p* acts to dephosphorylate Hog1p and/or Pbs2p, resulting in downregulation of the cascade. The isolation of *Ptc1* as an extragenic suppressor of a defect in a second MAPK cascade could indicate a role for *Ptc1* in the dephosphorylation of the MAPK/MAPKK homologues encoded by *MPK1*, *M KK1* or *M KK2*. Again, it could be proposed that dephosphorylation by *Ptc1p* reduces the activity of components of the MAPK cascade. A model for this is shown in Figure 11.

Our observation that the *pct1Δ mpk1A* double mutation is synthetically lethal was unexpected. Since *MPK1* and *Ptc1* were isolated as positive and negative factors in this cascade, respectively, it is odd that they have a combinatorial defect. If the sole role of *Ptc1p* in the Pkc1p kinase cascade is to dephosphorylate components of the cascade, deletion of *Ptc1* in an *mpk1Δ* background should cause no greater defect than either single deletion. It is possible that *Ptc1p* acts on an additional substrate that is important for cell wall maintenance. Alternatively, this effect could be related to the apparent role of *Mpk1p* in the regulation of *PTP2* gene.

**Figure 10.**—Northern analysis of *PTP2* transcript levels. Cells were grown at 30° in either YEPD or YEPD with 1 M glucose. Ten µg of total RNA was loaded in each lane. Strains analyzed include YKH12a (wild type, lane 1), YKH113 (*kes1A::HIS3*, lane 2), YKH140 (*pct1A::LEU2*, lane 3), YKH41 (*mpk1A::TRP1*, lane 4), and YKH29a (*pck1-4*, lane 5). The blot was first probed with a fragment of the *YKH41* glucose. Ten pg of total RNA was loaded in each lane. Strains analyzed include suppress both the ts and hyperrecombination phenotypes conferred by the *pck1-4* allele suggests that there are at least two substrates for *Pkc1p* in the cell that are also both targets of the *Pkc1p* phosphatase. We propose that a *pct1* defect compensates for a kinase with reduced activity. This is supported by our observation that mutation of *Ptc1* does not suppress the growth defect of the *pck1* null mutant. In addition, a *pct1* mutation fails to suppress the ts phenotype of a *pck1-2* strain at 34°, yet weakly suppresses the growth defect at 32° and suppresses the hyperrecombination defect conferred by the *pck1-2* allele at 30°. It is likely that the activity of the kinase encoded by the *pck1-2* allele decreases with increasing temperature, and the ability of a *pct1* mutation to compensate for the defects conferred by the *pck1-2* allele is similarly temperature dependent.

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**Figure 11.**—A potential model for the regulation of the Pkc1p-mediated recombination and cell wall metabolism cascades by Ptc1p. PKC1, BCK1, MKK1, MKK2 and MPK1 all encode putative serine-threonine protein kinases that have been proposed to act sequentially in a kinase cascade as diagrammed (Fields and Thorner 1991; Lee and Levin 1992; Irie et al. 1993; Lee et al. 1993b).
expression. Since a ptp2 mutation results in a synthetic growth defect in combination with a ptc1 mutation, it would be expected that deletion of a gene encoding a positive regulator of PTP2, such as MPK1, would have a similar effect. Because the phenotype of the ptc1 mpk1 double mutant is more severe than the ptc1 ptp2 double mutant, it is likely that deletion of MPK1 results in additional defects as well.

It is interesting that expression of PTP2 is dependent on the PKCl and MPK1 genes. Because we find no evidence for regulation of the Pkc1p MAPK cascade by Ptp2p, our data suggest that there is "crosstalk" between the Hog1p and Pkc1p MAPK cascades. We propose that one role of Mpklp is to activate the phosphatase that dephosphorylates Fus3 (DOI 1993). Expression of the gene encoding MPK1 is induced concurrently with activation of the MAPK pathway that it regulates (DOI 1993a). Unlike MPG5 and MPK1, Ptp2p is the first phosphatase whose synthesis appears to be regulated by a MAPK cascade that it does not modify.

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LITERATURE CITED


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