Transcriptional Profiling of the Protein Phosphatase 2C Family in Yeast Provides Insights into the Unique Functional Roles of Ptc1*

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Type 2C protein phosphatases are encoded in *Saccharomyces cerevisiae* by several related genes (PTC1–5 and PTC7). To gain insight into the functions attributable to specific members of this gene family, we have investigated the transcriptional profiles of *ptc1*–5 mutants. Two main patterns were obtained as follows: the one generated by the *ptc1* mutation and the one resulting from the lack of *PtC2–5*. *ptc4* and *ptc5* profiles were quite similar, whereas that of *ptc2* was less related to this group. Mutation of *PTC1* resulted in increased expression of numerous genes that are also induced by cell wall damage, such as *YKL161c, SED1*, or *CRH1*, as well as in higher amounts of active Slt2 mitogen-activated protein kinase, indicating that lack of the phosphatase activates the cell wall integrity pathway. *ptc1* cells were even more sensitive than *slt2* mutants to a number of cell wall-damaging agents, and both mutations had additive effects. The sensitivity of *ptc1* cells was not dependent on Hog1. Besides these phenotypes, we observed that calcineurin was hyperactivated in *ptc1* cells, which were also highly sensitive to calcium ions, heavy metals, and alkaline pH, and exhibited a random haploid budding pattern. Remarkably, many of these traits are found in certain mutants with impaired vacuolar function. As *ptc1* cells also display fragmented vacuoles, we hypothesized that lack of Ptc1 would primarily cause vacuolar malfunction, from which other phenotypes would derive. In agreement with this scenario, overexpression of *VPS73*, a gene of unknown function involved in vacuolar protein sorting, largely rescues not only vacuolar fragmentation but also sensitivity to cell wall damage, high calcium, alkaline pH, as well as other *ptc1*-specific phenotypes.

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7 The abbreviations used are: MAP, mitogen-activated protein; CFW, Calcofluor white; CWI, cell wall integrity; MAPK, MAP kinase.

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The on-line version of this article (available at http://www.jbc.org) contains supplemental Tables 1 and 2.

Ser/Thr protein phosphatases have been classically classified in four groups as follows: PP1, PP2A, PP2B, and PP2C. PP1, PP2A, and PP2B catalytic subunits are closely related in their primary sequence and define the PPP family. Type 2C phosphatases, which constitute the PPM family, are not related in sequence with PPP members, although their three-dimensional structures and catalytic mechanism appear to be very similar (1).

Protein phosphatase 2C represents an evolutionary conserved group of proteins that, in contrast with most members of the PPP family, are monomeric enzymes that apparently lack regulatory subunits. Five type 2C phosphatase genes (*PTC1–5*) have been classically defined in the budding yeast *Saccharomyces cerevisiae* (2), although a sixth member (*YHR076w/PTC7*) was recently added to the list (3). A putative seventh member (*YCR079w*) was reported some time ago, although its phosphatase activity *in vitro* has not been demonstrated (4).

Although the first reports on the characterization and biological role of type 2C phosphatases in yeast appeared more than 15 years ago, our knowledge on the specific functions of each isoform and how they are regulated is still very limited. It is commonly accepted that a major role for type 2C phosphatases in yeast is to negatively regulate the osmotically activated HOG pathway by dephosphorylating and inactivating the Hog1 MAP7 kinase (5–9). Most PP2C isoforms have been associated with this function, although with slightly different roles. Thus, it has been proposed that although Ptc1 would play a role in maintaining low levels of Hog1 activity under basal conditions and adaptation to osmotic stress (5), Ptc2 and Ptc3 would be necessary to limit an excessive activation of the kinase during stress (7). Ptc1 would be recruited to the scaffold upstream Hog1 kinase, Pbs2, through its interaction with Nbp2 (10). In addition to Ptc1–3, a role for Ptc4 in dephosphorylating Hog1 has been proposed recently (11).

Besides its regulatory role in the HOG pathway, diverse type 2C phosphatase isoforms have been related to a variety of specific functions. Thus, Ptc1 has been involved in the regulation of tRNA splicing (12) and in mitochondrial inheritance (13). Ptc2 and Ptc3 have been postulated as responsible for the dephosphorylation of cyclin-dependent kinases (4) and to be required for checkpoint inactivation after a DNA double strand break, which would confer to these specific isoforms an important role...
briefly, cell pellets were resuspended in 150 mM pre-chilled water, snap-frozen, and kept at 

interestingly, the ptc1 mutation was found to be synthetically lethal with that of slt2 (18), whereas overexpression of PTC1–4 suppressed the lethality of a cnb1 slt2 strain (11). Recent work in our laboratory has demonstrated that mutation of PTC1 (but not that of PTC2–5) confers sensitivity to lithium cations to yeast cells (19). Therefore, the current evidence defines a scenario in which type 2C phosphatases control a large number of processes in yeast cells, probably through a complex interplay of functions that in some cases could be rather specific but in many other cases appear largely overlapping. The available information, however, is rather fragmentary and does not provide a comprehensive understanding of the biological role of these important enzymes. We considered that a broader and more systematic overview could be obtained by comparative analysis of the transcriptomic profiles from cells deficient in each of these phosphatases. We have observed that cells lacking Ptc1 present a distinct and very specific expression pattern, reminiscent to that of cells suffering some kind of cell wall damage. Further characterization of ptc1 mutants revealed multiple, apparently unrelated phenotypic defects, suggesting a large variety of cellular functions. However, our results allow proposing a simple model that would explain most of the functions attributed to Ptc1.

**Experimental Procedures**

**Yeast Strains and Culture Conditions**—Yeast strain BY4741 was used as a wild type, and unless otherwise stated, the deletion mutants studied (Table 1) were in this same genetic background.

**Preparation of Yeast Extracts and Immunoblot Analysis**—For immunodetection of the MAP kinase Slt2, saturated cell cultures of the indicated strains in YPD medium were diluted to an A600 of 0.2 in fresh YPD and grown until A600 of 1.3–1.4. Cell cultures (5 ml) were harvested by filtration, washed once with pre-chilled water, snap-frozen, and kept at −80 °C. Total cell proteins were purified basically as described previously (20). Briefly, cell pellets were resuspended in 150 μl of lysis buffer (50 mm Tris-HCl, pH 7.5, 150 mm NaCl, 10% glycerol, 1% Triton X-100, and 0.1% SDS) containing phosphatase and kinase inhibitors (50 mm NaF, 1 mm sodium orthovanadate, 5 mm β-glycerol phosphate, 5 mm sodium pyrophosphate, 0.5 mm EDTA, pH 8.0), 1 mm phenylmethylsulfonyl fluoride, and a protease inhibitor mixture (Complete EDTA-free protease inhibitor mixture tablets; Roche Applied Science). One volume of acid-washed glass beads was added, and cells were broken at 4 °C by vigorous shaking in a Fast Prep cell breaker (Bio 101, Inc., Vista, CA; setting 5.5 for 25 s). After sedimentation at 16,000 × g, the cleared lysate was recovered and the protein concentration quantified by Bradford assay. Forty μg of total protein were fractionated by SDS-PAGE (using 10% polyacrylamide gels) and transferred to nitrocellulose membranes (Hybond C-Extra; Amersham Biosciences). Membranes were incubated for 2 h with either anti-phospho-p44/42 MAPK (Thr202/Tyr204) antibody (New England Biolabs), at 1:2000 dilution, or anti-GST–Slt2 antibody (21), at 1:10,000 dilution, to detect dually phosphorylated Slt2 or total Slt2, respectively. A 1:25,000 dilution of horseradish peroxidase-conjugated anti-rabbit antibody was used to detect the primary antibodies. ECL Advance Western blotting detection kit (Amersham Biosciences) was used to visualize the immunocomplexes. Chemiluminescence was detected using an LAS-3000 equipment (Fuji) and quantified using the Multi Gauge version 3.0 software.

**β-Galactosidase Reporter Assays**—Wild type strain BY4741 and its isogenic mutants (22) were co-transformed with the diverse β-galactosidase reporter constructs. Cells were grown to saturation on synthetic medium lacking uracil and then inoculated into YPD medium to give an A600 of 0.15. Growth was resumed until A600 of 0.8 was reached, and cells were then recovered by centrifugation, and β-galactosidase was measured as described previously (23).

**RNA Purification**—For RNA purification, 30 ml of yeast cultures were grown at 28 °C in YPD medium until A600 0.6–0.8. Yeast cells were harvested by centrifugation and washed with cold water. Dried cell pellets were kept at −80 °C until RNA purification. Total RNA was extracted using the RiboPure Yeast kit (Ambion) following the manufacturer’s instructions. RNA quality was assessed by electrophoresis in denaturing 0.8% agarose gel and quantified by measuring A260 in a BioPhotometer (Eppendorf). cDNA Synthesis and DNA Microarray Experiments—Transcriptional analyses were performed using DNA microarrays containing PCR-amplified fragments from 6014 S. cerevisiae open reading frames (24, 25). Amplified DNA was resuspended in 50% dimethyl sulfoxide and arrayed onto aminosilane-

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**Table 1**

| Yeast strains used in this work | Relevant genotype | Source/Ref. |
|-------------------------------|------------------|-------------|
| BY4741                         | MATa his3Δ1 leu2Δ met15Δ ura3Δ | 69          |
| MAR143                         | BY4741 ptc2::nat1 | 19          |
|                                | BY4741 ptc2::kanMX4 | 69        |
|                                | BY4741 ptc3::kanMX4 | 69        |
|                                | BY4741 ptc4::kanMX4 | 69        |
|                                | BY4741 ptc5::kanMX4 | 69        |
|                                | BY4741 slt2::kanMX4 | 69        |
| MAR154                         | BY4741 slt2::kanMX4 ptc1::nat1 | This work |
|                                | BY4741 slt2::kanMX4 ptc1::nat1 | This work |
| MAR210                         | BY4741 slt2::kanMX4 ptc1::nat1 | This work |
|                                | BY4741 mid2::kanMX4 | 69        |
| MAR211                         | BY4741 mid2::kanMX4 ptc1::nat1 | This work |
| MAR212                         | BY4741 mid2::kanMX4 ptc1::nat1 | This work |
|                                | BY4741 rom2::kanMX4 | 69        |
| MAR213                         | BY4741 slt2::kanMX4 ptc1::nat1 | This work |
|                                | BY4741 slt2::kanMX4 ptc1::nat1 | This work |
|                                | BY4741 rlm1::kanMX4 | 69        |
|                                | BY4741 rlm1::kanMX4 ptc1::nat1 | This work |
|                                | BY4741 yhl16c::kanMX4 ptc1::nat1 | This work |
|                                | BY4741 yhl16c::kanMX4 ptc1::nat1 | This work |
|                                | BY4741 log1::kanMX4 | 69        |
| MAR150                         | BY4741 log1::kanMX4 ptc1::nat1 | 19         |
|                                | BY4741 cnb1::kanMX4 | 69        |
| MAR168                         | BY4741 cnb1::kanMX4 ptc1::nat1 | 19         |
| JA100                          | Mata ura3-52 leu2-3,112 trpl-1 his4 can-1r | 70        |
| MAR161                         | JA100 ptc1::nat1 | 70          |
| JA100                          | JA100 slt2::LEU2 ptc1::nat1 | 70          |
| MAR215                         | JA100 slt2::LEU2 ptc1::nat1 | This work* |

* This strain was only viable in the presence of 1 μm sorbitol.
coated glass slides (UltraGAPS™, Corning Glass) using a MicroGrid II spotter (BioRobotics). Fluorescent Cy3- and Cy5-labeled cDNA was prepared from 8 µg of purified total RNA by the indirect dUTP-labeling method, using the CyScribe post-labeling kit (Amersham Biosciences). DNA fragments from 6014 open reading frames were PCR-amplified from yeast genomic DNA (25).

Pre-hybridization, hybridization, and washes were carried out as recommended by The Institute for Genomic Research with minor modifications. Briefly, prehybridizations of the DNA microarrays were carried out at 42 °C for 1 h in a solution containing 5× SSC, 0.1% SDS, 1% bovine serum albumin. For hybridization, dried Cy3- and Cy5-labeled probes were resuspended in 35 µl of hybridization solution (50% formamide, 5× SSC, 0.1% SDS) each and mixed. Five µg of salmon sperm DNA was added to the mix before denaturation for 3 min at 95 °C. DNA microarrays were hybridized in an ArrayBooster hybridization station (Sunergia Group) for 14 h at 42 °C. For each experimental condition (mutant versus wild type strain) a dye swapping was performed. The scanner ScanArray 4000 (Packard Instrument Co.) was used to obtain the Cy3 and Cy5 images after the hybridization and wash procedures.

Vacuole morphology was assessed as described previously (29). Ten ml of yeast cultures at A660 of 1.0 were harvested, washed, and resuspended in 0.5 ml of fresh YPD. Two hundred fifty-µl cultures at initial A660 of 0.01 were grown at 28 °C in YPD in the presence of the specified conditions for 12–14 h. Growth was monitored in an iEMS Reader MF (Labsystems) at 620 nm.

Vacuole morphology was assessed as described previously (29). Ten ml of yeast cultures at A660 of 1.0 were harvested, washed, and resuspended in 0.5 ml of fresh YPD. The fluorescent dye FM4-64 (Molecular Probes) was added at a final concentration of 20 µM, and cells were incubated for 15 min at 30 °C. The cells were then washed, resuspended in 3 ml of YPD, and incubated for 30–60 min to allow the internalization by endocytosis and accumulation of the dye within the vacuole.

Identification of the carboxypeptidase Y processed forms was achieved by immunodetection as follows. Extracts were prepared from 10 ml of yeast cultures (A660 of 1.0) in TEPI buffer (50 mM Tris, pH 7.5, 5 mM EDTA, 0.5% SDS, plus protease inhibitors). Two hundred µl of extracts were incubated for 5 min at 95 °C. Then 800 µl of TNET buffer (30 mM Tris, pH 7.5, 120 mM NaCl, 5 mM EDTA, 1% Triton X-100) were added, mixed, and then centrifuged for 10 min at 16000 × g. Supernatants were resolved by 10% SDS-PAGE before detection of carboxypeptidase Y by immunoblot.

Staining of bud scars for determination of budding pattern was performed in exponential cultures that were grown at either 30 or 37 °C, for 6–8 h. Cells were fixed with 3.7% formaldehyde for 1 h, washed with phosphate-buffered saline, and stained with 0.02 mg/ml CFW (Fluorescent Brightener F-6259; Sigma).

RESULTS

Expression Profile Analysis of PP2C Mutant Yeast Strains—In this work we have focused on the study of Ptc1–5 phosphatases. YCR079w has not been included because the encoded protein failed to show any phosphatase activity (4), and Ptc7 (YHR076w) was not considered because of its relatively remote similarity with other members of this family. Ptc1–5, as shown...
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A) Genes induced

B) Transposable Elements

Cell Wall-Related

PAU-Related

Phosphate and Iron Metabolism

Transcript changes: 0.15 -fold change to 7.0
in Fig. 1A, share a common catalytic domain that has specific amino-terminal or carboxyl-terminal extensions (in Ptc5 and Ptc2/Ptc3, respectively). Amino acid sequence alignment of these proteins shows that Ptc2 and Ptc3 have the highest degree of identity, whereas Ptc5 is only distantly related (Fig. 1A). To identify novel and possibly specific functions of the members of this family, we decided to analyze the alterations provoked in the expression pattern by the absence of every single PP2C gene. To this end, we compared the expression profile of the mutant strains with that obtained from wild type cells, and we considered a given gene to be induced when its expression was at least 1.8-fold higher in a mutant strain than in the wild type strain. A gene was catalogued as repressed when its expression was at least 1.8-fold lower in a mutant strain than in the wild type. We considered a given gene to be induced when its expression was at least 1.8-fold higher in a mutant strain than in the wild type strain. Fig. 1B shows the dendrogram generated from the DNA microarray experiments. It is remarkable that the classifications obtained by sequence analysis and by expression profiling were substantially different. For instance, the structural similarity between Ptc2 and Ptc3 did not translate in a similar transcriptional response, as the expression profile obtained from the ptc3 mutant was more related to the profile from the ptc4 and ptc5 strains than to the one from ptc2. Similarly, according to their amino acid sequence, it could be predicted that the ptc5 profile should be relatively unrelated from the rest of the ptc mutants. Lack of PTC5, however, induces a similar transcriptional modifications very similar to the ones provoked by the ptc4 deletion. Surprisingly, the expression profile of cells lacking Ptc1, a protein structurally related to Ptc2 and Ptc3, was largely different from any of the mutants studied here. In fact, when the correlation coefficient (r) was calculated for each pairwise comparison of the transcriptional profiles, the correlation between ptc1 and the rest of mutants was near zero in all cases (Table 2).

The number of genes induced and repressed in each ptc mutant strain is shown in Fig. 2A. It is worth noting that from a total of 32 genes whose expression was induced in a ptc1 strain (see Table 3 for further details), only one was also induced in other ptc mutants (MLS1 in ptc2). Mutation of PTC2 yielded a comparatively large response (80 genes induced, 36 repressed). In contrast, lack of Ptc3 or Ptc4 resulted in a relatively small number of induced (4 and 12, respectively) and repressed genes (4 and 5, respectively), which in most cases were also affected by mutation of PTC2. It is remarkable that mutation of PTC1 did not result in repression of any gene (besides PTC1, as expected), indicating again a very specific profile for this mutation. The complete list of genes induced or repressed by lack of Ptc2–5 can be found in supplemental Tables 1 and 2.

To identify co-regulated sets of genes, we analyzed expression profiles by hierarchical clustering using uncentered Pearson correlation matrix with average linkage (30). This analysis allowed us to identify several clusters enriched for genes that have common biological function (Fig. 2B). Two clusters were mainly composed of genes up-regulated in the ptc2, ptc5, ptc4, and ptc5 mutants and were rich in genes encoding proteins involved in phosphate and iron metabolism (cluster 1) and in transposable elements (cluster 2). Cluster 3 was rich in genes encoding proteins highly similar to the serinapepulin family and included genes down-regulated in the ptc2, ptc5, and, to less extent, in the ptc4 mutants. A fourth cluster, comprising most genes up-regulated in the ptc1 mutant and unchanged or barely expressed in the rest of ptc strains, was found to be enriched in cell wall-related genes (cluster 4). As shown in Table 3, a significant number of genes induced in the ptc1 strain code for proteins involved in maintenance of cell wall integrity (CWI). Interestingly enough, most genes induced in the ptc1 mutant have been also found to be induced in response to cell wall stress, in mutants lacking proteins required for cell wall synthesis or remodeling, as well as by exposure to high levels of calcium.

Remarkably, cluster 4 also included PHO89, encoding a Na+/phosphate transporter. Comparison of clusters 1 and 4 reveals that expression of PHO89 and that of several other members of the PHO regulon is differently affected by ptc mutations. Expression of PHO89 is induced 3-fold in cells lacking Ptc1 but does not significantly change in other ptc strains. In contrast, expression of PHO84, encoding a H+/phosphate transporter, is induced in the ptc2, ptc3, and ptc5 mutants but does not change in ptc1 cells. PHO11 and PHO12, coding for repressible acid phosphatases and VTC3, coding for a protein involved in vacuolar polyphosphate accumulation, present an expression pattern similar to that of PHO84.

Fig. 2B provides additional examples of gene families differentially affected by the absence of specific phosphatase genes. For instance, several genes encoding proteins involved in iron uptake in the form of siderophores, such as ARN1, SIT1, FIT2, and FIT3, are induced when Ptc4 or Ptc5 are absent, but they do not change (or even are slightly repressed) in the ptc1 mutant. Similarly, the expression of a set of transposable elements corresponding to the Gag proteins was increased in the ptc2, ptc3, ptc4, and ptc5 strains but underwent a modest decrease in ptc1 mutant cells. Finally, cluster 3 provides an example of genes specifically repressed in ptc2 and ptc5 strains. This cluster includes several members of the PAU1 gene family, which includes subtelomeric genes coding for a large group of proteins with a homology greater than 85%, in many cases induced by anoxia, but whose function remains largely unknown.

Analysis of Genes Differentially Expressed in the ptc1 Strain—Because the DNA microarray experiments clearly showed a striking differential expression pattern between ptc1 and the rest of phosphatase mutants, we wanted to further confirm these results. To this end, we selected two classes of genes for an independent analysis as follows: 1) two cell wall-related genes

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**FIGURE 2. Comparative transcriptional profiling of the PTC family.** A, Venn diagram representation of genes up-regulated or down-regulated in the different ptc mutants. The number of genes whose expression was induced more than 1.8-fold (left) or repressed at least 2.0-fold (right) in any of the analyzed ptc mutants is indicated. B, cluster analysis of the expression profiles of ptc mutants. The selected set of genes mentioned above were hierarchically clustered (average linkage clustering, uncentered correlation) using the Cluster software (version 2.11) and visualized using TreeView (version 1.60) (30). Subtrees containing a significant number of functional or structurally related genes (denoted in boldface) are shown in greater detail. The intensity of expression change can be inferred by comparison with the enclosed scale. Gray color indicates genes with very low expression, below the established threshold.
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TABLE 3
Genes induced in the ptc1 mutant
The selected genes were induced at least by 1.8-fold. Functional groups are based in FunCat (72). Genes induced in the pct1 mutant. The codes for the “Induced by” column are as follows: 1) induced in response to cell wall stress (Congo Red, CFW, or zymolase) (37, 73); 2) induced by activation of the CW1 signaling pathway (74); Ca and Ch indicate induced by Ca++ or chitosan, respectively (31, 75); f, g, and m indicate induced in fks1, gas1, or mnn9 mutants, respectively (45).

| Open reading frame | Gene | -Fold change | Induced by | Other ptc | Gene product |
|--------------------|------|--------------|------------|----------|-------------|
| Cell wall-related proteins | YKL161C | 6.78 | 1, 2, Ch, f, m | | Protein kinase implicated in the Slt2p MAPK signaling pathway |
| | YLR121C | 3.77 | 1, Ca, Ch, f, g, m | | GPI-anchored aspartic protease |
| | YDR077W | 2.66 | 1, 2, f, g, m | | GPI cell wall glycoprotein |
| | YGR189C | 2.38 | 1, 2, Ca, Ch, f, g, m | | Putative glycosidase of the cell wall. GPI protein |
| | YLR194C | 2.32 | 1, 2, Ca, Ch, f, g, m | | Hypothetical protein. GPI protein |
| | YHR030C | 2.29 | 1, 2, Ca, Ch, f, g, m | | Serine/threonine MAP kinase |
| | YDR055W | 2.16 | 1, 2, Ch, f, g, m | | Cell wall protein that contains a putative glycosylphosphatidylinositol attachment site |
| | YKL163W | 2.04 | 1, 2, Ch, f, g | | O-Glycosylated covalently bound cell wall protein PIR |
| | YOR134W | 2.00 | Ca | | GPI mannoprotein |
| | YMR096W | 1.91 | Ca, g | | Involved in vitamin B6 biosynthesis |
| | YEL058W | 1.82 | 1, g, m | | N’-Acetylgalactosamine-phosphatase mutase involved in the biosynthesis of chitin |
| | YIL159W | 1.80 | 1, 2, g | | PIR mannoprotein |
| Metabolism | YKR091W | 3.38 | 1, Ca, Ch, f, g, m | ptc5 | Suppresses the lethality of a rad53 null mutation |
| | YCR005C | 2.76 | Ca, g | | Citrate synthase |
| | YPL088W | 2.19 | 1, Ca, Ch, f, g, m | | Putative aryl alcohol dehydrogenase |
| | YNL117W | 1.86 | | | Malate synthase |
| Transport | YBR296C | 3.00 | 1, Ca | | Na+/P+ cotransporter |
| | YKR039W | 2.22 | Ca | | General amino acid permease |
| | YOR306C | 2.16 | 1, g, m | | Similarity to mammalian monocarboxylate permeases |
| Perception and response to stimuli | YBR072W | 3.06 | Ca, Ch | ptc2 | | Small heat shock protein |
| | YFL014W | 2.82 | 1, Ca, f | ptc2, ptc3 | | Plasma membrane-localized protein |
| | YDR085C | 2.63 | 1, Ch, f, g, m | ptc2, ptc3, ptc4, ptc5 | | α-Factor pheromone receptor regulator |
| | YIL117C | 2.53 | 1, 2, Ch, f, g, m | | Pheromone-regulated protein |
| | YBR295W | 1.93 | Ch, g | | P-type metal-transporting ATPase |
| Mitochondrial | YIL116C | 3.01 | 1 | | Regulate mitochondrial expression of subunits 6 and 8 of the F0F1-ATP synthase |
| Uncharacterized proteins | YIR042C | 3.43 | 1, Ca | | Hypothetical protein |
| | YHR209W | 3.02 | 1, Ca, Ch | | Putative S-adenosylmethionine-dependent methyltransferase |
| | YAL053W | 2.37 | 1, Ca, Ch, f, g, m | | Heme utilization factor |
| | YMR101C | 2.31 | Ch | | Hypothetical protein |
| | YIL107C | 1.96 | Ca, g | | Hypothetical protein |
| | YNL057W | 1.89 | | | Protein of unknown function |
| | YLR042C | 1.81 | 1, Ca | | |

(YKL161c and CRH1) plus PHO89, as representative of those induced in Ptc1-deficient cells; and 2) PHO84 and PHO12, which are induced in other ptc mutants but not in ptc1 cells. The promoters of each of these genes were fused to the lacZ gene placed in a multicopy plasmid, and the constructs were introduced into each ptc mutant strain to determine β-galactosidase activity as reporter of the promoter activity. YKL161c and CRH1 reporters were a generous gift of J. Arroyo, and details on their construction will be provided elsewhere. Other reporters have been previously published (23). As shown in Fig. 3, YKL161c, CRH1, and PHO89 were specifically induced in cells lacking Ptc1, whereas PHO12 was only induced in ptc2 and ptc3 mutants and PHO84 in ptc2, ptc3, and ptc5 cells. PHO5, also a member of the PHO regulon whose expression was undetectable by DNA microarray analysis, showed very low level of activity and did not display significant changes in any of the mutants tested. These results confirmed the information extracted from the DNA microarray data and corroborated that expression of PHO89 is specifically affected by mutation of PTC1 and, therefore, does not group with other members of the PHO regulon.

Lack of Ptc1 Mimics a Situation of Cell Wall Damage—The characteristic transcriptional profile of ptc1 cells described above was reminiscent of that observed after exposure of yeast cells to diverse cell wall-damaging conditions. We considered that this may reflect that the ptc1 mutation could negatively affect the integrity of the cell wall. To confirm this, we tested the sensitivity of ptc1–5 cells to diverse conditions considered to cause cell wall damage. As shown in Fig. 4A, ptc1 cells were hypersensitive to caffeine, CFW, Congo Red, or alkalinization of the medium, whereas the sensitivity of the rest of mutants did not differ from wild type cells. Mutation of PTC2 or PTC3 in a ptc1 background did not result in increased sensitivity to Congo Red or CFW. In fact, when ptc2 and ptc3 mutations were combined, the resulting strain was slightly more tolerant to these compounds than the wild type strain (not shown). Because it is known that cell wall damage causes activation of the Slt2 MAP kinase pathway, we speculated that if the ptc1 mutation would...
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![Graphs and diagrams](image)

**FIGURE 3.** Expression level from relevant promoters in the different ptc mutants. Wild type (WT) strain BY4741 and its isogenic ptc1–5 derivatives were transformed with multicopy plasmids containing the indicated promoters fused to β-galactosidase. Cells were grown and harvested as indicated under “Experimental Procedures,” and the β-galactosidase activity was determined. Results are means ± S.E. from 6 to 12 independent assays.

somehow mimic this insult, it might be reflected in an alteration in the activation state and/or the expression of Slt2. As shown in Fig. 4B, in cells lacking Ptc1 (but not in other ptc mutants) an increase in the phosphorylated form of Slt2, as well as in the total amount of the protein, can be detected. It must be noted that the simple increase in the amount of Slt2 does not necessarily imply an increment in the phosphorylated form, as cells expressing Slt2 from a multicopy plasmid accumulate a large amount of the protein, but it remains in the nonphosphorylated state (Fig. 4B).

The observation that lack of Ptc1 results in the activation of the Slt2 pathway prompted us to consider whether there could be a functional link between this activation and the increased expression of genes related to maintenance of CWI. To this end we combined the ptc1 mutation with that of different upstream and downstream components of the CWI pathway. It must be noted that a previous work reported that the slt2 and ptc1 mutations were synthetically lethal (18). However, we noticed that more recent large scale analysis projects failed to detect such phenotype. As this might reflect strain-to-strain differences, we attempted to construct this double mutant in various genetic backgrounds. Our results confirmed that in certain backgrounds, such as BY4741, the double mutant is viable in the absence of osmotic support, although in others (such as JA100) survival requires the presence of 1 M sorbitol. Therefore, we were able to include the double slt2 ptc1 mutant in our study. We then tested the activity of the YKL161c and CRH1 promoters in all these mutants. As shown in Fig. 5A, the increased expression produced by lack of Ptc1 was unaltered in cells lacking various elements upstream from the Slt2 MAP kinase module (perhaps with the only exception of the wsc1 mutant, which resulted in somewhat impaired expression). In contrast, lack of Bck1, Slt2, or the downstream transcription factor Rlm1 abolished the increased activity of the YKL161c and CRH1 promoters caused by deletion of PTC1, indicating that the observed transcriptional response was mediated by an activation of the MAP kinase module.

The effect of lack of different components of the CWI pathway on the sensitivity of the ptc1 mutant to several cell wall-damaging agents is presented in Fig. 5B. It is remarkable that mutation of some elements upstream of the MAP kinase module, particularly MID2, clearly improved tolerance of the ptc1 strain to Congo Red and CFW (but not to caffeine). Mutation of downstream elements, such as RLM1, also resulted in increased tolerance. Interestingly, this effect was not observed when cells were challenged with caffeine.

To further characterize in a more quantitative form the relationship between Ptc1 and the Slt2 MAP kinase, we tested the sensitivity of ptc1, slt2, and ptc1 slt2 mutants to caffeine, Congo Red, and CFW by its capacity to grow in liquid cultures. As shown in Fig. 6A, mutation of PTC1 confers a phenotype of sensitivity to caffeine stronger than that produced by lack of...
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![Graph A](image1.png)
![Graph B](image2.png)

FIGURE 5. Analysis of the functional relationship between Ptc1 and components of the Slt2 MAPK pathway. A, the indicated mutations were introduced in a wild type (WT) BY4741 strain (empty bars) or its ptc1 derivative (filled bars). Strains were transformed with the reporter plasmids pYKL161c-LacZ or pCRH1-LacZ, and β-galactosidase activity was determined. Data are means ± S.E. from 3 to 9 independent experiments. B, the mentioned strains were spotted on YPD containing different concentrations of the indicated drugs, and their growth was monitored after 2 days.

Slt2, and both effects were additive. ptc1 cells were much more sensitive to Congo Red or CFW (not shown) than slt2 cells, whereas the double mutant was slightly more sensitive to Congo Red than the ptc1 strain. The presence of 1 M sorbitol, which fully rescues the lysis induced by cell wall damage in the slt2 mutant, partially rescued the sensitivity of the ptc1 mutant to Congo Red but only increased very slightly the tolerance of the ptc1 strain to Congo Red or CFW (not shown).

Because Ptc1 has been related to the regulation of the Hog1 pathway, we wanted to evaluate the possible relevance of this pathway in the phenotypes described above. As shown in Fig. 6B, deletion of HOG1 did not increase sensitivity to caffeine, CFW, or Congo Red (not shown) in wild type cells (in fact, it does result in perceptible tolerance to the first two compounds). Lack of Hog1 did not increase caffeine sensitivity of the ptc1 strain and even increased its tolerance to CFW and Congo Red (not shown). As shown in Fig. 6C, lack of Hog1 did not block the increased expression from the YKL161c or CRH1 promoters that results from the mutation of PTC1. These results suggest that the Hog1 pathway is not related to the phenotypes derived from the lack of Ptc1 that are being characterized here.

Lack of Ptc1 Influences Calcineurin-dependent Gene Expression and Calcium Tolerance—To extract further information from the transcriptional profile defined for Ptc1-deficient cells, we performed a comparison between this profile and those produced by different stresses. This allowed us to identify a significant overlap between the group of genes induced by lack of this phosphatase and those identified after an increase in the concentration of extracellular calcium. As shown in Fig. 7A, 19 genes induced in ptc1 cells (almost 60% of the total number) are also induced by high calcium, whereas only 5 genes would be expected if both events were unrelated. This prompted us to examine in more detail the possible relationship between Ptc1 and calcium homeostasis. High calcium activates the protein phosphatase calcineurin that results, through the activation of the Crz1/Tcn1 transcription factor, in changes in the transcription of a substantial number of genes (31). Therefore, we selected a gene, PHO89, which is induced by both high calcium and lack of Ptc1 and tested whether the increased PHO89 expression because of the absence of Ptc1 was mediated by calcineurin. As shown in Fig. 7B, mutation of CNB1, encoding the regulatory subunit of calcineurin required for its phosphatase function, fully abolished the increased expression of PHO89 in a ptc1 strain. This result was compatible with the idea that lack of Ptc1 results in hyperactivation of calcineurin. To further test this possibility, we transformed wild type and ptc1 cells with plasmid pAMS366, which carries a tandem of four calcineurin-dependent response elements from the calcineurin-regulatable GSC2/FKS2 promoter, and we measured the transcriptional activity of this construct. As it can be observed (Fig. 7B) expression from this construct was about 5-fold higher in the ptc1 mutant than in the wild type strain, thus confirming our hypothesis. This increase was completely abolished in cells lacking Cnb1 or its downstream transcription factor Crz1.

It has been shown that cells lacking calcineurin are abnormally tolerant to high extracellular calcium concentrations. We considered that if calcineurin activity is abnormally high in ptc1 cells, they might be hypersensitive to high calcium. Fig. 7C shows that cells lacking Ptc1 are, indeed, extremely sensitive to calcium and that they could not grow when the cation in the medium reaches 100 mM. This phenotype was not observed in cells lacking Ptc2 or Ptc3 (data not shown). Interestingly, the tolerance of the double ptc1 cnb1 strain was as high as that of the cnb1 mutant until the concentration of calcium in the medium reached 150 mM, indicating that the extreme sensitivity of the ptc1 strain to calcium was mediated by the hyperactivation of calcineurin, although it became somewhat lower (matching the tolerance of the wild type strain) when higher amounts of calcium were present in the medium. These results clearly show that lack of Ptc1 interferes with mechanisms required for normal calcium homeostasis.
Deletion of PTC1 Modifies Budding Pattern in Haploid Cells—The suggestive relationship between deletion of PTC1 and increased expression of cell wall-related genes prompted us to re-investigate the early observation that Ptc1-deficient diploid cells, when grown at 37 °C, do not complete cell separation and accumulate daughter cells bound to the mother cell (12). We observed that haploid ptc1 cells showed a phenotype characterized by clumpy cells when growing at 37 °C but not at 28 °C (Fig. 8A). Isogenic wild type cells or a ptc2 mutant (not shown) did not display this phenotype. During the course of this study, we also observed in the ptc1 mutant growing at 37 °C a much higher number of cells presenting improperly localized buds. As shown in Fig. 8B, CFW staining of the haploid ptc1 strain BY4741 and its cnb1 and crz1 derivatives (open bars) were deleted for the PTC1 gene (filled bars) and transformed with plasmid pPHO89-LacZ or pAMS366 (which carries a tandem of four copies of the calcineurin-dependent response element found in the FK506 gene). The activity of these promoters was determined by measuring β-galactosidase activity. Data are mean ± S.E. from 6 to 9 independent experiments.

Vacuolar Alterations Observed in Ptc1-deficient Cells Could Be Responsible for Most ptc1 Phenotypes—A currently ongoing screen in our laboratory searching for genes that in high copy number could improve tolerance of the ptc1 mutant to CFW revealed several genes whose function could be related to the
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**DISCUSSION**

A common problem in identifying the biological roles of the different members of a gene family is the frequent occurrence of overlapping functions, which often impede precise functional assignments. In this work we have resorted to the DNA microarray technology to identify possible differential expression profiles, caused by the absence of five members of the type 2C protein phosphatase family, as a first step to define their possibly specific functions. Our data clearly show that lack of Ptc1 results in a transcriptional profile completely different from that of the rest of the family members, which, in contrast, display to some extent an overlapping response (Fig. 1B and Table 2). This was initially unexpected, on the basis that Ptc1 and several other PP2C isoforms have been shown to be involved in common cellular tasks, such as the negative control of the Hog1 MAP kinase in response to osmotic stress (5–8). Furthermore, the peculiar transcriptional profile of Ptc1 could not be anticipated from primary structure comparisons, which would predict related profiles for Ptc1, Ptc2, and Ptc3. Although a detailed study of the ptc2–5 response will be presented elsewhere, it is worth noting here that deletion of PTC2, -3, and -5 results in increased expression of several genes induced by phosphate starvation (PHO84, PHO11, and PHO12), whereas deletion of PTC1 specifically results in induction of another member of this family, PHO89. This is not completely unexpected, as there are examples of lack of co-regulation between PHO89 and other genes induced by low phosphate. For instance, it was shown that PHO84 is repressed about 5-fold at the diauxic shift, whereas PHO89 is induced 2.5-fold under the same conditions (35). Induction of PHO89 (but not other PHO-regulatable genes) in ptc1 cells fits well with the previous observation that PHO89 is transcriptionally sensitive to an increase in calcium levels (31, 36), and it is induced under cell wall damage conditions (37).

The transcriptional profile of ptc1 cells suggests that this mutant suffers some kind of alteration in its cell wall. In agreement with this notion, we found that ptc1 mutants are abnormally sensitive to Congo Red, and we confirm its sensitivity to other cell wall-damaging condition such as, caffeine, CFW, or high pH (22, 28, 38–40). Other laboratories have found ptc1 mutants to be sensitive to the drug caspofungin, which interferes with glucan synthesis and cell wall formation (41), and tolerant to the K1 killer toxin, a characteristic of certain mutants in genes involved in cell wall synthesis and regulation (42). Likewise, mutation of PTC1 exhibits a synthetically lethal phenotype with genes important for cell wall construction, such as FKS1, GAS1 and SMI1 (43, 44). In agreement with their transcriptional profile, none of the other Ptc mutants showed sensitivity to the conditions tested, pointing again to a very specific function for the Ptc1 isofrom on cell wall integrity.

A common trait for situations leading to long term cell wall damage is an increased expression of the Slt2 MAP kinase (37, 45). We also find here expression of the SLT2 gene specifically induced in ptc1 mutants, and we confirm, by immunoblot analysis, the presence of higher levels of the protein, thus providing further support to the notion that Ptc1 is important for cell wall integrity. Our data indicate that the transcriptional activation of cell wall-related genes in the ptc1 mutant is dependent of the Slt2 MAP kinase module and the downstream transcription factor Rlm1 (Fig. 5A). This requirement may suggest that the role of Ptc1 could be the regulation of the MAP kinase module.
additive to the
the
ptc1
classes (1–3 or more than 3 vacuoles/cell).

tion of the above-mentioned phenotype. At least 700 cells for each strain were classified into four different
VPS73
genes. For instance, deletion of
ground, although not very common, has a number of prece-
dence (18), we were able to generate a double
CEN.PK2 background (46). Similarly, in a W303-derived back-

For instance, Ptc1-deficient cells are unable to grow on nonfer-
mentable carbon sources and are highly sensitive to an alkaline
environment, phenotypes that have been observed in many
vacuolar mutants (28, 51–53). Together with high pH sensitiv-
ity, the inability to tolerate high levels of extracellular calcium
sensitivity is fully abolished (at least within a wide range of
external calcium concentrations) by mutation of the
CNB1
gene. Increased induction of certain genes in a ptc1 mutant can
be also explained by hyperactivation of calcineurin and activa-
tion of the Crz1 transcription factor (Fig. 7B).

Evidence collected in the last few years has pointed to a pos-
sible interaction between the Slt2 and the osmotically activated HOG
pathway (48, 49), as well as to a role of the Hog1 MAP kinase in cell wall
integrity. Thus, hog1 mutants were found to be more resistant to CFW
(50), a phenotype that we confirm here and extend to caffeine and
Congo Red treatments. Because Ptc1 has been shown to dephospho-
ylate and inactivate Hog1 (5), it could be hypothesized that the
hypersensitivity of the ptc1 strain to cell wall-damaging agents could be the
result of hyperactivation of Hog1. However, this possibility appears unlikely, as we show that a double
ptc1 hog1 strain still displays a strong sensitivity to CFW and Congo Red and that lack of the
Hog1 kinase does not improve at all the growth of the ptc1 mutant on
caffeine. Similarly, we have observed that lack of Nbp2, which is
necessary for Ptc1-Hog1 interaction through the Pbs2 scaffold (10), does
not improve sensitivity of the ptc1 strain to caffeine or CFW (not shown).

A survey of the existing literature, together with the evidence pre-
sented in this work, clearly indicates that lack of Ptc1 function results in
numerous cellular defects. This could be the result of the involve-
ment of Ptc1 in a large variety of regu-
lar pathways. However, it came
to our attention that many of the
phenotypes ascribable to lack of

(similarly to the negative regulation exerted by Ptc1 on Hog1).
However, our observations that the cell wall phenotypes of the
ptc1 mutant are 1) more severe than those of the slt2 strain, 2)
additive to the slt2 mutation, and 3) only marginally rescued by
1 M sorbitol strongly suggest that the phenotypes of the
phosphatase mutant are not simply mediated by the MAP kinase
pathway. It must be noted that, in contrast with reported evidence (18), we were able to generate a double
ptc1 slt2 mutant, demonstrating that, in certain genetic backgrounds, the double
mutation is viable (although we have confirmed in other back-
grounds the synthetic lethality of this combination). The
dependence of the lethality of a mutation on a given back-
ground, although not very common, has a number of prece-
dents. For instance, deletion of PKH2 (YOL100w) was found to
have no effect in the FY1679 strain, although it was lethal in the
CEN.PK2 background (46). Similarly, in a W303-derived back-
ground it was required the simultaneous deletion of PKH1 to
yield the lethal phenotype (47).

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**FIGURE 10.** A model accounting for the variety of phenotypes specifically derived from lack of the PP2C Ptc1 isoform. See main text for details.

other defects (i.e. sensitivity to heavy metals such as copper or zinc (58, 59)). This is also shared by ptc1 mutants, as we observe that they are sensitive to zinc (Fig. 9C) as well as to copper and cesium (not shown). Vacuolar mutants have been described as defective in sporulation and germination (60), similarly to what has been found previously for Ptc1-deficient cells (12, 60). We show here that ptc1 mutants are sensitive to diverse drugs that affect cell wall and that display an increased expression of a number of genes also induced by cell wall damage. Cell wall structural defects have been also associated to vacuolar malfunction (41, 44, 61). Interestingly, an early report showed that a diploid homozygous ptc1 strain accumulates multibudded cells when grown at 37 °C, as daughter cells failed to complete cell separation (12). We show that ptc1 haploid cells display a similar defect when grown at 37 °C but not at 28 °C, and in addition, they show a shift from the normal axial budding pattern to a random-like pattern (Fig. 8). A variety of proteins involved in vacuolar targeting or function are also defective in bud size selection in diploid cells (62), and it is suggestive that haploid vma4Δ cells have been described as prone to have a random budding pattern (61).

In addition to the functional similarities between ptc1 and vacuolar mutants described above, we confirm here the recent finding that Ptc1-deficient cells display abnormal vacuolar morphology, specifically fragmented vacuoles (32, 63). This alteration is reminiscent to the so-called class B vps mutants (64) that include, among others, vps5 and vps17 (which assemble together onto the membrane to promote vesicle formation), as well as vps66, vps71, vps72, and vps73, deficient in proteins of unknown function involved in vacuolar protein sorting (32, 64). It must be noted that a member of the type 2C gene family (ptc4Δ), rather similar to budding yeast Ptc1, was defined some time ago as necessary for correct vacuolar fusion in the fission yeast Schizosaccharomyces pombe (65). The striking similarity between ptc1 cells and certain vacuolar mutants allows us to propose a scenario that would explain many, if not all, Ptc1-related cells defects (Fig. 10). We propose that lack of Ptc1 would result primarily in a defect in vacuolar biogenesis. Loss of full vacuolar function would result, for instance, in the inability to properly acidify the vacuole, and hence the strong growth defect at high pH observed in ptc1 cells. Similarly, because the vacuole is the main calcium storage site in yeast (66, 67), improper vacuolar function would increase cytosolic calcium levels and activate calcineurin, thus explaining the strong calcium sensitivity of the ptc1 mutant and the increased expression from calcineurin/Crz1-activable promoters. Our proposal is strongly supported by the observation that overexpression of VPS73, a gene encoding a protein involved in vacuolar protein sorting (32), not only is highly efficient in rescuing the aberrant fragmented vacuolar morphology of the ptc1 mutant but also effectively rescues a large number of other phenotypic defects associated with loss of Ptc1 function, such as abnormal sensitivity to cell wall-damaging agents, heavy metals such as zinc cations, high calcium levels, or alkaline pH (Fig. 9). This novel perspective on Ptc1 function should be instrumental to redirect the efforts to identify the primary, specific target(s) for Ptc1.

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REFERENCES

1. Cohen, P. T. (2004) in Protein Phosphatases (Arino, J., and Alexander, D. R., eds) pp 1–20, Springer-Verlag, Heidelberg
2. Stark, M. I. (1996) Yeast 12, 1647–1675
3. Jiang, L., Whiteway, M., Ramos, C., Rodriguez-Medina, J. R., and Shen, S. H. (2002) FEBS Lett. 527, 323–325
4. Cheng, A., Ross, K. E., Kaldis, P., and Solomon, M. J. (1999) Genes Dev. 13, 2946–2957
5. Warinka, J., Hanneman, J., Lee, J., Amin, D., and Ota, I. (2001) Mol. Cell. Biol. 21, 51–60
6. Maeda, T., Tsai, A. Y., and Saito, H. (1993) Mol. Cell. Biol. 13, 5408–5417
7. Young, C., Mapes, J., Hanneman, J., Al Zarban, S., and Ota, I. (2002) Eukaryot. Cell 1, 1032–1040
8. Saito, H., and Tatebayashi, K. (2004) J. Biochem. (Tokyo) 136, 267–272
9. Martin, H., Flandez, M., Nombela, C., and Molina, M. (2005) Mol. Microbiol. 58, 6–16
10. Mapes, J., and Ota, I. M. (2004) EMBO J. 23, 302–311
11. Shitamukai, A., Hirata, D., Sonobe, S., and Miyakawa, T. (2004) J. Biol. Chem. 279, 3651–3661
12. Robinson, M. K., van Zyl, W. H., Phizicky, E. M., and Broach, J. R. (1994) Mol. Cell. Biol. 14, 3634–3645
13. Roeder, A. D., Hermann, G. J., Keegan, B. R., Thatcher, S. A., and Shaw, J. M. (1998) Mol. Biol. Cell 9, 917–930
14. Leroy, C., Lee, S. E., Vaze, M. B., Ochsenbien, F., Guerois, R., Haber, J. E., and Marsolier-Kergoat, M. C. (2003) Mol. Cell 11, 827–835
15. Marsolier, M. C., Rousset, P., Leroy, C., and Mann, C. (2000) Genetics 154, 1523–1532
16. Welihinda, A. A., Tiraspophon, W., Green, S. R., and Kaufman, R. J. (1998) Mol. Cell. Biol. 18, 1967–1977
17. Munoz, L., Simon, E., Casals, N., CLOTET, J., and Arino, J. (2003) Yeast 20, 157–169
18. Huang, K. N., and Symington, L. S. (1995) Genetics 141, 1275–1285
19. Ruiz, A., Gonzalez, A., García-Salcedo, R., Ramos, J., and Arino, J. (2006) Mol. Microbiol. 62, 263–277
20. Martin, H., Rodriguez-Pachon, J. M., Ruiz, C., Nombela, C., and Molina, M. (2000) J. Biol. Chem. 275, 1511–1519
21. Martin, H., Arroyo, J., Sanchez, M., Molina, M., and Nombela, C. (1993) Mol. Gen. Genet. 241, 177–184
