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PRS5, the Fifth Member of the Phosphoribosyl Pyrophosphate Synthetase Gene Family in *Saccharomyces cerevisiae*, Is Essential for Cell Viability in the Absence of either *PRS1* or *PRS3*

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In *Saccharomyces cerevisiae*, an open reading frame, YOL061w, encodes a polypeptide with sequence similarity to the four known 5-phosphoribosyl-1(α)-pyrophosphate synthetase (PRS) genes since it contains a divalent cation binding site and a phosphoribosyl pyrophosphate binding site. We regard YOL061w as the fifth member of the *PRS* gene family, *PRS5*. Loss of Prs5p has a significant impact on PRS enzyme activity, causing it to be reduced by 84%. On the other hand, Δ*prs5* strains are not affected in growth or in the size of their nucleotide pools. However, simultaneous deletion of *PRS1* and *PRS5* or *PRS3* and *PRS5* rendered the strains inviable, which implies that *PRS5* plays an important role in the maintenance of PRS function in *S. cerevisiae*.

The enzyme 5-phosphoribosyl-1(α)-pyrophosphate synthetase (PRS; ATP:D-ribose-5-pyrophosphotransferase; EC 2.7.6.1) catalyzes the biosynthesis of phosphoribosyl pyrophosphate (PRPP) from ribose-5-phosphate and ATP (9). PRPP is required for the production of purine, pyrimidine, and pyridine nucleotides and the amino acids histidine and tryptophan (13, 15). In *Saccharomyces cerevisiae*, there are at least four genes capable of encoding PRS (3). The *PRS2*-, *PRS3*-, and *PRS4*-predicted polypeptides are 318 to 320 amino acids long, whereas the *PRS1*-predicted polypeptide sequence is longer and more divergent since it contains an in-frame insertion of 105 amino acids bearing no similarity to any *PRS* product or any other known gene product. This insertion, which is neither an intron nor processed by protein splicing, has been named nonhomologous region 1-1 (NHR1-1) (3, 4). The contributions of the *PRS* gene products to the cell's well-being do not appear to be equal, but none of the genes per se is essential. Measurements of growth rates and enzyme activity suggested that Prs1p might well be the key member encoded by the *PRS* gene family (4).

The predicted polypeptide of YOL061w discovered on chromosome XV in the course of the European Yeast Genome Sequencing Project (10) is 496 amino acids long and contains the characteristic motifs of PRS enzymes, the divalent cation binding site (DCbs) and the PRPP binding site (PRPPbs) (2, 7), suggesting that it may be encoded by the fifth member of the yeast *PRS* gene family. This open reading frame (ORF) encodes two potential NHR regions, 116 and 70 amino acids long, which in analogy to Prs1p have been designated NHR5-1 and NHR5-2. NHR5-1 lies N-terminal to the DCbs, and NHR5-2 is located between the DCbs and the PRPPbs (Fig. 1A), the same relative position as that of NHR1-1. *PRS5* is transcribed to give an mRNA of approximately 1.6 kb; this is in accordance with neither NHR5-1 nor NHR5-2 being spliced, which suggests, as is the case for NHR1-1, that they are not introns. Furthermore, Northern analysis showed that deletion of *PRS5* had no effect on the transcriptional levels of the other four *PRS* genes and that *PRS5* transcription was not affected by deletion of any of the other *PRS* genes.

To investigate the role of Prs5p in vivo, we created a null mutant by targeted gene disruption. The entire *PRS5* ORF (1,490 bp) was replaced by either the *KanMX4* module (16) or its derivative, the *loxP-KanMX-loxP* cassette (6). Plasmid pUG6, containing the *loxP-KanMX-loxP* cassette and pFA6-*KanMX4*, shares the sequence of the multiple cloning site, allowing the same oligonucleotides to be used for PCR amplification of both cassettes. Two DNA fragments of 1,542 and 1,691 bp comprising the *KanMX4* module or the *loxP-KanMX-loxP* cassette flanked by 41 and 40 bp of DNA homologous to the regions immediately upstream and downstream of the *PRS5* coding sequence were obtained by PCR by using the corresponding plasmids as templates with the primers PRS5-SFH1 (5′-CTTTGTTGGAGGTTGCTACGAGGCTAGGAACGCAGTCTGGCAGCTGAAGCTTCGTACGCTG-3′) and PRS5-SFH2 (5′-CCCTATTTTTATCAATAAAAAAATGAACACATCAATGCCAATAGGCCACTAGTGGATCTG-3′) (12) (Fig. 1A). The PCR amplification products were used to transform (5) the yeast strains YN94-1 (MAT**a** *ade2-1 his3-11 leu2-3 trp1-1 ura3-1 can1-100*) and YN94-2 (MATα; isogenic to YN94-1), and the resulting transformants were selected on complete medium containing 200 mg of Geneticin G418 (Boehringer, Mannheim, Germany) per liter. The correct integration of the Kan^r cassette was verified by PCR and Southern hybridization (Fig. 1B). Thus, we obtained four *S. cerevisiae* strains: two Δ*prs5::KanMX4* strains (YN96-1 MAT**a** and YN96-2 MATα) and two Δ*prs5::loxP-KanMX-loxP* strains (YN96-54 MAT**a** and YN96-55 MATα). YN96-1 and YN96-2 were used for matings as described below, while YN96-54 and YN96-55 were transformed with plasmid pSH47, which carries on a *URA3*-based plasmid the Cre recombinase gene from bacteriophage P1 under the control of the inducible *GAL1* promoter (6). When grown in galactose-containing medium, the Cre recombinase is induced and the *KanMX* module is excised by a recombination event between the two *loxP* sites, leaving behind a single *loxP* site at the *PRS5* locus. The correct excision of the *KanMX* module was checked by PCR and Southern blotting. In this way, strains YN96-68 and YN96-69 (Δ*prs5*::*loxP*) in both mating types were obtained. The pSH47 plasmid (*URA3*$^+$) was removed from these strains by streaking

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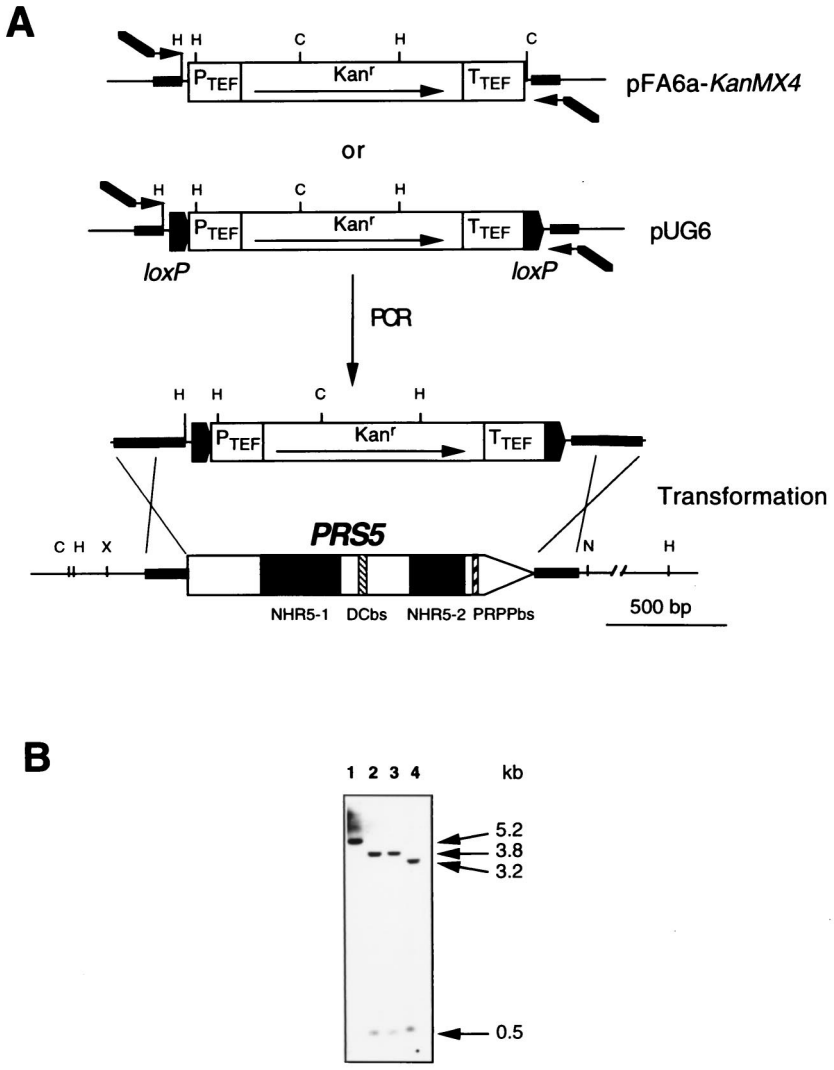

FIG. 1. *PRS5* disruption. (A) Schematic representation of the disruption cassettes and the *PRS5* locus. Arrows at either end of the modules represent the oligonucleotides used for PCR, arrowheads correspond to the cassette amplification regions (black bars), and thick lines of the arrows represent the 40- to 41-bp extension used to target the PCR fragment to the *PRS5* flanking sequences. These oligonucleotides were used to amplify the *KanMX4* plasmid pFA6a-*KanMX4* (16) or the *loxP-KanMX-loxP* plasmid pUG6 cassette (6, 12). Yeast wild-type strains were transformed with these DNA products (5). The Δ*prs5* strains were obtained by recombination (indicated by ×) between the DNA regions upstream and downstream of the *PRS5* locus and their homologous sequences flanking the corresponding cassette. The positions of NHR5-1 and NHR5-2 as well as those of the DCbs and PRPPbs are indicated. (B) Southern blot of *Hin*dIII/*Cla*I-digested genomic DNA of the wild-type strain, YN94-1 (lane 1), the Δ*prs5::loxP-KanMX-loxP* MAT**a** (YN96-54) and MATα (YN96-55) strains (lanes 2 and 3, respectively), and the Δ*prs5::KanMX4* MAT**a** strain (YN96-1) (lane 4) hybridized with an *Xba*I/*Nsi*I fragment containing the *PRS5* gene as shown in panel A. The wild-type strain gives a 5.2-kb signal corresponding to the *Hin*dIII genomic fragment containing the *PRS5* gene. When hybridized with the same probe, YN96-54 (lane 2) and YN96-55 (lane 3) give rise to two signals: a 0.5-kb fragment corresponding to the region between the *Hin*dIII site upstream of *PRS5* and the *Hin*dIII site in the promoter P_{TEF} of the *loxP-KanMX-loxP* module and a 3.8-kb fragment corresponding to the DNA contained between this *Hin*dIII site and the *Hin*dIII site downstream of *PRS5*. DNA from YN96-1 (lane 4) gives the same 0.5-kb upstream fragment, but the downstream fragment is smaller, 3.2 kb, since this cassette has an additional *Cla*I site present in the 3′ end of the *KanMX4*. Restriction enzyme abbreviations: C, *Cla*I; H, *Hin*dIII; N, *Nsi*I; X, *Xba*I. P_{TEF} and T_{TEF} are the promoter and terminator sequences, respectively, of the *Ashbya gossypii* TEF gene (16).

the cells onto plates containing 5-fluoroorotic acid (5-FOA; Sigma-Aldrich, Poole, Dorset, United Kingdom), which counterselects *URA3*$^+$ plasmids (1).

The creation of the *PRS5* null mutant strains shows that disruption of *PRS5* is not a lethal event. Furthermore, there is little or no effect on growth since the Δ*prs5* strains have doubling times of 1.9 to 2.0 h, virtually identical to that of the wild type, which has a doubling time of 1.7 to 1.9 h. PRS enzyme activity was measured in crude cell extracts prepared from mid-log-phase cultures of the Δ*prs5* strain (YN96-69) and assayed by thin-layer chromatography (4). The Δ*prs5* strain retained only 16% of the wild-type activity since it synthesizes 4.6 ± 0.3 nmol of PRPP min^{-1} mg of $protein^{-1}$ (mean ± standard deviation), in contrast to the wild type, which produced 28 ± 4 nmol of PRPP min^{-1} mg^{-1}.

By using high-performance liquid chromatography, we analyzed the effect of deleting each of the *PRS* genes on the nucleotide profile of the yeast cell. Total nucleotides were extracted from wild-type and Δ*prs::loxP* strains after growth of the strains in complete medium to approximately mid-log phase (11). The extracts were resuspended in 150 μl of 7 mM KH_2PO_4 (pH 4.0). Fifty microliters of the resuspended extract

TABLE 1. Nucleotide content of wild-type and Δ*prs* strains

Strain	% of nucleotides produced[a,b]			
	UXP	CXP	AXP	GXP
YN94-1 (wild type)	99 ± 0.8	100 ± 0.6	101 ± 1.5	99 ± 1.1
YN96-66 (Δ*prs1::loxP*)[c]	25 ± 7.0	12 ± 0.5	45 ± 9.0	37 ± 6.0
YN96-67 (Δ*prs3::loxP*)[c]	26 ± 4.0	31 ± 9.8	55 ± 4.0	38 ± 3.0
YN97-7 (Δ*prs2::loxP*)[c]	72 ± 13	97 ± 1.5	91 ± 9.5	80 ± 8.2
YN97-6 (Δ*prs4::loxP*)[c]	75 ± 15	102 ± 2.0	70 ± 15	86 ± 19
YN96-69 (Δ*prs5::loxP*)	101 ± 15	80 ± 13	89 ± 12	96 ± 10

[a] UXP, UMP + UDP + UTP; CXP, CDP + CTP (CMP is not detectable under the experimental conditions used); AXP, AMP + ADP + ATP; GXP, GMP + GDP + GTP.

[b] Values are the percentages of nucleotides produced by each strain with respect to that of the wild type and represent the average ± standard deviation of at least three independent determinations.

[c] These deletion mutant strains were created as described for Δ*prs5::loxP* in the text by using appropriate primers for each gene.

was used to determine the nucleotide pools as described by Strauch et al. (14). Nucleotide standards (98% pure) were from Sigma-Aldrich.

The nucleotide content of YN96-66 (Δ*prs1::loxP*) was drastically reduced in comparison to that of the wild type, and a deletion in *PRS3* (YN96-67) also had a dramatic effect. Δ*prs2*, Δ*prs4*, and Δ*prs5* strains (YN97-7, YN97-6, and YN96-69, respectively) had profiles differing only slightly from that of the wild type (Table 1). It is unlikely that the reduction in the nucleotide content observed for the Δ*prs1* and Δ*prs3* strains (YN96-66 and YN96-67) was caused by degradation of nucleotides, since all peaks were reduced and there were no abnormally high nucleoside monophosphate peaks, as would be expected if nucleoside di- and triphosphates were degraded. This finding was confirmed by adding known amounts of ATP and GTP to extracts of the wild-type strain and observing that no significant degradation of them occurred as the result of the extraction procedure. These results are in agreement with previous observations indicating that Prs1p and Prs3p apparently make a more important contribution to the yeast metabolism than the other members of the family (4).

To further characterize the possible role of Prs5p in the production of PRPP in yeast and to establish its relationship with products of the other members of the *PRS* gene family, we constructed strains disrupted in *PRS5* and one of each of the other four *PRS* genes. Double-disrupted Δ*prs2* Δ*prs5* (YN97-89) and Δ*prs4* Δ*prs5* (YN97-90) strains were constructed by transforming Δ*prs5::loxP* mutants with the appropriate disruption cassette in the same manner as described above. In spite of repeated attempts, it was not possible to obtain the double disruptants Δ*prs1* Δ*prs5* and Δ*prs3* Δ*prs5*. To determine the reason for this failure, we constructed heterozygous diploids (*PRS1*/Δ*prs1::HIS3* Δ*prs5::KanMX4*/*PRS5* and *PRS3*/Δ*prs3::TRP1* Δ*prs5::KanMX4*/*PRS5*) by crossing the corresponding haploid strains (YN94-5 [YN94-2 Δ*prs1::HIS3*] × YN96-1 and YN94-9 [YN94-2 Δ*prs3::TRP1*] × YN96-1). More than 130 tetrads were analyzed after sporulation of the diploids, but no viable HIS^+ $G418^r$ or TRP^+ $G418^r$ haploids were recovered. Microscopic examination of the spores corresponding to the double disruptants indicated that they had undergone germination but had not progressed beyond two or three cell divisions. This suggested that the double-mutant combinations Δ*prs1* Δ*prs5* and Δ*prs3* Δ*prs5* were lethal.

To confirm this result, a heterozygous diploid containing wild-type and deleted versions of *PRS1* and *PRS5* but with a copy of *PRS1* in a *URA3*-based plasmid (pVT1) was constructed. A similar experiment was performed for *PRS3* by using plasmid pVT3. As a result, viable Δ*prs1::HIS3* Δ*prs5::KanMX4* and Δ*prs3::TRP1* Δ*prs5::KanMX4* isolates were recovered, but they always contained the corresponding plasmid, pVT1 or pVT3. These strains were sensitive to media containing 5-FOA, indicating that any cells losing the plasmid were inviable (Fig. 2). Wild-type and single-deletion mutants carrying pVT1 and pVT3 gave rise to colonies on 5-FOA-containing media. These data confirm that the *PRS5* null mutant is synthetically lethal in combination with either Δ*prs1* or Δ*prs3*. Therefore, while *PRS5* is not an essential gene, the loss of Prs5p cannot be tolerated together with the loss of either Prs1p or Prs3p, indicating that the maintenance of the cell's requirement for PRPP is a complicated issue that could involve inter-

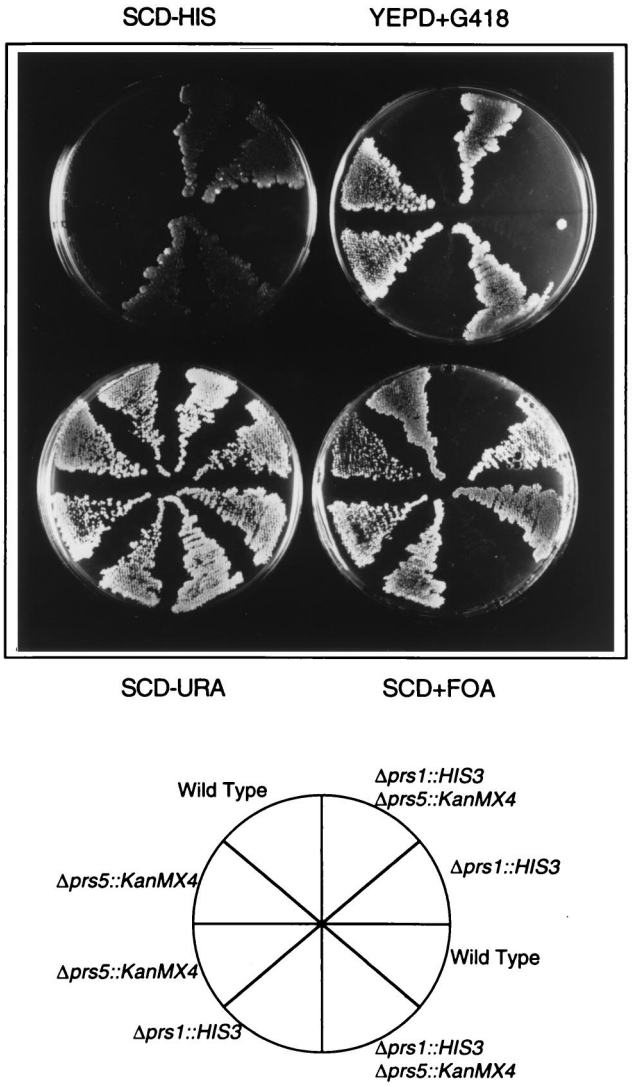

FIG. 2. Δ*prs5* is lethal in combination with Δ*prs1*. *PRS1*/Δ*prs1::HIS3* Δ*prs5::KanMX4*/*PRS5* diploids carrying pVT1 were sporulated, and tetrads were dissected. Two tetrads were streaked on media for selection of single disruptants (synthetic complete dextrose minus histidine [SCD-HIS] and yeast extract-peptone-dextrose plus Geneticin G418 [YEPD+G418]) and for the maintenance (SCD-URA) or loss (SCD+FOA) of plasmid pVT1. Yeast media were prepared as described by Kaiser et al. (8). Similar results were obtained with the combination of Δ*prs3* and Δ*prs5*.

action between Prs5p and Prs1p or Prs3p. Two-hybrid experiments to investigate this further are under way.

Our analysis of the *PRS* gene family in *S. cerevisiae* has shown that Prs1p and Prs3p may play a more important role in PRPP biosynthesis than the other members of the family (4). In this study, we have shown that either Prs1p or Prs3p is essential in the absence of Prs5p. To be able to understand the level of functional interaction among the *PRS* gene products, it will be necessary to analyze the phenotypes associated with strains bearing combinations of multiple disruptions of the *PRS* genes and to determine to what extent PRS activity is influenced by each member of this gene family.

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