Genetic Analysis and Enzyme Activity Suggest the Existence of More Than One Minimal Functional Unit Capable of Synthesizing Phosphoribosyl Pyrophosphate in Saccharomyces cerevisiae*

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The PRS gene family in Saccharomyces cerevisiae consists of five genes each capable of encoding a 5-phosphoribosyl-1-(α)-pyrophosphate synthetase polypeptide. To gain insight into the functional organization of this gene family we have constructed a collection of strains containing all possible combinations of disruptions in the five PRS genes. Phenotypically these deletant strains can be classified into three groups: (i) a lethal phenotype that corresponds to strains containing a double disruption in PRS2 and PRS4 in combination with a disruption in either PRS1 or PRS3; simultaneous deletion of PRS1 and PRS5 or PRS3 and PRS5 are also lethal combinations; (ii) a second phenotype that is encountered in strains containing disruptions in PRS1 and PRS3 together or in combination with any of the other PRS genes manifests itself as a reduction in growth rate, enzyme activity, and nucleotide content; (iii) a third phenotype that corresponds to strains that, although affected in their phosphoribosyl pyrophosphate-synthesizing ability, are unimpaired for growth and have nucleotide profiles virtually the same as the wild type. Deletions of PRS2, PRS4, and PRS5 or combinations thereof cause this phenotype. These results suggest that the polypeptides encoded by the members of the PRS gene family may be organized into two functional entities. Evidence that these polypeptides interact with each other in vivo was obtained using the yeast two-hybrid system. Specifically PRS1 and PRS3 polypeptides interact strongly with each other, and there are significant interactions between the PRS5 polypeptide and either the PRS2 or PRS4 polypeptides. These data suggest that yeast phosphoribosyl pyrophosphate synthetase exists in vivo as multimeric complexes.

The enzyme 5-phosphoribosyl-1-(α)-pyrophosphate synthetase (ATP:-ribose-5-phosphate pyrophosphotransferase; EC 2.7.6.1) (PRS) catalyzes the reaction at a key junction in intermediary metabolism. PRS transfers the pyrophosphate moiety released from ATP to ribose-5-phosphate, thus giving rise to phosphoribosyl-pyrophosphate (PRPP) (1), and the enzyme therefore directs ribose-5-phosphate from energy generated by the pentose phosphate pathway to the important biosynthetic intermediate PRPP. PRPP is a precursor for the production of purine, pyrimidine, and pyridine nucleotides and the amino acids histidine and tryptophan (2). PRPP is required for both the de novo and the salvage pathways of nucleotide metabolism (3). It has been shown that in Mycobacterium spp. PRPP is also required for the biosynthesis of polyprenylphosphate pentoses that contribute to the arabinosyl residues of the cell wall (4).

Five PRS genes have been cloned and sequenced from a variety of organisms; bacteria (5–9), mycoplasma (10), and protozoa (11) each contain apparently one PRS gene. In nematodes (12, 13) and the yeast Schizosaccharomyces pombe (14, 15) two PRS genes have been found so far, whereas in Spinacia oleracea four PRS cDNAs have been identified (16). PRS genes have also been cloned in rat (17–20) and human (21–23). Both rat and human have two ubiquitously expressed PRS genes, with a third testis-specific transcript found in humans (22).

The biochemistry of the rat liver enzyme has been widely studied and led to the identification of the genes encoding it. PRSI and PRSII encode the catalytic subunit, which consists of two highly homologous isoforms, PRSI and PRSII, each with a molecular mass of 34 kDa (17, 24). The enzyme has two additional components of 39 and 41 kDa (24), which have been designated PRPP synthetase-associated proteins (PAPs). A negative regulatory role has been proposed for these PAPs because their removal from the rat liver enzyme complex results in an increased enzymatic activity (25). cDNAs corresponding to the rat PAP39 and PAP41 have been obtained, the deduced amino acid sequences of both are very similar to each other and to those of the PRS catalytic subunits; therefore broadly speaking on the basis of sequence similarity PAP39 and PAP41 can be considered to be members of the PRS family (25, 26). In humans the situation appears to be comparable with that in rat because human PAP39 and PAP41 cDNAs have been obtained and found to be very similar to each other and to rat PAP39 and PAP41 and human PRSI and PRSII (27, 28).

Our analysis of the PRS gene family in Saccharomyces cerevisiae has identified five genes, each located on a different chromosome (29, 30). The five predicted PRS polypeptides contain the characteristic motifs of PRS: a divergent cation-binding site and a PRPP-binding site (5, 31). The predicted PRS2, PRS3, and PRS4 polypeptides are 318–320 amino acids long, whereas PRS1 and PRS5 polypeptides are longer and more divergent in their sequences because they contain in-frame insertions bearing no similarity to any known PRS and, in fact,
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to any known gene product. We have named these regions nonhomologous regions (NHRs). PRS1 contains one NHR (NHR1–1) that is neither an intron nor processed by protein splicing (29, 32), and PRS5 contains two NHRs, NHR5–1 and NHR5–2, that are not introns (30). Our preliminary analyses of the phenotypes associated with strains containing disruptions in each of the five PRS genes have shown that none of the five genes is essential, although the contribution of each to the cell’s metabolism does not appear to be equal because Prs1p and Prs3p make a more significant contribution than the other members of the family (30, 32). However, the PRS5 null mutation is synthetically lethal in combination with a disruption in either PRS1 or PRS3, implying an important role for Prs5p in the production of PRPP (30).

Because all PRS genes sequenced to date have a high degree of similarity, studies on PRS in S. cerevisiae could provide valuable information on the genetics and biochemistry of eukaryotic PRS. In the present study we have constructed a collection of strains bearing all the possible combinations of disruptions in the five PRS genes and analyzed their phenotypes. The data described here are consistent with the existence of two functionally different entities, one consisting of Prs1p and Prs3p and the other consisting of Prs2p, Prs4p, and Prs5p.

EXPERIMENTAL PROCEDURES

Standard DNA manipulations were carried out as described by Sambrook et al. (33). Preparation of yeast genomic DNA, total RNA, Northern blotting, and hybridization were performed as described previously (29, 32). In Northern analysis 32P-labeled actin-encoding DNA was used as the loading control. Unless otherwise stated yeast transformation was carried out according to Ebble (34). Quantification of radioactive signals were performed using a Fuji BAS-1500 phosphorimager.

Strains, Plasmids, and Growth Conditions—The S. cerevisiae strains used in this study are listed in Table I. The strains were grown at 30 °C in yeast extract peptone dextrose medium (YPED) or in YEPD containing 200 mg/liter of the aminoglycoside antibiotic geneticin G418 (Roche Molecular Biochemicals) (35) or synthetic complete medium (36). Tetrad were dissected using a Singer® micromanipulator, model MSM (Singer Instruments). 5-Fluoro-oorotic acid (5-FOA; Sigma-Aldrich) was used to select against uracil prototrophy (37).

S. cerevisiae strains containing disruptions in two or three PRS genes were obtained by one of the following methods. (i) A PCR-based method using theloxPKanMX-loxP module as a marker (38). DNA fragments containing the disruption cassette together with 40–45-nucleotide extensions at their ends that are homologous to the regions immediately upstream and downstream of the start and stop codons of each PRS gene were obtained by PCR (38). These DNA fragments were used to transform (39) S. cerevisiae strains already disrupted in a PRS gene (30), and the resulting transformants were selected on YEPD containing G418. The correct integration of the KanMX cassette was verified by PCR and Southern blotting. Derivatives from the strain HF7c (40) containing disruptions in each PRS gene were obtained by the same procedure. The KanMX marker was excised from the deletants by a recombination event between the two flankingloxP sites after transforming the corresponding strains with plasmid pSH47, which contains the Cre recombinase under the GAL1 promoter. Plasmid pSH47 (URA+) was removed from these strains by streaking the cells onto plates containing 5-FOA, which counterselects RecA+ plasmids (37). (ii) Strains YN84-22 and YN85-100 were obtained by appropriate crosses of available strains (32). The diploids obtained were sporulated, the resulting tetrads were dissected, and the spores were selected for the presence of the markers used for the disruptions. In all cases the disruptions were checked by PCR and Southern blotting.

Plasmids were amplified in Escherichia coli DH5α (supE44 ΔlacZΔM15 Gal–lac–I2179 [480 lacZam15] hsdR17 recA1 endA1 gyrA96 thi-1 relA1; Ref. 41). Plasmid pRS21 consists of the 2562-bp HindIII fragment containing the PRS2 coding sequence plus 1470 and 135 bp upstream and downstream, respectively (29), inserted in plasmid pRS416 (42). pRS41 is plasmid pRS416 containing the 2950-bp SalI/BgII fragment that corresponds to the PRS4 coding sequence plus 509 and 1429 bp upstream and downstream thereof (29). Plasmid pVT1 contains the 1346-bp PstI/SalI fragment containing the PRS1 coding sequence plus 42 and 21 bp upstream and downstream, respectively (29), inserted into the appropriately restricted plasmid pVT100–U (43). pVT3 is plasmid pVT100–U containing the 1113-bp SauBI fragment that corresponds to the PRS3 coding sequence plus 73 and 77 bp upstream and downstream thereof (29).

Determination of PRS Activity—Crude cell extracts were prepared from mid-log phase cultures. Activity of PRS was assayed by thin layer chromatography (44) as modified by Carter et al. (32). The relative amounts of radioactive material corresponding to ATP and PRPP were determined with a Packard Instant Imager 2024. Specific activity is expressed as nmol PRPP min–1 mg–1 protein. Protein content was determined according to Bradford (45) using bovine serum albumin as the standard.

Extraction and Quantification of Nucleotides—Total nucleotides were extracted from wild type and Δprs::loxP strains after growth in complete medium to approximately mid-log phase (46). The extracts were reconstituted in 150 μl of 7 mm KH2PO4, pH 4.0. Nucleotide pools were determined by high pressure liquid chromatography using 50 μl of the extract (47). The eluant was monitored with a diode array detector, and peaks were quantified by comparison of peak areas at 260 nm with those of known amounts of 98% pure standards (Sigma-Aldrich).

Yeast Two-Hybrid Analysis—PRS genes were cloned in the two-hybrid system (48) vectors pGAD424 and pGBT9 (CLONTECH, Palo Alto, CA) which contain the Gal4p activation domain or DNA-binding domain, respectively. For this purpose each PRS gene was amplified by PCR using primers that produced an EcoRI restriction enzyme site immediately upstream of the initiation codon and a PstI restriction endonuclease site immediately downstream of the stop codon (PRS1–4) or placed BomHI restriction endonuclease sites at both ends (PRS5). The DNA fragments obtained were cloned into appropriately restricted pGAD424 and pGBT9, and the constructs were sequenced to confirm the correct insertions and to check for PCR-induced errors.

The yeast reporter strain HF7c (40) was transformed with every possible pairwise combination of Gal4pAD-Prsp and Gal4pDBD-Prsp plasmids to trypanoth and leucine prototrophy. Crude cell extracts were prepared from mid-log phase cultures. Two-hybrid analyses were performed by assaying for β-galactosidase activity using a chemiluminescent substrate (Galacto-Light PlusTM, Tropix Inc, MA) according to the manufacturer’s instructions. Chemiluminescence was measured using a Lumac LB9501 luminometer (Berthold, Wildbad, Germany). Results were recorded as relative light units/μg protein and expressed relative to the values obtained with control strains containing empty two-hybrid vectors.

RESULTS

Construction of PRS Deletion Strains

A collection of S. cerevisiae strains containing all possible combinations of disruptions in the five PRS genes has been created by targeted gene disruption. TheloxPKanMX-loxP cassette together with the Cre recombinase expression system allows for repeated use of the KanMX marker and is therefore ideal for the functional analysis of gene families. The strains created by this system have the advantage that the only foreign DNA contained therein is theloxP site remaining after the Cre recombinase-induced event and all the deletants will be congenic (38). Using this procedure the viable double deletants listed in Table I were obtained. The viable double deletant strains Δprs2::loxP Δprs5::loxP (YN97-89) and Δprs4::loxP Δprs5::loxP (YN97-90) have been described previously, and we have shown that the double deletant strains Δprs1::loxP Δprs5::loxP and Δprs3::loxP Δprs5::loxP are inviable (30). The triple deletants Δprs1::loxP Δprs2::loxP Δprs3::loxP (YN97-169), Δprs1::loxP Δprs3::loxP Δprs4::loxP (YN97-170), and Δprs2::loxP Δprs4::loxP Δprs5::loxP (YN97-91) (Table I) were obtained by transformation of appropriate double deletant strains. Our success in constructing these deletant strains shows that the combined loss of these PRS genes is not a lethal event.

Synthetic Lethal Combinations

Repeated attempts were made to obtain the triple disruptants Δprs1 Δprs2 Δprs4 and Δprs2 Δprs3 Δprs4, but none was successful. This observation suggested that a double dis-
ruption of PRS2 and PRS4 might be synthetically lethal in combination with a disruption in either PRS1 or PRS3.

To analyze this possibility we performed a cross between the single deletant, and double deletant strains carrying the rele-

ting to produce PRPP, and nucleotide content.

Doubling Times—The analysis of growth rates showed that strains containing disruptions affecting PRS1 and PRS3 to-

together or in combination with disruption of other PRS genes are affected severely in their growth rates. The double disrup-

tants have doubling times between 3.5 and 4.0 h, and the triple deletants prs1 Δprs2Δprs3 (YN97-169) and Δprs1Δprs2Δprs4Δprs5 (YN97-170) have doubling times of 4.8 and 5.0 h, respectively. However, strains carrying disruptions in PRS2, PRS4, or PRS5 grow at the same rate as the wild type with a doubling time of 2 h (Table III).

PRPP Synthesizing Ability—We have examined PRS activity in five single, nine double, and three triple congenic deletant

| Strain | Relevant genotype | Reference |
|--------|-------------------|-----------|
| YN94–1 | Mata ade2–1 his3–11 leu2–3 trp1–1 ura3–1 can1–100 | M. J. R. Stark |
| YN94–2 | Mata ade2–1 his3–11 leu2–3 trp1–1 ura3–1 can1–100 | M. J. R. Stark |
| YN94–22 | Mata YN94–1 Δprs1::HIS3 Δprs4::LEU2 | This study |
| YN95–100 | Mata YN94–1 Δprs3::TRP1 Δprs4::LEU2 | This study |
| YN96–22 | Mata HF7c Δprs5::KanMX4 | This study |
| YN96–25 | Mata HF7c Δprs1::KanMX4 | This study |
| YN96–56 | Mata HF7c Δprs3::KanMX4 | This study |
| YN96–66 | Mata YN94–2 Δprs1::loxP | 30 |
| YN96–67 | Mata YN94–2 Δprs3::loxP | 30 |
| YN96–69 | Mata YN94–2 Δprs5::loxP | 30 |
| YN96–78 | Mata YN94–2 Δprs1::loxP-KanMX-loxP Δprs3::loxP | This study |
| YN96–79 | Mata HF7c Δprs4::loxP-KanMX-loxP | This study |
| YN97–3 | Mata YN94–2 Δprs2::loxP-KanMX-loxP | 30 |
| YN97–4 | Mata YN94–2 Δprs1::loxP Δprs3::loxP | This study |
| YN97–6 | Mata YN94–2 Δprs4::loxP | 30 |
| YN97–7 | Mata YN94–2 Δprs2::loxP | 30 |
| YN97–9 | Mata YN94–1 Δprs1::loxP-KanMX-loxP Δprs3::loxP | This study |
| YN97–12 | Mata YN94–2 Δprs2::loxP Δprs4::loxP-KanMX-loxP | This study |
| YN97–13 | Mata YN94–2 Δprs2::loxP Δprs4::loxP | This study |
| YN97–70 | Mata YN94–2 Δprs2::loxP Δprs4::loxP Δprs5::loxP-KanMX-loxP | This study |
| YN97–88 | Mata YN94–2 Δprs1::loxP Δprs4::loxP | This study |
| YN97–89 | Mata YN94–2 Δprs2::loxP Δprs5::loxP | 30 |
| YN97–90 | Mata YN94–2 Δprs4::loxP Δprs5::loxP | 30 |
| YN97–91 | Mata YN94–2 Δprs2::loxP Δprs4::loxP Δprs5::loxP | This study |
| YN97–140 | Mata HF7c Δprs4::loxP-KanMX-loxP | This study |
| YN97–142 | Mata YN94–1 Δprs1::loxP-KanMX-loxP Δprs4::loxP | This study |
| YN97–144 | Mata YN94–2 Δprs1::loxP Δprs2::loxP | This study |
| YN97–145 | Mata YN94–2 Δprs1::loxP Δprs4::loxP | This study |
| YN97–163 | Mata YN94–2 Δprs1::loxP Δprs2::loxP-KanMX-loxP Δprs3::loxP | This study |
| YN97–166 | Mata YN94–1 Δprs1::loxP Δprs3::loxP-KanMX-loxP Δprs4::loxP | This study |
| YN97–169 | Mata YN94–2 Δprs1::loxP Δprs3::loxP | This study |
| YN97–170 | Mata YN94–1 Δprs1::loxP Δprs3::loxP Δprs4::loxP | This study |
| YN97–171 | Mata YN94–2 Δprs2::loxP Δprs3::loxP-KanMX-loxP | This study |
| YN97–172 | Mata YN94–2 Δprs3::loxP Δprs4::loxP-KanMX-loxP | This study |
| YN98–5 | Mata YN94–2 Δprs2::loxP Δprs3::loxP | This study |
| YN98–6 | Mata YN94–2 Δprs3::loxP Δprs4::loxP | This study |
| HF7c | Mata ade2–1 his3–11 leu2–3 trp1–1 ura3–1 can1–100 | 1 mg

The double and triple deletants prs1 Δprs2Δprs3 (YN96-66) and Δprs1Δprs2Δprs4Δprs5 (YN96-67) produce 0.85 and 1.08 nmol PRPP min$^{-1}$ mg$^{-1}$, respectively. The double and triple deletants bearing disruptions in PRS1 or PRS3 together or in combination with disrup-
The heterozygous diploids Δprs1::HIS3 PRS2 Δprs4::LEU2 × PRS1 Δprs2::loxP-KanMX-loxP PRS4 and PRS2 Δprs3::TRP1 Δprs4::LEU2 × Δprs2::loxP-KanMX-loxP PRS3 PRS4 containing the indicated plasmid were sporulated and tetrad-dissected, and the resulting spores were incubated at 30 °C. PD, parental ditype; NPD, nonparental ditype; TT, tetratype; RS, random spores; TT or RS indicates the source of the triple mutants. pRS21 and pRS41 are pRS416 derivatives containing PRS2 and PRS4 DNA, respectively. pVT1 and pVT2 are PRS1 and PRS3 DNA cloned in pVT100-U, respectively.

| Cross | Number of tetrads | Random spores | Segregation pattern | Number of triple mutants |
|-------|-------------------|---------------|---------------------|-------------------------|
| [pRS21] | 18 | 16 | 1 | 2 (2 RS) |
| [pRS41] | 19 | 68 | 19 | 9 (6 TT/3 RS) |
| [pVT1] | 9 | 54 | 1 | 8 (3 TT/6 RS) |
| [prs2] | 2 | 22 | 2 | 7 (1 TT/6 RS) |
| [prs4] | 13 | 59 | 13 | 6 (5 TT/1 RS) |
| [pVT3] | 14 | 27 | 1 | 6 (TT/1 RS) |

Nucleotide Content—PRPP synthetase activity and growth of S. cerevisiae Δprs strains

The doubling times were determined graphically from the exponential phase of growth. PRS activity was measured in crude extracts of cells harvested at mid-log phase. The reaction products were separated using TLC. The specific activity of PRS was determined from the relative amounts of radioactivity in PRP and ATP spots. Data for each strain were obtained from at least three independent determinations. W.T., wild type.

| Strain | Relevant genotype | Doubling time | Specific activity | Relative activity |
|--------|-------------------|---------------|-------------------|-------------------|
| YN94–1 | a W.T. | 1.6 | 26.2 ± 0.8 | 100.0 |
| YN94–2 | a W.T. | 1.7 | 30.0 ± 0.4 | 100.0 |
| YN96–66 | a Δprs1 | 3.0 | 0.85 ± 0.2 | 2.8 |
| YN97–7 | a Δprs2 | 2.0 | 4.35 ± 0.66 | 14.5 |
| YN96–67 | a Δprs3 | 2.5 | 1.08 ± 0.14 | 3.6 |
| YN97–6 | a Δprs4 | 2.0 | 5.09 ± 0.45 | 16.9 |
| YN96–69* | a Δprs5 | 2.0 | 4.82 ± 0.2 | 16.0 |
| YN97–144 | a Δprs1 Δprs2 | 4.0 | 0.35 ± 0.06 | 1.2 |
| YN97–88 | a Δprs1 Δprs3 | 4.0 | 0.76 ± 0.08 | 2.9 |
| YN97–4 | a Δprs1 Δprs3 | 4.0 | 0.67 ± 0.18 | 2.2 |
| YN97–145 | a Δprs1 Δprs4 | 3.5 | 1.03 ± 0.02 | 3.9 |
| YN98–5 | a Δprs2 Δprs3 | 4.0 | 0.27 ± 0.07 | 0.9 |
| YN97–13 | a Δprs2 Δprs4 | 2.0 | 4.47 ± 0.8 | 14.9 |
| YN97–89 | a Δprs3 Δprs5 | 2.0 | 3.31 ± 0.24 | 11.0 |
| YN98–6 | a Δprs3 Δprs4 | 3.6 | 1.68 ± 0.38 | 5.6 |
| YN97–90 | a Δprs4 Δprs5 | 2.0 | 4.13 ± 0.27 | 13.7 |
| YN97–169 | a Δprs2 Δprs3 Δprs4 | 4.8 | 0.35 ± 0.08 | 1.2 |
| YN97–170 | a Δprs3 Δprs4 Δprs4 | 5.0 | 0.57 ± 0.11 | 2.2 |
| YN97–91 | a Δprs2 Δprs3 Δprs4 Δprs5 | 2.0 | 4.88 ± 0.74 | 16.2 |

* From Hernando et al. (30).

Nucleotide content of wild type and PRS deletant strains

The nucleotide content was determined by high pressure liquid chromatography as described under “Experimental Procedures.” Values are the percentage of nucleotides produced by each strain with respect to the wild type and represent the average of at least three independent determinations. UXP, UMP + UDP + UTP; AXP, AMP + ADP + ATP; GXP, GMP + GDP + GTP. The values for cytosine ribonucleotides are not included because under the experimental conditions used they are usually below the level of detection.

| Strain | Nucleotide content |
|--------|--------------------|
| YN94–1 (wild type) | 99 ± 1 |
| YN97–144 (Δprs1 Δprs2) | 20 ± 7 |
| YN97–4 (Δprs1 Δprs3) | 25 ± 3 |
| YN97–145 (Δprs1 Δprs4) | 19 ± 7 |
| YN97–6 (Δprs2 Δprs3) | 19 ± 9 |
| YN97–13 (Δprs2 Δprs4) | 84 ± 9 |
| YN97–89 (Δprs3 Δprs5) | 89 ± 19 |
| YN98–8 (Δprs4 Δprs5) | 24 ± 5 |
| YN97–90 (Δprs4 Δprs5) | 92 ± 9 |
| YN97–169 (Δprs1 Δprs2 Δprs3) | 21 ± 2 |
| YN97–170 (Δprs1 Δprs3 Δprs4) | 9 ± 1 |
| YN97–91 (Δprs2 Δprs3 Δprs4 Δprs5) | 111 ± 18 |

4.28

0.27

0.12

0.67

0.24

1.03

0.87

1.68

4.13

0.35

0.57

4.88

26.2 ± 0.8

30.0 ± 0.4

0.85 ± 0.2

4.35 ± 0.66

1.08 ± 0.14

5.09 ± 0.45

4.82 ± 0.2

0.35 ± 0.06

0.76 ± 0.08

0.67 ± 0.18

1.03 ± 0.02

0.27 ± 0.07

4.47 ± 0.8

3.31 ± 0.24

1.68 ± 0.38

4.13 ± 0.27

0.35 ± 0.08

0.57 ± 0.11

4.88 ± 0.74

100.0

100.0

2.8

14.5

3.6

16.9

16.0

1.2

2.9

2.2

3.9

0.9

14.9

11.0

5.6

13.7

1.2

2.2

16.2

0.27 to 1.68 nmol PRPP min⁻¹ mg⁻¹.
reduction in the nucleotide content observed for the strains containing disruptions in PRS1 and PRS3 was caused by degradation of nucleotides because known amounts of ATP and GTP added to extracts of the wild type strain were not significantly degraded (data not shown). These data are consistent with previous observations on the nucleotide content of the single disruptants that indicated that deletions of PRS1 or PRS3 significantly affect the nucleotide content of the yeast cell, whereas the nucleotide content of strains deleted in PRS2, PRS4, or PRS5 was slightly or not affected (30). Nucleotide pools were essentially identical in cells grown in YEPD (data not shown).

**Transcription of PRS Genes in the Deletant Strains**

Each PRS gene product shares between 40 and 90% identity with the other members of the family, and this may imply functional redundancy. One might expect the existence of a control mechanism of gene expression such that the absence of certain PRS mRNAs or their translation products may trigger an alteration in the amount of transcript of one and/or another of the remaining PRS genes. To test this possibility we performed Northern blot analysis with the wild type strain and representative disruptants. Northern blots were probed with 32P-labeled DNA from the coding region of each gene. The filters were stripped and reprobed with 32P-labeled actin-encoding DNA as the loading control. The wild type strain contained transcripts of each PRS gene (PRS1, 1.5 kilobases; PRS2, PRS3, and PRS4, 1.3–1.2 kilobases; PRS5, 1.6 kilobases). In the deletant strains we could detect only the transcripts of the genes not disrupted therein. After correcting for background hybridization the signal associated with each PRS transcript was estimated by comparison of its signal with the actin signal. There were no significant differences under the experimental conditions used between mRNA levels from wild type and Δprs strains (data not shown).

**Analysis of Physical Interactions by the Yeast Two-hybrid System**

We used the yeast two-hybrid system (48) to test for protein-protein interaction between the PRS gene products. H7c transformants containing all possible combinations of Gal4pDBD-Prs1-5p with Gal4pAD-Prs1-5p were obtained. The β-galactosidase activity associated with each pairwise combination is shown in Fig. 1A. Gal4pDBD-Prs1p shows interaction with Gal4pAD-Prs3p; this interaction is also detectable, although less pronounced, in the opposite combination. The interaction of Prs1p and Prs2p is still detectable but has not increased with respect to the wild type, and Prs5p interactions vary from strain to strain. In the Δprs1 and Δprs5 deletant backgrounds Prs5p interacts with all others, particularly with Prs4p and Prs2p, and the Prs5p interaction with itself observed above can no longer be measured. In the Δprs3 background Prs5p interacts clearly with Prs2p and Prs4p. Protein-protein interactions in Δprs2 and Δprs4 backgrounds are very similar; again we can detect strong interaction between Prs1p and Prs3p. In these backgrounds we can detect clear interaction between Prs1p and Prs2p. We can also detect interaction between Prs1p and Prs4p, and the strength of interaction of Prs5p with Prs2p and Prs4p has also increased. This enhancement of PRS polypeptide interactions in strains depleted for endogenous PRS polypeptides suggests that in vivo the PRS gene products interact to form multimeric complexes.

**DISCUSSION**

In _S. cerevisiae_ we have identified five PRS genes that constitute the PRS gene family. The existence of paralogous genes has been explained in different terms for different gene families. In some cases it has been shown that the product of each member contributes to the total activity of the corresponding enzyme, as is the case for the three members of the ATP1 gene family (49). In other cases, the products of the various members of the gene family are distributed to different cell compartments, for example the _HSP70_ gene family (50). A third explanation for a gene family is that the gene products may have different substrate specificities, as is the case for the five genes encoding acyl-CoA synthetases (51, 52). To elucidate the nature of the functional roles of the members of the PRS gene family, we have created a collection of strains containing all the possible combinations of disruptions in the five PRS genes and have performed a systematic phenotypic study of them. This analysis has revealed three phenotypes: (i) the least affected phenotype associated with strains bearing combinations of disruptions of PRS2, PRS4, and PRS5 that are capable of synthesizing PRPP at about 17% of the wild type (Table III) sufficient to support growth and nucleotide production as in the wild type; (ii) a severely affected phenotype that corresponds to strains containing disruptions in PRS1 and PRS3 together or in combination with any of the others. These strains produce between 1 and 6% of the wild type level of PRPP and are impaired in their growth and nucleotide production confirming previous results that PRS1 and PRS3 genes apparently make a more important contribution to yeast metabolism than the other members of the family (30); and (iii) a synthetic lethal phenotype that corresponds to a double disruption in PRS2 and PRS4 in combination with a deletion of either PRS1 or PRS3. Furthermore, a PRS5 null mutant is also synthetically lethal in combination with a disruption in either PRS1 or PRS3 (Table V). These findings suggest that the PRS gene products may be organized into two functionally discrete entities, one comprising Prs1p and Prs3p and the other comprising Prs2p, Prs4p, and Prs5p. In the absence of one entity or when some of its components are missing, the yeast cell could survive on the amount of PRPP produced by the other entity. However, when both functional entities are damaged, it represents such a burden for the cell that it is no longer able to survive. In this situation Prs2p and Prs4p, due to their high degree of identity, (89.3%) may be able to compensate functionally for each other, and only if the two are simultaneously absent would the functional entity be severely damaged. However, this interchange-
Fig. 1. Interaction of PRS polypeptides using the two-hybrid system. Gal4pDBD-Prs1-5p and Gal4pAD-Prs1-5p fusion protein interactions were measured in HF7c and PRS deletant strains derived therefrom. The β-galactosidase activity associated with strains containing all possible pairwise combinations of the two-hybrid plasmids was determined as described under “Experimental Procedures.” Values represent an activity relative to that of control strains containing empty two-hybrid vectors and are the average β-galactosidase activities of at least three independent determinations for each of three transformants. A, HF7c; B, YN96-25 (Δprs1); C, YN96-56 (Δprs3); D, YN96-22 (Δprs5); E, YN97-140 (Δprs2); F, YN96-79 (Δprs4).
Table V

Summary of multiple PRS delemt strains

| Deletants | Viable | Lethal |
|-----------|--------|--------|
| Double    | Δprs1 Δprs2 | Δprs1 Δprs5 |
|           | Δprs1 Δprs3 | Δprs1 Δprs5 |
|           | Δprs1 Δprs4 | Δprs1 Δprs5 |
| Triple    | Δprs2 Δprs3 | Δprs1 Δprs4 |
|           | Δprs2 Δprs4 | Δprs1 Δprs4 |
|           | Δprs2 Δprs5 | Δprs1 Δprs4 |
|           | Δprs3 Δprs4 | Δprs1 Δprs5 |
|           | Δprs3 Δprs5 | Δprs1 Δprs5 |

ability of Prs2p and Prs4p would only take place when the other entity is compromised because when either PRS1 or PRS3 is present, single and double disruptants of PRS2 and PRS4 all have a similar level of PRFPP. There are three viable triple deletant combinations, and each of these defines a minimal functional entity (unit) capable of sustaining the PRPP requirement of S. cerevisiae. These three minimal functional units are: (i) Prs1p and Prs3p and (ii) Prs5p in combination with either Prs2p or Prs4p (Table V).

The high degree of similarity of PRS polypeptides is suggestive of a certain degree of functional redundancy. However, our phenotypic analysis indicates that apart from the possibility of functional compensation between Prs2p and Prs4p, this is not the case. This interpretation is also supported by the apparent lack of a mechanism for the control of gene expression, at least at the mRNA level, which would compensate for the absence of a PRS gene transcript by increasing the amount of transcript from any of the remaining PRS genes. Nevertheless, we cannot exclude the possibility of post-transcriptional or post-translational modification that may be compensatory. In any case the compensation will never be to the full extent because we have shown that disruptions affecting the components of the Prs1p/Prs3p functional entity are associated with a more severe defect than when the deletions damage the other entity.

In rat four members of the PRS gene family have been identified (17, 25, 26). PRS1 and PRSII encode the catalytic subunits (24), whereas PAP39 and PAP41 polypeptide have been proposed to play a negative regulatory role in PRS because their removal from the rat liver complex causes an increase of PRS activity of the remaining subunits (25). Interestingly, PAP39 and PAP41 polypeptides both contain a short motif that are clearly detectable in the wild type and enhanced in the deletant strains (Fig. 1). However, one cannot say whether the two putative PRS subcomplexes interact as a larger complex and to establish the structure of a PRS complex, it will be necessary to purify the enzyme. In other organisms PRS has been shown to be a multimeric complex of varying composition. For example the rat liver enzyme exists in an aggregate form composed of 34-kDa (PRS1 and PRSII), 39-kDa (PAP39), and 41-kDa (PAP41) subunits in a ratio of 20:5:8:1 (55). In rubber tree latex the enzyme seems to be a tetramer (56), although the crystal structure of the Bacillus subtilis PRS has shown it to be hexameric (57) and in Salmonella typhimurium the smallest active form of PRS may be pentameric (58).

The work presented here represents a genetic dissection of the PRS gene family in S. cerevisiae. The collection of strains obtained in this study will be very useful in biochemical and kinetic studies of PRS and will allow us to define precisely the structure, function, and regulation of the PRS enzyme.

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