In *Saccharomyces cerevisiae*, impaired PRPP synthesis is accompanied by valproate and Li⁺ sensitivity

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**Abstract**

The biosynthetic intermediate PRPP (phosphoribosylpyrophosphate) has a central role in cellular biochemistry since it links carbon and nitrogen metabolism. Its importance may be reflected in the fact that, in the *Saccharomyces cerevisiae* (yeast) genome, there are five unlinked genes, *PRS1–PRS5*, each of which is theoretically capable of encoding the enzyme synthesizing PRPP. Interference with the complement of *PRS* genes in *S. cerevisiae* has far-reaching consequences for yeast physiology and has uncovered unexpected metabolic links including cell wall integrity and phospholipid metabolism.

**Key words**: CTP, metabolic networking, Prs (phosphoribosylpyrophosphate synthetase), *Saccharomyces cerevisiae*, valproate.

**Abbreviations used**: CFW, Calcofluor White; MAPK, mitogen-activated protein kinase; NHR, non-homologous region; Y2H, yeast two-hybrid; PRPP, phosphoribosylpyrophosphate; Prs, PRPP synthetase.

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Prs [PRPP (phosphoribosylpyrophosphate) synthetase] proteins in *Saccharomyces cerevisiae*

The metabolic intermediate PRPP provides the link between carbon and nitrogen metabolism and is required for the *de novo* and salvage synthesis of purine, pyrimidine and pyridine nucleotides. In bacteria and fungi, PRPP is also required for histidine and tryptophan synthesis [1]. The *S. cerevisiae* genome contains five unlinked paralogous genes, *PRS1–PRS5*, each capable of encoding Prs (EC 2.7.6.1; ATP:ribose-5-phosphate pyrophosphotransferase) [2,3]. Each of the gene products contains the characteristic motifs for Prs, a bivalent cation-binding site and a PRPP-binding site. Each of Prs2–Prs4 has a length of approx. 320 amino acids, whereas Prs1 and Prs5 are longer, and contain 427 and 495 amino acids respectively. The increase in length of Prs1 and Prs5 is due to the presence of NHRs (non-homologous regions), which are in-frame insertions and have no sequence similarity either to Prs polypeptides of other species or, indeed, to any other known protein. Prs1 contains an NHR, NHR1-1, extending from amino acid 199 to 309 that has been proven to be not an intron and was not removed by protein splicing [2]. Prs5, on the other hand, contains two such insertions, NHR5-1 and NHR5-2. NHR5-2 with a length of 62 amino acids is, like NHR1-1, located between the bivalent cation-binding and the PRPP-binding sites. NHR5-1, 110 amino acids long, is located 114 amino acids from the N-terminus of the protein.

The biological significance of the three NHR sequences is currently unknown; however, in a high-throughput analysis of the yeast phosphoproteome, Prs5 was identified as triply phosphorylated and the phosphorylation sites lie close to the C-terminus of NHR5-2 [4].

A systematic phenotypic analysis of our collection of strains representing all possible combinations of deletions of the five *PRS* genes has been carried out and we have defined three phenotypic classes: (i) a synthetically lethal phenotype when *PRS1* or *PRS3* was deleted in a *prs3Δ* strain. Simultaneous deletion of *PRS2* and *PRS4* in combination with loss of *PRS1* or *PRS3* also results in inviability; (ii) a second phenotype characterized by severe reduction in growth rate is encountered in strains containing deletions of *PRS1* and *PRS3* together or in combination with lack of *PRS2* or *PRS4*; and (iii) a third class with no apparent phenotype is found in strains carrying deletions of *PRS2, PRS4* or *PRS3* or combinations thereof [2,5,6]. Furthermore, three viable triple deletion combinations, namely *prs2Δ prs4Δ prs3Δ, prs1Δ prs3Δ prs4Δ* and *prs1Δ prs2Δ prs3Δ*, permitted the definition of three minimal subunits, *Prs1/Prs3, Prs2/Prs5* and *Prs4/Prs5*, suggesting that Prs activity in *S. cerevisiae* is carried out by heterodimeric complexes of Prs polypeptides.

An extensive Y2H (yeast two-hybrid) analysis suggested the existence of two interacting functional entities *Prs1/Prs3* and *Prs2/Prs4/Prs5* in the wild-type, which seem to be capable of compensating for each other since, in the absence of one entity or one of its components, the yeast cells still survive [3,5,6].

**Characteristics of prsΔ strains**

Table 1 summarizes some diagnostic characteristics of yeast strains carrying individual deletions of *PRS* genes, for instance the increased doubling time and drastically reduced enzyme activity associated with the deletion of either *PRS1* or *PRS3* [5,7]. The reduced nucleotide content of these two deletant strains is likely to be the consequence of their severely impaired Prs activity. Unexpectedly, both of these strains are sensitive to the purine analogue caffeine. As can be seen, deletion of *PRS2, PRS4* or *PRS5* has little influence on doubling time and the approx. 85% reduction in enzyme
Table 1 | Selected characteristics of PRS single deletants

Values are the percentages of nucleotides produced by each strain with respect to that of the wild-type (WT) and represent the means ± S.D. for at least three independent determinations. UXP = UMP + UDP + UTP; CXP = CDP + CTP (CMP was not detectable under the experimental conditions used); AXP = AMP + ADP + ATP; GXP = GMP + GDP + GTP.

<table>
<thead>
<tr>
<th>Relevant genotype</th>
<th>Doubling time (h)*</th>
<th>Prs relative activity (%)*</th>
<th>Caffeine conc. (mM)‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>1.6</td>
<td>100</td>
<td>99 ± 0.8 101 ± 1.5 99 ± 1.1 100 ± 0.6 6.0</td>
</tr>
<tr>
<td>prs1Δ</td>
<td>3.0</td>
<td>2.8</td>
<td>25 ± 7.0 45 ± 9.0 37 ± 6.0 12 ± 0.5 3.0</td>
</tr>
<tr>
<td>prs2Δ</td>
<td>2.0</td>
<td>14.5</td>
<td>72 ± 13 91 ± 9.5 80 ± 8.2 97 ± 1.5 6.0</td>
</tr>
<tr>
<td>prs3Δ</td>
<td>2.5</td>
<td>3.6</td>
<td>26 ± 4 55 ± 4.0 38 ± 3.0 31 ± 9.8 3.0</td>
</tr>
<tr>
<td>prs4Δ</td>
<td>2.0</td>
<td>16.9</td>
<td>75 ± 15 70 ± 15 86 ± 19 102 ± 2.0 5.5</td>
</tr>
<tr>
<td>prs5Δ</td>
<td>2.0</td>
<td>16.0</td>
<td>101 ± 15 89 ± 12 96 ± 10 80 ± 13 4.5</td>
</tr>
</tbody>
</table>

* Data taken from [5]. † Data taken from [6]. ‡ Data taken from [7].

activity caused no more than a 30% reduction in nucleotide content and, with the possible exception of the prs5Δ strain, there is no induction of caffeine sensitivity in comparison with the wild-type.

Prs and phospholipid metabolism

One striking fact shown in Table 1 is the extremely low CXP (CMP + CDP + CTP) content for a prs1Δ strain. Overexpression of either URA7 or URA8, the two S. cerevisiae genes encoding CTP synthetase [8], restored normal growth in a prs1Δ strain (results not shown). CTP in a reaction with PtdOH (phosphatidic acid) is required for the synthesis of the liponucleotide, CDP-DAG (CDP-diacylglycerol), which is the starting material for PtdCho, PtdIns and cardiolipin as well as for the synthesis of CDP-Etn (CDP-ethanolamine) and CDP-Cho (CDP-choline) [9–11]. In light of this possible connection of Prs with phospholipid synthesis, it was decided to examine whether the deletion of PRS genes influenced the cell’s ability to grow on valproate and LiCl. Both valproate, which is a branched fatty acid, and lithium, used for the treatment of bipolar effective disorder (a severe debilitating illness encountered in 1–2% of the population), influence molecular targets in phosphoinositide metabolism by reducing the ratio of PtdIns and PtdCho [12,13]. Different concentrations of valproate were tested and it was found that, at a concentration of 10 mM, both prs1Δ and prs3Δ showed a considerable reduction in growth, whereas neither the wild-type nor the remaining PRS deletant strains were affected. The wild-type, prs2Δ, prs4Δ and prs5Δ were capable of growing on 100 mM LiCl but the growth of prs3Δ was slightly retarded and that of prs1Δ severely retarded at the same concentration. In addition, deletion of PRS1 renders the cell sensitive to 100 mM NaCl and this phenotype is reversed by overexpression of CTP synthetase (results not shown). Interestingly, overexpression of CTP synthetase did not correct either LiCl or caffeine sensitivity. These observations suggest that deletion of PRS1 may have an impact on both phospholipid metabolism and on the cell wall integrity pathway, which is activated *inter alia* in the presence of caffeine.

Prs and the cell integrity pathway

The cell integrity pathway in S. cerevisiae as shown in Figure 1 relies on the protein kinase-mediated relay of
phosphorylation signals to a final MAPK (mitogen-activated protein kinase), Slt2 that in turn phosphorylates the transcription factors Rlm1 and Swi4/Swi6 [14–19]. This pathway is required for polarized cell growth, i.e. during budding and mating, and may be activated by membrane stretch, which could be a response to cell wall stress [14,16,17]. There is strong evidence that Rlm1 expression is diminished when any of the PRS genes is deleted. Furthermore, the activation of Rlm1 in response to elevated temperature is also impaired and this is particularly obvious when either PRS1 or PRS3 is deleted. Overexpression of Pkc1, a well-documented upstream regulator of the Slt2 pathway, can partially restore the temperature-dependent activation of Rlm1 transcription in \( \text{prs}1 \Delta \) and \( \text{prs}3 \Delta \) strains. This Pkc-mediated improvement of Rlm1 activation may explain why overexpression of Pkc1 counteracts \( \text{prs}1 \Delta \)- and \( \text{prs}3 \Delta \)-mediated \( \alpha \) factor sensitivity, an indication of disturbed polarized growth [20]. Interestingly, valproate has been postulated to inhibit phosphatidylserine synthase, thus limiting the production of PtdSer (phosphatidylserine), which is a known activator of Pkc [21], and this may well explain why \( \text{prs}1 \Delta \) and \( \text{prs}3 \Delta \) strains are sensitive to valproate (results not shown).

Evidence for the fact that cell wall synthesis is compromised in \( \text{prs}1 \Delta \) and \( \text{prs}3 \Delta \) strains was obtained by measuring the chitin content of these strains. Lack of \( \text{prs}1 \Delta \) or \( \text{prs}3 \Delta \) resulted in a 3-fold increase in chitin content and a highly reduced ability to increase chitin content following exposure to CFW (Calcofluor White). Perturbations in chitin synthesis are associated with CFW resistance and, indeed, \( \text{prs}1 \Delta \) and \( \text{prs}3 \Delta \) strains are resistant to CFW [20], a negatively charged fluorescent dye that binds to chitin [16].

There is therefore a significant body of evidence to suggest that cell integrity signalling is affected when either \( \text{prs}1 \Delta \) or \( \text{prs}3 \Delta \) is deleted. Measurement of the Slt2 phosphorylation level in \( \text{prs}1 \Delta \) and \( \text{prs}3 \Delta \) strains has shown that, in contrast with the wild-type, Slt2 is hyperphosphorylated under normal conditions as shown for zero time in the Western blot in Figure 2. In the \( \text{prs}1 \Delta \) strain, Slt2 phosphorylation did not increase following growth at 37°C for a period of 4 h. There did, however, appear to be a loss of signal after 5 h of growth at 37°C. The strength of the phosphorylation signal in the \( \text{prs}3 \Delta \) strain grown at 28°C is less than that documented in the \( \text{prs}1 \Delta \) strain but higher than the wild-type signal and it continued to increase when the strain was grown at 37°C for up to 4 h. Furthermore, there was apparently a decrease in signal strength at 5 h in both deletant strains (Figure 2).

This hyperphosphorylation of Slt2 was somewhat unexpected in light of the poor activation of Rlm1 in \( \text{prs}1 \Delta \) and \( \text{prs}3 \Delta \) deletants. The abnormally high degree of phosphorylation of Slt2 at ambient temperature may in fact be the cell’s response to stress caused by the low nucleotide content, for instance insufficient UTP and GTP, both of which are important for the biosynthesis of activated sugars involved in cell wall construction [22] and in phospholipid synthesis [9].

**Physical interaction of Prs polypeptides and Slt2**

There remains, however, the intriguing possibility that there is a direct interaction between Prs polypeptides and components of the cell signalling pathway, which when disrupted interferes with the phosphorylation of Rlm1 by Slt2 [17,23]. The possibility of a physical interaction between Prs polypeptides and the cell integrity pathway was examined by performing a Y2H analysis with each of the five Prs polypeptides and selected components of the pathway, including the target transcription factor Rlm1. No protein–protein interaction between the five members of the Prs family and the redundant MAPK kinases, Mkk1/Mkk2 polypeptides or Rlm1 was detected. However, a strong interaction of the MAPK Slt2 with Prs1, Prs2 and Prs3 was observed. The interactions between Slt2 and the three Prs polypeptides gave a positive result for all three reporter assays in the yeast strain used. There may be some slight interaction between Prs4 and Slt2, because in a reporter strain positive results, clearly above background, were obtained for two of the three reporter assays. Advantage was taken of our collection of truncated versions of Prs1 in Y2H vectors to investigate further the interaction between Prs1 and Slt2. Interestingly, deletion of NHR1-1 of Prs1, which has no influence on the Y2H interactions with Prs2 or Prs3, completely abolished the interaction of Prs1 with Slt2. When Prs1 was bisected at the BamHI restriction site located in the NHR1-1, both portions of Prs1 were still capable of interacting with Slt2, whereas neither interacted with Prs2 or Prs3 [20,24].

The expression of one of the known outputs of the cell integrity pathway, Fks2, a subunit of 1,3-β-glucan synthase, was examined in the absence of \( \text{prs}1 \Delta \) and \( \text{prs}3 \Delta \). There are two genes encoding 1,3-β-glucan synthase: \( \text{FKS}1 \), which is active under normal conditions, and \( \text{FKS}2 \), which comes into play at times of environmental stress. The transcription of \( \text{FKS}2 \) as measured by a β-galactosidase reporter gene was reduced by more than 70% in the \( \text{prs}1 \Delta \) strain as compared with the congenic wild-type strain under normal growth...
conditions but was still capable of increasing in response to elevated temperature. FKS2 transcription in the prs3Δ strain was only slightly lower than that in the wild-type and there was no increase in transcription following growth at 37°C [20,24].

This impact of PRS1 or PRS3 deletion on FKS2 transcription may or may not be due to the impairment of Rlm1 phosphorylation in these strains. It is known that Rlm1 is responsible for the activation of Fks2 at elevated temperature [25]. However, it may be that the low GTP content of the prs1Δ and prs3Δ strains as indicated in Table 1 plays a role in the activation of the FKS2-encoded 1,3-β-glucan synthase. The small GTPase Rho1, in addition to activating Pck1 that in turn activates the cell integrity pathway, is in fact the regulatory subunit of Fks2 and Fks1 [14,15,26].

Conclusions
Evidence described here shows that loss of either PRS1 or PRS3 has far-reaching consequences for the metabolism of S. cerevisiae, ranging from altered chitin synthesis, constitutive activation of the cell integrity pathway to an apparent disturbance in phospholipid metabolism. Interestingly, complementary observations have been made by M. Greenberg’s group in studies on PGS1, the committed step of cardiolipin synthesis. Deletion of PGS1 caused an abnormal distribution of chitin, severe growth defect at 37°C and a reduction in the content of the cell wall component β-1,3-glucan [27].

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References

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