Valproic acid- and lithium-sensitivity in prs mutants of Saccharomyces cerevisiae

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Introduction

Prs [PRPP (phosphoribosylpyrophosphate) synthetase] catalyses the transfer of pyrophosphate from ATP to ribose 5-phosphate, thereby activating the pentose sugar for incorporation into purine and pyrimidine nucleotides. The Saccharomyces cerevisiae genome contains five genes, PRS1–PRS5, whose products display characteristic PRPP and bivalent-cation-binding sites of Prs polypeptides. Deletion of one or more of the five PRS genes has far-reaching and unexpected consequences, e.g. impaired cell integrity, temperature-sensitivity and sensitivity to VPA (valproic acid) and LiCl. CTP pools in prs1Δ and prs3Δ are reduced to 12 and 31 % of the wild-type respectively, resulting in an imbalance in phospholipid metabolism which may have an impact on the intracellular inositol pool which is affected by the administration of either VPA or LiCl. Overexpression of CTP synthetase in prs1Δ prs3Δ strains partially reverses the VPA-sensitive phenotype. Yeast two-hybrid screening revealed that Prs3 and the yeast orthologue of GSK3 (glycogen synthase kinase 3), Rim11, a serine/threonine kinase involved in several signalling pathways, interact with each other. Furthermore, Prs5, an essential partner of Prs3, which also interacts with GSK3 contains three neighbouring phosphorylation sites, typical of GSK3 activation. These studies on yeast PRPP synthetases bring together and expand the current theories for the mood-stabilizing effects of VPA and LiCl in bipolar disorder.

Key words: bipolar disorder, lithium, phosphatidylinositol, phosphoribosyl pyrophosphate synthetase (Prs), Saccharomyces cerevisiae, valproic acid

Abbreviations used: BD, bipolar disorder; CDP-DAG, cytidine diphosphate diacylglycerol; DISC1, disrupted in schizophrenia 1; GSK3, glycogen synthase kinase 3; MIPS, 1-amino catalytic function. Interestingly, plant and fungal genomes contain paralogous sets of genes, with Spinacia oleracea containing four PRS genes and Arabidopsis thaliana [10] and Saccharomyces cerevisiae each containing five PRS genes [11]. X-ray crystallography has shown that, in Bacillus subtilis, whose genome contains only one PRS gene, the protein exists as a homohexamer [12]. An extensive genetic analysis of the PRS genes of Saccharomyces cerevisiae has shown that at least one of three minimal functional protein dimers, Prs1–Prs3, Prs2–Prs5 or Prs4–Prs5, must exist for the cell to survive [11]. Expression of these yeast-defined minimal functional units in an Escherichia coli PRPP-deficient strain confirmed the ability of these dimers to produce sufficient PRPP to permit growth of this mutant, even though only one of the combinations, Prs1–Prs3, exhibited enzyme activity [13]. Investigations in my group [11,14–16] revealed that sequence similarity of PRS2, PRS3 and PRS4 to each other was much greater than to either PRS1 or PRS5. The reason for this is that PRS1 and PRS5 contain NHRs (non-homologous regions) which increase their length by at least one third. NHRs are in-frame insertions which are not introns. NHR1-1 found in PRS1 is 105 amino acids in length and is located between the bivalent-cation-binding site and the PRPP-binding site. PRS3 contains two NHRs, NHR5-1 and NHR5-2, with the latter having a similar location to NHR1-1, whereas NHR5-1 is located towards the C-terminus of the protein and in fact contains three neighbouring phosphorylation sites [17]. Mutations in the human PRPS genes are associated with impaired neurological development. Specifically, Arts syndrome [18], an X-linked disorder characterized by ataxia, delayed motor development, hearing impairment and optic

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atrophy, has been associated with missense mutations in PRPS1. Interestingly, these amino acid changes are located in that region of PRPS1 which is highly conserved with the region of yeast Prs1 N-terminal to NHR1-1. The importance of this region is highlighted by the fact that an in vitro mutation in yeast Prs1 at amino acid 130 (H130A) compromising the bivalent-cation-binding site and which cannot complement a PRS1 deletion is located only three amino acids N-terminal from the mutation associated with Arts syndrome ([18] and L.M. Schweizer, unpublished work). Furthermore, Charcot–Marie–Tooth inherited neuropathy has also been linked to missense mutations in PRPS1 [19].

### The connection between Prs and BD (bipolar disorder) therapy

The manic–depressive illness BD occurs in approx. 1.5% of all populations regardless of ethnic and demographic boundaries [20]. This population risk increases with relatedness, reaching 10–15% for first-degree relatives [21]. Genome-wide association studies, copy number variations and molecular cytogenetics have revealed possible candidate genes, and one of these, DISC1 (disrupted in schizophrenia 1), has been subjected to extensive analysis since it was first reported in a Scottish family with a high loading of major mental illness. DISC1 is a scaffold protein which has been shown to interact with many proteins which can be classified into the following groups: cytoskeleton, cell cycle, signal transduction, intracellular transport and CNS (central nervous system) development [22].

During investigation of our collection of PRS mutants in *S. cerevisiae*, we discovered that they were compromised in cell integrity and we therefore screened a large number of substances including the well-known psychotherapeutics, lithium and VPA (valproic acid) for their effects on the growth of these mutants. Several of the mutants are sensitive to both drugs. We have reported previously that PRS mutants are sensitive to 10 mM VPA and 100 mM LiCl in complex media [23]. However, when the experiments are carried out in defined media, the sensitivity to VPA is observed at a concentration of 1 mM, which is closer to the therapeutic range. Simultaneous deletion of *PRS1* and *PRS3* resulted in higher sensitivity to both substances (results not shown).

With regard to VPA at a concentration of 2.5 mM, *prs1Δ* and *prs3Δ* strains ceased growth at a dilution of 10⁻⁵. The influence of LiCl is less pronounced with the wild-type capable of growth at a concentration of 150 mM, whereas the *prs1Δ*, *prs3Δ* and *prs5Δ* strains show growth inhibition at a concentration of 125 mM LiCl (Figure 1A). Combination of the various *PRS* deletions resulted in a marked increase in sensitivity to lithium with a *prs1Δ prs3Δ* strain showing almost total growth inhibition at 100 mM LiCl. Likewise sensitivity to VPA is increased in strains carrying double and triple deletions (results not shown). The different sensitivities of the mutants to lithium and VPA may be associated with their ability to interfere with inositol synthesis. It has been observed that rat brain displays diminished levels of inositol after long-term administration of either VPA or lithium [24]. Both yeast MIPS (1-D-myo-inositol-3-phosphate synthase) and human MIPS expressed in yeast have been shown to be inhibited indirectly by VPA [25]. Lithium causes inositol depletion by inhibiting IMPase (myo-inositol monophosphatase), the final step in the recycling of inositol phosphates in the phosphoinositide cycle and which is also required for the de novo synthesis of inositol (Figure 2). Although inositol depletion is consistent throughout administration of VPA, the acute response and the chronic effects of VPA on yeast cells differ greatly with regard to inositol-responsive genes and phospholipid synthesis. VPA is thought to inhibit the conversion of the lipid nucleotide intermediate CDP-DAG (cytidine diphosphate diacylglycerol) into PtdSer (phosphatidylserine) which interferes with the activation of PKC (protein kinase C), causing decreased phosphorylation and consequent activation of Op1, the negative regulator of MIPS encoded by INO1 [26,27]. The acute response of INO1 could not be observed in cells exposed to lithium under the same conditions.

The involvement of the lipid nucleotide intermediate CDP-DAG and PKC in the regulation of the inositol pool as a result to exposure of lithium or VPA may well explain the lithium- and VPA-sensitivity of *PRS* mutants. As part of the investigation of our *PRS* deletants, we measured their nucleotide content and found that, in *prs1Δ* and *prs3Δ* strains, all four nucleotide pools were reduced and *prs1Δ* had a particularly low CTP pool: 88 % reduction in comparison with the wild-type [11,16]. CTP is both a biosynthetic intermediate and a regulatory entity for phospholipid synthesis. CTP and PA (phosphatidic acid) are essential for the synthesis of CDP-DAG, the starting point for the synthesis of PtdIns, PtdCho (phosphatidylcholine) and cardiolipin [28]. CTP is also required for the Kennedy or salvage pathway which feeds into the CDP-DAG pathway for the production of PtdCho. A reduction in the CTP pool as measured in a *prs1Δ* strain has an impact on the cellular content of PA and flux through the Kennedy pathway as well as interfering with the interconversion of PA and DAG which is regulated by CTP and CDP-DAG [29–31]. Indeed, we have measured a 50 % increase in the amount of PA in the steady-state phospholipid composition of a *prs1Δ* strain (P. Gricic, unpublished work) as determined by labelling with [32P]orthophosphate [32,33]. Furthermore, double-labelling studies [30] which quantify the contributions of the methylation and Kennedy pathways to PtdCho synthesis revealed a 20 % increase in the contribution of the Kennedy pathway to phospholipid synthesis in the same strain (P. Gricic, unpublished work). Interestingly, a *prs4Δ* strain which has a CTP pool of wild-type dimension and the wild-type strain showed identical contributions of the CDP-DAG and Kennedy pathways to phospholipid synthesis.

Attempts to replenish the CTP pool(s) of the *prs1Δ* strain by individual overexpression of the two genes encoding *S. cerevisiae* CTP synthetase had no effect on the lithium- or VPA-sensitivity of *prs1Δ*. On the other hand, the *prs3Δ* strain whose CTP pool is less affected than that of a *prs1Δ* strain is rendered more sensitive to lithium and VPA.
Figure 1 | Sensitivity of prs mutants to lithium and VPA

(A) Growth of single PRS deletant strains (1Δ, 2Δ, 3Δ, 4Δ, 5Δ) in the presence of LiCl and VPA. Serial dilutions of yeast strains were spotted on to synthetic complete medium (SC) containing the indicated concentrations of LiCl or VPA. (B) Overexpression of CTP synthetase influences both lithium and VPA sensitivity of a prs1Δ prs3Δ (1Δ 3Δ) strain. Cultures of transformants containing the Ura7, Ura8 or the appropriate control plasmids were spotted at the dilutions indicated. The plates were photographed after 3 days of incubation at 30°C. wt, wild-type.

by overexpression of Ura8 (results not shown). This result is surprising since it is known that Ura7 is more highly expressed than Ura8. Interestingly, we also observe increased sensitivity to lithium and VPA when Ura8 is overexpressed in a prs1Δ prs3Δ strain. However, overexpression of Ura7 partially alleviated VPA sensitivity (Figure 1B).

The inositol-depletion theory [34,35] may well explain the therapeutic effects of lithium in certain cases of BD. However, the mood-stabilizing effects for VPA cannot be so readily explained by this theory. The reason is that VPA decreases intracellular levels of inositol indirectly [25] because it only inhibits de novo synthesis of inositol and not both de novo synthesis and recycling of inositol [36]. It has recently been shown that GSK3 (glycogen synthase kinase 3) is required for the maintenance of the inositol pool [37]. There are four yeast homologues of mammalian GSK3, and,
Figure 2 | Role of CTP and lithium and VPA targets in relation to the pathways of phospholipid synthesis

The URA7/8 genes encode CTP synthethase enzymes, with Ura7 being more highly expressed than Ura8. CTP is required for *de novo* and salvage synthesis of phospholipids. In addition, it is an allosteric regulator (CTP*) of the interconversion of PA and DAG, with the latter being an intermediate of the salvage pathway [46]. The acute target of VPA is PtdSer synthase which catalyses the committed step from CDP-DAG by replacing CMP from CDP-DAG with l-serine. This reduction in PtdSer results in attenuated expression of INO1 which encodes the rate-limiting step of *de novo* inositol synthesis (MIPS) and consequently results in a lowering of the inositol pool (not shown) [47]. Chronic administration of lithium and VPA depletes the intracellular levels of inositol by indirectly inhibiting the INO1-encoding enzyme MIPS and the inositol monophosphatase (Inm1) [48]. The PA concentration in the cell is also influenced by phospholipase D (Spo14). Abbreviations: Cho, choline; CL, cardiolipin; DGPP, diacylglycerol pyrophosphate; Etn, ethanolamine; Glucose-6-P, glucose-6-phosphate; Inositol-3-P, inositol-3-phosphate; P-Cho, phosphocholine; P-Etn, phosphoethanolamine; PtdEtn, phosphatidylethanolamine; PtdGro, phosphatidylglycerol; PtdGroP, phosphatidylglycerolphosphate; PtdME, phosphatidylmonomethylethanolamine; PtdDME, phosphatidyltrimethylethanolamine; TAG, triacylglycerol.

in a large-scale interaction study of *S. cerevisiae* proteins by high-throughput MS protein complex identification, Rim11, one of these homologues of mammalian GSK3, was found to bind to three of the five Prs proteins encoded by the yeast genome [38]. The same multimeric complex also contained Cki1, a choline kinase involved in the Kennedy pathway as well as Ira2, a GTPase-activator for Ras proteins [23,39]. Rim11 plays a role in the expression of meiotic genes by phosphorylating Ime1 and Ume6 [40] and is also involved in promoting the binding of the stress-response protein Msn2 to DNA [41]. We have confirmed the interaction between Rim11 and Prs2, Prs3 and Prs5 by specific yeast two-hybrid experiments in which it was shown that the interactions were measurable when Rim11 was fused to either the binding or activation domain of the test plasmids and, in both instances, all three reporter systems, histidine prototrophy, adenine prototrophy and β-galactosidase activity, were positive (Figure 3) [42]. In the meantime, it was shown that the quadruple mutant of GSK3 has a growth defect at elevated temperature which is reversed by the addition of inositol [43]. A further connection of Prs polypeptides to inositol metabolism, specifically phosphoinositides, the collective term for PtdIns and its phosphorylated derivatives, is that loss of PRS1, PRS3 or PRS5 renders yeast sensitive to the phosphoinositide 3-kinase inhibitor wortmannin ([44,45] and L.M. Schweizer, unpublished work). This provides a further link to the DAG–InsP3–Ca2⁺-signalling second messenger system that is essential to neural signalling [35]. We therefore believe that the imbalance in phospholipid metabolism and the interaction between the GSK3 orthologue, Rim11, and components of the PRPP-synthesizing machinery of yeast may reduce the inositol pool, thereby resulting in the unexpected sensitivity of prs mutants to lithium and VPA. Clearly, the results tell us nothing about the causes
The coding sequence of the RIM11 gene was cloned in-frame with the binding domain of GAL4 and each of thePRS gene-coding sequences were cloned in-frame with the activating domain of GAL4 on the respective plasmids. Growth on media lacking adenine (-ADE) or the amino acid histidine (-HIS) was tested together with the ability of the strain to produce β-galactosidase in the β-galactosidase filter test (A). β-Galactosidase was also quantified using the ONPG (o-nitrophenyl β-D-galactopyranoside) test (B). Results are means±S.D. for three independent transformants. 

Figure 3 | Yeast two-hybrid assay showing the interaction between Rim11 and members of the Prs protein family

of BD, but serve to illustrate the complexity of networks impinging on inositol and phospholipid metabolism.

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References


16 Hernando, Y., Pari, A. and Schweizer, M. (1998) PR55, the fifth member of the phosphoribosylpyrophosphate synthetase gene family in Saccharomyces cerevisiae, is essential for cell viability in the absence of either PRS1 or PRS2. J. Bacteriol. 180, 6404–6407


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