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

Anna Kleineidam*, Stefano Vavassori†, Ke Wang†, Lilian M. Schweizer†, Peter Griac‡ and Michael Schweizer†[1]

*Henry Wellcome Laboratories for Integrative Neuroscience and Endocrinology, University of Bristol, Bristol BS1 3NY, U.K., †School of Life Sciences, Heriot-Watt University, Edinburgh EH14 4AS, U.K., and ‡Institute of Animal Biochemistry and Genetics, Slovak Academy of Sciences, Bratislava, Slovak Republic

Abstract

Prs [PRPP (phosphoribosyl pyrophosphate) 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 *prs1*Δ and *prs3*Δ 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 *prs1*Δ *prs3*Δ 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.

Introduction

Prs [PRPP (phosphoribosyl pyrophosphate) synthetase] (EC 2.7.6.1) occupies a central position in metabolism linking carbon and nitrogen biosynthesis since it is responsible for the transfer of the terminal pyrophosphate of ATP to ribose 5-phosphate. The product synthesized, PRPP, is essential for the synthesis of purine, pyrimidine and pyridine nucleotides, and, in yeast, it is also required for the synthesis of the amino acids histidine and tryptophan [1]. In general, bacteria contain one gene capable of encoding Prs [2], whereas the human and fungal genomes contain several PRPP-encoding genes. In humans there are three genes: *PRPS1* and *PRPS2*, both located on the X-chromosome, and *PRPS1L1* located on chromosome 7, which is believed to be testis-specific [3–5]. Identification of PAPs (Prs-associated proteins) in rodents and humans increases the complexitity of PRPP synthesis and expression in these organisms [6–9]. The PAPs show a high degree of similarity to the amino acid sequences of the corresponding PRPS proteins in the same species, but they display mutations in residues conserved in the PRPS proteins of other organisms, thus suggesting that they have no catalytic function. Interestingly, plant and fungal genomes contain paralogous sets of genes, with *Spinacia olereacea* 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. *PRS5* 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

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-D-*myo*-inositol-3-phosphate synthase; NHR, non-homologous region; PA, phosphatidic acid; PAP, phosphoribosyl pyrophosphate synthetase-associated protein; PKC, protein kinase C; PRPP, phosphoribosyl pyrophosphate; Prs, PRPP synthetase; PtdCho, phosphatidylcholine; PtdSer, phosphatidylserine; VPA, valproic acid.

[1]To whom correspondence should be addressed (email M.Schweizer@hw.ac.uk).

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].

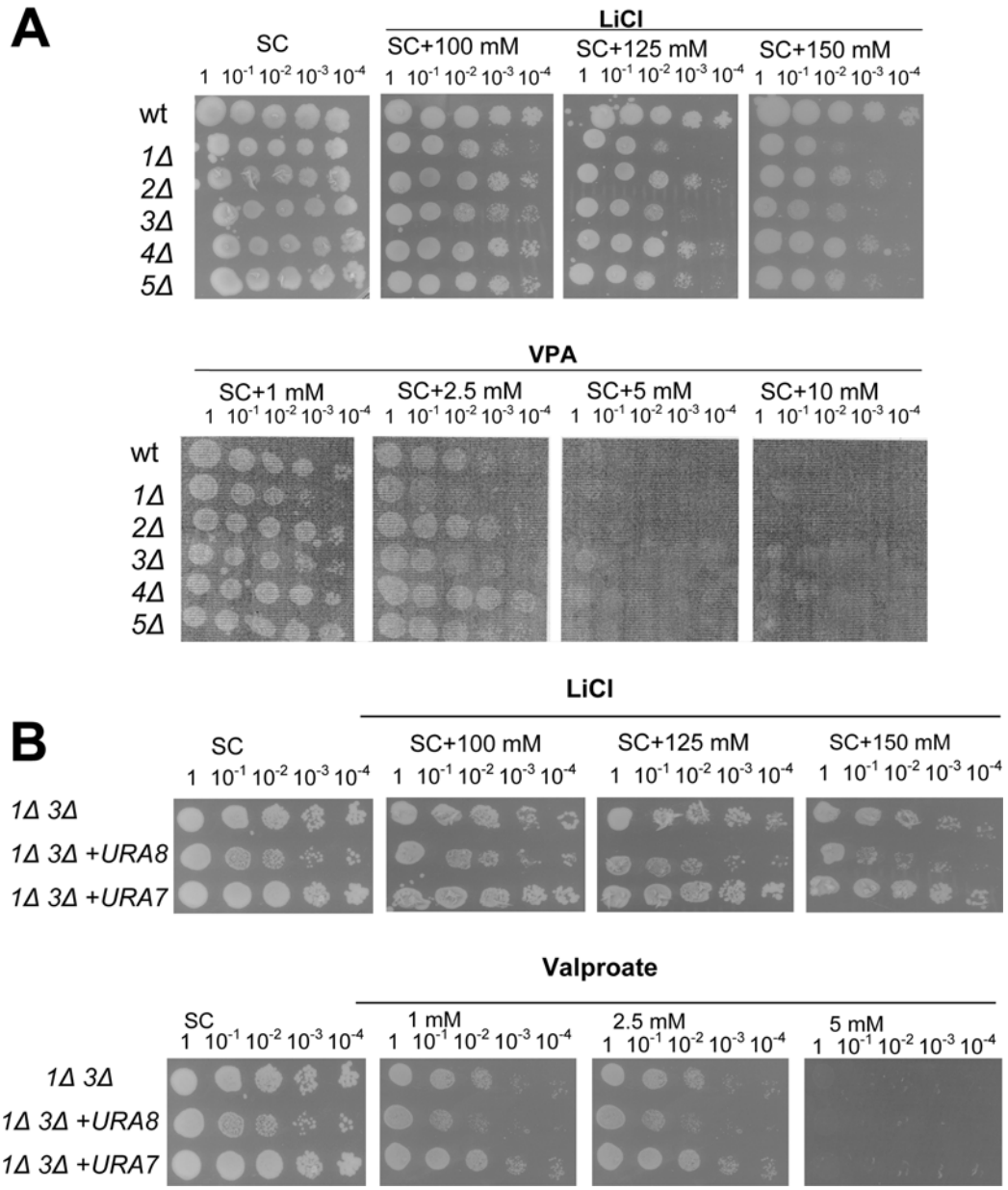
During investigation of our collection of *PRS* mutants in *S. cerevisiae*, we discovered that they were compromised in cell integrity and we therefore screened a large number of substances including the well-known psychotherapeutics, lithium and VPA (valproic acid) for their effects on the growth of 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^{-3}. 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 Opi1, 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. Griac, unpublished work) as determined by labelling with [^{32}P]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. Griac, 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.

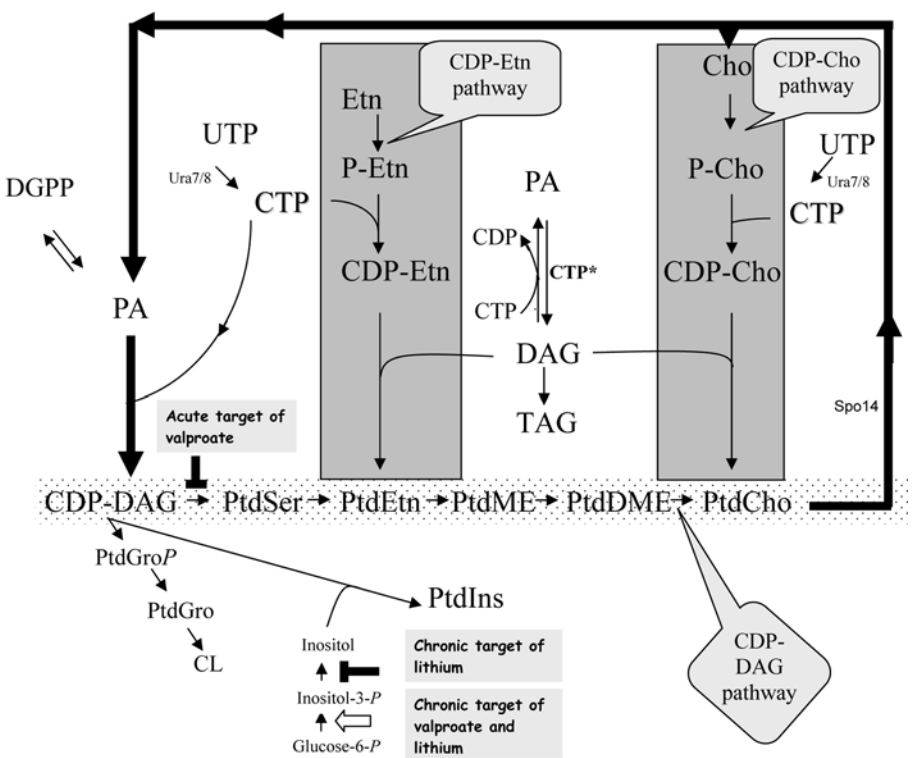


by overexpression of Ura8 (results not shown). This result is surprising since it is known that Ura7 is more highly expressed than Ura8. Interestingly, we also observe increased sensitivity to lithium and VPA when Ura8 is overexpressed in a *prs1Δ prs3Δ* strain. However, overexpression of Ura7 partially 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 deceases 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; PtdGro*P*, phosphatidylglycerolphosphate; PtdME, phosphatidylmonomethylethanolamine; PtdDMe, phosphatidlydimethylethanolamine; TAG, triacylglycerol.

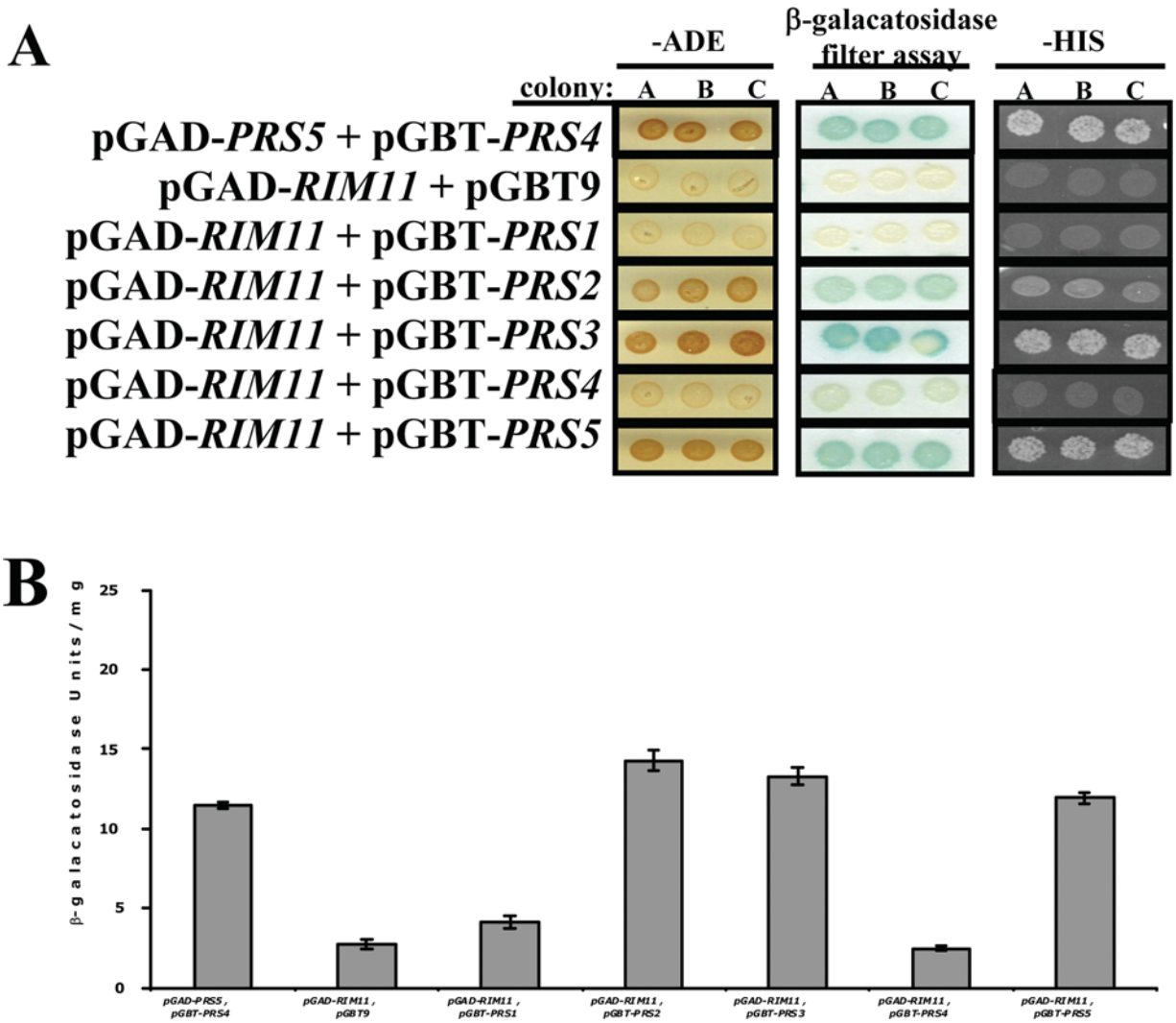


in a large-scale interaction study of *S. cerevisiae* proteins by high-throughput MS protein complex identification, Rim11, one of these homologues of mammalian GSK3, was found to bind to three of the five Prs proteins encoded by the yeast genome [38]. The same multimeric complex also contained Cki1, 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–InsP_3–Ca^{2+}-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

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

The coding sequence of the *RIM11* gene was cloned in-frame with the binding domain of *GAL4* and each of the *PRS* 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.



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

Funding

This work was supported by the Eda Lady Jardine Trust (S.V.) and the Henry Lester Trust Ltd (K.W.).

References

1 Khorana, H.G., Fernandes, J.F. and Kornberg, A. (1958) Pyrophosphorylation of ribose 5-phosphate in the enzymatic synthesis of 5-phosphorylribose 1-pyrophosphate. J. Biol. Chem. **230**, 941–948

2 Hove-Jensen, B., Harlow, K.W., King, C.J. and Switzer, R.L. (1986) Phosphoribosylpyrophosphate synthetase of *Escherichia coli*: properties of the purified enzyme and primary structure of the *PRS* gene. J. Biol. Chem. **261**, 6765–6771

3 Becker, M.A. (2001) Phosphoribosylpyrophosphate synthetase and the regulation of phosphoribosylpyrophosphate production in human cells. Prog. Nucleic Acid Res. Mol. Biol. **69**, 115–148

4 Taira, M., Iizasa, T., Shimada, H., Kudoh, J., Shimizu, N. and Tatibana, M. (1990) A human testis-specific mRNA for phosphoribosylpyrophosphate synthetase that initiates from a non-AUG codon. J. Biol. Chem. **265**, 16491–16497

5 Taira, M., Kudoh, J., Minoshima, S., Iizasa, T., Shimada, H., Shimizu, Y., Tatibana, M. and Shimizu, N. (1989) Localization of human phosphoribosylpyrophosphate synthetase subunit I and II genes (*PRPS1* and *PRPS2*) to different regions of the X chromosome and assignment of two PRPS1-related genes to autosomes. Somat. Cell Mol. Genet. **15**, 29–37

6 Katashima, R., Iwahana, H., Fujimura, M., Yamaoka, T., Ishizuka, T., Tatibana, M. and Itakura, M. (1998) Molecular cloning of a human cDNA for the 41-kDa phosphoribosylpyrophosphate synthetase-associated protein. Biochim. Biophys. Acta **1396**, 245–250
7 Kita, K., Ishizuka, T., Ishijima, S., Sonoda, T. and Tatibana, M. (1994) A novel 39-kDa phosphoribosylpyrophosphate synthetase-associated protein of rat liver: cloning, high sequence similarity to the catalytic subunits, and a negative regulatory role. J. Biol. Chem. **269**, 8334–8340
8 Kita, K., Otsuki, T., Ishizuka, T. and Tatibana, M. (1989) Rat liver phosphoribosyl pyrophosphate synthetase: existence of the purified enzyme as heterogeneous aggregates and identification of the catalytic subunit. J. Biochem. (Tokyo) **105**, 736–741
9 Tatibana, M., Kita, K., Taira, M., Ishijima, S., Sonoda, T., Ishizuka, T., Iizasa, T. and Ahmad, I. (1995) Mammalian phosphoribosyl-pyrophosphate synthetase. Adv. Enzyme Regul. **35**, 229–249
10 Krath, B.N. and Hove-Jensen, B. (1999) Organellar and cytosolic localization of four phosphoribosyl diphosphate synthase isozymes in spinach. Plant Physiol. **119**, 497–506
11 Hernando, Y., Carter, A.T., Parr, A., Hove-Jensen, B. and Schweizer, M. (1999) Genetic analysis and enzyme activity suggest the existence of more than one minimal functional unit capable of synthesizing phosphoribosyl pyrophosphate in *Saccharomyces cerevisiae*. J. Biol. Chem. **274**, 12480–12487
12 Eriksen, T.A., Kadziola, A., Bentsen, A.K., Harlow, K.W. and Larsen, S. (2000) Structural basis for the function of *Bacillus subtilis* phosphoribosyl-pyrophosphate synthetase. Nat. Struct. Biol. **7**, 303–308
13 Hove-Jensen, B. (2004) Heterooligomeric phosphoribosyl diphosphate synthase of *Saccharomyces cerevisiae*: combinatorial expression of the five *PRS* genes in *Escherichia coli*. J. Biol. Chem. **279**, 40345–40350
14 Carter, A.T., Beiche, F., Hove-Jensen, B., Narbad, A., Barker, P.J., Schweizer, L.M. and Schweizer, M. (1997) *PRS1* is a key member of the gene family encoding phosphoribosylpyrophosphate synthetase in *Saccharomyces cerevisiae*. Mol. Gen. Genet. **254**, 148–156
15 Carter, A.T., Narbad, A., Pearson, B.M., Beck, K.F., Logghe, M., Contreras, R. and Schweizer, M. (1994) Phosphoribosylpyrophosphate synthetase (PRS): a new gene family in *Saccharomyces cerevisiae*. Yeast **10**, 1031–1044
16 Hernando, Y., Parr, A. and Schweizer, M. (1998) *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*. J. Bacteriol. **180**, 6404–6407
17 Ficarro, S.B., McCleland, M.L., Stukenberg, P.T., Burke, D.J., Ross, M.M., Shabanowitz, J., Hunt, D.F. and White, F.M. (2002) Phosphoproteome analysis by mass spectrometry and its application to *Saccharomyces cerevisiae*. Nat. Biotechnol. **20**, 301–305
18 de Brouwer, A.P., Williams, K.L., Duley, J.A., van Kuilenburg, A.B., Nabuurs, S.B., Egmont-Petersen, M., Lugtenberg, D., Zoetekouw, L., Banning, M.J. et al. (2007) Arts syndrome is caused by loss-of-function mutations in *PRPS1*. Am. J. Hum. Genet. **81**, 507–518
19 Kim, H.J., Sohn, K.M., Shy, M.E., Krajewski, K.M., Hwang, M., Park, J.H., Jang, S.Y., Won, H.H., Choi, B.O., Hong, S.H. et al. (2007) Mutations in *PRPS1*, which encodes the phosphoribosyl pyrophosphate synthetase enzyme critical for nucleotide biosynthesis, cause hereditary peripheral neuropathy with hearing loss and optic neuropathy (cmtx5). Am. J. Hum. Genet. **81**, 552–558
20 Craddock, N. and Sklar, P. (2009) Genetics of bipolar disorder: successful start to a long journey. Trends Genet. **25**, 99–105
21 Porteous, D. (2008) Genetic causality in schizophrenia and bipolar disorder: out with the old and in with the new. Curr. Opin. Genet. Dev. **18**, 229–234
22 Chubb, J.E., Bradshaw, N.J., Soares, D.C., Porteous, D.J. and Millar, J.K. (2008) The DISC locus in psychiatric illness. Mol. Psychiatry **13**, 36–64
23 Vavassori, S., Wang, K., Schweizer, L.M. and Schweizer, M. (2005) Ramifications of impaired PRPP synthesis in *Saccharomyces cerevisiae*. Biochem. Soc. Trans. **33**, 1418–1420
24 O'Donnell, T., Rotzinger, S., Nakashima, T.T., Hanstock, C.C., Ulrich, M. and Silverstone, P.H. (2000) Chronic lithium and sodium valproate both decrease the concentration of *myo*-inositol and increase the concentration of inositol monophosphates in rat brain. Brain Res. **880**, 84–91
25 Ju, S., Shaltiel, G., Shamir, A., Agam, G. and Greenberg, M.L. (2004) Human 1-D-*myo*-inositol-3-phosphate synthase is functional in yeast. J. Biol. Chem. **279**, 21759–21765
26 Ju, S. and Greenberg, M.L. (2003) Valproate disrupts regulation of inositol responsive genes and alters regulation of phospholipid biosynthesis. Mol. Microbiol. **49**, 1595–1603
27 Carman, G.M. and Henry, S.A. (2007) Phosphatidic acid plays a central role in the transcriptional regulation of glycerophospholipid synthesis in *Saccharomyces cerevisiae*. J. Biol. Chem. **282**, 37293–37297
28 Schweizer, M. (2004) Lipids and membranes. in The Metabolism and Molecular Physiology of *Saccharomyces cerevisiae* (Dickinson, J.R. and Schweizer, M., eds), pp. 140–223, CRC Press, Boca Raton
29 Carman, G.M. and Han, G.S. (2009) Regulation of phospholipid synthesis in yeast. J. Lipid Res. **50** (Suppl.), S69–S73
30 Ostrander, D.B., O'Brien, D.J., Gorman, J.A. and Carman, G.M. (1998) Effect of CTP synthetase regulation by CTP on phospholipid synthesis in *Saccharomyces cerevisiae*. J. Biol. Chem. **273**, 18992–19001
31 Chang, Y.-F. and Carman, G.M. (2008) CTP synthetase and its role in phospholipid synthesis in the yeast *Saccharomyces cerevisiae*. Prog. Lipid Res. **47**, 333–339
32 Atkinson, K., Fogel, S. and Henry, S.A. (1980) Yeast mutant defective in phosphatidylserine synthesis. J. Biol. Chem. **255**, 6653–6661
33 Steiner, S. and Lester, R.L. (1972) Studies on the diversity of inositol-containing yeast phospholipids: incorporation of 2-deoxyglucose into lipid. J. Bacteriol. **109**, 81–88
34 Berridge, M.J. and Irvine, R.F. (1989) Inositol phosphates and cell signalling. Nature **341**, 197–205
35 Hallcher, L.M. and Sherman, W.R. (1980) The effects of lithium ion and other agents on the activity of *myo*-inositol-1-phosphatase from bovine brain. J. Biol. Chem. **255**, 10896–10901
36 Azab, A.N., Agam, G., Kaplanski, J., Delbar, V. and Greenberg, M.L. (2008) Inositol depletion: a good or bad outcome of valproate treatment? Future Neurol. **3**, 275–286
37 De Sarno, P., Li, X. and Jope, R.S. (2002) Regulation of Akt and glycogen synthase kinase-3β phosphorylation by sodium valproate and lithium. Neuropharmacology **43**, 1158–1164
38 Ho, Y., Gruhler, A., Heilbut, A., Bader, G.D., Moore, L., Adams, S.L., Millar, A., Taylor, P., Bennett, K., Boutilier, K. et al. (2002) Systematic identification of protein complexes in *Saccharomyces cerevisiae* by mass spectrometry. Nature **415**, 180–183
39 Parrini, M.C., Jacquet, E., Bernardi, A., Jacquet, M. and Parmeggiani, A. (1995) Properties and regulation of the catalytic domain of Ira2p, a *Saccharomyces cerevisiae* GTPase-activating protein of Ras2p. Biochemistry **34**, 13776–13783
40 Xiao, Y. and Mitchell, A.P. (2000) Shared roles of yeast glycogen synthase kinase 3 family members in nitrogen-responsive phosphorylation of meiotic regulator Ume6p. Mol. Cell. Biol. **20**, 5447–5453
41 Hirata, Y., Andoh, T., Asahara, T. and Kikuchi, A. (2003) Yeast glycogen synthase kinase-3 activates Msn2p-dependent transcription of stress responsive genes. Mol. Biol. Cell. **14**, 302–312
42 Vavassori, S. (2005) An investigation of the influence of impaired PRPP production on the physiology of *Saccharomyces cerevisiae*, Ph.D. Thesis, Heriot-Watt University, Edinburgh, U.K.
43 Azab, A.N., He, Q., Ju, S., Li, G. and Greenberg, M.L. (2007) Glycogen synthase kinase-3 is required for optimal *de novo* synthesis of inositol. Mol. Microbiol. **63**, 1248–1258
44 Cameroni, E., De Virgilio, C. and Deloche, O. (2006) Phosphatidylinositol 4-phosphate is required for translation initiation in *Saccharomyces cerevisiae*. J. Biol. Chem. **281**, 38139–38149
45 Fruman, D.A., Meyers, R.E. and Cantley, L.C. (1998) Phosphoinositide kinases. Annu. Rev. Biochem. **67**, 481–507
46 Wu, W.I. and Carman, G.M. (1994) Regulation of phosphatidate phosphatase activity from the yeast *Saccharomyces cerevisiae* by nucleotides. J. Biol. Chem. **269**, 29495–29501
47 Vance, J.E. and Steenbergen, R. (2005) Metabolism and functions of phosphatidylserine. Prog. Lipid Res. **44**, 207–234
48 Murray, M. and Greenberg, M.L. (2000) Expression of yeast *INM1* encoding inositol monophosphatase is regulated by inositol, carbon source and growth stage and is decreased by lithium and valproate. Mol. Microbiol. **36**, 651–661

Received 29 April 2009
doi:10.1042/BST0371115