The whys and wherefores of phosphate removal

Meeting on The Biology of Phosphatases

Daimark Bennett¹, R. James Matthews² & Jean G. Sathish³

¹Oxford University, Oxford, UK, ²Cardiff University, Cardiff, UK, and ³Liverpool University, Liverpool, UK

The EMBO conference/FEBS advanced course, 'Europhosphatases Conference 2005', on The Biology of Phosphatases, was held in Churchill College, Cambridge University, UK, between 10 and 14 July 2005. The conference was organized by D. Alexander, J. Arino and E. Da Cruz e Silva.

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interacting partners, mechanisms of regulation, physiological substrates, biological roles and their links to disease remain substantial. The timing of the conference was particularly opportune because 2005 marked the 50th anniversary of the seminal discovery, by Edmond Fischer and Edwin Krebs, of reversible protein phosphorylation—a control mechanism now appreciated to pervade all aspects of cell physiology. In this overview of the meeting, we describe the current status of the protein phosphatase field and highlight some of the emerging themes.

Unlike protein kinases, which derive from a common ancestor, protein phosphatases have evolved from distinct progenitors and therefore have no common co-factor requirements and no shared structural features. Protein phosphatases were originally characterized as hydrolysing phosphoryl groups from proteins, but it is now understood that a limited number of protein phosphatases have the capacity to remove phosphate from either phospholipids or mRNA. Protein phosphatases can be broadly classified, on the basis of their structure, into the PPP and PPM families (Barford, 1996), which dephosphorylate phosphoserine (pSer) and phosphothreonine (pThr), and into the cysteine-based PTPs, most of which dephosphorylate either phosphotyrosine (pTyr) exclusively or pTyr and pThr. The catalytic mechanism for the protein phosphatases involves a surface nucleophile, which is provided by a metal ion-activated water molecule in the case of the PPP and PPM families and by a conserved cysteine for the cysteine-based PTPs. In addition, a new class of haloacid dehalogenase (HAD)-like protein phosphatases has been described that is dependent on a conserved nucleophilic aspartate for the dephosphorylation of pSer or pTyr on protein substrates. The protein phosphatases are further distinguished by their subunit structure and sub-cellular localization. The PPPs are universally located in the cell and comprise a small number of simple catalytic units, each able to bind to an extensive number of regulatory proteins. By contrast, the individual PTPs are located either in the plasma membrane or inside the cell and comprise a diverse family of multi-domain, single-chain proteins.

The phosphatasome

For each of the main classes of protein phosphatase, the requirements for defining the phosphatasome have been distinct. N. Tonks (Cold Spring Harbor, NY, USA) described how a principal achievement for the PTP field has been the annotation of each of the 106

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Introduction

"Now is not the end. It is not even the beginning of the end. But it is, perhaps, the end of the beginning". This quotation from Sir Winston Churchill had resonance at the recent 'Europhosphatases Conference 2005' held at Churchill College, Cambridge. Although the identity of each of the protein phosphatase genes in the human genome is now known, the challenges of determining the enzymes'

¹Department of Zoology, Oxford University, South Parks Road, Oxford OX1 3PS, UK
Tel: +44 1865 281 520; Fax: +44 1865 310 447;
E-mail: daimark.bennett@zoo.ox.ac.uk

²Infection and Immunity, Department of Medical Biochemistry and Immunology, Henry Wellcome Building for Biomedical Research in Wales, School of Medicine, Cardiff University, Heath Park, Cardiff CF14 4XX, UK
Tel: +44 29 2074 2484/2074 5006; Fax: +44 29 2074 4001;
E-mail: matthewsrj@cardiff.ac.uk

³Pharmacology & Therapeutics, Sherrington Buildings, Liverpool University, Ashton Street, Liverpool L69 3GE, UK
Tel: +44 151 794 6615; Fax: +44 151 794 5540;
E-mail: jean.sathish@liverpool.ac.uk

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functional PTP and PTP-like genes (Alonso *et al.*, 2004; Andersen *et al.*, 2004). The PTP gene family includes 38 classical tyrosine-specific phosphatases, 43 dual-specificity phosphatases (DSPs) that dephosphorylate pTyr and pSer/pThr, 16 myotubularin-related phosphatases, five inositol phosphatases, three CDC25 phosphatases and one low-molecular-weight (LMW) phosphatase. All of the cysteine-based PTPs are dependent on a conserved cysteine for their catalytic activity with catalysis requiring the formation of a phospho-cysteine intermediate. The tyrosine-specific PTPs can be further categorized as transmembrane or intracellular with 21 and 17 human genes of each type, respectively. The DSP sub-family encompasses a much less tightly conserved set of PTPs, including: 11 protein phosphatases that are specific to the mitogen-activated protein kinases (MAPKs) as they have MAPK targeting motifs; three 'phosphatase of regenerating liver' protein phosphatases implicated in cell proliferation, invasion and metastasis; and three slingshot (named after altered bristle morphology in a *Drosophila* mutant) protein phosphatases, involved in dephosphorylating the LIM kinase and cofilin components of the actin cytoskeleton. The catalytic activity of the slingshot protein phosphatases is tightly governed by their phosphorylation state, which is in turn regulated by the interaction of PAK4 kinase and PP2A phosphatase, exemplifying the close interdependence of kinases and phosphatases. A. Gohla (Düsseldorf, Germany) described the purification of a second type of cofilin phosphatase called chronophin (CIN), which is necessary for the regulation of cofilin-dependent actin dynamics during cell division. CIN is a new HAD-type phosphotransferase, an unusual class of enzyme that forms an intermediate containing a mixed anhydride with an essential aspartate. Cofilin is the first phospho-serine-containing protein substrate of a HAD phosphatase to be identified in mammals (Gohla *et al.*, 2005).

A.-C. Gingras (Seattle, WA, USA) described how proteomic approaches are being successfully used to define the breadth of regulatory proteins able to interact stably with the limited number of PPP catalytic subunits (Fig 1). Networks of at least 63 proteins in mammals and 43 proteins in yeast were found surrounding PP2A, PP4 and PP6. These PP2A-like catalytic subunits are found in distinct complexes, reinforcing the idea that specificity is conferred by the precise composition of the associated complexes (Gingras *et al.*, 2005). The results from yeast two-hybrid screens have further expanded the repertoire of PP1-binding proteins, of which there are now estimated to be over 100 in mammalian cells. E. da Cruz e Silva (Aveiro, Portugal) described the identification of 46 human testis-specific proteins that interact with PP1 γ 1 and/or PP1 γ 2 and that might be involved in the control of sperm motility. It is not yet known whether any of these interactors show isoform-specific binding, but M. Bollen (Leuven, Belgium) reported that arginine 20 of PP1 γ is crucial for nucleolar targeting, indicating that at least one nucleolar PP1 γ -specific interacting protein must exist. D. Bennett (Oxford, UK) presented the identification of a PP1 β -specific binding protein from *Drosophila*. A total of 40 proteins were identified as binding *Drosophila* PP1 in a yeast two-hybrid screen, but only one—MYPT-75D—discriminated between the different PP1 isoforms. Experiments using MYPT-75D peptides incapable of binding PP1 suggest that MYPT-75D might mediate the essential role of PP1 β in non-muscle myosin regulation in flies by stimulating the dephosphorylation of non-muscle myosin II regulatory light chain (Vereshchagina *et al.*, 2004).

Fig 1 | Stable interactions within the PP4/Pph3 module, as detected by tandem-affinity purification and yeast two-hybrid assay. Mammalian PP4 components (shown in capitals) and yeast Pph3 components (shown in italics) are represented. Interactions detected by co-precipitation (solid lines) and yeast two-hybrid assay (dashed lines) are depicted, with arrows indicating the direction of the assay. The blue node (PP4R1) and arrow indicate a mammalian-specific protein and interaction. Green nodes and arrows indicate interactions detected only in yeast (note that orthologues of Rad53, Spt4 and Spt5 also exist in mammalian cells). The yellow diamond indicates an evolutionarily conserved protein complex, which is involved in cisplatin sensitivity. Other interacting partners for the human PP4 catalytic subunit have been detected using other approaches (reviewed in Cohen *et al.*, 2005). CCT, chaperonin-containing t-complex. Figure courtesy of A.-C. Gingras.

Regulatory mechanisms and substrate identification

The PTPs are distinguished by their structure, in which individual PTP domains are fused to extracellular domains or one or more distinct non-catalytic modular domains that are implicated in protein or phospholipid binding. T. Mustelin (La Jolla, CA, USA) presented results for the intracellular PTP, PTP-MEG2, which show how the non-catalytic domains of PTPs can influence their function. PTP-MEG2 localizes to the secretory vesicle membrane where it regulates the formation of immature secretory vesicles. PTP-MEG2 has a Sec14 homology domain at its amino-terminus that binds to phosphatidylinositol-3,4,5-trisphosphate *in vitro* and the same lipid is thought to enhance the catalytic activity of PTP-MEG2 *in vivo*. The characterization of physiological substrates for the PTPs has been particularly challenging, but for PTP-MEG2 this problem seems to have been resolved with the identification of N-ethylmaleimide-sensitive factor (NSF) as a target for the enzyme (Huynh *et al.*, 2004). In an elegant series of experiments, Mustelin described how the site for PTP-MEG2 dephosphorylation on NSF has been mapped to Tyr83, which when mutated to phenylalanine, mimics the spontaneous secretory vesicle fusion detected with PTP-MEG2 over-expression. Furthermore, in terms of a molecular mechanism, the phosphorylation of Tyr83 correlates with the increased ATPase activity of NSF and its functional inactivation.

Tonks also discussed how the controlled generation of reactive oxygen species (ROS) has the capacity for fine-tuning signalling

pathways by the transient inhibition of PTP activity. The conserved cysteine in the active site of the PTPs constitutes not only the crucial nucleophile of the catalytic mechanism, but because it exists as a thiolate anion at neutral pH, it is also a potential regulatory target through reversible oxidation. The ready reversal of the reaction in physiological reducing agents ensures that the oxidation of the catalytic cysteine leads to only a short-lived loss of activity. For the classical PTPs, the formation of a cyclic sulphenamide guarantees that the oxidation event remains reversible, whereas for the DSPs, CDC25 and LMW-type PTPs, the availability of a second cysteine residue within the active site facilitates the formation of a reversible disulphide bond on oxidation (Salmeen *et al*, 2003). An unresolved question in this field is whether ROS such as H_2O_2 can be delivered in a controlled manner to PTPs. Evidence exists to suggest that the insulin-triggered generation of ROS specifically affects T-cell PTP (TC-PTP) and PTP1B (Meng *et al*, 2004); J. den Hertog (Utrecht, The Netherlands) showed that classical PTP domains might be differentially influenced by reversible oxidation. Furthermore, the sensitivity of PTPs to oxidation correlated with the specific orientation of the conserved arginine in their active site.

Regulation of PPPs is mainly conducted by associated non-catalytic subunits. However, as J. Goris (Leuven, Belgium) and E. Ogris (Vienna, Austria) discussed, studies from several groups have revealed that reversible methylation at the carboxyl group of the C-terminal leucine of PP2A-type catalytic subunits has an important role in the biogenesis of functional PP2A holoenzymes by coordinating their assembly and activation. The picture emerging from these studies is that a yeast methyltransferase, Pme1 (PME1 in humans), stabilizes an inactivated pool of PP2A (Longin *et al*, 2004), whereas a methyltransferase, Ppm1 (PPMT1 in humans), promotes association of PP2A with its regulatory subunits. As the Ogris group first showed, Rrd1/Ypa1 and Rrd2/Ypa2 (PTPA in humans) have an essential role in the activation of heterotrimeric PP2A complexes. This probably occurs by facilitating the insertion of divalent cations into the phosphatase active site, thereby preventing non-physiological dephosphorylation of substrates by the free catalytic subunit (Fellner *et al*, 2003). Crystallographic studies have revealed that non-catalytic domains also have important roles in regulating PPP activity. D. Barford (London, UK) reported that the regulatory tetratricopeptide repeat (TPR) domain in PP5 engages with the catalytic channel of the phosphatase domain and restricts access to the catalytic site (Yang *et al*, 2005). The mechanism of autoinhibition—which involves steric interactions between glutamic acid 76 and tyrosine 451 of PP5—resembles the inhibition of other PPPs, such as PP1 by toxins and PP2B by its autoinhibitory domain (Fig 2).

Non-catalytic subunits modify PPP catalytic activity or subcellular localization and are thought to be more specific for individual processes than the catalytic subunits. However, several non-catalytic subunits have pleiotropic functions, some of which do not seem to be dependent on binding to PPPs. Hal3 is a conserved protein that binds to and inhibits the yeast PP1-like Ppz1. J. Arino (Barcelona, Spain) presented data showing that cells lacking Ppz1 or overexpressing Hal3 had increased expression of the Na^+ -ATPase-encoding *ENA1* gene owing to the activation of a calcineurin/Crz1-signalling pathway. This is consistent with a physiological role for Hal3 in Ppz1 regulation. However, genetic complementation studies using Hal3 mutants that were unable to bind or inhibit Ppz1, showed that Hal3 and the Hal3-related protein Vhs3 might have important Ppz1-independent functions. M. Bollen (Leuven, Belgium) discussed the

Fig 2 | Structure of human Ppp5. Ribbon representation of Ppp5 with the tetratricopeptide repeat (TPR) and phosphatase domains coloured green and pink, respectively. The C-terminal sub-domain, including the α -J-helix is in red. Metal ions of the binuclear centre are shown as blue spheres. Figure courtesy of D. Barford.

role of the nuclear scaffold protein NIPP1, which is a potent PP1 inhibitor and is involved in the translocation and retention of PP1 in the nucleus (Lesage *et al*, 2004). NIPP1 has at least two other functions—as a splicing factor and as a potent Polycomb Repressive Complex 2-dependent transcriptional repressor. Although NIPP1 can perform these functions in the absence of functional PP1-binding sites, PP1 may facilitate NIPP1 function during splicing by regulating the maternal embryonic leucine zipper kinase (MELK), which binds to NIPP1 and prevents recruitment of NIPP1 to spliceosomes. Bollen reported that PP1 could bind with high-affinity to MELK and inactivate it by dephosphorylation. These studies highlight the need for increased understanding of interactions between components of PPP-containing complexes, and the identification of PPP substrates within them. Few physiologically relevant phosphoprotein substrates have been identified for the PPP holoenzymes, and this remains one of the greatest challenges for the field. I. Mansuy (Zurich, Switzerland) described how immobilized metal ion affinity chromatography is being used with mass spectrometry to investigate the role of PP1 in learning and memory. In a preliminary study of total phosphoproteins, more than 1,000 phosphorylation sites on about 500 synaptic proteins were identified, demonstrating the potential of phosphoproteomics to address this problem and revealing the scale of the task ahead.

Drug development

The success of the protein tyrosine kinase (PTK) inhibitory drug, imatinib mesylate (Gleevec), for the treatment of chronic myeloid leukaemia and gastrointestinal stromal tumours has spurred efforts to generate equivalent inhibitors for the PTPs. Z.-Y. Zhang (Indianapolis, IN, USA), however, described two significant hurdles that have

proven problematic in the PTP inhibitor field. First, the conserved nature of the PTP active site has so far hindered the development of specific inhibitors and second, the charged nature of pTyr mimetics reduces their cell-membrane permeability. The current focus is therefore on nonpeptidic PTP inhibitors with less negatively charged groups that can achieve inhibition by binding to both the active site and less conserved sites on the PTP surface. Several anticancer drugs—such as cantharadin and fostriecin—have long been known to inhibit PPPs. P.T.W. Cohen (Dundee, UK) and Gingras reported how Ppp4c, in complex with regulatory subunits R2 and R3, might regulate sensitivity to the commonly used anticancer agent, *cis* di-amine-di-chloroplatinum (cisplatin; Cohen *et al*, 2005; Gingras *et al*, 2005). Experiments in yeast showed that deletion of the orthologues of Ppp4c, R2 and R3 result in cisplatin hypersensitivity and that the mammalian R3 can substitute for its yeast counterpart in cisplatin-sensitivity assays. Furthermore, a *Drosophila* R3 hypomorph also exhibits cisplatin hypersensitivity, indicating that the activity of this PP4 complex in the DNA-damage response is conserved in metazoans. Therefore, combined therapies of cisplatin and an agent that targets this complex could have the potential to decrease the incidence of cellular resistance to cisplatin, or its analogues, and improve the efficacy of these drugs.

Phosphatases in the immune system

Protein phosphatases have pivotal roles in the immune system, not only influencing the magnitude of immune-cell responses, but also affecting the quality of the signal elicited by various stimuli. This point was reinforced in presentations implicating PTPs in diverse biological functions ranging from T-cell–antigen-presenting cell (APC) interactions to the regulation of thymocyte intracellular pH. D. Alexander (Cambridge, UK) emphasized the far-reaching effects of perturbing the PTP–PTK balance in T cells. All mice that are deficient in the receptor PTP, CD45, and which express the intracellular PTK transgene, LckY505F, develop thymic tumours. The presence of oncogenic Lck prevents the apoptosis that is a normal consequence of DNA damage and Alexander discussed the mechanism through which apoptosis is blocked. Surprisingly, the DNA damage-induced deamidation of Bcl-X_L is not caused enzymatically but by an increase in intracellular pH. This pH change derives from the increased expression of the Na⁺/H⁺ exchanger NHE1. However, by preventing the upregulation of NHE1, oncogenic Lck seems to inhibit Bcl-X_L deamidation, thereby preserving its pro-survival functions. Another PTP able to dephosphorylate PTKs in T cells is PTPα. C. Pallen (Vancouver, BC, Canada) demonstrated that PTPα^{−/−} thymocytes show hyperphosphorylation of several proteins under basal conditions, which correlates with an increase in the activity of the PTK, Fyn. Interestingly, PTPα is present in lipid rafts and crucially the activity of Fyn in the lipid rafts of PTPα^{−/−} thymocytes is increased, whereas non-raft Fyn is normal. The selective dephosphorylation of substrates within discrete cellular spatial locations might be a widespread mechanism to regulate signalling.

Key roles are also performed by intracellular PTPs in immune cells. The vaccinia H1-related (VHR) DSP is a regulator of T-cell signalling and A. Alonso (Valladolid, Spain) elaborated on the intimate connection between the PTK ζ-associated protein of 70 kDa (ZAP-70) and VHR. After T-cell receptor (TCR) stimulation, VHR is phosphorylated on Tyr38 and Tyr138 by ZAP-70, which leads to an upregulation of VHR activity. Active VHR is able to dephosphorylate and inactivate extracellular signal-regulated kinase 2 and c-Jun N-terminal kinase

(JNK1). Given the central role of the ERK pathways in TCR signalling, VHR functions as a key control element. However, the physical nature of the interaction between VHR and ZAP-70 has so far been unclear. Using a yeast two-hybrid approach, Alonso has now identified a new VHR-interacting protein, the POZ-domain containing protein POZTIV, which is upregulated in activated T cells.

Using mouse embryonic stem cells, in which the expression of mutant intracellular SHP1 could be regulated, M. Welham (Bath, UK) provided convincing data to implicate SHP1 in multiple stages of haematopoietic cell differentiation and proliferation (Paling & Welham, 2005). J. Matthews (Cardiff, UK) highlighted an additional mode of SHP1 action in peripheral T cells. Using SHP1-deficient T cells expressing a transgenic TCR, Matthews showed that SHP1 exerts its effects on TCR-signalling thresholds by reducing the adhesive interaction between the T cell and the APC. Importantly, SHP1-deficient cytotoxic T cells have an enhanced *in vivo* killing capacity, which could potentially enhance adoptive T-cell immunotherapy strategies. T. Tiganis (Monash, Vic, Australia) described the central role of the ubiquitously expressed TC-PTP in negatively regulating the inflammatory response and specifically in controlling tumour necrosis factor-α (TNF-α) signalling. TC-PTP associates with the adaptor, TNF-receptor-associated factor 2, and acts upstream of MAP3K and selectively regulates TNF-α-induced ERK activation while sparing NFκB signalling. The suppressive function of TC-PTP can be attributed to its action on the PTK, Src. Src was revealed to be a TC-PTP substrate by use of substrate-trapping isoforms of TC-PTP and consistent with this observation, thymocytes deficient in TC-PTP exhibit increased Src phosphorylation.

Phosphatases in health and disease

Given the central importance of protein phosphatases to cell biology, it is not surprising that this family of enzymes has been directly implicated in several human disorders and is an attractive target for pharmacological intervention in alleviating disease. Indeed, a functional polymorphism in the gene encoding the PTP Lyp has recently been linked to susceptibilities to four autoimmune disorders: systemic lupus erythematosus, Graves' disease, rheumatoid arthritis and type I diabetes (Bottini *et al*, 2004). PTP1B has been highlighted as a potential target for the treatment of type II diabetes because increased insulin sensitivity and obesity resistance are two significant phenotypic hallmarks of induced PTP1b-deficiency in mice. Furthermore, the enhanced sensitivity to insulin in these mice correlates with an increased and prolonged tyrosine phosphorylation of the insulin receptor (IR). It has been established that PTP1B dephosphorylates the IR directly; however, given that PTP1B localizes to the endoplasmic reticulum (ER), the results presented by M. Tremblay (Montreal, Quebec, Canada) on impaired ER-stress signalling in the absence of PTP1B suggest an additional scenario. ER stress—as triggered by factors associated with obesity—leads to inositol requiring kinase (IRE1) activation and the subsequent activation of JNK1, an antagonist of IR-signal propagation. The failure to induce JNK1 activation in Ptp1b^{−/−} mouse embryonic fibroblasts after the induction of ER stress is intriguing because it suggests that PTP1B might have an additional substrate in the ER that enables IRE1 activation. Further explanation of the mechanism by which PTP1B influences IRE1-mediated ER-stress signalling might also be of direct relevance to the development of new therapeutics for type I diabetes, as ER stress triggered by high glycaemia can lead to the apoptosis of insulin-producing pancreatic β-cells.

Learning and memory are complex functions involving many signalling pathways regulated by serine/threonine and tyrosine phosphorylation. Most excitatory synapses in the mammalian brain are found on specialized membrane protrusions called dendritic spines. Neurabin (Nrb), a conserved regulator of PP1, has an important role in spine development and maturation. S. Shenolikar (Ann Arbor, MI, USA) reported that disrupting the functions of a Nrb-PP1 complex hindered the morphological and functional maturation of dendritic spines (Terry-Lorenzo *et al*, 2005). He also reported that Nrb/PP1 has measurable effects on long-term potentiation and depression of excitatory synaptic transmission, indicating that Nrb-mediated targeting of PP1 is important for events underlying synaptic plasticity. I. Mansuy (Zurich, Switzerland) reported the effects of modulating PP1 activity on excitatory postsynaptic potentials at CA1-CA3 synapses in hippocampal slices under ischemic conditions. Inhibition of PP1 activity using pharmacological, genetic and physiological approaches worsened the effects of ischemia on post-synaptic potentials, whereas elevation of PP1 activity improved recovery, indicating that PP1 has a neuroprotective role.

PP1 and PP2A have been implicated in the control of the circadian rhythm, which is crucial for the optimization of behaviour and metabolism during daily light-dark cycles. D. Virshup (Salt Lake City, UT, USA) reported that the stability of the circadian regulator protein, PER2, and the length of the circadian period are reciprocally regulated by casein kinase 1 ϵ (CK1 ϵ) and PP1. Phosphorylation by CK1 ϵ results in PER2 degradation, mediated by the ubiquitin ligase adaptor protein β -TrCP (Eide *et al*, 2005). PER2 is a direct substrate of PP1 and inhibition of PP1 accelerated β -TrCP-mediated PER2 degradation. However, PP1 levels do not seem to fluctuate over a 24-h period, so attention is now being turned towards regulators of PP1 that might oscillate in a circadian pattern. Several lines of evidence indicate that increased PP1 activity might contribute to cardiac hypertrophy and heart failure. A. DePaoli-Roach (Indianapolis, IN, USA) reported that overexpression of a functional C-terminally truncated form of the heat-stable PP1-binding protein Inhibitor-2 reduced PP1 activity, improved cardiac performance (Kirchhefer *et al*, 2005) and rescued the cardiac defect of mice overexpressing PP1 (Carr *et al*, 2002). This suggests that PP1 could be a target for therapeutic intervention. Overexpression or knockout of other PP1 regulatory subunits, including the glycogen-binding subunits PTG and RGL, is being pursued to establish the role of PP1 in metabolic control.

Although several PTPs have been linked to the development of solid tumours (Wang *et al*, 2004), SHP2 is the first PTP to be implicated in leukaemogenesis. B. Neel (Boston, MA, USA) showed that somatic SHP2 missense mutations have been found in 35% of cases of sporadic juvenile myelomonocytic leukaemia and a lower but significant incidence in paediatric acute myelogenous leukaemia, myelodysplastic syndrome and B-cell acute lymphoblastic leukaemia. Germ-line mutations in SHP2 have also been described in the autosomal dominant, Noonan and multiple lentigenes LEOPARD syndromes, which share similar features including congenital heart defects and facial and skeletal abnormalities. In its basal state, SHP2 is held in a catalytically inactive configuration by the insertion of residues from its amino-terminal SH2 (N-SH2) domain into the PTP domain but engagement of pTyr-containing ligands by the SH2 domains of SHP2 leads to an allosteric release of this autoinhibition. For the Noonan syndrome and leukaemia patients, gain-of-function SHP2 mutants have

been identified that primarily interfere with the N-SH2-PTP domain inhibitory interface but some mutants also seem to increase the sensitivity of SH2-domain ligand engagement (Keilhack *et al*, 2005). There is a reasonable correlation between the level of PTP activity and the type of SHP2-mediated disease, with leukaemia-inducing SHP2 mutants more potent in myeloid cell transformation assays (Mohi, 2005). Intriguingly, the LEOPARD syndrome mutations seem to have reduced catalytic activity and it will be important to establish how both loss- and gain-of-function SHP2 mutants can give rise to similar syndromes. Significantly, mouse models of Noonan syndrome and SHP2-induced leukaemia have been established (Araki *et al*, 2004; Mohi *et al*, 2005) that will constitute invaluable resources for establishing the molecular mechanisms of SHP2-associated pathology and evaluating therapeutic treatments.

Concluding remarks

'Europhosphatases Conference 2005' is the latest in a series of biennial conferences that, over the past two decades, have brought together investigators from the diverse corners of protein phosphatase biology. The success of these meetings is in part a reflection of the dynamic nature of the protein phosphatase field. The protein phosphatase family of enzymes, including their interacting regulators, represent a significant component of the human proteome that is potentially amenable to pharmacological manipulation. Furthermore, the marked phenotypes that result from the genetic manipulation of protein phosphatase expression and the direct association of protein phosphatases to human disease highlight the importance of protein phosphatases as regulators of cellular function. The molecular characterization of individual protein phosphatases and a precise definition of their *in vivo* functional roles provide the promise of new biological paradigms and the development of pharmaceuticals for the treatment of many human diseases.

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REFERENCES

- Alonso A, Sasin J, Bottini N, Friedberg I, Osterman A, Godzik A, Hunter T, Dixon J, Mustelin T (2004) Protein tyrosine phosphatases in the human genome. *Cell* **117**: 699-711
- Andersen JN, Jansen PG, Echwald SM, Mortensen OH, Fukada T, Del Vecchio R, Tonks NK, Moller NP (2004) A genomic perspective on protein tyrosine phosphatases: gene structure, pseudogenes, and genetic disease linkage. *FASEB J* **18**: 8-30
- Araki T *et al* (2004) Mouse model of Noonan syndrome reveals cell type- and gene dosage-dependent effects of Ptpn11 mutation. *Nat Med* **10**: 849-857
- Barford D (1996) Molecular mechanisms of the protein serine/threonine phosphatases. *Trends Biochem Sci* **21**: 407-412
- Bottini N *et al* (2004) A functional variant of lymphoid tyrosine phosphatase is associated with type 1 diabetes. *Nat Genet* **36**: 337-338
- Carr AN *et al* (2002) Type 1 phosphatase, a negative regulator of cardiac function. *Mol Cell Biol* **22**: 4124-4135

- Cohen PT, Philp A, Vazquez-Martin C (2005) Protein phosphatase 4—from obscurity to vital functions. *FEBS Lett* **579**: 3278–3286
- Eide EJ, Kang H, Crapo S, Gallego M, Virshup DM (2005) Casein kinase I in the mammalian circadian clock. *Methods Enzymol* **393**: 408–418
- Fellner T, Lackner DH, Hombauer H, Piribauer P, Mudrak I, Zaragoza K, Juno C, Ogris E (2003) A novel and essential mechanism determining specificity and activity of protein phosphatase 2A (PP2A) *in vivo*. *Genes Dev* **17**: 2138–2150
- Gingras AC, Caballero M, Zarske M, Sanchez A, Hazbun TR, Fields S, Sonenberg N, Hafen E, Raught B, Aebersold R (2005) A novel, evolutionarily conserved protein phosphatase complex involved in cisplatin sensitivity. *Mol Cell Proteomics* **4**: 1725–1740
- Gohla A, Birkenfeld J, Bokoch GM (2005) Chronophin, a novel HAD-type serine protein phosphatase, regulates cofilin-dependent actin dynamics. *Nat Cell Biol* **7**: 21–29
- Huynh H *et al* (2004) Control of vesicle fusion by a tyrosine phosphatase. *Nat Cell Biol* **6**: 831–839
- Keilhack H, David FS, McGregor M, Cantley LC, Neel BG (2005) Diverse biochemical properties of Shp2 mutants: implications for disease phenotypes. *J Biol Chem* **280**: 30984–30993
- Kirchhefer U *et al* (2005) Enhanced cardiac function in mice overexpressing protein phosphatase inhibitor-2. *Cardiovasc Res* **68**: 98–108
- Lesage B, Beullens M, Nuytten M, Van Eynde A, Keppens S, Himpens B, Bollen M (2004) Interactor-mediated nuclear translocation and retention of protein phosphatase-1. *J Biol Chem* **279**: 55978–55984
- Longin S *et al* (2004) An inactive protein phosphatase 2A population is associated with methylesterase and can be re-activated by the phosphotyrosyl phosphatase activator. *Biochem J* **380**: 111–119
- Meng TC, Buckley DA, Galic S, Tiganis T, Tonks NK (2004) Regulation of insulin signaling through reversible oxidation of the protein-tyrosine phosphatases TC45 and PTP1B. *J Biol Chem* **279**: 37716–37725
- Mohi MG *et al* (2005) Prognostic, therapeutic, and mechanistic implications of a mouse model of leukemia evoked by Shp2 (PTPN11) mutations. *Cancer Cell* **7**: 179–191
- Paling NR, Welham MJ (2005) Tyrosine phosphatase SHP-1 acts at different stages of development to regulate hematopoiesis. *Blood* **105**: 4290–4297
- Salmeen A, Andersen JN, Myers MP, Meng TC, Hinks JA, Tonks NK, Barford D (2003) Redox regulation of protein tyrosine phosphatase 1B involves a sulphenyl-amide intermediate. *Nature* **423**: 769–773
- Terry-Lorenzo RT, Roadcap DW, Otsuka T, Blanpied TA, Zamorano PL, Garner CC, Shenolikar S, Ehlers MD (2005) Neurabin/protein phosphatase-1 complex regulates dendritic spine morphogenesis and maturation. *Mol Biol Cell* **16**: 2349–2362
- Vereshchagina N, Bennett D, Szoor B, Kirchner J, Gross S, Vissi E, White-Cooper H, Alphey L (2004) the essential role of PP1 β in *Drosophila* is to regulate nonmuscle myosin. *Mol Biol Cell* **15**: 4395–4405
- Wang Z *et al* (2004) Mutational analysis of the tyrosine phosphatome in colorectal cancers. *Science* **304**: 1164–1166
- Yang J, Roe SM, Cliff MJ, Williams MA, Ladbury JE, Cohen PT, Barford D (2005) Molecular basis for TPR domain-mediated regulation of protein phosphatase 5. *EMBO J* **24**: 1–10

D. Bennett [author], J. Arino [organizer], J.G. Sathish [author], E. Da Cruz e Silva [organizer], R.J. Matthews [author] & D. Alexander [organizer]