EMBO CONFERENCE REPORT

Protein phosphorylation and protein phosphatases De Panne, Belgium, September 19–24, 1999

Stanislaw Zolnierowicz and
Mathieu Bollen^{1,2}

Intercollegiate Faculty of Biotechnology UG-MUG, Gdansk, Poland
and ¹Afdeling Biochemie, Faculteit Geneeskunde, Katholieke
Universiteit Leuven, B-3000 Leuven, Belgium

²Corresponding author
e-mail: Mathieu.Bollen@med.kuleuven.ac.be

This report is dedicated to Matthew L. Thomas (St Louis, MO), who
was invited as a speaker to Europhosphatase 99, but who died
suddenly on his way to the conference.

Introduction

About one-third of all proteins in eukaryotic cells are thought to be phosphorylated at any one time. An understanding of how a particular cellular process is regulated by protein phosphorylation requires an insight into (i) the identity of the phosphoproteins and their phosphorylation sites, (ii) the effects of their phosphorylation, (iii) the nature of the protein kinases (PKs) and protein phosphatases (PPs) involved, and (iv) the mechanisms that determine when and where these enzymes are active. The dynamic nature of protein phosphorylation implies that phosphorylation levels can be modulated by changes in the activities of either PKs or PPs. These enzymes show a great structural diversity, and their activities are tightly regulated by hormones, growth factors and metabolites. The recent progress in our understanding of the structure and regulation of protein phosphatases and of their role in various cellular processes was the subject of an EMBO conference that was organized in De Panne, Belgium (September 19–24, 1999) by M. Bollen, D. Barford and S. Klumpp. This 'Europhosphatase' conference attracted 170 participants from 25 different countries.

Novel protein phosphatase (regulators)

Protein phosphatases are classified into three families, based on the structure of their catalytic domains. The PPP family includes the phosphoserine/phosphothreonine-specific protein phosphatases PP1, PP2A, PP2B (calcineurin), PP4 and PP5. The PPM family comprises Mg²⁺-stimulated protein phosphatases, such as PP2C, which also dephosphorylate phosphoserine and phosphothreonine residues. Protein-tyrosine phosphatases and 'dual-specificity' protein phosphatases, which dephosphorylate all three phosphoamino acids, belong to the PTP family. S. Klumpp (Marburg, Germany) reported on the purification of a histidine phosphatase from rat liver (14 kDa) that is insensitive to classical phosphatase inhibitors except P_i. Peptide sequencing did not show any homology with known protein phosphatases. This enzyme is therefore likely to represent the first member of a

new family, putatively termed PHP for protein-histidine phosphatases.

The genome of *Saccharomyces cerevisiae* (~6100 genes) encodes 33 catalytic subunits. *Caenorhabditis elegans*, with a 3-fold larger genome, also has about three times more genes that encode PPs. An extrapolation to mammalian genomes (70 000–140 000 genes) yields 350–700 PP-encoding genes. The known PP catalytic subunits only comprise a minor fraction of this estimate. Surprisingly, no novel PP catalytic subunits were described at Europhosphatase 99, with the exception of a dual-specificity PTP involved in spermatogenesis (H. Shima, Sapporo, Japan). In contrast, various new non-catalytic subunits were discussed (see below). This could indicate that higher eukaryotes simply have less PP-encoding genes than estimated. Possibly, the diversity of PP holoenzymes in higher eukaryotes is increased in other ways, e.g. by the use of splice variants and by the ability of the catalytic subunits to form complexes with a large variety of regulatory proteins.

The haemochromatosis candidate gene V product (HCG V) was identified as a specific inhibitor (14 kDa) of PP1, with properties similar to that of the cytoplasmic inhibitors-1 and -2, hence its name inhibitor-3 (Zhang *et al.*, 1998). On the other hand, inhibitor-3 is similar to the inhibitor NIPP1 in that they are both nuclear and inactivated by phosphorylation (E. Lee, Valhalla, NY). David Brautigan (Charlottesville, VA) described an inhibitor of PP1 that inhibits holoenzymes as efficiently as free catalytic subunit (PP1_C) and was therefore termed PHI, for 'phosphatase holoenzyme inhibitor'. PHI is structurally related to the smooth muscle inhibitor CPI-17, but has a broader tissue distribution. CPI-17 and PHI are activated by phosphorylation with protein kinase C and lack the 'RVXF' motif for interaction with the catalytic subunit (PP1_C), which is present in nearly all other regulators of PP1.

PP2A consists of a constant dimeric core, i.e. the catalytic subunit (PP2A_C) and the A-subunit (PR65), and a variable B-subunit (Figure 1). The C-terminal leucine of PP2A_C is methylated reversibly *in vivo* and this may represent a signal for the binding of a B-subunit to the dimeric core. J. Goris (Leuven, Belgium) reported on the purification and cloning of both a methyltransferase (De Baere *et al.*, 1999) and a methylesterase acting on PP2A_C. The latter was identical to that recently described by Ogris *et al.* (1999). Two novel B-subunits of PP2A were described, i.e. PR59 (R. Bernards, Amsterdam, The Netherlands) and PR48 (M. Mumby, Dallas, TX), both with functions in the cell cycle (see below). Nucleoredoxin, which displays an oxidoreductase activity *in vitro*, was also identified as a novel PR65-binding protein (K. Lechward, Gdansk, Poland).

PP4 is a predominantly nuclear phosphatase, but is also

Fig. 1. The diversity of PP2A-interacting proteins.

associated with the centrosomes and is involved here in the initiation of microtubule growth. T.Cohen (Dundee, UK) described the purification of two mammalian PP4 holoenzymes, which represent heterodimers between the catalytic subunit (PP4_C) and regulatory subunits of 105 kDa (PP4_{R1}) and 51 kDa (PP4_{R2}). PP4_{R1} was also described recently by Kloeker and Wadzinski (1999).

Crystal structures

The PR65 subunit of PP2A, which binds both PP2A_C and a variable B-subunit, consists of 15 copies of tandemly repeated HEAT motifs of 39 residues. Its crystal structure revealed that the HEAT motifs are composed of a pair of antiparallel α -helices that assemble in a linear fashion to form an elongated protein characterized by a double layer of α -helices (Groves *et al.*, 1999). Likewise, the tetratricopeptide repeats (34 residues) of PP5 consist of a pair of antiparallel α -helices that fold, however, into a right-handed superhelical structure (Das *et al.*, 1998). Due to a slide projection problem, we also caught a glimpse of the crystal structure of the eukaryotic release factor 1 (eRF1), which is involved in the termination of translation and targets PP2A to the ribosomes (D.Barford, London, UK).

The catalytic domain of the MAP-kinase phosphatase MPK3/PYST3 was shown to adopt a protein-tyrosine phosphatase fold with a distorted, inactive catalytic site in the absence of substrate (Stewart *et al.*, 1999). N.McDonald (London, UK) also presented data indicating that substrate binding results in a conformational change that is associated with an activation of the catalytic domain.

Table I. Subcellular targeting of PP1_C

Targeting protein	Subcellular localization (substrate)
AKAP220	vesicles and peroxisomes
Inhibitor-1/DARPP-32	cytoplasm
Inhibitor-3	nucleus
MYPT1	myofibrils (myosin)
NIPP1	nuclear speckles (CDC5L or associated protein)
Neurabin I	F-actin in neural lamellipodia
PNUTS/p99/R111	nucleus
Reg1p	cytoplasm (hexokinase II)
Sds22	nucleus + cytoplasm [mitotic protein(s)]
SARA	plasma membrane (TGF- β receptors)
Spinophilin/neurabin II	synaptic spines (AMPA-glutamate channels)
R _{GI} /R _M	glycogen (glycogen synthase, phosphorylase)
Yotiao	sarcoplasmic reticulum (phospholamban)
	plasma membrane (N-methyl-D-aspartate receptor)

The table only shows the PP1_C-interacting proteins that were discussed at the conference and that have an established subcellular localization. The presumed substrates of the corresponding PP1 holoenzymes are indicated in parentheses.

Subunit interaction sites

Type-1 protein phosphatases consist of a constant catalytic subunit and one or two variable regulatory subunits that control the activity, substrate specificity and subcellular localization of the holoenzymes (Table I). PP1_C contains various binding sites for its regulators. These include the β 12- β 13 loop near the catalytic site (residues 269-279),

which is essential for the inhibition by various toxins and protein inhibitors (S.Shenolikar, Durham, NC). A different site on PP1_C binds the so-called 'RVXF' motif, which is present in most regulatory proteins of PP1. An emerging agreement between various speakers was that the regulatory proteins often have multiple sites of interaction with PP1_C. M.Bollen (Leuven, Belgium) provided initial evidence that NIPP1 and inhibitor-2 share at least two PP1_C-binding sites. He proposed that the specific effects of the regulatory subunits can be accounted for by their binding to a specific combination of sites on PP1_C. This 'shared-site' model could explain how regulatory proteins can interact with the catalytic subunit in many different ways, in spite of the existence of only a relatively small number of interaction sites. The model also suggests that the hormonal or metabolic regulation of PP1 holoenzymes may involve a control on the number and identity of interaction sites between the subunits. An additional level of regulation of PP1 may be provided by the presence of subcellular targeting domains in the non-catalytic subunits. Examples that were discussed include the targeting of MYPT1 to myosin (D.Hartshorne, Tucson, AZ), of NIPP1 to the cell cycle and pre-mRNA splicing regulator CDC5L (A.Boudrez, Leuven, Belgium), and of spinophilin to the synaptic spines in neurons (A.Nairn, New York, NY). A recurring theme was also the regulation of PP1 holoenzymes by phosphorylation. A striking example of the complexity of this regulation is represented by the myosin-associated phosphatase in smooth muscle, which is activated by the phosphorylation of MYPT1 with a mitotic kinase but is inactivated by phosphorylation of MYPT1 with a Rho kinase (D.Hartshorne, Tucson, AZ).

W.Hendriks (Nijmegen, The Netherlands) showed that PTP-BL contains a FERM domain that targets the enzyme to the apical side of epithelial cells. PTP-BL also contains PDZ domains that are involved in the binding and clustering of multiple proteins, including RIL. PTP-BL-deficient mice are viable, but the adult male mice have an increased body weight.

PTP substrates

PTP-mediated catalysis occurs via a cysteinyl-phosphate enzyme intermediate. N.Tonks (Cold Spring Harbor, NY) has pioneered the use of 'substrate-trapping' mutants to identify the physiological substrates of PTPs (Flint *et al.*, 1997; Table II). Using this approach, his group has recently been able to identify p52Shc and the epidermal growth factor receptor as substrates of the 45 kDa variant of protein-tyrosine phosphatase TC-PTP (TC45). Likewise, the cell cycle regulator VCP (p97/CDC48) was identified as a substrate of the band 4.1-related PTP-H1, and p130^{cas} as a substrate of the cytosolic PTP-PEST. Using a slightly different substrate-trapping procedure, J.Den Hertog (Utrecht, The Netherlands) also identified p130^{cas} as one of the substrates of the transmembrane receptor-like RPTP α . A.Elson (Rehovot, Israel) showed that the voltage-gated Kv2.1 channel associates with a substrate-trap mutant of the cytoplasmic form of PTPe. Also, Schwann cells from mice with a disrupted PTPe gene show a decreased myelination and hyperphosphorylation (activation) of the Kv channel. G.Peters (Lyngby, Denmark) reported that C-terminally located residues in phospho-

Table II. Protein-tyrosine phosphatases and their substrates

Protein-tyrosine phosphatases	Substrate(s)
CD45	p56 ^{lck}
Cdc25	cyclin B-Cdc2
Cytoplasmic LMW-PTP	PDGF receptor
Cytoskeleton-associated LMW-PTP	p190 ^{Rho-GAP}
MPK3/ PYST1	ERK MAP kinases
PTEN	phosphatidylinositol 3,4,5-P ₃
PTPe	Kv2.1 channel
PTP-1B	insulin receptor, EGF receptor
PTP-PEST	p130 ^{cas} , paxillin, FAK
4.1-related PTP-H1	VCP (p97/CDC48)
RPTP α	p130 ^{cas} , fyn
TC-PTP (TC45)	p52Shc, EGF receptor

The table shows some of the recently identified substrates of PTPs. LMW-PTP, low molecular weight PTP; FAK, focal adhesion kinase; EGF, epidermal growth factor; PDGF, platelet-derived growth factor.

tyrosine-containing peptides are important determinants of the substrate specificity of PTPs.

Protein kinase-phosphatase signalling complexes

Numerous PKs and PPs are targeted to their substrates and regulators through interaction with specific anchoring proteins (Colledge and Scott, 1999). Some of these anchoring proteins bind both PKs and PPs, as has been elegantly shown by J.Scott (Portland, OR). For example, AKAP79 binds to and inhibits protein kinases A and C as well as PP2B, and thereby targets these proteins to the plasma membrane. Similarly, AKAP220 acts as a vesicular and peroxisomal targeting protein of PP1 and PKA. Yotiao is an *N*-methyl-D-aspartate (NMDA) receptor-associated protein that binds protein kinase A and PP1 (Westphal *et al.*, 1999b). Interestingly, yotiao also acts as a substrate-specifying subunit of PP1, since the associated PP1_C is active and actually dephosphorylates the NMDA channel much faster than does free catalytic subunit, thereby limiting NMDA channel activity. Activation of PKA overcomes this constitutive dephosphorylation of the NMDA channel.

B.Wadzinski (Nashville, TN) showed that PP2A is associated with the p70 S6 kinase and with the p21-activated kinases PAK1 and PAK3 (Westphal *et al.*, 1999a). His group also isolated a stable and stoichiometric complex of PP2A with the Ca²⁺- and calmodulin-dependent kinase IV. These data fit nicely with accumulating evidence that PP2A is a major regulator of protein kinase cascades because of its ability to dephosphorylate protein kinases (Millward *et al.*, 1999). Some of these protein kinase cascades, including the protein kinase B and Ndr kinase signalling pathways, were reviewed by B.Hemmings (Basel, Switzerland).

Protein kinase-phosphatase complexes also play a role in signalling through tyrosine (de)phosphorylation. S.Arkininstall (Geneva, Switzerland) summarized data on the association of the MAP-kinase phosphatase MKP3/ PYST1 with ERK MAP-kinases, which triggers their catalytic activation. The transmembrane RPTP α dephosphorylates and thereby activates protein-tyrosine kinases of the src family. L.Vaughan (Zürich, Switzerland)

showed that RPTP α and the intracellular protein-tyrosine kinase fyn interact with the neuronal glycosylphosphatidylinositol-linked receptor contactin, which is involved in the development of the nervous system. It was suggested that RPTP α acts as a bridge between contactin and the intracellular kinase.

Insulin signalling

Mice lacking a functional *PTP-1B* gene display an increased insulin sensitivity and show an increased phosphorylation of the insulin receptor in liver and muscle (Elchebly *et al.*, 1999). This suggests that the insulin receptor is a substrate of PTP-1B. Remarkably, these animals are also resistant to a fat-induced weight gain, which correlates with an increased expression of the uncoupling protein UCP-1 in brown adipocytes, resulting in the dissipation of metabolic energy as heat (M.Tremblay, Montreal, Canada).

A.DePaoli-Roach (Indianapolis, IN) described a murine knock-out of the muscle-type glycogen-binding subunit R_{G1}/R_M. These mice have a severely decreased level of muscle glycogen, which can be accounted for by a hyperphosphorylation of glycogen synthase and phosphorylase. Surprisingly, these mice still respond to an administration of insulin with a normal activation (dephosphorylation) of glycogen synthase, suggesting that this insulin effect is mediated by the inhibition of a glycogen synthase kinase and/or by the stimulation of a different glycogen-synthase phosphatase.

Cell cycle

DNA damage activates cell cycle checkpoints that block the G₁/S and G₂/M transitions. P.Russell (La Jolla, CA) showed that DNA damage in *Schizosaccharomyces pombe* causes the activation of protein kinase Chk1, which phosphorylates the dual-specificity protein phosphatase Cdc25 (Lopez-Girona *et al.*, 1999). The phosphorylated Cdc25 binds to Rad24, a 14-3-3-like protein, which functions as an attachable nuclear export signal and promotes the nuclear export of the complex. Since Cdc25 initiates mitotic entry by dephosphorylating the nuclear cyclin B-dependent protein kinase Cdc2, its removal from the nucleus can account for the DNA damage-induced block of mitosis. I.Hoffmann (Heidelberg, Germany) reported that DNA damage also resulted in a marked down-regulation of the human Cdc25A activity, which is involved in the G₁/S transition and has cyclin E- and cyclin A-dependent kinases as its direct targets (Blomberg and Hoffmann, 1999). The down-regulation of Cdc25A was also correlated with its binding to 14-3-3 proteins, and the association with 14-3-3 proteins could be mimicked *in vitro* by phosphorylation of Cdc25A with Chk1. Thus, Chk1 appears to be involved in the DNA damage checkpoints of both the G₁/S and the G₂/M transitions. J.Maller (Denver, CO) showed that Cdc25C, which triggers the dephosphorylation and activation of cyclin B-Cdc2 in higher eukaryotes, is activated by the Ser/Thr-specific polo-like kinase Plx1 in *Xenopus laevis*. Plx1 itself is activated by a novel kinase termed *Xenopus* polo-like kinase kinase (xPlkk1), which in turn appears to be

activated by a hitherto unidentified polo-like kinase kinase.

E.Ogris (Vienna, Austria) described a 169 kDa protein that associates with the chromosomes in a phosphorylation-dependent manner and was also shown to bind to the B-subunit of PP2A. M.Mumby (Dallas, TX) identified a new member of the B''/PR72 family of PP2A regulators, PR48, which targets the DNA replication factor Cdc6 for dephosphorylation by PP2A, resulting in its nuclear import (Yan *et al.*, 1999). R.Bernards (Amsterdam, The Netherlands) discussed his recent work on the retinoblastoma (pRb) protein and the structurally related p107. Phosphorylation of these growth inhibitors in late G₁ results in the release and activation of the E2F transcription factors. A novel B'' subunit of PP2A (PR59), which is structurally related to PR48, was shown to target p107 specifically for dephosphorylation by PP2A (Voorhoeve *et al.*, 1999). In contrast, the previously characterized PR72 subunit was found to be involved in the specific dephosphorylation of pRb.

Various lines of evidence also implicate PP1 in the dephosphorylation of pRb. N.Berndt (Los Angeles, CA) showed that the α -isoform of PP1_C is phosphorylated on Thr320 during the M phase and around the G₁/S transition. Interestingly, the phosphorylation of PP1_{C α} by cyclin E-Cdk2 during the G₁/S transition only involves the fraction of catalytic subunit that is associated with pRb (Liu *et al.*, 1999). Since PP1_{C α} is inactivated by phosphorylation, this modification was proposed to keep pRb phosphorylated until the G₁/S transition and/or the S phase are complete. In accordance with this view, the expression of the T320A mutant of PP1_{C α} causes a G₁ arrest.

Glucocorticoids induce a G₁ growth arrest through induction of p21^{Waf/Cip1}, an inhibitor of cyclin-dependent protein kinases. Using a novel antisense approach, R.Honkanen (Mobile, AL) convincingly showed that PP5 promotes cellular proliferation by inhibiting the expression of p21^{Waf/Cip1} (Zuo *et al.*, 1999). PP5 also affected the phosphorylation of the growth suppressor p53, which is involved in the expression of p21^{Waf/Cip1}.

Growth inhibition and viral transformation

Since many proto-oncogenes encode PKs, it seems logical to assume that at least some PPs may function as tumour suppressors. This is certainly true for the protein phosphatase PTEN, which is mutated in a large number of cancers (Cantley and Neel, 1999; Maehama and Dixon, 1999). Also, germline mutations of PTEN are believed to be the cause of Cowden disease, which is associated with an increased risk for the development of various tumours. Interestingly, PTEN contains the PTP consensus sequence HC(X)₃R, but acts very poorly on protein substrates. Several independent lines of evidence, reviewed by T.Maehama from J.Dixon's laboratory (Ann Arbor, MI), T.Mak (Toronto, Canada) and N.Tonks (Cold Spring Harbor, NY), unequivocally show that PTEN functions as a phosphatidylinositol phosphatase *in vivo*. Through its ability to hydrolyse phosphatidylinositol 3,4,5-P₃ at position 3, PTEN inhibits cell growth and survival signalling through the protein kinase PKB/AKT pathway.

SARA (Smad anchor for receptor activation) recruits Smads to the transforming growth factor- β (TGF- β)

receptors in the plasma membrane and thereby promotes Smad phosphorylation and activation. L.Alphey (Oxford, UK) showed that SARA also interacts with PP1_C in *Drosophila*, and that this interaction requires a specific motif in SARA. PP1 is likely to be a negative regulator of TGF- β signalling since the elimination of PP1_C from the TGF- β receptor complex potentiates the action of TGF- β . Signalling through TGF- β results in the activation of TAK1, which belongs to the MAP kinase kinase kinase superfamily. Activation of TAK1 results in its association with the adaptor protein TAB1. S.Tamura (Sendai, Japan) showed that PP2C binds to and dephosphorylates TAK1.

T.Kleinberger (Haifa, Israel) reported that the adenovirus type 5 E4orf4 protein induces a p53-independent apoptosis that is mediated by an interaction with the PR55/B-subunit of PP2A (Shtrichman *et al.*, 1999). PP2A also interacts directly with the Vpr protein encoded by the human immunodeficiency type-1 virus, and thereby promotes viral transcription (A.Garcia, Paris, France).

Cell adhesion

Mice lacking the α -isoform of PP2A die during embryonic development, before mesoderm formation (J.Götz, Zürich, Switzerland). The total level of PP2A_C is not affected in these embryos because of a compensatory increase in the concentration of the β -isoform. This indicates that, in spite of their high degree of identity (97%), the PP2A_C isoforms cannot functionally replace each other. It was suggested that the embryonic lethality stems from defects in cell adhesion since E-cadherin and β -catenin were less membrane associated in the knock-outs. In the absence of PP2A_{C α} , β -catenin also failed to show a nuclear translocation, which is in accordance with a recent report that implicates PP2A in the Wnt- β -catenin signalling pathway (Seeling *et al.*, 1999).

The targeted disruption of PTP-PEST is also lethal and is associated with tyrosine hyperphosphorylation of p130^{cas}, paxillin and the focal adhesion kinase FAK (M.Tremblay, Montreal, Canada). PTP-PEST^{-/-} fibroblasts display defects in motility, migration and cytokinesis (Angers-Loustau *et al.*, 1999).

M.Streuli (Boston, MA) described proteins that interact with transmembrane PTPs of the LAR family. One of these proteins, liprin- α 1, co-localizes with LAR at discrete ends of focal adhesions. Another LAR-binding protein, Trio, possesses two rho/rac guanine nucleotide exchange factor domains. Trio appears to be involved in cytoskeleton organization as well as in the distribution of focal contact sites (Seipel *et al.*, 1999).

G.Ramponi (Florence, Italy) discussed the role of low molecular weight PTPs (LMW-PTP) in signalling by platelet-derived growth factor (PDGF). A cytoplasmic form of LMW-PTP binds to and dephosphorylates the PDGF receptor. On the other hand, a cytoskeleton-associated LMW-PTP, which is phosphorylated and activated by protein-tyrosine kinase Src following stimulation with PDGF, dephosphorylates p190^{Rho-GAP} and thereby promotes cell adhesion and migration.

Immune response

CD45 is a transmembrane protein-tyrosine phosphatase that is expressed on haematopoietic cells. In CD45^{-/-} mice, the

level of mature T-cells in the periphery is decreased by 90–95% as a result of defects in thymic development (D.Alexander, Cambridge, UK). Thymocytes lacking CD45 show an increased phosphorylation of the T-cell receptor (TCR)-associated protein-tyrosine kinase p56^{lck}. Backcrossing of CD45^{-/-} mice to mice expressing an active p56^{lck} transgene (Y505F) reverses most of the defects in CD45^{-/-} mice. This result is consistent with a role for CD45 in regulating TCR thresholds by dephosphorylating the inhibitory Tyr505 of p56^{lck} (Pingel *et al.*, 1999).

J.Matthews (Cardiff, UK) reported on an examination of T-cells from the SHP-1-deficient 'motheaten' mouse, which revealed that this cytosolic PTP negatively regulates TCR signalling pathways in immature thymocytes and in mature peripheral T-cells (Johnson *et al.*, 1999). In macrophages, SHP-1 and CD45 coordinately regulate adhesion events (B.Neel, Boston, MA). This regulation includes an integrin-induced tyrosine phosphorylation of the SHP-1-associated proteins SHP1-1 and PIRB. The cytosolic protein-tyrosine phosphatase SHP-2 regulates the coupling of the TCR to the Ras/MAP kinase pathway or a parallel pathway, and this coupling appears to be mediated by association with the docking protein Gab-1 (D.Alexander, Cambridge, UK) or Gab-2 (B.Neel, Boston, MA).

The release of histamine from mast cells requires PP2A (A.Sim, Callaghan, Australia). The antigen-stimulated secretion of histamine is associated with a translocation of PP2A to a membrane fraction. However, this translocation of PP2A does not appear to be the trigger for mast cell secretion.

Pleiotropic functions of protein phosphatases in yeast

J.Ariño (Barcelona, Spain) demonstrated that Ppz1, a PP1-related phosphatase that is involved in the maintenance of cell integrity and regulation of salt tolerance in *S.cerevisiae*, also regulates the G₁/S transition (Clotet *et al.*, 1999). In this respect, Ppz1 opposes the action of another protein phosphatase, Sit4, a homologue of mammalian PP4. Ppz1 is inhibited by the Hal3 protein and also binds to the *TEF5* gene product, an EF-1 β elongation factor.

Saccharomyces cerevisiae only contains one gene encoding PP1_C. M.Stark (Dundee, UK) analysed the functions of yeast PP1_C (Glc7) by the characterization of temperature-sensitive alleles. He reported that the Glc7-10 mutant F135L shows a defect in kinetochore function, which is correlated with hyperphosphorylation of the kinetochore protein Ndc10 (Sassoon *et al.*, 1999). This mutant also displays a defect in vacuolar membrane fusion (Peters *et al.*, 1999) and in protein kinase Pkc1-controlled cell wall integrity.

T.Haystead (Charlottesville, VA) explored the effect of a deletion of the gene encoding the PP1-interacting protein Reg1p on the yeast phosphoproteome. Two-dimensional phosphoprotein mapping and mixed peptide sequencing enabled his group to identify hexokinase II as one of the hyperphosphorylated proteins (Alms *et al.*, 1999). Additional experiments confirmed that Reg1p targets hexokinase II for dephosphorylation by Glc7 and that a functional RVXF motif is essential for the Reg1P–Glc7 interaction.

Conclusions

Europhosphatase 99 has revealed once more that protein phosphatases rival protein kinases in their structural diversity and complexity, and are equally tightly regulated by hormones, growth factors and metabolites. It is also evident that the malfunctioning of protein phosphatases can result in severe defects such as the development of cancer. It seems likely that a major focus in the coming years will be to identify the physiological substrates of specific protein phosphatases. The two most promising approaches in this respect are the use of substrate-trapping mutants and phosphoproteome analysis. Another essential point will be to understand exactly how the regulatory proteins affect the properties of the catalytic subunits. Undoubtedly, a lot of this information will come from crystal structure analysis.

The anonymous evaluation forms that were filled out by the participants of Europhosphatase 99 show that the format and content of the conference were highly appreciated. There was also unanimous support for the continuation of the Euroconference series on a bi-annual basis. As a matter of fact, S.Klumpp has already agreed to organize the 7th Europhosphatase conference in Marburg (Germany) in 2001. We sincerely hope that the continuing success of the Europhosphatase meetings will stimulate our colleagues working on protein kinases to organize Eurokinase conferences in the alternating years.

Acknowledgements

The authors are supported by grants from the Howard Hughes Medical Institute (#75195-544001 to S.Z.) and by a Flemish Concerted Research Action (to M.B.).

References

Alms,G.R., Sanz,P., Carlson,M. and Haystead,T.A. (1999) Reg1p targets protein phosphatase 1 to dephosphorylate hexokinase II in *Saccharomyces cerevisiae*: characterizing the effects of a phosphatase subunit on the yeast proteome. *EMBO J.*, **18**, 4157–4168.

Angers-Loustau,A., Cote,J.F., Charest,A., Dowbenko,D., Spencer,S., Lasky,L.A. and Tremblay,M.L. (1999) Protein tyrosine phosphatase-PEST regulates focal adhesion disassembly, migration and cytokinesis in fibroblasts. *J. Cell Biol.*, **144**, 1019–1031.

Blomberg,I. and Hoffmann,I. (1999) Ectopic expression of Cdc25A accelerates the G₁/S transition and leads to premature activation of cyclin E- and cyclin A-dependent kinases. *Mol. Cell. Biol.*, **19**, 6183–6194.

Cantley,L.C. and Neel,B.G. (1999) New insights into tumor suppression: PTEN suppresses tumor formation by restraining the phosphoinositide 3-kinase/AKT pathway. *Proc. Natl Acad. Sci. USA*, **96**, 4240–4245.

Clotet,J., Gari,E., Aldea,M. and Arino,J. (1999) The yeast ser/thr phosphatases sit4 and ppz1 play opposite roles in regulation of the cell cycle. *Mol. Cell. Biol.*, **19**, 2408–2415.

Colledge,M. and Scott,J.D. (1999) AKAPs: from structure to function. *Trends Cell Biol.*, **9**, 216–221.

Das,A.K., Cohen,P.W. and Barford,D. (1998) The structure of the tetratricopeptide repeats of protein phosphatase 5: implications for TPR-mediated protein-protein interactions. *EMBO J.*, **17**, 1192–1199.

De Baere,I., Derua,R., Janssens,V., Van Hoof,C., Waelkens,E., Merlevede,W. and Goris,J. (1999) Purification of porcine brain protein phosphatase 2A leucine carboxyl methyltransferase and cloning of the human homologue. *Biochemistry*, **38**, 16539–16547.

Elchebly,M. et al. (1999) Increased insulin sensitivity and obesity resistance in mice lacking the protein tyrosine phosphatase-1B gene. *Science*, **283**, 1544–1548.

Flint,A.J., Tiganis,T., Barford,D. and Tonks,N.K. (1997) Development of 'substrate-trapping' mutants to identify physiological substrates of protein tyrosine phosphatases. *Proc. Natl Acad. Sci. USA*, **94**, 1680–1685.

Groves,M.R., Hanlon,N., Turowski,P., Hemmings,B.A. and Barford,D. (1999) The structure of the protein phosphatase 2A PR65/A subunit reveals the conformation of its 15 tandemly repeated HEAT motifs. *Cell*, **96**, 99–110.

Johnson,K.G., LeRoy,F.G., Borysiewicz,L.K. and Matthews,R.J. (1999) TCR signaling thresholds regulating T cell development and activation are dependent upon SHP-1. *J. Immunol.*, **162**, 3802–3813.

Kloeker,S. and Wadzinski,B.E. (1999) Purification and identification of a novel subunit of protein serine/threonine phosphatase 4. *J. Biol. Chem.*, **274**, 5339–5347.

Liu,C.W., Wang,R.H., Dohadwala,M., Schonthal,A.H., Villa-Moruzzi,E. and Berndt,N. (1999) Inhibitory phosphorylation of PP1 α catalytic subunit during the G₁/S transition. *J. Biol. Chem.*, **274**, 29470–29475.

Lopez-Girona,A., Furnari,B., Mondesert,O. and Russell,P. (1999) Nuclear localization of Cdc25 is regulated by DNA damage and a 14-3-3 protein. *Nature*, **397**, 172–175.

Maehama,T. and Dixon,J.E. (1999) PTEN: a tumour suppressor that functions as a phospholipid phosphatase. *Trends Cell Biol.*, **9**, 125–128.

Millward,T.A., Zolnierowicz,S. and Hemmings,B.A. (1999) Regulation of protein kinase cascades by protein phosphatase 2A. *Trends Biochem. Sci.*, **24**, 186–191.

Ogris,E., Du,X., Nelson,K.C., Mak,E.K., Yu,X.X., Lane,W.S. and Pallas,D.C. (1999) A protein phosphatase methyltransferase (PME-1) is one of several novel proteins stably associating with two inactive mutants of protein phosphatase 2A. *J. Biol. Chem.*, **274**, 14382–14391.

Peters,C., Andrews,P.D., Stark,M.J., Cesaro-Tadic,S., Glatz,A., Podtelejnikov,A., Mann,M. and Mayer,A. (1999) Control of the terminal step of intracellular membrane fusion by protein phosphatase 1. *Science*, **285**, 1084–1087.

Pingel,S., Baker,M., Turner,M., Holmes,N. and Alexander,D.R. (1999) The CD45 tyrosine phosphatase regulates CD3-induced signal transduction and T cell development in recombination-deficient mice: restoration of pre-TCR function by active p56^{lck}. *Eur. J. Immunol.*, **29**, 2376–2384.

Sassoon,I., Severin,F.F., Andrews,P.D., Taba,M.R., Kaplan,K.B., Ashford,A.J., Stark,M.J., Sorger,P.K. and Hyman,A.A. (1999) Regulation of *Saccharomyces cerevisiae* kinetochores by the type 1 phosphatase Glc7p. *Genes Dev.*, **13**, 545–555.

Seeling,J.M., Miller,J.R., Gil,R., Moon,R.T., White,R. and Virshup,D.M. (1999) Regulation of β -catenin signaling by the B56 subunit of protein phosphatase 2A. *Science*, **283**, 2089–2091.

Seipel,K., Medley,Q.G., Kedersha,N.L., Zhang,X.A., O'Brien,S.P., Serra-Pages,C., Hemler,M.E. and Streuli,M. (1999) Trio amino-terminal guanine nucleotide exchange factor domain expression promotes actin cytoskeleton reorganization, cell migration and anchorage-independent cell growth. *J. Cell Sci.*, **112**, 1825–1834.

Shtrichman,R., Sharf,R., Barr,H., Dobner,T. and Kleinberger,T. (1999) Induction of apoptosis by adenovirus E4orf4 protein is specific to transformed cells and requires an interaction with protein phosphatase 2A. *Proc. Natl Acad. Sci. USA*, **96**, 10080–10085.

Stewart,A.E., Dowd,S., Keyse,S.M. and McDonald,N.Q. (1999) Crystal structure of the MAPK phosphatase Pyst1 catalytic domain and implications for regulated activation. *Nature Struct. Biol.*, **6**, 174–181.

Voorhoeve,P.M., Hijmans,E.M. and Bernards,R. (1999) Functional interaction between a novel protein phosphatase 2A regulatory subunit, PR59 and the retinoblastoma-related p107 protein. *Oncogene*, **18**, 515–524.

Westphal,R.S., Coffee,R.L., Jr., Marotta,A., Pelech,S.L. and Wadzinski,B.E. (1999a) Identification of kinase-phosphatase signaling modules composed of p70 S6 kinase-protein phosphatase 2A (PP2A) and p21-activated kinase-PP2A. *J. Biol. Chem.*, **274**, 687–692.

Westphal,R.S., Tavalin,S.J., Lin,J.W., Alto,N.M., Fraser,I.D., Langeberg,L.K., Sheng,M. and Scott,J.D. (1999b) Regulation of NMDA receptors by an associated phosphatase-kinase signaling complex. *Science*, **285**, 93–96.

Yan,Z., Fedorov,S.A., Mumby,M.C. and Williams,R.S. (2000) PR48, a novel regulatory subunit of PP2A, interacts with Cdc6 and modulates DNA replication in human cells. *Mol. Cell. Biol.*, in press.

Zhang,J., Zhang,L., Zhao,S. and Lee,E.Y. (1998) Identification and characterization of the human HCG V gene product as a novel inhibitor of protein phosphatase-1. *Biochemistry*, **37**, 16728–16734.

Zuo,Z., Urban,G., Scammell,J.G., Dean,N.M., McLean,T.K., Aragon,I. and Honkanen,R.E. (1999) Ser/Thr protein phosphatase type 5 (PP5) is a negative regulator of glucocorticoid receptor-mediated growth arrest. *Biochemistry*, **38**, 8849–8857.

Received November 12, 1999; revised and accepted December 8, 1999